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OP 01 Topic Highlights

OP01

PRELIMINARY RESULTS OF A PHASE 1 STUDY OF XL184, A MET, VEGFR2 AND RET KINASE INHIBITOR (TKI), ADMINISTERED ORALLY TO PATIENTS WITH MEDULLARY THYROID CANCER (MTC)

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Background: XL184 is an oral TKI that strongly inhibits cell proliferation in MTC-derived cells harboring activated RET. Pharmacodynamic studies showed substantial inhibition of RET and MET phosphorylation in a MTC xenograft model.

Methods: Adults with advanced solid tumors received XL184 in a 3+3 study design. Primary objectives include evaluation of safety and pharmacokinetic parameters and maximum tolerated dose (MTD) determination. RECIST response was assessed on day 28 and every 8 weeks. Markers of anti-angiogenic therapy, tumor markers, and RET status are being analyzed. Adverse events as of the December 1, 2008 data transfer (AEs) and pharmacokinetic data are reported for all patients. Pharmacodynamic data and efficacy are reported for the 37 patient MTC subgroup.

Results: Eighty-five patients were enrolled. XL184-related AEs were primarily Grade 1/2. Grade 3 related-AEs in ≥2 patients include fatigue (9%), palmar-plantar erythema (PPE, 8%), diarrhea, increased AST and decreased weight (4% each), and increased ALT and hypertension (3% each). One report of a related Grade 4 AE included pulmonary embolism. The MTD of 175mg QD was determined based on dose-limiting toxicities of PPE, mucositis, transaminitis and increased lipase. The terminal half-life is ~100 hrs. XL184 induced changes in biomarkers of angiogenesis including PIGF, VEGF-A, sVEGFR2, and sMET. Of the MTC patients with measurable disease, 14 of 34 (41%) had a PR (9 confirmed, 26%) and the disease control rate (PR + SD > 3 months) was 84%. Response in MTC patients appears independent of RET status and most MTC patients have had reductions in plasma calcitonin and CEA.

Conclusions: XL184 is generally well tolerated, the MTD is defined. In MTC patients with measurable disease, 41% achieved a PR as a best response and the majority derived clinical benefit. A global phase 3 pivotal study of XL184 in MTC is ongoing.
THE THYROID HORMONE RECEPTOR ALPHA LOCUS AND BONE: A ROLE FOR THE CIRCADIAN CLOCK GENE REV-ERB-ALPHA

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Objectives: Thyroid hormone (TH) action is mediated via the TH receptors alpha (TRα) and beta. TRα is the predominant receptor in bone. A knock-in mutation, which leads to a 10-fold reduction in ligand-binding of TRα, results in delayed bone development and osteosclerosis in mice (Vennström et al., 2002). However, no human data on genetic variation in TRα and bone are available. We therefore studied the TRα locus in relation to bone mineral density (BMD), bone geometry and fracture risk in nearly 6000 Caucasian subjects.

Methods: 15 Polymorphisms were selected that covered THRA and the 10 kb surrounding region, which includes Rev-Erb-alpha, a circadian clock gene located on the opposite chromosomal strand partially overlapping TRα. We genotyped 5974 persons aged 55 years and older, of whom data on bone endpoints were available.

Results: All polymorphisms were in HWE and had no associations with TH levels. Male TRα-rs1568400-G allele carriers had a lower lumbar spine BMD (1.16±0.006 (mean ±SE) vs 1.18±0.006, p=0.018), a lower femoral neck BMD (0.89±0.006 vs 0.91±0.005, p=0.024) and a higher buckling ratio (14.0±0.05 vs 13.8±0.04, p=0.020), an indicator of bone instability.

Rev-Erb-alpha-rs939347-A allele homozygotes had a 1.41 times higher BMD-loss (p=0.003) and a 1.56 times higher risk of vertebral fractures (OR=1.64(1.08-2.48)) compared to carriers of the wildtype allele.

Rev-Erb-alpha-rs2269457-G allele carriers had a 1.12 times higher overall fracture risk (HR=1.16(1.02-1.32)) compared to non-carriers. Additionally, these carriers had a lower section modulus (1.14±0.006 vs 1.16±0.005, p=0.006), an indicator of bone bending strength.

Conclusions: Although replication is needed, we show that TRα-rs1568400 is associated with BMD and bone geometry. Moreover, polymorphisms in the TRα antisense-gene Rev-Erb-alpha (rs939347 and rs2269457) are associated with BMD-loss, bone geometry and fracture risk. Rev-Erb-alpha expression has been shown to influence splicing and expression of TRα, suggesting that the effects of Rev-Erb-alpha on bone might be TRα-mediated.
Objective: Due to the limitations of the cytopathological classification of thyroid nodules, the preoperative assessment of malignancy is a major clinical problem. microRNAs (miRNAs) are small (about 22 nucleotides), non-coding, and single-stranded RNAs. miRNAs regulate the translation and stability of specific messenger RNAs (mRNA) mainly by imperfect base pairing with 3' untranslated regions of the mRNA. In this way miRNAs are believed to regulate about 30% of all protein-coding genes in mammals. Since miRNAs are frequently changed during malignant progression, the identification of differentially expressed miRNAs could improve the diagnosis of malignant tumors. We therefore aimed to generate a miRNA-based classifier, which could distinguish between thyroid follicular adenomas and carcinomas.

Methods: The expression of miRNAs was examined on frozen tissue samples in 15 follicular adenomas, 12 fetal adenomas and 14 follicular carcinomas by microarray analysis and classifiers were generated by leave-one-out cross-validation. We have used Ncode™ Human miRNA Microarray V3 (mirBase 10,0) and Ncode™ Rapid miRNA Labelling System. Upon hybridization and washing, scanning was performed with an Agilent DNA Microarray Scanner.

Results: We show that a three-microRNA signature is sufficient to differentiate between follicular adenomas and carcinomas, with 92% sensitivity and 93% specificity for malignancies. Due to similarities in the miRNA expression patterns, it was not possible to differentiate between fetal adenomas and follicular carcinomas. For validation we examined eight follicular tumours (six adenomas and two carcinomas) originating from a different hospital. All, except one adenoma, which was classified as a carcinoma, were classified according to its original histopathological diagnosis.

Conclusion: A miRNA classifier can differentiate between follicular adenomas and carcinomas. For future perspectives we intend to apply the classifier upon the fine-needle aspirate as an additive diagnostic tool, trying to solve the clinical dilemma associated with follicular thyroid lesions.
OP04
CHARACTERISATION AND PLASTICITY OF ADULT STEM/PROGENITOR CELLS IN THE HUMAN THYROID
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We recently isolated a population of adult stem/progenitor cells in the normal human thyroid. The method was based on the enzymatic digestion of fresh surgical thyroid specimens, followed by culture of cells in the presence of EGF and bFGF. Self-replicating spheroid lines composed of clonally derived cells were obtained.

Objectives: We aimed to characterise spheroids and prove their plasticity.

Methods: Immunophenotyping by FACS analysis and RT-PCR were employed to characterize 12 generated lines. Spheroids were seeded in 3D collagen gel cultures in differentiation medium to verify their plasticity toward the thyrooidal phenotype. Co-culture with a neuroblastoma cell line was used to verify the plasticity toward the neuronal lineage. Experiments of mesenchymal differentiation of spheroids were also attempted. Spheroids were injected either subcutaneously or intra-organ in SCID mice to assess tumorigenicity.

Results: Among thyroid differentiation markers the different lines expressed in variable percentage thyroglobulin (Tg) and thyroperoxidase (TPO). TSH-receptor (TSH-R) and sodium iodide symporter (NIS) were never detected. Five lines lacked the expression of thyroid differentiation markers. Calcitonin was not detected, while somatostatin was expressed in 3/4 lines. Among the 'so-called' stem cell markers a subset of CD34+ CD45- cells was identified. Among 'stroma cell markers' CD73 was positive in 2/3 lines. mRNA of nestin and pluripotency markers Oct-4 and Nanog was detected. In differentiation conditions spheroids generated follicles secreting T4; they were positive for Tg and TPO, but negative for TSH-R expression. When co-cultured with neuroblastoma cells, spheroids produced progeny expressing the neuronal marker tubulin beta III. Cells appeared enlarged in their cytoplasmic content, sometimes with elongated shape, resembling the neuronal phenotype. Spheroids underwent adipogenic but not osteogenic differentiation. Xenografted SCID mice did not develop tumours.

Conclusions: A predominant functional type of stem/progenitor cells is resident in the human thyroid with intrinsic ability to generate thyroidal and non-thyroidal cells.
Background: Previous randomized trials have suggested an association between radioiodine treatment for Graves' hyperthyroidism and thyroid associated ophthalmopathy (TAO).

Methods: 313 patients with Graves' hyperthyroidism were randomized to iodine-131 (163 patients) or 18 months of medical treatment (150 patients). Patients were followed for four years. L-thyroxine was given early after start of treatment to prevent hypothyroidism in both groups. The primary endpoint was worsening or development of TAO. Possible predictive factors for ophthalmopathy, e.g. smoking were also analyzed.

Results: Worsening or development of TAO was significantly more common in the iodine-131 treated group (63 patients; 38,7 %) compared with medical treatment (32 patients; 21,3 %). The risk for de novo development of TAO was greater in patients treated with iodine-131 (53 patients) than with medical treatment (23 patients). However, worsening of TAO in the 41 patients (13,1 %) who had ophthalmopathy already before start of treatment, was not more common in the radioiodine group (10 patients) than in the medical treatment group (9 patients). Smoking was shown to influence the risk of worsening or development of TAO and smokers treated with radioiodine had the overall highest risk for TAO. However, the significantly higher risk of worsening or development of TAO in the radioiodine group compared to the medical treatment group was shown only in non-smokers, but not in smokers.

Conclusions: Radioiodine treatment is a significant risk factor for development of TAO in Graves' hyperthyroidism. Smokers run the highest risk for worsening or development of TAO irrespective of treatment modality.
A TWO-STEP CANDIDATE-GENE ASSOCIATION STUDY IDENTIFIES FOXE1 AS A LOW PENETRANCE GENE ASSOCIATED TO THYROID CANCER AND REVEALS THE UNDERLYING MECHANISM

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Thyroid cancer is believed to have a strong genetic component, but no high penetrance gene has been reported so far. In order to identify genetic factors related to thyroid cancer susceptibility we adopted a candidate gene approach, studying tag- and putative functional Single Nucleotide Polymorphisms (SNPs) in genes involved in thyroid cell differentiation and proliferation and genes found to be differentially expressed in thyroid carcinoma. A total of 768 SNPs in 97 genes were studied, using the Illumina GoldenGate Genotyping platform, in a Spanish series of 609 cases and 504 controls, the former comprising the largest collection of patients with this pathology from a single population. Association tests were performed on single SNPs and haplotypes to define susceptibility Papillary Thyroid Carcinoma (PTC) loci. SNPs in a Linkage Disequilibrium (LD) block spanning the entire FOXE1 gene showed the strongest evidence of association with PTC susceptibility (for the best tagSNP OR[per-allele]=1.47; 95%CI=1.23-1.75; \(P=2.4\times10^{-5}\)). This association were validated in a second stage of the study that included an independent Italian series of more than 400 patients and over 500 controls, genotyped by KASPar probes. In-silico analyses suggested a FOXE1 promoter variant to affect gene transcription, so functional assays focused on that variant. DNA-binding assays demonstrated that exclusively the sequence containing the polymorphic allele recruited the USF1/USF2 transcription factors. In addition, these proteins were part of a regulatory network involving CREB/CREM/DREAM transcription factors. Transfection studies
showed an allele-dependent transcriptional regulation of FOXE1. We propose a FOXE1 regulation model dependent on the promoter polymorphism genotype, demonstrating that this SNP is a causal variant in thyroid cancer susceptibility. These results not only confirm the recent suggestion of FOXE1 involvement in PTC, but also identify the causal variant and the underlying mechanism. Overall, our study attests to the efficacy of candidate-gene approaches in the GWAS era.
MILD IODINE DEFICIENCY: A PERSISTENT PUBLIC HEALTH PROBLEM IN BELGIUM

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Introduction: Previous surveys in Belgium have consistently shown that our country remains affected by mild iodine deficiency (MID). A majority of these surveys concerned children. The nutritional profile of children, however, differs from that in adults.

Objectives: To reassess the iodine status and thyroid function in an adult population of different ethnic origins residing in Brussels. The number of thyroidectomies for multinodular goiter (MNG) is also reported, as an indirect indicator of the impact of MID.

Methods: A stratified random sample of 1000 healthy subjects of Belgian, Moroccan, Turkish and Congolese descendant was obtained in Brussels. In a subset of 401 individuals, the iodine status and thyroid function was determined. The number of thyroidectomies for MNG, between 1999-2006, was obtained from a central governmental database.

Results: The median urinary iodine concentration (UIC) was 68 µg/L. The frequency of UIC values < 100 µg/L was 73.2 %, < 50 µg/L 31.4% and < 20 µg/L 1.5 %. There was no difference for UIC between subjects from different ethnic origins. The frequency of UIC < 50 µg/L was significantly higher when comparing the Fall-Winter to Spring-Summer periods (p=0.004). This season-related difference was confirmed after controlling for age and sex by multivariate analysis. Total number of thyroidectomies for MNG was 17.919 for the entire country and was 2-fold greater in the Southern, compared with the Northern part of Belgium.

Conclusion: The prevalence of MID remains elevated in the adult population of Brussels. MID constitutes a burden not only for individuals by affecting thyroid function, but also for the social security system, by the cost generated by treatment and follow-up of MNG. Therefore, a policy is urgently needed in Belgium to regulate and optimize the dietary iodine intake.
OP08
ASSESSMENT OF SUSTAINABILITY OF THE OBLIGATORY IODINE PROPHYLAXIS IN POLAND

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In 1997 the obligatory iodine prophylaxis, based on household salt iodization, has been introduced in Poland. Changing nutritional habits of the society may result in inadequate iodine consumption and reduced effectiveness of the prophylaxis. In 1999 the monitoring of the current prophylaxis model has been started.

The aim of the study was to assess the effectiveness and sustainability of the Polish obligatory iodine prophylaxis model.

Methods: The study included 7425 schoolchildren (3832 girls and 3593 boys) aged 6-12 years from the primary schools, investigated in 1999-2008. Thyroid volume was assessed by ultrasound (“Thyromobil” van provided by Merck KGaA, Darmstadt, Germany, equipped with Siemens Sonoline ultrasound system with 7,5 MHz linear probe). The thyroid volume normal values by Delange et al. have been applied. The iodine concentration in the casual urine morning samples was evaluated using Sandell-Kolthoff method.

Results: 4,6% of examined children (girls - 4,5%, boys - 4,7%) were diagnosed with goiter. Increased in the goiter frequency has been observed in the peripubertal children (girls aged 9-12 years - 6,5%, aged 6-8 years - 2,7%; boys: 5,3% and 4,2%; total: 5,9 and 3,4%, respectively). Median urinary iodine concentration was 92,5 ug/l. Urinary iodine concentration above 100 ug/dl was observed in 44,9% of examined children. The median urinary iodine concentration in year 1999, 2000, 2001,2002, 2003, 2005, 2006 and 2008 was: 84, 118, 84, 81,7, 95,7, 98,7 and 93,2 ug/dl respectively.

Conclusions: Polish model of the obligatory iodine prophylaxis resulted in decrease in goiter frequency in school-children below the endemic level, particularly in younger children. However the desired values of iodine urinary concentration have not been achieved, probably due to changed nutritional habits. Establishing of the new iodine sources is necessary to increase that microelement consumption.
Introduction: Thyroid hormones are essential for the proper development of the central nervous system. Thyroid and iodine deficiency during pregnancy are related to poorer development outcome of the progeny.

Objectives: To evaluate the influence of maternal thyroid function and iodine status throughout pregnancy on children's psycho-motor development.

Subjects and methods: The study was carried out at the Centro Hospitalar do Alto Ave - EPE, Guimarães, Portugal, between January of 2003 and December of 2005. We invited 131 pregnant women who were attending the antenatal clinic to assess thyroid function during pregnancy. Permission was given by the mothers to enroll their babies in the study. Demographic and clinical information were collected. The study was approved by the local research ethical committee.

Thyroid function (TT4, FT4, TT3, FT3, TSH, anti-TPO and anti-Tg antibodies) was evaluated by RIA and urinary iodine was measured by the ammonium persulfate method.

Infant development was assessed by the Bayley Scale of Infant Development at 12, 18 and 24 months, measuring Mental Development Index (MDI) and Psychomotor Development Index (PDI).

Statistical analyses were performed using the SPSS 15 software package. Comparisons were made using the t-test or the nonparametric Mann-Whitney test.

Results: Children from mothers displaying the lower levels of FT4 in the first trimester of pregnancy have lower PDI at 18 and 24 months; those from mothers with lower urinary iodine also in first trimester presented lower PDI at 12 months.

TT3 levels in the second trimester of pregnancy seem to have a clinical impact on children's PDI at 18 and 24 months.

Concentrations of TT4, FT4, FT3 and UI in the third trimester of pregnancy have significant impact on MDI and PDI at 12, 18 and 24 months.

Conclusions: Maternal thyroid function and iodine deficiency during all pregnancy influence the progeny's cognitive and motor development.
PREVALENCE OF HYPOTHYROIDISM IN ADULT SURVIVORS OF CHILDHOOD MALIGNANCY

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\textbf{Objectives:} Overall survival following treatment of childhood cancer has increased from 20 - 30\% during the 1960's to 75\% today. With the improvement of survival long-term follow-up of patients and their monitoring concerning endocrine complications of cancer therapy, including thyroid dysfunction, are of increasing importance. The aim of the present study is to determine the frequency of hypothyroidism amongst the patients within the British Childhood Cancer Survivor Study (BCCSS).

\textbf{Methods:} The BCCSS is a population based, long term follow-up study of childhood cancer survivors diagnosed between 1940 and 1991, and surviving at least five years. Self reported information on thyroid status collected by questionnaire was available for 10,091 patients.

\textbf{Results:} Amongst the whole cohort 7.7\% were hypothyroid. The patients at greatest risk were those treated for Hodgkin's disease (HD) (19.9\%), CNS neoplasms (15.3\%), Non-Hodgkin's lymphoma (6.2\%) and leukaemia (5.2\%). Patients were more likely to develop hypothyroidism if they had received radiotherapy for HD (p=0.0001) or a CNS neoplasm (p<0.00001) but not leukaemia (p=0.3). In these three patient groups the frequency of hypothyroidism was similar in men and women. Patients treated for HD, CNS neoplasm and leukaemia who remained on long-term follow-up were more likely to be diagnosed with hypothyroidism, 25.9\% vs 16.7\% (p=0.004), 30.5\% vs 8.0\% (p<0.0001) and 7.3\% vs 3.4\% (p<0.0001) respectively.

\textbf{Conclusions:} Hypothyroidism is a surprisingly common finding amongst adult survivors of childhood malignancy, particularly those who have received radiotherapy. The reduced frequency of hypothyroidism amongst patients not on long-term hospital follow up is of concern as it suggests that the diagnosis may be missed in a substantial number of patients cared for outside of specialized centres for follow-up of cancer late effects.
ANNUAL PATTERN OF SERUM TSH IN A RETROSPECTIVE CROSS-SECTIONAL AND LONGITUDINAL ANALYSIS OF OVER 20,000 RECORDS IN THYROXINE TREATED AND NON-TREATED SUBJECTS. EVIDENCE FOR SEASON-DEPENDENT REQUIREMENT FOR THYROXINE IN HYPOTHYROIDS

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Objectives: To evaluate whether thyroxine replacement should be adequately adjusted in line with season changes, we analyzed TSH monthly variation as an index of optimal therapy in patients with hypothyroidism.

Methods: A seven-year retrospective cross-sectional analysis was performed in over 20,000 TSH measurements. Data were grouped by months and included records from hypothyroid patients requiring substitutive or suppressive doses of thyroxine and euthyroid subjects referred for thyroid evaluation. A total of 237 thyroxine-treated patients who had two hormone measurements in both January/February and August/September while on the same thyroxine dose were also evaluated as a longitudinal study.

Results: Monthly median TSH values in thyroxine-treated patients varied from 1.30 mU/L in January to 0.70 mU/L between August to October (Kruskal Wallis test followed by Dunn’s test, P< 0.001), with no gender variations. No significant seasonal variation was found for FT4. Values of FT3 were significantly lower in January/February compared to August/September (P< 0.01). Longitudinal analysis confirmed the cross-sectional results. Euthyroid untreated patients did not show significant yearly TSH fluctuations (mean of medians = 1.34 mU/L). Thyroxine overtreatment (TSH < 0.1 mU/L for cancer patients, TSH < 0.4 mU/L for hypothyroids) was more frequent in August/September than in January/February whereas undertreated patients (TSH >0.4 mU/L for thyroid cancer, TSH >2.5 mU/L for hypothyroids) were more prevalent in winter months.

Conclusions: In a large cohort of thyroxine treated patients we observed a significant seasonal TSH fluctuation while no changes were detected in euthyroid untreated subjects. Unawareness of these seasonal variations may expose patients to unintentional over- or under- treatment, the latter being of special concern with thyroid cancer patients. A yearly TSH measurement may not be adequate for monitoring optimal thyroxine therapy throughout the seasons.
OP12

REPLACEMENT THERAPY IN HYPOTHYROIDISM: COMPARISON OF "HIGH-NORMAL" AND "LOW-NORMAL" TSH LEVEL

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Objective: The objective of this study was to analyze the features of adjustment of L-T4 dosage, aiming for a serum TSH concentration in the lower reference range (< 2 mU/L) in comparison with a serum TSH concentration in the upper reference range (> 2 mU/L).

Methods: We conducted a cohort study in 116 patients (18-55 y.o.) with primary hypothyroidism. Patients were divided into two groups: Group A (N = 68) with “low-normal” and Group B (N = 48) with “high-normal” TSH level. The scores for the Short-Form 36 (SF-36), Zung Self-rating Anxiety Scale, Beck Depression Inventory, symptoms of hypothyroidism, C-reactive protein and lipid profiles were analyzed.

Results: No significant difference (p>0.05) between groups of patients was demonstrated on lipid profile, prevalence of dyslipidemia, C-reactive protein, symptoms score, anxiety and depression levels. We revealed lower levels nearly of all scales of quality of life (except of mental health) (p < 0.05) among patients with low-normal and high-normal TSH level in comparison with euthyroid patients. There was no difference between patients with low-normal TSH level and control group in scale of mental health measured by SF-36 (p>0.05). And in group of patients with high-normal TSH the level of mental scale was lower than in euthyroid subjects (p< 0.05).

Conclusion: Compared with high-normal TSH level, replacement treatment with maintenance a serum TSH concentration in the lower reference range shows higher level of mental health, but no advantages in hypothyroid symptoms, lipid profile, C-reactive protein level and psychological tests.
OP13
DOSE-DEPENDENT ACUTE EFFECTS OF RECOMBINANT HUMAN TSH (RHTSH) ON THYROID SIZE AND FUNCTION. COMPARISON OF 0.1, 0.3 AND 0.9 MG OF RHTSH
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Context: Recombinant human TSH (rhTSH) can be used to augment the effect of radioiodine therapy for nontoxic multinodular goitre. Reports of acute thyroid swelling and hyperthyroidism warrant safety studies evaluating whether these side-effects are dose dependent.

Objective: To determine the effects on thyroid size and function of various doses of rhTSH.

Design: In nine healthy male volunteers the effect of placebo, 0.1, 0.3 and 0.9 mg of rhTSH was examined in a paired design including four consecutive study rounds.

Main outcome measure: Changes in thyroid volume (TV), determined by planimetric ultrasound, and thyroid function by serum TSH, freeT3, freeT4 and Tg levels.

Results: Following placebo or 0.1 mg rhTSH the TV did not change significantly from baseline at any time. At 24 and 48 hours after administration of 0.3 mg rhTSH TV increased by 37.4±12.3(SEM)% (p=0.03) and 45.3±16.1% (p=0.05), respectively. After 0.9 mg rhTSH TV increased by 23.3±5.8% (p=0.008) and 35.5±18.4% (p=0.02), respectively. The largest TV increase was observed between day 1 and 4 and showed considerable inter-individual variation indicating that some individuals are more prone to develop thyroid enlargement than others. The largest TV increase was positively correlated with the peak level of Tg (p=0.003), but not with the peak serum TSH, FT3 and FT4 levels. The increase in serum freeT3, freeT4, and Tg was greater when administering 0.3 mg compared to 0.1 mg (p=0.02) and when administering 0.9 mg compared to 0.3 mg (p=0.02). After 0.1 mg rhTSH the increase in freeT3 and Tg was not significantly different from placebo whereas the freeT4 increase was significantly higher (p=0.02 compared to placebo).

Conclusions: The effect of rhTSH on thyroid size and function is rapid and dose dependent. Adverse effects are unlikely to be of clinical significance, following doses of 0.1 mg or less.
IS REGULATION OF ENERGY EXPENDITURE BY THE NOVEL BILE ACID (BA) - THYROID HORMONE LINK ALSO RELEVANT FOR HUMANS?

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Rationale: A novel concept indicating a signalling role of BA in the control of energy metabolism was conceived by Watanabe and colleagues (Nature, 2006). The group reported that selected BA induce energy expenditure (EE) by promoting intracellular thyroid hormone activation in murine brown adipose tissue. The required G-protein coupled TGR5 receptors and enzymes are also expressed in human skeletal muscle. In the current study we investigated possible relationships between BA and EE in healthy subjects and in patients with liver cirrhosis who present with increased BA levels.

Methods: Ten healthy subjects (8m/2f, 43±9 y; BMI 26.4±3.5 kg/m²) and eight patients with liver cirrhosis and in situ porto-systemic stent shunt (6m/2f, 47±12 y, BMI 25.0±2.6 kg/m²) were investigated at baseline and after oral nutrition (300 kcal). EE was determined by indirect calorimetry (Deltatrac). BA and serum thyroid hormones (TSH, fT3, fT4) were assessed from the cubital vein in controls, and in patients additionally from the mesenteric vein and radial artery.

Results: Resting EE and thyroid hormones were normal in healthy subjects and patients. Baseline BA related to resting EE in healthy subjects (r= 0.746, p = 0.013) and in patients (venous: r=0.738, p=0.037; arterial: r=0.786, p=0.021, mesenteric: r=0.833, p=0.010). But only in patients EE related positively to the postprandial cumulative BA increase 45 min after the meal (mesenterial blood, r=0.715, p=0.071). TSH significantly decreased within the first 60 min postprandially in healthy subjects (p < 0.01) and in patients (p < 0.05). fT3 and fT4 did not change during the postprandial period.

Conclusion: Our data support serum BA as new players in human energy metabolism even in healthy individuals with normal BA levels. The comparable delayed TSH response to a nutritional BA stimulation in patients with liver cirrhosis and healthy subjects suggests a subacute central impact of BA on thyroid axis.
UNEXPECTEDLY WIDE RANGE OF TRANSCRIPTION ACTIVITY MEDIATED BY THYROID-HORMONE RESPONSE ELEMENTS IN THE MOUSE HEART IN VIVO

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Objectives: Key cardiac genes such as Myosin Heavy Chain α (MHCα) and sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2a) are transcriptionally regulated by thyroid hormone (T3) acting through its receptor bound to T3-response-elements (TRE). Since numerous other trans-acting factors modulate transcription, the potential of T3-signaling to contribute to the expression of these genes is a matter of debate. This issue is relevant given the recently described local regulation of T3 activity in the heart. The aim of this study was to investigate the dynamic range of cardiac T3-dependent transcription mediated by TRE's in vivo.

Methods: We determined cardiac T3-dependent transcription activity in mice by in vivo transfection of cardiomyocytes with a novel T3-response plasmid (pT3R). pT3R contains Firefly (FLuc) and Renilla (RLuc) luciferase genes driven by the same minimal promoter, except for the presence of two TRE's in the case of FLuc. The range of T3 responsiveness of pT3R was assessed by direct injection of the plasmid in the left ventricular (LV) wall of mice with serum T3 levels ranging from 0.02 nM (hypothyroid) to 90 nM (hyperthyroid). After three days, FLuc activity was determined and normalized to RLuc activity.

Results: FLuc expression increased 2-fold between 0.02 nM and the euthyroid T3 level of 0.5 nM. Unexpectedly, a further 5-fold stimulation was found over the entire serum T3 range up to the supra-physiological levels. MHCα mRNA expression, measured by RT-PCR, showed the same dependency on serum T3 levels, but this did not apply for SERCA2a.

Conclusions: Cardiac T3-dependent transcription activity was measured in vivo. A correlation was found with serum T3 up to supra-physiological levels, which reflects a potential dynamic range of endogenous T3-dependent promoters of at least some T3 responsive genes, however other factors must be expected to selectively interfere with T3-dependent stimulation of this gene.

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**OP16**

**IDENTIFICATION OF ROS-GENERATING NADPH OXIDASE NOX4 IN THYROID: ROLE IN DNA-DAMAGE RESPONSES INDUCED BY ONCOGENIC H-RAS**

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**Background:** Reactive oxygen species (ROS) are involved in different biological processes. Among ROS-producing systems, there are the NADPH oxidases NOX1, 2, 3, 4 and 5, DUOX1 and 2. To date, only DUOX1/2 have been described in the thyroid. p22phox, a sub-unit functionally associated to NOX1, 2, 3, 4, was found to be expressed in the thyroid suggesting that one of these NOX could be functional in this tissue. Moreover, it was shown in some cell types that activation of certain oncogenes such as Ras lead to an increase in intracellular ROS and that some NOX are involved in carcinogenesis. The constitutively activated forms of three Ras oncogenes (H, K and N-Ras) have been identified in thyroid tumors.

**Objective:** Evaluate the role of oncogenic Ras in ROS production catalysed by NOX/DUOX and their participation in the DNA damage signals induced by Ras.

**Results:** Nox4 mRNA expression analysed by real-time PCR, was detected in normal tissue and found significantly increased in differentiated cancers. NOX4 and its partner the p22phox protein displayed an identical pattern staining by immunohistochemistry. Using the human non tumoral thyroid cell line (Nthy-ori 3.1) with a conditional expression of H-Ras under the control of doxycycline, we observed that the expression of p22phox is positively regulated by H-Ras. Only Nox4 is expressed in these cells. Interestingly, knocking down p22phox expression with specific siRNA suppressed ROS production measured after addition of doxycycline confirming the involvement of p22phox in a ROS-generating system. Implication of p22phox in the activation of DNA-damage response was analysed. Phosphorylated histone H2A.X (γ-H2A.X), induced by H-Ras was inhibited by p22phox siRNA as well as other DNA-damage response markers including phospho-ATM and phospho-chk1.

**Conclusion:** An NADPH oxidase having p22phox as functional partner (Nox4 probably) plays a crucial role in the DNA-damage response induced by H-RasV12 in thyroid cells.
IDENTIFICATION OF SUMOYLATION SITES ON CCDC6, THE PRODUCT OF FIRST AND MOST FREQUENTLY OBSERVED RET-FUSED GENE, UNCOVER A MODE OF REGULATING CCDC6 FUNCTION

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Objectives: Sumoylation regulates the activity of substrates through covalent attachment to lysine residues of target proteins Here we describe a sumo-mediated regulation of CCDC6 gene product functions.

Methods: We identify three highly conserved SUMO modification sites in CCDC6, the product of first and most frequently observed RET-fused gene, also found rearranged with PDGFRβ in CMML (LK266NE, LK74IE and FK424RP). By site-directed mutagenesis K266R, K74R, K424R CCDC6 mutants were generated. CCDC6-SUMO-2 conjugates were detected by immunoblotting after co-immunoprecipitation in HEK293 cells. YFP-SUMO2 and CFP-CCDC6 interactions have been evaluated by fluorescence confocal analysis. TUNEL assay, Annexin V immuno-staining and PARP cleavage have been performed to detect apoptosis in CCDC6 wt and mutant expressing cells together with FLAG-tagged SUMO2. Luciferase assays and ChIP analysis have been performed in order to assess the reported CCDC6 mediated co-repression function and DNA binding of ATF members transcriptional activity, upon sumoylation.

Results: We detected sumoylated forms of CCDC6 at 95, 110 and 125 kDa, as a minor form. The mutations of all predicted lysines (KKK74/266/424RRR) resulted in the abolition of SUMO2/CCDC6 association, indicating that all residues are necessary for CCDC6 sumoylation. Sumoylation delocalize CCDC6 in the cytosol, and impairs CCDC6 apoptotic activity. Moreover, we observed that sumoylation serves to limit ATF2-mediated CCDC6's repressive function (Leone V and Fusco A, 2007, manuscript in preparation).

Conclusions: The N-terminus of CCDC6 is essential for activating TK domains of fusion proteins, upon chromosomal translocation, and acts as a dominant negative on the CCDC6 wt protein functions and localization. Then, we hypothesized that CCDC6 loss of function in thyroid and hematological tumors could contribute to the neoplastic transformation process. Indeed, it will be instructive to determine a potential role for Sumo-mediated post-translational modifications of the CCDC6 N-terminal fragments in the oncogenic activity of the described fusion proteins.
SHORT OLIGODEOXYNUCLEOTIDES (DGOLIGOS) TARGETING OF THE THRB GENE 5'-UTR MARKEDLY INCREASE THE EFFICIENCY OF TRBETA PROTEIN TRANSLATION - A THERAPEUTIC POTENTIAL OF SENSE/ANTISENSE BASED DGOLIGOS AS GIBB'S FREE ENERGY MODULATORS OF 5'-UTR STEM-LOOP STRUCTURE

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T3-action is mediated by TRalpha and TRbeta, which act as hormone-inducible transcription factors that regulate T3 target gene expression. T3 controls gene networks that regulate cell proliferation and differentiation during development. Levels of TRbeta are reduced in several tumors including liver, renal and thyroid carcinomas. Consequently, THRB has been identified as a tumor suppressor and reduced TRbeta protein levels are implicated in tumorigenesis. We previously showed that alternative splicing of the human TRbeta1 5'-UTR results in expression of mRNA variants that possess multiple upstream AUG codons located in folded regions of complex secondary structure. The upstream AUGs and folded regions inhibit TRbeta1 protein translation, and alternative splicing of the 5'-UTR may account in part for varying levels of TRbeta1 protein in different tissues.

The aim of this study was to develop strategies to increase TRbeta1 protein translation by targeting inhibitory folding regions in the THRB 5'-UTR. We designed short oligonucleotides complementary to THRB 5'-UTR sequences (dGoligos) that inhibited formation of secondary structures of the highest Gibb's free energy. This resulted in unwinding of 5'-UTR hairpin loops and reduced the accessibility of upstream AUGs to the translational machinery. To assess the effect of dGoligos on translation efficiency, we used pGL3 and pKS vector constructs containing THRB 5'-UTR variants upstream of a luciferase reporter. The effect of dGoligos was investigated in rapid translation system assays and in transient transfection studies using Caki-2 human renal carcinoma cells. Specific dGoligos resulted in an up to 6-fold increase in luciferase activity compared to control. Thus, sense/antisense based oligonucleotides targeting of the 5'-UTR facilitates increased protein expression from the human THRB gene.

This strategy has important implications in the development of novel gene therapy approaches aimed at increasing expression of inhibitory proteins such as tumor suppressors and complements other available genetic technologies such as oncogene silencing.
OP19
DIFFERENCES IN HYPOTHALAMUS-PITUITARY-THYROID (HPT) AXIS SETPOINT REGULATION BETWEEN GPB5 (THYROSTIMULIN) KNOCK OUT (GPB5-/-) AND WILD TYPE (WT) MICE
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Introduction: Recently, two novel glycoprotein hormone subunits were discovered: GPA2 and GPB5. The heterodimer of these subunits, coined thyrostimulin, activates the TSH receptor. Although adult GPB5-/- do not have an overt endocrine phenotype, it is unknown at present whether thyrostimulin plays a role setpoint regulation of the HPT-axis.

Objective: The aim of the present study was to evaluate the role of thyrostimulin in thyroid hormone metabolism during hypo- and hyperthyroidism in juvenile GPB5-/- and WT mice.

Methods: Hypothyroidism was induced by PTU/low iodide diet for three weeks. Hyperthyroidism was induced by giving T4 (5 mg/l) in drinking water for two weeks. Mice were killed at the age of six weeks. Serum T3 and T4 was measured using RIA. PreproTRH, deiodinase type 2 (D2) and 3 (D3) mRNA expression was measured in the hypothalamic periventricular region (PE), while TSH-β, D1 and D2 mRNA expression was measured in the pituitary.

Results: Surprisingly, serum T4 was 20% lower in euthyroid GPB5-/- compared to WT, whereas no differences were observed any of the other parameters. During hypothyroidism (similarly decreased serum T4 and T3 and hypothalamic D3 mRNA expression in both groups) the increase in pituitary TSH-β mRNA expression was 2-fold lower in GPB5-/- compared to WT. Hypothyroidism decreased pituitary D1 mRNA expression markedly in GPB5-/- but not in WT. During hyperthyroidism serum T4 was 25% lower in GPB5-/- compared to WT. However, pituitary TSH-β and D2 mRNA expression was suppressed to a similar extent in both strains. In the PE, hyperthyroidism resulted in a pronounced increase in D3 mRNA expression which was similar in both genotypes.

Conclusion: The observed differences between juvenile GPB5-/- and WT mice point to differential effects of thyrostimulin in setpoint regulation of the HPT axis.
ENDOSCOPIC DECOMPRESSION SURGERY IN 150 CONSECUTIVE PATIENTS WITH SEVERE GRAVES’ ORBITOPATHY

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Background: Disfiguring proptosis and optic neuropathy lead to cosmetic impairment and loss of vision in severe Graves’ orbitopathy (GO). Surgical decompression of the crowded orbit offers a valid therapeutic option.

Methods: 150 consecutive patients (median age 54 years, 113 female, 280 orbits) with severe GO had combined endonasal-transpalpebral decompression surgery at a university joint thyroid-eye-clinic. A complete multidisciplinary endocrine and ophthalmic assessment was performed pre-, three and 12 months post-operatively.

Results: Cosmetic reasons (196 orbits, 73%), dysthyroid optic neuropathy (DON, 67, 24%), and one corneal ulceration (0.4%) were the indications for surgery. Preoperatively, relative afferent pupillary defects and vision field defects were noted in 16% and in 43% of DON-patients but in 8% only after 12 months (p< 0.001). Median proptosis decreased from 23 mm (13-32) to 20 mm (10-30) and 19 mm (11-30) at 3 and 12 months, respectively (p< 0.001). Upgaze intraocular pressure dropped from 23 mmHg (10-44) by 4 mmHg at 12 months (p< 0.001). Median severity score (NOSPECS) declined from 7 points (2.5-13) to 4 (1-11) and 3.5 points (1-7) at 3 and 12 months (p< 0.001). NOSPECS and intraocular pressure decreased less in GO smokers vs. non-smokers (both p< 0.001). Two patients (1.3%) only complained about new-onset constant diplopia three months after decompression, while 22/150 (15%) patients reported de-novo inconstant or intermittent diplopia. Prisms and/or subsequent squint surgery corrected all cases of diplopia. Preoperative coexistent sinusitis (n=97, 65%) was successfully treated by decompression surgery in all cases. No major side-effects were registered.

Conclusion: The combined endonasal-transpalpebral orbital decompression is a safe and efficient therapy for severe GO.
PREGNANCY IS A NEGATIVE OUTCOME PREDICTOR IN DIFFERENTIATED THYROID CANCER

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Objective: Differentiated thyroid cancer (DTC) represents the second more frequent tumor among those diagnosed during pregnancy. In order to evaluate the outcome of DTC diagnosed during pregnancy, three groups of patients affected with DTC in relation to the timing of tumor diagnosis were studied:

Group 1 including 47 women with diagnosis of DTC at least 1 year after the delivery;
Group 2 including 15 women diagnosed during pregnancy and submitted to thyroidectomy during the second trimester or in the first year after delivery;
Group 3: 61 women diagnosed and treated before pregnancy or nulliparous.

The 3 groups did not differ as far as age and tumor staging concerns. In addition, immunohistochemical studies of ERalpha were performed in 39 PTC tissues from the 3 Groups.

Results: A significant better outcome was observed in patients of Gr. 1 and 3 compared to patients of Gr. 2 (P< 0.0001). Accordingly, at the multivariate analysis including well known outcome predictors and the belonging to Gr. 2 as input variables, and persisting/relapsing disease as end-point, the diagnosis of DTC during pregnancy or in the first year post-partum, resulted to be the more significant indicator of persistent disease (P=0.001). Interestingly, ER alpha expression significantly differ among the 3 Groups, being detected in 5/16 (31%) of Gr. 1, in 7/8 (87.5%) of Gr.2, and in 0/14 of Gr.3 tumors (P=0.01).

Conclusions: Thyroid cancer diagnosed during pregnancy was found to be significantly associated with persistence or relapse of the disease compared to patients, diagnosed before pregnancy or more than 1 year after the delivery, strongly suggesting that pregnancy has a negative impact on the outcome of thyroid cancer. The presence of ER in the majority of tumors during pregnancy firstly indicate that the poorer outcome of these cases could be related to the estrogen-mediated stimulus to grow.
TREND OF SERUM TG MEASURED WITH ULTRASENSITIVE ASSAY IN THE FOLLOW-UP OF DIFFERENTIATED THYROID CARCINOMA (DTC) PATIENTS WITH NO EVIDENCE OF DISEASE

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After initial treatment (thyroidectomy and 131I-ablation) some patients with DTC have basal low detectable serum thyroglobulin (Tg) levels without any evidence of disease (NED). The clinical significance of these Tg concentrations is unclear. Using new ultrasensitive Tg (US-Tg) assays the number of patients in this condition may even increase. It is generally believed that for decision making, the time trend of serum Tg levels is of paramount importance.

Objective: To analyze the time trend of serum US-Tg levels in 141 consecutive DTC patients with NED using US-Tg assay (Tg Access, functional sensitivity: 0.1 ng/ml).

Results: In the first evaluation (median 33.7 months after 131I ablation) 110/141 patients (78%, Group 1) had undetectable basal serum US-Tg levels (< 0.1 ng/ml) and 31/140 (22%, Group 2) had detectable serum Tg levels (range 0.11-0.91 ng/ml, median 0.17 ng/ml). All of them had negative Tg antibody and neck ultrasound. At the last follow-up (median 21 months after first evaluation) basal US-Tg was still undetectable in 108/110 patients (98,2%) of Group 1, with NED. In 2 patients (1,8%) serum Tg converted from undetectable to detectable (0.13 and 0.23 ng/ml) still with NED. In Group 2 (31 patients with basal serum Tg >0.1 ng/ml), at the last follow-up (median 21 months after first evaluation) serum Tg converted from detectable to undetectable in 18/31 patients (58%) while remained detectable in the same range in 13/31 patients (42%). Also in this Group there was NED.

Conclusions: Undetectable basal levels of serum US-Tg after initial treatment had a very good negative predictive value (98%) in the 21 months follow-up. Conversely, low detectable levels of serum US-Tg after initial treatment converted spontaneously to undetectable in more than 50% suggesting that low detectable US-Tg levels (< 1.0 ng/ml) have little clinical significance in patients without evidence of disease.
Medullary Thyroid Carcinoma (MTC) can be in sporadic (75%) or familial (25%) form. About 95% of familial and 50% of sporadic MTC present a germline or a somatic activating point mutation of RET proto-oncogene. The RET non mutated cases are still “orphans” of an oncogenic driver alteration although several mechanisms have been recently suggested. In this study we investigated both RET and chromosome 10 copy number variations in a large series of hereditary and mutated or not mutated sporadic MTCs, in order to identify possible mechanisms of RET activation in RET negative cases. We studied 63 MTC (13 familial and 50 sporadic) and RET mutation analysis on blood and frozen tissue DNA was performed by PCR and sequencing. Fluorescence in Situ Hybridization (FISH) for RET and for the centromeric region of chromosome 10 was then performed and results were validated by Real-time PCR. A RET germ line mutation was found in 13/13 (100%) familial cases and in 22/50 (44%) sporadic cases. After FISH analysis, RET and chromosome 10 alterations were found in 18/63 (28.5%) MTC: Four/13 (31%) familial and 14/50 (28%) sporadic MTC (p=NS). Among sporadic MTC, 11/22 (50%) were mutated and 3/28 (10.7%) were not (p< 0.003). The only type of alteration found in familial cases was the RET gene amplification. Data obtained were confirmed by Real-time PCR analysis. In conclusion, in our large series of MTC, we found several alterations either in RET gene and/or in chromosome 10 copy number and they were significantly more frequent in MTC harbouring a RET mutation. RET activating point mutation might induce a considerable rate of genomic instability causing an alteration of RET gene itself and/or entire chromosome 10. We can finally exclude that RET gene amplification could be an alternative mechanism of RET activation.
SULINDAC-SULFIDE ACTIVATES FOXO3A VIA INHIBITION OF THE PI3K/AKT AXIS AND INDUCES APOPTOSIS IN THYROID CARCINOMA CELLS

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**Background:** Overactivation of the PI3K/Akt pathway has emerged as a pivotal trigger of thyroid carcinogenesis. Recent findings suggest that PI3K/Akt mediated tumour-initiation critically involves the inactivation of the transcription factor FOXO3a contributing to an increased cellular proliferation of thyroid cancer cells.

**Objectives:** We sought to restore the transcriptional activity of FOXO3a with help of the non-steroidal anti-inflammatory drug Sulindac-Sulfide (SS). Furthermore, we assigned the appropriateness of SS to inhibit cellular proliferation in thyroid carcinoma cells.

**Results:** We demonstrate that SS exerts anti-proliferative effects via the activation of FOXO3a. Using an in-vitro model of thyroid epithelial cells (FRTL-5/FTC-133 cells) we found that SS promotes the nuclear accumulation of FOXO3a in thyrocytes with combined mRNA induction of the anti-proliferative FOXO-target genes Gadd45a and p27kip¹ and the proapoptotic FOXO-target gene Bim. Furthermore, we provide first evidence that SS mediated activation of FOXO3a depends on inhibition of the PI3K/Akt signalling axis. Intriguingly, we found that SS induces apoptosis and alters cell cycle distribution in thyrocytes. Finally, we show that these SS mediated effects are amplified by overexpression of FOXO3a and blunted after silencing FOXO3a.

**Conclusion:** Our data suggest that the chemopreventive action of SS involves the restoration of FOXO-activity via an inhibition of the PI3K/Akt signalling cascade. Therefore, treatment with SS might represent a promising tool for the clinical management of thyroid neoplasia with inherent overactivation of PI3K/Akt signalling. Finally, our results might help to uncover the molecular actions of SS, which are still incompletely understood.
ACTIVATION OF TYRO3/AXL TYROSINE KINASE RECEPTORS IN THYROID CANCER

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Objectives: We have previously shown that the chemokine receptor CXCR4 is overexpressed in human thyroid cancer and that the stimulation of CXCR4-positive thyroid cancer cells with CXCL12/SDF-1, one of the CXCR4 ligands, induces proliferation, resistance to apoptotic stimuli and invasive behaviour. To understand the molecular mechanisms underlying CXCR4-SDF-1 biological activity in thyroid cancer, we sought to identify CXCR4-transcriptional targets in thyroid cancer cells.

Methods: By using global gene profiling on human papillary thyroid carcinoma cells (TPC-1), we identified, as SDF-1 targets, two tyrosine-kinase receptors: TYRO3 and AXL. Their expression levels on human PTC samples and cell lines were evaluated by RT-PCR, IHC and western blot analyses. Receptor phosphorylation in cell lines was evaluated by TYRO3/AXL immunoprecipitation followed by western blot analysis with anti-phosphotyrosine antibodies. Activation of downstream signaling pathways was evaluated by using phospho-specific antibodies to ERK1/2, Akt, PLCγ. The function of TYRO3/AXL and of their ligand GAS6 in thyroid cancer cells was assessed by using blocking tools (Ab, RNAi) in proliferation and apoptosis assays.

Results: We found that TPC1 cells constitutively express TYRO3/AXL, and SDF-1 could increase their protein levels and tyrosine phosphorylation. The majority of the thyroid cancer cell lines we tested express TYRO3/AXL and, in some cell lines, the two receptors display high levels of tyrosine-phosphorylation, due to constitutive expression of GAS6. TYRO3/AXL are also overexpressed in human thyroid carcinoma samples with respect to normal thyroid. The inhibition of TYRO3 and AXL-mediated signaling by blocking reagents or RNA interference targeting the receptors or the ligand decreased cell proliferation and resistance to apoptotic stimuli. Accordingly, we show that the stimulation of thyroid cancer cells with GAS6 increased their proliferation and survival.

Conclusions: These results show that autocrine stimulation of TYRO3/AXL sustain proliferation and survival of thyroid cancer cells.
OP26
TRANSCRIPTOME OF MICRODISSECTED PAPILLARY THYROID CANCER CELL
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Aim: The transcriptome of papillary thyroid cancer has been well characterized by microarray studies, however, analysis of gross specimens, with significant content of stromal tissue, lymphocytic infiltrate and normal thyrocytes makes the interpretation of gene expression profile difficult. Until now only scarce data on gene expression in pure tumor cell populations are available.

Methods: Microdissection was performed on papillary thyroid cancer (PTC) fragments and normal thyroid tissue of 26 patients by PALM laser microdissector. PTC cells, normal thyrocytes, stromal tissue, lymphocyte infiltrates and medium-sized vessel populations were obtained. In more than half of them scarce amount of RNA was obtained. From 41 samples cRNA was synthesised by 3' IVT Express Kit (Affymetrix) , samples were hybridised to HG-U133 PLUS 2.0, (Affymetrix). QPCR was carried out by Universal Probe Library system (Roche).

Results: Both RNA and aRNA showed significant degradation of the material. Up to now, 12 microarray hybridisations were performed. Normalization of highly divergent microarray studies was a challenging task; thus, in the first step we analyzed non-normalized profiles. Up to now, QPCR performed in microdissected PTC cell populations confirmed the significant over-expression of RXRG, FN1, HIF1A, SPARC, ANXA1 and ANXA2 in PTC in comparison to normal thyrocytes, but not VEGF, CDH3, BNIP3, HGD, LRP4.

Conclusions: Among genes specifically changed in PTC cells, as shown by microarray study in microdissected cell populations, we indicate on the specific over-expression of annexins 1 and 2 (ANXA1 and 2), SPARC and HIF1. We confirm the over-expression of RXRG and FN1, genes previously shown to be specifically up-regulated in PTC cells. Over-expression of VEGF, CDH3 and LRP4 and down-regulation of BNIP3 and HGD in PTC seem not to be directly related to their mRNA content in pure PTC population. Supported by Ministry of Science and Higher Education (2P05A02230), M.O-W and A.R. contributed equally
The activating mutation BRAFV600E is a frequent genetic event in papillary thyroid carcinoma (PTC) that has been associated to extrathyroid extension and loss of NIS expression leading to subsequent radioiodide-refractory metastasis. Epithelial to mesenchimal transition (EMT) is required for tumour cells to invade and metastasize being TGFbeta a key regulator of this process. We have previously demonstrated that BRAF induces functional TGFbeta secretion in thyroid cells, mediating BRAF-induced NIS repression. The aim of this work was to study the role of this autocrine TGFbeta loop in EMT and invasion. Using PCCl3 thyroid cells conditionally expressing BRAFV600E, we analyzed mRNA, protein levels and subcellular localization of the main components that regulate EMT. Our preliminary results demonstrate that at least gain of vimentin and fibronectin, defining features of EMT, is strongly induced by BRAFV600E and this effect was blocked after inhibiting TGFbeta signalling. Similarly, BRAF induced matrigel invasion was also impaired after a TGFbeta inhibitor. Consistent with this process, TGFbeta and other key components of its signalling, such as TbetaRII and p-Smad 2, are overexpressed in a series of 52 cases of human PTC, suggesting a widespread activation of this pathway. In addition we observed that increased expression of TGFbeta is associated with PTC invasion, nodal metastasis and BRAF status. Moreover, in a subset of PTCs that were widely invasive, TGFbeta was preferentially expressed in the invasive front whereas NIS was expressed only in the centre of the tumour in an unequivocally inverse correlation between both proteins. We conclude that TGFbeta regulates EMT and tumour invasion in thyroid cancer.

Supported by Grants from the Spanish Ministry of Science and Innovation: SAF2007-60614, RD06/0020/0060 and Acción Transversal del Cáncer (FIS, Instituto de Salud Carlos III) (to P.S), by FIS ISCIII PI06-1231 (to A.DV)
THE ANALYSIS OF SILENCING MECHANISM OF THE TUMOUR SUPPRESSOR GENES - P16(INK4A), CDH1 AND RASSF1A - IN THYROID NEOPLASMS

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Background: Protein products of p16(INK4A), CDH1 and RASSF1A genes are involved in cell proliferation, differentiation and apoptosis via direct transcription regulation and initiation of the proapoptotic signalling pathways. Silencing of the suppressor gene expression by the promoter hypermethylation, can lead to abnormal cell divisions. Epigenetic silencing of p16(INK4A), CDH1 and RASSF1A genes has commonly been observed in human thyroid neoplasms.

The aim of the study: Assessment of the expression levels of studied genes in the benign and malignant neoplasms and testing of correlation between the expression levels, promoter hypermethylation and tumour type.

Materials & methods: Thyroid tissue samples (50-100 mg) were obtained from patients who underwent total thyroidectomy. Genomic DNA and total RNA was isolated. Expression levels of the studied genes were assessed in real-time PCR reaction, on ABI PRISM 7900 Sequence Detection System, using TaqMan probes. Evaluation of the promoter region methylation levels was conducted using CpG WIZ® Amplification Kit and direct sequencing on 3130xl Genetic Analyzer, after DNA bisulfide treatment. The analysis involved 3 groups: papillary thyroid carcinoma - PTC (15 cases), follicular adenoma - FA (8 cases) and medullary thyroid carcinoma - MTC (5 cases).

Results: The decreased expression levels of RASSF1A and CDH1 levels were observed in all analyzed groups when compared to controls (nodular goitre - NG). The differences between PTC group and FA and/or NG groups were statistically significant for both RASSF1A and CDH1. The expression level of p16(INK4A) was not suppressed. The presence of hypermethylation in promoter region of p16(INK4A), CDH1 and RASSF1A was found.

Conclusions: The obtained results indicate the role of epigenetic influence in the regulation of tumour suppressor gene expression in thyroid tumourigenesis.
AURORA B INHIBITOR AZD1152 INDUCES MITOTIC CATASTROPHE IN ANAPLASTIC THYROID CARCINOMA CELLS AND ENHANCES THE CELL KILLING ACTIVITY OF THE ONCOLOYTIC ADENOVIRUS DL922-947

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The Aurora proteins (A-B and-C) constitute a family of serine/threonine kinases that are essential for mitotic progression. Aurora-B kinase is one of the chromosomal passenger proteins that play a pivotal role in the control of spindle checkpoints and cytokinesis. Another function exerted by Aurora B is the phosphorylation of histone H3 on Ser 10. Aurora B gene is overexpressed in several human cancers. We have previously shown a high expression of Aurora B in anaplastic thyroid carcinomas.

AZD1152 is a specific Aurora kinase inhibitor with high selectivity for Aurora-B. The treatment of ATC cells induces an accumulation in G2/M, polyploidy and subsequent cell death by mitotic catastrophe, as suggested by the presence of micronuclei. Phosphorilation of Ser 10 on Histone H3 is significantly inhibited. The growth of xenograft tumours was significantly reduced.

We have already demonstrated that the replication selective oncolytic adenovirus dl922/947 is active against ATC cells. Cell cycle analysis of ATC cells infected with dl922/947 also show G2/M accumulation and polyploidy. Therefore, we have evaluated the effects of the combined treatment showing that AZD 1152 treatment enhances the cell killing activity of dl922-947. A 24 h pre-treatment with the drug is also able to potentiate the effects of the virus.

In treated cells, an increase of viral entry was not observed whereas an increase in sub G1 and ≥4N phase was detected. Combined treatment almost completely abrogates H3 phosphorilation on Ser 10.

Our data indicate the Aurora B inhibitor AZD1152 could be useful for the treatment of ATC alone or in combination with the oncolytic virus dl922-947.
Background: According to embryological studies thyroid follicles develop from aggregates of unpolarized precursor cells which must polarize, assemble cell-cell junctions, and form lumena. Tight junctions (TJs) are dynamic structure, subject to modulation and remodelling at different stages of epithelial tissue development playing an essential role in maintaining the integrity and the physiological function of thyroid follicles. Claudins (CLDNs), integral transmembrane proteins, have been identified as major components of TJs. Tissues are characterized by individual CLDNs patterns which composition and expression levels change during differentiation and tumor formation. To date, no data exist about CLDNs expression during thyroid ontogenesis.

Aim: To examine CDLN-1,3,4,5, and 7 immunohistochemical distribution and staining pattern in human fetal thyroid glands.

Materials and methods: Eighteen thyroid glands were obtained from human fetuses (gestational age range: 15-22 weeks). Immunostaining was performed using a panel of polyclonal (CLDN-1,-3) and monoclonal (CLDN-4,-5 and 7) antibodies.

Results: CDLN-7 was constantly expressed in all samples showing strong, diffuse and linear basolateral positivity. CDLN-4 and 5 immunostaining was similar to CDLN-7 but to a lesser degree of intensity. CDLN-1 exhibited a weak membranous staining in focal areas at periphery of the gland. CDLN-3 immunoreactivity was negative.

Conclusions: CDLN-7 and 4 are constitutively expressed in thyroid epithelium during ontogenesis at a level approximately equal from foetal up to adult thyroid tissue, thus suggesting an essential role in architectural stability of follicular cells. CLDN-1 immunostaining was expressed at the periphery of the foetal gland where the first follicles, containing colloid, are localized. Conversely, CLDN-1 is absent in adult normal tissue while up regulated in thyroid cancer, thus emerging as an oncofetal antigen and a potential marker of thyroid cancer. Our study also demonstrates CLDN-5 expression in thyroid gland, rising the need to evaluate its possible role in normal and neoplastic adult thyroid tissue.
Microchimerism is the presence of small populations of cells from one individual in another genetically distinct individual. This phenomenon can arise from pregnancy, organ transplantation, blood transfusion or from bidirectional cell trafficking between twins in utero. Microchimerism has recently been proposed to play a role in the pathogenesis of thyroid autoimmunity (TA). In that case, twins from opposite sex pairs (OS) should have an increased risk of TA.

**Aim:** To compare the frequency of thyroid autoantibodies in twin individuals from OS and same sex monozygotic (MZ) twin pairs.

**Design:** A case-control study of 240 individuals (120 females and 120 males) from OS twin pairs (cases) and 568 control individuals from MZ pairs (284 females and 284 males), matched for age.

**Methods:** Antibodies towards thyroid peroxidise (TPOAb), thyroglobulin (TgAb), and the TSH receptor (TSHRAb) were measured by routine commercial kits and considered positive if >60 U/ml, >60 U/ml and > 1.0 U/l, respectively.

**Results:** In both females and males, the frequency (%) of positive TPOAb, TgAb and TSHRAb was higher in twins from OS pairs (cases) than in twins from MZ pairs (controls), see Table.

<table>
<thead>
<tr>
<th></th>
<th>TPOAb</th>
<th>p-value</th>
<th>TgAb</th>
<th>p-value</th>
<th>TSHRAb</th>
<th>p-value</th>
<th>Any thyroid Ab.</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Female cases</td>
<td>15.0</td>
<td></td>
<td>5.0</td>
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<td>4.2</td>
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<td>18.3</td>
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<td>Female controls</td>
<td>7.4</td>
<td>0.018</td>
<td>1.1</td>
<td>0.023</td>
<td>0.7</td>
<td>0.026</td>
<td>8.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Male cases</td>
<td>5.0</td>
<td></td>
<td>4.2</td>
<td></td>
<td>3.3</td>
<td></td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>Male controls</td>
<td>1.4</td>
<td>0.034</td>
<td>1.8</td>
<td>0.171</td>
<td>1.1</td>
<td>0.203</td>
<td>3.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

In females, the odds ratio (95% limits) for the association between being an OS twin and positive TPOAb, TgAb, TSHRAb status was 2.1 (1.1-4.1), 4.7 (1.2-19.3) and 5.9 (1.1-31.2), respectively. Essentially similar results were obtained in males.

**Conclusion:** Both female and male twins from OS pairs, as opposed to MZ pairs have an increased risk of TA. These data indicate a pathogenic role of microchimerism in TA.
Human monoclonal autoantibodies (MAbs) to the TSH receptor (TSHR) with the characteristics of patient serum autoantibodies are valuable tools for studying TSHR-TSHR autoantibody interactions and to date, two such human MAbs, one with stimulating activity (M22) and one with blocking activity (5C9) have been isolated from different patients. We now describe two new human MAbs (one stimulating and one blocking) isolated at the same time using peripheral blood lymphocytes from a single patient with hypothyroidism who previously presented with hyperthyroidism. Heterohybridoma cell lines were prepared using standard techniques and two clones stably expressing antibodies that inhibit TSH binding to the TSHR were obtained, a human MAb (K1-18; IgG1/kappa) with TSHR stimulating activity and a human MAb with TSHR blocking activity (K1-70; IgG1/lambda).

Both K1-18 and K1-70 IgGs bind to the TSHR with high affinity (0.7x10\(^{10}\)L/mol and 4x10\(^{10}\)L/mol respectively) and inhibit binding of TSH, M22, 5C9 and each other to the receptor. Binding of both K1-18 and K1-70 to the TSHR is inhibited by patient sera with TSHR stimulating or blocking autoantibodies. K1-18 IgG has the ability to stimulate cyclic AMP production in CHO cells expressing the TSHR from 3.9x basal at 1ng/mL to 54x basal at 100ng/mL. K1-70 IgG inhibits TSH stimulation of cyclic AMP production in CHO-TSHR cells with 50ng/mL showing a clear effect. Furthermore, K1-70 has the ability to block the stimulating activities of M22, K1-18 and patient sera (15/15) with thyroid stimulating autoantibodies but has no effect on basal TSHR activity.

V region gene analyses indicated that K1-18 and K1-70 originated from different B cell clones and evolved independently from each other. Our study provides the first direct evidence that a mixture of TSHR stimulating and blocking autoantibodies can be produced by a patient at the same time.
Previous studies have indicated that a TSHR fragment consisting of amino acids 22-260 (TSHR260) is sufficient for high affinity binding of the thyroid stimulating monoclonal autoantibody (M22) and we now describe the interaction of a panel of TSHR autoantibodies (TRAb) positive patient sera with TSHR260. The procedure was based on a TSHR260-alkaline phosphatase fusion protein (TSHR260-AP) expressed in insect cells and a bridging-type ELISA. In the assay, test samples were incubated with full length TSHR coated onto ELISA plate wells (via a monoclonal antibody to the TSHR C terminus). After a wash step TSHR260-AP was added and this bound to the free binding site on any divalent TRAb bound (monovalently) to TSHR coated wells. After a further wash step, TSHR260-AP bound was detected by addition of substrate and reading at 405nm. 48/50 sera positive for TRAb by commercially available assays based on inhibition of TSH and/or M22 binding to full length TSHR were positive in the bridge ELISA and results correlated well with inhibition of M22 binding ($r=0.904$, $n=57$) and inhibition of TSH binding ($r=0.913$, $n=57$). Also, 5/5 sera with TSHR stimulating activity and 5/5 sera with TSHR blocking activity (both activities measured using TSHR transfected CHO cells) reacted well in the TSHR260 ELISA.

The two sera found non-reactive in the TSHR260-AP bridge ELISA were investigated further using TSHR260 coated onto ELISA plate wells (using a monoclonal antibody to TSHR260). Both sera inhibited M22 peroxidase binding to the TSHR260 coated wells strongly indicating that they reacted well with TSHR260 and the presence of IgG4 TRAbs (essentially monovalent) in these two sera could explain the discrepancy.

Overall therefore, TSHR260 contains major epitopes for TSHR autoantibodies in all TRAb positive patient sera studied irrespective of whether the sera have blocking or stimulating activities.
OP34
PPAR-ALPHA AGONISTS MODULATE TH1 AND TH2 CHEMOKINES IN THYROCYTES, FIBROBLASTS AND PREADIPOCYTES FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY
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Aims: Serum CXC Th1-chemokine CXCL10 is high in patients with active Graves' ophthalmopathy (GO). Human thyrocytes, orbital fibroblasts, and preadipocytes from GO patients produce large amounts of CXCL10 when stimulated by IFN-gamma and TNF-alpha; furthermore, PPAR-gamma agonists dose-dependently suppressed IFN-gamma +TNF-alpha-induced CXCL10 release, and it has been demonstrated that PPAR-alpha agonists may have an anti-inflammatory action.

Methods: The effects of IFN-gamma and TNF-alpha stimulation and of increasing pharmacological concentrations of PPAR-alpha agonists (ciprofibrate, fenofibrate, gemfibrozil) on Th1-chemokines CXCL9, CXCL10, CXCL11 and Th2-chemokine CCL2 secretion in primary cultures of thyrocytes, fibroblasts, and preadipocytes from GO patients were tested.

Results: In primary thyrocytes, fibroblasts and preadipocytes cultures, from patients with GO, CXCL9, CXCL10 and CXCL11 were undetectable in the supernatant. IFN-gamma dose-dependently induced CXCL9, CXCL10 and CXCL11 release, whereas TNF-alpha alone had no effect on them. However, the combination of TNF-alpha and IFN-gamma had a significant synergistic effect on CXCL9, CXCL10 and CXCL11 secretion. IFN-gamma or TNF-alpha or the combination of TNF-alpha and IFN-gamma significantly stimulated also the secretion of CCL2. Treatment of thyrocytes with ciprofibrate, fenofibrate or gemfibrozil (added at the time of IFN-gamma and TNF-alpha stimulation) dose-dependently inhibited the release of CXCL9 (by 44%, 36% and 23%, respectively; p< 0.001; % with respect to control), CXCL10 (by 49%, 41% and 27%, respectively; p< 0.001), CXCL11 (by 44%, 33% and 20%, respectively; p< 0.001) and CCL2 (by 47%, 35% and 21%, respectively; p< 0.001). Similar results were observed in fibroblasts or preadipocytes. PPAR-alpha agonists alone had no effect and did not affect cell viability or total protein content in thyrocytes, retrobulbar fibroblasts and preadipocytes.

Conclusion: In GO:
1) thyrocytes and retrobulbar cell types participate in the self-perpetuation of inflammation by releasing CXCL9, CXCL10, CXCL11 and CCL2 chemokines under the influence of cytokines;
2) PPAR-alpha activation plays an inhibitory role in this process.
CIRCULATING SELENOPROTEIN P CONCENTRATIONS ARE DECREASED IN PATIENTS WITH GRAVES' DISEASE AND CORRELATE INVERSELY TO SEVERITY OF ORBITOPATHY

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Selenium (Se) is an essential trace element for mammals and of prime importance for the regular functioning of the immune system. Recent studies have shown that Se supplementation is effective in reducing autoantibody load in patients with autoimmune thyroid disease. In general, Se in blood is composed of three main fractions, i.e., small compounds and selenosugars, selenomethionine-containing proteins and selenocysteine-containing selenoproteins. The latter are known to mediate most of the biological functions of Se in the mammalian organism. Selenoprotein P (SEPP) is the main circulating selenoprotein in mammals and controls Se transport via the blood. In order to characterize the Se status in patients with Graves' Orbitopathy (GO), we have analyzed their SEPP concentration and correlated it to the severity of the orbitopathy.

Serum samples from age and sex matched control subjects (n=92) and GO-positive Graves' disease patients (n=110, on average 4 control points per patient) were analysed by a sandwich assay developed recently in our lab. GO patients displayed significantly lower concentrations of circulating SEPP compared to controls (2.1 ± 0.6 versus 3.0 ± 0.5 mg/L, p< 0.0001). A weak inverse correlation was observed between disease activity (according to the CAS score) and SEPP concentrations (r=-0.22, p = 0.02). These data indicate that the Se status of the patients might be of functional relevance for disease progression. We conclude that the analysis of the Se status of GO-positive patients via SEPP determination yields a meaningful diagnostic parameter. Low SEPP concentrations identify those patients who are in need of the trace element and who might profit from an adjuvant Se supplementation.

Supported by the Deutsche Forschungsgemeinschaft and Deutsche Krebshilfe.
OP36
AUTOANTIBODIES TO THE INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR IN PATIENTS WITH GRAVES’ ORBITOPATHY
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Graves’ Orbitopathy (GO) occurs in about half of the patients with autoimmune hyperthyroidism and reflects a more severe course of Graves’ disease (GD). Autoantibodies to the TSH-receptor (TRAb) represent well-established pathogenic agents involved in both the deregulated thyroid growth and the typical orbital changes. Some evidence has been presented recently implicating autoantibodies to the IGF-1 receptor (IGF-1R) which is expressed on orbital fibroblasts as an additional pathogenic component of disease progression. In order to verify this theory, we have developed a new assay for the analysis of these autoantibodies and compared serum samples from age and sex matched controls (n=92) to GO-positive GD patients (n=110).

The assay is based on recombinant expression of human IGF-1R cDNA as fusion protein with firefly luciferase as reporter (IGF1R-LUC). Human embryonic kidney cells (HEK293) were stably transfected and used for immunoprecipitation analyses. Successful expression and predicted extracellular localization of the reporter on the cell membrane were verified during characterization of positive HEK293 clones. The recombinant fusion protein was suited for the specific detection and quantification of IGF-1R autoantibodies. The signals obtained from human sera proved to be stable upon dilution and repeated freeze-thaw cycles. The concentrations of IGF-1R autoantibodies varied strongly between individuals ranging from 200 to 150000 relative light units (rlu). Albeit, the average (median) IGF-1R autoantibody concentrations were not different (p=0.94) in controls (1016 rlu) and GO patients with untreated active disease (1065 rlu). In conclusion, circulating IGF-1R autoantibodies display a wide concentration range in both control individuals and patients. Additional disease parameters need to be compared to the IGF-1R autoantibody concentrations to test for pathophysiological meaningful interactions. In view of the large interindividual differences of this potentially growth-modifying immune parameter, further analyses are warranted to characterize its potential diagnostic value.

Supported by the Deutsche Forschungsgemeinschaft and Deutsche Krebshilfe.
Hashimoto's thyroiditis (HT) is sometimes associated with vitiligo and other autoimmune disorders. A prevalent proapoptotic Th-1 effector pathway of CD4+ lymphocytes characterizes cellular immunity in patients with HT. Vitiligo is an acquired hypomelanotic disorder whose autoimmune pathogenesis is controversial and is not known whether its presence may modify the T lymphocytes asset of HT.

**Objective:** This study was aimed at analysing and comparing intracellular cytokine profiles (Th1: IL-2 and IFN-γ; Th2: IL-4) in patients with HT isolated or associated with vitiligo and/or other autoimmune diseases.

**Methods:** Vitiligo has been diagnosed in 4.7% of 806 patients with HT and in 16.7% of 228 patients in whom HT was associated with other autoimmune disorders. Seventy patients (57W/13M; median age=39yrs) were enrolled in this study: 33 patients had isolated HT (group A), 11 had HT associated with vitiligo (group B) and 26 had HT associated with other autoimmune disorders (group C). Intracellular cytokines were identified and analyzed in peripheral lymphocytes by multiparameter flow cytometry.

**Results:** The number of IL-2+-cells was high in all patients but median values were similar in all groups (A:34.4%; B:32.8%; C:36.3%; p=ns). Patients with vitiligo and HT were characterized by a lower number of IFNγ+-cells than patients with HT isolated or associated with other autoimmune disorders (median= 13.5% vs 19.0% vs 29.9%; ANOVA p=0.0005). Interestingly, the number of IL-4+-cells was in the normal range in patients with isolated HT while clearly supranormal in both patients with HT and vitiligo (median: 5.0 vs 20.60%; p=0.0032) and in those with other autoimmune disorders (16.0%; p< 0.0001).

**Conclusions:** In the presence of vitiligo, a modified cytokines pattern in peripheral lymphocytes indicated a shift from the typical Th1 pattern of HT to Th1/Th2 pattern. These results also support the immune pathogenesis of vitiligo, at least when associated with HT.
PARATHYROID HORMONE-RELATED PEPTIDE IS DOWN-REGULATED AND APOLIPOPROTEIN L DOMAIN-CONTAINING 1 IS UP-REGULATED BOTH IN ACTIVE AND CHRONIC PHASE OF GRAVES’ OPHTHALMOPATHY

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Background: In the active phase of Graves’ ophthalmopathy (GO) an autoimmune response with infiltration of orbital tissue by immunocompetent cells triggers inflammation resulting in orbital edema and expansion of adipose tissue. These processes decline in the chronic phase where fibrosis predominates.

Objective: The aim was to identify genes which were up- or down-regulated in both active and chronic phase of ophthalmopathy.

Material and methods: Intraorbital adipose/connective tissue was collected from 10 patients with active GO operated within 1 year and 10 patients with chronic GO operated 4-12 years after the onset of GO. 10 thyroid healthy individuals undergoing restorative eye surgery were used as controls. Gene expression was studied with microarray using the HG-U133 Plus 2.0 GeneChip from Affymetrix and with real-time RT-PCR.

Results: In a pool of orbital mRNA from GO patients analysed with microarray we found 2 genes upregulated (fold change >2) and 8 genes downregulated (fold change > 2) in both chronic and active phase of GO compared to thyroid healthy controls. PTHrP (parathyroid hormone-related peptide) and APOLD1 (apolipoprotein L domain-containing 1) were confirmed with real-time RT-PCR in 10 individuals with GO in active or chronic phase and in 10 controls. APOLD1 was up-regulated 2-3 times (p< 0.05) and PTHrP was downregulated three times (p< 0.05) in both chronic and active phase as compared to the controls.

Conclusion: PTHrP is a multifunctional protein which may have evolved to regulate local tissue functions in contrast to PTH with systemic hormonal effects. One function of PTHrP is inhibition of adipogenesis and if downregulated it may increase adipogenesis, APOLD1 has been suggested a role in vascular permeability. Whether these observations are a consequence of the disease process or contribute to the pathogenesis of GO has to be further investigated.
IDENTIFICATION OF A NEW CLUSTER OF TRANMEMBRANE RESIDUES THAT ARE ESSENTIAL FOR SWITCHING THE THYROTROPIN RECEPTOR TO THE ACTIVE STATE

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For the thyrotropin receptor (TSHR), a G-protein coupled receptor (GPCR), numerous constitutively activating mutations (CAMs) have been identified as naturally occurring mutants or by site-directed mutagenesis.

In a previous study we identified two CAMs in extracellular loop 2 and transmembrane helix 6 (TMH6) of the TSHR. Their wild type amino acids interact with each other and they are involved in regulation of TSHR activation. Based on these findings and molecular models we hypothesised that other essential molecular switch(es) probably exist in close vicinity.

Indeed, we identified seven new CAMs (V421I; Y466A; T501A; M637C, W; S641A; Y643F; Y667A) in close proximity to the extracellular portion of the transmembrane helix bundle using modelling-driven site-directed mutagenesis. These CAMs are clustered together and are likely to be involved in molecular switches triggering between the non-active and active receptor conformations. Here, we highlight one particular position. Met637 is located at position 6.48 in TMH6, where in most other GPCRs a highly conserved tryptophan is well known to function as a side-chain rotamer switch to the active conformation. We describe an alternative rotamer switch, where a methionine performs a rotamer activation comparable to that of tryptophan. Despite the striking sequence difference between the TSHR and other GPCRs, our CAMs C637W and M637C show that parts of the transmembrane activation mechanism of the TSHR proceed at corresponding locations and in a similar manner to other GPCRs.

All of the identified amino acids are located in close proximity to the potential transmembrane binding pocket of low molecular weight (LMW) ligands for the TSHR. Thus, they indicate locations where a LMW agonist may interact and switch the receptor to an active conformation.

www.ssfa-gphr.de. These mutations lead to an active receptor conformation. Locations and properties of the mutated residues provide clues about differences between the non-active and active conformations.
THYROID STIMULATING HORMONE (TSH) IS A NOVEL, LOCALLY PRODUCED REGULATOR OF HUMAN EPIDERMAL BIOLOGY THAT UNDERLIES CLASSICAL HYPOTHALAMUS-PITUITARY-THYROID AXIS CONTROLS

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Context: Several elements of the hypothalamic-pituitary-thyroid axis (HPT) reportedly are transcribed by human skin cell populations, and human hair follicles express functional receptors for thyroid stimulating hormone (TSH); Thyrotropin releasing hormone (TRH), Thyroid hormone (TH).

Objective: Therefore, we asked whether the epidermis of normal human skin is yet another extra-thyroidal target of TSH, and whether epidermis even produces TSH. If so, we wished to clarify whether intraepidermal TSH expression is regulated by TRH and/or thyroid hormones, and whether TSH alters selected functions of normal human epidermis in situ.

Methods: TSH and TSH receptor (TSH-R) expression were analysed in the epidermis of normal human scalp skin by immuno-histochemistry and PCR. In addition, full thickness scalp skin was organ-cultured and treated with TSH, thyrotropin-releasing hormone (TRH) or thyroid hormones, and the effect of TSH treatment on the expression of selected genes was measured by Q-PCR and/or quantitative immunohistochemistry.

Results: Here, we show that normal human epidermis expresses TSH at the mRNA and protein levels in situ, and transcribes TSH-R. Intra epidermal TSH immuno-reactivity is up-regulated by TRH and down regulated by thyroid hormones. Though TSH-R immuno-reactivity in situ could not be documented within the epidermis, but in the immediately adjacent dermis, TSH treatment of organ cultured human skin strongly up regulated epidermal expression and activity cytochrome-c oxidase (COX).

Conclusions: Thus, normal human epidermis in situ is both, an extra pituitary source and an (indirect?) target of TSH signalling, which regulates defined epidermal parameters. Intra epidermal TSH expression appears to be regulated by the classical endocrine controls that determine the systemic HPT axis.
Thyroid stimulating hormone (TSH) regulates endocrine function of the thyroid gland within the hypothalamus-pituitary-thyroid axis via the TSH receptor (TSHr). TSHr is predominantly located on the follicular epithelial cells of the thyroid. However TSHr is also expressed in several other tissues. Recently using genome-wide expression profiling we found upregulation of expression of the TSHr during differentiation and lineage commitment in the human thymus, suggesting a role for TSH in T-cell development. RQ-PCR analysis confirmed that mRNA of the TSHr is present in thymocytes. mRNA of the hormone TSH was not present in the thymus. TSHr expression was absent on stem cells, mature B and T cells and monocytes.

Moreover stimulation of thymocytes with 100nM TSH activated signaling pathways of the TSHr measured by calcium flux indicating that the expression of the TSHr on thymocytes is functional.

To further investigate the functional role of TSH in T-cell development we used an advanced culture system, the fetal thymic organ culture (FTOC), in which human stem cells are cultured in mouse fetal thymic tissue. Cells were cultured for 14 days with TSH added biweekly. The effects of TSH on differentiation, apoptosis and proliferation were investigated.

After 14 days of culture stem cells were fully T cell committed with clear differentiation into immature single positive (ISP) and double positive (DP) thymocytes. Addition of TSH to the culture system resulted in an increase of ISP and DP thymocytes in 6 out of 7 cultures. Moreover, apoptosis was clearly reduced in cultures with TSH. No differences in proliferation were found.

The expression of the TSHr on human thymocytes is functional and within the haematopoietic system specific for thymocytes. TSH stimulates differentiation and thymic output and decreases cell death of human thymocytes in vitro. Thus, TSH acts as a previously unrecognized growth factor for human thymocytes.
DELIMITATION AND FUNCTIONAL CHARACTERIZATION OF THE BIDIRECTIONAL THOX-DUOXA PROMOTER REGIONS

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Objectives: The ThOX and DUOXA genes encode components of the oxidative machinery involved in thyroid hormone biosynthesis. Both of these genes are duplicated in mammalian genomes and are positioned in a head-to-head configuration, ThOX1 facing DUOXA1 and ThOX2 facing DUOXA2 respectively. In both cases, the intergenic region is small-sized which raises the question about what sequences control the transcription of each of the two genes within the pair.

Methods: The transcription start sites of all four genes were localized using the RLM-RACE technique. The intergenic regions were inserted in a specifically designed vector harbouring two distinct reporter genes facing each other for their functional investigation in transfection experiments conducted in the rat thyroid cell line PCC13. The binding of transcription factors was analyzed by EMSA using PCC13 cell extracts.

Results: The transcription start sites of ThOX1-DUOXA1 and ThOX2-DUOXA2 genes are separated by 63 and 170 base pairs respectively. Both of these intergenic regions exhibit bidirectional promoter activity in the transfection assay. Only the DUOXA2 transcription unit is preceded by a canonical TATA-box. Transcription of the ThOX2 gene initiates within the context of an INR element. The ThOX1-DUOXA1 intergenic region is highly GC-rich and contains a high affinity binding site for the transcription factor Sp1. The mutation of this Sp1 binding site dramatically reduces transcriptional activity in both directions.

Conclusions: The ThOX-DUOXA intergenic regions, particularly the ThOX1-DUOXA1 intergenic region, are of very limited size, however both of these regions are sufficient to promote transcription bidirectionally. As a single Sp1 binding site appears to play an identical role in the control of transcription of both ThOX1 and DUOXA1 genes, it suggests that the two genes share a common bidirectional promoter instead of relying on the action of distinct promoter elements embedded within each other in the intergenic space.
OP43
REGULATION OF THE THYROID DUAL OXIDASE ACTIVITY BY ESTROGEN, THYROTROPIN AND IODINE

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Objectives: Estrogen, TSH and iodine exert profound effects on thyroid gland physiology, thus we aimed to evaluate their possible effects on thyroid dual oxidase activity.

Methods: Wistar female rats were ovariectomized (OVX) or sham-operated (control) and daily treated with either 0.7mg (OVX+0.7) or 14mg (OVX+14) estradiol benzoate/100g body weight, s.c. After 21 days, animals were sacrificed by decapitation. Wistar male rats were divided into the control (water ad libitum), MMI (0.03% methimazole in the drinking water, 10 days, elevated TSH), and T4 (10mg/100g body weight, subcutaneously, 10 days, decreased TSH). Iodine overload was achieved by NaI treatment (0.05% NaI in the drinking water for 1, 4 and 6 days).

Results and conclusions: Serum estradiol levels were increased in both OVX and intact rats treated with the highest dose of estradiol. DuOx activity was reduced in OVX rats, when compared to control, while estradiol treatment of OVX rats normalized DuOx activity. When compared to control male rats, DuOx activity was significantly greater in control female rats. Our results show that estradiol might positively regulate DuOx H2O2 generating activity, which could contribute to the higher prevalence of thyroid nodules among women. DuOx activity was reduced in the thyroid of rats treated with MMI and increased when treated with T4. Therefore, the in vivo regulation of DuOx by TSH seems to be different from other thyroid differentiation markers, such as TPO and NIS. Treatment with NaI reduced DuOx activity, which was significant after 4 days of treatment. So, similarly to other proteins involved in thyroid hormone biosynthesis, iodine overload reduced DuOx activity. Thus, our data suggest a possible role of Duox in the escape from autoregulation by iodine, since the decrease in Duox activity by iodine treatment was transient.

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DUOX2 is the oxidase that generates $\text{H}_2\text{O}_2$ in the thyroid, a key step in the synthesis of thyroid hormone. Known regulators of DUOX2 function are TSH-induced cAMP signals, intracellular calcium and cytokine Interferon-$\gamma$. RNA editing is a posttranscriptional process that overwrites the encoded information of RNA to modify gene function or regulation. Most edited nucleotides are within intronic or untranslated regions. Only 13 mammalian genes have been discovered so far where editing results in an amino acid change. Hence, studies are scarce on the biological significance of single amino acid changes on the activity of “edited” proteins.

We identified a discrepancy between genomic (gDNA) and complementary (cDNA) DNA sequences of human DUOX2 at nucleotide position 74, where adenine (74A) is found in gDNA, and guanine (74G) in cDNA. This modification changes asparagine at position 25 (25N) into serine (25S). With the exception of chimpanzee, 25N/S is not conserved in DUOX2 mammalian orthologs.

In mRNAs from human thyroid (n=5), liver (n=2), kidney (n=3), skeletal muscle (n=3) and fibroblasts (n=2) guanine (74G) was identified. However, heterozygous signal (A and G) was present in RT-PCRs from lung (n=2), suggesting cell type-specific or incomplete editing in certain tissues. In a functional assay, the edited 25S-DUOX2 protein is 60% more active in $\text{H}_2\text{O}_2$ production than unedited 25N-DUOX2 protein, suggesting functional advantage of the mRNA editing process.

In conclusion, a functionally critical position (N/S site) of the human DUOX2 oxidase is controlled by Adenine to Inosine (A-to-I) RNA editing. This editing seems a relatively recent achievement in mammalian evolution. Complete RNA editing was found in thyroid and 4 additional tissues, but heterozygous A/G is present in lung, suggesting “relaxed” or cell-specific editing of DUOX2 in cellularly heterogeneous tissues. In the thyroid, 25S-DUOX2 represents a more efficient generation of hydrogen peroxide, a rate-limiting step in thyroid hormonogenesis.
Thyroid-stimulating hormone (TSH) is the primary regulator of mammalian thyroid function and growth. In previous studies, goitrogen treatment of amphibian tadpoles was shown to increase the expression of known TSH-responsive genes and to cause thyroid hyperplasia, suggesting a conserved role of TSH in non-mammalian vertebrates. In this study, we used ex vivo organ culture of thyroids from X. laevis tadpoles to study the role of TSH signalling in the regulation of thyroidal gene expression.

Thyroids from prometamorphic tadpoles were pre-incubated for 16-18 h in serum-free medium followed by incubation in serum-free medium containing bovine TSH, forskolin or cAMP analogues. We further analyzed the effects of insulin (10 µg/ml) supplementation on TSH response patterns. Gene expression profiles were analyzed using real-time PCR. In thyroids cultured without TSH, differentiation marker (slc5a5, tpo, tshr) expression decreased rapidly, whereas TSH addition (1 mU/ml) rapidly increased their expression and concentration-dependent increases were evident for 0.25-1.0 mU/mL TSH. In contrast, TSH treatment did not change expression of proliferation markers (mcm2, pcna, kif2c). Histology further showed absence of hyperplastic changes in TSH-treated thyroids. Insulin supplementation did not alter the TSH response of differentiation markers, but enhanced the TSH-inducibility of various other transcripts. Basal expression of proliferation markers was increased by insulin supplementation, but TSH still failed to increase their expression. Forskolin mimicked the effects of TSH. Co-incubation of thyroids with TSH and Rp-8-Br-cAMP (PKA inhibitor) diminished the TSH-induction of differentiation markers but, surprisingly, resulted in increased proliferation marker expression compared to TSH alone. Our results demonstrate a central role for the TSH-cAMP-PKA pathway to regulate differentiation marker expression in amphibian thyroids. In our model, insulin supplementation resulted in a TSH-induced expression profile that closely reflected the in vivo effects pattern. Further, our data indicate a unique regulation of genes related to cell proliferation in the amphibian thyroid.
TGF-beta signaling pathway is a negative regulator of follicular cell growth and how thyroid cancer cells evade its inhibitory signal remains unclear. Micro RNAs (miRNA) are a new class of small non-coding RNAs, involved in posttranscriptional gene regulation through imperfect pairing on the 3'UTR region of the target mRNA. MiRNAs play crucial roles in cancer acting as oncogenes and tumor suppressor genes. Our group is particularly interested in miRNAs that could modulate the TGF-beta pathway and questioned whether miR-146b, which is overexpressed in papillary thyroid cancer can contribute to deregulate this pathway. Firstly we performed a computational search for predicted targets of miR-146b using TargetScan and Pictar programs, and found that miR-146b putatively binds the 3'UTR region of Smad4, an important member of TGF-beta signaling pathway. Then we generated a stable rat follicular cell line, PC-146b, which harbors a tetracycline(TET)-responsive system, that conditionally drives the expression of miR-146b through the addition of TET to culture medium. Treatment of PC-146b cells with TET induced more than 4-fold miR-146b expression. MiR-146b over-expression significantly decreased Smad4 at mRNA (-55%) and protein (-50%) levels, when compared to untreated cells, and also down-regulated the expression of Smad2 and Smad7, but not Tgf-beta1. Since there are reports of over-expression of miR-146b in TPC-1, papillary thyroid carcinoma cell line, we used an anti-miRNA oligonucleotide that specifically inhibited miR-146b expression in TPC-1 (93% of inhibition). Depletion of miR-146b induced significant increase of Smad4 (+83%), Smad2 (+42%) and Smad7 (+76%) mRNA levels. 

**In conclusion:** The controlled induction of miR-146b in follicular cells allowed us to detect the influence of this miR-146b on TGF-beta signaling pathway, also confirmed by RNA interference approach in papillary thyroid cancer cells. Our data suggests an oncogenic role of miR-146b in thyroid follicular cells.

Supported by FAPESP and CNPq Grants.
Objective: To describe the epidemiology of subtypes of hyperthyroidism.

Design: A prospective population-based study, monitoring a well-defined cohort in Denmark, Aalborg with moderate iodine deficiency (n = 311,102) and Copenhagen with mild iodine deficiency (n = 227,632).

Methods: A laboratory monitoring system was used to identify subjects with thyroid function test suggesting overt hyperthyroidism. For all subjects we collected information on medical history and therapy, thyroid scintigraphy, and thyroid hormone receptor antibody (TRAb) measurements in order to verify primary overt hyperthyroidism and to subclassify hyperthyroidism into nosological disorders.

Results: During a four year period (2,027,208 person-years of observation) we verified 1,682 new cases of overt hyperthyroidism. Incidence rate (standardized to the Danish population, SIR, per 100,000 person-years) was 81.6 in the combined cohort, higher in Aalborg (96.7) compared to Copenhagen (60.0), corresponding to a standardized incidence ratio SIRR (95%-confidence-interval) of 1.6 (1.4-1.8). Nosological types of hyperthyroidism: multinodular toxic goitre (MNTG) with no measurable TRAb 43.6%, Graves´ disease 37.3%, solitary toxic adenoma 6.5%, TRAb+ MNTG 5.4%, subacute thyroiditis 2.3%, post-partum thyroid dysfunction 2.2%, amiodarone-associated hyperthyroidism 0.8%, hyperthyroidism after previous thyroid radiation 0.7%, lithium-associated hyperthyroidism 0.7%, and hyperthyroidism caused by various other factors 0.6%. The higher incidence of thyrotoxicosis in Aalborg (moderate ID) compared to Copenhagen (mild ID) was caused by higher occurrence of MTNG without positive TRAb (SIRR = 1.9(95%CI=1.6-2.2)), TRAb+ MNTG (4.7(3.2-13.3)), STA (2.4(1.4-3.3)) and amiodarone-associated thyrotoxicosis (7.2(1.1-65.5)), whereas no difference was observed with regards of the other subtypes of thyrotoxicosis. Lifetime risk for hyperthyroidism was 6.5% (female-male-ratio: 10.5%/2.4%). Female preponderance was observed for all subtypes except for amiodarone-associated hyperthyroidism (more men) and STA (no difference).

Conclusion: The occurrence of overt hyperthyroidism was relatively high in Denmark. The higher incidence of hyperthyroidism in the region most iodine deficient, was quantitatively due to more cases with MNTG and STA.
VARIATION IN PHENOTYPIC APPEARANCE OF GRAVES' DISEASE: EFFECT OF GENETIC ANTICIPATION AND DURATION OF COMPLAINTS

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Objective: Both genetic and environmental factors contribute to susceptibility of Graves' disease. In this study we evaluated whether the duration of symptoms or a positive family history of autoimmune thyroid disease (AITD) are related to specific phenotypes in patients with a first episode of Graves' hyperthyroidism (GH).

Design: Cross-sectional multicentre observational study.

Patients: 263 consecutive untreated patients with a first episode of GH were included. Biochemical and clinical severity of GH was evaluated. Participants were asked to complete questionnaires about environmental factors (smoking behaviour, use of estrogens, stress etc.), the duration of symptoms (interval between start of symptoms and date of referral) and family history for AITD. We ascertained the autoimmune nature of thyroid disease in affected relatives. Family History Scores (FHS; high score indicating a close genetic relationship and/or a large number of affected relatives) were calculated for patients with a positive family history for AITD.

Results: The peak incidence for the diagnosis of GH was 2-3 months after onset of symptoms (32% of patients). Duration of symptoms was negatively associated with age (P for trend = 0.04). A positive family history for AITD was present in 42.6% of patients. Patients with the highest FHS were more often male (P=0.01) while age at onset was lower (P=0.02) compared to patients with a lower FHS. Among patient groups with different FHS, no differences were found in exposure to environmental factors, nor in clinical or biochemical severity of hyperthyroidism.

Conclusion: Our study does not support the hypothesis that a short duration of thyrotoxic symptoms until diagnosis is related to more severe hyperthyroidism in Graves' disease. We have found supporting evidence for the existence of genetic anticipation in Graves' disease by means of a lower age of onset in the group with the highest FHS.
OP49
COMPARISON OF R-HUMAN-TSH AND MONOCLONAL ANTIBODY-M22 BASED ASSAYS FOR THE MEASUREMENT OF TRAB IN PATIENTS WITH THYROID DISEASE

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The best biochemical marker of Graves´ disease (GD) is the presence in serum of autoantibodies to the TSH receptor (TRAb).

Aim: The aim of this study was to compare the clinical performances of two sensitive TRAb assays in widespread clinical. In one assay (M22-TRAb) TRAb compete with a biotin labelled monoclonal antibody (M22) against the TSH-receptor in binding to immobile porcine TSH receptors. In the other assay (H-TRAb) TRAb compete with labelled bovine TSH on binding to immobile recombinant humane TSH-receptors.

Method: H-TRAb and M22-TRAb were measured in patients with new hyperthyroidism due to GD (n = 106), or multinodular toxic goitre (n = 93), and patients with new primary autoimmune hypothyroidism (n = 100), and healthy controls (n = 100). Patients were consecutively included from a population survey.

Results: ROC curves indicated a high sensitivity and specificity of the two assays (area under curve, H-TRAb: 0.977 (CI: 0.954-1.00); M22-TRAb: 0.979 (CI: 0.957-1.00). The two assays identified nearly the same TRAb positive patients, though large differences in TRAb values were obtained in some individual patients. Values were in average 2.5 times higher with the H-TRAb assay compared to the M22-TRAb assay corresponding to recommended cut-off values of 1.0 IU/L and 0.4 u/L. The H-TRAb assay had a considerably lower intra-assay CV of 3.8 %; M22-TRAb: 9.5 % (p < 0.01).

Conclusion: Both assays had a high sensitivity and specificity for diagnosing GD. In individual patients the ratio between results obtained using the two assays varied widely. Thus, results obtained using one assay can not be quantitatively transformed to values obtained using the other assay. H-TRAb had a considerably lower intra-assay CV.
RADIOIODINE THERAPY COMBINED WITH LITHIUM CARBONATE INCREASES CURE RATE IN PATIENTS WITH GRAVES' DISEASE

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Background: Radioiodine (RAI) is a well known and effective treatment for Graves' hyperthyroidism. Lithium carbonate (Li) can decrease the release of iodine from the thyroid gland increasing its intrathyroidal time of permanence. We have previously reported that RAI+Li was associated with a prompter restoration of euthyroidism. However, it is unknown whether patients treated with RAI+Li have a higher cure rate of hyperthyroidism than those treated with RAI alone. The aim of the study was to compare the cure rate of RAI alone versus RAI+Li in a large cohort of patients with Graves' disease.

Patients and methods: We prospectively enrolled 650 patients (506 women, 244 men; F/M ratio 2:1), aged 17-77 years (mean age 44±13 years), with Graves' disease of recent onset with mild or absent Graves' ophthalmopathy between January 2005- December 2007. Three hundreds and fiftyfour patients were treated with RAI alone (group 1) and two 296 patients were given RAI+Li (group 2) (900 mg/day 5 days before RAI and continued 7 days thereafter). All patients received metimazole to control hyperthyroidism, which was withdrawn 5 days before RAI. Thyroid function was frequently evaluated in all patients for at least 12 months after RAI. Cure rate was evaluated at 12 months after RAI by the Fisher's exact test; rapidity of cure was evaluated by Kaplan-Meyer analysis.

Results: Cure of hyperthyroidism was obtained in 303 of 354 patients (86%) of group 1 and in 271 of 296 patients (93%) of group 2 (p=0.04). The median time to reach euthyroidism was 90 days in group 1 and 45 days in group 2 (p< 0.0001). No major side effects were observed in group 2 patients.

Conclusions: Lithium increases the efficacy of RAI in patients with Graves' disease, increasing the cure rate of hyperthyroidism; in addition, it can be reached in a shorter period than in patients treated with RAI alone.
SURGERY RATES AND USE OF RADIOIODINE FOR BENIGN THYROID DISORDERS FOLLOWING IODIZATION - A NATIONWIDE STUDY

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Objectives: Iodine fortification was introduced in Denmark in 1998, but not effectively implemented until 2000. Denmark was previously an area of established mild to moderate iodine-deficiency (ID). The aim of this study was to monitor the effect of iodization on the utilization rate of surgery and radioiodine (¹³¹I)-therapy for benign thyroid disorders.

Methods: Nationwide register study covering the period 1990-2007. Information on surgery was obtained from the Danish National Patient Registry, and information on use of ¹³¹I-therapy was subtracted from the National Institute of Radiation Protection. Nationwide treatment rates are presented for surgery and for ¹³¹I-therapy, both separately and as a combined rate. Furthermore rates are presented for regions of prior mild and moderate ID.

Results: The combined treatment rate increased 20% in the area of previous moderate ID in the first years of iodine fortification (1998-2001). After 2002, the number decreased, declining below pre-fortification level in 2004, without reaching a new steady state. In the region of mild ID a slight decline was observed from 1994 and throughout the period. When looking at surgery and ¹³¹I-therapy separately, it appears that the changes are primarily seen for radioiodine and only in the region of prior moderate ID, whereas surgery rates are almost constant.

Conclusions: Iodization caused a temporary increase in use of radioiodine in the area with moderate ID prior to iodine fortification, probably as a result of iodine induced hyperthyroidism. Even though prior studies have shown a regression in thyroid size after iodization, this decrease is probably too small to affect a distinct decrease in use of surgery. It may take a generation before the positive effects of iodization become evident as regards surgical treatment. Iodization should be regarded as a preventive measure not as treatment.
Large toxic nodular goitre represents a limitation for radioiodine treatment (RAI): typically nodules larger than 5 cm undergo surgical intervention; large nodules, furthermore, even smaller than 4 cm, often require activities of 131I greater than 600 MBq, and cannot be treated on an out-patient basis. To overcome this problem we developed a protocol combining Percutaneous Laser thermal Ablation (PLA) with 131-Iodine.

16 patients (10 females and 6 males, mean age 62 ± 15 years) at high surgical risk (11) or refusal of surgery (5) with large autonomous nodules were recruited. Thyroid function (serum free T4, free T3 and TSH) was assessed before and after PLA, and after RAI.

SPET thyroid scans with 99mTc-pertechnetate were performed to determine the volume of active thyroid parenchyma. At time of entry 12 patients were treated with anti-thyroid drugs (ATD) and 4 had TSH levels very low. After PLA, hormonal pattern, SPET thyroid scan and 131-Iodine uptake were repeated.

In 3 patients PLA was followed by euthyroidism and RAI was not necessary. In the remaining patients active volume was reduced of 27% ± 8% (from 48 ± 16 ml before PLA to 35 ± 13 ml after PLA). Uptake values at 24h showed a reduction from 60% ± 17% to 54% ± 16%, due to smaller volume of functioning thyroid tissue. A significant reduction in radioiodine dose (20,5% ± 7,1 %) was obtained. All patient could be treated on an out-patient basis with doses lower than 600 MBq. After a follow-up of 27 ± 8 months 12 out of 13 patients and the 3 pts who did not undergo RAI had persistent euthyroidism; only one needed L-Thyroxine therapy.

PLA and RAI showed to be an effective alternative to surgery, especially in older patients and in patients at high surgical risk.
LONG TERM OUTCOME OF GRAVES’ OPHTHALMOPATHY (GO) TREATED WITH GLUCOCORTICOID (GC) FOLLOWING TOTAL THYROID ABLATION (TTA): FOLLOW-UP OF A RANDOMIZED CLINICAL TRIAL

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Objectives: The pathogenesis of GO may be related to autoimmunity against antigens expressed by thyroid and orbital tissues. Complete elimination of thyroid antigens may be beneficial for GO. We previously investigated this issue in a randomized clinical trial in patients with GO subjected to near-total thyroidectomy (TX) or TTA (near-total thyroidectomy followed by radioiodine). Findings at 3 and 9 months after iv glucocorticoids (GC) indicated a beneficial effect of TTA. Here we investigated the long term effects of TTA in the same patients.

Methods: In the original study, 60 patients with Graves' disease and mild to moderate, active GO, had been randomized to TX or TTA, followed by ivGC. At baseline, the 2 groups were homogeneous for gender, age, thyroid and GO features. Six patients (3 in TTA and 3 in TX group) were excluded or lost to follow-up after enrollment. To evaluate the long term effects of TTA, patients were asked to undergo a re-evaluation; 32 accepted: 15 in TTA group (6 males, 15 females; age 40.2±12.1 yr) and 17 (6 males, 11 females; age 35.6±8.4 yr) in TX group. Duration of follow-up after GC was 84.9±14.3 mo. (range 54-116) in TTA and 85.9±19.9 mo. (range 49-113) in TX group.

Results: Although a general trend to a beneficial effect of TTA was still observed, there was no statistical difference between the two groups concerning the overall outcome of GO and of individual GO features (proptosis, eyelid width, diplopia and clinical activity score).

Conclusions: Given the limitations due to the low number of patients, our findings seem to indicate that TTA has a more pronounced effect on GO in the short term. The lower effect in the long term may reflect the natural history of GO with its tendency to improve spontaneously. Further studies are needed to confirm these conclusions.
DECREASED SERUM TSH LEVELS ARE NOT ASSOCIATED WITH MORTALITY IN THE ADULT NORTHEAST GERMAN POPULATION

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Objective: Results of studies on the association between decreased serum TSH levels and mortality are conflicting. Some studies demonstrated an increased mortality risk in subjects with decreased serum TSH levels, others did not. Even meta-analyses revealed contradictory results. We undertook the present study to investigate the association between decreased serum TSH levels and mortality in the large population-based Study of Health in Pomerania (SHIP).

Design and methods: Data from 3651 individuals from SHIP without known thyroid disorder or thyroid treatment were analyzed. Serum thyrotropin (TSH), free triiodothyronine, and free thyroxin levels were determined by immunochemiluminescent procedures. Decreased TSH was defined as serum TSH levels below 0.25 mIU/l. The median duration of mortality follow-up was 7.2 years (13,913 person years). Cox regression was used to associate decreased TSH levels with mortality.

Results: During follow-up, 299 individuals (6.9 %) died corresponding to a death rate of 9.92 deaths per 1000 person-years. Survival times were shorter in subjects with decreased serum TSH levels compared to euthyroid individuals. After adjustment for age and sex, however, there was no association between decreased serum TSH levels and all-cause mortality (hazard ratio: 0.95; 95%-confidence interval: 0.67; 1.36). Likewise, decreased serum TSH levels were neither associated with cardiovascular nor with cancer mortality.

Conclusion: There is no association of decreased serum TSH levels with all-cause, cardiovascular and cancer mortality in the adult Northeast German population.
OP 08 Models of Thyroid Disease

OP55

THE THYROID HORMONE RECEPTOR α MODULATES LPS-INDUCED CHANGES IN PERIPHERAL THYROID HORMONE METABOLISM

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Introduction: Acute inflammation is characterized by low serum T₃ and T₄ levels accompanied by decreased liver type 1 deiodinase (D1) mRNA and increased muscle type 2 deiodinase (D2) mRNA. It is unknown at present whether the thyroid hormone receptor α (TRα) plays a role in altered peripheral thyroid hormone metabolism during acute illness in vivo.

Aim: To evaluate the role of TRα in peripheral thyroid hormone metabolism during acute illness in mice.

Methods: TRα₀/₀ and Wild type (WT) mice (3F/3M) received LPS (a bacterial endotoxin) or saline. Mice were killed after 4, 8 and 24h. Serum fT₃ and fT₄ levels were measured. Liver D1 mRNA and activity, liver TRβ1 mRNA, muscle D2, D3 and uncoupling protein 3 (a T₃-responsive gene, UCP3) mRNA expression were measured using qPCR. Muscle and liver interleukin (IL)-1β mRNA were measured as a reflection of the inflammatory response.

Results: Basal levels: Liver D1 activity and muscle D3 mRNA expression were significantly lower in TRα₀/₀ mice compared to WT.

Response to LPS: In both liver and muscle the IL-1β mRNA increase in WT and TRα₀/₀ mice was similar. By contrast, the decrease in serum fT₃ and fT₄ and in liver D1 mRNA and activity was attenuated in TRα₀/₀ mice compared to WT. Muscle D2 and UCP3 mRNA increased similarly in both strains, whereas muscle D3 mRNA decreased less pronounced in TRα₀/₀ mice.

Conclusion: Although the inflammatory response was similar in both strains, lacking the TRα gene attenuated the LPS-induced changes in peripheral thyroid hormone metabolism significantly. These results indicate an important role of TRα in the LPS induced changes in peripheral thyroid hormone metabolism.
Thyroid hormone (T3) is known to play a critical role in lipid homeostasis. However, the molecular mechanism by which T3, via thyroid hormone receptors (TRs), regulates adipogenesis remains to be elucidated. Two TR genes, alpha and beta, encode four T3 binding TR isoforms (beta1, beta2, beta3, and alpha1). Using the loss-of-function approach, we created knockin mutant mice harboring a dominantly negative PV mutation targeted to the TRalpha gene (TRalpha1PV mouse) or TRbeta gene (TRbetaPV mouse) to understand TR actions in vivo. The PV mutation was identified in a patient with thyroid hormone resistance. We found that white adipose tissue (WAT) mass is markedly reduced in TRalpha1PV mice but, interestingly, not in TRbetaPV mice, indicating distinct regulation of adipogenesis by TR isoforms. We therefore generated 3T3-L1 cells stably expressing TRalpha1 (L1-alpha1PV cells) or TRbetaPV (L1-betaPV cells) to understand the molecular mechanisms of TR mutant isoform actions in adipogenesis. T3-dependent adipogenesis in parental 3T3-L1 cells was significantly inhibited as evidenced by decreased lipid droplets in L1-α1PV and L1-βPV cells (95% and 75%, respectively), indicating differential impairment of adipogenesis by TR mutant isoforms. Analyses of the expression of the two key regulators of adipogenesis, PPARgamma and C/EBPalpha, showed that TRalpha1PV was a more potent repressor of their expression than was TRbetaPV. Consistently, the expression of several PPARgamma downstream target genes - the lipoprotein lipase, adipsin, and aP2 involved in adipogenesis - was more repressed by TRalpha1PV than by TRbetaPV (90-95% and 40-50%, respectively). Using ChIP assays, we found that TRalpha1PV was more avidly recruited to the promoter of C/EBPalpha than was TRbetaPV to differentially repress its expression. These results indicate that mutant TR isoforms differentially regulated the expression of key regulators, resulting in distinct impairment in adipogenesis. The present study provides direct evidence to indicate that TR does regulate adipogenesis, but in an isoform-dependent manner.
OP57
THE ROLE OF HES1 FOR NORMAL THYROID DEVELOPMENT: A KNOCK-OUT MICE STUDY
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Introduction: Notch signaling and Hes1 have been shown to be involved in endocrine pancreas and pituitary development, and recently in thyroid cancer. Thus, we reasoned that this might also be the case for thyroid endocrine development.

Objectives: This study aimed to determine
1) the expression profile and
2) the function of hes1 in the developing mouse thyroid.

Methods: Expression of hes1 was investigated by RT-qPCR and immunohistochemistry in micro-dissected embryonic (from E13.5 to E18.5) and in adult WT mouse thyroids. Thyroids of hes1⁻/⁻ and WT mice were analyzed by immunohistochemistry in order to quantify thyroid area, morphology, cellular composition, proliferation and apoptosis in E9.5, E11.5, E13.5, 15.5 and E 16.5 embryos using antibodies against hes1, nkx2.1, pax8, T4, mash1 and calcitonin.

Results: In WT mice, hes1 expression is upregulated from E13.5 to E18.5 as compared to adult thyroid. Fusion of the median anlage and the ultimobranchial bodies was delayed in hes1⁻/⁻ mice by 3 days (E16.5 vs. E13.5 in WT). In hes1⁻/⁻, mice the thyroid area was significantly smaller (-40 to -60%) compared to WT at all investigated stages. Within the hypoplastic thyroids we found a significantly decreased calcitonin (~65% vs. WT) and T4 (~78% vs. WT) labelling surface if normalized to the nkx2.1 labelling surface. Apoptosis and proliferation ratios were similar in wild-type and hes1⁻/⁻ thyroids from E11.5 to E16.5. Very interestingly, the number of nkx2.1 positive progenitor cells in the median anlage of hes1⁻/⁻ at E9.5 and in the ultimobranchial bodies at E11.5 were markedly decreased compared to WT (P< 0.05).

Conclusions: During thyroid development hes1 is required
1) for maintenance of follicular cell and C-cell progenitors and when mutated resulting in severely hypoplastic thyroids,
2) for correct fusion of the median and the lateral anlagen, and
3) for adequate endocrine function of follicular cells and C-cells.
CLEAVAGE OF THE TSHR IN CELLS DEFICIENT IN ADAM (A DESINTEGRIN AND METALLOPROTEASE) 17 AND ADAM 10

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The TSHR undergoes a post-translational cleavage and the mature receptor comprises an extracellular $\alpha$ and a transmembrane $\beta$ subunits held together by disulfide bridges. Part of the $\alpha$ subunit is shed from thyroid and transfected cells in vitro (1). We have shown that the cleavage occurs at the cell membrane by a metalloprotease sharing properties with the ADAM (A Desintegrin And Metalloprotease) family (2). This cleavage yields in the excision of part of the extracellular domain (residues 314-378) of the receptor. We had provided arguments showing that ADAM 17 did not seem to be involved (1). In this study we used cells derived from ADAM knock-out mice to study the involvement of candidate ADAMs. We used SV40 immortalized mouse embryonic fibroblastic cells derived from mice deficient for ADAM 17 (or TNF$\alpha$ converting enzyme) and ADAM 10. Cells were transfected with a TSHR expression vector and maturation of the TSH receptor was studied by western Blots. In control and deficient cells, the mature $\alpha$ and $\beta$ subunits were observed using monoclonal antibodies recognizing respectively the extracellular and the intracellular domains of the receptor, and precursor extended $\beta$ subunits were detected using the antibody recognizing the cleaved-off region of the receptor. Monomeric precursors were also observed in similar amounts in control and deficient cells. This allows to conclude that ADAM 10 and ADAM17 do not seem to play a major role in the maturation of the TSH receptor.

Additional studies with cells lacking widely expressed and catalytically active ADAMs (ADAM 8, 9/12/15, 17 and 19) are currently under study to evaluate their contribution in the maturation of the TSHR.

References:
We have recently demonstrated induction of significant tumor-selective iodide uptake activity in neuroblastoma tumors after systemic polyplex-mediated sodium iodide symporter (NIS) gene delivery. After intravenous application of NIS-conjugated polyplexes neuroblastoma tumors accumulated 8-13% ID/g $^{123}$I with a biological half-life of 13 h. The aim of the current study was to evaluate the efficacy of these synthetic nanoparticle vectors based on pseudodendritic oligoamines for systemic NIS gene transfer in a hepatocellular carcinoma (HCC) mouse model. Therefore, in the current study we used the same synthetic nanoparticle vectors characterized by high intrinsic tumor affinity to target a NIS-expressing plasmid (CMV-NIS- pcDNA3) to hepatoma (Huh7) cells in vitro and in vivo. In vitro incubation with NIS-conjugated nanoparticles resulted in a 44-fold increase in perchlorate-sensitive iodide uptake activity in Huh7 cells as compared to mock-transduced cells. After establishment of subcutaneous Huh7 tumors in SCID mice, NIS-conjugated nanoparticle vectors were injected via the tail vein followed by analysis of radioiodine distribution after i.p. injection of 18.5 MBq $^{123}$I using gamma camera imaging. After systemic NIS gene delivery Huh7 tumors accumulated 6-11% ID/g $^{123}$I with a biological half-life of approximately 6 h, while tumors transduced with the control vector showed no specific iodide uptake. Moreover, iodide uptake in NIS transduced tumors completely abolished upon i.p. injection of sodium perchlorate, confirming NIS-specificity. In addition, non-target organs like liver, lung, kidneys and spleen exhibited only mild or no significant iodide uptake as shown in ex vivo biodistribution experiments.

These results clearly demonstrate that systemic in vivo NIS gene transfer using non-viral pseudodendritic polyplexes is capable of inducing tumor-specific iodide uptake, which represents a promising innovative strategy for NIS-mediated radionuclide therapy of metastasized extrathyroidal tumors.
The aim of the study was to reveal chromogranin A (CgA) as a specific target molecule for a cytotoxic immune response against neuroendocrine tumor cells in a transgenic mouse model (Ret/Cal mice) with a H2-Kb phenotype. Six different amino acid-modified and -non-modified CgA peptides were used for dendritic cell vaccination. Tetramer analyses of inguinal lymphocytes showed a large increase of CgA-specific CD8+ T cells up to 0.80% compared to control mice. Immunization with modified vs. non-modified CgA peptides revealed a significant higher number of tetramer positive CD8+ T cells in the CgA-modified group (0.20% ± 0.35%) compared to the control group (0.09% ± 0.15%). In order to evaluate the in vivo effects we also applied tumor cells. Since no medullary thyroid carcinoma cell line with a H2-Kb phenotype exists we had to use an alternative murine neuroendocrine tumor (pheochromocytoma) cell line. Immunohistochemistry of applied tumor cells showed a strong infiltration of CD8+ T cells in treated mice. These cells also had a strong in vitro lysis capacity up to 58.3% which could almost completely be blocked by coincubation with MHC I antibodies. Cold targeting analyses revealed that lysis activity of cytotoxic T cells was mostly mediated by CD8+ T cells specific for peptide 4 covering amino acid positions 391 to 398. Importantly, CgA peptide-immunized mice showed a largely diminished tumor outgrowth (-87.4%) following application of tumor cells. These results are of major impact, as they are the first to establish a role for (amino acid-modified) chromogranin A peptides as target molecules for immunotherapy in medullary thyroid carcinoma.
3,5-diiodo-L-thyronine (T2), contemporarily administered to rats receiving a high-fat diet (HFD) for 1 month, has been shown to reduce adiposity and body weight gain by increasing hepatic fatty acid oxidation. This process has been associated with AMPK-activated protein kinase (AMPK) phosphorylation.

Aim: To gain insight into the cellular events that underlie the effects of T2 on fat handling, we studied the temporal relationship between fatty acids oxidation in liver and their release from white adipose tissue (WAT). Moreover, the role of AMPK phosphorylation in the organ specific response was assessed.

Results: T2 rapidly (within 6h) stimulated mitochondrial fatty acid oxidation in liver without induction of AMPK Thr172/acetyl CoA carboxylase (ACC) Ser79 phosphorylation and of carnitine palmitoyl transferase (CPT) activity. This effect persisted also after inhibition of AMPK phosphorylation with Compound C. In the white adipose tissue, on the other hand, phosphorylation of AMPK and its target residue Ser565 of hormone sensitive lipase HSL (inhibiting its activity) as well as Ser563 (activating HSL) was rapidly increased (1 day), but no increase in lipolysis was observed. After 1 week, as a consequence of a decrease in phosphorylation of AMPK as well as HSL Ser565, an increase in both HSL phosphorylation at Ser563 and lipolysis was observed.

Conclusions: The administration of T2 to HFD rats is able to affect lipid handling both in liver and WAT in a organ specific manner. AMPK was affected earlier in WAT and later in liver, possibly playing different roles.
OP62
ASSESSMENT OF HEPATIC GENE EXPRESSION IN TYPE 3 DEIODINASE-DEFICIENT MICE SUBJECTED TO BACTERIAL INFECTION
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Introduction: We have recently observed that systemic infection in mice deficient in the type 3 deiodinase (D3KO mice) results in significant decreases in serum T4, T3 and TSH levels similar to that noted in wild type (WT) animals. To begin to define the effects of infection and the resultant metabolic changes on gene expression in the liver, we have measured hepatic mRNA levels for selected genes in WT and D3KO mice infected with Streptococcus (S). pneumoniae.

Objectives: To assess the effects of systemic infection on gene expression patterns in the liver of normal and D3KO mice.

Methods: We infected WT and D3KO mice by intranasal inoculation of 5x10^6 CFU of S. pneumoniae. Control mice of both genotypes received saline. All mice were sacrificed after 48h, their livers harvested and mRNA expression of selected hepatic genes were studied by qPCR.

Results: Hepatic type 1 deiodinase (D1) and TRα2 mRNA levels in control D3KO mice were only 25% and 60%, respectively, of that in WT animals. Bacterial infection resulted in a significant decrease in these parameters in WT animals, whereas no further decrease was noted in the D3KO. Similarly, liver TRα1 mRNA expression decreased approximately 20% (p< 0.05) in infected WT animals, but did not change in infected D3KO mice.

Infection significantly decreased liver PPARα, PGC-1β, malic enzyme and Spot14 mRNA expression to similar extents (approximately 40%, 70%, 60% and 85%, respectively) in both WT and D3KO mice.

Conclusion: Acute bacterial infection results in alterations in hepatic gene expression in mice, with significant differences noted between WT and D3KO animals with regards to the regulation of D1 and the TRα isoforms. This altered pattern of gene regulation in the liver of adult D3KO mice may be caused by the transient hyperthyroidism experienced by these animals during the developmental period.
Germline activating mutations of the RET proto-oncogene are associated with inherited medullary thyroid cancer (MTC) and can be also detected in about 10% of apparently sporadic MTC cases. In the present study, 4 novel RET mutations, located in the extracellular domain (A510V, E511K and C531R) and in the intracellular juxtamembrane region (K666N), all identified at the genetic screening on apparently sporadic MTC cases, are reported and functionally characterized. RET plasmids carrying Ret9-WT (the short isoform of protoRet gene) and RET mutants, obtained by site-directed mutagenesis, were transiently transfected in HEK 293T cells. Ret9-C634R (the protoRet gene containing a MEN2A causing mutation) was used as positive control. The tyrosine phosphorylation level was evaluated by immunoprecipitation and Western blot analyses. The extracellular variants A510V, E511K and C531R were found to harbour autophosphorylation levels higher than Ret9-WT, but significantly lower than Ret9-C634R. Differently, the K666N variant, located few residues downstream the transmembrane domain displayed a high kinase activity. Computational analysis predicted non conservative alterations in all the mutant proteins consistent with a possible modification of the receptor conformation. In particular, K666N mutant leads to a significant alteration of the transmembrane alpha-helix, likely changing the secondary structure of the protein. In conclusion, functional analyses on four novel germline RET mutations are reported. The K666N variant is associated with a high kinase activity indicating that alterations in the juxtamembrane region can strongly activate RET in a ligand independent manner. Therefore, screening of family members and strict follow-up starting at a young age for mutation carriers in this novel “hot” region appear to be mandatory. Finally, present data confirm the need to routinely perform the genetic screening for RET in apparently sporadic MTC and to extend the molecular analyses to regions other than the cysteine residues and other classical “hot” spots.
The majority of thyroid cancers can be effectively treated by radioiodine ablation. However, about 20% of differentiated thyroid carcinomas do not take up radioiodine, resulting in a poor prognosis. Previous studies have shown this is due to reduced sodium iodide symporter expression caused by loss of TSH receptor expression and/or aberrant activation of the ERK and Akt signalling pathways, which have both been shown to negatively regulate NIS expression. We investigated whether treatment with BAY 43-9006 (B-RAF inhibitor) alone or in combination with Wortmannin (PI3K inhibitor) could rescue NIS gene expression.

Serum starved TPC1 or NThy cells were treated with 25 ng/ml EGF for 2hrs, with or without 10 µM BAY 43-9006, 20 µM Wortmannin or both. Western blotting of whole cell lysates from treated cells detecting phospho-ERK and phospho-Akt, determined the activation status of the ERK and Akt pathways. Transient transfection of these cells with NIS promoter luciferase reporters was performed to determine the effect of these treatments on NIS expression.

The treatment of TPC1 cells with BAY 43-9006, Wortmannin or both resulted in a 1.5, 2.4 and 2.6-fold induction of NIS reporter expression. Similar results were obtained with the NThy cells.

We show that combined inhibition of the ERK and Akt pathways resulted in a greater induction of the NIS promoter than inhibition of either pathway alone. We are now investigating the effect of combined treatment on NIS function (i.e., iodine uptake). Such treatment may prove beneficial to cases where loss of TSH receptor expression has rendered the tumour unresponsive to TSH treatment.
Objectives: Functional chemokine receptors are expressed in many malignant tumors, including papillary thyroid carcinoma (PTC). These receptors promote tumor growth and metastasis in response to endogenous chemokines. The purpose of this study was to examine the expression of the chemokine SDF-1 and its receptor, CXCR4, in a series of PTCs considered as low risk for small tumor size (< 2cm).

Methods: CXCR4 and SDF-1 expression was assessed in 48 PTCs using a semiquantitative measurement of immunohistochemical (IHC) staining and quantitative RT-PCR (Q RT-PCR). Expression level was compared with clinicopathological features.

Results: CXCR4 and SDF-1 expression is increased in all tumor samples vs samples of normal thyroid tissue. In particular, CXCR4 is expressed in PTC tumor cells with heterogenous intensity and in stromal-derived cells, particularly in macrophage-like cells and lymphocytes cells. High-intensity IHC staining for CXCR4 correlated with smaller tumor size (p=0.003), classical histological variant (p=0.02) and presence of lymph-node metastases at initial diagnosis (p=0.01). SDF-1 is abundantly expressed both in PTC tumor cells and in stromal fibroblasts, and SDF1 expression correlated with female gender (p =0.05).

Conclusions: Our study is the first to demonstrate the co-expression of both SDF1 and its receptors in the early PTC stage, using Q RT-PCR and IHC. This suggests an autocrine-paracrine loop that can be an early event in PTC, providing a proliferative advantage for SDF-1 sensitive cells. Further studies are necessary to define these mechanisms and to determine potential prognostic and therapeutic implications.
OP66
COMBINED ANALYSIS OF CLAUDIN-1 EXPRESSION AND 1513A/C POLYMORPHISM OF THE P2X7 RECEPTOR GENE: PROGNOSTIC ROLE IN PAPILLARY THYROID CANCER

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Introduction: Claudins (CLDN) are integral constituents of tight junctions. In several human cancers, including thyroid carcinoma (TC), an increased or decreased expression pattern has been reported. The modulation of the P2X7 receptor (P2X7R) may be involved in human carcinogenesis. Recently, we observed a strong relationship between 1513A/C (loss of function) polymorphism of P2X7R gene and both the follicular variant of papillary thyroid carcinoma (PFV) and more aggressive cancer behaviour.

Aim: To analyze CLDN1 expression and the presence of the 1513A/C polymorphism in different TC samples and to evaluate their potential role as combined markers of the disease.

Materials and methods: Fifty-seven thyroid cancer specimens [34 papillary classical variant (PCV), 12 PFV, 7 tall cell variant (PTV) and 4 poorly differentiated carcinomas (PPD)], with diverse TNM staging (class I/II: 50.9%; class III: 32.1%; class IV: 17.0%) were immunostained with a polyclonal antibody (Zymed, CA) against CLDN1 as compared to normal contralateral thyroid tissue. The presence of 1513A/C polymorphism was also evaluated by PCR amplification followed by restriction fragment length polymorphism (RFLP) analysis.

Results: CLDN1 expression was significantly reduced in PTV and PPD samples (p=0.01 vs PCV and PFV). The minor allele frequency of the 1513A/C polymorphism resulted significantly higher in PFV, PTV and PPD than PCV samples (p=0.003). A significant positive relationship was found between 1513A/C polymorphism and either CLDN1 expression or disease staging (p=0.04; rho=0.34, for both). Considering the joint presence of reduced CLDN1 expression and P2X7R polymorphism, a significant correlation with histological tumour aggressiveness was also found (p=0.02).

Conclusion: Our preliminary data show an association between both the molecular markers and aggressive cancer behaviour, suggesting a role in selecting patients with different clinical outcome. The combined appraisal of CLDN1 expression and 1513A/C polymorphism might become a potential tool for modulating the therapeutic approach to thyroid cancer patients.
Objective: The management of clinically apparent lymph nodes metastases is agreed upon most Centers, but controversy exists about the role of prophylactic pretracheal/paratracheal lymph node dissection (CLND, VI level) in differentiated thyroid cancer. Aim of the study was to evaluate the prognostic role of prophylactic CLND in 324 patients with PTC (294 F, 30 M; mean age 45.5±14.78) referred to our Centre from 1995 to 2008. All patients were treated by total thyroideectomy (TT), while radioiodine ablation was performed only in tumors >2 cm or in the presence of extrathyroidal extension and lymph node/distant metastasis. Starting from September 1998, CLND was associated to TT in all cases with preoperative diagnosis of malignancy. Persistence/recurrence or remission were evaluated according to the European consensus guidelines.

Results: At multivariate analysis, including well known outcome predictors as input variables, only lymph nodal metastases and extrathyroidal invasion were significantly associated with a poorer outcome (P=0.03 and P=0.01). Concerning therapeutic actions, CLND was found to be significantly associated with a better outcome (P=0.03). In particular, 54% of patients who underwent prophylactic CLND were found to have at histology at least on lymph node metastasis, being all these patients free of disease at follow-up. Transient or permanent hypoparathyroidism was recorded in 7.2 and 3.6% of patients submitted to TT alone and in 8.8 and 1.5% of cases treated by TT+CLND. Temporary or permanent laryngeal nerve paralysis was observed in 3.1 and 2.4% of patients treated by TT alone and in 2.7 and 1.5% of cases treated by TT+CLND.

Conclusions: Present data demonstrate the major impact of prophylactic CLND on prognosis and suggest to routinely associate it to TT in cases with a preoperative diagnosis of malignancy. It is worth noting that, if performed by skilled surgeons, CLND is not associated with an increased risk of complications.
The thyroid axis 'setpoints' are significantly altered after long-term suppressive LT4 therapy

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Objective: To investigate the changes in setpoints of the thyroid axis after long-term suppressive LT4 therapy for differentiated thyroid carcinoma (DTC).

Patients and methods: 99 patients were reviewed. All patients were treated for DTC, and had at least one known TSH-level >0.01 mU/l (lower detection limit) and < 1.0 within 2 years of initial treatment (time1) and had at least one TSH-value >0.01 mU/l and < 1.0 mU/l after continuous LT4 therapy for a minimum of 5 years (time2).

Results: At time1 the mean LT4 dosage / kg bodyweight, TSH, FT3 and FT4 levels were significantly higher than at time 2, while bodyweight was lower. At time2, the FT4-to-FT3 conversion rate had dropped significantly (P< 0.001) compared to time1, while the dose of LT4/kg to FT4 ratio remained unchanged (P=0.32).

At time1, on average 0.59 years after initial treatment, patients would require on average 2.94 micrograms / kg bodyweight to reach total TSH-suppression; FT3 levels should be 8.02 pmol/l and FT4 levels 34.6 pmol/l. After a minimum of 5 years of suppressive of LT4 therapy, the dose of LT4/kg required for suppression can be lowered by about 0.05 micrograms/kg bodyweight each year. At time2, after a mean of 12.7 years of continuous suppressive LT4 therapy, patients would require 2.25 micrograms / kg bodyweight (-23.5%) to reach total TSH-suppression while FT3 levels should be 5.28 pmol/l (-34.2%) and FT4 levels 27.3 pmol/l (-21.1%).

Conclusion: After long term suppressive LT4 therapy, the FT4-to-FT3 conversion rate has dropped significantly. Nonetheless, TSH levels are suppressed at lower FT3 and FT4 levels than is the case early in the treatment of DTC. As a result, the dosage of LT4 per kilogram bodyweight can be lowered after long-term follow-up.
Objective: To assess the additional value of SPECT-CT scanning over planar imaging and ultrasound only for the detection of cervical lymph node metastases around I-131 ablation.

Methods: A planar whole-body imaging study with detailed images of the cervical region was acquired and a SPECT-low-dose-CT scan was recorded from the mandibula to below the sternoclavicular joints in 55 patients.

Results: Overall, 27/55 (49%) patients were judged to be negative for the presence of cervical lymph node metastases, 22/55 (40%) positive and 6 patients (11%) indeterminate for the presence of lymph node metastases based on planar imaging. After SPECT-CT imaging these numbers were 35/55 (64%), 20/55 (36%) and 0/55, respectively. 4 patients who were negative on planar imaging were positive SPECT-CT, and positive 6 patients were negative in SPECT-CT. In all, SPECT-CT was of clinical benefit in 27/55 (49%) patients. In ultrasound 19 patients showed enlarged lymph nodes on ultrasound of the neck; in only one of these patients the enlarged lymph nodes were classified as likely to be malignant. Only 7 of these patients showed lymph node metastases on SPECT-CT, additionally 9 other patients that did not show enlarged lymph nodes on ultrasound were found to have cervical lymph node metastases.

Conclusion: SPECT-allows a greater rate of detection of lymph node metastasis, more precise determination of lesion dignity as well as more precise localisation of lymph node metastases than planar imaging and / or ultrasound. It is of clinical benefit in half the patients undergoing ablation.
EVALUATION OF THE USEFULNESS OF NAF-PET IN DIAGNOSIS OF BONE METASTASES FROM THYROID CANCER IN COMPARING WITH THAT OF CONVENTIONAL TC-99M-DIPHOSPHONATE BONE SCINTIGRAPHY

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Object: Bone metastases from differentiated thyroid carcinoma (DTC) are sometimes difficult to visualize on bone X-ray films. Conventional Tc-99m-diphosphonates bone scintigraphy (CBS) is used for detecting bone metastases of DTC. 18F-NaF has been used in PET as a bone seeking agent. We evaluated the effectiveness of NaF-PET in comparison with CBS for the diagnosis of bone metastases in DTC.

Materials and methods: Thirteen subjects (7 men and 6 women; mean age, 64 years; range, 43-81 years) underwent 14 NaF-PET studies. Three men and six women received I-131 therapies once for DTC bone metastases. A woman received twice. Two men had prior I-131 treatment and 2 men were healthy volunteers. Twelve subjects also underwent CBS. Whole body images of both NaF-PET and CBS were obtained 60 minutes after intravenous injection of 185MBq NaF and 180 minutes after injection of 740MBq Tc-99m-HMDP, respectively. An Eminence B PET scanner (Shimadzu Corp., Japan) and an ADAC Forte camera (ADAC Corp., USA) were employed. Diagnostic abilities of F-18 NaF-PET and CBS were examined visually based on the following criteria: ability to distinguish lesions from normal bone structure; visual resolution; and ability to distinguish uptake by normal soft tissue. We measured the maximum RI counts of abnormal uptake by bone metastases and osteoarthritis in the ROIs.

Results: The overall accuracy of bone PET was higher in all subjects in all three criteria. In 11 of the 12 cases, CBS was accurate in distinguishing lesions from normal bone, but did not perform well in the other two criteria. In the quantitative evaluations with the ratios to the normal bones, significant differences between the groups of bone metastases (mean, 3.6 ± 1.4) and osteoarthritis (mean, 2.8 ± 1.0) were found (p< 0.05) in PET studies. In CBS studies, significant differences of the them were not found.
Some cases of congenital hypothyroidism (CH) are associated with goiter or a gland of normal size.

**Objective:** To identify DUOX2 mutations in children with CH or isolated hyperthyreotropinemia and a eutopic thyroid gland.

**Patients:** 17 neonates with CH and a eutopic thyroid gland and 5 children with isolated hyperthyreotropinemia. In all the children LT4 was stopped to verify thyroid function when they were 3 yo. In 7 children a partial and in 2 children a complete organification defect was shown after 123-I scintigraphy and perchlorate test. In children with the organification defect TPO, DUOX2, DUOXA2 genes were analyzed while in the others DUOX2, DUOXA2, PAX8 and TSH receptor genes were studied. The functional activity of the DUOX2 variants was studied after expression in eukaryotic cells.

**Results:** No TPO mutations were identified. Direct sequencing of the DUOX2 gene revealed a monoallelic deletion S965FsX994 in three children. Four children showed H678R, R701Q, P982A heterozygous mutations. One child was compound heterozygous for S911L and C1052Y substitutions. The functional studies confirmed that the deletion was responsible for a complete defect in H2O2 production while only S911L and 1052Y caused a partial defect. Only polymorphisms of the TSH receptor and PAX8 were identified.

**Conclusions:** We performed a genetic analysis in 22 children with CH or isolated hyperthyreotropinemia with a eutopic thyroid gland. Three children with partial organification defect harbored the S965FsX994 deletion, while in one child two new mutations were identified in DUOX2, which are responsible for the partial deficit in the organification process.
NKX2-1, also known as TITF-1, TTF-1 or T/ebp, is a member of the homeodomain-containing NK-2 transcription factor gene family and is expressed in early development of thyroid, lung and forebrain. Targeted inactivation of Nkx2.1 in mice revealed a neonatal lethal phenotype due to lung agenesis, thyroid dysgenesis and forebrain malformations, particularly the basal ganglia and hypothalamus. To date, only few NKX2-1 mutations were identified in patients with variable congenital hypothyroidism, choreothetiform movement defect and pulmonary symptoms matching the mouse phenotype. Here we report the systematic molecular screening analysis of NKX2-1 for mutations and DNA copy number changes of 102 patients with thyroid dysfunction combined with movement disorders and pulmonary affection aiming to define in more detail the clinical spectrum of NKX2-1 deficiency.

DNA of all patients was analyzed by direct sequencing and customized high-resolution oligo array-CGH, covering the region of interest with a resolution of 200-300 bp, was applied to a subset of 48 patients.

In our series 28 novel alterations were identified, comprising 10 heterozygous deletions of the NKX2-1 and adjacent regions and 18 intragenic point mutations, whereas neither a common microdeletion nor a mutational hot spot could be revealed. NKX2-1 variations were most likely to occur in patients with choreothetosis, hypothyroidism and pulmonary dysfunction (42.3 %) and less frequently in patients with neurological and thyroid dysfunction (29.2 %) or separate choreothetosis (18.8 %). In conclusion, NKX2-1 deficiency does not necessarily entail the triad of neurological, thyroid and pulmonary dysfunctions, but also results in choreothetosis with or without hypothyroidism. NKX2-1 analysis of patients with movement disorders like choreothetosis who are clinically difficult to classify should include screening for genomic aberrations within the NKX2.1 genomic region.
Several mutations of Pax8 and TSHR have been reported in congenital hypothyroidism (CH) due to thyroid hypoplasia or dysfunction either in sporadic and in familial cases.

**Aim of the study:** To search for Pax8 mutations in a large cohort of CH patients and to test the Pax8/ TSHR digenism hypothesis.

**Patients and methods:** 104 patients were analysed by direct sequencing of the binding domain of Pax8 (exons 2 to 4). For Pax8 mutated patients, we then sequenced the 10 coding exons of the TSH receptor.

**Results:** A total of three Pax8 heterozygous mutations were identified in 7 patients: The previously described R31H mutation (Macchia, 1998) was found in one parent affected by severe thyroid hypoplasia and one child with radiologically proven ectopy in 2 different families (n=4). Two novel missense mutations were identified in 3 sporadic CH cases with normal thyroid gland: R31C, a de novo mutation (n=2) and I47T inherited from the mother with subclinical hypothyroidism (n=1). Functional studies confirmed the alteration of transactivation capacity of R31C mutated protein on Tg promoter. Among these 7 patients with Pax8 mutation, extrathyroidal malformation was found in one patient who displayed unilateral kidney agenesis and TSHR analysis revealed 2 novel heterozygous mutations. These 2 patients were affected by either athyreosis or normal sited gland with minor radiological distinctive features.

**Conclusion:** Pax8 mutations are rare in CH patients but could appear with a range of phenotypical variation, including for the first time, radiologically proven ectopic thyroid gland. The combined Pax8/ TSHR heterozygous mutations found in 2 cases may suggest a digenism as a cause of the phenotypic variability.
Congenital Hypothyroidism (CH) is the third congenital disorder for prevalence, affecting 1:1500-2500 newborns. CH aetiology is still largely unknown, but several data indicate a prevalent genetic component. In order to find novel CH genes, the recent research is looking for candidates based on their common involvement in the development of the thyroid gland and of organs whose defects are frequently associated with CH (e.g. heart, eye). In a previous microarray study we identified new pathways under TSH regulation in thyroid cells. Particular interest was raised by the presence of elements of the Notch pathway, a highly conserved signalling cascade with fundamental roles in the development and differentiation of many organs, among TSH-regulated genes. Defects in several elements of the Notch pathway are associated with different diseases. Among these, the Alagille Syndrome (ALGS) is an autosomal multisystemic disorder characterized by variable defects of several organs (mainly liver, heart, eye, bones). Mutations in the JAG1 gene, a ligand of the Notch receptor, are the main cause of ALGS. Here, we conducted a genetic screening of the JAG1 gene in three distinct groups: A) 22 patients with isolated CH; B) 50 patients with isolated heart developmental defects (HDD); C) 18 patients with CH and HDD. The screening identified 1 genetic variation, already described in a patient with ALGS, in 2/18 patients of group C. No genetic variations were found in the other groups. Functional studies are indicative of a reduced activity of this variant compared to WT JAG1. Since concomitant studies of our group demonstrate a role for the Notch pathway in the development of the thyroid gland in the zebrafish, these results suggest a potential involvement of the Notch pathway in the pathogenesis of CH, particularly in cases with a complex phenotype, such as those associated with malformations common to the ALGS.
Background: Follicular cell-derived thyroid cancers (termed Non Medullary Thyroid Cancer - NMTC) occur mostly sporadically but, intriguingly, NMTC has the highest familial relative risk amongst all cancer types. This epidemiological observation is strengthened by the clinical occurrence of NMTC in familial aggregation (FNMTC) and by the detection of chromosomal loci in linkage with the disease phenotype. However, up to date, no predisposing gene could be identified. We have previously found that LOH was frequent in tumours from familial clusters of NMTC at 19p13.2 and 2q21, in a pattern that, in some families, was consistent with the inactivation of a tumour suppressor gene (TSG).

Aim: To try to identify genes located at 19p13.2 and 2q21 that may prove to be relevant to sporadic or familial NMTC.

Material and methods: We have performed global gene expression in normal thyroid and tumour samples from a FNMTC patient. From the 78 genes located at 2q21, only one gene was down-regulated by more than 3-fold. We searched afterwards for somatic inactivation of this putative TSG in sporadic thyroid cancer. For this purpose, we analyzed mRNA expression, promoter methylation, mutation and microRNAs (miRNA) in thyroid cancer cell lines and human tumours.

Results: We have found frequent mRNA silencing (8/8 cell lines, and about 50% of primary tumours). We have detected promoter hypermethylation in 3/10 cell lines and in about 1/3 of the tumours. We have identified a frameshift deletion encompassing exons 1 and 2 (1/5 tumours), as well as overlapping recurrent 5'UTR deletions (2/5 human tumours). We have found that overexpression of a specific miRNA was highly correlated with the level of mRNA down-regulation in thyroid cancer cell lines that did not present promoter methylation.

Conclusions: Our findings validate the existence of a “new” TSG in sporadic thyroid tumours.
OP76
SCREENING FOR CHROMOSOMAL ABERRATIONS BY ARRAY CGH IN 80 PATIENTS WITH CONGENITAL HYPOTHYROIDISM DUE TO THYROID DYSGENESIS
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Congenital Hypothyroidism occurs in 1:3500 live births and is therefore the most common congenital endocrine disorder. A spectrum of defective thyroid morphology, termed thyroid dysgenesis, represents 80% of permanent CH cases. Although several candidate genes have been implicated in thyroid development, comprehensive screens failed to detect mutation carriers in a significant number of patients with non-syndromic TD. Due to the sporadic occurrence of TD, high rates of de novo chromosomal rearrangements are conceivably representing one of the molecular mechanisms participating in its aetiology. Recently, the use of array CGH technique has provided the ability to map these variations with high resolution. We performed a screen of 80 TD patients with genomewide array CGH to determine the role of copy number variants in the aetiology of the disease. DNA of all patients was analyzed by array-CGH using a 36K whole human genome tiling path BAC-array with a resolution < 1Kb. We identified novel CNVs in 8.75% of all patients that are to date not described in the healthy population. Affected patients presented with athyreosis or thyroid hypoplasia and in one case with associated heart malformation. None of the yet established genes in the aetiology of TD were located within the aberrant chromosomal regions. Analysis of the positional candidates identified, revealed a gene that is partially disrupted by a 75 Kb deletion and which we proved to be expressed in fetal and adult thyroid tissues in mouse and human. Although a recurrent rearrangement is to be excluded as a major pathogenic factor in the disease we could describe the possible role of a novel gene in normal as well as in impaired development of the thyroid.
GENETIC HETEROGENEITY IN PATIENTS WITH CONGENITAL HYPOTHYROIDISM WITH TOTAL AND/OR PARTIAL IODIDE ORGANIFICATION: HIGH FREQUENCY OF MONOALLELIC MUTATIONS AND NEW GENE SEQUENCE ALTERATIONS
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Objectives: To identify TPO gene mutations in patients with CH with total (DIIT) or partial (DIIP) iodide organification defects and to perform in silico analysis of the mutations.
Patients and methods: We included thirty six patients (detected at the Neonatal Screening Program). The perchlorate discharge test was conducted at 2-4 years of age: 15 patients with DIIT (iodide discharge >51%) and 21 with DIIP (iodide discharge between 25-50%). Amplification and sequencing of the promoter (3000bp) and 17 exons of TPO gene were performed.
Results: Eight new sequence alterations were identified: Leu68Ile, Gly319Glu, Ala426Gly, Arg584Gln, Val618Met, Pro883Leu, Ala909Thr, and A909fsX49 as well as four previously described mutations: 396fsX76, Gln660Gly, Arg665Trp, Cys838Ser were detected. Homozygous mutations were identified in two patients with DTII. Two patients (one with DTII and one with DPII) carried compound heterozygous mutations. TPO monoallelic mutations were identified in 60% of patients whom harbor TPO mutations (3 with DIIP and 3 with DIIT). TPO mutations were not found in 100 normal controls. Sequence alignment studies and in silico structural analysis of the TPO protein suggested that the new mutations are bona fide mutations.
Conclusions: High frequency of TPO monoallelic mutations was detected in patients with DIIT and DIIP. TPO Monoallelic expression may explain the positive perchlorate discharge test and the hypothyroidism of the patients. In both groups of patients the mutations were located all throughout the gene, in the extracellular as well as in the intracellular domain. Only two mutations have been identified in more than one patient, indicating the genotypic heterogeneity in this group of newborns with CH.
DIFFERENCES IN THE GENETIC PROFILE OF RISK FOR GRAVES' DISEASE IN A BRAZILIAN AND IN A DUTCH POPULATION

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Individual response to environmental factors such as viruses and chemicals might be related to the observed differences in Graves' Disease (GD) incidence all over the world. We previously demonstrated that genes involved in the detoxification of toxicants such as the ones existing in cigarette smoke were associated to the risk of GD in the Brazilian population. Also, we observed an increased prevalence of HHV7, but not HHV6, infection in Brazilian GD patients. In order to investigate the effect of CYP1A1m1, GSTP1, 72TP53 polymorphisms, in addition to HHV7 infection, in different populations, we compared 400 GD Brazilian patients paired on the basis of gender, age, and ethnicity to 574 Brazilian healthy individuals to a population from the Netherlands including 250 GD patients and 143 controls. All 1367 individuals were genotyped for CYP1A1m1, GSTP1 and 72TP53 using PCR-RFLP assays and investigated for HHV7 infection using a nested-PCR. We observed a similar rate of HHV7 infection in the control groups from Brazil (43.6%) and the Netherlands (42%; p=0.768). However, HHV7 was much more frequent among Brazilian (61%) than among Dutch (32%) GD patients (p< 0.0001). HHV7 infection increased the risk for GD in the Brazilian population more than 3 times (OR=3.133;95% CI=1.959-5.011), but did not affect the Dutch population. The inheritance of Pro/Pro TP53 (p< 0.0001), GSTP1 (p< 0.0001), and CYP1A1m1 (p=0.0072) gene variants was increased in GD Brazilian patients compared to controls, whereas in the Dutch population Pro/Pro TP53 (p=0.0061) but not GSTP1 (p=0.1272) nor CYP1A1m1 (p=0.5395) were overrepresented. We conclude that HHV7 infection is a factor of risk for GD in the Brazilian but not in the Dutch population. Differences in the profile of genes involved in the metabolism of toxic compounds may also contribute to epidemiologic and clinical differences observed in these populations.
POSTNATAL EARLY OVERNUTRITION CHANGES THE LEPTIN SIGNALING PATHWAY IN THE HYPOTHALAMUS-PITUITARY-THYROID AXIS OF YOUNG AND ADULT RATS

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Postnatal early overnutrition (EO) is a risk factor for obesity. Rats raised in small litter develop hyperinsulinaemia, hyperphagia, hyperleptinaemia and hypertension when adults. Leptin regulates the hypothalamic-pituitary-thyroid axis. We studied leptin signaling pathway in pituitary and thyroid glands on the postnatal EO model. To induce EO, at the 3rd day of lactation, litter size was reduced to 3 pups/litter (SL group) and controls (NL group) were adjusted to 10 pups/litter. Rat offspring were killed at 21 (weaning) and 180 days-old. Plasma TH, TSH and leptin were measured by radioimmunoassay. Proteins of leptin signaling pathway were analyzed by Western Blotting. Body weight of SL group was higher since the 7th day of lactation (+33%, p< 0.05) until 180 days-old (+18%, p< 0.05). SL group showed higher visceral fat mass at 21 and 180 days-old (+176%, +52%, p< 0.05, respectively), but plasma leptin was higher only at 21 days (+88%, p< 0.05). SL offspring showed higher plasma TSH, T3 and T4 at 21 days (+60%, +91%, +68%, p< 0.05, respectively), while the opposite was observed at 180 days regarding TH (T3: -10%, T4: -30%, p< 0.05), with no difference in TSH. In hypothalamus, no change was observed in leptin signaling pathway at 21 days. However, lower JAK2 and p-STAT3 content were detected in adulthood. In pituitary, SL group presented higher Ob-R, JAK2 and p-STAT3 content at 21 days and lower JAK2 and STAT3 content at 180 days-old. Thyroid Ob-R expression was lower in young SL rats, while the adult SL group presented higher Ob-R and JAK2 content. We evidenced that postnatal EO induces short and long-term effects in the hypothalamus-pituitary-thyroid axis, suggesting interplay between thyroid function and leptin action. These changes may help to explain a future development of metabolic and endocrine dysfunctions, such as metabolic syndrome and hypothyroidism.
Thyroid hormone is important for brain development, acting through regulation of gene expression. Thyroid hormone-regulated genes have been identified during the postnatal period in the rat brain, but only a few genes during the foetal period.

**Objectives:** To identify thyroid hormone-regulated genes in the foetal rat brain at term, and to analyze the respective roles of the foetal or maternal-derived hormones.

**Methods:** Thyroidectomized pregnant dams were given MMI to produce maternal and foetal hypothyroidism. Cerebral cortex RNA from GD21 foetuses from 10 hypothyroid and 10 control litters was isolated and hybridized to Agilent cDNA microarrays. Statistical analysis of microarray data was performed using the limma package.

**Results:** Using a cut-off of $P < 0.01$ and a change of at least 1.6 fold, the expression of 47 genes were increased and of 53 genes were decreased in the hypothyroid foetuses compared to normal. Twenty selected genes were confirmed by real time PCR in a different group of animals. In addition, T3 stimulated the expression of 9 out of the 20 genes analyzed, at least two-fold 24 hours after addition to primary cultures of cerebral cortex neurons, and are therefore candidates as primary responses to T3. Expression of selected genes was normal in foetuses from thyroidectomized dams without MMI administration.

**Conclusions:** This work fills a gap in the knowledge of thyroid hormone action in the brain by identifying gene targets of thyroid hormones in the foetal rat cerebral cortex using a global approach. Furthermore, some of the identified genes are most probably direct targets of T3, since they respond to the hormone in primary cultured neurons. In addition, we also observe that the main regulator of gene expression in the foetal rat brain at term is the hormone produced by the foetal thyroid gland.
THE REGULATION OF THE MITOCHONDRIAL ENERGY METABOLISM IN HUMAN HAIR FOLLICLES (HF) UNDERLIES THE CLASSICAL HYPOTHALAMUS-PITUITARY-THYROID (HPT) AXIS

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Objectives: Human HFs reportedly express and are controlled by elements of the HPT-axis, namely: Cellular components of HFs have functional receptors for TRH, TSH, THs which modulate hair cycle, hair growth, apoptosis, and melanogenesis. Since the mitochondrial cytochrome-c oxidase (MTCO1) differentially triggers cell signalling factors such as Bax (pro-apoptotic) and/or Bcl-xl (anti-apoptotic), we investigate here the effects of the HPT-axis on (MTCO1) expression and activity in HFs.

Methods: Human anagen HFs were isolated from scalp skin of euthyroid females undergoing face-lift surgery. HFs were cultured in serum free medium, supplemented with/without TRH, TSH and TH for 6 days. MTCO1 protein was detected by immuno-histochemistry on frozen HF sections, while enzyme activity was measured in HF homogenates using the COX activity assay kit (SigmaAldrich).

Results: We show here that TRH, TSH and TH up-regulate MTCO1 protein expression and enzyme activity. MTCO1 immunoreactivity detected in the HF epithelium increased after TRH, TSH and TH administration by 21.6%(p< 0.01), 24.5%(p< 0.001) and 25.5% (p< 0.01) respectively. COX activity was also stimulated: 20.2±3.4mU/ml vs. 47.4±8.4mU/ml (p< 0.01); 30.0±2.71mU/ml vs 51.5±5.54 mU/ml (p< 0.01) and 27.4±3.1mU/ml vs 54.5 ±7.2mU/ml (p< 0.01) respectively.

Conclusions: The HPT axis appears to regulate specific functions in human hair follicles by activation/de-activation of the mitochondrial energy metabolism which subsequently modulates proliferation, apoptosis or cell cycle arrest.
Monocarboxylate transporter 8 (MCT8) is an important thyroid hormone transporter in the brain. The MCT8 (SLC16A2) gene is associated with the Allan-Herndon-Dudley Syndrome (AHDS), with a presentation of severe X-linked psychomotor retardation, in combination with elevated serum T3 levels and low-normal serum T4 levels. To understand the pathophysiology of the mutations and to determine a genotype-phenotype association, we studied seven newly identified mutations found in AHDS patients. We introduced the changes by site-directed mutagenesis in our human MCT8 cDNA.

<table>
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<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Reported by</th>
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<tr>
<td>Patient 7</td>
<td>G558D</td>
<td>Frints et al (2008)</td>
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The MCT8 mutants were functionally analyzed in vitro using transiently transfected JEG3 and COS1 cells. We measured the uptake of 125I-T3 and T4 after 10 min in cells co-transfected with wild-type or mutant MCT8 and mu-crystallin. Both in JEG3 and COS1 cells, all mutants were inactive except for some residual transport activity for G282C and G558D in COS1 cells. Immunoblots of the mutants using our C-terminal MCT8 antibody, showed less intensity of the insV238 and G558D proteins in both cell lines, suggesting a lower stability of these mutant proteins. It is yet unknown whether the mutants are properly expressed at the plasma membrane.

We demonstrated an almost complete decrease in uptake of thyroid hormone by the mutants, which could explain the severe psychomotor retardation caused by a low T3 uptake in the neurons.
OP83
LARGELY DIFFERENT GENE EXPRESSION PROFILES IN CELLS FROM MCT8 PATIENTS

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Objectives: MCT8 facilitates T3 transport across the plasma membrane in various cell types. Mutations in MCT8 result in psychomotor retardation and elevated serum T3 levels. The phenotype probably results from decreased intracellular T3 levels in developing central neurons, resulting in altered transcription of T3-responsive genes and impaired brain development. This hypothesis was tested using fibroblasts from MCT8 patients as a model.

Methods: Skin fibroblasts were cultured from 4 patients with MCT8 mutations and from 3 controls. Following incubation for 24h in medium containing 0, 1 or 10 nM T3, RNA was isolated and gene expression profiles were analysed using microarrays.

Results: Over 2000 genes showed a >1.5-fold different expression in patients vs controls, of which 45% were upregulated. About 500 genes showed similar responses to T3 treatment in patients and controls, whereas only ~70 genes responded differently to T3. The largest differences in the patients' fibroblast transcriptome were T3-independent. The most significantly changed gene networks are involved in (embryonic) cellular development. In-depth analysis showed that in patients' fibroblasts, T3 receptor alpha was markedly downregulated, which likely affects numerous T3-responsive genes. A large decrease was observed in transcripts involved in GABA and glutamate receptor signalling as well as in transcripts for several neurotrophic factors like brain-derived neurotrophic factor.

Conclusions: The expression of a myriad of genes, including those highly expressed in brain, were affected in fibroblasts from MCT8 patients. The observation that only a small proportion of differentially expressed genes between patients and controls are T3-responsive may be explained by
1) lasting effects of defective T3 uptake, or
2) a role of MCT8 in addition to T3 transport such as the transport of another ligand which is essential for normal human brain development.
Our data present novel insights in the pathogenesis of the MCT8 syndrome, which may have implications for therapy.
EFFECTS OF AMIODARONE AND DRONEDARONE TREATMENT ON CENTRAL THYROID HORMONE METABOLISM IN RATS

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Background: The potent antiarrhythmic drug amiodarone (AM) has an inhibitory effect on the binding of T3 to the thyroid hormone receptors TRα1 and TRβ1 whereas dronedarone (Dron) has an inhibitory effect on TRα1 only. In this study we investigate the effect of AM and Dron on thyroid hormone metabolism in the pituitary and hypothalamic periventricular region (PE).

Objective and methods: Three groups of male rats received either Dron, AM or vehicle by daily intragastric administration for two weeks. Plasma T3, T4 and TSH were measured. Type 2 deiodinase (D2) activity and D1 and D2 mRNA expression were measured in the pituitary. D2, D3 and TRH mRNA expression was measured in the PE (mRNA expression by qPCR). Results are expressed as mean ± SEM, differences between groups were evaluated using Mann-W or t-test (*p< 0.01; #p=0.055).

Results: In AM treated rats plasma T3 was lower (C:1.29±0.03; Dron:1.25±0.06; AM:0.85 ±0.05* nmol/L), and T4 (C:81±2.3; Dron:85±3.2; AM:168±5.8* nmol/L) and TSH (C:1.57 ±0.21; Dron:1.25±0.14; AM:5.76±1.19* ng/ml) were higher compared to Dron treated and control rats. No differences were seen in pituitary D1 and D2 mRNA between the groups. However, AM treated rats showed decreased pituitary D2 activity (C:33.3±2.3; Dron:38.8 ±3.3; AM:25.0±3.4* fmol/mg*hr)) which was correlated to increased plasma T4 levels (p=0.001). TRH mRNA expression decreased in both Dron and AM treated rats compared to controls, whereas no changes in hypothalamic D2 and D3 mRNA were observed between groups.

Conclusion: Amiodarone treatment in rats decreases pituitary D2 activity in association with high plasma T4 levels. The decrease in TRH mRNA in the PE in both AM en Dron treated rats suggests a role of TRα1 in TRH regulation which is independent of plasma thyroid hormone levels.
OP85
CARDIAC METABOLISM OF 3-IODOTHYRONAMINE
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Objectives: 3-iodothyronamine (T1AM) is an endogenous relative of thyroid hormone which interacts with plasma membrane receptors known as trace amine-associated receptors, and produces functional effects like hypothermia and reduced cardiac contractility. In principle T1AM can be produced from thyroxine by enzymatic decarboxylation and deiodination, but its catabolism is unknown. The present study aimed at determining the catabolism and uptake of exogenous T1AM in rat heart.

Methods: Isolated working rat hearts were perfused with T1AM (50 nM) and analysis was performed in the recirculating buffer and in cardiac homogenate. Similar experiments were performed in isolated cardiomyoblasts (H9C2 cells). In the latter model both incubation medium and cell lysate were collected at different times and submitted to analysis. The analytical method included reverse phase HPLC coupled to tandem mass spectrometry (ESI-MS-MS), and unabled contemporary detection of T1AM, 3-iodothyroacetic acid (TA1), thyronamine (T0AM) and thyroacetic acid (TA0). Experiments were repeated in the presence 0.1 mM iproniazide, an inhibitor of monoamine oxidases and semicarbazide-sensitive amine oxidases.

Results: In both models T1AM concentration in the perfusion buffer decreased exponentially over time (the half life was on the order of 20 min in isolated heart perfused with 200 ml of recirculating buffer). T1AM could be detected in cardiac homogenate and in cell lysate, showing that significant uptake occurred. We also detected TA1, a product of T1AM oxidative deamination, which significantly accumulated in cell lysate and cardiac homogenate. Pretreatment of H9C2 cells or isolated hearts with iproniazide significantly inhibited T1AM conversion to TA1. Deiodinated derivatives (i.e. T0AM and TA0) were not detected in any model.

Conclusions: We conclude that T1AM is taken up by cardiomyocytes and can be catabolized to TA1 through iproniazide-sensitive amine oxidases. HPLC-ESI-MS-MS proved to be an effective and quantitative technique to elucidate T1AM metabolism.
Cardiac myosin heavy chain (MHC) α and β generate three myosin isoforms which regulate myocardial contractile activity. Although the studies of TR-null mice suggest that thyroid hormone (T3) and its receptor (TR) are important for both MHC genes expression, the molecular mechanism of especially trans-repression of MHCβ gene is completely unknown. Here, we report that transcription enhancer factor (TEF) is an important activator of MHCβ gene, which is suppressed by T3/TR. Taking notice that α1-adrenergic receptor signaling activates the MHCβ gene expression as well as TEF family and that the MHCβ gene promoter harbors two M-CAT elements and an A/T-rich sequence which are recognized by TEF, we investigated the role of TEF in MHCβ gene activation. In CV1 cells, expression of TEF family (TEF-1, DTEF-1 or RTEF-1) enhanced the activity of MHCβ (nt. -295/+125)-CAT reporter gene by 5-20 folds. Reporter analyses and gel shift assays revealed that two M-CAT elements and an A/T-rich sequence in MHCβ promoter are all required for the full activation of the gene by TEF. Another transcription factor, MEF2 (myocyte enhancer factor-2), involved in cardiac morphogenesis also enhanced the MHCβ promoter activity and co-expression of TEF and MEF2 dramatically increased it. Their synergistic effects may be via protein-protein interaction, since the DNA binding of MEF2 is not proven. Interestingly and importantly, T3-bound TR suppressed the TEF-induced activation of MHCβ promoter in a dose dependent manner of the ligand and receptor. Deletion analyses showed that the DNA-binding domain of TR is critical for the suppressive activity. Immunoprecipitation study using CV1 cell extracts expressed with FLAG-tagged TR and myc-tagged TEF-1 demonstrated in vivo association between TR and TEF-1, suggesting that T3-bound TR inhibits the MHCβ gene activity by interfering with the function of the activator TEF through interacting with it.
P001
PROLIFERATION AND SURVIVAL MOLECULES IMPLICATED IN THE INHIBITION OF BRAF PATHWAY IN THYROID CANCER CELLS HARBOURING DIFFERENT GENETIC MUTATIONS
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Background: Thyroid carcinomas show a high prevalence of mutations in the oncogene BRAF which are inversely associated with RAS or RET/PTC oncogenic activation. The possibility of acting on the BRAF pathway using inhibitors has become of increasing interest for therapeutic purposes. The underlying signalling pathways implicated on the cellular effects of BRAF pathway inhibition in thyroid cancer cells are not well established.

Objectives: To evaluate the alterations at proliferation and survival pathways, as well as of their associated molecules, induced by BRAF inhibition in thyroid carcinoma cell lines harbouring distinct genetic backgrounds.

Methods: Suppression of BRAF pathway in thyroid cancer cell lines (TPC-1, 8505C and C643) using RNA interference (RNAi) for BRAF and the kinase inhibitor, sorafenib. BrdU and TUNEL assays to assess proliferation and apoptosis, respectively.

Results: Both BRAF siRNA and sorafenib inhibit proliferation in all the thyroid cell lines, although decreased ERK1/2 phosphorylation, increased p27Kip1 and lower cyclin D1 levels, were particularly evident in the cell line with mutated BRAF (8505C). In addition sorafenib was able to induce apoptosis in cells harbouring BRAFV600E mutation. The mechanism by which sorafenib induces apoptosis seems to be due to a balance of the anti apoptotic proteins Mcl-1 and Bcl-2, which was more relevant in cells with BRAFV600E mutation. Specific inhibition of BRAF by RNAi had no effect on apoptosis in cells with BRAFV600E mutation.

Conclusion: Our results show that BRAF signalling pathway provides major proliferation signals in thyroid cancer cells through ERK1/2, p27Kip1 and cyclin D1. We have shown that, in thyroid cancer cells harbouring BRAFV600E, sorafenib induces apoptosis by affecting Mcl-1 and Bcl-2 anti-apoptotic proteins. Sorafenib may be particularly useful in the treatment of thyroid carcinomas harbouring BRAF mutations that are refractory to conventional treatment.
HIGH IODIDE CONCENTRATION REGULATES NIS AND SELENOPROTEINS EXPRESSION IN THYROID CELLS

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It has been well established that high doses of iodide inhibit thyroid hormone biosynthesis, the Wolff-Chaikoff effect. Moreover, iodide itself blocks iodide transport into the cell (by inhibiting NIS expression), reestablishing intracellular iodide concentrations leading to the escape of the W-C effect. It is suggested that iodide induces cell oxidation, and this is responsible for NIS modulation. The aim of this study was to investigate a possible role of NIS and selenium-dependent anti-oxidant proteins in the effects of W-C on the thyroid cell.

NIS mRNA expression was inhibited not only by high I- concentration (40%), but interestingly also by Se (35%), and this inhibitory effect was synergic (75%). However, there was no modulation on NIS promoter activity. I- treatment inhibited both protein expression and functional NIS. The inhibitory effect was not observed after the Se treatment and the treatment with both I- and Se did not inhibit functional NIS more than I- alone. These data suggest that I- and Se concentrations may interfere with NIS mRNA stabilization.

We observed that iodide (I-) increased the oxidative status of the PCCl3 thyroid cell, and this effect was maintained even when selenium (Se) was added into the medium. We determined the expression of several selenoproteins in thyroid (Txn, TxnRd1 and 2, Gpxs1-5, Selm, Selk, Sels, Sepp1, Sep15 and Sbp2) and observed that Se increased their mRNA expression in some cases. Interestingly, this effect was blocked by iodide administration. These results suggest that high concentrations of I- modulate selenoprotein expression induced by Se increasing the oxidative status of the cell. These data suggest that in addition to NIS, several selenoproteins are involved in the effects of high iodide doses on the thyroid cell, however, further studies are needed to elucidate the specific role of these selenoproteins on the W-C effect.
To precise the role of oxidative metabolism in the genesis of follicular thyroid carcinoma, we looked for the most important pathway controlling mitochondrial functions and biogenesis. Among the PGC-1 family of transcriptional coactivators, PRC has provided a mechanistic framework relating mitochondrial biogenesis to cell growth program. We explored in pathological thyroid tissues the role of two signaling pathways activated in normal tissue that could also induce PRC expression: the TSH/cAMP/PKA and the nitric oxide (NO) pathways.

We selected two differentiated follicular thyroid carcinoma cell lines, FTC-133 and RO82-W1, for their mitochondrial behaviour. By quantitative PCR we studied the expression of mitochondrial target genes (ND5, CYT C) and two actors involved in the coordination of mitochondrial biogenesis (ERR\(\alpha\), PRC). We tested TSH (1 à 100 mU/ml) or SNAP (NO donor, 50 µM) effects. We explored the interaction between the transcriptional factor ERR\(\alpha\) and PRC by their transient transfection in RO82-W1 and their inhibition in FTC-133 by XCT790 and siRNA for ERR\(\alpha\) and PRC respectively. The impact of such inhibitions was estimated measuring 2 mitochondrial enzymes activities: the cytochrome oxidase (COX) and the citrate synthase (CS).

In our two models, TSH addition had no effect on mitochondrial biogenesis. Treatment with SNAP increased PRC and CYT C expression in FTC-133 and RO82-W1 cells while ERR\(\alpha\) expression was induced in RO82-W1 only. When we transfected RO82-W1 with ERR\(\alpha\) and PRC, we found an increase of COX and CS activities, whereas the inhibition of ERR\(\alpha\) and PRC decreased COX and CS activities in FTC-133.

In the follicular thyroid carcinoma, mitochondrial biogenesis is induced by NO but not by the TSH/cAMP/PKA pathway. This NO effect could be mediated by the complex PRC - ERR\(\alpha\). This new signaling pathway open the way for new therapeutic targets.
Objectives: CDH-16/Ksp-cadherin was characterized as a kidney-specific adhesion molecule belonging to the 7-D cadherin family. More recently, alpha B-crystallin was identified as a binding partner of its cytosolic domain and it was suggested to mediate the connection to the actin cytoskeleton. We decided to investigate the expression of CDH-16 in thyroid cells and its potential role in cell differentiation and transformation.

Methods: the expression of CDH-16 and alpha B-crystallin in thyroid gland and in thyroid cells was investigated by microarray analysis and by qRT-PCR. Protein localization in the thyroid gland was determined by immunofluorescence and confocal microscopy. TSH-dependent expression of CDH-16 was investigated in rat thyroid cell cultures by qRT-PCR and western blot. CDH-16, E-cadherin and alpha B-crystallin expression in human thyroid tumors was determined by qRT-PCR and by TMA immunofluorescence and confocal microscopy analysis.

Results: CDH-16 and alpha B-crystallin were expressed in the thyroid gland, at the basolateral plasma membrane of thyrocytes. CDH-16 was expressed in the mouse thyroid already at E15 and it co-localized with E-cadherin. The expression of CDH-16 in thyroid cell cultures was TSH dependent as most thyroid differentiation markers. In human thyroid tumors CDH-16 and E-cadherin expression was progressively lost. Alpha B-crystallin expression was also markedly decreased. By confocal microscopy, E-cadherin-negative tumor cells were always negative for CDH-16. However, CDH-16-negative cells could be positive for E-cadherin staining.

Conclusions: CDH-16 is expressed in the thyroid gland during embryonic development and in the adult, and TSH regulates its expression in thyroid cell cultures. CDH-16, as well as E-cadherin, is lost in thyroid tumors, but the former is the first to be lost during tumor progression. Alpha B-crystallin is also down-regulated in thyroid tumors. Overall these results are suggestive of a role of CDH-16 in thyroid cell differentiation and transformation.
Background: The increasing thyroid cancer incidence may be due to environmental carcinogens. However, specific thyroid carcinogens have not been identified because of the lack of experimental models. The discovery of stem cells has allowed the “stem cell hypothesis” for human thyroid cancer, since thyroid cancer cells express stemness markers, such as p63.

Aim of the present study: Preparation of thyroid cell precursors (thyrospheres) for in vitro studies on thyroid carcinogenesis.

Results: Fresh normal thyroid tissue was collected at surgery and digested by collagenase IV. Cells were seeded onto anti-adherent plates in stem cell medium culture (without fetal bovine serum and with the addition of growth factors). Thyrospheres started to appear after 7 days of cultures as floating cell clumps. They were then trypsinized and replated in either undifferentiating (unadherent, with stem cell medium) or differentiating conditions (adherent, with complete medium containing 6H mixture and TSH). Real Time PCR indicated that under undifferentiating conditions cells expressed stem cell markers including p63, Oct4 and HNF4alfa, whereas they did not express thyroid specific markers such as Tg, TSH-R, TPO, and NIS. The opposite occurred under differentiating conditions. The same procedure was also performed to isolate thyroid cancer stem cells. The effect of environmental carcinogens including BPA and cadmium was tested. Interestingly, incubation with BPA and cadmium increased cell proliferation. Western Blot analysis indicated that BPA and Cadmium increased Akt but not ERK phosphorylation. Moreover, cells exposed to BPA and Cadmium underwent differentiation with lower efficiency than control cells.

In conclusion, these results indicate that human thyrospheres are aggregates of thyroid cell precursors sensitive to the proliferative and de-differentiating effects of some environmental carcinogens including BPA and Cadmium. They appear, therefore, a suitable in vitro model for studying thyroid carcinogenesis.
OVERACTIVATION OF THE PI3K/AKT/MTOR PATHWAY IS OBSERVED IN THYROID BENIGN DISEASES

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Introduction: A mouse strain with the Pten gene selectively deleted in thyroid follicular cells (PtenL/L; TPO-Cre mice) has been previously described as having a constitutively active PI3K/AKT/mTOR pathway which leads to the development of thyroid hyperplasia and adenoma (1). Inhibition of mTOR with RAD001 was proved to be efficient in restoring the normal proliferation index in this model (1).

Aim: To determine whether the mTOR pathway is activated in human thyroid benign proliferative diseases.

Material and methods: One hundred and ten thyroid tissues including 61 nodular goiters, 27 thyroid adenomas and 22 adjacent normal thyroid tissues were analysed by immunohistochemistry for mTOR upstream (PTEN, pAKT Ser473, pAKT Thr308) and downstream (pS6 Ser235/236, p4E-BP1 Thr37/46) molecules.

Results: Increased expression of the pathway inhibitor PTEN was observed in both types of thyroid lesions when compared with normal tissues. Overexpression of the unphosphorylated and phosphorylated form of mTOR (Ser2448) and of its upstream activators, pAKT Ser473 and pAKT Thr308, was also detected in both types of benign thyroid lesions. Furthermore, in thyroid adenomas, overexpression of p4E-BP1 was observed. When we compared the two types of benign lesions, pAKT Ser473 and mTOR were observed more expressively in adenomas than in nodular goiter.

Conclusions: In conclusion, the mTOR pathway seems to be in part overactivated in thyroid nodular hyperplasia and benign tumours, particularly in follicular adenomas. Targeting of mTOR could be a valuable strategy to develop an efficient treatment for these disorders.

EFFECT OF VITAMIN D RECEPTOR DISRUPTION ON THYROID MORPHOLOGY AND FUNCTION
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Background: High levels of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the active form of vitamin D, inhibit proliferation of thyroid cancer cells. Vitamin D receptor (VDR) polymorphisms have been associated with autoimmune thyroid disease. Vitamin D deficiency has been reported to modulate autoimmune thyroid disease. High levels of 1,25(OH)2D3 decrease calcitonin production in medullary cancer cells in vitro, and in rats in vivo.

Objective: To investigate the effect of vitamin D receptor (VDR) disruption on thyroid morphology and function, focussing on both follicular cells (thyrocytes) and parafollicular cells (C cells).

Methods: After weaning, Leuven VDR knockout and sex-matched wildtype control mice were kept either on a normal diet or on a calcium-rich diet to control for effects induced by hypocalcemia. At 10-12 weeks, mice were sacrificed and thyroid and serum were isolated.

Results: Serum TSH, measured as a first indication of thyroid functionality, was not different between knockout and control mice. Morphometric analysis of the thyroid sections revealed no difference in thyrocyte size nor colloid/thyrocyte ratio. Also no evidence was present for autoimmune thyroiditis. Further functional analysis was performed by immunostainings for iodinated thyroglobulin (B1), T4, 4-hydroxynonenal (4-HNE), the NADPH oxidase DUOX, caspase 6 and calcitonin. There was no different staining for B1, T4, 4-HNE and caspase 6. DUOX tended to be located more cytoplasmic and more apically respectively in the knockout and control mice irrespective of the diet. In the parafollicular C cells a clearly stronger calcitonin staining was found in the knockout mice, irrespective of the diet.

Conclusion: Vitamin D receptor disruption did not induce major differences in thyroid morphology and thyrocyte function. However, a clear increase of calcitonin expression was observed in the parafollicular C cells and persisted after correction of serum calcium, indicative for an calcemia-independent inhibitory effect of 1,25(OH)2D3 on calcitonin-producing parafollicular C cells through the VDR.
SOMATOSTATIN RECEPTOR 2 EXPRESSION IN COLD THYROID NODULES

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Aim: The specificity and cellular origin of the SSRS findings in benign cold (CN) and hot thyroid nodules (HN), papillary carcinomas (PCs) and Graves' disease (GD) are currently contradictory and difficult to understand in the context of the well defined action of somatostatin on thyroid cell signaling. Therefore we systematically evaluated SSTR2 expression in various neoplastic and nonneoplastic diseases of the thyroid by means of immunohistochemistry.

Material and methods: Tissue sections from 29 HN, 22 CN, 19 PC and their tissue surroundings and 8 GD thyroids were immunostained for SSTR2 with an affinity-purified rabbit polyclonal antibody against SSTR2 (Bio Trend, Cologne, Germany) in a final dilution of 1:1000. Membranous SSTR2 staining was quantitated by evaluating 10 high power fields (HPF) systematically distributed along the largest diameter of the tissue section.

Results: The area covered by thyroid epithelial cells in 10 HPF expressed as median in mm² was 0.53 for CN, 0.44 for HN, 1.5 for PC, 1.3 for GD and 0.3 for the tissue surroundings. The SSTR2 staining density determined by dividing the area of SSTR2 positively stained thyroid epithelial cells in mm² by the area of all thyroid epithelial cells in mm² in 10 HPF was 0.1662 for CN, 0.0204 for HN, 0.0369 for PC and 0.0386 for GD.

Conclusions: SSTR2 receptor expression is inhomogeneous in thyroid disease, with the highest density detected in CN. The high SSTR2 receptor density in CN has to be considered when using SSRS for the diagnosis and localization of radioiodine negative thyroid cancer. In addition to the immunocompetent cells infiltrating the thyroid gland in Graves’ disease also the thyroid epithelial cells in Graves’ disease express SSTR2 receptors. The repeated SSRS detection in PC is mostly related to SSTR2 expression on thyroid epithelial cells and not dependent on their lymphocytic infiltration.
ELECTRIC AND MAGNETIC FIELD DO NOT MODIFY THE BIOCHEMICAL PROPERTIES OF FRTL-5 CELLS

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Electric and magnetic fields are invisible lines of force that surround any electrical device. Magnetic fields result from the flow of current through wires or electrical devices and increase in strength as the current increases. The effects of electromagnetic fields on the human body depend not only on their field level but on their frequency and energy.

The aim of the present study was to evaluate the effects of electric and magnetic fields (EMF) on the biochemical properties of the FRTL-5 cells (differentiated thyroid cell line), in particular on their ability to trap iodine and to produce cAMP.

FRTL5 were grown in Coon's medium supplemented with 5% calf serum, gentamicyn and six-hormone mixture containing bovine TSH (6H). Cells were seeded in 3 cm dishes, at confluence were maintained in 5H medium (without bTSH) for 7 days and were subsequently irradiate for 24, 48 and 96 hours with EMF (800-900 MHz, power-frequency of mobile communication systems). Cells were then maintained in 6H for 2 days before to perform iodide uptake and cAMP assay. To measure iodide uptake cells were incubated for 45'at 37°C with Na¹²⁵I, solubilized and the radioactivity was counted in g-counter. To measure cAMP production cells were incubated for 1h at 37°C in hypotonic buffer containing bTSH 10mU/ml and IBMX and cAMP was determined by RIA assay.

Our data demonstrate that FRTL-5 cells exposed for 24, 48 and 96 hours to EMF do not show modifications of the vitality, iodide uptake and bTSH-stimulated cAMP production.

In conclusion, EMF do not seem to be able to interfere with the biochemical properties of FRTL5 cells.
P02 Genetics of Benign Thyroid Disease 1

P010
NON-GOITROUS AND NON-AUTOIMMUNE HYPERTHYROIDISM CAUSE BY A HETEROZYGOUS TSHR MUTATION

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Context: Activating mutations in the TSHR gene were found in patients suffering from non-autoimmune hyperthyroidism. So far it was assumed that thyroid hyperplasia in these patients is due to constitutive activation of the Gs/adenylyl cyclase signaling pathway, however, the physiological role of the Gq/11 pathway in this context remains obscure.

Objective: The objective of this study was to investigate the TSHR gene in a patient with non-autoimmune and non-goitrous hyperthyroidism.

Results: We detected a heterozygous mutation in exon 10 of the TSHR gene leading to an exchange of a highly conserved Cys residue in transmembrane helix 6 for Trp at amino acid position 636. Functional characterization of the mutant receptor revealed constitutive activation of the Gs mediated signaling pathway. Cell surface expression of the mutant receptor is comparable to the wild-type receptor, but maximal hormone binding properties are slightly reduced. Interestingly, investigation of the Gq/11 phospholipase C pathway revealed a nearly complete loss-of-function after bTSH stimulation. High conservation of a cysteine at this position in the G-protein coupled receptor family 1 and our molecular homology modeling studies suggest a fundamental role of this residue in activity regulation not only for the TSHR.

Conclusion: This is the first case report of a patient in whom a TSHR mutation leads to non-autoimmune hyperthyroidism due to a mutation that constitutively activates the Gs signaling pathway, but additionally inhibit the Gq/11 pathway completely which could explain the lack of goiter in the patient. These findings point to a concept of receptor activation in which the activation of each signaling pathway needs its specific conformation for activation.
HIGH SUSCEPTIBILITY HAPLOTYPES OF THE TSHR GENE IN GRAVES-BASEDOW DISEASE

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The mechanisms triggering autoimmunity in the Graves-Basedow disease (GBD) are unknown, although the evidence for a genetic predisposition exists.

**Objectives:** To identify the main alleles/haplotypes of the TSHR gene, and to analyze the existence of TSHR susceptibility alleles to the GBD.

**Methods:** To establish the main alleles of TSHR gene, 54 polymorphisms (53 SNPs and 1 DIP) were selected including:
1) SNPs identified by sequencing the promoter and 3'UTR regions, and
2) TagSNPs capturing most of the genetic variability of the gene.

TagSNPs have been selected using the Haploview software and available data from HapMap project. These 54 polymorphisms were genotyped, using SNPlex technology, in 329 gDNA samples from: 192 control subjects and 137 GBD patients. The relation between haplotypes and age at the GBD start was analyzed.

**Results:** After the multiple tests correction, a set of 10 SNPs showed a significantly different distribution between cases and controls (p< 0.05). The odds ratio obtained ranged from 2.08 to 5.15. All these significant SNPs were located in a region that covers from the position -6200 in the promoter, to the intron 1. The main haplotypes of the TSHR gene were established, using the different blocks of linkage disequilibrium (LD). Two highly significant haplotypes, one protective and one predisposing to the disease, were identified. The polymorphism more strongly associated (SNP11) influence the age of the process start: Homozygote patients for the allele of risk present a half age of GBD start lower than the homozygote for the protective variant (p = 0.03).

**Conclusions:** A set of SNPs located in the 5' region of TSHR gene (from the promoter up to intron 1) significantly associated to GBD was identified. The existence of LD among the associated SNPs allows defining the main haplotypes, two of them conferring susceptibility or protection to the disease.
Mutations in the MCT8 gene coding for the monocarboxylate thyroid hormone transporter 8, have been recently associated with Allan-Herndon-Dudley syndrome (AHDS), an X-linked condition characterized by severe mental retardation, dysarthria, athetoid movements, muscle hypoplasia and spastic paraplegia and a peculiar thyroid phenotype (low T4, increased T3 and slightly increased TSH). The relationship genotype/phenotype of this conditions still remains to be fully elucidated. We describe in detail the clinical and biochemical features of a 5 year old boy affected by AHDS with a unexpectedly low TRH-stimulated serum TSH and severe neurological abnormalities and a novel MCT8 mutation, a 1343-1344insGCC denovo insertion, resulting in a truncated protein lacking the last four transmembrane domains as well as the carboxyl cytoplasmic end. Clinical features included mental retardation, axial hypotonia, hypertonia of arms and legs, paroxysmal dyskinesias, seizures. The endocrine phenotype showed low serum total (58.4 nmol/L) and free (7.6 pg/ml) T4, very elevated total (>12 nmol/L) and free (13.2 pg/ml) T3 and markedly increased (>180 nmol/L) serum SHBG, in keeping with the recognized liver hyperthyroid status of AHDS. Unexpectedly, serum TSH was normal (0.925 mU/L) with a blunted response (1.93 mU/L) to TRH, while normal to increased basal and TSH-stimulated TSH has been so far reported in AHDS. This finding suggests the presence in our case a subtle T3-mediated thyrotoxic condition even at the pituitary level and constitutes a remarkable contribution to better characterize this disorder and to elucidate the functional consequences of MCT8 gene mutations.
Recent studies suggest that both germline and somatic mutations of the GR gene may affect glucocorticoid (GC) sensitivity and its role in several human diseases. GR mutations have been associated to resistance to GC action (ER22/23EK), while others to increased sensitivity (N363S, BCII). Aim of this study was to analyze the distribution of GR polymorphisms in GO patients treated with intravenous steroid therapy (IVGC) in relation to the disease response and side effects. Sixty patients with active GO, 44 women and 16 men, aged 55±13 (SE) years, were treated with IVGC (500 mg of methylprednisolone weekly for 12 weeks; cumulative dose 7.5 g). A complete ophthalmological assessment was performed to determine the CAS and NOSPECS score at baseline and response to therapy was assessed 5 months after IVGC as a decrease of the CAS≥2 points of NOSPECS classes 2-4≥1 point. Impairment of glucose metabolism and liver function and occurrence of depression were considered as major side effects of therapy whereas insomnia, dyspepsia, flushing, myalgia, asthenia, nausea as minor side effects. Thirty-nine patients (65%) responded to IVGC treatment, while 16 and 5 patients had persistence or recurrence of active GO, respectively. 46% of patients reported major and 54% minor side effects. Either N363S or ER22/23EK polymorphisms were found in heterozygosis in three patients (5%), while BCII variant was observed in 23 (38.3%) and 6 (10%) patients in heterozygosis and homozygosis, respectively. Only one patient showed the association of N363S and BCII. No significant association was observed between the prevalence of any of the three polymorphisms and response to therapy (P=NS) or occurrence of side effects and (P=NS). In conclusion, these preliminary data suggest that polymorphisms in GR gene do not influence GO patients' sensitivity to steroid therapy and the occurrence of side effects.
THE BCL1 GLUCOCORTICOID RECEPTOR GENE POLYMORPHISM AND TREATMENT IN SEVERE GRAVES' OPHTHALMOPATHY (GO)

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Objectives: Glucocorticoid activity may be involved in autoimmune thyroid disease. It is not known whether it is involved in GO. The aim of this study was to examine possible associations between the Bcl1 polymorphism of the glucocorticoid receptor (GlucR) gene, and clinical parameters of GO. This polymorphism may be associated with increased sensitivity to glucocorticoids.

Methods: We studied 172 patients of Dutch ethnic origin who had Graves' disease and GO, and attended the outpatients’ clinic, dept of Endocrinology and Orbital Center of the Academic Medical Centre, Amsterdam. CAS score, exophthalmometry measurements always by the same investigator, lid oedema etc were recorded. Autoantibody levels (both antiTPO and TRab) were measured. The type of therapy (glucocorticoids, orbital irradiation or surgery) which was used to treat GO was recorded in 127 patients.

Results: Allele frequency was 38.97%. There was no difference in the distribution of the polymorphism between Graves' disease patients with GO and a historical healthy control previously genotyped by us (34.89%). Of those who did not need treatment (n=116) 67.2% were Bcl1 carriers and 32.8% were non-carriers, while of those who needed glucocorticoid treatment only 42.9% were carriers and 57.1% were non-carriers (p=0.013, Fisher's exact). No significant differences were found in proptosis, CAS or TRab levels between Bcl1 carriers and non-carriers.

Conclusions: Patients with GO, who also carry the Bcl1 GlucR gene polymorphism, need less frequently therapeutic intervention compared to non-carriers. Because this polymorphism is associated with increased sensitivity to glucocorticoids, this finding may reflect a protective role of increased endogenous glucocorticoid activity in this autoimmune process.
Graves' ophthalmopathy (GO) is an autoimmune disorder affecting the orbit of up to 50% of Graves' disease (GD) patients with varying degrees of severity. T cells infiltrating the retrobulbar tissues are considered to play an important role in the inflammatory process affecting the extraocular muscles and the orbital fatty/connective tissue. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a key negative regulator of T-cell activation and proliferation during the immune response, but its role in the susceptibility to GO is poorly understood. Also, there is a lack of information on CTLA-4 relationship with other genes including detoxification genes involved in the metabolism of toxic compounds such as the ones found in smoke, a well recognized factor of susceptibility to GO. We studied CTLA-4-318 polymorphism in 393 individuals previously genotyped for CYP1A1 m1 and GSTP1 polymorphisms. There were 193 GD patients (160 women and 33 men, 39.3±10.7 years old) matched on the basis of gender, age, and ethnicity to 200 healthy individuals (125 women and 75 men, 40.4±15 years old). GO was present in various degrees in 96 patients. CTLA-4-318 variants had a similar distribution in GD patients (24.4%) and controls (18.5%), but GSTP1 variants were more frequent in GD patients (61.7%) than in controls (41.12%; p=0.0007) as well as CYP1A1 variants (47.3% versus 28.2%; p< 0.0001). CTLA-4-318 variants were more frequent among GO (33.4%) than in GD patients with no eye disease (15.5%; p=0.005). Indeed, the inheritance of a CTLA-4-318 variant increased the susceptibility to GO more than 2 times (OR=2.652; 95% CI=1.350-5.321). A multiple logistic regression analysis also identified a higher risk for GO in males (p=0.0108; OR=2.77; 95% CI=1.24-6.2), tobacco consumers (p=0.0017; OR=2.65; 95% CI=1.43-4.9) and GD patients with large goiters (p=0.001; OR=1.75; 95% CI=1.18-2.58). Our data suggest that CTLA-4-318 is an important determinant of susceptibility for eye disease in GD patients.
STUDY OF A POLYMORPHISMS IN THE PROMOTER REGION OF THE SEPS1 GENE AND RISK OF HASHIMOTO THYROIDITIS

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Introduction: Selenoprotein S is an endoplasmic reticulum (ER) resident protein which is known to promote cell survival by regulating the ER stress. Selenoprotein S has also been implicated in inflammation. Genetic variation in the selenoprotein S gene (SEPS1) was strongly associated with circulating levels of TNF-alpha, IL-6, and IL-1β and it was suggested a link influencing the proinflammatory cytokine profiles observed in some common human disorders.

Aim: The aim of our study is to determine whether a pro-inflammatory promoter polymorphism associated with impaired expression of SEPS1 is associated with the risk to develop Hashimoto Thyroiditis.

Materials: The G/A -105 polymorphism in the promoter region of SEPS1 was studied in DNA extracted from 380 blood samples: 188 from patients with HT and 192 from control subjects. The polymorphism was analyzed by PCR and confirmed by sequencing.

Results: The GA genotype was 2 times more frequent in patients with HT than in control (P =0.0027; 95% confidence interval [CI] 1,269 to 3,108). There were significant differences regarding gender. Although, there were only 11 HT males, the GA genotype in male patients with HT was increased (72,73% in HT and 17,91% in controls ) giving a 11 times higher risk (P =0.0016; 95% CI 2,452 to 46,397).

Conclusions: The significant differences of SEPS1 allele frequencies between disease group and controls suggest that the SEPS1 -105A allele may be a risk factor for HT. According to our results SEPS1 -105G/A polymorphism is significantly more frequent in males patients with HT than in healthy controls, representing, in males, a 11 fold risk for developing HT, but larger studies are necessary in order to confirm these findings.
CHARACTERIZATION OF A NOVEL LOSS OF FUNCTION MUTATION OF PAX8 ASSOCIATED WITH CONGENITAL HYPOTHYROIDISM

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Congenital hypothyroidism (CH) is the most frequent congenital endocrine disorder, occurring with an incidence of 1/3000-4000 newborns. In 80-85% of cases CH is associated with thyroid dysgenesis (TD) with a gland that is either absent (athyreosis), ectopically located or severely reduced in size. In approximately 15% of the patients, hereditary disorders in thyroid metabolism (dyshormonogenesis) are found. In a small percentage of cases, mutations in NKX2.1/TITF1, FOXE1/TITF2, PAX8, NKX2.5 or TSHr genes have been associated with the disease indicating that other genes may be responsible for at least some cases of TD.

Whereas most cases of TD are sporadic, mutations have been found in genes involved in thyroid development including several thyroid transcription factors and the TSH receptor gene. So far, five mutations in the PAX8 gene have been identified in patients with TD. Herein, we report a novel loss of function heterozygous mutation in the PAX8 gene in a family where both the mother and her child are affected by CH diagnosed by neonatal screening. Ultrasound of the thyroid in the mother showed a hypoplastic thyroid in normal position. The 123-I-uptake in the boy was low and remained unchanged after perchlorate administration. Mother and son are currently on thyroid hormone substitution.

The mutation consists in a T to G transversion at nucleotide 165 in exon 2 of the PAX8 gene and is responsible for the changing of a conserved histidine at position 55 with a glutamine (H55Q) in the DNA-binding domain.

The mutation was cloned and transfection experiments demonstrated that PAX8-H55Q is unable to activate transcription from the thyroglobulin gene promoter.

Our data confirm that loss of function mutation in the PAX8 gene may be responsible for congenital hypothyroidism and contribute to add a new piece into the unsolved puzzle of the molecular basis of thyroid dysgenesis.
The single nucleotide polymorphism C1858T within the PTPN22 gene was recently associated with autoimmune thyroid disease (AITD). The purpose of this study was to examine the joint association of this polymorphism with the AITD.

Materials and methods: In this association study 358 subjects were genotyped for the C1858T polymorphism PTPN22 gene. The study population included 108 Novosibirsk patients with Grave’s disease (GD) and 107 Hashimoto’s thyroiditis (HT), and 143 healthy controls. The presence of biochemical hyperthyroidism together with either the presence of dysthyroid eye disease or a diffuse goiter and a significant titer of microsomal, or TSH receptor autoantibodies defined GD. The diagnosis of Hashimoto’s thyroiditis was established by positive thyroid peroxidase antibodies, reduced echogenicity on thyroid ultrasound, and elevated thyrotropin levels. The male:female ratio of patients with GD, HT, was 1:2.9, and 1:9.7 respectively. The average ages (in years) of patients with GD, HT, were 41.67±1.22, 44.11±1.18, respectively. All control subjects had normal thyroid function and were thyroid autoantibody negative.

Results: No differences in genotype frequencies were observed between GD and controls for the C1858T polymorphism PTPN22 gene in population of Novosibirsk. The PTPN22 1858 T-allele frequency was strongly increased in patients with HT 24.3% versus controls 12.9%, \( \chi^2=10.8, (\alpha=0.001 \text{ OR} =2.16 \text{ 95%- CI 1.36-3.44} ) \). The T-allele frequency was 24.7% in women with HT and 12.1% in the control group, \( \chi^2=7.62, (\alpha=0.006) \). The T-allele were associated with the increased risk for HT in women (odds ratio OR=2.39 95% CI 1.27-4.89).

Conclusion: The PTPN22 gene is a joint susceptibility locus for HT.
Lack of Genotype / Phenotype Correlations for Somatic TSH-Receptor Mutations in Hot Thyroid Nodules

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The large spectrum of markedly different clinical severities of hyperthyroidism (CSH) of hot thyroid nodules (HNs) has repeatedly led to speculations about possible correlations of the in vitro activity (IVA) of the different TSH receptor (TSHR) mutations with the CSHs. We analyzed published IVAs determined as linear regression analysis (LRA) of constitutive activity as function of TSHR expression, basal cAMP and basal IP accumulation for possible correlations with clinical activity indicators of HNs described for 75, 56 and 28 patients reported by Trulzsch et al. 2001, Gozu et al. 2006 and Krohn et al. 2008. Moreover, we tested for significant differences of the clinical activity indicators of HNs with and without TSHR mutations.

The largest diameter of the HNs determined by ultrasound and the preoperative PTU dose required to maintain euthyroidism showed no correlation with LRA value, basal cAMP or IP level. A weak (Spearman's rho: r=-0.277) but significant (p=0.05, for n=75) correlation of the patient's age at time of surgery with the LRA value but not with the basal cAMP or IP level was observed. No significant differences for the HNs largest diameter, the patient's age at time of thyroid surgery or the preoperative PTU dose was found for patients with or without TSHR mutations.

The three examined clinical indicators of the clinical activity of a somatic TSHR mutation in HNs namely largest diameter of the HN, age of patient at time of thyroid surgery and the preoperative PTU dose showed no clear correlation with the IVA of the TSHR mutation characterized by the LRA value, the basal cAMP accumulation or the IP activity. The three indicators showed no significant differences for HNs with or without a TSHR mutation. Therefore, epigenetic and environmental factors are likely to be important determinants of the clinical activity of somatic TSHR mutations.
A higher frequency of skewed X chromosome inactivation (XCI) is found in patients with autoimmune thyroid disease (AITD) than in controls. Although goitre is often present in AITD, a recent study failed to show an association between XCI and clinically overt non-toxic goitre. However, the aetiology of overt goitre is complex, and the mechanisms influencing thyroid volume may involve fewer factors than the mechanisms underlying overt goitre. We have therefore examined the relationship between thyroid volume and XCI in a sample of euthyroid female twin pairs.

**Objective and design:** In order to examine the impact of XCI on thyroid volume, we studied whether within cohort and within twin pair differences in XCI are correlated with differences in thyroid volume.

**Subjects:** 138 euthyroid female twin individuals (age, 19-51 years) distributed in 69 same-sex pairs.

**Methods:** XCI was determined by PCR analysis of a polymorphic CAG repeat in the first exon of the androgen receptor gene. Thyroid volume was determined by ultrasound.

**Results:** Neither in the within cohort nor in the within twin pair analysis could we demonstrate a statistically significant association between XCI and thyroid volume: Regression coefficient (β) = 0.023 (95% confidence interval, -0.062-0.108), p = 0.592 and β = 0.038 (-0.080-0.156), p = 0.521, respectively. Controlling for potential confounders such as zygosity, age, TSH, smoking habits and use of oral contraceptives did not change the findings.

**Conclusion:** In a sample of euthyroid Danish female twins, we found no evidence of a relationship between XCI pattern and thyroid volume.
EVIDENCE FOR A MORE PRONOUNCED EFFECT OF GENETIC PREDISPOSITION THAN ENVIRONMENTAL FACTORS ON GOITROGENESIS BY A CASE CONTROL STUDY

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Objektives: Family and twin studies as well as linkage analyses and a genome-wide scan in 18 euthyroid goiter families suggest a genetic predisposition of euthyroid goiters. However, iodine deficiency and smoking are important exogenous factor for the development of goiters. To simultaneously asses the influence of both genetic and environmental factors and to reduce the influence of possible confounders we investigated goiter predisposition by a matched case control study.

Methods: 376 patients providing written consent were included in the study. We matched 188 patients with euthyroid or subclinically hyperthyroid goiter (TSH 4.20-0.05 mU/l) with 188 euthyroid controls without thyroid enlargement for age and gender. Family history of the patients was recorded using a standardised questionaire and thyroid ultrasound was performed for patients with goiter and controls.

Results: 50.5% of patients with goiters showed a positive family history for goiter. In contrast, only 25% of patients with normal thyroids had a positive family history for goiter (p< 0.001; OR=3.1). Patients with goiters had a significantly higher proportion of parents (p< 0.001; OR=3.6) or siblings (p=0.004; OR=2.5) with goiters. Children of parents with goiters showed a 2.7-fold increased risk for goiter and had a goiter prevalence of 73.3%. Patients with goiter positive family history had a 4.1-fold increased risk for goiter (p< 0.001). The contribution of smoking and obesity to goiter development was less important than the genetic predisposition (OR=1.7; p=0.06; OR=1.67; p=0.13).

Conclusion: The significantly higher rate of positive family histories in patients with goiters as compared to the matched controls as well as the increased goiter prevalence in children of parents with goiters indicate the importance of genetic factors in goiter development. Genetic factors play an important etiologic role in the development of goiters which appear to be more important than most environmental factors.
THE CHEMOKINE CXCL10 DYSREGULATION IN PAPILLARY THYROID CANCER

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Aims: In papillary thyroid carcinomas (PTCs), oncogenes activate a transcriptional program including upregulation of the CXCL10 chemokine, which stimulates proliferation and invasion. Furthermore, PPARgamma activators thiazolidinediones modulate CXCL10 secretion in normal thyroid cells (TFC), and inhibited PTC growth. Until now, no study has evaluated the effect of cytokines on CXCL10 secretion in PTCs, nor the effect of PPARgamma activation.

Methods: The effects of IFNgamma+TNFalpha stimulation on CXCL10 secretion in primary cells from PTCs and TFC were tested. Furthermore, the effect of PPARgamma activation by thiazolidinediones, on CXCL10 secretion and proliferation in these cell types was studied.

Results: In primary cultures of TFC and PTCs CXCL10 production was absent under basal conditions; a similar dose-dependent secretion of CXCL10 was induced by IFNgamma in both cell types. TNFalpha alone induced a slight but significant CXCL10 secretion only in PTCs. The stimulation with IFNgamma+TNFalpha induced a synergistic CXCL10 release in both cell types; however, a more than ten times higher secretion was induced in PTCs. Treatment of TFC with the thiazolidinediones dose-dependently suppressed IFNgamma+TNFalpha-induced CXCL10 release, while stimulated CXCL10 secretion in PTCs. A significant antiproliferative effect by thiazolidinediones was observed only in PTCs.

Conclusions: A dysregulation of the CXCL10 secretion has been shown in PTCs. In fact, a more than ten times higher CXCL10 secretion has been induced by IFNgamma+TNFalpha in PTCs with respect to TFC. Moreover, thiazolidinediones inhibited CXCL10 secretion in TFC and stimulated it in PTCs. The effect of thiazolidinediones on CXCL10 was unrelated to the significant antiproliferative effect in PTCs.
P023
GENE EXPRESSION PROFILING ASSOCIATED WITH THE PROGRESSION TO POORLY DIFFERENTIATED THYROID CARCINOMAS

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Objectives: Despite the high overall survival associated with the majority of thyroid tumours, poorly differentiated (PDTC) neoplasia represent a phenotype which may have an aggressive behaviour, often with a fatal outcome. Although the molecular pathogenesis of these carcinomas is still controversial, previous findings suggest a continuum of dedifferentiation from well-differentiated carcinomas (WDTC) to PDTC. To characterise the molecular mechanisms involved in such progression and identify novel therapeutical targets, we compared the genome-wide expression of normal thyroid tissues, WDTC and PDTC cases.

Methods: Gene expression profiles of normal thyroid tissues and 24 tumour cases - 7 classic papillary (cPTC), 8 follicular variants of papillary (fvPTC), 4 follicular (FTC) thyroid carcinomas and 5 PDTC - were assessed using the GeneChip HG-U133 Plus 2.0 Array, and correlated with RAS and BRAF mutations, and with RET/PTC1,-2,-3 and PAX8-PPARG rearrangements. Selected genes were further confirmed by quantitative RT-PCR in an independent set of 28 tumours.

Results: Hierarchical clustering and principal components analysis showed that distinct profiles separated FTC from other histotypes (PTC and PDTC). Gene expression similarity was higher between PDTC and fvPTC, particularly for tumours harbouring RAS gene mutations. PDTC presented molecular signatures related to cell cycle and proliferation, poor prognosis, mitotic spindle assembly checkpoint and cellular adhesion. Compared to normal tissues, PTC had 307/494 (60%) genes over-expressed, whereas FTC had 137/171 (80%) genes under-expressed. Nonetheless, the highest proportion of under-expression (92/107; 86%) was found in PDTC, suggesting that gene down-regulation is involved in tumour dedifferentiation. The validated genes were significantly deregulated in PDTC samples and also in PDTC cell lines, compared to normal tissues.

Conclusions: Our findings suggest that fvPTC, particularly the cases with RAS mutation, are potential precursors of PDTC. The genes confirmed by quantitative RT-PCR have essential roles in DNA methylation or in cell invasion, and so have potential therapeutic uses.
BRAF IN PRIMARY AND RECURRENT PAPILLARY THYROID CANCERS: RELATIONSHIP WITH THE 131-IODINE (131-I) AND 2-[18F]-FLUORO-2-DEOXI-D-GLUCOSE (18-FDG) UPTAKE CAPABILITY

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Activating mutations of BRAF represent the most frequent genetic event in adult papillary thyroid cancers (PTCs) -36-48% of cases- and a possible marker of poor prognosis. In a previous report we showed that PTC recurrences with no 131-I uptake carried a high frequency of BRAF mutations, a low expression of thyroid-related genes (TPO, Pendred's Syndrome gene, Tg) and a high expression of GLUT-1 transporter gene.

Objectives: To extend previous observations to a larger series of recurrent PTCs, considering paired primary and recurrent PTC cases.

Patients and methods: In 53 recurrent cancers, BRAF analysis was conducted by direct sequencing and mutant allele-specific PCR amplification (MASA), in paired primary and recurrent PTCs when available (19 cases); 31/55 patients underwent 18-FDG-PET-TC.

Results: BRAF mutations were present in 55% (29/53) of recurrent PTCs, of which 86% (25/29) with no 131-I uptake and 14% (4/29) with 131-I uptake. The V600E BRAF mutation was present in all cases but one where a new oncogenic event, leading a serine to phenylalanine substitution at codon 602 (S602F), was showed. 70% (13/19) of recurrent PTCs carrying BRAF mutations were 131-I negative and 18-FDG positive, 10% (2/19) were simultaneously 131-I and 18-FDG negative, 10% (2/19) were 131-I and 18-FDG positive and 10% (2/19) were 131-I positive and 18-FDG negative. In paired primary and recurrent PTCs, BRAF mutations were present in 37% (7/19) of primary cancers and in 58% (11/19) of their recurrences during follow-up.

Conclusions: BRAF mutations seem to be more frequent in thyroid recurrences with no radioiodine uptake by comparison with radioiodine-positive ones; they correlate with the capacity to concentrate the 18-FDG and they can appear as a de novo event in recurrent PTCs.
Our Aim was to relate mitochondrial richness and tumor aggressiveness to human Death Associated Protein 3 (DAP3) expression in thyroid tumors.

DAP3 is a GTP-binding constituent of the mitochondrial ribosome; its proapoptotic function has also been described. hDAP3 is a marker of aggressiveness in the thymoma, another endocrine tumor. Mitochondrial characteristics of thyroid oncocytoma suggest this tumor type as a model to explore the links between cellular growth and mitochondrial biogenesis. In a previous 2-step study (differential display and macroarray), we found the hDAP3 gene 1.5-fold upregulated in oncocytic adenoma (n=6) compared to paired normal tissue. Analysing the hDAP3 promoter using bio-informatic tools, we showed that the 9 best scored transcription factors presenting enriched motifs are implicated in cellular growth (ELK1, ELK4, RUNX1, HOX11-CTF1, TAL1-TCF3) and mitochondrial biogenesis (NRF1, GABPA, PPARG-RXRA, ESRRA).

We investigated the hDAP3, ELK1 and ESRRA expression (quantitative PCR) in 10 macrofollicular adenomas, 10 papillary carcinomas, 10 oncocytic tumors and 10 control tissues. We also explored the DAP3 protein level (immunohistochemistry) in 95 independent thyroid tumors presenting various mitochondrial contents.

The DAP3 expression is upregulated in thyroid oncocytoma, higher in carcinomas (1.9-fold) than in adenomas (1.5-fold), compared to the normal tissue. ELK1 and ESRRA were also upregulated in these tumors. The overexpression of DAP3, ELK1 and ESRRA in thyroid oncocytic tumors are validated by analysis of a normalised public thyroid microarray dataset. At the protein level, the DAP3 expression was found correlated with a mitochondrial antigen (COX IV) expression (rho=0.65, p< 0.01).

Our results demonstrate DAP3 may play a role in mitochondrial homeostasis on one hand, and in the process of tumorigenesis, on the other. If the thyroid tumorigenic process calls for increased mitochondrial biogenesis, a defect in even one of the proteins of the mitoribosome, such as DAP3, could lead to cell apoptosis.
BRAF MUTATION AND FUSION ONCOGENES (RET/PTC-1, RET/PTC-3, AKAP9/BRAF AND PAX8/PPARγ) EXPRESSION IN THYROID NODULAR DISEASE IN CHILDREN: ANALYSIS OF 119 ASPIRATES FROM BIOPSY

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Objectives: The genetic abnormalities in childhood versus adult thyroid cancer are different and therefore it requires to search the other candidate genes as diagnostic markers of malignancy.

Methods: Preoperative material from fine-needle biopsy (aspirates) of childhood thyroid nodules was studied. Both, DNA (for BRAF gene mutation) and RNA (for RET/PTC-1, RET/PTC-3, AKAP9/BRAF, PAX8/PPARgamma expression analysis) were isolated. From the total DNA, the exon 15 of BRAF gene was amplified to investigate the T1799A (V600E) mutation based on PCR-sequencing technique. Total RNA underwent reverse-transcription and polymerase-chain-reaction (RT-PCR) to obtain cDNA and to analyse the expression of markers of thyroid cancer (candidate genes)(Qiagen OneStep RT-PCR). In all samples the analysis of control genes: GAPDH (for the qualitative and quantitative evaluation of RNA) and thyroglobulin (control marker of thyroid cells) were performed. Aspirates used for studies were verified histologically in 75 patients and represented all histological types of thyroid disease including 11 PTC, 1 FTC and 2 MEN2A.

Results: 119 samples were analysed. T1799A (V600E) BRAF mutation was negative in all samples. Among fusion oncogenes the positive result for RET/PTC-1 was seen in 3 samples of 119 (only in PTC), in 4 samples/119 was positive for RET/PTC-3 [PTC, follicular adenoma and in 2 aspirates not verified yet (multinodular goiter - colloid and a solitary nodule - colloid nodule)]. PAX8/PPARγ was positive in only 1 patient with congenital hypothyroidism and coexisting thyroid nodules in both lobes (histologically - dyshormonogenetic goiter). AKAP9/BRAF was negative in all analysed aspirates.

Conclusions:
1. BRAF gene mutations are not responsible for thyroid cancer in the studied children.
2. The incidence of RET/PTC rearrangements is in agreement with previous published data and more common RET/PTC-1 rearrangements prove the sporadic nature of this type of thyroid cancer in the studied group.
Thyroid cancer is one of the malignancies most strongly associated with ionizing radiation in humans. Epidemiological research has found that risk of papillary thyroid cancer (PTC) among atomic-bomb survivors significantly increased with radiation dose. A major early event in papillary thyroid carcinogenesis is the constitutive activation of MAPK signaling pathway caused by one of such gene alterations as \( \text{RET/PTC} \), \( \text{NTRK1} \) and \( \text{BRAF} \) rearrangements, and \( \text{RAS} \) and \( \text{BRAF} \) gene point mutations.

To clarify relationship between radiation exposure and development of PTC, we attempted to identify preferentially occurring gene alterations in radiation-associated PTC. Toward this end, we analyzed \( \text{RET/PTC} \), \( \text{NTRK1} \) and \( \text{BRAF} \) rearrangements and \( \text{BRAF} \) and \( \text{RAS} \) point mutations in 73 cases of adult-onset PTC (52 exposed patients and 21 non-exposed patients) among atomic-bomb survivors, in relation to radiation dose as well as years elapsed since atomic-bomb radiation exposure. The gene alterations detected in the exposed PTC cases were mutually exclusive. Therefore, when dividing the subjects into three groups with chromosomal rearrangements, point mutations, or no detected gene alterations, radiation-dose (tertiles) responses of these groups differed: Relative frequency of chromosomal rearrangements significantly increased with increased radiation dose \( \left( P_{\text{trend}} < 0.001 \right) \), while point mutations showed significantly decreased relative frequency with radiation dose \( \left( P_{\text{trend}} < 0.001 \right) \). Furthermore, non-detected gene alterations tended to be more frequent with increased radiation dose \( \left( P_{\text{trend}} = 0.05 \right) \), suggesting that radiation-associated gene alterations other than rearrangements of \( \text{RET} \), \( \text{NTRK1} \) and \( \text{BRAF} \) might be involved in adult-onset PTC cases among atomic-bomb survivors exposed to high radiation dose. In addition, PTC cases with chromosomal rearrangements developed cancer earlier after exposure than did the cases with point mutations \( \left( p = 0.04 \right) \). Taken together, these results indicate an important role of chromosomal rearrangements, especially \( \text{RET/PTC} \) rearrangements, in radiation-associated PTC carcinogenesis.
Background: The mechanisms underlying aneuploidy in follicular thyroid tumours remain unclear. We showed an association between the presence of the H-RAS81T→C polymorphism and occurrence of aneuploidy in thyroid tumours, suggesting a possible role of H-RAS in their aneuploidization. The link between the H-RAS81T→C and aneuploidy could be that the allele81C is associated with higher amounts of total H-RASmRNA and/or higher relative amounts of p21RAS /p19RAS.

Aims: To confirm the association between the H-RAS81T→C polymorphism and aneuploidy and to verify if there is an association between the presence of C allele and the H-RAS isoforms expression.

Material and methods: H-RAS81T→C polymorphism was studied in 53 follicular tumours (34 FTA and 19 FTC, previously characterized in terms of DNA content by flow cytometry) by PCR/SSCP. Colony-RT-PCR and sequencing technique was used in 3 tumours and in a thyroid cell line heterozygous for the polymorphism to study the association between the alleles and the RAS isoforms.

Results: H-RAS81T→C polymorphism was significantly(p=0.0084) more frequent in aneuploid tumours than in diploid tumours. The same holds true regarding the alleletype distribution(p=0.027).Tumours with the polymorphism had a significantly higher(p=0.025) G2/S fraction than those from tumours without the polymorphism. This difference was also significant(p=0.004) when we compared, only within the group of aneuploid tumours those with and without H-RAS81T→C polymorphism, thus meaning that the increase of G2/S phase is not only due to the ploidy but the polymorphism is also playing a role. We also confirm the association between the C allele and the p21RAS expression (p=0.0023).

Conclusion: We confirmed the association between the H-RAS81T→C polymorphism and the aneuploidy of the tumours, as well as the association between the presence of C allele and the p21RAS expression. Further studies addressing the p21RAS and p19RAS are necessary for better understanding the role of these two isoforms in cancer and aneuploidy.
A NEW PCR/DIGESTION METHOD FOR THE SCREENING OF V600E BRAF MUTATION IN THYROID TISSUE AND CYTOLOGICAL SAMPLES

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Thyroid tumors are the most common endocrine malignancy. Papillary thyroid carcinomas (PTC) accounts for almost 80% of all thyroid cancers. This malignancy frequently has genetic alterations leading to activation of the MAPK (mitogen-activated protein kinase) pathway. The genetic alterations commonly seen in PTC include RET/PTC rearrangements. Recent studies demonstrate that the point mutation T1799A (V600E) of BRAF gene is present in about 45% of PTC adult patients, representing the most common genetic alteration in PTC. Fine-needle aspiration biopsy (FNAB) is the primary means to distinguish benign from malignant nodules and select patients for surgery. However, adjunctive diagnostic tests are needed because in 20-40% of cases the FNAB result is uncertain. Therefore, the detection of the V600E BRAF mutation represents a useful tool for the diagnosis, prognosis and therapies in thyroid cancer. The aim of this study was to set up an accurate and sensitive method to detect V600E BRAF mutation. The mutation was evaluated by PCR and digestion by restriction enzyme HpyCH4-IV. The digestion analysis was performed by means of an artificially created HpyCH4-IV restriction site in PCR product, corresponding to V600E BRAF mutation. We analyzed genomic DNA extracted from about 100 PTC patients (80% FNAB - 20% paraffin-embedded histological samples) already characterized by cytological examination. Our method allowed us to detect heterozygous V600E BRAF mutation in 40% of the PTC samples analyzed. To confirm the results obtained with PCR/digestion method, we performed a sequence analysis on all the samples by the automated ABI PRISM DNA sequence (Applied Biosystem). The DNA sequencing gave completely overlapped results. These data indicate that our method to detect V600E BRAF mutation is sensitive, accurate and easy. Thus it is a useful tool for the diagnostic and prognostic assessment of PTC in clinical setting, especially for those patients with cytological suspicious/indeterminate thyroid nodules.
Routine BRAF Mutation Analysis in Thyroid Cancer Patients; Patient Acceptability and Relationship of Clinicopathologic Features - Preliminary Study

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Objective: Recently, studies about BRAF v600E mutations are actively underway concerning thyroid cancers. Most of these researches relate these mutations with clinicopathologic features that are considered as poor prognostic factors. However, some researchers reported that BRAF mutation has no relationship with poor prognostic factors. In this study, we checked the reactions of the patients scheduled for thyroid cancer operations after full explanation about proceeding with gene studies, and tried to find out the relationship between the presence of BRAF mutation and clinicopathological features after the operation.

Methods: From October 2008, Catholic University of Korea, we are receiving an informed consent from patients scheduled for operation of thyroid cancers about carrying out gene studies. Until February 2009, 126 patients underwent the operation after getting an explanation about the gene studies including the BRAF gene. The operative method was total thyroidectomy with central compartment dissection. All patients were investigated with clinicopathologic factors. BRAF mutation analysis was done using the Direct sequencing method.

Results: 126 patients underwent operation. 112 patients (88.9%) agreed with performing gene studies. BRAF mutation was found in 75 patients (67%). Age, node metastasis, tumor size, multifocality, and tumor number showed no statistical significance between the BRAF positive and the negative group. Extrathyroidal extension and p53 was slightly high in BRAF mutation group (p=0.04).

Conclusion: In this study, thyroid cancer patients were fully explained about performing the genetic study and underwent with BRAF gene study with those who agreed. BRAF mutation group showed relatively low distribution. Except extrathyroidal extension, it had little statistical significance with the pathologic factors representing poor prognosis. Although limitations of a small number of patients and short follow-up period, further investigations seem to be needed to prove that BRAF mutation, which was considered as a poor prognostic factor.
The Na⁺/I⁻ symporter (NIS) is a glycoprotein integrated in the basolateral membrane that actively mediates iodide transport into thyroid cells. The activity of NIS could be regulated by many factors and it has been demonstrated to be related to the degree of thyroid tumor differentiation. Post-Chernobyl thyroid tumors have been considered as more aggressive than sporadic even if children properly treated were cured as well as those affected with sporadic tumors.

In this study we investigated NIS protein expression in 51 Ukrainian post-Chernobyl tumors (27 papillary carcinomas [PTC] and 24 follicular adenomas [FA]). The mean age of patients (21±5 yrs and 23±7yrs) and mean latency period (14±1 yrs) were similar in both groups.

NIS protein expression was detected by immunohistochemistry using a monoclonal antibody kindly gifted by BRAHAMS.

We found that 13/24 (54.2%) FA were positive for NIS and 11 (45.8%) were negative. Among positive cases 9/13 (69.2%) showed a diffuse or spotted positivity of membrane while 4/13 (30.8%) showed a cytoplasmic staining. Cytoplasmic NIS expression was observed only in oxyphilic cells adenomas. Seven out of 27 (26%) PTC were positive for NIS and 20 (74%) were negative. Among positive cases 3/7 (42.8%) exhibited diffuse cytoplasmic immunostaining while 4/7 (57.2%) showed a spotted membrane immunoreactivity. Cytoplasmic NIS expression was found only in oxiphilic tumors. Membrane positivity was mainly localized in tumoral burden and in the foci of tumoral spreading. Statistical analysis showed that NIS expression was higher in FA with respect to PTC.

In conclusion we found a higher expression of NIS in FA than in PTC, a prevalent cytoplasmic staining in oxiphilic benign and malignant tumors and a lower, but not significant, percentage of membrane staining in PTC than in FA. These findings are not different from those reported on sporadic thyroid tumors.
STATUS OF GNAQ (GUANINE NUCLEOTIDE-BINDING PROTEIN-Q) GENE IN THYROID TUMOURS

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Background: Thyroid tumours and melanoma show frequent activation of common genetic events. BRAF and NRAS somatic mutations and PTEN loss are frequent events associated with malignant transformation in both types of neoplasias. A recent study by Van Raamsdonk et al. (1) reported a high rate of somatic mutations in the heterodimeric G protein α-subunit q (GNAQ) in melanocyte associated lesions. These authors found a mutation occurring exclusively at codon 209 in the GNAQ Ras-like domain in blue naevi (83%) and uveal melanoma (46%). This mutation is likely to induce a constitutive activation of GNAQ with subsequent activation of the mitogen-activated protein (MAP) kinase pathway. The appearance of GNAQ as a dominant oncogene providing an alternative route for MAP kinase activation in uveal melanoma, and the genetic similarities found in thyroid tumours and melanomas, led us to identify GNAQ as a promising target for somatic mutations in thyroid tumours.

Objective: To determine the mutational status of GNAQ gene in thyroid neoplasias and cell lines.

Material and methods: PCR/ direct sequencing of exon 5 of GNAQ gene in 82 benign and malignant thyroid neoplasias and 8 thyroid cell lines.

Tumour histotypes and cell lines
Microfollicular Adenoma Fetal type (n=10)
Follicular Thyroid Carcinoma (n=14)
Conventional Papillary Thyroid Carcinoma (n=16)
Follicular Variant of Papillary Thyroid Carcinoma (n=17)
Poorly Differentiated Thyroid Carcinoma (n=8)
Anaplastic Thyroid Carcinoma (n=8)
Medullary Thyroid Carcinoma (n=9)
Thyroid Cell Lines (n=8)

Results: No mutations were found in the exon 5 of GNAQ gene.

Conclusion: The absence of mutations suggests that GNAQ constitutional activation does not play a crucial role in thyroid carcinogenesis.

P033
CLINICAL VALUE OF M22 BASED ASSAYS FOR TSH-RECEPTOR ANTIBODY (TRAB) IN THE FOLLOW-UP OF ANTITHYROID DRUG TREATED GRAVES’ DISEASE: COMPARISON WITH THE SECOND GENERATION HUMAN TRAB ASSAY

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Background: We compared the clinical performances of two new M22 based assays for TSH-receptor antibody (TRAb) with those of the conventional human TRAb assay (hTRAK) in the follow-up of antithyroid drug Graves’ disease.

Patients and methods: Sera were obtained from 128 Graves’ disease patients at the end of an 18-month treatment with antithyroid drugs. TRAbs concentrations were measured using the hTRAK assay, by electrochemiluminescence (ECLIA) with the automated Cobas® or by ELISA using the Medizym® TRAb clone kit.

Results: Results were significantly higher (Z= -9.6, p< 10⁻⁴) for the ECLIA (median: 2.7 IU/L, range:1.1-18.5 IU/L) or lower (Z=8.7, p< 10⁻⁴) for the ELISA (median: 0.56 IU/L, range:0.22-14.8 IU/L) than those obtained with the hTRAK (median:1.5 IU/L, range: 0.9-9.8 IU/L). The use of cut-off limits at 1.9 IU/L, 3.2 IU/L and 0.94 IU/L gave similar sensitivity, specificity, positive and negative predictive values for the TRAKh, ECLIA and ELISA, respectively. At these cut-offs, the prevalence of TRAbs (62.9% vs 16.7%; 61.3% vs 15.2%; 59.7% vs 15.2%) was significantly higher in patients who relapsed compared to those in remission (chi ²= 18.51, p< 10⁻⁴; 18.29, p< 10⁻⁴ or 19.78, p< 10⁻⁴) for the TRAKh, ECLIA and ELISA, respectively. However, some patients remained misclassified in each group of patients in remission or relapse.

Conclusion: The M22 based TRAb assays did not improve the predictive value of the hTRAK assay in the treatment outcome of Graves’ disease. The 3rd generation methods display no clinical superiority over the currently available 2nd generation assays in the follow-up of treated Graves'disease. High intermethod variability requires harmonization for correct interpretation of results.
THE PREVALENCE OF THYROID CANCER IN PATIENTS WITH NODULAR HASHIMOTO'S THYROIDITIS

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Objective: The risk of thyroid malignancy in the nodules of Hashimoto Thyroiditis (HT) is debatable. No causal relationship has been established for the HT-thyroid cancer coexistence till now. The reported cancer prevalences in the HT population generally depend on surgical series and are retrospective. We aimed to design a prospective study to demonstrate the prevalence of thyroid cancer in the nodules of HT patients.

Methods: The patients diagnosed HT with thyroid nodules of at least 10 mm diameter in the outpatient clinic of our department between May 2006 and February 2009 were included in the study. HT was diagnosed with conventional autoimmune serology and ultrasonography (US). Fine needle aspiration biopsy (FNAB) was performed at least once to all of the nodules of 10 mm and higher diameter using a standard procedure of our department. US and FNAB were performed by the same endocrinologist.

Results: 128 HT cases (116 female, 12 male) participated in the study. Among the 142 nodules of the cases, 128 turned out to be benign (90.2%), 6 indeterminate (4.2%), 6 inadequate (4.2%), and 2 malignant (1.4%) after FNAB. Nodules with inadequate cytology were found to be benign after the second biopsy performed. The surgery of the 6 indeterminate nodules revealed that they were benign. The two cytologically malignant nodules were determined to have papillary thyroid cancer histopathologically.

Conclusion: The thyroid nodules of HT patients do not seem to have increased risk for malignancy; they may even bear a decreased risk when compared to the nodular goiters. Autoimmunity may contribute to a possible decreased risk of malignancy. Using more flexible criteria may be feasible when selecting the nodules to biopsy of patients with HT in daily practice.
A ROLE FOR AUTOANTIBODIES IN ENHANCEMENT OF PRO-INFLAMMATORY CYTOKINE RESPONSES TO THYROID PEROXIDASE

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Objective: Thyroid peroxidase (TPO) antibodies (TPOAbs) are predictive of the development of autoimmune thyroid disease, comprising Hashimoto's thyroiditis (HT) and Graves' disease. Although HT is considered a T-cell mediated disease, virtually all patients with active HT are seropositive for TPOAbs. However, the pathogenic role of TPOAbs in HT remains unclear.

Methods: We selected sera with high TPOAb-concentrations from eleven patients with HT, ten healthy monozygotic twins to HT patients, and nine healthy individuals with no familiar disposition to AITD. Mononuclear cells (MNCs) from a healthy donor was challenged with TPO or a control antigen, tetanus toxoid (TT), in the presence of each serum. Moreover, we assessed the influence of immunoglobulin G (IgG), isolated from five HT patients, on normal MNCs' response to TPO, in the presence and absence of antibodies to the Fcgamma-receptors CD16, CD32 and CD64.

Results: The TPO-induced production of pro-inflammatory cytokines (TNF-alpha, IL-6 and IFN-gamma), and the anti-inflammatory cytokine IL-10, correlated with the TPOAb content of the serum present in the MNC cultures (p=0.0002-0.05). Enrichment of foetal calf serum-containing media with IgG from HT patients enhanced the production of TNF-alpha, IL-6, IFN-gamma and IL-4 by normal MNCs (p<0.0002-0.05), in a dose-dependent manner. The enhancement of TNF-alpha-, IL-6- and IFN-gamma responses was counteracted by Fcgamma-receptor blockade.

Conclusions: Taken together, these data demonstrate a role for TPOAbs of IgG isotype in promoting the TPO-elicited release of pro-inflammatory cytokines from phagocytic cells, on the one hand, and in enhancing the activity of antigen-presenting cells, and thereby inducing T cell responses to TPO, on the other.
Numerous clinical and experimental data indicate close, bi-directional relations between immunological and endocrine systems. During the last decade, an increasing interest, is observed, in the role of dendritic cells (DCs) in formation and development of autoimmunological diseases.

**Aim:** The aim of the present study was a complex analysis of the dendritic cell subsets and fenotypes in patients with chronic thyroiditis (ChT) as well as in the patients monitored for differentiated thyroid cancer (DTC).

**Patients and methods:** Blood samples were collected from patients suffering from ChT before and after treatment with L-T4 (N=18). Moreover, to investigate the influence of thyroid hormones, blood samples for ex vivo analysis were collected from thyroidectomised (because of differentiated thyroid carcinoma) patients (n=21) at two time points: (i) after withdrawal of L-T4 treatment group before treatment, and (ii) during 2 months of L-T4 administration in order to suppress TSH concentration group after treatment. FACS analysis of expression of selected molecules on the blood DCS was performed. Furthermore, the investigation of the DC cell culture was performed in the conditions of deficiency and excess of T3.

**Results:** We found that the percentage of pDCs and mDCs in peripheral blood was dependent on thyrometabolic state and that in patients with ChT this regulation was partially impaired. Additionally, we observed lower expression of CD86 on mDCs in hypothyroid ChT patients as compared to thyroidectomised patients. Interestingly this difference was attenuated by L-T4 treatment. The effect of thyroid hormones on surface expression of co-stimulatory molecules was then confirmed in vitro in experiments with freshly sorted human DCs.

**Conclusions:** Results of our study indicate that thyroid hormones influence the biology of peripheral blood DCs. This regulatory effect was furthermore affected by chronic thyroiditis. This observation might be of great importance for understanding of immune disorders of endocrine system.
Nearly 4% of patients diagnosed with AT and normal thyroid function become subclinical or clinical hypothyroid each year. While data on the prognostic factors predicting the transition from subclinical hypothyroidism to overt hypothyroidism are available (TSH levels and TPO levels) few studies explored the predictor factors of conversion from euthyroidism to subclinical hypothyroidism.

In this retrospective study, we aimed to define prognostic factors that predict the possibility to develop a subclinical hypothyroidism in 382 women (mean age 53±16 yr; range 10-86 yr) with AT, who had normal function at the time of the first diagnosis, after a mean follow-up of 39.8 months (range 24-68 months).

At the end of follow-up 50/382 (13%) patients developed subclinical hypothyroidism (SC), while 332/382 (87%) remained euthyroid (EU). The risk to become subclinical hypothyroid was significantly related to higher TSH levels at baseline (p< 0.001) (2.7±0.8 mU/ml in SC vs 1.7±0.8 mU/ml in EU), lower FT3 (p=0.0005) (2.9±0.4 pg/ml in SC vs 3.1±0.4 in EU), lower FT4 (p=0.0004) (10.1±2.3 pg/ml in SC vs 11.4±2.3 pg/ml in EU), higher levels of TPO antibodies (p=0.003) (537±394 U/ml in SC vs 366 ±363 U/ml in EU), lower thyroid volume (p=0.0008) (10±4.8 ml in SC vs 16±14 in EU) and younger age (p=0.01) (48±15 in SC vs 54±16 in EU).

However, by multivariate regression analysis, only baseline TSH and FT3 levels were significant variables. In particular, by ROC curve analysis a baseline of serum TSH levels above 2.3 mU/ml was the best predictor of probability to develop subclinical hypothyroidism (sensitivity: 75%, and specificity: 76%).

In conclusion, our study indicate that about 4% of patients with AT for year develop a subclinical hypothyroid and that serum TSH and FT3 in the upper or lower normal range respectively, are the main indicators of subclinical hypothyroidism risk.
An association between breast cancer (BC) and thyroid autoimmunity has been reported by several authors. High incidence of anti-thyroperoxidase antibodies (TPOAb) has been detected both in treated and untreated BC patient and recently, an independent positive predictive role of TPOAb in high aggressive BC has been demonstrated. BC cells and thyroid cells share similar properties such as the ability to uptake the iodine and to oxidase intracellular inorganic iodine. The aim of this study was to evaluate the possible expression of thyroperoxidase (TPO) gene in BC. TPO gene expression was evaluated by Reverse-Transcriptase PCR after isolating total mRNA from frozen tissues. TPO protein was detected by indirect immunofluorescence incubating slides of tissues with TPOAb positive serum and then with a fluoresceinated sheep antihuman IgG. TPOAb free serum were used as negative control.

The expression of TPO mRNA was evaluated in 4 BC tissues and the results were compared to 2 peritumoral breast tissues (PT), 1 breast dysplasia (BD) and 3 pancreas ductal adenocarcinoma (PDA). Normal thyroid tissue (T) was used as positive control. TPO gene expression was detected in 4/4 (100%) BC and in 2/3 (66%) PDA tissues. No TPO mRNA was found in PT and in BD tissues. The TPO protein was detected in all BC samples and no expression was found in PDA, PT and BD tissues. TPO mRNA and protein were strongly expressed in thyroid tissue.

Our data indicate that TPO mRNA and protein are expressed by BC cells. This observation may contribute to explain the known association between BC and thyroid autoimmunity and the positive predictive role of TPOAb in breast malignancy.
IMMUNOHISTOCHEMICAL DETECTION OF C CELL HYPERPLASIA AND CEA TISSUE EXPRESSION IN AUTOIMMUNE DISORDERS OF THE THYROID GLAND

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Introduction: Reactive C cell hyperplasia with increased serum calcitonin in thyroiditis Hashimoto and increased carcinoembryonic (CEA) serum levels in Grave's disease have been reported. Our aim was to evaluate the presence and degree of C cell hyperplasia and the tissue expression of CEA in both thyroid autoimmune disorders.

Methods: Paraffin sections from 107 Hashimoto's thyroiditis, 29 Grave's disease and 20 nodular hyperplasia have been immunohistochemically investigated for the detection of chromogranin-positive C cells and for epithelial expression of CEA. Primary antibodies: Monoclonal chromogranin (DAKOM867), CEA(DAKOM707), Detection kit DAKO Real Envision K5007. The hyperplasia was scored as absent, mild, moderate, severe and the CEA expression evaluated semiquantitatively as +−+++. The findings have been correlated to the disorders, age, sex, weight of the gland and the presence of oxyphilic cells.

Results: C cell hyperplasia was absent in nodular hyperplasia and present in 12.1% in thyroiditis Hashimoto and in 6.8% in Graves disease in a slightly higher degree in males and in older age. CEA was found to be expressed in the parafollicular as well as in the follicular cells, frequently in a luminar location in 33.8% in autoimmune disorders, whereas absent in nodular hyperplasia. Inflammatory infiltrates did not express CEA. In Graves disease CEA exhibited a tissue expression in 44.8% and in Hashimoto's thyroiditis in 30.8%. The CEA expression was higher in the oxyphilic type of thyroiditis Hashimoto and might be stimulated by cytokine secreted by the oxyphilic cells.

Conclusions: C cell hyperplasia and CEA expression were absent in thyroid tissue from nodular hyperplasias and present in different degree in the tissue from autoimmune thyroid disorders, with C cell hyperplasia being more prominent in Hashimoto's thyroiditis, males and older patients and the CEA expression more prominent in Grave's disease and the oxyphilic type of thyroiditis Hashimoto.
DOES PARATRACHEAL LYMPH NODE HAVE CLINICAL VALUE IN PATIENTS WITH AUTOIMMUNE THYROIDITIS?

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Objective: To assess the clinical value of paratracheal lymph nodes (PLNs) as a novel sonographic finding in various autoimmune thyroiditis (AT) compared with healthy subjects.

Method: A total of 135 consecutive patients with newly diagnosed AT and 30 healthy subjects were included in the study. All patients underwent thyroid sonographic examinations, thyroid function tests, anti-TSH receptor antibody (TR-Ab), anti-thyroperoxidase antibody (TPO-Ab) and anti-thyroglobulin antibody (Tg-Ab). Patients who have thyroid nodule were excluded.

Results: One hundred-fourteen of 135 patients were diagnosed as Hashimoto Thyroiditis (HT) (82/114 were thyroid autoantibodies (ATA) +, and 32/114 were ATA-), and 21 were Graves' Disease (GD) (14/21 were TR-Ab positive, and 7/21 were TR-Ab negative). PLNs were seen in 64 of 82 patients (78%) in the ATA+ group and in 17 of 32 patients (53%) in the ATA- group, 9 of 14 patients (64.3%) TR-Ab positive GD, and 5 of 7 patients (71.4%) TR-Ab negative GD, and 6 of 30 (20%) subjects in the control group (p< 0.001). PLNs in control group were fewer (p < 0.001) and smaller (p< 0.001) compared to patients group. PLNs were detected more frequently in the ATA+ group than ATA- group (p=0.008). While number of PLNs in ATA- group was fewer (p= 0.03) than ATA+ group there was no difference in volume and size between the two groups (p= 0.132, p= 0.079, respectively). Frequency of PLNs was similar in the TR-Ab negative and positive GD groups (p=0.572). There is no difference in PLNs volume, and size in the TR-Ab negative and positive GD groups (p= 0.132, p= 0.079, respectively).

Conclusion: Paratracheal lymph nodes are often present in patients with autoimmune thyroiditis. However, there is need for prospective randomized studies to determine whether the existence of PLNs is helpful for diagnosis of antibody negative autoimmune thyroiditis patients.
INDICES OF THYROID AUTOIMMUNITY AND CORTISOL LEVELS: ASSOCIATIONS WITH AGE IN AN ELDERLY AMBULATORY POPULATION

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Objectives: Several reports indicate a lower prevalence of thyroid autoimmunity in the very old. We have previously reported that in patients with autoimmune thyroid disease and in normal children, increased levels of thyroid autoantibodies (Th-Ab) are associated with lower glucocorticoid activity. We investigated whether this association is also present in an elderly ambulatory population, in whom we have previously reported positive association of cortisol levels with age.

Methods: 321 ambulatory subjects (age 51-90 years, median 70, 207 female). Thyroid function tests, cortisol, glucose, insulin and biochemical parameters were measured. A modified overnight dexamethasone suppression test (0.25mg) was performed; this test is proposed as an index of glucocorticoid sensitivity.

Results: Forty subjects had positive anti-TPO and 36 positive anti-TG antibodies. Mean basal cortisol levels were significantly lower in the Th-Ab(+) groups (anti-TPO(+): 11.6±4.4 vs. anti-TPO(-): 13.3±4.5, p=0.007 and anti-TG(+): 11.6±4.7 vs. anti-TG (-): 13.2±4.5 mcg/dl, p=0.011, Mann-Whitney). TSH levels range was 0.4 to 24.9 µUI/ml; 2 subjects were diagnosed with hypothyroidism (TSH >10). Mean TSH levels were higher in Th-Ab(+) subjects (anti-TPO(+): 3.4±4.3 vs. anti-TPO(-): 1.6±2.0 µUI/ml, p<0.001, anti-TG(+): 2.5±1.9 vs. anti-TG(-): 1.8±1.8, p=0.038). T3, FT4, post dexamethasone cortisol levels, C-Reactive Protein, HOMA-IR index and BMI did not differ between these groups. Mean age of Th-Ab(+) subjects was lower compared to the Th-Ab(-) group (anti-TPO(+): 67±7.8 vs. 71±8.8, p = 0.019 and anti-TG(+): 67.2±7.7 vs. 71.1±8.8 years, p=0.018).

Conclusions: Reduced glucocorticoid activity is associated with an increased prevalence of Th-Ab positivity in older ambulatory subjects. Subjects without Th-Ab in this population sample are relatively older. It is not known if this is related to increasing glucocorticoid tone with age.
Background: Autoimmune hypophysitis can result in growth hormone deficiency (GHD). Although autoimmune hypophysitis is uncommon, it is associated with other autoimmune endocrine diseases like autoimmune hypothyroidism (AIH). Recent studies suggest a high prevalence (5%) of GHD in AIH, which could contribute to the reduced quality of life frequently observed in patients with AIH despite adequate treatment with thyroxine.

Objective: To establish the prevalence of growth hormone deficiency in patients with AIH.

Patients: We included patients with AIH (TPO-Ab ≥ 100 kU/L), who were adequately treated with thyroid hormone suppletion (TSH 0.2 - 5.0 mU/L). Exclusion criteria were prior I131 treatment, thyroid surgery, or a history of hypothalamic or pituitary disease. Patients were recruited via our outpatient clinics and via patient self-help organizations. A total number of 837 patients applied for the study.

Research design and methods: We measured TSH, FT4, TPO-Ab and IGF-I. If the IGF-I concentration was below the 10th percentile of age specific reference values, a GHRH/GHRP-6 test was done. GHD was defined as a growth hormone peak after GHRH/GHRP-6 below the 2.5th percentile according to age specific reference values.

Results: From 837 patients who applied for the study, 515 (476 female, 39 male) were included. 322 were not included (157 because TPO-Ab < 100 kU/L, 165 because TSH < 0.2 or > 5.0 mU/L). The IGF-I concentration was < 10th percentile in 49 of 515 patients (9.5%). These 49 underwent a GHRH/GHRP-6 test. 2 patients had a growth hormone peak < 2.5th percentile.

Conclusion: Surprisingly, we found a prevalence of GHD in autoimmune hypothyroid patients of only 0.4% (2 out of 515 patients), whereas other studies suggest a higher prevalence.
AVIDITY OF THYROGLOBULIN ANTIBODY IN SERA FROM PATIENTS WITH HASHIMOTO'S THYROIDITIS WITH DIFFERENT THYROID FUNCTIONAL STATUS

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Objective: The mechanism of disease progression in Hashimoto's thyroiditis (HT) is still unclear. Thyroglobulin antibody (TgAb) is a diagnostic hallmark of HT. The aim of our study was to evaluate the avidity of TgAb in sera from patients with HT with different thyroid functional status.

Methods: Sera from 50 patients with newly diagnosed HT were collected and they were divided into three groups according to thyroid function: patients with hypothyroidism (H, n = 18), subclinical hypothyroidism (sH, n = 18) and euthyroidism (EU, n = 14). Titres of TgAb were determined by using serial serum dilutions in ELISAs and expressed as logarithm value (lgT). TgAb avidity was also assessed by ELISAs, and avidity constant (aK) was determined as the reciprocal value of the thyroglobulin molar concentration in the liquid phase resulting in 50% inhibition of TgAb binding to thyroglobulin in solid phase ELISA.

Results: The titre and avidity of TgAb were of significant low levels in sera from patients with EU than those with H and sH. The mean lgT of TgAb in sera from patients with H, sH and EU were 4.19±0.6, 3.77±0.63, 3.29±0.64, respectively (P=0.001). The mean aK of TgAb were 4.06×10⁹, 2.91×10⁹, 5.55×10⁸, respectively (P=0.001). Weak correlation were found between TgAb titre and avidity (r=0.594, P< 0.01).

Conclusions: Our study indicated that HT patients with high avidity of TgAb might be at high risk to develop subclinical hypothyroidism, even to overt hypothyroidism.
EFFECT OF SELENIUM ON THYROID PEROXIDASE ANTIBODY ASSAY

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Selenium(Se) has been shown to reduce the titre of antithyroid peroxidase antibodies (TPOAb) in patients with Hashimoto’s thyroiditis and postpartum women. The mechanism of reduction is not clear. Could Se interfere with the TPOAb assay?

**Objective:** To assay TPOAb in known positive sera before and after addition of Se

**Methods:** Using the Bayer TPOAb automated assay (-ve TPOAb 32kU/L/\textless). 10 sera (TPOAb -ve in 2, 48.1-884.3 in 4 and \textgreater1300 in 4) were selected. Sera were from 10 females aged 17-72 yrs. One had high TSH (13mU/L). FT4 ranged from 11.8-20.1pmol/L. 2 were pregnant and 4 were receiving levothyroxine. Se selenite was added to produce concentrations of 1.0, 1.5 and 2.0 micromols/L before reassaying TPOAb concentrations.

**Results:** Se did not alter -ve Ab results in the 2 sera with initial -ve TPOAb. In 1 low titre positive sera Se addition resulted in a 31% increase in assay titre; in 3 sera with initial higher titres (>1300kU/L) Se addition led to changes of \pm3% in assay titre. In 4 sera with very high initial titres no change was observed after Se addition.

**Conclusions:** These preliminary data do not suggest that selenium addition to TPOAb+ve sera interferes with antibody assay significantly. The mechanism of reduction of TPOAb titre by selenium remains unclear.
MEASUREMENT OF INTERLEUKIN - 12 IN SERA OF PATIENTS WITH HASHIMOTO'S THYROIDITIS

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The severity of autoimmune Hashimoto's thyroiditis (HT) vary among patients. Various cytokines may play role in initiation and persistence of autoimmune process. Interleukin - 12 is a proinflammatory cytokine known for inducing the differentiation of CD4+ T lymphocytes from a Th0 to Th1 phenotype and is mainly produced by activated macrophages, dendritic cells and granulocytes. To clarify the role of interleukin - 12 in different stages of HT we investigated sixty-six patients with autoimmune thyroiditis: 24 patients with euthyroid HT (Group I), 18 patients with hypothyroid HT (Group II), and 24 subjects treated with Levothyroxine (Group III). Twenty-two healthy subjects were included as controls. Concentrations of interleukin - 12 in the serum samples of patients and controls were evaluated by ELISA kits. Serum thyrotrophin, thyroid hormones and antiTPO antibodies were also measured.

Results: Interleukin - 12 was significantly higher in euthyroid HT patients (103.74 ± 14.4 pg/ml, mean ± SE) in comparison with controls (70.98 ± 10.83 pg/ml, p=0.04). However, no difference was observed in interleukin - 12 levels between hypothyroid HT (79.62 ± 13.8 pg/ml) and normal controls. Normalization of thyroid function with Levothyroxine treatment in patients with hypothyroidism increased serum interleukin - 12 concentrations (93.62 ± 7.89 pg/ml), but not statistically significant. These results suggest that interleukin - 12 may have an important role in the initiation of autoimmune response in HT and thyroid hormones might influence the production of interleukin - 12.
The thyroid-stimulating hormone (TSH)-receptor has been found in a variety of cell types, including preadipocytes and adipocytes. In vitro, TSH-mediated preadipocyte and adipocyte responses include proliferation, differentiation, survival and lipolysis. The aim of this study was to measure the response of serum leptin to exogenous administration of recombinant human TSH (rhTSH) in vivo. One hundred patients (77 females and 23 males) with differentiated thyroid cancer treated with total thyroidectomy and \(^{131}\text{I}\) remnant ablation were recruited. Patients were scheduled to receive a standard rhTSH dose for measurement of thyroglobulin in the follow-up of their disease. Mean age was 47 ± 13 years (range 16-76), mean body mass index (BMI) was 26.8 ± 6.3 Kg/m² (17.4-62.4). Patients were under a variable dose of L-T4 from substitutive to suppressive. Blood samples were taken for the dosage of TSH and leptin before the first administration of rhTSH (time 0) and after 24 h (time 1), 48 h (time 2), 72 h (time 3) and 96 h (time 4). Basal values of leptin were (mean+SD) 11.6 ± 10.9 ng/ml (range 0.33-76.8). Mean serum leptin increments, with respect to basal value, were 16.3%, 13.9%, 18.2%, 11.6% at time 1, 2, 3 and 4 respectively. The effect of rhTSH stimulus was studied by a time-average analysis [area under the curve (AUC)]. Significant positive correlations of leptin-AUC with basal leptin levels (R = 0.411; p< 0.0005) and BMI (R = 0.368; p< 0.0005) were observed. In conclusion, rhTSH administration induces leptin secretion in vivo. The rise in serum leptin after rhTSH is proportional to the adipose mass, and indicates the presence of a functioning TSH receptor in human adipocytes. The role that TSH receptor activation in adipocytes might play in physiological and pathological conditions remains a matter of investigation.
INTERACTIONS BETWEEN AGE AND CARDIOVASCULAR PARAMETERS IN PATIENTS WITH AUTOIMMUNE THYROIDITIS AND SUBCLINICAL HYPOTHYROIDISM

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Objectives: Subclinical hypothyroidism (SH) might be associated with various alterations in cardiac and arterial function. It is less clear as to whether and how age modifies the cardiovascular response to mild hypothyroidism. The aim of this study was to investigate interactions between age and cardiovascular abnormalities in patients with new, untreated primary SH.

Methods: We studied segmental left ventricular diastolic function by tissue Doppler imaging (TDI), arterial stiffness by ultrasound assessment of distensibility coefficient (DC) and Young’s elastic modulus, as well as biochemical parameters in 95 women aged 20 - 50. 47 of them had autoimmune thyroiditis and SH, 48 were euthyroid (controls). Patients of each group were categorized according to their age into 3 categories, and SH versus controls compared (12 and 13 patients aged 20 - 30, 12 and 10 aged 31 - 40, 23 and 25 aged 41 - 50, respectively).

Results: Among the youngest, SH patients presented higher Young’s elastic modulus (p=0.048), lower DC (p=0.052) and elevated dysfunctional segments number revealed by TDI (p=0.036). TSH values positively correlated with the number of dysfunctional segments (R=0.55, p=0.010), and negatively with DC (R=-0.53, p=0.022). Among the middle-aged women, SH patients demonstrated higher Young’s elastic modulus (p=0.049), whereas segmental LV diastolic function did not differ from controls (p=0.32). Among the oldest there were no differences in regional diastolic function and arterial wall stiffness between the patients having SH and controls (p>0.05).

Conclusions: Age modifies the cardiovascular response to mild thyroid failure. In younger subjects, SH is associated with the impairment of regional diastolic function and increased arterial wall stiffness, possibly, indicating to the increased cardiovascular risk. In older patients, cardiovascular effects of mild hypothyroidism appear to play a lesser role compared to other risk factors.
P048
INCREASED TSH AND DECREASED MITOCHONDRIAL FUNCTION IN OBESE ADOLESCENT COMPARED TO LEAN CONTROL PERSONS

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Objective: The aim of the present study was to compare (TSH) and mitochondrial activity in a group of obese adolescents and a lean control group.

Methods: A group of 29 obese adolescents (age < 16 yrs) (BMI above the age-related 95th percentile, mean age 12 yrs range 8-15yrs) and 30 lean adolescents (age < 16 yrs) ((BMI below the age-related 95th percentile, mean age 12 range 8-15yrs) were recruited from the outpatient clinic and from the staff of the paediatric department. None of the participant received medication. Mitochondrial mass and membrane potentials (MMP (Δψ)) were measured in isolated mononuclear blood cells, by a FACSCanto II Flow Cytometer after staining with MitoTracker Green (MTG) and tetra methyl rhodamine methyl ester (TMRM). Serum TSH was determined using immunochemiluminometric technology (Advia Centauer Ready-Pack, Bayer Corporation) and fT4 by electrochemical luminescence (ELECSYS with Roche Modular E system). Normally distributed values were compared with students t test, whereas comparison of TSH were done with the Mann-Whitney test.

Results: TSH was significantly increased in obese adolescents (THS= 3.06 mu/l (2.00-3.61), median and range) compared to lean persons (TSH = 2.33 mU/l (1.70-2.81, p = 0.04) and fT4 tended to be lower (fT4=14.5± 1.9 mean± SD) compared to lean persons (fT4= 15.9 ±2.0 mean± SD, p= 0.09). The mitochondrial mass was significantly decreased (relative emission = 6385±1462 a.u. compared to 7605 ±2388 a.u., mean± SD, p = 0.03) and also mitochondrial membrane potential was decreased in obese adolescents compared to lean adolescents (relative emission= 11427±3861 vs 14017±5536, mean± SD, p = 0.04).

Conclusion: Obese adolescents have decreased thyroid hormone stimulation of mitochondrial activity. Although it is not yet known if this alteration is a basal defect in obesity, mitochondrial dysfunction caused by decreased thyroid hormone stimulation of mitochondria, may be an important factor for maintenance of obesity.
IMPAIRED L-T4 ABSORPTION IN SEVERE OBESITY
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Objectives: The precise replacement dose or suppressive therapy with levothyroxine (L-T4) is depending mainly on lean body mass and results in higher demands of dosing in obese patients. This is due to many reasons. Our aim was to study L-T4 absorption in obese patients.

Methods: 37 euthyroid volunteers, aged 18-50 years, were studied; 13 with severe obesity (BMI >40kg/m) and 24 with BMI+15% of the ideal one. Following a 12-hours overnight fast, we administered a dose of 600µg L-T4 as an oral solution, which was prepared by a powder (Faran company) that diluted in absolute alcohol and watered down with final concentration 600mcg levothyroxine per 240 ml. Blood samples were collected to measure serum T4 at -0.5; -0.25; 0; 0.5; 1; 1.5; 2; 2.5; 3 and 4 hours post dose. The mean value of serum T4 concentrations of -0.5, -0.25 and 0 was considered as baseline. T3; TSH; and abTPO were measured at baseline. To evaluate the L-T4 absorption, the Area Under the Curve from baseline to 4h(AUC), peak T4 Concentration (Cmax), and Time to peak concentration (Tmax) were estimated.

Results: The baseline mean values of T3 and TSH were higher in obese patients compared to controls T3: 1.38±0.14 vs 0.88±0.34ng/ml; (p< 0.01); TSH: 2.1±0.83 vs 1.25±0.94µIU/ml; (p< 0.05). The baseline T4 concentrations were similar T4: 8.04±0.95 vs 7.75±1.67µg/dl ; (ns). The AUC and Cmax of the mean concentrations of T4 were lower and Tmax was delayed in obese patients compared to controls 43.25±6.83 vs 49.23± 8.61; (p< 0.05); 11.8 ± 1.98 vs 13.97±2.55 µg/dl; (p< 0.05) and 2.42± 0.93 vs 1.85± 0.79 hours; (p=0.05), respectively.

Conclusions: Patients with severe obesity appear to have impaired L-T4 absorption. This may result in higher L-T4 dose demands in replacement or suppression therapy in those patients.
WHETHER ROUTINE MEASUREMENT OF TSH IN ALL ADOLESCENT WITH OBESITY IS NECESSARY?

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Objectives: In recent years it is established that children and adolescents with obesity have higher TSH level than children with normal weight. At the same time, high level of TPOAbs is more often detected in adolescents with obesity and high TSH level. Autoimmune thyroid disorders are probably connected stronger with increased TSH than with obesity.

Materials and methods: 1179 adolescents (542 boys) aged from 13 till 16 years were evaluated. In all adolescents thyroid ultrasound («FUCUDA-750XT», Japan), as well as measurement of TSH and TPOAbs levels in serum (Axsym®, Abbott Diagnostic Division, USA) were performed. The overweight and obesity were defined according to the international criteria including body mass index, age and sex [Cole T.J., 2000]. 28 patients with thyroid disorders (26 - autoimmune thyroiditis, 1 - Grave's disease and 1 - nodular toxic goiter) were excluded from study. Remaining adolescents were divided into 3 groups: 1) normal body weight (1060: f/m=564/496); 2) overweight (74: f/m=43/31); 3) obesity (17: f/m=8/9).

Results: No significant differences of TSH level between adolescents with normal weigh and overweight were found (2,3 [1,7; 3,0] mU/l and 2,4 [1,8; 3,2] mU/l respectively, *<0,373). In obese persons TSH level (3,1 [2,5; 4,1] mU/l) was significantly higher, than in other groups (p < 0,005), although it was always inside the reference range.

Conclusion: In obese adolescents without obvious clinical signs of hypothyroidism routine TSH level measurement is not necessary. Casual revealing of moderate TSH level increase in this group of adolescents has no clinical value.
EFFECT OF LEVOTHYROXINE REPLACEMENT ON INTIMA-MEDIA THICKNESS, ENDOTHELIAL FUNCTION AND CARDIOVASCULAR RISK FACTORS IN SUBCLINICAL HYPOTHYROIDISM

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Subclinical hypothyroidism (sHT) is a prevalent disease for which exact therapeutic approaches have not yet been established. We assessed carotid artery intima-media thickness (IMT), flow-mediated vasodilatation (FMD) of the brachial arteria and other cardiovascular risk factors in 50 sHT patients [mean age (SD) 47.82 ± 11.31 yr; TSH: 6.4 (2.9) mUI/ml] at baseline and after 12 months of randomized L-thyroxine (L-T4) replacement. In comparison with 23 age- and body mass index (BMI)-matched controls, sHT patients had elevated Lpa (p = 0.01). Significant positive relationships were found between TSH and apoprotein B (rs = 0.23, p = 0.05). Significant negative relationships were found between free T4 and apoprotein A (rs = -0.3, p = 0.01) and FMD (rs = - 0.3, p = 0.05). In addition to age (r = -0.5, p = 0.004) and waist diameter (r = -0.43, p = 0.02), FMD correlated significantly with reactive C protein (r = -0.36, p = 0.004) and IMT (r = -0.32, p = 0.008). FMD reduced in the group without L-T4 treatment from 18.24 ± 10.95 % to 14.38 ± 7.78 % (p= 0.03) and augmented in L-T4 treatment group from 17.75 ± 7.66 % to 19.18 ± 7.94 %, although it was not significant (p=0.59). We also noticed that the change in FMD between the 2 groups were significant (p=0.052). We concluded that minimal thyroid dysfunction poses no adverse effects on endothelial function in the studied population of s-HT patients with TSH levels below 10 mUI/ml. Large multicenter, placebo-controlled prospective trials are necessary to address if s-HT patients with higher levels of TSH have endothelial dysfunction and the effectiveness of L-T4 treatment. We could observe in this study a beneficial effect of thyroid hormone substitution on endothelial function of a group of s-HT patients.
FREQUENCY OF HYPOTHYROIDISM IN MALES AND FEMALES WITH AND WITHOUT METABOLIC SYNDROME

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Many cardiovascular risk factors have been recognized - increased waist circumference, arterial hypertension, dyslipidemia, glucose intolerance, as well as hypothyroidism.

Material and methods: Two thousand four hundred and four subjects (1343 female, mean age 48.68 ±14.4 y and 1061 male, mean age 46.51±14.49 y, NS) were included in the study. All participants filled a questionnaire form, underwent clinical examination (height, waist circumstance, blood pressure) and blood was collected for TSH, blood glucose, oGTT, total cholesterol.

Results: The waist circumference was >94 cm in 675 (63.3%) of total 1067 males and >80 cm in 811 (60.2%) of 1348 females or overall 1486 participants in this study (61.5%) were with increased waist, i.e. with abdominal obesity. Metabolic syndrome (MetS) according to the IDF criteria (2005) was confirmed in 743 out of 2404 individuals (30.91%), of whom 349 of 1348 females (25.9%) and 394 of 1061 males (37.1%), p< 0.001.

Hypothyroidism was found in 6.33% (n=152) of subjects (serum hsTSH≥4.2 mUI/l). Waist circumference was increased in 69.6% of the patients with hypothyroidism, cholesterol level was >5.2 mmol/l in 63.5% and arterial hypertension was found in 53.9%. Increased waist circumference was found in 71.9% and arterial hypertension - in 64.5% of males with hypothyroidism. Of the females with hypothyroidism 50% had arterial hypertension and in 68.3% - abdominal obesity. Cardiovascular disease was observed in 13.2% (30/219) of the patients with hypothyroidism. Elevated TSH levels reached 8.2% (61/743) in subjects with MetS and 5.4% (91/1662) in those without MetS, NS. The study of the data by sex revealed that males with MetS had higher frequency of hypothyroidism compared to those without MetS (8.5% v.s. 3.6%, p< 0.02). In females no such relation was found (8.9% v.s. 7.1%, NS).

Conclusion: Together with the features of metabolic syndrome, hypothyroidism is a serious cardiovascular risk factor in men.
P053
TSH AND FREE THYROXINE IN AN OBESE POPULATION BEFORE AND AFTER SURGICAL WEIGHT LOSS
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Introduction: The knowledge that adipocytes and preadipocytes possess thyrotropin receptors, hormone that could induce adipogenesis and adipokines production, leads to the hypothesis that TSH contributes to obesity.

Objectives: To assess changes in levels of TSH and free thyroxine (FT4) in an obese population before and after surgical weight loss.

Methods: Patients who underwent bariatric surgery at our institution, had no previous diagnosis of thyroid disorder, were not taking medication that could affect the thyroid function evaluation were included in this retrospective evaluation. It was investigated the association between the values of TSH and FT4 with BMI (body mass index) and with % FM (fat mass) before and after bariatric surgery; it was evaluated the relationship of TSH and FT4 at 6, 12 and 24 months after surgery. It was assessed the variation of TSH and FT4 with % EBMI (excess body mass index) loss and with % FM loss.

Results: A total of 53 patients met the study criteria. When comparing the value of preoperative TSH and 6, 12 and 24 months, it is verified that its value decreases (respectively, p = 0,028, 0,018 and 0,191); there was no statistically significant difference in the value of FT4. Regarding changes in hormonal levels with % FM, there was only statistically significant difference in the TSH value at 12 months (p = 0,046). There was no statistically significant difference between the values of TSH and FT4 and the different classes of obesity (at 0, 6, 12 and 24 months), or between the values of these hormones and % EBMI loss. There was no statistically significant difference between the values of TSH and FT4 and the % FM loss.

Conclusions: There is still a need for further research into the causes of the changes in thyroid hormones in obesity and weight loss.
Objectives: To examine whether autoimmune thyroiditis (AIT), with and without hypothyroidism is associated with increased of cardiovascular risk factors.

Methods: We recorded thyroid function tests, BMI, insulin resistance markers comprising the Homeostasis Model Assessment for insulin resistance (HOMA-IR), the Quantitative Insulin Sensitivity Check Index (QUICKI), HISI (Hepatic Insulin Sensitivity Index), WBISI (Whole-Body Insulin Sensitivity Index), IGI (Insulinogenic Index) and the levels of total cholesterol (TC), HDL, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein(a) (Lp[a]), homocysteine, CRP (C-reactive protein), folic acid and vitamin B12 levels, in 150 patients with AIT and hypothyroidism, and in 100 patients with AIT without hypothyroidism. The patients with AIT and hypothyroidism were treated with levothyroxine, in order to normalize the T3, T4 and TSH levels. A 75-g OGTT was performed in the morning, and blood samples were obtained every 30min for 120min for measurements of plasma glucose, insulin, and C-peptide. Statistical analysis was performed with ANOVA and Pearson's correlations test. Results are expressed as means ±SD or percentages. A two-tailed p value < 0.05 was considered significant.

Results: We found that patients with TAI and hypothyroidism had significantly higher levels of insulin (10.44±7.49 vs 7.45±3.97mU/ml, p=0.02), C-peptide (2.94±1.29 vs 2.50±0.76ng/ml, p=0.03), CRP (0.47 ±0.58 vs 0.37±0.26mg/dL; p=0.04), HOMA-IR (1.94±0.19 vs 1.67±0.94, p=0.02), and IGI (0.48 ±0.25vs0.36±0.14, p=0.03). In the group of patients with TAI and hypothyroidism there were significant positive correlations between TSH and insulin (R=0.251; P=0.01), C-peptide (R=0.257; p< 0.01), TC (R=0.401; p< 0.001), LDL (R=0.352; P< 0.001), TG (R=0.302 P< 0.01), and ApoB (R=0.279; P< 0.01).

Conclusions: Thyroid function is associated to serum lipids and insulin resistance even in patients with AIT classified as being euthyroid. These findings are consistent with an increased cardiovascular risk in subjects with low normal thyroid function.
Introduction: Thyroid function was already demonstrated to be in association with lipids, as well as other metabolic syndrome components, either in sub-clinic hypothyroidism or in normal range thyroid hormones. Therefore these seem to be crucial risk factors for atherosclerosis.

Objectives: Investigate the association between thyroid hormones and lipid profile, in a sample of euthyroid stroke patients, and assess if thyroid function predicts stroke outcome at short term.

Methods and design: Cohort study, with both retrospective and prospective character, of patients admitted to our hospital, for ischemic stroke. Inclusion criteria included no previous history of thyroid diseases, not taking drugs known to influence thyroid function, and having a thyroid stimulating hormone (TSH) measurement on first 48h of admission. Correlation of TSH and cholesterol was evaluated by Pearson’s test. Prediction of length of stay, and vital status at discharge, and stroke recurrence, was assessed by linear and logistic regression, respectively. Significance level at 0,05.

Results: We followed 284 patients, with mean age 68,6 years and male preponderance (59,3%). Among them, almost 98% had major cardiovascular risk factors. Left medial cerebral artery was the most affected vascular territory (41,4%), being mainly of lacunar type. About 7% were submitted to thrombolysis. NIHSS on admission ranged between 0 and 25 points. Mean TSH was 1,64 µU/L (±1,38), total cholesterol, 179,5mg/dl (±48,6), HDL, 35,7mg/dl (±14,5), LDL, 116,2 mg/dl (±36,7) and triglycerides, 135,8mg/dl (±77,2). TSH was inversely correlated with HDL, already not statistically significant. Stay length was, also inversely related to TSH, which explained near 2% of its variability. TSH value didn’t predict stroke recurrence, but there was a trend to increase the odds of intra-hospital death for each 1µU/L increment, regardless of being statistically insignificant.

Conclusions: TSH correlates with lipid profile and might be an important predictor among stroke patients.
Surgical difficulties in removing nodal recurrences of thyroid carcinoma are well known, mainly in areas previously surgically treated with total thyroidectomy and neck dissection. A surgical technique based on ultrasound intra-operative nodes' visualization and staining was assessed aiming to easily identify intraoperatively the recurrences with low risk of complications. This technique has been used at European Institute of Oncology (Milan) since July 2007. This technique was used for identifying nodal recurrences of thyroid carcinoma resistant to radiiodine treatment, assessable at US scan but not clinically.

**Methods:** Just before surgery, thirteen patients underwent ultrasound neck scan under general anesthesia. Methylene blue dye (0.01 ml) was injected with a 22 gauge needle into the metastatic nodes under US control. The nodal recurrence could therefore be easily localized and visualized during surgery.

**Results:** This technique was successfully used in all thirteen patients. Among them two patients had undergone unsuccessful explorative cervicotomy in another center 30 days before our operation. All patients were considered definitely cured after this procedure, and recurring nodal disease was safely and completely removed in previously surgically treated neck areas.

**Conclusions:** This technique allows faster and easier localization of the recurrent disease during surgery. It minimizes the surgical field and the related risks of damage of neurovascular structures, thus reducing the morbidity of secondary surgical intervention and the risk of surgical failure. The descriptive aspects of ultrasound scan have developed into a concrete surgical approach, leading to a more precise procedure in a minimal surgical field.
PRELIMINARY RESULTS OF INTRAOPERATIVE PHOTODYNAMIC VISUALIZATION OF PARATHYROID GLANDS WITH PER OS USE OF 5-AMINOLEVULINIC ACID
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Aim of the study: To evaluate the usefulness and tolerance of intraoperative photodynamic visualization of parathyroid glands with use of 5-aminolevulinic acid (5-ALA) administered orally.

Methods: 1.5 g of 5-ALA was administered orally to 31 patients with various thyroid diseases 1-3 hours before operation. Patients were randomized for different regimes of ALA administration (1 or 2 doses; 1, 2 or 3 hours before operation). Conventional and videoassisted thyroidectomy was performed. During the operation neck cavity was examined in blue light (380-440 nm) with use 5-mm endoscope and optical filter. Pink fluorescence of parathyroids with various intensity was revealed in 27 cases. There was no fluorescence from the other tissues of the neck. In 27 (87%) of patients it was possible to visualize normal parathyroid glands under fluorescence guiding.

Results: All patients tolerated 5-ALA use well. There were no signs of allergy and photodermatitis, levels of liver transaminases and bilirubin remained unchanged in all the patients. PTH level slightly increased in 3, 24 levels after operation and became normal from 72 hours after operation.

Conclusion: Photodynamic visualization of normal parathyroid glands with 5-ALA use is effective method which can be useful during surgery for primary hyperparathyroidism and thyroid surgery. Cost-effectiveness and complication rates of this technique should be subjects of further investigation.
Aim: To evaluate the safety of central neck dissection with intraoperative photodynamic visualization of parathyroid glands with use of 5-aminolevulinic acid (5-ALA).

Methods: 24 patients with papillary thyroid cancer and no signs of lymph node involvement were included in this study. Diagnosis of papillary thyroid cancer was based on fine needle biopsy results. Patients were randomized for use of prophylactic central neck dissection (CND) before operation. Total thyroidectomy with CND was used in 14 patients. Patients from CND group were randomized for use of 5-ALA during surgery. After randomization 1.5 g of 5-ALA was administered orally to 6 patients 2 hours before operation. During the operation neck cavity was examined in blue light (380-440 nm) with use 5-mm endoscope and optical filter. Pink fluorescence of parathyroids with various intensity was revealed in all cases. In a group with 5-ALA use in 5 patients all 4 parathyroids were visualized, in 1 case - 3 parathyroids. There was no fluorescence from the other tissues of the neck including lymph nodes. In a group without 5-ALA use during CND in 4 cases 4 parathyroid glands were visualized, in 2 - 3 glands, in 2 - 2 glands.

Results: All patients tolerated 5-ALA use well. There were no signs of allergy and photodermatitis, levels of liver transaminases and bilirubin remained unchanged in all the patients. PTH level was stable after operation in 5-ALA group in 5 cases and decreased in 1 case, in a group without 5-ALA use in 3 of 8 cases PTH level decrease was revealed. Ionized calcium level decreased after the operation in 1 patient from 5-ALA group and in 2 patients from the group without 5-ALA administration.

Conclusion: Photodynamic visualization with 5-ALA use can be a useful method for intraoperative visualization of parathyroid glands during thyroid operations followed by CND.
CONTRALATERAL CENTRAL LYMPH NODE METASTASES IN PAPILLARY THYROID CARCINOMA WITH UNILATERAL LYMPH NODE METASTASIS IN THE LATERAL NECK

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Objectives: To investigate the frequency, pattern and predictive factors for contralateral central lymph node (LN) metastases in papillary thyroid carcinoma (PTC) patients with unilateral lateral neck LN metastasis.

Summary background data: There have been few studies assessing the frequencies and predictive factors for the presence of metastases to the contralateral central neck compartment in these patients.

Methods: We reviewed the medical records of 58 consecutive PTC patients with unilateral positive lateral neck who have initially received total thyroidectomy and comprehensive neck dissection including bilateral central LN dissection from 2003 to 2008. The relationships between LN metastases to the contralateral central neck compartment and preoperative image findings, clinco-pathologic factors such as age, sex, location or size of primary tumor, perithyroidal invasion, lymphovascular invasion, capsular invasion and the extent of lateral neck involvement were analyzed.

Results: Central neck LN metastases were present in 82.8%, in which 36.2% had bilateral central neck involvement and 46.6% had unilateral ipsilateral central neck involvement. Isolated contralateral central LN metastases without ipsilateral central LN metastases were not found. Univariate analysis showed that the rate of contralateral central LN metastases was significantly higher in patients with multifocal thyroid tumor, perithyroidal invasion, ipsilateral positive central LN and metastases in all lateral neck levels (level II, III, IV) (P< 0.05). Multivariate analysis showed that the presence of metastases in all lateral neck levels was an independent risk factor for the presence of contralateral central LN metastases (P=0.043, odds ratio 3.957). Additionally, clinically suspicious ipsilateral central LN in preoperative imaging studies was an independent predictive factor of pathologic contralateral central LN metastasis (P=0.011, odds ratio 7.046).

Conclusion: Simultaneous lymphatic metastases in all lateral neck levels (II + III + IV) predict the presence of contralateral central LN metastases in PTC patients with clinically evident LN metastasis in the lateral neck.
Objective: Nodal metastasis of papillary thyroid cancers (PTCs) occurs in a sequential fashion: from the thyroid gland to the central and lateral lymph node compartments. But this sequential pattern of lymph node metastasis does not occur in all cases. Skip metastasis is not uncommon in PTCs and the frequency varies between 11.1% and 37.5%. We aim to investigate the frequency and pattern of skip metastasis in the PTCs and determine the relationship between skip metastasis and primary tumor site.

Methods: We undertook a retrospective review of 87 patients, 102 neck dissection cases treated between January, 2004 and December, 2008 for PTCs by total thyroidectomy and central neck dissection (n=87) with lateral neck dissection. We defined “Skip metastasis” as negative ipsilateral central and positive ipsilateral lateral compartment lymph node of primary tumor site. Preoperatively all the patients underwent ultrasonography and were classified into upper 1/3, middle 1/3, lower 1/3 groups according to primary tumor location.

Results: The frequency of ipsilateral nodal metastasis from primary tumor site was 73.7% at level III, 63.5% at level IV, 55.0% at level II. Skip metastasis were found in 5 of 102 neck dissection cases (4.2%). For the skip metastasis, all of them had primary tumor on upper 1/3 of thyroid gland and metastatic site were at level III (4/5), level II (2/5), level IV (1/5).

Conclusions: Cervical lymph node skip metastasis was 4.2%, which is much lower than prior reports. All of the skip metastasis had their primary tumors in upper part of thyroid gland. These results show that skip metastasis would be direct lymphatic spread from upper part of thyroid gland rather than skip metastasis. It can be assumed that actual skip metastasis will be much lower than expected. Therefore central neck dissection should be recommended when lateral nodal metastasis is clinically suspected in PTCs.
Background: The extent of lateral neck dissection for a clinically evident lateral neck in a papillary thyroid cancer (PTC) patient continues to remain controversial.

Methods: We retrospectively reviewed the medical records between March 2005 and March 2008 of 70 PTC patients who underwent therapeutic lateral neck dissections (level II ~ V) to establish indications for omission of level V lymphadenectomy. All patients in the study did not have a clinically positive level V lymph node. Neck dissection specimens were obtained for histologic analysis for node metastasis with respect to separate neck levels.

Results: Thirty-four (49%), 52 (74%), and 48 (69%) patients had histological positive lymph nodes in level II, III, and IV, respectively. Occult metastases in level V were observed in 11 (16%) patients. Isolated positive level V lymph nodes were never found, while all patients with positive level V lymph nodes had simultaneous positive level IV lymph node occurrence. There was no instance of pathological positive in level V without suspicious metastastic lymph node in level IV by preoperative ultrasonography. In multivariate analysis, simultaneous positive lymph node multilevel involvement (level II, III, and IV) was significantly associated with level V metastasis.

Conclusion: Level V lymphadenectomy may be omitted in the treatment of PTC patients if positive nodes were not observed in level IV.
Background: Although some lymph nodes (upper para-esophageal lymph nodes) that exist between the right recurrent laryngeal nerve and esophagus and/or prevertebral fascia should be involved routine central compartment lymph node dissection (CLND) in patients with papillary thyroid cancer (PTC), this procedure can cause some injury to the nerve, resulting from traction and elevation during the removal of these lymph nodes.

Objective: We were to assess the incidence and pattern of and prognostic factors related with right upper para-esophageal lymph node metastasis and to determine whether or not the resection of this area for a routine CLND in patients with PTC.

Study design: Retrospective chart review

Patients and methods: From March 2007 to February 2008, a total of 123 consecutive patients (102 women and 21 men) with PTC underwent total thyroidectomy with routine CLND. We evaluated the relation between the metastasis of upper para-esophageal lymph nodes and several potential predictors.

Results: Fourteen (11.4%) of 123 patients exhibited nodal metastasis at right upper para-esophageal lymph nodes. The metastasis of right upper para-esophageal lymph nodes were usually occurred in lesion of right thyroid lesion, comparative large tumor size (more than 1 cm), and patients with lateral cervical lymph node metastasis (p< 0.05).

Conclusions: Right upper para-esophageal lymph node should be removed during dissection for patients with the lesion of right thyroid gland, comparative large tumor size (>1cm), and lateral cervical lymph node metastasis.
Objective: The authors have retrospectively analysed their experience concerning the treatment of the Thyroid Papillary Carcinoma, with the aim of evaluating the systematic treatment performed by our Unit.

Materials and methods: We studied a total of 73 patients with papillary carcinoma, which might or not have been the cause of the surgery. Whenever the diagnosis occurred before the surgery, a total thyroidectomy with central neck dissection was performed as minimum procedure. If not, the patient would be submitted to a totalization, had the previous surgery been a hemithyroidectomy.

Results: 32 total thyroidectomies with central neck dissection were performed. The remaining cases were found after histologic evaluation of tissues coming from the total thyroidectomy or from the hemithyroidectomy (in each case, recurring to totalization). From the 73 carcinomas found, 25 were less than 1 cm (microcarcinoma) and the others 48 had more than 1 cm. Multifocality in the same lobe (2) and in the contralateral lobe (11) was visible in 13 of the 48 tumors with more than 1 cm and in 9 cases, 5 contralateral and 4 homolateral, of the 25 tumors less than 1 cm. Lymph node metastases was found in 13 of the 26 central neck dissection perform in the group of the patients with carcinoma more than 1cm in diameter; and in 3 of 6 central neck dissection perform in the group of patients with carcinoma less than 1 cm.

Conclusions: The high probability of multifocality (30,1%) and lymph node metastases in the central compartement (50% neck dissections), allows us to say that total thyroidectomy is the appropriate and adequate procedure for its treatment, regardless the size of the tumour. We also recommend systematic central neck dissection as a way of diminishing the probability of a local recidive in such patients.
CENTRAL LYMPH NODE METASTASES IN UNILATERAL PAPILLARY THYROID MICROCARCINOMA

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Background: The indications and appropriate extent of prophylactic central lymph node (CLN) dissection for clinically node-negative patients with unilateral papillary thyroid microcarcinomas (PTMC) is unknown.

Methods: The frequency, patterns, and predictive factors for CLN metastases from 86 unilateral PTMC patients with clinically node-negative necks were analyzed with respect to the following variables: age, sex, MACIS score, tumour size, number and location of tumours, presence of ipsilateral CLN metastases, and presence of lymphovascular or capsular invasion. All patients underwent total thyroidectomy and CLN dissection.

Results: Twenty-seven of 86 patients (31%) had metastatic CLN: 18 ipsilateral and 9 bilateral. Although univariate analysis suggested males (N=4) and tumour size > 0.5 cm to be significant factors in predicting ipsilateral CLN metastases, only ipsilateral nodal positivity was a significant predictor of contralateral CLN metastases (P=0.007)

Conclusion: Central lymph node metastases are relatively common in PTMC.
SURGICAL TREATMENT OF THYROID CARCINOMA USING MINIMALLY INVASIVE OPEN APPROACH AND HARMONIC SCALPEL

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The use of harmonic scalpel in thyroid surgery broadens in recent years.

**Aim:** To present our results in the surgical treatment of thyroid carcinoma using harmonic scalpel and minimally invasive open approach.

**Patients and methods:** For one year period 27 patients with thyroid carcinoma have been operated on using harmonic scalpel through a minimally invasive approach in our institution (23 females and 4 males, age from 21 to 49 years). Patients have been included in this group on the basis of criteria accepted in our clinic discussed in previous papers. Thyroid resections were performed by harmonic scalpel (Ethicon Endo-Surgery). The extent of resection, amount of hemostatic instruments, stitches and drains used in the procedure as well as the operative time, intra- and postoperative complications, length of postoperative hospital stay have been evaluated.

**Results:** In 24 patients papillary thyroid carcinoma and in 3 patients follicular thyroid carcinoma was found, from 0.5 to 2 cm in size. Lymph node metastases in the central cervical compartment were detected in 4 patients. Thyroidectomy through a central collar incision, 2 to 3 cm in length has been used in all cases without use of hemostatic instruments and stitches because of the safe hemostasis achieved with the harmonic scalpel. All procedures were finished without need of draining. Complications were not observed. All patients leaved the hospital until the 24 hour after the procedure. Postoperative thyroid scans were negative in all patients.

**Conclusions:** Harmonic scalpel is a very effective tool giving the opportunity to perform safe and adequate thyroidectomy through a minimally invasive open approach in selected patients with thyroid carcinoma.
DUOX1 AND DUOX2 ACTIVITIES ARE DIFFERENTIALLY REGULATED BY THE TWO MAIN THYROID SIGNALING PATHWAYS

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Dual oxidases are identified as NADPH oxidases producing H₂O₂ necessary for thyroid hormone biosynthesis. Mutations in DUOX2 gene of patients suffering from partial iodide organification defect implies the crucial role played by Duox2 in thyroid hormone synthesis. The function of Duox1 in the thyroid remains unclear. Duox activity has been measured by co-expressing Duox1 or Duox2 with their respective maturation factors, DuoxA1 and DuoxA2, to compare their intrinsic enzymatic activities under stimulation of the major signaling pathways active in the thyroid in relation to their membrane expression. We showed that basal activity of both Duox isoenzymes depends on calcium and functional EF-hand motifs. However, the two oxidases are differentially regulated by activation of intracellular signaling cascades. Duox1 but not Duox2 activity is stimulated by forskolin (EC₅₀ = 0.1 µM) via protein kinase A-mediated Duox1 phosphorylation on serine 955. In contrast, phorbol esters induce Duox2 phosphorylation via protein kinase C activation associated with high H₂O₂ generation (PMA-EC₅₀ = 0.8 nM). These results were confirmed in human thyroid cells suggesting that Duox1 is also involved in thyroid hormonogenesis. Our data provide, for the first time, detailed insights into the mechanisms controlling calcium-dependent activation of Duox1/2 proteins revealing additional phosphorylation-mediated regulations.
THYROTROPIN RECEPTOR (TSHR) ACTIVATION INCREASES HYALURONAN (HA) PRODUCTION IN PREADIPOCYTE-FIBROBLASTS; EXPLANATION FOR HA ACCUMULATION IN THYROID DYSFUNCTION?

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The thyrotropin (TSH) receptor (TSHR) is expressed during lineage-specific differentiation (e.g. adipogenesis) and is activated by TSH, thyroid stimulating antibodies (TSAB) and gain-of-function mutations (TSHR*). Comparison of gene-expression profiles of non-modified human preadipocytes (n=4) with the parallel TSHR* population revealed significant upregulation of 27 genes including hyaluronan (HA) synthases (HAS) 1 & 2. Array data were confirmed by QPCR of HAS1 & HAS2, and ELISA measurement of HA, all significantly increased (p< 0.03) in TSHR* expressing preadipocytes (n=10). Preadipocytes (n=8) treated with db-cAMP display significantly increased HAS1 & HAS2 transcripts, HAS2 protein (western blot) and HA production (p< 0.02). HAS1 siRNA treatment of db-cAMP stimulated preadipocytes (n=4) produced 80% knockdown in HAS1 transcripts (compared with scrambled) but had no effect on HAS2; the corresponding HA production was reduced by 49%. Reporter assays using A293 cells transfected with HAS1 promoter driven plasmids containing/not the proximal CRE and treated with db-cAMP, revealed that it is functional. Chromatin immunoprecipitation, using a CREB antibody, of db-cAMP treated preadipocytes (n=4) yielded products for HAS1 and HAS2 with relative fold-increases of 3.3±0.7 and 2.3±1.4 respectively. HA accumulates in adipose/connective tissues of patients with thyroid dysfunction. We investigated the contributions of TSH and TSAB and obtained small (9-14%) but significant (p< 0.02) increases in preadipocyte HA production with both ligands. Similar results were obtained with a TSHR monoclonal lacking biological activity (p< 0.05). We conclude that TSHR activation is implicated in HA production in preadipocytes which, along with thyroid hormone level variation, explains the HA over-production in patients with thyroid dysfunction.
IN ADDITION TO HYDROPHILIC ALSO HYDROPHOBIC RESIDUES OF THE TSHR HINGE REGION ARE INVOLVED IN RECEPTOR SIGNALING AND HORMONE BINDING

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The hinge region links the extracellular hormone binding LRR domain of the TSHR with the transmembrane domain. Crystallographic studies deciphered a main part of the three-dimensional structure of the LRR domain for the TSHR and FSHR. However, these data lack structural information for the complete hinge region. The precise molecular mechanisms of hormone binding, signal transduction and receptor activation are only partly known. However, in vitro and in vivo data provide evidence for a functional importance of the TSHR hinge region for hormone binding and receptor activation. Beside the well characterized positions S281 and Y385 we identified several hydrophilic residues of the hinge region (E297, E303, D382, D403, E404, N406) as new and important determinants for TSH binding and/or signaling. To further characterize the TSHR hinge region we substituted hydrophobic positions between I292-V397 by slight side chain alterations to maintain the hydrophobic property, which has a strong influence on receptor conformation. The generated single mutations were functionally characterized by determination of cell surface expression, bTSH binding, cAMP and IP signaling. All mutants were expressed at the cell surface and showed TSH binding comparable to the wt TSHR except F381V. This mutant showed an expression of 100% but a strong decrease of bTSH binding to 38% compared with the wt. None of the mutants revealed an increased basal cAMP or IP signaling but 8 of 14 substitutions exhibited a decrease of the maximal cAMP and/or IP signaling. Our data suggest, that the analyzed hydrophobic residues are most likely not involved in maintaining the basal wt receptor conformation, whereas several hinge positions seem to be involved in signal transmission after TSH binding. Moreover, F381 as a direct neighbor of the negatively charged D382, which was shown to be involved in TSH binding via charge-charge interaction, also appears to participate in hormone binding.
Growth and function of the thyroid are controlled by TSH. While the activation of the TSH receptor (TSHR) primarily leads to stimulation of the adenylyl cyclase via the Gs protein, at higher TSH concentrations an activation of the phospholipase C cascade by Gq has also been observed. Activating TSHR mutations are a major cause of toxic thyroid adenoma (TTN) and familial hyperthyroidism. In-vitro, TSHR mutations differ in their degree of constitutive activation of the cAMP and inositol phosphate cascades. However, the in-vitro potency does not correlate with the severity of the clinical phenotype either in TTNs or familial hyperthyroidism. Variable phenotypes associated with the same TSHR mutation could be the result of influences on signaling downstream of the TSHR. In this work we aimed to elucidate the molecular changes in signal transduction causing the differential biological activity of three activating TSHR mutations (A623I, L629F and Del613-621) and the TSHR wildtype (WT) on the proteome level. We analyzed cellular lysates of untreated/TSH or IBMX treated FRTL-5 cells with stable expression of the respective TSHR mutations or the WT by 2D-Fluorescence Difference Gel Electrophoresis (2D-DIGE). We here describe first global changes of protein expression after TSH or IBMX stimulation with a primarily oppositional stimulation of the PKA independent cAMP/Epac1/Rap1 pathway and the cAMP/mTOR/p70s6k pathway in FRTL-5 cells carrying the TSHR-WT compared to TSHR mutants.
Previous studies have shown that alterations in thyroid status may lead to changes in serum leptin and adiponectin, both in humans and rodents. The mechanisms, especially for adiponectin, are unclear. In the present study, we evaluated the effect of T3 on expression and secretion of adiponectin and leptin from white adipose tissue (WAT) explants obtained from normal rats. Adiponectin and leptin were quantified by radioimmunoassay in incubation medium of 50mg explants (in slices of 10mg) of inguinal (subcutaneous) and epididimal (visceral) fat pads of euthyroid rats cultured with iodothyronin-free fetal bovine serum, at 37º C in CO2 atmosphere in the presence or absence of T3, rosiglitazone or dexametasone. After incubation, adiponectin mRNA was evaluated by real time PCR, and tissue integrity was confirmed by light microscopy of histological sections stained with hematoxilin-eosine dye. T3 at 10 nM was not able to modify the secretion of adiponectin in explants of both WAT depots incubated for 24 and 48 hours. However, T3 reduced the adiponectin mRNA expression by 40% only in inguinal explants. Rosiglitazone at 10 µM induced a 2-fold increase (p< 0.05) in adiponectin mRNA abundance in inguinal WAT incubated for 48 hours, and conversely, the same tissues exhibited a significant reduction in adiponectin release. The secretion of leptin was reduced by 10 nM T3 (40% p< 0.001) in explants of epididimal, but not inguinal WAT, cultured for 48h, while 10nM dexametasone potently stimulate leptin secretion from both WAT explants depots. Both results agree with other studies employing cultured isolated adipocytes, and reinforces the view that T3 acts directly at adipose tissue to inhibit leptin secretion. However, the present study suggests that T3 is a potential inhibitor of adiponectin mRNA expression specific at a subcutaneous depot, although had no influence on adiponectin secretion in white adipose tissue, and therefore, probably other mechanisms are also involved in the variations of serum adiponectin of hypo- and hyperthyroid rodents reported in previous studies.
The TSH receptor (TSHR) is a G protein-coupled receptor (GPCRs) which presents a large extracellular domain responsible for high affinity hormone binding and a carboxyl-terminal serpentine region, shared by all GPCRs, implicated in the transmission of the activation signal. The glycoprotein hormones (GPHs [TSH, FSH, LH and hCG]) are heterodimeric proteins comprising a common alpha subunit ($\alpha_1$) and specific beta subunits ($\beta_1$ to 4) having substantial sequence similarity. In addition to activation by TSH, TSHR can be stimulated by hCG, which can reach concentrations during the first trimester of pregnancy at which it displays some thyrotropic activity, bringing most pregnant women on the fringe of hyperthyroidism. A fifth glycoprotein hormone was recently identified. This hormone harbors specific alpha and beta subunits ($\alpha_2/\beta_5$) which confer to this protein the prototypical cystine-knot structure, despite a weak similarity (30%) with the amino acids sequence of other GPHs. In addition, this $\alpha_2/\beta_5$ shows a strong thyrotropic activity in vitro (but no LH or FSH like activities) and was therefore named “Thyrostimulin”. Nevertheless, whether or not the TSHR is the natural endogenous receptor of the Thyrostimulin remains an open question.

Objectives and methods: To identify the residues on the TSHR which are involved in the Thyrostimulin binding, we produced and purified recombinant Thyrostimulin and we generated series of mutants of the extracellular domain the receptor. The mutants were tested functionally for their sensitivity to TSH and/or Thyrostimulin and we identified residues shared by both binding sites.

Results/conclusions: Based on the structure of the TSH receptor/TSH model and studies generated by site-directed mutagenesis, we provide in the present work a robust molecular model of the interaction of Thyrostimulin with TSHR. In addition, by comparison with FSH receptor/FSH and LH receptor/LH-CG complexes, we propose a molecular explanation to the specific thyrotropic activity of the Thyrostimulin.
Upon activation by thyrotropin, the thyrotropin receptor (TSHR) transduces the extracellularly initiated signal into the cell primarily by cAMP stimulation via activation of G-protein-syntype Gαs (cAMP accumulation). The phenomenon of G-protein coupled receptors (GPCRs) also being capable of coupling multiple different G-protein subtypes was for TSHR previously assumed to be an in vitro effect. Recently it was demonstrated that to maintain thyroid function TSHR-initiated stimulation of phospholipase via Gαq/11-activation (IP accumulation) is also of high physiological importance. Therefore, we combined structural and functional investigations to reveal insights into the selectivity patterns and molecular determinants distinguishing the molecular interactions between TSHR and Gαs / Gαq.

Utilizing the recently published X-ray structure of opsin (an active GPCR structure) complexed with the C-terminal peptide of transducine, we generated homology models of an activated conformation of the TSHR in complex with Gαs and Gαq peptides as well as in complex with heterotrimers Gβγ of the respective subtypes. Furthermore, functional characterisation of those amino acids of unknown contribution (as suggested by our models) in the intracellular region, especially in the intracellular loop 1 (ICL1) was carried out by site-directed mutation.

In detail, mutations of amino acids I438, S442 and R450 in ICL1 impair both Gαq and Gαs activation, while L440A, T441A and H443A are selectively decreasing IP accumulation. In our homology models the structural differences between interaction of the particular Gαs or Gαq portions and the TSHR are consistent with data from mutagenesis of the ICLs by explaining different binding modes between the TSHR and Gαq or Gαs.

This study provides general information about the selective interaction between GPCRs and Gαs or Gαq.
IODIDE TRANSPORTER MODULATION IN THE PLACENTA

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The iodide transporters- sodium iodide symporter (NIS) and Pendrin (PDS) have been demonstrated in human placental trophoblasts and have been shown to have a role in iodide accumulation by the placenta. The capacity of the placenta to accumulate and store iodine under the control of the pregnancy related hormones hCG, Oxytocin and Prolactin has been demonstrated by our group. The aim of this study was to investigate the effect on iodide accumulation by placental primary cultures of combinations of these hormones and to study the mechanisms by which these increases in uptake occur. The most effective combination of these hormones was that of 17β Estradiol -Progesterone-Prolactin-Oxytocin at upper physiological concentrations which increased uptake by 82%. Less effective were combinations of Estradiol-Progesterone-Prolactin and Estradiol-Progesterone-Prolactin-Oxytocin while Estradiol-Progesterone produced no significant effect. RNA was isolated from placental trophoblasts preincubated with various hormones both alone and in various combinations using the Quiagen RNA extraction kit. Taqman real time RT-PCR with NIS and PDS probes was carried out to determine the levels of expression of both transporters. NIS and PDS were normalised against the 18s RNA endogenous control. In all treatments where iodide uptake was increased there were corresponding significant increases in expression level of NIS mRNA as compared with control non-treated trophoblast mRNA (hCG 1.42; Oxytocin 1.28 & Prolactin 1.26). There were also increases in NIS expression levels in the combination hormone treated cells (17β Estradiol -Progesterone-Prolactin-Oxytocin 1.56). In contrast the expression levels of PDS remained unchanged in any of the hormone treated cells indicating that increased NIS expression was solely responsible for the increase in iodide uptake. This study demonstrates the involvement of pregnancy associated hormones in promoting placental iodide uptake and supports a potential role for the placenta in protecting against iodine deficiency in the fetus.
The trace elements: Se, Zn and Cu are the essential components or cofactors required to activate numerous enzymes and proteins, playing crucial role in various physiological processes.

**The aim of the study:** The aim of the study was to evaluate the influence of selected trace elements: Se, Zn and Cu on development of thyroid diseases.

**Material and methods:** In 17 patients aged 35.8±11.7 years with Hashimoto Disease (HD), 25 patients with papillary thyroid cancer (PTC) aged 51.6±13.4 years and 13 patients with follicular thyroid cancer (FTC) aged 52.5±15.3 years, and in 20 healthy controls, aged 37.7±9.3 years serum TSH, fT4, fT3, Se, Zn, Cu, the ferric reducing ability of plasma (FRAP) and Glutathione Peroxidase (GPX3) activity were determined.

**Results:** The Cu plasma concentration was significantly above normal in patients with PTC: (19.6µM/L, v.s.17.3µM/L respectively). Zn/Cu was higher in HD and controls than in PTC and the differences were statistically significant. No statistically significant differences were found in plasma Zn and Se concentration between patients with thyroid diseases and healthy controls.

The GPX3 activity was highest in controls: 267 U/L, in HD- 258 U/L in PTC 254 U/L and in FTC 247 U/L respectively. The mean FRAP activity was highest in patients with PTC: 672 µM Fe/L and FTC 671 µM Fe/L, in controls 657 µM Fe/L and in HD 622 µM Fe/L, but the differences were not significant. Only in HD we detected correlations between age and Se level, GPX3 and FRAP activity. There was positive correlation between age and FRAP activity in patients with FTC.

**Conclusions:** The metabolism of trace elements is abnormal in thyroid diseases. Selenium and selenium- dependent enzymes play an important role in Hashimoto Disease. It is suggested, that copper can play a role as an antioxidant molecule in oxidative stress in different thyroid diseases.
**P13 Genetics of Thyroid Cancer**

**P075**

**WDR3 GENE IS ASSOCIATED WITH THYROID CANCER RISK AND IT SHOWS AN ABERRANT EXPRESSION IN HUMAN THYROID CANCER CELLS**

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**Objectives:** Genetic predisposition together with environmental factors are important in the carcinogenesis processes of the thyroid. We found different susceptibility markers in the 1p12 region that are associated with thyroid cancer. One of them, the SNP 4658973, is in the **WDR3** gene. This gene could be involved in cell cycle progression and signal transduction. Our aim was to investigate the possible implication of **WDR3** in thyroid cancer by association studies and analysis of expression in human thyroid cancer cell lines.

**Methods:** We performed a case-control association study by genotyping six SNPs that cover the **WDR3** gene, in 162 control and 118 thyroid cancer patients of a Spanish population. The expression of the gene was analysed by RT-PCR and western blot in 12 human cell lines. In addition, methylation of the promoter is been analysed by bisulfite sequencing.

**Results:** Haplotype analysis using the combination of the six SNPs has identified six common haplotypes. Three of them have showed strong association with thyroid cancer: Hap T-C-T-A-C-T, OR= 14.59, 95% CI= 6.37-33.44 (*P* < 0.0001); Hap C-A-C-G-T-G, appeared with 0.028 frequency in patients, but did not appear in the control group (*P* < 0.0001) and Hap T-C-T-A-C-G, OR= 0.38, 95% CI= 0.22-0.66 (*P*= 0.0006).

Both messenger RNA and protein expression were altered in six of the ten thyroid cancer cell lines analysed, indicating the implication of **WDR3** in the aetiology of thyroid cancer. The analysis of promoter methylation and its relation to **WDR3** expression will also be presented.

**Conclusions:** **WDR3** gene is a genetic factor for thyroid cancer risk, found in the association studies. Moreover, the altered expression of **WDR3** in thyroid cancer cell lines would suggests the implication of this gene in thyroid cancer development.
GERMLINE SDHD VARIANTS MODULATE THE DEVELOPMENT OF MEDULLARY THYROID CARCINOMA

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Background: Medullary thyroid carcinoma (MTC) develops from the neural-crest derived C-cells of the thyroid. These are rare, but aggressive, thyroid tumours and are generally associated with oncogenic mutations in the RET oncogene, which give rise to multiple endocrine neoplasia type 2 (germline RET mutations) or sporadic MTC (somatic RET mutations). The succinate dehydrogenase genes (SDHB, SDHC and SDHD) were recently found to be tumour-suppressor genes for the development of hereditary paragangliomas and phaeochromocytomas; in addition, germline SDHD alterations were found to be associated with the development of familial C-cell hyperplasia, a condition thought to precede MTC, being over-represented in MTC patients, as well as associated with younger age at diagnosis of MTC.

Aim: In this study, we have analysed a series of 46 MTC patients for germline alterations in SDHB and SDHD in order to confirm that SDHB and SDHD alterations may modulate MTC tumourigenesis.

Results: Five different germline SDHB (two) and SDHD (three) alterations were identified in nine patients (20%). One patient harboured a double alteration in SDHB - A6A in exon 1 and ISV2-36 G>T in intron 2 - and eight patients were found to have coding alterations in SDHD: three harboured the H50R alteration in exon 2, one harboured the G12S alteration in exon 1 and four patients presented both the G12S and the S68S alteration in exon 3. No pathogenic mutations were disclosed. We observed a trend for over-representation of germline SDHD alterations in MTC patients when compared to a control population (p=0.07) and for a lower mean age at diagnosis in MTC patients with germline SDHD alterations than in those without SDHD alterations (41 vs. 55 years, p=0.025).

Conclusions: We conclude that germline alterations in SDHD may modulate MTC tumourigenesis.
Point mutations in the RET oncogene were found in sporadic and familial cases of medullary thyroid carcinoma (MTC) and Hirschsprung’s disease (HSCR). We have previously described a large Brazilian kindred with a missense mutation (1597G>T) within exon 8 of RET gene that leads to a Gly^{533}Cys substitution in the cystein-rich domain of RET. To evaluate the biological and biochemical effects of the RET mutant, PCCL3 were stably transfected with a vector encoding RET G533C, RET wild type and RET C634Y. Clones expressing RET mutants were evaluated for their ability to induce proliferation and anchorage-independent growth in PCCL3 cells in comparison to wild type. Additionally, we investigated the effects of G533C on activation of MAPK pathway and the expression of genes previously identified as target of RET. Our results show that G533C confers to PCCL3 cells a more malignant phenotype which was accompanied by activation of MAPK, changes in expression of thyroid specific genes and in potential RET target genes. Additionally, an increased genetic instability was observed in cells expressing RET G533C, which is a hallmark of cancer. Our findings indicate that cells expressing RET G533C have a more aggressive phenotype when compared to RET wild type and RET C634Y. This study reinforces the importance of functional analysis of RET mutations and its clinical implications.
HETEROGENEOUS PATTERN OF COPY NUMBER VARIATIONS IN COLD THYROID NODULES DETECTED BY HIGH-DENSITY SNP ARRAYS

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The molecular etiology of cold thyroid nodules (CTNs) is still largely unknown. Structural changes of chromosomes such as aneuploidies, translocations, copy number variations (CNV) and point mutations are common tumor hallmarks. Chromosomal CNV in regions coding for oncogenes and tumor suppressor genes may affect cell growth, proliferation, apoptosis, and metastasis. Therefore, the identification of tumor-specific CNV can provide clues about the molecular basis of tumorigenesis and will identify new oncogenes and tumor suppressor genes.

Genome-wide CNV was analyzed in 10 CTNs in comparison to their surrounding tissues using Affymetrix 500k SNP arrays. This array set contains probes for 500,568 SNPs covering most of the human genome with an average spacing of 5.88 kb. CNAG software was used for data analysis. Six hemizygous deletions were detected at 2p, 6q, 15q, 21q, and 22q. Moreover, 34 amplified regions at 1p, 2q, 4q, 5q, 6p, 7p, 7q, 9p, 9q, 10q, 11q, 12q, 13q, 14q, 15q, 16p, 16q, 17p, 20p, 20q, 22q, and Xp were identified. Trisomy of chromosome 7, 16, and X was detected once. Whereas one amplification at 15q occurred in 2 CTNs all other CNV were detected only once. While CNV were described by classical CGH in the region or near to the region of 25 CNV described above, the remaining 15 deletions and amplifications have not been previously reported in benign nodules. The aberrated regions contain several genes with known functions in thyroid pathophysiology. Interestingly, the detected pattern of CNV is very heterogeneous. Hemizygous deletion at 2p is in line with the previous finding of loss of heterozygocity within or near to the TPO locus in CTNs by Krohn and Paschke (Thyroid, 2001). Further studies have to validate the newly identified regions for CNV and should further examine the respective candidate genes.
CDKN1B encodes the cyclin-dependent kinase (Cdk) inhibitor p27 (Kip1). This is an important cell-cycle regulatory protein that controls the progression from G1 to the S phase by interacting with cyclinE/Cdk2 and cyclinD1/Cdk4 complexes and stimulating their degradation or inhibition. Loss of p27 function may contribute to tumorigenesis. A total of 21 single nucleotide polymorphisms in CDKN1B have been described. Of these, 11 have low allelic frequency (5%) and 9 occur within the noncoding regions of the gene. Only one single nucleotide polymorphism (T3G) causes an amino acid substitution, a glycine for valine at codon 109. This V109G polymorphism affects p27 degradation in vivo and appears to be associated with advanced prostate cancers, suggesting its role in tumor progression. Furthermore, expanded pedigree analysis shows that p27(Kip1) mutations are associated with the development of a MEN-like phenotype in multiple generations. We evaluated the frequency of the CDKN1B V109G polymorphism in medullary thyroid carcinomas (MTC) negative for RET mutations and correlated with the disease progression and outcome.

**Methods:** The V109G single nucleotide variant was examined on the germline DNA from 35 patients affected by sporadic MTCs, using PCR amplification of exon 1, followed by direct sequencing of the amplicon.

**Results:** 50% of the samples analyzed were positive for the polymorphism, a significantly higher frequency than that reported (20%) in other neoplasias. The value was even higher than that found in the control population (circa 30%, n=50). The polymorphism was associated with better prognosis, absence of recurrences and/or metastatic spreading as compared to wild type sequence bearing patients.

**Conclusions:** These data suggest that CDKN1B V109G polymorphism may play a critical role in the aggressiveness of MTC not addressed so far. Moreover, its detection may be used as a prognostic factor, especially if associated with total protein level detection and subcellular localization.
MOLECULAR GENETIC ANALYSIS OF MEDULLARY AND PAPILLARY THYROID CARCINOMAS: UNUSUAL CASES

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Objectives: Medullary thyroid carcinoma (MTC) is a rare thyroid malignancy which is derived from the parafollicular cells and is caused by germline and somatic mutations in the RET proto-oncogene. The most frequent thyroid cancer is a papillary thyroid carcinoma (PTC) originated from the follicular cells which BRAF mutations and RET/PTC rearrangements are typical for.

Cases: Molecular genetic analysis comprised 284 families with MTC and 242 PTC patients among them we registered several unusual cases. In two patients, we report the simultaneous occurrence of medullary and papillary thyroid carcinoma. Both patients are women who underwent total thyroidectomy with respect to elevated basal calcitonin level and histology revealed MTC and papillary microcarcinoma. Molecular genetic analysis detected a heterozygous germline mutation Tyr791Phe in exon 13 in the RET proto-oncogene in the first patient. In the second patient, neither alterations in the RET proto-oncogene nor the BRAF gene or RET/PTC rearrangement were detected. The germline mutation Tyr791Phe in the RET gene was also found in a patient who was operated on for PTC and histology revealed C-cell hyperplasia as well. Other interesting two cases are patients with histologically confirmed MTC who also have typical genetic alterations for PTC - RET/PTC1 rearrangement and BRAF mutation Val600Glu, respectively.

Conclusions: The co-existence of medullary and papillary thyroid carcinoma occurs very rarely which is evidenced by only at around 50 cases described in the literature. We describe the molecular and clinicopathological findings in 5 patients with simultaneous thyroid cancer or atypical results of molecular genetic analysis that were recruited from our large cohort of patients with thyroid carcinomas.

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SNP RS2145418 A CONSERVED REGION OF 1P12 IS ASSOCIATED WITH SUSCEPTIBILITY TO THYROID DISEASES

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Objective: Currently it is postulated that susceptibility genes and environmental factors may play an important role in thyroid carcinogenesis. Our recent studies show that chromosome 1p12 is associated with thyroid cancer susceptibility, the risk marker rs2145418 maps to an empty gene area. However, Sequence Data Base shows that rs2145418 is located in an evolutionary conserved region. Our association studies will be extended to a German population of thyroid cancer patients and to a Tunisian population of hyperthyroidism and Hashimoto patients. In addition, to identify the susceptibility region marked by rs2145418 we’ll genotype three additional SNPs linked to rs2145418 covering 2 kb region in a Spanish population of thyroid cancer.

Methods: Case-control design was performed for association studies. RFLP-PCR was used to genotype the rs2145418 in both German and Tunisian populations. The three SNPs around rs2145418 were genotyped by MassArray technique at the Genotype National Centre of Spain using a Spanish population of thyroid cancer patients.

Results: A significant genotype OR for rs2145418 [3.29, 95% CI (1.98-5.44) and 2.82, 95% CI (0.97-8.17) for GT and GG, respectively, P< 0.0001] in the German population was found, which agree with our previous Spanish population results. Moreover, in the Tunisian population, rs2145418 showed a high association with hyperthyroidism [0.25, 95% CI (0.14-0.42) and 0.86, 95% CI (0.44-1.69) for GT and GG, respectively, P< 0.0001] and Hashimoto disease [0.20, 95% CI (0.09-0.44) and 0.23, 95% CI (0.06-0.83) for GT and GG, respectively, P=0.0004]. Haplotypes analysis of the three SNPs linked to rs2145418 and their relation with clinicopathological characters in thyroid cancer patients of the Spanish population will be also presented.

Conclusions: rs2145418 is a new susceptibility marker for thyroid diseases and the fact that it maps to a conserved region could suggest that a regulatory sequence in the 1p12 region maybe related to thyroid diseases.
ASSOCIATION OF A432A RET POLYMORPHISM WITH RET/PTC IN PATIENTS WITH PAPILLARY THYROID CARCINOMA

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Background and aims: RET/PTC rearrangements of the RET proto-oncogene are responsible for development of papillary thyroid carcinomas (PTC). Some studies reported an association among single nucleotide polymorphisms (SNPs) of the RET gene and PTC. We investigated the frequency of RET polymorphisms in Czech patients with PTC and studied possible association of SNPs with RET/PTC and with clinicopathological features.

Methods: We analyzed 102 patients with PTC and 172 healthy controls. RNA and DNA were extracted from fresh frozen thyroid samples and blood. RNA was reversely transcribed to cDNA and the presence of RET/PTC was detected on agarose gel. Polymorphisms in the RET gene - A432A, G691S, S836S and S904S were detected using specific TaqMan probes. Haplotypes were generated using PHASE software. Presence of RET/PTC and RET polymorphism was correlated with clinicopathological parameters.

Results: RET/PTC was detected in 26 PTC patients (25.5%) - 2 RET/PTC1 (2%), 2 RET/PTC3 (2%) and 22 RET/PTCX (21.5%). RET/PTCs were more frequent in males with PTC, but this was not statistically significant. The minor allele A of A432A (G/A) was under-represented in PTC patients carring RET/PTC compared with the patients without this alteration (19.2% vs. 40.7%, \(P = 0.007\)). No significant difference of SNPs and haplotypes frequencies in the patients versus normal controls was found. However, the distribution of haplotypes significantly differed in PTC patients related to presence of RET/PTC (\(P = 0.047\)) and to sex of patients (\(P = 0.025\)).

Conclusions: The frequency of RET/PTC is 25.5% in Czech patients with PTC and this alteration is more prevalent in males. Minor allele of RET gene polymorphism A432A seems to be protective against RET/PTC development.

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PHENOTYPIC CHARACTERIZATION OF A RET PROTO-ONCOGENE MUTATION WITH DISTINCT DISTRIBUTION IN THE CENTRAL REGION OF PORTUGAL IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A

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Background and aims: Multiple endocrine neoplasia type 2 (MEN2) syndromes result from an autosomal dominantly inherited germline mutation in the RET proto-oncogene. Previous studies have shown a significantly increased frequency of Cys611Tyr mutations in the central region of Portugal in MEN2A families. The aim of this study was to further characterize the phenotypic presentation of this rare codon 611 mutation.

Methods: We evaluated the clinical phenotype of the members of five families carrying a mutation at RET codon 611 resulting in a cysteine for a tyrosine substitution, identified by PCR-DNA sequencing. Age of presentation of C cell hyperplasia and MTC; sex; penetrance of MTC; additional features of MEN2A and clinical evolution were analyzed.

Results: Fifteen mutation carriers were identified. Ten were female (64.67%). The mean age at MTC diagnosis was 45.75 ± 14.97 years (range: 18-62 years), with a mean calcitonin of 2034.8±4112.5pg/ml. A 63 year old carrier of the mutation was not submitted to prophylactic thyroidectomy due to the absence of any manifestation of MEN2A. Two other individuals were asymptomatic and had prophylactic thyroidectomy. The mean follow-up time for the remaining operated patients was 10.5 ±12.6 years. Two were lost to follow-up; two patients with persistent disease died of unknown cause, one died from metastatic disease (9%). Three patients (27.2%) remain in remission while three have persistent disease. Three cases had primary hyperparathyroidism (20%) and four (26.7%) presented with pheochromocytoma, one, prior to MTC.

Conclusions: RET mutation screening allowed early diagnosis of disease in six of nine carriers. Our clinical findings indicate that this mutation is associated with a relatively late disease onset and an intermediate aggressive course. The explanation for the lack of clinical disease in a 63 year old mutation carrier may lie in other genetic or epigenetic events triggering tumorigenesis.
ROLE OF WNT4 IN THYROID CANCER

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By using an inducible system of Ras-induced thyroid cells neoplastic transformation (FRTL-5/ERTM-Ras cells), we analyzed gene expression profile changes at early times after oncogene activation. We observed that Ras activation is able to rapidly reorganize gene expression, by inducing profound changes in the expression of dozens of genes in few hours after being activated. Among these, one of the most strongly repressed is wnt4. Wnt4 is a soluble cytokine that through its binding to cell surface receptors, is able to activate canonical beta catenin-dependent signalling as well as noncanonical pathways, depending on the cell and receptor context. Although its role in cancer has not been deeply investigated, wnt4 has been found either overexpressed or downregulated in different types of cancer. In our in vitro transformation system its repression is associated with the acquisition of the neoplastic phenotype. Ras in fact is able to repress its expression between one and six hours after its activation, and to chronically repress wnt4 expression in stably transformed cells. To study the role of repression of wnt4 in thyroid transformation, we generated FRTL-5/ERTM-Ras cell lines constitutively expressing wnt4 in order to ectopically maintain its expression when Ras abolishes the expression of the endogenous wnt4. Forced expression of wnt4 does not interfere with the ability of Ras in inducing loss of differentiation and TSH-independent cell proliferation. However, we observed that, in the presence of forced expression of wnt4, the ability of Ras to induce increased migration in FRTL-5 cells is severely impaired. Preliminary data obtained by measuring by real-time RT-PCR the expression of wnt4 in a panel of human thyroid tumors of different histotypes, show that wnt4 expression is significatively lower in cancer respect to the normal thyroid tissue. We are currently testing the possibility that wnt4 could interfere with xenografted thyroid tumor formation in mice.
IMMUNOHISTOCHEMICAL EXPRESSION OF CLAUDIN-1, 7 AND GALECTIN-3 IN PAPILLARY THYROID CARCINOMA: CORRELATION TO DIFFERENT HISTOLOGICAL PATTERNS AND AGGRESSIVE CLINICAL BEHAVIOUR

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Background: Claudins (CLDNs), integral membrane proteins, are the major components of tight junction. An abnormal expression of CLDN1 and 7 has been reported in several human cancer, including thyroid carcinoma. According to previous studies, CLDN1 is expressed at higher levels in papillary thyroid carcinoma (PTC) compared to other histotypes while CLDN7 gene expression is reported in both normal and neoplastic thyroid tissue. Galectin-3 (GAL3) is a well recognized marker of PTC and its overexpression may be associated with invasive and metastatic properties.

Aim: To evaluate CLDN1, 7 and GAL-3 protein expression in different PTC subtypes with aggressive course (persistent disease/locoregional or distant metastases) and to analyze the possible relationship with BRAFV600E mutation.

Material and methods: Ninety-four PTC [53 classic (PCL), 25 follicular (PFV), 11 tall cell variant (PTV), and 5 PTC with poorly differentiated (PD) areas] were selected for immunohistochemical analysis. BRAFV600E mutation was examined by SSPC-PCR followed by DNA sequencing in 58 samples.

Results: Most of PCL and PVF showed strong, diffuse and linear CLDN1 positivity at basolateral membrane surfaces. CLDN1 intensity staining was decreased in PTV and in PTC with PD areas (frequently weak, discontinuous or absent). CLDN7 immunoreactivity showed high heterogeneity both between and within different tumor subtypes. GAL-3 cytoplasmic immunostaining was found in 92.5% of PTC with variable intensity from weak to moderate or strong. Expression was slightly decreased in PVF including solid growth pattern areas. No relationship was detectable with BRAFV600E mutation.

Conclusions: Our study confirms that CLDN1, frequently up-regulated in PTC, may represent a novel marker for this tumor according to what is described for GAL3. Neither GAL3 nor CLDN7 expression was associated with different histological patterns. Decreased CDLN1 reactivity is associated with “aggressive” histological variants of PTC (p=0.003). This finding could suggest possible prognostic implications of this membrane protein in PTC.
We have recently demonstrated that patients with the familial form of papillary thyroid cancer (FPTC) have significantly shorter telomeres and increased expression and activity of telomerase (hTERT), both in cancer tissues and in the blood compared to sporadic PTC patients. The Shelterin complex is a family of six proteins that shapes and safeguards human telomeres by actively changing the architecture of telomeric DNA. In particular 3 shelterin subunits (Trf-1, Trf-2 and Pot1) directly recognize telomeric TTAGGG repeats, with POT1 proving a dual role on telomere stabilization: by allowing telomerase activity or by preventing telomerase-dependent telomere elongation.

**Objective and methods:** Aim of our study was to investigate, by semi-quantitative PCR, the expression of Trf-1 and Pot1 in the blood of 20 FPTC patients (5 males and 15 females, mean age at diagnosis 48.3 ± 3.6) already found to have short telomeres and high telomerase activity, and in 42 sporadic PTC (10 males and 32 females, mean age at diagnosis 43.9 ± 13.8).

**Results:** We found that POT1 expression was significantly lower (p< 0.0001, Mann Whitney test) in familial cases (OD POT1/GADPH: 0.6 ± 0.2) compared to sporadic cases (OD POT1/GADPH: 0.8 ± 0.01), while Trf-1 expression was not different (p=0.5) in the two groups. We observed that POT1 expression was inversely correlated with hTERT gene expression (R2=-0.14; p=0.002) and positively correlated with relative telomere length (R2=0.16; p =0.01).

**Conclusions:** Our results, although preliminary, suggest that the shelterin family (Pot1 in particular) may play some role in the abnormalities of telomere-telomerase complex previously described in our laboratory.
DIFFERENTIAL EXPRESSION OF THE HGF/C-MET LIGAND/RECEPTOR SIGNALING AND THREE DOWNSTREAM MOLECULES (STAT3, PI3K, RHO) IN BENIGN AND MALIGNANT THYROID NODULES

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Objective: Upon binding with the ligand HGF, c-met induces morphogenetic, scattering and growth effects in the cell by utilizing three distinct pathways. Specifically, morphogenetic effects are mediated by STAT3, while scattering and growth are mediated by PI3K and RHO, respectively. HGF, c-met and STAT3 are expressed with very high frequency in papillary thyroid carcinomas (PTC), suggesting a major functional role of HGF/c-met/STAT3 signaling in this histotype. We are unaware of studies that have simultaneously evaluated the expression of these five molecules in benign and malignant thyroid nodules. Such a study would be interesting because c-met was indicated as a target for novel drugs to medically treat DTC.

Methods: We have evaluated the immunohistochemical expression of c-met, HGF, STAT3, PI3K and RHO in an initial series of 42 thyroid lesions: 10 colloid nodules (CN), 10 follicular hyperplasia (FH), 7 follicular adenomas (FA), 4 oncocytic adenomas (OA), 7 PTC, 3 follicular carcinomas (FTC) and 1 anaplastic carcinomas (ATC).

Results: All PTC and two FA (29%) were HGF/c-met/STAT3/PI3K/RHO+ive. In contrast, all FTC and the remaining 71% of FA expressed only PI3K. Two CN (20%) and two FH (20%) were HGF/c-met/PI3K/RHO+ive, while two OA (50%) were HGF/c-met/PI3K+ive. The proportion of c-met+ive thyrocytes in PTC (26±13%) was 9- to 26-fold greater than in thyrocytes of the other c-met+ive nodules. The proportion of PI3K+ive thyrocytes in FTC (33±6%) was 2- to 16-fold greater than in thyrocytes of the other PI3K+ve nodules. Regardless of histotype, HGF, PI3K and RHO immunoreactions were both epithelial and stromal while c-met+ive and STAT3+ive immunoreactions were epithelial only.

Conclusions: Because of the postulated therapeutic implications, if our data will be confirmed, c-met for PTC and PI3K for FTC emerge as potential in vitro markers to test the effectiveness of novel drugs for DTC.
MUC1 expression does not predict differentiated thyroid carcinoma patients' outcome

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MUC1 has been related to bad outcome in a variety of epithelial malignancies including differentiated thyroid cancer (DTC). In order to evaluate MUC1 gene expression clinical utility, we used immunohistochemistry (IHC) to analyze MUC1 protein in 303 thyroid cancer patients including 259 papillary thyroid cancer (PTC): 162 classic (CPTC), 20 tall cell variant (TCV), and 77 follicular variant (FV); and 44 follicular thyroid carcinomas (FTC). The staining characteristics of the tumor were determined by the cytoplasmic localization of MUC1 and by a score, from 0 to 7, determined by the percentage of stained cells plus the intensity of this staining. We considered positive tumors staining above 5. In addition, we quantified MUC1 mRNA using Real Time-PCR in 75 out of the 259 PTC patients' tumor tissues. All patients were submitted to a similar management protocol and followed-up for 67±39 (median=73 months). The patients were classified according to clinical, laboratorial (Tg and TgAbs), and image data as: free-of-disease (201 cases); and bad outcome (elevated Tg, local and/or distant recurrence=35; death=13 cases). Only 2% of the FTC were positive whereas 39% of the PTC stained positive for MUC1 (p<0.0001). There was no relationship between MUC1 protein expression and any clinical or histological feature of aggressiveness. On the contrary, MUC1 protein expression was more frequently observed in classic (60.5%) than in more aggressive PTC histological variants (p<0.0001). Also, mRNA did not correlate to parameters of aggressiveness and was overexpressed in 41% of the patients without metastasis at the diagnosis but in only 8% of the patients with metastasis and (p=0.0041). There was no relationship between MUC1 protein and mRNA expression. We concluded that MUC1 gene expression is a poor parameter of DTC patients' prognostic.
Iodide uptake in thyrocytes by the sodium/iodide symporter (NIS) is an important step in thyroid hormone synthesis. NIS is also a marker gene for the differentiation of thyrocytes. Moreover, NIS protein function became the basis for the radiotherapy of thyroid cancer. However many undifferentiated thyroid cancers do not sufficiently express NIS. Therefore transcription of NIS mRNA and translation into NIS protein is of clinical interest and increasing the options of pharmacological treatment causing stronger NIS expression would be valuable.

In our study we determined the effect of increased TSH receptor signalling, transfection of thyroid transcription factors (PAX8 and TITF1), demethylating agents and histone deacetylation on NIS expression in follicular thyroid carcinoma cells (FTC133). Compared to primary thyrocytes NIS mRNA expression is low in FTC 133. However, a combination of several factors induces NIS mRNA up to 60-fold. But this strong induction is restricted to the mRNA level because we do not detect a concomitant increase of protein expression.

To find a possible explanation for the lack of protein induction we studied reporter constructs expressing luciferase under the control of the endogenous NIS near upstream promoter element or tandem repeated cAMP response elements. While such reporters are induced under conditions that also induce NIS mRNA, transferring sequences from the 3'UTR of the NIS gene to the reporter constructs strongly reduces luciferase activity in FTC133 compared to HEKgt cells. This suggests that NIS expression is in part under the control of unknown 3' regulatory elements that are the focus of further studies.
INHIBITION OF ARGINASE II MEDIATES APOPTOSIS IN FOLLICULAR THYROID CARCINOMA CELL LINE

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Background: We have previously shown increased expression of Arginase II (ARGII) in follicular thyroid carcinomas and papillary thyroid carcinomas compared to normal thyroid and benign lesions. Although ARGII expression is associated with a malignant phenotype, its role in the pathogenesis of thyroid tumors is still unknown. ARGII participates on the urea cycle competing with NOS (nitric oxide synthase) for L-Arginine.

Objective: The aim of the present study was to better understand the biological and biochemical role of ARGII on the pathogenesis of thyroid cancers.

Methods: Small interfering RNA-based strategy was used to knockdown ARGII in human follicular thyroid carcinoma cell line (WRO).

Results: Using quantitative PCR and Western blot analysis, we confirmed that three distinct regions of ARGII were effective on the knockdown of the targeted mRNA by 50-95% between 48 and 96 hours post-transfection. We observed that silencing ARGII reduced Ki67 expression concomitantly with increased apoptosis, reduced viability and ERK phosphorylation and modulated expression of genes associated with oxidative stress. Additionally, we found that the knock down of ARGII caused reduction of ARGII activity with simultaneous higher levels of NO (nitric oxide).

Conclusions: Considering the dual action of NO in either promoting or impairing programmed cell death, our results suggests that expression of ARGII may be involved in pathways tumor cells use to escape apoptosis.
Dysregulation of apoptosis with aberrantly extended cell viability is a hallmark of cancer, facilitating the insurgence of transforming mutations and resistance to therapy. Survivin is a recently described apoptosis inhibitor selectively over-expressed in most tumors and associated with aggressive disease, unfavourable prognosis and abbreviated survival. A potential role of survivin as prognosis predictor was investigated in differentiated thyroid cancer.

**Methods:** Survivin expression was investigated by immunohistochemistry of formalin fixed, paraffin-embedded tissue blocks. Statistical significance was determined using one-way analysis of variance.

**Results:** By immunohistochemistry, survivin was present in 30/30 cases of thyroid cancer studied. The mean level of survivin expression was 67% in T1 thyroid tissues (n= 16), while significantly increased (p< 0.05) up to 83% in T2-T3 samples (n=14). Moreover survivin was expressed at high level (range 80-100%) in metastatic lymph nodes.

**Conclusions:** Our preliminary data showed that survivin expression was significantly associated with advanced stages thyroid cancers suggesting that patients with low survivin expression could have better prognosis than patients with high survivin expression. Survivin over-expression are probably early events in tumorigenesis of thyroid papillary carcinoma but their full role in the process of tumor progression and their clinical value need further investigations.
Introduction: Angiogenesis plays an essential role in the embryonic and tumoral development. VEGF, one of the best known pro-angiogenic factors, is increased in thyroid cancers, especially in papillary carcinomas. However, other regulating mechanisms refine the answer to VEGF, as the NOTCH family ligands and receptors, in particular the ligand delta-like 4 (Dll-4). Dll-4 can play either anti-, or pro-tumoral role in the development of various tumours. Nevertheless, its role has not been yet investigated in the thyroid. The purpose of our study was to analyze its expression in some benign and malignant thyroid lesions.

Materials and methods: The expression of Dll-4 was analyzed by immunohistochemistry, qRT-PCR, and Western-blot (WB) in normal thyroids (NT), hyperplasic thyroids from patient with Graves' disease (GD), microcarcinomas, classical papillary carcinomas (PTC), and follicular variant of papillary carcinomas.

Results: The immunohistochemical expression of Dll-4 was highly variable in thyreocytes from NT and GD. In contrast, the staining of Dll-4 in tumours was homogeneous and often more intense, whatever the size and tumour type. Dll-4 negative thyreocytes were significantly more frequently observed in normal than neoplastic tissues. Only carcinomas contained significantly more Dll-4 strongly positive cells than NT; and PTC more than other carcinomas. This increased expression of Dll-4 in carcinomas compared with the neighbouring normal tissue was confirmed by qRT-PCR and WB. However, only capillary endothelial cells of GD were positive, the expression remaining restricted to large vessels in carcinomas and NT.

Conclusions: The detection of Dll-4 in thyreocytes and its regulation in various pathologies suggest that Dll-4 plays a role in the thyroid. Moreover, the variability in expression among GD and carcinomas suggests a different role for dll4 in benign and malignant angiogenesis.
P093
HYPERMETHYLATION OF P16/CDKN2, RIZ1, AND FHIT IN THYROID PAPILLARY CANCER
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Objectives: Inactivation of tumor suppressor genes (TSGs) by genetic and epigenetic pathways plays a significant role in human carcinogenesis. Transcriptional silencing of TSGs by methylation of CpG rich promoter regions has been reported in many kinds of human cancers. The aim of this study was to assess the frequency of promoter hypermethylation of p16/CDKN2, RIZ1, FHIT in thyroid papillary cancer.

Methods: We performed methylation-specific PCR using frozen tissue samples from 30 thyroid papillary cancers and 20 normal samples obtained from our department.

Results: DNA methylation of the p16/CDKN2 promoter was detected in 8 of 30 (26%) thyroid cancers. The respective values DNA methylation of the RIZ1 and FHIT promoter were 7 cancers (23%) and 6 cancers (20%), respectively. Hypermethylation of two or more gene is detectable in 33% of thyroid cancers. Methylation induced gene silencing appears to affect multiple genes in thyroid tissue and increases with cancer progression.

Conclusions: Our results support the notion that promoter methylation is an important mechanism of p16/CDKN2, RIZ1, FHIT gene inactivation and occurs frequently in thyroid papillary cancers. Moreover, p16/CDKN2, RIZ1, FHIT methylation represents a new molecular marker for targeted diagnostic and therapeutic approaches in thyroid cancers.
INTEGRIN (α6β4) REGULATION OF ANAPLASTIC THYROID CARCINOMA PROGRESSION IN VITRO AND IN VIVO

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Context: The α6β4 integrin, an epithelial-specific integrin, functions as a receptor for the members of the laminin family of extracellular matrix proteins. While the primary known function of integrin α6β4 is to contribute to tissue integrity through its ability to mediate the formation of hemidesmosomes (HD), there is growing evidence suggesting that this integrin also plays a pivotal role in functions associated with carcinoma progression in gastric, colonic and breast carcinoma. In aggressive and metastatic carcinoma cells, the host-tumor microenvironment induces the relocalization of integrin α6β4 from HD into F-actin in lamellipodia and filopodia, where this integrin becomes signaling competent by functionally interacting with other growth factor receptors and G-protein coupled receptors. We evaluated the role of integrin α6β4 in thyroid cancer.

Objective and method: We evaluated the expression pattern of integrin α6β4 in follicular thyroid cancer cell line (FTC236), papillary cancer cell line (NPA), and anaplastic cancer cell lines (FRO and ARO). Proliferation, migration and invasion assay were performed using FRO and ARO cells with or without treatment of shRNA to knock down integrin α6β4. Finally, we also examined in vivo tumor-forming ability after implantation into nude mice.

Results: Higher proliferative index and migration ability were associated with expression of integrin α6β4 in FRO and ARO cells. FRO cells transduced with shRNA to block integrin α6β4 grew slower than control FRO cells in vitro and in vivo.

Conclusions: We found that integrin α6β4 plays an important role in the aggressiveness of anaplastic thyroid cancer and integrin α6β4 could be a target for the treatment of anaplastic thyroid cancer.
The study of the molecular profile of papillary thyroid tumors (PTC) and the identification of new molecular markers is of relevance to improve the diagnosis and the prognosis of the tumor. Recently we demonstrated the existence of an important cooperation between TAZ (Transcriptional co-activator with PDZ-binding motif) and both transcription factors Pax8 and TTF-1 in the modulation of thyroid gene expression, suggesting a role of TAZ in the control of genes involved in thyroid development and differentiation.

In this study we analyzed the expression of TAZ/WWTR1 in papillary thyroid carcinoma and we demonstrated an important deregulation of its expression. Specifically, we evaluated the expression of TAZ mRNA in tissue specimens of papillary thyroid carcinoma by a quantitative RT-PCR assay and we demonstrated that the papillary thyroid carcinoma samples express much higher TAZ specific mRNA levels in comparison to normal thyroids. TAZ expression was also evaluated in normal and pathological human thyroids by immunohistochemical analysis and the increase of TAZ expression in papillary thyroid carcinoma was confirmed. Interestingly, in papillary thyroid neoplasia immunohistochemical staining was observed preferentially in the cytoplasm.

In addition, we used an inducible system consisting of FRTL-5 cells expressing a conditional RAS oncoprotein and we showed that the activation of the MAPK signaling pathway is involved in the deregulation of TAZ.

These observations suggest that the activated effectors of the RAS/RAF/MEK/ERK signaling pathway are involved in the increased expression of TAZ, supporting the idea that this may also occur in thyroid papillary carcinoma. In addition, the predominant cytoplasmatic localization of TAZ in PTC cells underlies the hypothesis that a loss of nuclear function might be the cause of the transformed phenotype. In conclusion, our data revealed that the transcriptional coactivator TAZ is likely to contribute to the development of well differentiated thyroid carcinomas.
DIPEPTIDYL PEPTIDASE IV AS MARKER IN THE SCREENING FOR DIFFERENTIATING AGENTS IN TRANSFORMED THYROCYTES

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Objectives: Differentiated thyroid carcinoma can be cured by surgery followed by radioiodine therapy remnant ablation. This treatment is very specific but primary or secondary resistance often decrease its efficacy and screening studies for re-differentiating agents to increase iodide uptake are ongoing. Dipeptidylpeptidase IV (DPP IV) is over-expressed selectively in differentiated thyroid carcinoma and aminopeptidase N (APN) is linked to progression in thyroid carcinoma. We hypothesize that differentiation can be assessed by the relation of the activity of these transmembrane proteases.

Methods: Proteolytic activities were measured by cleavage of specific synthetic peptides in normal porcine and human thyroid tissue, in cultured normal porcine thyrocytes and in human thyroid carcinoma cells lines (FTC 133 and 238) before and after treatment with retinol as re-differentiating agent. In parallel, 125I-iodide uptake was measured. Dipeptidylpeptidase II (DPP II), a lysosomal protease not linked to malignancy, was also studied.

Results: DPP II, DPP IV and APN activities were detected in tissues and cultures of normal porcine thyrocytes and in FTC 133 and 238 cells. In thyrocytes of normal human tissue only DPP II activity was detected. Upon treatment of FTC 238 cells with retinol DPP IV activity decreased significantly, APN activity remained constant and activity of DPP II slightly increased. In normal porcine thyrocytes no changes in the activities of these proteases upon retinol treatment occurred.

Conclusions: The difference in the proteolytic activity of DPP IV and APN between transformed human and normal porcine thyrocytes, which are often used as model for normal human cells, suggests a species- and cell specific expression of transmembrane proteases activity. Although changes were more pronounced in iodide uptake than DPP IV activity, our data suggest that the evaluation of DPP IV in conjunction with APN may serve as a marker for the assessment of re-differentiating action in transformed thyrocytes.
Effects of Enalapril in Orbital Fibroblasts from Patients with Graves' Ophthalmopathy

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Objectives: In spite of the efforts in the basic and clinical fields, treatment of Graves' Ophthalmopathy (GO) remains a difficult challenge because of the poor knowledge of its pathogenesis. Inhibition of fibroblast proliferation or glycosaminoglycan (GAG) production is a possible therapeutic approach. Several candidate drugs may exert a similar action, among which Enalapril was previously found to inhibit fibroblast proliferation in keloid scars. Enalapril is a common anti-hypertensive drug and it belongs to the family of angiotensin-converting enzyme (ACE) inhibitors. Here we investigated its effects in orbital fibroblasts.

Methods: Primary cultures of orbital fibroblasts from GO patients and control subjects were treated with enalapril or with control compounds (captopril and lisinopril) at various concentrations (0 to 5 mM) and for various time periods (3-5 days). The following parameters were tested:

i) Cell proliferation, measured using a commercial assay based on the determination of BrdU incorporation during DNA synthesis;
ii) Necrosis, determined using a commercial kit based on measurement of lactate dehydrogenase (LDH) release upon cell lysis;
iii) Apoptosis, tested using a commercial assay based on the determination of cytoplasmic histone-associated-DNA-fragments after induced cell death; and
iv) hyaluronic acid (HA) production, measured in cell media using a commercial kit.

Results: Enalapril reduced cell proliferation in orbital fibroblasts, regardless of whether cells had been taken from GO patients or from patients without GO. Reduction was due to a necrotic effect of the drug. Thus, LDH release increased in a dose dependant manner whereas apoptosis was not increased. HA release was markedly reduced by enalapril, regardless of whether fibroblasts had been taken from GO patients or from patients without GO.

Conclusions: Based on these findings, we concluded that enalapril inhibits proliferation and HA release in orbital fibroblasts, although its actions are not specific for GO fibroblasts.
PATIENTS WITH GRAVES' DISEASE EXPERIENCE IMPAIRMENT IN MAJOR AREAS OF QUALITY OF LIFE, REGARDLESS OF THYROID DYSFUNCTION. A MULTIVARIATE ANALYSIS INCLUDING PATIENTS WITH NON-TOXIC GOITRE AS CONTROLS

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Objective: To evaluate thyroid-specific quality of life in patients with Graves' hyperthyroidism, compared to patients with non-toxic goitre.

Methods: The newly developed ThyPRO questionnaire evaluating thyroid-specific quality of life multidimensionally in 13 scales was used to measure quality of life in 160 patients with Graves' hyperthyroidism and 258 patients with non-toxic goitre (control group) from two endocrine outpatient clinics. Patients were sampled cross-sectionally across the entire disease- and treatment span. Mean scale scores on the 13 scales were compared using SAS PROC TTEST. A multivariate comparison, controlling for sex, age, education and non-thyroid disease was performed using SAS PROC GLM. Effect of dysthyroidism was evaluated by including it as a covariate in the multivariate analysis.

Results: In univariate analyses, patients with Graves' hyperthyroidism had significantly worse QoL on hyperthyroid symptoms, cognitive complaints, emotional susceptibility, impaired social life, daily life and sex life scales in the univariate analysis. Graves' patients had better scores on the goitre symptoms scale. No differences were found on hypothyroid symptoms, eye symptoms, tiredness, anxiety, depressivity or cosmetic complaints. The same results were found in the multivariate analyses controlling for background variables, except regarding anxiety, where Graves' patients now had significantly worse scores. When including dysthyroidism as a covariate, the differences on the psychological scales (cognitive impairment, anxiety and emotional susceptibility) disappeared, whereas they persisted on the symptom and impairment (social, daily and sex-life) scales.

Conclusion: Patients with Graves' disease had reduced quality of life, when compared cross-sectionally to patients with non-toxic goitre. Not surprisingly, they experienced more hyperthyroid symptoms, worse mental health and greater impairment in important aspects of daily functioning. When controlling for dysthyroidism, the greater symptom burden as well as daily functioning impairment persisted, whereas the reduced mental health was no longer observed.
Optic neuropathy (ON) is a sight-threatening clinical manifestation of Graves' orbitopathy (GO) which generally requires urgent surgical therapy. High doses intravenous steroids (IVGC) have been used in an attempt to treat ON, but may not change the outcome of the disease. Aim of the present study was to evaluate retrospectively the effectiveness of therapy of ON with two regimens of IVGC. Twelve patients, 7 females and 5 males were studied. The diagnosis of ON was made based on the patient's visual acuity, visual field, color perception and saturation, visual evoked potentials and crowding of orbital apex at CT scan. Patients were treated with IVGC (5 with 500 mg and 7 with 1000 mg methylprednisolone, three weekly infusions for 2 weeks) and monitored. After steroid therapy, surgical orbital decompression was the outcome in 8 patients, 3 of 7 treated with 1000 mg IVGC and all 5 treated with 500 mg IVGC (mean±SD dose 687.5± 258.8 mg), whereas in 4 patients, all treated with 1000 mg IVGC, normal optic nerve function was permanently restored. There was a significant association between the mean administered dose of IVGC and ON outcome, the lower dose (500 mg) being always associated to orbital decompression (ANOVA p=0.04). While no significant association was found between the outcome and the cumulative dose (P=NS), the better outcome was significantly associated to a longer duration of GO (20.3±8.7 vs. 10.1±4.6 months, P=0.022). These results suggest that even when treated with high dose IVGC, ON progresses in about 2 thirds of patients who require urgent surgical decompression. Steroid therapy may be successful in restoring normal optic nerve function in about 30% of patients with GO duration > 15 -18 months, when treated with IVGC and a mean infusion dose of 1000 mg. A larger, multicenter study is necessary to confirm these data.
Most patients with GO suffer from hyperthyroidism, and many are treated with ATD for 1-2 years. In severe GO, ATD withdrawal often leads to relapse of hyperthyroidism with worsening of autoimmunity. **Objective:** A prospective follow-up of prolonged low dose ATD therapy of patients with severe GO and hyperthyroidism.

**Methods:** 158 consecutive patients with severe GO and hyperthyroidism were offered remission induction therapy (ATD+Glucocorticoids+Cyclosporine+orbital irradiation+cure from smoking). Many patients opted for prolonged ATD therapy to maintain stability.

**Results:** 88 patients were ATD treated for at least 3 years, with a mean duration of 88 months (median 80, range 36-180). One had relapse of hyperthyroidism on low dose medication after 30 months, whereas 87 were well controlled. 81 received Methimazole+L-T4 during the entire period, 7 had been changed from Methimazole to Propylthiouracil during initial therapy on suspicion of side effects. The mean dose of Methimazole at last evaluation was 6.6 mg/day and L-T4 was 110 microgram/day. During long term therapy most patients were controlled once a year. The 3 latest s-TSH values (264 samples) were within 0.2-4.5 mU/L in 92.8%, 4 were above (5.1 -6.7 mU/L), eight were 0.1-0.2 mU/L, and 7 were < 0.1. None showed overt thyroid dysfunction.

Mean TRAb was 13.7 U/l at admission and 1.7 U/L after 3 years. One Propylthiouracil treated patient developed ANCA positive vasculitis of the skin after 6 years. It disappeared after cessation of therapy.

**Conclusion:** Patients treated for severe GO and hyperthyroidism will remain in a stable euthyroid state during long-term therapy with a small dose of Methimazole + L-T4. Such therapy may be an attractive alternative to stop of medication after 1-2 years or to ablative therapy.
CENTRAL SEROUS CHORIORETINOPATHY AS SIDE EFFECT OF INTRAVENOUS PULSED METHYLPREDNISOLONE FOR DYSTHYROID OPTIC NEUROPATHY

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Context: Dysthyroid optic neuropathy (DON) is best treated with intravenous pulsed methylprednisolone (IVMP). Steroids have been implicated in the pathogenesis of Central serous chorioretinopathy (CSC) and might lead to a further reduction in visual acuity. CSC is defined clinically as a detachment of the sensory retina. There is no information regarding CSC in patients with DON in the literature.

Objective: To describe the concomitant occurrence of systemic cortico-steroid treatment and the development of CSC in a patient treated with IVMP for DON.

Material and methods: Clinical evaluation, computerized visual field, MRI, visual evoked potential test (EVP), measurements of serum non-organ-specific autoantibodies, serum anti-TSH-receptor antibodies and routine blood tests.

Case: The patient was a 47-year-old man who complains of loss of visual acuity (measured as 20/80 and 20/60 in left and right eyes, respectively) noted after 6 months of stop the treatment for graves disease. The patient presented reduced colour vision and swelling of the optic nerve together with enlargement of extraocular muscles at MRI and abnormal VEP test. The perimetry showed combined peri and paracentral scotomas. The Clinical Activity Score (CAS) was 2. Management was once-weekly pulses of intravenous glucocorticoids over a 12-week period, and then by oral prednisone treatment for 2 months (steroid cumulative dose of 9g). The patient was found to have steroid-induced CSC, and observation was the management choice without resolution. With fluorescein angiography, the diagnosis of CSC was confirmed. The prednisone dose was reduced. At the 10-month follow-up, visual acuity had gradually not returned to normal but stabilized at 20/60. All others parameters for DON have responded to steroid therapy.

Conclusion: This case demonstrates that steroids might be responsible for the occurrence of CSC mimicking refractory DON and we must be aware of this unusual complication of steroid therapy.
Objectives: To evaluate the possibilities of the orbital MSCT in diagnostics of the GO activity and severity.

Methods: 96 orbits with the GO symptoms were studied. The activity and severity of GO were defined with CAS scale and EUGOGO protocol, accordingly. The orbits were divided into the 3 groups by the severity: mild, moderately and very severe, and into the 2 groups by activity: active, inactive disease. The control group contained 16 orbits without eye pathologies. All patients were examined by the orbital MSCT in 3 projections.

Results: In active GO (CAS ≥3) the extraocular muscle (EOM) density varied from -40 to 0 (8,5%), 0 - 29 (40,3%), 30 to 50 (34%) to 64 HU (17%). Orbital fat tissue (OFCT) density varied from -118 to -4 HU. Optic nerve (ON) density from 3 to 76, lacrimal gland (LG) density from 0 to 88. In the control group EOM, OFCT, ON, LG densities varied from 16 to 50, from -110 to -92, from 16 to 57, from 15 to 56, accordingly. In non-active phase EOM density rose to 98 HU and amounted from -39 to 0 (11,5%), 0 to 29 (38,9%), from 30 to 50 (31,5%), to 98 (18%). OFCT, ON, LG densities varied from -154 to +38, from 4 to 91, from -10 to +92, accordingly. Among patients with CAS ≥3, the group (46 orbits) without signs of the activity rise, with EOM density more than 50, but less than 70, was found. GO severity correlated with the EOM thickness, proptosis, ON length, diameter of ON in 1 cm from the macula, relation ON length/diameter, LG width, OFCT minimal density, apical crowding.

Conclusions: The orbital MSCT data allow to improve the diagnostics not only of the severity, but also of the activity of GO.
Background and aims: Graves' disease is a common metabolic disorder that is associated with prominent cardiovascular manifestations, like marked reduction in peripheral vascular resistance and increased total blood volume and heart rate. Hyperthyroidism can consequently exacerbate preexisting cardiac disease or cause de novo cardiovascular abnormalities, such as atrial fibrillation and heart failure. Recent reports suggested a potential link between hyperthyroidism and pulmonary hypertension (PHT). But, the potential pathogenic mechanisms of hyperthyroidism-related PHT remain unclear. This study was performed to investigate the prevalence of PHT and the hemodynamic changes using echocardiographic measurements in untreated patients with Graves' disease and to determine the relation between PHT and thyroid autoantibodies.

Subjects and methods: We performed serial echocardiographic examinations in 69 patients with newly diagnosed or relapsing GD without or with less than 4 weeks of treatment with antithyroid drugs to estimate pulmonary artery systolic pressure (PASP), cardiac output (CO), total vascular resistance (TVR), and left ventricular filling pressure. Examinations were performed at baseline and 6 months after initiation of antithyroid treatment. Thyroid autoantibodies titer was measured in sera from the patients. Results were compared with 37 age- and sex-matched healthy controls. PHT was defined as PASP of at least 35 mmHg.

Results: 31 patients (45%) from 69 untreated patients with Graves' disease had PHT. The presence of systemic hypertension was associated with PHT. There was no relationship between PHT and thyroid autoantibodies in untreated patients with Graves' disease. Mitral regurgitation was more prevalent in Graves' patients with PHT.

Conclusion: In our study the prevalence of PHT using echocardiographic measurements was 45%. These data show that PHT is prevalent in patients with Graves' disease and thyrotoxicosis itself, not underlying autoimmune process, might contributes to the pathogenesis of PHT related to Graves' disease.
USE OF $^{131}$I IN PATIENTS WITH GRAVES’ DISEASE: EARLY CLINICAL EVALUATION

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In Graves’ disease radioiodine ($^{131}$I) is a safe and effective treatment for hyperthyroidism as alternative to thyroidectomy when medical therapy fails.

**Objectives:** Our objective was to evaluate thyroid function of hyperthyroid patients treated with $^{131}$I after 45 and 90 days.

**Methods:** 163 patients affected by Graves’ disease (GD) with hyperthyroidism, mild goiter and mild or absent ophthalmopathy received a fixed dose of 555 MBq of $^{131}$I. Before treatment 3$^{rd}$ and 24$^{th}$ hours radioiodine thyroid uptake (RAIU) was performed. Free thyroid hormones, TSH, AbTg, AbTPO, TRAb, urinary iodine and thyroid ultrasound were performed at baseline and 3 months after $^{131}$I. Serum FT4, FT3 and TSH was measured 45 days after treatment.

**Results:** Basal thyroid volume was 20 ± 9 ml and 3 months after $^{131}$I was reduced to 8.6 ± 6.3 ml. 45 days after treatment 57% patients were hypothyroid (34% overt hypothyroid); 22% were euthyroid and 21% still were hyperthyroid. Final evaluation, 90 days after treatment, showed 26% subjects euthyroid, 70% hypothyroid (57% of them were already treated with L-thyroxine therapy) and 11% still hyperthyroid. Basal thyroid volume was directly correlated with final thyroid function. Persistent hyperthyroidism was correlate with larger basal volume (p < 0.01) and with higher basal FT3 (p < 0.01). No correlation was found between final thyroid function and 24$^{th}$ RAIU. The serum levels of TRAb were increased compared with those before the $^{131}$I treatment in patients with persistent hyperthyroidism, but no significantly differences were observed with hypothyroid or euthyroid patients.

**Conclusions:** We suggest to use a fixed $^{131}$I dose to obtain early hypothyroidism in patients with Graves’ hyperthyroidism and mild or small goiter.
STANDARD TREATMENT WITH A SINGLE FIXED DOSE OF 10 MCI RADIOACTIVE IODINE THERAPY IN GRAVES' DISEASE

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Objective: To describe the outcome of a fixed dose of 10 mCi of radioactive iodine (RAI) therapy delivered to a population of Graves' Disease (GD) patients treated at a District Thyroid Clinic.

Methods: Retrospective analysis of the clinical and the laboratorial data over the last 8 years, and comparison with the literature.

Results: A total of 49 patients, 41 female an 8 male, with a mean age at diagnosis of 38.78 years, 38.5 and 40.14 respectively, were identified. Graves' ophthalmopathy (GO) affected 30.6% of cases. RAI therapy was administered at a mean age of 40.92 years, 39.95 for women and 45.5 for men. Pretreatment with antithyroid drugs was realized in 87.75% of cases, of which 67.4% with propylthiouracil. The cure rate reached 73.5% after a single dose of RAI, and definitive hypothyroidism emerged in 76% of patients in the first year. A second dose induced this same side effect in 75% in the same period. Pretreatment with propylthiouracil and male sex where more likely to predict treatment failure. Post-RAI treatment with glucocorticoids prevented progression in 80% of GO. None developed radiation thyroiditis.

Conclusion: A single dose of 10 mCi RAI appears to be safe, and could be more effective if metimazole was the antithyroid drug of choice for pretreatment. Higher doses should be considered in man and larger goiters. Hypothyroidism is generally an inevitable side effect that is easily managed with thyroid hormone substitution therapy.
IATROGENIC HYPOTHYROIDISM AND EXACERBATION OF ORBITOPATHY DURING THE TREATMENT OF GRAVES' HYPERTHYROIDISM

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Objectives: The precipitating factors of the exacerbation of orbitopathy during the treatment of Graves' hyperthyroidism was evaluated.

Materials and methods:
1) Clinical characteristics of 18 patients (M3:F15, aged 36-75) who showed exacerbation of orbitopathy during the treatment of Graves' hyperthyroidism were evaluated during 2004 - 2008.
2) The prevalence of iatrogenic hypothyroidism during the antithyroid drug treatment of Graves' hyperthyroidism was evaluated depending on the initial dosage of methylmercaptoimidazole (MMI) (15mg vs 30mg) during 1983 -2007.

Results:
1) After the initiation of MMI therapy, exacerbation of exophthalmos, palpebral edema and/or double vision was observed within one year in 8 patients (44%), after 1-10 years in 4 (22.2%) and after more than 10 years in 6 (33%). Two patients were after Radioactive Iodine Therapy and 3 patients after subtotal thyroidectomy. Nine or 50% of the patients were overtly and 2 or 11% were latently hypothyroid at the time of exacerbation of orbitopathy. Two or 11% were slightly thyrotoxic and five or 28% of the patients were euthyroid. TSH receptor antibody was strongly positive in 7 (>50IU/L or TSab>1000%) and apparent stress was experienced in 4 patients. Eight patients were heavy smokers.
2) Iatrogenic hypothyroidism was observed in 45 or 13.1% of 343 patients or in 26 or 3.3% of 796 patients initially treated with 30 or 15mg MMI, respectively.

Discussion & conclusion: Overtreatment iatrogenic hypothyroidism seems to be the precipitating factor of exacerbation of orbitopathy, rather than the relapse of Graves' hyperthyroidism. Even smaller initial dosage of anti-thyroid drug could cause iatrogenic hypothyroidism. Therefore, intensive control of thyroid function is necessary in order to avoid iatrogenic hypothyroidism, not only after Radioactive Iodine Therapy or thyroidectomy but also during antithyroid drug therapy.
Purpose: To investigate the effect of early thyroidectomy versus conventional antithyroid drug treatment (ATD) on the course of Graves’ orbitopathy (GO).

Patients and methods: Two GO patient cohorts were retrospectively evaluated. The patients either received an early thyroidectomy (Tx group) 6 months after the beginning of antithyroid drug (ATD) therapy or this therapy was continued for another 6 months (ATD group). Patients who stayed in remission 12-30 months after cessation of ATD were excluded. GO was evaluated (activity, severity, TSH-receptor-antibodies = TRAb, inactivation rate, antiinflammatory therapy) at baseline and after 6 months follow up.

Results: At baseline severity of GO was similar in both groups (median NOSPECS score 6 (Tx) versus 7 (ATD) [p=0.72]). GO was significantly more active (median CAS=6) in the early Tx group in comparison to the ATD group (median CAS=4) [0.02]. Median TRAb were higher in the Tx group (median 19.5 IU/l) than in the ATD group (median 9.3 IU/l) [p=0.06]. There were no differences concerning age, sex, smoking behaviour prior antiinflammatory therapy. After 6 months of follow up, patients of both groups had received equal amounts of steroid and radiotherapy. 100% of the patients had received steroids and 75%/72% orbital radiotherapy. Severity scores decreased in comparable amounts in both groups to 5 in the Tx group and to 6 in the ATD therapy group. However, GO was inactivated in 87.5% of the patients after early thyroidectomy in comparison to only 66.7% during further ATD therapy [p=0.1].

Conclusion: Residual GO symptoms are not different 6 months after early thyroidectomy in comparison to patients who received further ATD therapy. However the rate of inactivation of GO is higher after early thyroidectomy (although more active at baseline). This allows an earlier beginning of rehabilitative surgery for patients with early thyroidectomy, which may influence the quality of life.
Antithyroid drugs are usually prescribed for 12-18 months in Graves Disease (GD) to achieve long-term remission. However, chances of relapse are high. Surgery and radiiodine are effective therapies usually leading to iatrogenic hypothyroidism.

**Objectives:** To evaluate treatment options for GD, and investigate their relative efficacy and outcome prognostic indicators for antithyroid drug treatment.

**Methods:** This retrospective study included 162 patients (mean age= 38.8 years old, 89.5% female, 10.5% male) with newly diagnosed GD. The first treatment option, thyrotoxicosis severity, goitre volume, thyrotropin receptor antibodies (TRAb) titre, duration of antithyroid drug treatment, outcome and percentage of relapse were analysed.

**Results:** 8 (5%) patients were immediately referred to surgery (very large goitres and severe ophtalmopathy); 39 (24%) had radiiodine as first therapy option - 35 became hypothyroid, 4 needed further treatment; 115 (71%) were treated with antithyroid drugs - 38 (33%) reached euthyroid state (median treatment period of 16.9 months, median follow-up after drug withdrawal of 60 months) and we had 77 (67%) patients that failed; of these, 37 remained hyperthyroid after a median treatment period of 22.7 months whilst the other 40 had a relapse within a median period of 10.9 months after drug withdrawal. There were no significant differences in disease severity, TRAb titre and duration of treatment between the failure and the successful group to antithyroid drug therapy. Graves’ patients with no goitre or small goitre had a significantly greater remission rate than those with medium size or large goitre (p=0.0025). The former had higher rates of euthyroidism with antithyroid drug treatment than the latter.

**Conclusions:** Medical treatment is still the preferred first line therapy for GD. Despite a remission rate of 33% with antithyroid drugs it is difficult to predict which patients would respond adequately. Our data suggests that goitre volume is the only statistically significant prognostic indicator.
Introduction: Graves' disease is one of the most common thyroid diseases in areas of iodine deficiency. The circulating autoantibodies specific to the disease are directed against the TSH receptor (TSHRabs).

Objective: To evaluate the past history, type of treatment and outcomes of a population of patients with Graves' disease.


Results: 84% were female and 16% male; 24.3% had family history of thyroid disease. The age at diagnosis (mean±SD) was 38.5±13.6 years. The mean TSH levels were (mean±SD): 0.049 ±0.1832uUI/ml (N=0.4-4.0); 37 patients had levels below the detection limit of the laboratory. TSHRabs were (mean±SD) 51.4±61.08U/L (N<9) at diagnosis.

133 patients were initially treated with antithyroid drugs (AT); only one had corticotherapy and one radioactive iodine (RAI) as the first line therapy. 29.3% were treated with tiamaizol and 65.2% with propylthiouracil. There was one case of febrile neutropenia, three skin reactions, two cases of gastrointestinal intolerance and two toxic hepatitis.

59 patients were submitted to RAI therapy; nine of these had a 2nd dose treatment and one was submitted to surgery. Until RAI therapy, the average time of AT therapy was 3.5 years. After the RAI, two patients developed acute thyroiditis and other two showed deterioration of pre-existing ophthalmopathy; 32 became hypothyroid.

Tree patients underwent surgery after AT therapy. The four surgeries performed were all total thyroidectomies. There were three more cases of surgical treatment of Graves' disease reported in our department before 2000.

Conclusion: There is a tendency to use AT as first line therapy for Graves' disease, although 43.6% of these ultimately require treatment with RAI. Surgery is rarely used. The incidence of side effects is low, and the most serious occurred with AT.
P110
CLINICAL EFFICIENCY OF GRAVE'S DISEASE RADIOIODINE TREATMENT
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Aim: To assess the safety and efficacy of radioiodine treatment (RIT) of Grave's disease (GD).

Methods: We have analyzed the data of 90 patients with GD (77 women, 13 men; age 46.5[39;57] years old) received RIT. ATD was stopped 10 days before RIT. Radioiodine dose was calculated individually according to thyroid volume (TV) and uptake of 131I. Mean dose of I-131 was 9[6.5;14]mCi.

Results: Median of TV before RIT was 29.5[21.5;43.2]ml; medians of TSH 0.08[0.02;0.4] mlU/l and fT4 19.8[15;30] pmol/l (normal ranges 0.2 - 4.0 mlU/l for TSH and 11.5-23.0 pmol/l for fT4). In 6 weeks the medians of TV was 16.7[12.8;20.8] ml; TSH 0.13 mlU/l (p>0.05), fT4 16.6 pmol/l (p< 0.05) and 30 (33.3%) patients remained thyrotoxic, 49 (54.5%) became euthyroid, 11(12.2%)- hypothyroid. For patients with evident thyrotoxicosis were prescribed small doses of ATD. In 12 months TV significantly decreased to 7[4.9;7.3] ml, TSH increased to 0.8[0.2;2.8] mlU/l, fT4 decreased to 16.4[14.3;18.5] pmol/l (p< 0.05) and 49 (54.4%) patients were hypothyroid and needed of prescribing of L-T4, 28 (31.1%) were euthyroid without any medications and 14(15.5%) were thyrotoxic. The patients with remained thyrotoxicosis had a larger TV (35.4 ml v.s. 28.6 ml, p< 0.05), fT4 level (26.7 vs 18.6 pmol/l) and 131I uptake (86% vs 78%) in comparison with successfully treated patients. Serious adverse events of RIT were absent.

Conclusion: RIT is effective in most patients with Graves disease, especially with small goiter
Introduction: Greece is considered to be iodine-sufficient. There is a paucity of data whether the increased iodine needs in pregnancy are being met. Thiocyanate (SCN), from food and tobacco, and perchlorate (ClO₄⁻), from the environment, as NIS inhibitors, potentially decreasing thyroid hormone synthesis.

Aim: To assess iodine (I), SCN and ClO₄ status in pregnant women in Athens and determine whether SCN and ClO₄ effected thyroid function in this population.

Subjects-methods: We prospectively studied 55 healthy pregnant women aged 30±4 y. Serum thyroid function tests and urine I, SCN and ClO₄ were measured in each trimester.

<table>
<thead>
<tr>
<th></th>
<th>1st trimester (n=55)</th>
<th>2nd trimester (n=20)</th>
<th>3rd trimester (n=9)</th>
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<tr>
<td>TSH (µIU/mL)</td>
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<td>FT4 (ng/dL)</td>
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<td>3.96±0.48+</td>
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<tr>
<td>I- mcg/L</td>
<td>106.4±60.7</td>
<td>107.9±53.5</td>
<td>111.0±32.1</td>
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<tr>
<td>ClO₄⁻ (mcg/L)</td>
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<td>2.3/3.1/4.2**</td>
<td>3.6/4.6/5.9</td>
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<tr>
<td>SCN (mcg/L)</td>
<td>429.4±297.1</td>
<td>476.4±262.8</td>
<td>384.9±229.9</td>
</tr>
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</table>

[Results]

*p< 0.05 between 2nd or 3rd trimester vs 1st trimester
+p< 0.05 among all trimesters
**p=0.05 between 1st or 3rd trimester vs 2nd trimester

Iodine deficiency (< 100mcg/L) occurred in 56%, 45% and 33% during the trimesters. A weak positive correlation was found between TSH and SCN levels (r=0.25, p< 0.05). No effect of ClO₄⁻ on thyroid function was observed.

Conclusion: A striking proportion of pregnant women residing in Athens were iodine-deficient. No major differences in I-, ClO₄⁻ or SCN over the three trimesters were noted. There was a weak positive correlation between TSH and SCN. However, no effect of ClO₄⁻ on thyroid function was observed.

These latter findings are similar to our recent studies in pregnant women residing in Italy, Wales, Argentina and the United States.
Introduction: Iodine is essential for the synthesis of thyroid hormones and its intake modulates the physiology and physiopathology of thyroid gland. In Portugal, endemic goiter has been practically eradicated. However, some data indicate that iodine intake, as in other European areas, is not sufficient. Taking into account the potential harmful effects of moderate iodine deficiency during pregnancy, when needs are increased, and the absence of recent data on iodine intake in Portugal, a countrywide study on urine iodine was undertaken in pregnant women and school children. The results of this study in pregnant women are presented.

Material and methods: Target Population-Pregnant women from maternity hospitals and school children, from strategic geographical areas (coast line and inland) and from the portuguese islands of Açores and Madeira; 3586 urines from 17 maternity hospitals were analysed.

Urinary iodide - A fast colorimetric method (Gnat et al, Clin Chem 49:1,186-188, 2003) is being used

Statistical methods - Central methods and proportional comparison tests

Global results: Median urinary iodide concentration was 82.9µg/L, being 25.3% below 50µg/L. 15.8% had values above 150µg/L

Results by hospital: Median urinary iodine varied from 49 to 124µg/L; 13.9% to 51.2% of women had values below 50µg/L and 1.2 to 34% had values above 150 µg/L.

Conclusions: Our results point out to an inadequate iodine intake in pregnant women assisted in most portuguese maternity hospitals. More detailed analysis is warranted in order to explain the observed differences between regions. Considering the potential deleterious effects of inadequate iodine supply during pregnancy, iodine supplementation is recommended in this period of life.
IS THE IMPACT OF CONTROLLED OVARIAN HYPERSTIMULATION (COH) ON THYROID FUNCTION DIFFERENT IN WOMEN WITH - AND WITHOUT THE HYPERSTIMULATION SYNDROME?

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Context: COH has an important impact on thyroid function, which is more pronounced in women with concomitant thyroid autoimmunity (TAI). The hyperstimulation syndrome (OHSS) is a complication of COH and leads to higher estradiol (E2) levels than those in COH.

Objective: To evaluate whether thyroid function in infertile women without TAI, developing OHSS was different compared to that in women with COH and whether this had an impact on the pregnancy outcome.

Patients and methods: Prospective study with determination of thyroid function (TSH, FT4) before, 2, 4 and 6 weeks after COH. Seventy seven women underwent COH of whom 25 developed OHSS. Women with TAI (i.e the presence of TPO - or Tg-abs) before COH were excluded from the study. The diagnosis of OHSS was based on clinical, biological and echographical criteria.

Results: Serum TSH and FT4 significantly increased 2 weeks after COH in both groups compared to the prestimulation levels. In the COH group: TSH 2.1 ± 1.1 vs. 2.6 ± 1.9 mU/L; p=0.009 and fT4 13.1 ± 1.7 vs. 13.8 ± 1.6 ng/L; p< 0.001. In the OHSS group: TSH 1.9 ± 0.8 vs. 3.1 ± 1.9 mU/L; p< 0.001 and fT4 12.3 ± 13.4 ng/L; p=0.001. Nor the increment in TSH and FT4, nor the AUC over the follow-up period were different between both groups. At 2 weeks of pregnancy, there was a significant positive correlation between TSH and E2 (p=0.018) and a negative between fT4 and E2 (p=0.016) in both groups. The pregnancy outcome was comparable between both groups (live birth rate of 77% in the COH and 93% in the OHSS group).

Conclusions: In women without TAI, OHSS has a significant impact on thyroid function, that is comparable to that in women with COH and did not alter the pregnancy outcome.
THYROID FUNCTION IN THE THIRD TRIMESTER OF PREGNANCY AND AFTER DELIVERY IN AN IODINE-SUFFICIENT REPUBLIC OF SLOVENIA

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Objectives: Studies measuring both free thyroid hormones during pregnancy and after delivery in iodine-sufficient areas are scarce. Our aim was to determine thyroid function during pregnancy and after delivery with regard to urinary iodine concentration in an iodine-sufficient area.

Methods: We followed 127 women with the mean age 30.9 ± 4.1 years in the third trimester of pregnancy (mean 31.6 ± 1.7 weeks) and after delivery (mean 15.9 ± 3.9 weeks). Concentrations of TSH, free T4 (fT4), free T3 (fT3) and urinary iodine (UIC) were measured on both occasions. All women were negative for thyroid autoantibodies, as established in the first trimester of pregnancy.

Results: TSH concentration during pregnancy (mean 1.78 ± 0.85 mU/L) was significantly higher (p<0.001) than after delivery (mean 1.48 ± 0.69 mU/L). On the contrary, concentration of fT4 (mean 11.26 ± 1.65 pmol/L) was significantly lower (p<0.001) during pregnancy than after delivery (mean 13.6 ± 2.11 pmol/L). Similarly, concentration of fT3 (mean 3.83 ± 0.32 pmol/L) was significantly lower (p<0.001) during pregnancy than after delivery (mean 4.57 ± 0.47 pmol/L). UIC during pregnancy (median 171 microg/g creatinine) did not differ significantly from the value after delivery (median 146.5 microg/g creatinine, p=0.095). We found no correlation between TSH concentration and UIC during pregnancy (r=0.023, p=0.803) and after delivery (r=-0.009, p=0.921). Similarly, no correlation between fT4 and UIC during pregnancy (r=-0.044, p=0.634) and after delivery (r=-0.163, p=0.074), and between fT3 and UIC during pregnancy (r=-0.067, p=0.466) and after delivery (r=0.081, p=0.377) was found.

Conclusions: In an iodine-sufficient area, the observed significant decrease of both free thyroid hormones in the third trimester of pregnancy is most likely due to reasons that are not related to iodine supply.
INCREASED LEVOTHYROXINE DOSAGE DURING PREGNANCY IN WOMEN WITH CENTRAL HYPOTHYROIDISM

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Objective: Evaluate levothyroxine substitution in pregnant women with central hypothyroidism.

Design: Multicenter, descriptive clinical study

Patients: 13 women, mean age 30.4 (27 - 41) years and mean BMI 25.7 (21-31) kg/m² presented partial or complete hypopituitarism with central hypothyroidism due to congenital defect (n = 1), empty sella syndrome (n = 1), Sheehan's disease (n = 3), post-partum hypophysitis (n = 1), post surgical treatment of craniopharyngioma (n = 2), post surgical or post-radiotherapy treatment of secreting (n = 4) or non secreting (n = 1) pituitary adenoma. In 8 women levothyroxine dose was increased upon confirmation of conception whereas in others the dose was adjusted to free T4 monitoring.

Results: Among the 19 pregnancies observed in those 13 women with central hypothyroidism, 5 miscarried and the others gave birth to healthy babies (one twin pregnancy). In women with central hypothyroidism, mean levothyroxine dose was 93 µg/day and 100 µg/day before pregnancy and in post partum period, respectively. On average the entire group had an increase in levothyroxine dosage of 17.7%, 14.9% and 11.7% during the first, second and third trimester, respectively. The mean free T4 level was 19 pmol/l before pregnancy. Despite the increased levothyroxine dosage (+51%), free T4 values were low or in the 1/3 inferior of the normal range in 2/9 (22%), 10/12 (83%) and 9/12 (75%) during the first, second and third trimester of pregnancy, respectively, whereas the objective was to obtain free T4 in the upper third of normal range.

Conclusion: In these patients with central hypothyroidism, we demonstrated that a significant increase of levothyroxine dosage was not sufficient to maintain the objective. Therefore, vigilant and monthly monitoring of free T4 concentration during pregnancy is mandatory in order to optimize the levothyroxine treatment in women with central hypothyroidism.
SOME ASPECTS OF THYROID SCREENING IN PREGNANCY

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Introduction: Questions regarding thyroid disease and pregnancy have been discussed in recent years. The determination of the reference intervals for TSH in the first trimester of pregnancy, and the cut-off for anti-TPO is necessary for the conditions of iodine sufficient region of the Czech Republic.

Methods: In the study group of non-selected 5,520 pregnant women (PW) in their 9th-11th week of pregnancy the TSH, TPOAb and FT4 were assayed (ADVIA® Centaur™).

Results: We selected an acceptable reference interval for TSH in PW such as the 2.5th percentile and 97.5th percentile (0.06-3.67 mIU/l). Out of all PW, 2.93% had a lower level and 4.48% had TSH over the reference interval. The limit for endocrinological examination was calculated to be 143 kIU/l TPOAb or TSH out of the reference interval. Based on this, 11.2% of PW were TPOAb-positive. In the group with TSH>3.67 mU/l were 44.1% of women TPOAb-positive compared to 10.1% in the group with TSH<0.06 mU/l and 9.1% in the group with TSH in normal reference interval (9.1%). The negative correlation between TSH and hCG in PW with suppressed TSH was significant. The level of TPOAb in (subclinical) hypothyroidism was higher (P<0.050) as compared to TSH suppression or TSH in normal reference interval. The average age of all PW was 31.3 (±4.6). No statistically significant differences in the average age between groups with different TSH and/or TPOAb were found.

Conclusions: The prevalence of thyroid dysfunction in Czech PW is 4-5%, the TPOAb are present in 11-12%. Systematic cooperation of all specialists involved in the care of PW should be established in order to solve laboratory normal intervals, pregnancy timing for examination, unification of diagnostic procedures, and the correct interpretation of the results.

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GENERAL SCREENING FOR THYROID DYSFUNCTION IN PREGNANCY IS NECESSARY UNDER CONDITIONS OF CZECH REPUBLIC

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Objectives: Thyroid dysfunction in pregnancy compromises maternal and fetal health. Guidelines for management of thyroid dysfunction during pregnancy and postpartum (Abalowich et al. 2007) recommend not universal but only case finding screening. The aim of the study was to assess the value of this recommendation.

Method: The data from 5200 pregnant women with sufficient iodine intake in central Czech Republic screened for thyroid function were available. Women were supplemented by 100-150 µg of iodide daily. The blood for TSH (thyroid stimulating hormone), TPOAb (antibodies to thyroid peroxidase) and FT4 (free thyroxine) estimation was collected in 9-11 week of pregnancy. Physical examination, family and personal history and laboratory data were analysed in 318 from 822 positively screened women (TSH>3.67 mIU/l and/or positive TPOAb), and thyroid ultrasound (TUS) was realised.

Results: Positively screened women were informed and half of them visited the endocrinologist within 1-4 weeks, the rest between 8-20 weeks. Subclinical hypothyroidism was diagnosed in 32.7%, overt hypothyroidism in 3.7% and hyperthyroidism in 2.1% of 318 women. There was no difference in TPOAb titres between women with positive and negative TUS. All women with elevated TSH were asymptomatic. Family and/or personal history of thyroid diseases and/or autoimmune diseases were present in less than 20%. Serum concentrations of FT4 were lower in TPOAb-positive as compared to TPOAb-negative women (medians: 13.79 vs. 15.18 pmol/l, P< 0.001) and differences of FT4 in euthyroid women with suppressed, normal and elevated TSH (medians: 17.89 vs. 13.98 vs. 12.91 pmol/l, P< 0.05) were found.

Conclusions: In Czech Republic, case finding screening is able to disclose less than 20% of asymptomatic mild or deep hypothyroidism or women with positive TPOAb in pregnancy. Investigation of combination of TSH and TPOAb is necessary. Effectiveness of general screening is tested in pilot project of Health care system in Czech Republic.
Objective: To evaluate the thyroid function in women with a history of preeclampsia and/or HELLP syndrome at least two years after delivery.

Design: Observational study.

Setting: University Medical Center Groningen, the Netherlands.

Population: Women with a history of preeclampsia and/or HELLP syndrome (n=310) or an uncomplicated pregnancy (n=363), between January 1990 and February 2003.

Methods: The measurement of Thyroid Stimulating Hormone (TSH) and antibodies to thyroid peroxidase and the use of a questionnaire about relevant history, history and family history on autoimmune diseases related to thyroid disease. Main outcome measures Prevalence of thyroid dysfunction and antibodies to thyroid peroxidase.

Results: Mean serum TSH values were not significantly different between the preeclampsia group and the control group (1.64 vs 1.81 mU/l). The percentage of women who have (had) hypothyroidism and hyperthyroidism respectively did not differ significantly between the preeclampsia and the control group (3.3% vs 6.1% and 10.0% vs 7.7%). Furthermore the prevalence of antibodies to thyroid peroxidase was not significantly different (6.1% vs 7.7%).

Conclusion: Preeclampsia and/or HELLP syndrome are not associated with an increased risk of thyroid dysfunction in later life.
ASSESSMENT OF THE THYROID HORMONE’S PROFILE DURING PREGNANCY
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Introduction: In accordance with a rising number of pregnant women with thyroid gland dysfunctions, The Thyroid Gland’s Disorders Outpatient Clinic for Pregnant Women has started its activity in our Endocrinology Department in January 2008. Patients with or without thyroid gland dysfunction in history have been under medical care.

During pregnancy thyroid gland is prone to the number of physiological changes, which cause difficulties in the interpretations of thyroid hormones results. Changes in estrogens level and TBG concentration, activity of hCG make TSH level inadequate. Transitory increase of FT4 and FT3 levels in first trimester is observed. However, in second and third trimester mainly FT4 level could be slightly decreased. Increased aTPO level detected during pregnancy has been shown to be associated with 2-4x higher risk of miscarriage.

Aims:
1. Attempt of establishing the referential norms of the FT3, FT4 in each trimester.
2. Establishing the referential norms of TSH in each trimester.
3. Morphological changes in thyroid gland in usg examination during pregnancy
4. Evaluation of the aTPO/hTrab level for post partum thyroid disease risk assessment

Method: Assay of the FT3, FT4, TSH level and aTPO/ hTrab in blood serum (ECL) during 1st, 2nd and 3rd trimester of pregnancy, thyroid USG.

Results: 253 pregnant women were examined from January 2008 till present day. Mean value of TSH for healthy women was as follows: 0,85 uIU/ml (± 0,61), 1,2 uIu/ml(±0,58), 1,32uIU/ml (±0,52) in 1st 2nd, 3rd trimester respectively. Abnormal result of aTPO have 53 patients (20,93%).

Conclusion: Establishment of reference values for each trimester is fundamental for correct assessment of thyroid function in pregnancy. About ¼ patients have increased result of aTPO and required further observation. Our researches need to be continued.
Introduction: Nodular thyroid disease often occurs in female subjects and is frequently first detected during pregnancy. Several factors contribute to this situation, namely the stimulation by b-HCG in the first trimester and the frequent contact with the medical team, which facilitates previously undetected pathology. Many authors postulate a medical approach that is similar to the nonpregnant women, and in particular the performance of fine needle aspiration biopsy (FNA) if the nodule is larger than 1 cm.

Case description: We report here about a 28 year old woman, with previous multinodular goiter, medicated with l-thyroxin 0.1 mg id. A dominant right thyroid nodule found 3 years before the patient was observed led to FNA which yielded a diagnosis of colloid. After the 21st week of pregnancy she repeated ultrasound guided FNA which led to the detection of a right nodular structure of 2.6 cm and a left 1.2 cm nodule in the inferior left pole, hypoecogenic and less well defined. Both nodules were punctured and analysis yielded papillar cancer of the 1.2 cm nodule and colloid in the 3 cm nodule. The patient decided to postpone the surgical intervention to the postpartum period. A clinical scenario of increased anxiety ensued. The patient was submitted to total thyroidectomy 8 weeks after delivery. The outcome of pathological analysis was nodular hyperplasia with adenomatous transformation.

Conclusion: Diagnosis of tumoral pathology during pregnancy is often experienced with great anxiety. Postponing FNA to the postpartum period in the case of nodules without suspicious ecographic characteristics is an option we do favour, given the experience in the follow up of pregnant women with nodular disease of the thyroid.
EVALUATION OF THYROID FUNCTION AND ANTROPOMETRIC PARAMETERS IN NEWBORNS FROM MOTHERS AFFECTED BY THYROID DISEASES

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Thyroid function is essential for the physiological development of newborns, both from a somatic and a neuropsychical point of view.

Aims of this study was to verify the role of maternal thyroid diseases and of L-tiroxine treatment on antropometric parameters and thyroid function of newborns, and to evaluate the correlations between neonatal thyroid function at the third day of extrauterine life and antropometric parameters of newborns.

Our results showed that neonatal thyroid function and antropometric parameters were not different between newborns from mother affected by different thyroid diseases (nodular goiter or chronic autoimmune thyroiditis), nor between newborns from mother treated with L-tiroxine during pregnancy and the ones from non-treated women. No correlation between neonatal serum concentration of autoantibodies (antithyroglobuline and anti-thyroperoxidase) and neonatal thyroid function was demonstrated.

Analysing all the newborns as a unique group, we demonstrated a direct significant correlation between gestational age and serum concentrations free thyroid hormones (FT4 and FT3) at the third day of extrauterine life. More, serum concentrations of FT4 and FT3 were significantly higher in newborns from a vaginal delivery in comparison with newborns from a cesarean delivery, also after correction for gestational age.

In conclusion, when maternal thyroid function is normal, both the presence of an autoimmune thyroiditis and L-tiroxine treatment do not interfere with neonatal antropometric parameters and thyroid function at the third day of extrauterine life. Serum concentrations of FT4 and FT3 at the third day of extrauterine life depend both on gestational age and on the mode of delivery.
Objective: According to the current protocol of systematic calcium replacement in immediate post-operative patients who underwent a total thyroidectomy, which allows us to give early discharge to the patients, a prospective study was carried out aiming at the determination of ionised calcium and intact parathormone (PTH) in pre-operatory and post-operatory phases until the normalisation of values. Thus we have tried to determine whether PTH values can be used to predict the calcium values of these patients.

Methods: Fifty patients who had undergone a total thyroidectomy (regardless the cause of such surgery) have been evaluated. Patients submitted to surgery because of an existing neoplasmy have also undergone anterior (or central) neck dissection. Each of the patient's PTH and ionised calcium values have been determined in the immediate pre-operatory phase so as to determine a basal value, having this procedure been repeated 12, 24, 72 hours after the surgery, and every 3 days until the normalisation of the values.

Results: With the current calcium replacement protocol in the Unit, none of the patients in this study needed to be hospitalised for more than 24 hours. We found a late transitory symptomatic hypocalcaemia which appeared 4 days after the surgery. We are able to establish a straight relation between the intact PTH values and the hypocalcaemia seriousness, so as to say that if the PHT values have been considered to be normal within the following 12 hours after the patient's admission, then the patient can safely be discharged.

Conclusions: A calcium replacement scheme and the determination of intact PHT values in the first hours after the surgery can allow the patient to undergo a total thyroidectomy in ambulatory surgery or with a hospitalisation period of less than 24 hours, in both cases ensuring patient safety.
PROSPECTIVE RANDOMIZED STUDY OF THYROID SURGERY USING THE HARMONIC SCALPEL

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Background-objective: Although the harmonic scalpel has been shown to be safe and effective in thyroidectomies, several surgeons consider the previously available instruments to be large and cumbersome, especially in terms of dissection capabilities. This has led to an innovative technical improvement of the device for thyroid surgery which has very recently been implemented and has been made available in 2008. Utilization of this new device, however, has not been evaluated in any study. We hypothesized that this instrument may result in further operative time reduction due to its greater tissue grasping and dissection capability. The objective of this study was to compare the results of thyroid surgery using the new harmonic scalpel (FOCUS) to that with the previously available device (HARMONIC ACE).

Methods: This is a prospective randomized study of all patients undergoing thyroid surgery in our endocrine surgery department between January and August 2008. Cases submitted to concurrent surgical procedures, such as parathyroidectomy or cervical lymph node dissection, were excluded. Patients (n=130) were randomized into those undergoing thyroid surgery with FOCUS (group A, n=65) and those with HARMONIC ACE (group B, n=65).

Results: No significant differences were identified between the two groups in terms of demographics, hyperthyroidism, type of thyroidectomy (total, near-total, subtotal or hemithyroidectomy), reoperative thyroid surgery, thyroid gland weight and volume, pathologic diagnosis, preoperative and postoperative calcium and parathyroid hormone levels, complications, hospital stay, and final outcome. On the contrary, mean operative time was significantly shorter in group A than group B (61.3±5.6 min vs 75.6±7.4 min, p=0.007).

Conclusion: The new harmonic scalpel device is a very useful adjunct to the thyroid surgeon's armamentarium. It is safe, effective, hand-friendly and offers great tissue delicate grasping and dissection capabilities. Utilization of this device significantly reduced operative time compared to the previously available instrument.
APPLICATION OF 2-OCTYLICYANOACRYLATE TISSUE ADHESIVE IN TOTAL THYROIDECTOMIES WITH A MODIFIED TECHNIQUE COMPARED TO SUBCUTICULAR SUTURING: A PROSPECTIVE RANDOMIZED STUDY

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Background-objective: 2-octylcyanoacrylate is a safe and effective skin closure modality. A completely dry wound is, however, required for its correct application. The aim of this prospective randomized study was to evaluate a modified, two-step 2-octylcyanoacrylate application technique completed on the first postoperative day compared to subcuticular wound closure.

Methods: Patients submitted to total thyroidectomy were randomized into those with cervicotomy closure with subcuticular suturing in the operating theatre (group A, n=30) and those with 2-octylcyanoacrylate application (group B, n=30). Exclusion criteria were previous irradiation to the neck, corticosteroid consumption within a year before surgery, previous neck incisions, diabetes mellitus, bleeding diathesis, personal history of keloid formation or scar hypertrophy, and allergies to 2-octylcyanoacrylate or suture material. In group B, the skin incision was closed in the operating theatre with 3 interrupted nonabsorbable sutures and 2-octylcyanoacrylate was applied the next morning after sutures removal. Mobility of the neck (neck stiffness) was assessed by all patients using a linear 1-10 visual analogue scale (VAS) score at 48 hours and 2 weeks after surgery. At 12 weeks postoperatively, cosmetic appearance was evaluated using a VAS score by the patients as well as 2 independent blinded physicians.

Results: The two groups of patients were homogenous in terms of age, gender, thyroid gland specimen weight, volume and pathologic diagnosis, complications and length of stay. Comparison between the two groups revealed no statistically significant differences in terms of VAS scores of neck stiffness and cosmetic result. No wound complications occurred in both groups.

Conclusion: The modified, two-step application technique of 2-octylcyanoacrylate in cervicotomy has comparably excellent results with standard subcuticular suturing while the surgeon benefits from the advantage of a dry wound on the first postoperative day.
MINIMALLY INVASIVE VIDEO-ASSISTED THYROIDECTOMY (MIVAT) - PRELIMINARY EXPERIENCE

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Introduction: The minimally invasive videoassisted thyroidectomy without gas insufflation (MIVAT) is a technique that combines the benefits of open and endoscopic surgery. The potential advantages are better recognition of the anatomic entities during dissection, improved hemostasis, reduction of procedure-related adverse events and post-operative pain with better cosmetic results.

Aim: Report the technique and preliminary results of MIVAT performed in an Endocrine Surgery Unit.

Methods: Selection criteria were: thyroid volume < 20ml, nodules not exceeding 3cm, absence of thyroiditis, previous neck surgery or previous irradiation.

The procedure is carried out with the patient in a supine position, without hyperextension of the neck. A 1.5 to 3cm horizontal incision is made 2cm above the sternal notch; a video-assisted dissection is performed using a 30 degree 5mm endoscope tube, ultrasonic scalpel, spatula-shaped aspirator and conventional material.

Results: The surgical indication of the 25 patients (24♀ / 1♂) were: follicular tumor (14), nodular goiter (8) and papillary carcinoma (3). The operation consisted of a lobectomy in 17 patients and a total thyroidectomy in 8 patients. Conversion to open surgery was not necessary. 4 transient recurrent nerve palsy and 1 perforation of the trachea (solved conservatively) were the complications observed.

Conclusions: MIVAT is a recent technique among us that requires a learning curve. It should be considered a valid option in selected surgical centers and selected group of patients. The small number of cases of our series cannot be inferred with statistical significance, conclusions regarding the outcome of patients.

Bibliography:


PREOPERATIVE VITAMIN D LEVELS AND POSTOPERATIVE HYPOCALCEMIA IN TOTAL THYROIDECTOMY

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Introduction and aim: Hypocalcemia is a common complication after thyroid surgery (1,6-83%). In literature, low preoperative 25-hydroxyvitamin D levels are suggested to be predictive for this postoperative event, however this finding was not confirmed in all studies. The aim of this study was to investigate the possible correlation between low preoperative vitamin D levels and postoperative hypocalcemia.

Material and methods: A retrospective analysis was performed in the files of 148 patients who underwent total thyroidectomy between January 2007 and December 2008. The preoperative 25-hydroxyvitamin D level was measured in 50 patients. The lowest calcium level during postoperative hospital stay was taken into account. Univariate regression analysis was chosen for statistical evaluation.

Results: Postoperative hypocalcemia, defined by a total calcium level less than 4mEq/l, occurred in 57 patients (38,5%). Of these, nineteen patients (12,8%) required oral vitamin D (1), oral calcium (7) or a combination of both (11). Persistent hypocalcemia more than a year was found in two patients. No statistically significant correlation between low preoperative vitamin D levels and low postoperative calcium levels was found (p=0,9168).

Conclusions: In this small study, no trend towards a predictive value of preoperative vit D levels for postoperative hypocalcemia was found.
GRANISETRON VS TROPISETRON IN THE PREVENTION OF POSTOPERATIVE NAUSEA AND VOMITING AFTER TOTAL THYROIDECTOMY

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Background-objective: Postoperative nausea and vomiting (PONV) are frequently encountered after thyroidectomy. We report on the efficiency of the serotonin receptor antagonists, particularly 5-hydroxytryptamine 3 (5-HT3) receptor antagonists, granisetron and tropisetron, on the prevention of PONV in patients undergoing total thyroidectomy.

Methods: This is a prospective randomized study of all patients scheduled for total thyroidectomy in our university endocrine surgery department from January 2006 until January 2007. Patients (n=127) were randomized to receive intravenously, prior to induction of anesthesia, tropisetron 5mg (n=40), or granisetron 3mg (n=45), or normal saline (5ml N/S 0.9%, n=42). All patients received additionally 0.625mg droperidol.

Results: The three groups were homogenous in terms of demographics, hyperthyroidism, reoperative thyroid surgery, thyroid gland weight and volume, pathologic diagnosis, operative and total anesthesia time, preoperative and postoperative calcium levels, complications, hospital stay, and final outcome. Nausea visual analogue scale (VAS) score was lower in tropisetron and granisetron groups than the control group at all measurements (p<0.01) except for the 8-hour measurement for tropisetron (p=0.07). Moreover, granisetron performed better than tropisetron (p<0.01) at all points of time apart from the 2-hour measurement. Vomiting occurred in 22.2%, 27.5% and 37.5% in granisetron, tropisetron and control groups, respectively (p=0.43). No drug-related adverse events were reported.

Conclusion: We conclude that the combination of the 5-HT3 antagonists with droperidol given before induction is well tolerated and superior to droperidol alone in preventing nausea but not vomiting after total thyroidectomy. Better results were observed with granisetron than tropisetron.
VIDEO ASSISTED NECK SURGERY FOR THYROID TUMORS

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We report our cases with thyroid tumors who received endoscopic thyroid surgery. Sixty patients with thyroid tumor are included in this study. They received endoscope assisted thyroid resection from August, 2006 to March, 2009. Each number of tumors was as follows; Forty nine patients of follicular adenoma and 5 of nodular goiter, 4 patients of incidental papillary microcarcinoma and 2 of follicular carcinoma with minimally invasion. Now, we usually perform Video Assisted Neck Surgery using an anterior neck-skin lifting method for thyroid diseases. In this method, we preserve all anterior cervical muscles with 3 cm skin incision on anterior chest wall under the subclavicular line without the skin incision of the neck area using wire hook retractor. This wire hook retractor is our original tool, and it brings the excellent endoscopic view and working space in the narrow neck space. We perform the operation with this method for the patients of follicular tumor, a symptomatic nodular goiter and Grave's disease. We usually exclude the cases of papillary carcinoma from the indication criteria of endoscopic thyroidectomy, even if it is microcarcinoma less than 1cm. We should discuss the indication of papillary carcinoma carefully because of its high frequency of lymph node metastases. If we obey the adequate indication criteria, endoscopic neck surgery that results in a better cosmetic appearance is a excellent surgical treatment for thyroid tumors.
Background: The optimal treatment of Graves’ disease (GD) is still controversial. Surgery is one treatment option along with radioactive iodine (RAI) and antithyroid medication.

Objective: The aim of this study was to evaluate the results of the surgical treatment of patients with GD.

Methods: Clinical and follow-up data were obtained by a retrospective review of medical records. A total of 27 consecutive patients underwent surgical treatment for GD from February 2006 to February 2009. Two pediatric patients were excluded. The surgical outcomes were reviewed with regard to mortality and morbidity.

Results: 25 patients were included; 8 men and 17 women. Mean age: 35.1 years (19 - 65 years). The most common surgical indications were: failure of medical therapy; medical therapy adverse effects; compressive symptoms and ophthalmopathy. Compressive symptoms were documented in 9 patients and 6 patients had ophthalmopathy. Total thyroidectomies were performed in all patients. A standardized operative technique was adopted and practiced among all surgeons in our department. The median postoperative hospital stay was 2 days. There were no recurrent laryngeal nerve injuries. Five patients developed transient hypocalcaemia requiring calcium supplementation. No patient had persistent hypocalcaemia. Local haematoma requiring revision and haemostasis was encountered in 2 patients. Four patients had papillary microcarcinoma and one patient had a follicular adenoma discovered on histological examination.

Conclusion: Our results indicate that total thyroidectomy is safe and effective for patients with GD.
THYROID SURGERY AUDIT IN AN ENDOCRINE AND BREAST SURGERY UNIT

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The surgical endocrine and breast unit of the surgery department of the H.S. João hospital perform regular audits to the surgeons activity.

Objectives and methods: Presentation of data reviewed in clinical reports of the patients submitted to thyroid surgery in 2008.

Results: 507 surgeries were performed in 498 patients (429 women; 69 men) with a mean age of 50.8 years old.
Total thyroidectomy - 394 (77.6%) with 42.4% of malignancy (isolated papillary thyroid microcarcinoma in 19.7%).
Pre-operative malignant cytology was identified in 65 (48.5%) patients and in 33 (24%) the cytological diagnosis was follicular tumour
Lobectomy - 78 patients. Malignancy was identified in 21 (26.9%) with completion of surgery in 14 (17.9%) of which 10 were cytological follicular tumours.
Completion thyroidectomy - 35 patients, 20 of them with goitre recurrence.
The mean hospital stay was 2.7 days (national average 6.0 days)
Morbidity and mortality was studied and compared with results from literature.

Conclusions: Published data show a trend towards the use of total thyroidectomy in thyroid diseases rather than less than total. Widely accepted are low complication rates of the surgery when performed in experience centres.
The analysis of our data fall under other published by differentiated centres.
Background: The association between the volume of an operation performed and outcome has important implications for enhancing quality and reducing the cost of healthcare. This has been widely proved in pancreatic and esophageal cancer. In thyroid surgery, although complications are infrequent, they can be ominous and should not be forgotten. A number of authors described an inverse relation between complication rate and the number of procedures performed in a specific department, suggesting that some difficult cases should be addressed to high-volume centers.

Objectives: To evaluate the experience of our Hospital in 13 years of thyroid surgery.

Patients and methods: The study group included our Hospital series of 407 patients submitted to thyroid surgery for both benign and malign diseases for a 13 year period. Retrospective data were evaluated.

Results: The experience of a regional Hospital is described in terms of clinicopathologic and operative data and a correlation is established with its surgical morbidity. Hypoparathyroidism, hemorrhagic complications and recurrent laryngeal nerve dysfunction were the most common complications recorded, particularly in bilateral procedures and neoplastic disease.

Conclusion: Our experience in thyroid surgery produced the expected results and a low surgical morbidity rate. The outcome of our regional series parallels what is extensively described in the literature, supporting that this type of intervention can be safely performed outside high-volume centers.
A NOVEL PATHWAY FOR MODULATION OF HEPATIC GLUCOSE PRODUCTION BY THYROID HORMONE INVOLVING THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS AND THE SYMPATHETIC NERVOUS SYSTEM

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Thyrotoxicosis is associated with increased endogenous glucose production (EGP) and hepatic insulin resistance. We have recently shown that these alterations can be modulated by selective hepatic sympathetic and parasympathetic denervation, pointing to neurally mediated effects of thyroid hormone on glucose metabolism. Here, we investigated the effects of central triiodothyronine (T3) administration on EGP.

We used stable isotope dilution to measure EGP before and after intracerebroventricular (icv) bolus infusion of T3 or vehicle in euthyroid rats. To study the role of hypothalamic pre-autonomic neurons, bilateral T3 microdialysis in the paraventricular nucleus (PVN) was performed during 2 h. Finally, we combined T3 microdialysis in the PVN with selective hepatic sympathetic denervation to delineate the involvement of the sympathetic nervous system in the observed metabolic alterations.

T3 microdialysis in the PVN increased EGP by 11±4% (p=0.020) while EGP decreased by 5±8% (ns) in vehicle treated rats (T3 vs Veh p=0.030). Plasma glucose increased by 29±5% (p=0.0001) after T3 microdialysis versus 8±3% in vehicle treated rats (T3 vs Veh p=0.003). Similar effects were observed after icv T3 administration. Effects of PVN T3 microdialysis were independent of plasma T3, insulin, glucagon and corticosterone. However, selective hepatic sympathectomy completely prevented the effect of T3 microdialysis on EGP.

We conclude that stimulation of T3-sensitive neurons in the PVN of euthyroid rats increases EGP via sympathetic projections to the liver, independently of circulating glucoregulatory hormones. This represents a novel central pathway for modulation of hepatic glucose metabolism by thyroid hormone.
SHORT AND LONG-TERM EFFECTS OF MATERNAL NICOTINE EXPOSURE DURING LACTATION ON BODY ADIPOSY AND THYROID FUNCTION OF RAT OFFSPRING

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Smoking affects thyroid function, especially during gestation and lactation. Epidemiological and experimental studies suggest an increase of obesity in the progeny of mothers exposed to cigarettes. We evaluated the short and long-term effects of maternal nicotine exposure during lactation upon body composition, lipid profile, leptinaemia and thyroid function of the offspring during development. Two days after birth, osmotic minipumps were implanted in lactating rats, divided into: NIC- nicotine subcutaneous infusion (6mg/Kg/day) for 14 days; C- saline. Offspring were killed at 15, 21 and 180 days-old, when blood, visceral fat mass (VFM) and carcass were collected. All presented data were significant at the level of p< 0.05. NIC pups (15 days-old) showed higher VFM (63%) and total fat (30%). At weaning (21 days-old), they presented only higher body total protein (+33%). At 180 days-old, NIC offspring showed higher BW (+10%), VFM (+32%), total body fat (+36%) and protein content (+11%). Lipid profile was unchanged in adulthood. NIC offspring presented hyperleptinaemia (+103%) at 180 days-old, with no changes in food intake. The 15 days-old NIC offspring showed lower serum T3 (33%) and T4 (31%) with higher TSH (39%). At 21, they showed only lower serum TSH(-32%). The 180 days-old NIC offspring exhibited lower serum TSH (-35%), T3 (-25%) and T4 (-11%). Thus, early nicotine exposure leads to a neonatal primary hypothyroidism and programs for a higher adiposity, hyperleptinaemia and secondary hypothyroidism in adulthood. These data corroborate epidemiological studies that show a higher prevalence of obesity in children from mothers that smokes. Our study suggests that nicotine can be one of the tobacco compounds responsible for later obesity and identifies the lactation period as critical to nicotine programming, and the hypothyroidism as a contributing factor.
TRI-IODOTHYRONINE (T3) CHANGES SULPHATIDE METABOLISM IN RAT PANCREATIC BETA-CELLS AND MIGHT EXPLAIN THE T3-INDUCED REDUCTION IN INCIDENCE OF DIABETES MELLITUS IN BB RATS

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Objectives: Tri-iodothyronine (T3) has been shown to decrease spontaneous diabetes mellitus (DM) in BB rats and increase the relative beta-cell mass in non-diabetes prone Wistar rats. Sulphatide, a glycosphingolipid present in pancreatic beta-cells, shown to facilitate insulin folding and monomerisation and preserve insulin crystals, has been linked to development of DM. Sulphatide is produced by addition of a sulfate group to galactosylceramide (GalCer) by the enzyme Ceramidegalactosyl sulphotransferase (CST). GalCer is produced by UDP-Galactose-ceramide galactosyltransferase (CGT). As changes in sulphatide levels in pancreatic beta-cells seem to influence the development of DM, we speculated that T3 might act through changes in sulphatide levels.

Methods and results: In diabetic BB rats, treated by T3 from the age of 5 to 9 weeks and killed 19 weeks old, the level of sulphatide (thin layer chromatography) was reduced to 36 % (n=5) compared to controls (n=5, p=0.008). To further explore the influence of T3 on pancreatic sulphatide, T3 was added to the drinking water of Wistar rats from age 5 to 9 weeks. Pancreatic weight increased to 175% (n=16) compared to controls (n=27, p< 0.001), and protein content to 193% compared to controls (p< 0.0001). Sulphatide content was reduced to 0.96±0.10 nmol (mean±SEM) compared to 2.50±0.29 nmol in controls (p< 0.0005). The sulphatide/mg pancreas was 0.92±0.09 nmol/mg compared to 4.175±0.46 nmol/mg (p< 0.0001). Likewise, sulphatide/protein was reduced to 20.1% compared to control (p< 0.0001). Finally, T3 treatment reduced the mRNA (quantitative PCR) expression of CST by 35% (n=3) compared to controls (n=5, p= 0.047) whereas mRNA expression of CGT was unaffected.

Conclusion: T3 treatment reduced the amount of sulphatide in both diabetic BB rats and Wistar rats, possibly due to a specific downregulation of the amount of CST. The T3 induced reduction of DM incidence might thus be connected to these findings.
3,5-diiodothyronine (T2) rapidly affects skeletal muscle (SKM) fatty acid oxidation and thermogenesis when acutely injected into hypothyroid rats, suggesting a potential role of this iodothyronine in protecting SKM from lipotoxicity.

The utilization of free fatty acid (FFA) by SKM, although sensitive to blood FFA levels, is also regulated at the tissue level, with FAT/CD36 playing a crucial role. Subcellular redistribution of FAT/CD36 from its endosomal compartment to the plasma membrane and to the mitochondria modulates FFA import into the tissue and their oxidation rate, respectively.

**Aim:** As FAT/CD36 redistribution may underlie the effect of T2 on SKM fatty acid metabolism in this study we examined the ability of T2, when acutely and chronically injected into hypothyroid rats, to influence

i) FAT/CD36 redistribution in SKM cells and

ii) serum and SKM FFA levels.

**Results:** Within 1 hour, T2 promotes FAT/CD36 translocation to sarcolemma and mitochondria and reduces SKM FFA (-40%), without affecting serum FFA, suggesting that in the first hour of T2 action FFA oxidation exceeds their tissue import. Following T2 chronic administration, a reduction of FFA serum level is observed (-63%) which can be explained by the constant higher capacity of SKM to import and to oxidise FFA (revealed by persistently higher plasmalemma and mitochondria FAT/CD36 levels). In the same animals SKM FFAs become not significant different from hypothyroid control ones, suggesting that in the long term action of T2 FFAs import balances their oxidation. These changes are accompanied by a reduced respiratory quotient which supports the data on tissue FFA utilization.

**Conclusion:** The translocation of FAT/CD36 to the plasma membrane and to mitochondria in a coordinated fashion contributes to the up-regulation of SKM lipid metabolism induced by T2 in hypothyroid rats.
3,5-DIODO-L-THYRONINE PREVENTS HIGH FAT DIET-INDUCED INSULIN RESISTANCE BY REDUCING TRIGLYCERIDES ACCUMULATION

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High-fat diet induced insulin resistance (IR) is accompanied by ectopic lipid deposition in peripheral tissues and, in particular, by accumulation of intramyocellular triglycerides. 3,5-diiodo-L-thyronine (T2), a naturally occurring iodothyronine, when administered simultaneously with a high-fat diet (HFD), by stimulating fatty acid oxidation in liver, prevents the development of liver steatosis and body weight gain without inducing thyrotoxicity in rats. T2 stimulates hepatic fatty acid oxidation leading to decreased plasma and hepatic lipid content. These actions induced us to hypothesize that T2 may also have similar effects in muscle, thus alleviating IR.

Objectives: Here we studied the effects that T2 elicits on systemic IR induced by HFD and evaluated the ability of T2 in preventing fat accumulation in skeletal muscle and improving insulin signalling in this tissue.

Results: In keeping with the notion that HFD leads to IR, glucose and insulin tolerance tests performed in HFD rats showed lower kinetics of glucose clearance when compared with control standard diet fed rats (N). T2 administration to HFD rats prevented development of IR. Indeed, glucose and insulin tolerance tests, which were altered in HFD rats, were normal in T2-treated ones. Skeletal muscle triglyceride content significantly increased in the HFD rats compared to N (3.4 ± 0.46 and 1.76 ± 0.22 mg/ml, respectively) while there was no change following T2 treatment, being the value (2.41 ± 0.55 mg/m) not significantly different from N. Compared to N rats, insulin-induced AKT phosphorylation, which induces glucose uptake and glycolysis, was significantly decreased in HFD rats, whereas it was increased in T2-treated ones. An upregulation of adipocyte differentiation-related protein (ADRP) was observed following T2 treatment in accordance with an unaltered insulin signalling pathway in skeletal muscle.

Conclusion: The results indicated that by reducing triglycerides accumulation and preserving insulin sensitivity, T2 has therapeutic potential for preventing IR-related metabolic disorders.
AGE-DEPENDENT REGULATION OF GLUTATHIONE S-TRANSFERASE IN THYROID HORMONES-RESISTENT MICE

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Objectives: Glutathione S-transferase (GST) enzyme is downregulated by thyroid hormones (THs), with high levels of expression in hypothyroidism and low ones is hyperthyroidism. We study GST and deiodinase type 1 (D1) expression in kidney and liver of animals, expressing 337T mutation on TR-β gene, which prevents T3 binding.

Methods: Hypothyroidism was induced in 8 week-old mice in wild-type (wt) and homozygous (ho) by 0.15 % 5-propyl-2-thiouracil (PTU) diet. After 5 weeks, animals received daily injections of low (0.2µg/100g BW), medium (0.5µg/100 g BW), or high (1.0µg/100g BW) dose of L-T3 for 7 days each throughout PTU treatment. We evaluated D1 and GST mRNA expression using Real-Time-RT-PCR.

Results: Were normalized for 36B4 RNA. Wt values were considered 1. As we expected, in 9 days after birth -old and adult homozygous mice D1 expression was decreased (wt:1±0.03,ho:0.02±0.01, p< 0.001) in both tissues, confirming peripheral resistance to thyroid hormones in these animals. T3 was able to induce an increase of D1 mRNA levels in wt mice (hypo:1+0.2,hyper:13.6+0.2,p< 0.05), but homozygous animals were resistant to its effects (hypo:0.3+0.04 hyper:1.6+2.4,NS). GST expression was higher in the liver of adult Ho (wt: 1+0.24,ho:3.8+1.7, p< 0.05). However, nine days after birth and after induction of hypothyroidism and treatment with TH, results were unexpected. Interestingly, levels of GST mRNA were reduced. In the kidney, GST mRNA levels was lower in homozygous (wt: 1.0 +0.13; ho:0.5+02) and after treatment with TH, we observed a raise in GST expression in response to T3 (wt hypo:1.0+0.1;ho hypo:3.3+1.3).

Conclusions: This study shows that GST gene has different patterns of expression in these two studied tissues. In the liver of 9 day-old animals and kidney of adult ones, decreased GST expression suggests negative regulation mediated by TR-α1. On the other hand, high levels of hepatic GST mRNA indicate a predominant role of isoform TR-β1.
Accurate measurement of D2 in muscle is complicated by low levels of enzyme, the potential presence of D3 and possible non-specific deiodination of labelled substrate. The latter may make quantitation of iodide release unreliable as an index of T4-to-T3 conversion. Our goal was to develop a reliable, sensitive method for this assay.

Microsomes from transgenic mice expressing myocardial human D2 were used as an endogenous D2 source. We investigated incubation times and cofactors (dithiothreitol (DTT), GSH, NADPH, thioredoxin (TRX)) and muscle cytosol. We also analyzed T4 vs. rT3 as substrate and evaluated T3 degradation. Reaction products were identified by HPLC.

Overnight incubation was used to maximize T3 production. D2 activity with 20 mM DTT was 1.2 fmol/min/mg protein. Myocardial cytosol did not stimulate D2 and caused non-saturable T4 and rT3 deiodination. This was almost completely blocked by 10-20 mM DTT but not by lower amounts of DTT or GSH, NADPH or TRX nor did these cofactors activate D2. Labelled T3 was not degraded. Homogenates could also be used for D2 assay. Surprisingly, adding 0.1 µM unlabeled T3 to prevent potential inner ring deiodination of T4 or T3 inhibited D2 activity by 50% while PTU had no effect. Using 20 mM DTT, we found no D2 activity in homogenates of adult murine gastrocnemius or anterior tibialis but found significant D2 in the same muscles from young mice (0.02 to 0.1 fmol/min/mg protein). Despite optimization of assay conditions, we were unable to establish the presence of D2 in adult murine muscle, though it was present in young animals. Non-specific iodide release is low but significant even with higher levels of DTT. D3 levels in murine muscle are not high enough to require the routine addition of cold T3 given its potential to block D2.
Recently, thyronamines have been described as endogenously occurring, rapidly acting, iodine bearing, hormone-related signaling molecules. Their great structural similarity to thyroid hormones suggests biosynthesis from thyroid hormones as precursors. Such biosynthetic pathways would require decarboxylation and one or more deiodination steps to convert e.g. the pro-hormone thyroxine (T4) into a thyronamine. We have previously reported that thyronamines are avid substrates for human deiodinases and suggested several possible pathways to convert T4 into the most potent iodothyronamine (3-T1AM). However, the decarboxylating enzyme involved remained elusive. The pyridoxal-5-phosphate-dependent aromatic L-amino acid decarboxylase (AADC), also known as dopa decarboxylase (DDC), represents a promising candidate for this pathway. It catalyzes the conversion of L-DOPA to dopamine or 5-hydroxytryptophan to serotonine. In analogy, we reasoned that thyronamines could similarly result from decarboxylation of iodothyronines catalyzed by AADC. We tested purified human recombinant AADC for decarboxylation of rT3 and other iodothyronines and varied incubation conditions according to pH, reaction time, and temperature. Conversion of L-DOPA to dopamine served as positive control. Products were analyzed by a sensitive and robust SRM (selected reaction monitoring) based LC-MS/MS (liquid chromatography-tandem mass spectrometry) method. Our first results reveal that the human recombinant AADC apparently does not efficiently catalyze the decarboxylation when rT3 is used as substrate under our experimental conditions. Now, more potential substrates and other candidate decarboxylating enzymes need to be investigated to test the suggested thyronamine biosynthesis pathway and to identify means for pharmacological intervention in thyronamine production, action and metabolism.

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REGULATION OF THE THERMOGENIC FUNCTION BY THE NUCLEAR T3 RECEPTOR ISOFORMS, TRβ1 AND TRα1, IN BROWN ADIPOCYTES

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Brown adipose tissue (BAT) thermogenesis is activated in response to cold and diet. T3 increases the adrenergic stimulation of UCP1, a BAT-specific protein. UCP1 is also involved in the maintenance of body weight. In BAT, T3 is locally produced by D2 deiodinase, stimulated adrenergically in parallel to UCP1. Most T3 actions are mediated by its binding to its nuclear receptors (TRα and TRβ). Both isoforms are present in BAT. Studies in mice suggest that TRβ is involved in UCP1 induction, while TRα regulates body temperature.

Aim: To compare the effect of T3 and GC1 and CO23, selective agonists of TRβ1 and TRα1, respectively, on the adrenergic induction of UCP1 and D2 Deiodinase.

Methods: Primary cultures of rat brown adipocytes were used. Experiments were done in differentiated adipocytes. D2 activities were determined using 2 nM T4. UCP1 and D2 mRNAs were analyzed by quantitative Real Time PCR.

Results: GC1 (TRβ1 agonist) and CO23 (TRα1 agonist) are less potent than T3 in the adrenergic stimulation of UCP1 and D2 expression at low doses (0.2-10 nM), while at higher doses GC1 and T3 had a similar potency. In the absence of adrenergic stimulation the agonists did not increase UCP1, nor D2. The adrenergic stimulation of D2 activity reached a peak using 2-10 nM T3, but at higher doses was inhibited by 50%. No inhibition was observed using GC1 (up to 400 nM). The stimulation using CO23 was poorer (0.5-200 nM). We analyzed if the differences found between T3 and GC1 were due to modulation of the adrenergic pathways (mostly β3+α1-adrenergic). cAMP levels increased in the presence of T3, GC1 and CO23.

Conclusions: The adrenergic stimulation of UCP1 and D2 activity and expression are regulated by T3, preferentially via the TRβ1. The TRβ agonists might be a useful tool to increase energy expenditure.
T2 AND T3 EFFECTS IN INS-1E CELLS AND HUMAN ISLETS
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Aims: It has been recently shown that 3,5-diiodo-L-thyronine (T2), a naturally occurring iodothyronine, has biological effects, but no data to our knowledge are present about the T2 effect on beta cells.

Methods: INS-1e cells were incubated (for 48 hours) in fresh RPMI 1640 medium containing 11 mM glucose in the presence or absence of different concentrations of triiodothyronine (T3) (0.1nM-1nM-0.1uM-1uM-10uM) and T2 (0.1nM-1nM-0.1uM-1uM-10uM). After washing, the cells were incubated for 60 min with KRB-HEPES buffer containing 3.3mM, 7.5mM, 11.0mM and 20mM glucose. The supernatants were collected for insulin determination, while cellular insulin contents were determined from acid-ethanol extracts. Moreover, the prototype Th1 chemokine CXCL10 and Th2 chemokine CCL2 were measured in the supernatants.

Results: Insulin secretion and insulin content were significantly (p< 0.01; ANOVA) stimulated (11.0mM and 20mM glucose) with T2 (1nM-0.1uM), while an inhibition was observed with higher concentrations (1uM-10uM). T3 showed a similar pattern of stimulation, in fact insulin secretion and insulin content were significantly (p< 0.01; ANOVA) stimulated (11.0mM and 20mM glucose) with T3 0.1uM-1uM, while an inhibition was observed at 10uM. CXCL10 and CCL2 secretion by INS-1e cells were not significantly changed. Then, pancreatic islets from human donors were washed and incubated at 37 °C for 45 min in KRB solution containing 3.3 mmol/l glucose, either with or without the addition of T3 or T2 (0.1nM, 0.1uM, 1uM). Insulin secretion was significantly (p< 0.01; ANOVA) stimulated with T2 (0.1nM-0.1uM), while an inhibition was observed with higher concentrations (1uM). A slight increase of insulin secretion was observed with T3 1uM.

Conclusions: These results show that T2 is able to stimulate insulin secretion in INS-1e cells and human islets at concentrations lower than T3. T2 and T3 are not able to modulate basal chemokines secretion in INS-1e cells.
Objective: 3-iodothyronamine (T1AM) derives from thyroid hormone through decarboxylation and deiodination, and binds to plasma membrane receptors known as trace amine-associated receptors. Since we have previously observed that T1AM produces a negative inotropic effect in rat heart (IC_{50} close to 20 microM), in this work we determined the effect of T1AM on calcium homeostasis.

Methods: Isolated adult rat cardiomyocytes were used to evaluate the calcium transient by the fluo-3 technique and to record L-type calcium current, by whole cell patch clamp. In additional experiments sarcoplasmic reticulum (SR) vesicles were obtained from isolated rat hearts perfused in the absence or in the presence of T1AM. After vesicle loading with 45Ca, the rate constant of calcium release was determined by quick filtration technique. Ryanodine binding was also assayed, since ryanodine is a specific ligand of the SR calcium channel, and interventions affecting channel function usually modify ryanodine binding.

Results: Acute exposure to 20 microM T1AM decreased the amplitude of the calcium transient (0.06 ±0.01 vs 0.10±0.02 arbitrary units, P< 0.05) and reduced its decay time (86±26 vs 176±40 msec, P< 0.01), while sarcolemmal calcium current density was unchanged. In SR experiments the release rate measured in the presence of 10 microM unlabeled calcium was not significantly modified after perfusion with T1AM. However perfusion with T1AM determined a significant increase in the rate constant of calcium release measured under conditions promoting channel closure, i.e. exposure to 0.3 mM tetracaine and 1 mM EGTA (0.32±0.03 vs 0.13±0.04 sec^{-1}, P< 0.01). Ryanodine binding decreased after perfusion with T1AM, due to reduced Bmax (265±19 vs 381±19 fmol/mg of protein, P< 0.05) with unchanged Kd.

Conclusions: We conclude that T1AM affects calcium homeostasis and suggest that its negative inotropic action is due to a diminished pool of SR calcium as a result of increased diastolic leak.
Objectives: Years ago we found a novel TRβ isoform which was deposited in Genbank with the acceptance name of TRβ Δ. TRβ Δ was highly homologous with TRβ1 with the only difference of a 36 amino acid peptide inserted between K128 and G129 of TRβ 1. In this article some characteristics of this TRβ Δ were investigated with TRβ1 as control.

Methods: After being cloned into plasmid pET-Duet, recombinant proteins of TRβ Δ and TRβ1 were prepared from bacteria E.coli. Their DNA binding abilities were determined by EMSA, while ligand binding affinity was tested with radioactive labelled T3. Tissue distribution of TRβ Δ and TRβ1 were detected by western blot with purchased antibody recognizing β Δ+β1, and self-generated antibody specifically recognized β Δ .Expression levels of these two TRβ isoforms in multi tissues were evaluated by real-time RT-PCR with three pairs of primes disigned for amplifying β Δ plus β1, β1 only and β Δ only.

Results: For DNA binding, recombinant TRβ Δ can bind DR-4 DNA sequence , a TRE of TRβ, with same ability of TRβ1. For ligand binding, rTRβ Δ binded T3 with the affinity constant of 2.54±0.13×10^9 L/mol, no significant difference with 2.32±0.14×10^9 L/mol of rTRβ1. Western blot showed only one band could be detected by anti β Δ+ β1 antibody, which was rebloted successfully by anti-β Δ antibody. Quantitative RT-PCR revealed that , in various tissues of adult rats, β Δ+ β1 and β Δ transcript coulded be detected in a large amount while β1 transcript was hardly detected.

Conclusions: TRβ Δ has both DNA and ligand binding ability, is a functional TRβ isoform. In adult rats, TRβ Δ might be the major TRβ isoform in multi tissues.
THYROGLOBULIN MEASUREMENT IN A SENSITIVE IMMUNOASSAY HAS COMPARABLE SENSITIVITY TO HYPOTHYROIDISM STIMULATED THYROGLOBULIN IN THE FOLLOW-UP OF LOW-RISK DIFFERENTIATED THYROID CANCER PATIENTS BUT NOT IN HIGH-RISK PATIENTS

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Undetectable stimulated serum thyroglobulin (Tg), after initial treatment of differentiated thyroid cancer (DTC) in patients with negative Tg autoantibody (TgAb), implies the absence of residual/relapsing DTC. However, recent data have shown that low-risk DTC patients with undetectable Tg on T4 treatment (onT4-Tg) using high-sensitivity (HS) assays do not need to be submitted to Tg stimulation. The present study was undertaken to compare onT4-Tg with a HS assay and the hypothyroidism TSH-stimulated Tg (offT4-Tg) in patients on low and high risk TNM score. We evaluated 110 consecutive DTC patients (65 low-risk, 45 high-risk) treated by total thyroidectomy and radioiodine ablation. Inclusion criteria were undetectable onT4-Tg for one year after primary treatment (functional sensitivity of 1.0 ng/mL), and negative TgAb and US. All patients underwent onT4-Tg HS assay (functional sensitivity of 0.2 ng/mL), 4 weeks offT4-Tg measurement, 131I whole body scan (WBS) and neck US. Measurement of onT4-Tg with a HS assay was undetectable in 91/110 patients. OffT4-Tg was under 2 ng/mL in 85/91 and 2/85 presented metastases seen on US. From the 6/91 patients with offT4-Tg above 2 ng/mL, 2 had cervical metastases. From the 4 recurrent patients with undetectable onT4-Tg, 3 were classified as high risk patients according TNM score. Furthermore, 19/110 patients presented detectable Tg onT4-Tg (0.2 to 0.9 ng/mL) and 12/19 patients also presented offT4-Tg above 2 ng/mL; in 8/12 metastases were detected. A combination of US-FNAC with a high-sensitivity onT4-Tg assay detected 11 out of 12 recurrent patients. The sensitivity and negative predictive value of the HS onT4-Tg assay plus US-FNAC was 92% and 99%, respectively. Our data indicate that measurement of onT4-Tg with a HS method combined with neck US and FNAC may avoid stimulated Tg in low-risk DTC patients, after the initial treatment and during follow-up, but not in TNM high-risk patients.
EMPIRICAL HIGH DOSE RADIOACTIVE IODINE THERAPY AND POST-TREATMENT WHOLE SCAN ARE NOT HELPFUL IN THE FOLLOW UP OF THE PATIENTS WITH ELEVATED SERUM THYROGLOBULIN AND NEGATIVE NECK ULTRASONOGRAPHY AND FDG-PET

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Backgrounds: It is well known that some patients who had elevated thyroglobulin (Tg) but negative whole body scan (WBS) after initial therapy of differentiated thyroid carcinoma may have benefit after empirical high dose radioactive iodine (RAI) therapy. However, the reported cases dealt with those patients with negative diagnostic WBS and routine neck ultrasonography (USG) and/or FDG-PET were not applied, which are now considered as diagnostic procedure of choice in case of elevated Tg.

Aim: This study aims to evaluate the effectiveness of empirical high dose RAI therapy in patients with elevated stimulated Tg (sTg) level, and a negative USG/PET after initial therapy for papillary thyroid carcinoma (PTC).

Patients and methods: Patients with elevated sTg, and a negative USG/PET at 1 year after initial treatment of PTC were enrolled. Empirical RAI therapy was done in treatment group and iodine uptake at post-therapeutic WBS (RxWBS) and the change of sTg levels between before and a year after I-131 therapy were evaluated. Control groups were followed up with only thyroxine suppression.

Results: Thirty-nine patients (Age 10-69 years, M:F=7:32) were enrolled and 14 patients underwent empirical RAI therapy. There were no significant difference in clinicopathological parameters between treatment group and control group. None of 14 patients in treatment group displayed iodine uptake on RxWBS. Five of 14 patients in treatment group had recurrence and 8 of 25 patients in control group had recurrence during 37 months of follow-up(p=0.88). There was no significant difference in the change of sTg levels between two groups.

Conclusion: Empirical RAI therapy and RxWBS were not useful diagnostic and therapeutic approach in patients with a positive sTg, and a negative USG/PET after initial treatment of PTC.
Aim: Thyroid cancer patients are exposed to radiation ($^{131}$I) and this can produce undesirable consequences, as the apparition of second primary tumours, related to therapeutic treatment. To reduce the undesirable effects, the applied dose should be reduced, but without affecting the therapeutic results. Since large variability is observed between patients, to know the radiosensitivity of each patient is a very important issue since this information can allow us to adjust the dose to be applied.

Methods: To detect the genetic damage induced by the irradiation, the frequency of micronuclei (MNi) in peripheral blood lymphocytes has been used. In recent diagnosed patients, the frequency of MNi was determined before and after the administration of radioiodine. From the follow up patients, MNi were determined before and after the application of 0.5 Gy to lymphocyte cultures. To look for the genetic basis of the observed radiosensitivity, SNPs of genes involved in single and double strand repair genes were genotyped.

Results: The frequency of MNi in in vivo therapeutically irradiated patients shows a significant increase. In the in vitro irradiation experiments, a significant increase in the frequency of MNi is also observed in the cultures after 0.5 Gy irradiation. High variability is observed between cancer patients, for both in vivo and in vitro exposure. Individual characterizations for the XRCC1, OGG1, XRCC2, XRCC3, Rad51 genotypes have been obtained.

Conclusions: The sensitivity test carried out in thyroid cancer patients, using the MN assay, has shown to be very useful, detecting large variability between patients. This would be a useful method to be applied in determining the radioiodine dose. Although the genetic individual characteristics can modulate the individual sensitivity, the MN assay distinguishes easily and clearly, the individual response to irradiation and, as consequence is a better indicator of secondary effect related to the therapeutical $^{131}$I treatment.
P147
PREDICTORS OF RADIOIODINE-REFRACTORY METASTATIC DISEASE IN PAPILLARY THYROID CARCINOMA
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Treatment of papillary thyroid carcinoma (PTC) is usually successful but there are patients with metastatic disease that have lost the ability to accumulate iodine and thus are unresponsive to radioiodine (131I) treatment, predicting a poor prognosis. Our aim was to study which clinical and genetic factors were associated to 131I-refractory metastases. For this purpose we retrospectively collected data about clinicopathological characteristics and clinical courses of 159 PTC patients between 1998 and 2004, and their tumour BRAF mutation status was determined. The mean follow-up after surgery was 58 months. Associations of initial tumour characteristics and BRAF mutation with both 131I-responsive and 131I-refractory recurrences were analyzed separately. 131I-responsive recurrences were associated with extrathyroidal extension [RR 8.77, CI 95% (2.58-29.16)], unilateral [RR 5.87, CI 95% (1.71-20.20)] and bilateral [RR 12.74, CI 95% (2.15-75.01)] lymph node metastases and tumor size (3.94 ± 2.52 vs 2.30 ± 1.44 cm, p= 0.001). No association was observed with age older than 45, gender, personal history of non-thyroid cancer, lymphocyte infiltration, diffuse hyperplasia, multifocality, histological subtype or BRAFV600E. Multivariate analysis found that extrathyroidal invasion, lymph node metastases and tumour size were independent prognostic factors that increased the risk of 131I-responsive recurrences. On the other hand, personal history of non-thyroid cancer [RR 3.73, CI 95% (1.04 - 13.38)], extrathyroidal invasion [RR 5.78, CI 95% (2.22 - 15.01)], bilateral lymph node metastases [RR 10.60, CI 95% (2.28 - 49.34)] and BRAFV600E mutation [RR 4.23 (1.21 - 14.79)] were significantly associated with 131I-refractory recurrences. Interestingly, only BRAFV600E remained significant on multivariate analysis. Although not significant, the majority of the patients with recurrences that had positive thyroglobulin antibodies during the follow-up were 131I-refractory. Our preliminary results show that there are specific factors associated to 131I-refractory recurrences and that BRAFV600E mutation is an independent predictor of these kind of progressive recurrences in PTC.
OFF-LABEL USE OF SORAFENIB IN PATIENTS WITH METASTATIC THYROID CANCER UNRESPONSIVE TO CONVENTIONAL THERAPIES: A SPONTANEOUS STUDY

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Objectives: Patients with advanced thyroid cancer that lose the ability to take up iodine have poor therapeutic options. Sorafenib is an oral-administered kinase inhibitor with multiple targets, including BRAF, approved for treatment of renal and hepatocellular carcinomas. 40% of aggressive thyroid tumors show BRAF mutation. We performed a clinical trial to evaluate the efficacy of Sorafenib (400 mg bidaily) in patients with aggressive thyroid tumors.

Methods: We enrolled six patients (Pt). Median duration of treatment was 3.5 months. CT scan was performed after 1 and 3 months. Sorafenib was discontinued or reduced in case of progressive disease or severe adverse events (AE). An informed consent was signed before enrollment.

Results: Pt-1 showed important reduction (PR) of a frontal bone lesion after 1 month; due to a severe rash the treatment was interrupted and the patient died for progressive disease 2 months later. Pt-2 showed significant reduction of the lung metastases after 2.5 months but new disease progression after 3 months. Pt-3 showed 50% reduction of the lung metastases after 1 month, confirmed 3 months later. Pt-4, with rapid progression showed the stabilization of the disease (SD) despite the reduction of dosage for AE. Pt-5 showed clinical progression (PD) after 1 month and the treatment was discontinued. Pt-6 showed important clinical benefit (resolution of dyplopia due to a large sphenoid lesion) within 1 month; at the present he is still under therapy.

Conclusions: We observed PR in 2/6 patients, SD in 2/6 and initial PR followed by a PD in 1/6. 1/6 patients did not show any response to the drug. With the exception of a diffuse and severe rash, the other AE could be managed by reducing the dose of the drug. Our data suggest a possible role of Sorafenib in the treatment of thyroid tumors unresponsive to conventional therapy.
RECOMBINANT TSH BEFORE RADIOIODINE ABLATION GIVES BETTER SUCCESS RATES AS WELL AS LOWER WHOLE BODY AND BLOOD DOSE AS COMPARED TO THYROID HORMONE WITHDRAWAL

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Introduction: This study looks at the efficacy of rTSH compared to THW with respect to success of thyroid remnant ablation.

Methods: Patient demographics, serum TSH and thyroglobulin (TG) prior to radioiodine, and at follow up were collected from the Electronic Patient Record along with whether the patient received rTSH or not. TG < 1 at 1 year was the surrogate marker used for successful ablation. Data for whole body dose and blood dose was collected from the medical physics records.

Results: 241 patients from 1999 to 2008 were identified. This included 180 (75%) females, 61 (25%) males. 204 (85%) had THW, 37 (15%) had rTSH. All patients in rTSH group and 72% patients in THW group had a TSH > 30 prior to radioiodine administration. TG results at 1 year post ablation were available for 172 (71%) patients and ablation was successful in 141/172 (82%). All patients in rTSH group had successful ablation while in 31 patients from THW group ablation failed (p=0.016). TSH levels pre-radioiodine had no significant effect on the success of ablation (p=0.122) while patients with lower TG pre-radioiodine had a significantly higher rate of successful ablation (p< 0.001). The Area under the curve (AUC) data for whole body dose was available in 199 patients (35 rTSH, 167 THW). The median AUC for the rTSH group was 1956 MBq.h and was significantly smaller than the median of 2325 MBq.h for THW group (p< 0.001). Patients in the THW group had a significantly higher whole body dose (0.198 vs. 0.155 Gy, p< 0.001) and blood dose (0.307 vs. 0.233 Gy, p=0.004).

Conclusion: rTSH is effective in raising TSH prior to ablation and has higher success rate that THW. The whole body and blood dose is significantly lower in patients who receive rTSH due to faster clearance of radioiodine from the body.
P150
PROGNOSTIC VALUE OF SERUM THYROGLOBULIN DETERMINATIONS BEFORE RADIOIODINE ABLATION IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

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The value of serum thyroglobulin (Tg) to detect recurrence in the follow-up of differentiated thyroid cancer after total thyroidectomy and radioiodine ablation is well established. Some studies have suggested that the Tg level measured after thyroidectomy and just before 131I ablation therapy might be useful as a prognostic marker. The aim of our study was to evaluate the clinical significance of endogenous TSH-stimulated serum Tg measurement determined before 131I ablation.

Patients with aggressive variants of differentiated thyroid carcinoma or positive anti-Tg antibodies were excluded. An endogenous TSH-stimulated (TSH above 30 µIU/mL) Tg measurement was obtained before 131I ablative therapy. Serum thyroglobulin was assayed by an immunoradiometric assay with a functional sensitivity of 0.9 ng/mL. All patients had undergone a total or near-total thyroidectomy. Periodic follow-up included clinical and ultrasound examination, 131I whole body scintigraphy and serum Tg measurements.

Two hundred eighty-five patients (215 women and 70 men) were included in the analysis. Mean age at diagnosis was 46.5 ± 15.3 years (range, 9-87 years). Two hundred fifty-eight patients had papillary carcinoma and 27 had follicular carcinoma. Two hundred thirty-eight patients had no evidence of persistent or recurrent thyroid cancer during follow-up. None of the 86 patients with a Tg below 1.0 ng/mL had persistent disease or developed a local recurrence or distant metastases. All 27 patients with a Tg higher than 50.0 ng/mL had persistent disease.

We conclude that a low (< 1.0 ng/mL) endogenous TSH-stimulated Tg determined before radioiodine ablation is highly predictive of absence of disease and a high Tg level predicts the persistence or recurrence of the thyroid cancer.
P151
RETINOIC ACID TREATMENT FOR ADVANCED THYROID CARCINOMA- 6 YEARS OF FOLLOW-UP
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Introduction: Retinoic acid (RA) administration to patients with advanced differentiated thyroid cancer might be of value for the control of disease progression. However, until today, there are limited studies analyzing the effectiveness of this drug.

Objective: We performed a clinical prospective study to assess the value of 13-cis-RA treatment (one or multiple cycles of treatment) in patients with advanced thyroid carcinoma and its real impact in major outcomes such as tumor regression and cancer related death with a long-term follow-up of patients submitted to radioiodine (131I) therapy after retinoic acid administration.

Patients: Sixteen patients (12 females and 4 males, from 28 to 79 years old) with inoperable disease and no significant radioiodine uptake on post-therapy scan were selected for this prospective study. 6 patients received more than one cycle of RA with an interval of at least 6 months.

Results: No matter the patients used one or multiple cycles of treatment, an objective partial response rate (defined as a decrease of 30% in the sum of the longest diameter measurements by RECIST) of 25% (four patients), a stable disease rate (defined as a decrease between 0% and 30% in the sum of the longest diameter by RECIST) of 25% (four patients) and a progression disease rate of 50% (eight patients). Five patients died (62.5%) in the group classified as progression of disease. Responses ranged from progressive disease to a decrease in target lesions of 75% by RECIST criteria. Progression free survival rate (PFS) ranged from 72 to 12 months with median PFS of 26.5 months.

Conclusion: Although our study has a limited number of patients, we show that RA might be an option for advanced de-differentiated thyroid cancer, due to the low rate of side effects.

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REVISITATION TO SOME ASPECTS OF LOW-IODINE DIETS (LID) FOR $^{131}$I ABLATION IN PATIENTS WITH THYROID CANCER: A) A SPOT URINARY IODINE SAMPLE IS EQUIVALENT TO A 24-HOUR URINE IODINE COLLECTION TO EVALUATE ITS ADEQUACY AND COMPLIANCE, AND B) THE USE OF L-T4 BY PATIENTS INCREASES THE IODINE POOL

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Treatment of thyroid cancer includes total thyroidectomy and $^{131}$I ablation. One of the strategies to increase $^{131}$I uptake is the depletion of plasma inorganic iodine by a low-iodine diet (LID) (30-50 ug/day/iodine). The evaluation of patients’ compliance to LID is usually done by 24-hour urinary iodine measurement (U24h); however, this procedure is not practical. In addition, there is a disagreement about if the amount of L-T4 used by patients will increase the iodine pool.

Therefore, the objectives of this work are a) to compare the correlation between U24h and spot sample (Ui) urinary iodine measurement in healthy individuals and in patients receiving L-T4 before and after a LID performed during 15 and 30 days.

A total of 307 patients with thyroid cancer were included in this prospective study. All patients performed a restricted iodine diet (15 or 30 days) and collected U24h and Ui samples previous to perform a WBS or a $^{131}$I ablation.

Our patients present high levels of iodine intake (21% of the healthy group had an above normal levels of iodine according WHO/ICCIDD) and the patients using L-T4 had even higher significant levels. The correlation between iodine in Ui and U24h was moderate before diet (r:0.38) and very strong after diet (r:0.71). We found that the average of iodine in Ui and U24h samples before diet were 27.09 and 25.27ug/dL and after diet 10.49 and 12.76 ug/dL, which corresponds to a decrease of 61% and 49.5%, respectively. There were no difference between 15 and 30 days when the decrease of iodine was evaluated. In conclusion, urinary iodine in spot samples correlated with the 24 hours sample, avoiding the discomfort of 24 hours collection. In addition, L-T4 used by patients increased the iodine pool.
MANAGEMENT OF PATIENTS WITH MILD HYPERCALCITONINEMIA AND SPORADIC THYROID DISEASE

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Background: Surgery in patients with mild hypercalcitoninemia (MHCT) and sporadic thyroid disease is questionable. The aim of this study was to evaluate the results of our experience of managing these patients.

Patients and methods: MHCT was defined as the elevation of basal CT (bCT) and stimulated CT (sCT) that does not exceed 30 pg/ml and 200 pg/ml respectively. In 15 years, 125 patients (97 men (77.6%) and 28 women (22.4%), mean age 53.2±13.4 years) with MHCT and sporadic thyroid disease were observed. Surgery was only indicated in patients presenting nodular goiter responsible for local pressure symptoms. All patients were reviewed or contacted by letter. bCT measurement in non-operated patients was repeated every 6 months during the first 2-3 years and then annually. Mean follow-up was 44.2±44.5 months (range 1-170 months).

Results: Mean bCT level was 17.6±5 pg/ml and mean sCT level was 79.1±51.1 pg/ml. 73 patients were operated on: 55 total thyroidectomies for multinodular goiter and 18 unilateral lobectomies for solitary nodule. Medullary microcarcinoma (1.6±0.7 mm) was found in 6 patients (8.2%): 2 females (20%) and 4 males (6.3%), and C-cell hyperplasia in 54 cases (74%). In 13 other patients (17.8%), no C-cell pathology was observed. No patients were operated on for elevation of CT levels. bCT levels were undetectable after total thyroidectomy and detectable (mean bCT-9±3.5 pg/ml and sCT-64±39 pg/ml), but stable during follow-up, after lobectomy. 52 patients were not operated on. Unexpectedly 17 (32.7%) had normalized CT level during follow-up. Mean bCT and sCT levels were 16.3±4.8 and 68.5±42 pg/ml respectively at initial measurement and 10.6±6.9 and 63.9±48 pg/ml at the end of follow-up.

Conclusions: The risk of missing medullary microcarcinoma leads to perform surgery in females, whereas systematic surgery in males is debatable.
THE BENEFIT OF MEASURING BASAL SERUM CALCITONIN IN TO DETECT MEDULLARY THYROID CARCINOMA IN A DANISH POPULATION WITH A HIGH PREVALENCE OF THYROID NODULES

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Background: Routine measurement of basal serum calcitonin (CT) to detect medullary thyroid carcinoma (MTC) is fiercely debated. Less attention has been paid to the positive predictive value of this method. We examined the validity of CT for detection of MTC in patients with nodular goitre living in a Danish region with mild to moderate iodine deficiency.

Subjects and methods: We collected, retrospectively, data on clinical findings, cytology, histopathology, biochemistry, and treatment from 959 consecutive patients with non-toxic nodular goitre examined at a secondary/tertiary university clinic from 1996 to 2003. CT was measured in 702 patients, and thyroidectomy was performed in 307 patients. The fate of the study cohort was examined by cross-checking our data prospectively with the Danish Thyroid Cancer Database, which covers all patients in Denmark with a malignant thyroid disorder.

Results: Thirty-nine patients had elevated CT, six of whom (five women, one man) had MTC detected by the initial diagnostic set-up; a prevalence of 0.63%. No additional patient in the cohort was registered in the Danish Thyroid Cancer Database, reflecting that all patients with MTC were classified correctly initially. The sensitivity of CT for detection of MTC using the upper limit for the reference interval (0.10 µg/L) was 100%, the specificity 95.3%, the positive predictive value 15.4%, and the negative predictive value 100%.

Conclusion: Basal calcitonin has a high sensitivity, specificity, and negative predictive value for detection of MTC and this method detects some cases of MTC overlooked by other diagnostic methods, among these fine needle aspiration biopsy. Nevertheless, the low positive predictive value, reflecting many false positive results, might lead to unnecessary thyroid surgery. Thus, the result of CT measurement should always be interpreted in the context of other clinical variables.
SERUM CT MEASUREMENT IS THE MOST IMPORTANT DIAGNOSTIC TOOL FOR THE EARLY DIAGNOSIS AND THE CURE OF MEDULLARY THYROID CANCER

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Early diagnosis of medullary thyroid carcinomas (MTC) is fundamental for its cure. We previously demonstrated that MTC patients diagnosed by serum calcitonin (CT) had a better outcome with respect to an historical group diagnosed at hystology or citology. One criticism to that study was that the worse outcome of the hystorical group could be due to the different era of diagnosis (i.e before neck ultrasound), different length of follow-up (i.e. much longer for the historical group) and the limited knowledge about MTC in the '80s.

The aim of this work was to confirm those results in two groups of MTC patients more homogeneous both for the era of diagnosis and the length of follow-up.

We studied 251 patients (146 females,105 males) with sporadic MTC diagnosed between 1991 and 2004 and followed-up to December 2008. They were subdivided in group-1 (n=123) diagnosed by CT screening and group-2 (n=128) diagnosed by cytology or histology: they were comparable for follow-up, age and sex distribution.

The comparison of the stage at the diagnosis showed that 78 patients of group-1 and 40 of group-2 had intrathyroid disease, 24 of group-1 and 27 of group-2 had lymph-node metastases and 21 of group-1 and 56 of group-2 had distant metastases or extrathyroidal infiltration (p< 0.0001).

The comparison of the disease status at the end of follow-up showed that 71 patients of group-1 and 35 of group-2 were free-of-disease, 21 of group-1 and 31 of group-2 had biochemical disease, 24 of group-1 and 39 of group-2 had evidence of metastases, 7 of group-1 and 23 of group-2 were dead for MTC (p< 0.0001). The Kaplan-Mayer analysis showed a significantly better survival (p=0.03) of group-1.

In conclusion, the routine CT screening has been confirmed an important tool for the early diagnosis and, as consequence, the cure of MTC.
DIFFICULTIES IN THE PRE-SURGICAL DIAGNOSIS OF THE MEDULLARY CARCINOMA OF THE THYROID

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Introduction: The medullary carcinoma of the thyroid (MCT) is a neuroendocrine tumor originated in the parafollicular (C) cells. It accounts for 2% to 5% of thyroid neoplasias. It may be sporadic (80%) or hereditary (20%).

Objective: To describe the difficulties encountered in the pre-surgical diagnosis of MCT in the Thyroid Centre.

Material and methods: Retrospective, descriptive study. In the period 1975-2008, one thousand eight hundred and ten surgeries were performed for thyroid pathology, 503 (27%) of which corresponded to carcinomas. Out of these, only 11 were MCT (2,2%).

Results: The average age of patients was 50 years old (30-37), being 9 of them female. Nine patients showed thyroid nodules (81%), one in the form of an easily perceptible cervical linfadenopathy (9,5%) and another one like bone metastasis (9,5%). Pre-surgical calcitonine was dosed in 3/11; in two of the patients, under the suspicion of a family disease, they were positive. A fine needle aspiration biopsy (FNAB) was performed in 9 patients, a ganglionar biopsy in one and a bone biopsy in the remaining one, which rendered findings compatible with MCT in only one FNAB (in agreement with the pre-surgical suspicion of hereditary neoplasia) and in the bone biopsy. The findings from the biopsy by freezing made MCT diagnosis possible in four cases, confirming the FNAB in one case and the bone biopsy in another. The other two cases were false negatives of FNAB. The eleven cases were confirmed by deferred biopsy and inmunohistochemistry.

Conclusion: The pre-surgical diagnosis of MCT is difficult. In our experience, it could only be established in two cases: one under the suspicion of hereditary illness and the other one by bone biopsy.
THE DIFFERENTIATED THYROID CARCINOMAS (DTC) DIAGNOSED IN THE LAST 15 YEARS SHOW DIFFERENT EPIDEMIOLOGICAL, CLINICAL AND PATHOLOGICAL FEATURES BUT THE SAME PROGNOSTIC FACTORS WHEN COMPARED WITH DTC DIAGNOSED BEFORE

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In the last years a relevant increase in the incidence of differentiated thyroid cancer (DTC) has been documented worldwide. This study was designed to evaluate the differences between “old” and “new” DTC in a large series treated at the University Hospital of Pisa (Italy) in the last 35 years. We analyzed 4187 DTC patients (3166 females, 1021 males), both as total series and separated into two groups: Group1 (n=1215) and Group2 (n=2972) diagnosed before and after 1990, respectively. A significant increase of small papillary thyroid cancer was observed in Group2. Age at diagnosis was similar while male gender was increased in Group2. A greater association with multinodular goiter and thyroiditis and a lower number of irradiated cases was found in Group2. The detection of a thyroid nodule was the presenting symptom in most cases of both groups. Larger tumors, lymph node, distant metastases and macro-extrathyroidal invasion at diagnosis were significantly less frequent in Group2. At variance, multifocality and bilaterality were more frequent in Group2. A statistically significant increase of the survival rate was observed in Group2 (98.7%) with respect to Group1 (91.4%). At univariate analysis gender, age, histotype, tumor size, extrathyroidal extension, lymph nodes, distant metastases and clinical class showed a statistically significant influence on survival both in the total series and in the two groups. At multivariate analysis only the older age at diagnosis and the presence of metastases (either lymph node or distant) independently correlated with a lower survival.

In conclusion, several epidemiological, clinical and pathological features of DTC patients are changed in the last 15 years and the “new” DTC appear to be less advanced at diagnosis and to have a better prognosis. However, as in the past, an advanced age and/or the presence of metastases, still represent poor prognostic factors for the survival of these patients.
PAPILLARY THYROID CARCINOMA ASSOCIATED WITH THYROID AUTOIMMUNITY: CLINICAL AND MOLECULAR CHARACTERIZATION

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Objectives: The clinical behaviour of papillary thyroid cancer (PTC) associated with thyroid autoimmunity is still debated and its genetic background is not known. To investigate this topic, a large series of PTCs associated or not with thyroiditis has been clinically evaluated and the genetic background of these two Groups of tumors was studied. In some cases, the thyroid tissue of the lobe controlateral to the tumor was also analyzed by means of RET and BRaf molecular analyses.

Results: No significant differences were found between the two Groups regarding either the clinical and pathological features, or the outcome. Interestingly, the molecular defects were significantly different among patients with PTC associated or not with thyroiditis (P=0.001), being ret/PTC1 significantly more represented in patients with PTC and autoimmunity, and BRaf in patients with PTC alone. A ret/PTC rearrangement was also found in 41% of non-neoplastic thyroiditis tissues controlateral to tumors harbouring either ret/PTC or BRaf or none mutations. In addition, the expression of the chemokines CCL20 and IL-8 and the adhesion molecule L-selectin, has been tested in tumor and normal tissues. These genes were found to be significantly up-regulated in tumor samples, independently either from the oncogene involved or the association with thyroiditis, with respect to normal tissues (P< 0.05).

Conclusions: The present findings extend the knowledge about the tight relationships between oncogenes, thyroiditis and thyroid cancer. A significantly different genetic background between PTCs with or without associated autoimmunity was firstly demonstrated. The up-regulation of genes involved in inflammation and tumor invasion, indicates that PTC activates a transcriptional program related to inflammation, well in agreement with recent findings in human primary thyrocytes (PNAS, 2005). Moreover, the presence of ret/PTC in inflammatory tissues associated with non-ret/PTC tumors, indicate that inflammation could predispose to carcinogenesis even if this is driven by different genetic alterations.
DIFFUSE SCLEROSING VARIANT OF PAPILLARY THYROID CARCINOMA, A 30-YEAR RETROSPECTIVE STUDY AT A SINGLE INSTITUTION

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Objectives: Diffuse sclerosing variant (DSV) is a rare variant of papillary thyroid carcinoma (PTC). The aim of this study was to evaluate the clinical data and outcome of a large cohort of patients with DSV.

Methods: We retrospectively analyzed the clinicopathologic features and outcome of patients diagnosed with DSV referred to our Institute between 1974 and 2004.

Results: Twenty four patients (19 women and 5 men) with DSV of PTC were identified, representing less than 1% of the patients with PTC referred to our Institute in the same period of time. Patients presented with a mean age of 26 years (± 13.6 years), with a mean follow up of 13.4 years. None of the patients had family history of PTC. One patient had previous history of cervical irradiation. At presentation 62% of patients had tumour size of T4a, 88% had lymph nodes metastases (LNM) and 8% had lung metastases. Seventy five percent underwent total thyroidectomy (TT) and bilateral lymphadenectomy for bilateral LNM, 12.5% underwent total thyroidectomy and unilateral lymphadenectomy and 12.5 % underwent total thyroidectomy only. Seventy nine percent of the patients were treated with radiodine. After primary therapy 63% (n= 15) had disease recurrence. Eight of these patients had LNM, two had lung and LNM, two had local recurrence and one patient had bone, lung and LNM. Only this last patient died of the disease. At the last observation 83% had no evidence of disease.

Conclusions: DSV presents mainly in young patients and with a higher prevalence of LNM and lung metastases. Such aggressive behaviour frequently requires more than one surgical intervention in some patients. Despite disease recurrence the outcome is usually favourable.
RESULTS OF 10 YEARS FOLLOW UP OF MORE THAN 1500 SURGICALLY TREATED THYROID CANCER PATIENTS

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Objectives: Considering favorable prognosis for patients radically operated on thyroid carcinoma (TC) real evaluation of treatment efficacy needs careful and long term follow up of large clinical group using standard protocol and database.

Methods: Results of surgical treatment of 1366 (76%) from 1797 primary cases of TC in single institution within 1996-2005 were analyzed. Survival, relapses and distant metastases were calculated separately for each morphologic form of TC and for each clinical stage of patients (TNM, 2002). Mean time of follow up was 6.9±2.2 years (3-12 years). Papillary and follicular carcinomas comprise 85% and 9%, medullary - 4.5%, anaplastic and poorly differentiated - 1.5% of all cases of TC.

Results: Good overall 10-years survival (92%) was contributed by favorable outcomes of papillary (93% survival) and follicular (90%) TC. Survival for medullary carcinoma was 80% and 67% at 5 and 10 years after operation. Only 4 from 7 patients with poorly differentiated TC survived more then 1 year and no one with anaplastic TC. Rate of distant metastases was 4.6%, 9.7%, 34% and 100% among papillary, follicular, medullary and poorly/undifferentiated TC correspondingly.

All patients with I stage of medullary TC achieved biochemical cure (calcitonin negative), whereas 40% and 86% patients of II and III stage shown elevated serum calcitonin. There were no significant differences in outcomes of stage adjusted groups radiation-induced and sporadic cases of TC.

Conclusions: The vast majority of TC cases (94%) was represented by differentiated papillary and follicular carcinomas with good long term outcomes unless older patients with T3-4 or N1b or M1 tumors. Adequate radical primary operation and radioiodine treatment facilitate management and provide better results. Early detection and radical operation of patients with medullary TC have the major impact on disease free survival. Poorly differentiated and anaplastic TC still demonstrate extremely bad prognosis.
THYROID CANCER RISK IN A COHORT IRRADIATED IN THE CHILDHOOD FOR TINEA CAPITIS TREATMENT

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Introduction: Scalp irradiation to induce epilation for tinea capitis treatment was applied to a cohort of 5358 children in the northern Portugal in the years 1950-1960.

Objective: Due to the well known carcinogenic effect of ionizing radiation our aim was to study the prevalence of thyroid pathology in this cohort in both retrospective and prospective analyses.

Material and methods: From a cohort composed of 5358 elements, 2488 individuals were contacted by mail or phone and a clinical observation was proposed to register the clinical history. To every individual a thyroid ultrasound scan and a serum calcium determination were advised. A FNAB was suggested to the individuals presenting clinical thyroid nodules smaller than 15 mm with scan suspicious characteristics, and to all the individuals with nodules larger than 15 mm.

Results: So far, we have clinically observed 800 individuals that did not differ significantly from the original cohort regarding gender and irradiation dose; they differ, however, regarding the age at irradiation [we have observed more individuals with younger age at time of irradiation (p = 0.0008)]. The analysis of ultrasound scans, FNAB reports and clinical characteristics of the nodules (dimensions, calcifications, hipoecogenicity), led to the suggestion of 27 thyroidectomies (or thyroidectomy completion). In total, 53 from the 800 (6.6%) observed individuals were submitted to thyroidectomy, having 37 thyroidectomies been performed previously to our clinical observation. Histology reports showed that 19 out of the 800 individuals (2.4%) had papillary thyroid cancer, 9 of which were diagnosed in the present study.

Conclusion: The high prevalence of papillary thyroid carcinoma found in this study of irradiated individuals is in accordance to the higher risk referred by others in irradiated patients and justify a close follow-up of the cohort in order to be able to identify the lesions in an early phase.
P162
PAPILLARY THYROID CARCINOMA AND THYROGLOSSAL DUCT CYSTS: A RETROSPECTIVE ANALYSIS
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Objectives: To evaluate papillary thyroid carcinomas (PTC) associated with thyroglossal duct cysts (TDC), their staging, management, long term follow-up and prognosis.

Materials and methods: Clinical files from our database since 1974 through 2008, were evaluated, taking into account medical and pathology records.

Results: From 3458 patients (pts) with PTC, 22 pts (0,63%) had PTC of TDC: 4 male and 18 female; mean age: 36.9 years (range 15-63 years).
All pts underwent Sistrunk procedure, 14 underwent thyroidectomy (64%) and from those 11 were ablated with Iodine-131.
Seven out of 22 pts proved to have tumour both in thyroid gland and TDC (32%).
Tumour staging: pT1 (n=8), pT2 (n=6), pT3 (n=1), pN0 (n=1), pN1a (n=2), pN1b (n=2), pNx (n=17), M0 (n=21), M1 (n=1).
Lymph node metastases were found in 4 of 5 pts (80%).
After a mean follow-up of 5 years (range 1-23 years), 14 pts are still under evaluation. Eight pts were lost to follow-up.
Ultrasound, when performed, did not show evidence of local recurrence.
Only 1 pt had lung metastases.
No tumour associated mortality was observed.
Serum thyroglobulin (Tg) levels at the last evaluation of 17 pts were: < 0,2 ng/ml in 10 pts, < 2 ng/ml and > 0,2 ng/ml in 2 pts and > 2 ng/ml in 5 pts.
From the 14 pts who underwent thyroidectomy (n=14) only 1 pt had Tg>2 ng/ml (14,1 ng/ml).

Conclusions: In the present series TDC carcinoma is a rare malignant tumour in agreement with the literature.
It is usually diagnosed postoperatively and sometimes co-existing with carcinoma in the thyroid gland.
The prognosis in the vast majority of pts with carcinoma in the TDC is good.
Differentiated thyroid cancer (DTC) is a rare disease in general population and also in children. As compared to adult forms, larger sizes, multifocality and bilaterality, extensive disease with frequent neck lymph and lung metastases at diagnosis are more commonly observed in them. However, prognosis of DTC in children and adolescent isn't as bad as it would be expected to be regarding the initial aggressive behaviour and a low mortality rate with high disease free rate has been described.

Objective: The aim of this retrospective study was to describe the management and outcome of 70 patient with DTC diagnosed during childhood and followed during a long period of time in order to verify that good prognosis.

Patients and methods: We retrospectively reviewed the medical records of 70 patients in whom DTC was diagnosed from 1975- 2005, all under 20 years old. Total thyroidectomy was performed and postoperative radiiodine ablation was given in all cases. Lymph node dissection was performed if enlarged nodes were present. We report age, sex, histological features of tumour, staging following TNM criteria for CDT, long term management and situation of disease. We defined complete remission when WBS (posttherapy and diagnostic) do not evidenciated disease and serum TG level in hypothyroidism was < 2 ng/ml.

Results: There were 51 females (female/ male ratio 5:1); mean age 17.12±3.31years. Papillary histology was present in 79.7%, follicular 16.9%. We found lymph node metastases in 44.8 % of patients. 31.1% were classified as Stage II TNM, in of all them metastases were pulmonary ones. We followed them for a mean of 186.25 months. There were no deaths and to date 56.7% are disease free.

Conclusions: CDT in childhood and adolescent has a good prognosis in spite of his aggressive initially behaviour. The response to treatment is also good, especially small lung metastases.
CLINICAL AND HISTOLOGICAL FEATURES OF PTC DIAGNOSED DURING 15 YEARS (1990-2004)

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Differentiate thyroid carcinomas are the most common endocrine malignancy accounting for 1% of all malignancies diseases and represent the 7th cancer most common in women. Papillary Thyroid Carcinoma (PTC) when diagnosed and treated early has good prognosis with survival of 90% at 10 years.

Objective: We evaluate clinical and histological features in 1066 PTC diagnosed during 15 years.

Materials: 1066 PTC comprised 486 PTC<1cm (groupA) and 580 PTC>1cm (groupB) and, using logistic regression analysis, we asses the associations between 2 group of PTC and the following variables:
1) age at diagnosis;
2) sex;
3) years of surgery;
4) multi-focal tumor;
5) infiltration of extra-thyroid tissue;
6) metastatic lymph-nodes.

The chi-square and student's t tests were used to describe the observations.

Results: Descriptive analysis showed that the percentage of males in groupB was significantly higher compared in groupA (21,6% vs 14,0% p=0002) even more frequently in women; The mean age at diagnosis in groupB was significantly lower than in groupA (44,5 vs 47,5 years p< 0001); Cases whit a PTC ≤ 1 cm were significantly more frequent after 1997 (61,1% vs 46,9% p< 0001); multi-focal tumor, infiltration of soft tissue and lymph-nodes metastases were significantly (p< 0001) more frequent in groupB than in groupA (22,4% vs 32,1%, 27,8% vs 48,4%, 17,5% vs 30,7%). ODDS-Ratios show that multi-focal tumor (OR:1,98; CI:1,36-2,88; p< 0001) and infiltration of extra-thyroid tissue (OR:2,91; CI:2,1-4,0; p< 0001) were significantly associated to PTC>1cm. On the contrary, the interaction between multifocal tumor and infiltration of extrathyroid tissue was significantly associated to PTC ≤ 1 cm (OR:0,46; CI:0,26-0,82; p< 0009).ODDS-ratios we estimated adjusted for gender and age at diagnosis.

Conclusion:
1) According to what is already present in the literature, our data show a strong association among multifocality, extra-thyroid tissue infiltration and the larger size of PTC
2) A reduction of tumor-size at diagnosis in the years following 1997 could be explained by the introduction, at this time, of improved ultrasound probe with more diagnostic sensitivity.
INCIDENTAL VERSUS NON INCIDENTAL PAPILLARY THYROID MICROCARCINOMA (PTMC) IN A SERIES OF 259 PATIENTS

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Introduction: The recognition of thyroid microcarcinoma has increased exponentially in recent years probably as a result of the widespread use of ultrasound-guided fine-needle aspiration biopsies (FNAB) of small non palpable nodules and possibly of a more extensive histological examination.

Objectives: To describe and compare histological and clinical characteristics of papillary thyroid microcarcinoma between patients with incidental and non incidental PTMC.

Methods: Retrospective analysis of clinical and pathological data of all patients operated on for thyroid cancer at our institution between 2006 and 2008. We defined incidental PTMC as those found in patients operated on for nodular goiter or Graves disease and non incidental PTMC as those found in patients with a FNAB diagnostic or suspicious of malignancy or false negative. Statistical analysis was done with SPSS 16.0 for Windows.

Results: During this period, 526 patients were diagnosed and treated for thyroid cancer at our institution and among these 259 (47.5%) had a thyroid cancer ≤ 1cm. These included 169 patients with Incidental PTMC (I) and 184 with NonIncidental PTMC (NI). When comparing these groups according to gender, age at diagnosis (< 45 or ≥45 years), tumour diameter (< 5mm or ≥5 mm), coexistence of Hashimoto’s thyroiditis, multifocality/multicentricity, capsular invasion, vascular invasion, extrathyroidal extension and lymph node involvement we found differences between them in tumour size [120 (60.6%)< 5mm in I vs 78 (39.4%) in NI; p< 0.001], vascular invasion [7 in I (18.4%) vs 31 (81.6%) in NI; p< 0.001], extrathyroidal extension [8 in I (22.9%) vs 27 in NI (77.1%), p< 0.005], multifocality [55 in I (26.2%) vs 155 in NI (73.8%); p< 0.001].

Conclusions: In this series PTMC is a prevalent condition and patients with nonincidental PTMC have a higher frequency of multifocality/multicentricity, vascular invasion and extrathyroidal extension.
Introduction: Differentiated thyroid carcinoma (DTC), although rare, is the most common endocrine malignancy in children.

Objective: To characterize the clinical presentation, treatment and outcome of DTC in patients who were less than 21 years and received treatment and follow-up at the Portuguese Cancer Centre, in Lisbon, from January 2001 to December 2004.

Material and methods: The authors searched the hospital-based tumour registry and undertook a retrospective analysis of clinical records of 32 patients aged ≤ 20 years.

Results: 87.5% were girls. The median age at diagnosis was 18 ± 3.9 years (range, 5-20 years); four children were ≤ 10 years. There was a history of prior irradiation in two cases and two patients had a family history of thyroid cancer. An asymptomatic neck tumour was the most common presentation. Results of preoperative aspiration cytology were available in 27 patients (papillary carcinoma - 19, follicular neoplasia - 7, benign - 1). Total thyroidectomy, in one or two steps, was performed in all but one case with concomitant neck dissection in 17 cases (bilateral in 5). Histopathologic subtypes included papillary (97%) and follicular (3%) carcinomas. Neck lymph nodes were positive in 38% and extrathyroidal extension occurred in 31%. Adjuvant therapy with $^{131}$I was offered to 26 patients. Distant metastases (lungs in all cases) were documented in post therapeutic studies in 11%. Median follow-up (n=28) was 72 ± 16.7 months. Four patients were submitted to further surgeries; 12 had two or more $^{131}$I therapies. At last observation, 57% were free of disease; 14% had residual disease (detectable tyroglobulin and negative ultrasonography) and 29% had persistence of disease; 12,5 % developed hypoparathyroidism.

Comments: Childhood DTC is associated with more locally aggressive disease and more frequent distant metastases. Lungs are almost the sole distant metastatic site. Total thyroidectomy and $^{131}$I therapy are effective treatments.
Objective: The risk of secondary carcinoma following radiotherapy can be difficult to quantify with few series being published. We aim to review cases of secondary thyroid carcinoma and the therapy-associated risk factors.

Methods: All patients were on long term follow up at The Christie Hospital, following childhood radiotherapy to the head and neck region. Patients were referred via the thyroid carcinoma multidisciplinary team for radioiodine treatment. Notes were reviewed and treatment regimens studied. All cases were checked to ensure that Cahan's criteria as a definition of radiotherapy induced secondary malignancy were met with all thyroid malignancies occurring within the treatment field.

Results: 11 patients identified with radiotherapy given between 2 months to 24 years old (average 9.2 years). 5 patients had initial diagnosis of acute lymphoblastic leukaemia (2 of these also received intrathecal Methotrexate) with others having lymphosarcoma, metastatic Wilms' renal carcinoma, Astrocytoma, Hodgkins, Haemangiolyphangioma and Medulloblastoma. Thyroid carcinoma developed with a latency of 16 to 44 years (average 26.6 years). Most cases were detected clinically with only one patient having regular ultrasound scans for hypothyroidism. The thyroid malignancy identified was mainly papillary carcinoma (9 cases), follicular or mixed in 1 case each. 10 cases stage 1 and 1 case stage 3. Average radiotherapy dose received was 24 Gy in 2 Gy per fraction with thyroid in the field. Dose regimens ranged from 4Gy in 1 fraction to 42.5Gy in 16 fractions. All patients are currently still alive except 1 who died of carcinomatosis, unknown primary.

Conclusions: The majority of our patients presented as early stage 1 papillary carcinoma, detected clinically with no biochemical abnormality. These results indicate the importance of clinical examination and early detection of thyroid carcinoma which, in our series occurred on average 24 years following initial radiotherapy.
PAPILLARY THYROID MICROCARCINOMA: A STUDY OF 187 CASES
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Introduction: Papillary thyroid microcarcinoma (PTMC), defined as papillary thyroid carcinoma measuring 10 mm or less in diameter, is being diagnosed with increasing frequency.

Objectives: To investigate the clinical behaviour of PTMC.

Methods: We retrospectively analysed the clinical records of 187 patients with PTMC followed in our department, for a minimum period of 1 year. Data analysis was performed using Fisher’s test; P value below 0.05 was considered significant.

Results: The female-to-male ratio was 4.5/1.0 and the mean age at diagnosis was 49.8±26.0 years (range: 19-79 years). One hundred and seventy-four patients (93%) were treated with total or near-total thyroidectomy. Radioiodine ablation was performed in 76 (40.6%). We found multifocal disease in 56 (30%) and extrathyroidal extension in 17 (9.1%). At presentation, lymph node metastases were observed in 39 patients (20.9%). After a mean follow-up of 7.1±8.2 years (range: 1.3-35.9 years), 180 patients (96.2%) were living without evidence of disease and 5 (2.7%) had persistent disease. There was not disease-specific mortality. Among the 5 patients with persistent disease, all were older than 45 years at diagnosis, 4 had lymph node metastases, 3 had multicentre disease and 2 had extrathyroidal extension at histology. Lymph node metastases were associated with higher risk of persistent disease (p< 0.008). Seven patients underwent further treatment (radioiodine, lymphadenectomy or external radiotherapy). We found distant metastases (bone and lung) in 1 patient.

Conclusions: Our study confirmed that PTMC has an excellent prognosis and was not associated with any mortality. The presence of lymph node metastases at diagnosis was associated with higher risk of persistent disease and can be considered an unfavourable prognostic factor. Distant metastases are extremely rare.
TRENDS IN THE INCIDENCE OF DIFFERENTIATED THYROID CANCER OVER A PERIOD OF 13 YEARS IN A SINGLE INSTITUTION

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Background: Recently it has been reported, both in Europe and North America an increased incidence of differentiated thyroid cancer, mainly due to an increased detection of small tumours.

Objectives: The purpose of this study was to investigate the relation between rates of detection, tumour size, age and sex of patients diagnosed with thyroid cancer at a large university hospital in the North of Portugal, during a period of 13 years (1996 - 2008).

Methods: We evaluated retrospectively the pathology reports of all patients with a definitive diagnosis of thyroid cancer submitted to surgery in our institution during the last thirteen years. Tumours were classified by size, according to TNM classification 6th edition. Statistical analysis was done with SPSS 16.0 for Windows.

Results: During this period a total of 1472 patients had a pathological diagnosis of thyroid cancer, 1273 women and 199 men, with a mean age of 49,5±14,5 and 51,5±15,3 years old, respectively. Tumour size was ≤ 2 cm in 1037 (70,6%), 2-4 cm in 293 (19,9%), > 4 cm in 139 (9,4%) and unknown in 3 cases. Age at diagnosis was ≤45 years in 572 (38,9%) and > 45 years in 900 (61,1%) patients. As expected the number of cases increased over this period. The proportion of small tumours (≤2 cm) increased from 48,1% in 1996 to 74,3% in 2008 (p=0,025). When examining differences in tumour detection rate by age and sex we found a significant increase in the number of tumours detected in men and in patients older than 45 years.

Conclusions: These findings suggest an increased detection rate of small tumours, as we have previously demonstrated, which probably reflects the widespread use of diagnostic imaging and greater detection of small, nonpalpable thyroid nodules.
Amiodarone-induced thyrotoxicosis (AIT) due to destructive thyroiditis (type 2) is frequently treated with glucocorticoids; however, recent surveys showed that only 45-50% of expert thyroidologists from ETA, ATA or LATS considered glucocorticoids as a first-line therapy in type 2 AIT.

Patients and methods: Forty-two untreated type 2 AIT patients were included in a historical prospective study. 21 patients were treated with metimazole and KClO₄ (initial dose 40 mg/day and 1 g/day respectively) (GROUP 1) and 21 patients were treated with prednisone (initial dose, 0.5 mg/kg/day) (GROUP 2) for 40 days. Patients were selected according to the formula obtained from a multiple regression models previously described, featuring a mean cure probability ≤ 40 days. The two groups did not differ as for mean age, weight, height, BMI, thyroid volume (measured by ultrasonography), basal serum FT4 and FT3 concentration, thyroidal RAIU (3°h and 24°h), urinary iodine excretion, duration of amiodarone treatment and cumulative dose of amiodarone. Group 1 patients with persistent thyrotoxicosis after 40 days were treated with glucocorticoids and followed accordingly.

Results: In 16 out of 21 patients treated with glucocorticoids (76.2%) euthyroidism was restored within 40 days at variance with 3 out of 21 patients treated with MMI + KClO₄ (14.3%) (p< 0.0001). Eighteen group 1 patients who were not cured with antithyroid drugs were treated with prednisone. Among the latter 18 patients, 16 reached euthyroidism within 40 days of glucocorticoid therapy; one patient was treated with total thyroidectomy after 40 days owing to worsening of underlying cardiac disease and another patient became euthyroid after 90 days of glucocorticoid therapy.

Conclusion: Glucocorticoids are more effective than metimazole and potassium perchlorate in restoring euthyroidism in patients with type 2 AIT and they should be considered as first-line treatment of this drug-induced destructive thyroiditis.
Introduction: A limitation for $^{131}$I therapy of compressive non-toxic goitres is a low thyroid radioiodine uptake (RAIU), often encountered in these patients. The thyroid RAIU is for a major part determined by the dietary iodine intake while the interaction between other factors is incompletely understood.

Methods: We examined prospectively 170 patients (146 females; age: 22-87 yrs.) with a compressive nodular goitre (median 64 ml, range: 20-464 ml) who were selected for $^{131}$I therapy. The thyroid RAIU was determined at 24h and 96h after oral administration of a tracer activity of 0.5 MBq $^{131}$I. The goitre volume was measured by ultrasound (n=127), or by MRI (n=43, patients with a goitre size >100 mL).

Results: The 24h and the 96h RAIU were 34.2±9.8(SD)% (range:11.4-66.0%) and 34.0±10.0% (range:10.5-60.9%), respectively, and were highly correlated ($r = 0.935$, p< 0.001). Overall, s-FT4index was positively correlated to the 24h RAIU ($r=0.20$, p=0.011), while age correlated negatively to this variable ($r=-0.18$, p=0.02). S-TSH was unrelated to the 24h RAIU ($r=-0.05$, p=0.51), as was the thyroid volume ($r=-0.04$, p=0.57). The latter variable was highly related to age ($r=0.31$, p< 0.001). In a regression analysis - including age, s-FT4index, s-FT3index, s-TSH, thyroid volume, sex, and smoking - s-FT4index was positively, and age negatively correlated with the 24h RAIU, as the only independent determinants. A similar finding was seen with the 96h RAIU.

Conclusion: In patients with a compressive nodular non-toxic goitre, the level of serum T4 and age - among other routine clinical variables - are the major determinants of the thyroid RAIU. A low- or sub-normal serum TSH is not a marker of a compromised thyroid RAIU but reflects a slight hyperthyroid state due to autonomously functioning nodules. However, the thyroid RAIU in these cases may be confined to a few 'hot spots' surrounded by dormant thyroid tissue.
OUTCOME OF PATIENTS WITH AUTONOMOUS THYROID NODULES AFTER RADIOIODINE THERAPY: A COMPARISON BETWEEN PATIENTS WITH SUPPRESSED AND NON-SUPPRESSED EXTRANODULAR THYROID TISSUE

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Objectives: Radioiodine represents an effective therapy for hyperthyroidism due to hot nodules, although the risk of hypothyroidism is a possible outcome. The purpose of this work is to access the impact of scintigraphic extranodular radioiodine uptake in the incidence of hypothyroidism in two groups of patients with autonomous nodules, one with suppressed extranodular thyroid 131I uptake and the other with extranodular activity.

Methods: From May 2004 to December 2007, 76 patients (67 women, 9 men; 26-91 years, average 63.9 years) with nodular hyperthyroidism received radioiodine treatment, 52 with solitary autonomous nodule and 24 with toxic nodular goiter. Patients were divided into two groups according to the scintigraphic findings: group I of 51 patients with hot nodules that inhibits extranodular uptake and group II including 25 patients with nodules that fails to suppress normal thyroid tissue. The mean calculated activity of 131I was 492,1MBq (13,3mCi). Thyroid function was followed up with TSH, FT3 and FT4.

Results: In group I, 41/51 patients (80,4%) achieved euthyroidism within 6 months, 2/51 (3,9%) maintained hyperthyroidism and 8/51 (15,7%) became hypothyroid (6 of these with subclinical hypothyroidism with only a slightly increased in TSH) but the latter had large goiter with extensive multinodular disease. In group II we found 14/25 euthyroid patients (56%), 1/25 (4%) hyperthyroid and 10/25 (40%) hypothyroid.

Conclusions: Radioiodine treatment for toxic thyroid nodules is related to a high incidence of euthyroidism, with an overall incidence of 72,3%. The failure rate is similar in both groups (~4%) but the incidence of hypothyroidism is significantly higher in group II due to irradiation of incomplete suppressed normal thyroid tissue. In patients with this scintigraphic pattern, hypothyroidism could be the outcome after 131I therapy.
Objectives: The aim of this study was to determine an optimal radioiodine treatment by comparing the clinical and biochemical outcome of patients with Graves disease treated with estimate doses of radioiodine to those treated with fixed doses.

Material and methods: A total of 116 consecutive patients of thyrotoxicosis treated with J-131 were divided into two groups according to the amount of J-131 administered: I group - 67 patients who received estimated dose of 111-550 MBq (mean 230 MBq), II group - 49 patients who received fixed dose of 370 MBq. All patients were treated with antithyroid drugs and were stopped one week before using radioiodine therapy. In 41.4% (48/116) patients with recurrent disease were given an additional dose (total dose 710 MBq). The outcome were followed from 6 months to 18 years (median 11.3 years).

Results: At six months after single dose of radioiodine therapy, euthyroid state was achieved in 36% patients in the fixed dose group and 51% patients in estimated dose group. There was significant difference in the rate of development of euthyroid state in respect to the given dose of J-131 (p< 0.05). 39% patients in the fixed dose group became hypothyroid, compared to 20% patients in estimated dose group. The median time to hypothyroidism was 26 weeks. 23% patients in fixed dose group remained hyperthyroidism six months after radioiodine therapy, compared with 29% patients in the estimated group. In that group significantly more patients who received low (150-220 MBq) doses remained hyperthyroid, compared to those who received high (< 370 MBq).

Conclusions: There was significant difference in outcome between the two treatment regimes. Better control of thyroid states can be achieved by estimated J-131 dose, while fixed radioiodine offers an affective treatment option where is required rapid control of thyrotoxicosis and long term hypothyroidism is a recognized outcome.
P174
THE BLOOD IN THYROTOXICOSIS BEFORE AND AFTER ACHIEVEMENT OF EUTHYROIDISM
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Thyrotoxicosis may affect blood in several ways, although clinically important abnormalities are usually rare.

Aim: To evaluate changes in blood and coagulation - hemostasis parameters in hyperthyroidism.

Patients and methods: Twenty-five thyrotoxic patients (P), 14 females (F) and 11 males (M) 41.6 ±13.6 yrs, and 17 controls (C), 6 F, 11 M of similar age were investigated. Anti-platelet and anti-white blood cells antibodies were negative both in P before treatment (PB) and C. The following parameters were measured in all individuals studied: HT, HB, red cell morphology (RCM), PTL, WCC, POL, LYM, bleeding time (BT), coagulation time (CT), partial thromboplastin time (PTHRT), prothrombin time ratio (PTR), thrombin (THR), antithrombin (ATHR), factor VIII, fibrinogen (FB), fibrinogen degradation factor (FDF), erythropoietin (ERP), ferrum economy indexes (FEI: ferum, total iron binding capacity, ferritin) and vitamin B12 (VB). In thyrotoxic patients, all the above measurements were repeated after they reached euthyroid stage (PA), either with RAI (P1 = 14) or antithyroid drugs (P2 = 11 patients).

Results: All the results were within normal range. However, when we compared the results of P with C some significant differences were observed (Table). There were also some negative significant correlations between TT3 and TT4 with CT, BT, PTL, TT3 with POL and TT4 with HB and positive between TT3, HT, HB and PTR.

<table>
<thead>
<tr>
<th></th>
<th>WCC</th>
<th>POL</th>
<th>LYM</th>
<th>CT</th>
<th>PTR</th>
<th>ATHR</th>
<th>VIII</th>
<th>FDF</th>
<th>ERP</th>
</tr>
</thead>
<tbody>
<tr>
<td>C vs. PB</td>
<td>C&gt;P*</td>
<td>C&gt;P*</td>
<td>C&lt;P+</td>
<td>C&gt;P+</td>
<td>C&gt;P*</td>
<td>NS</td>
<td>C&gt;P#</td>
<td>C&lt;P*</td>
<td>C&lt;P*</td>
</tr>
<tr>
<td>C vs. PA</td>
<td>C&gt;P+</td>
<td>C&gt;P+</td>
<td>C&lt;P+</td>
<td>NS</td>
<td>C&gt;P#</td>
<td>C&gt;P*</td>
<td>C&gt;P+</td>
<td>NS</td>
<td>C&lt;P#</td>
</tr>
<tr>
<td>PB vs. PA</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>PA&gt;PB*</td>
<td>NS</td>
<td>PB&gt;PA*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

[+=p< 0.05, #=p≤0.01, *=p≤0.001, NS=non significant]

Conclusions: Thyrotoxicosis does not produce essential hematological abnormalities but only minor alterations in the formed elements of the blood and also in some parameters and factors of hemostatic mechanism. These changes require no specific intervention or treatment.
IMPEDANSOMETRICAL ESTIMATION OF INTRAHEPATIC BLOODCIRCULATION CHANGES IN PATIENTS WITH THYROTOXICOSIS

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Background: The increase of metabolic necessity in patients with thyrotoxicosis leads to the development of hepatical ischaemia and is accompanied by morfofunctional changes in hepatocells.

Aim: Assess the changes of intrahepatical bloodcirculation in patients with thyrotoxicosis.

Equipment and methods: 21 patients with thyrotoxicosis aged 31-68 years were examined. Clinical investigation included the observation over the levels of thyrotropic hormone (TSH), free thyroxin (fT4) and free triiodothyronine (fT3). The intrahepatical bloodcirculation was examined with diagnostic complex "Polyreocardiograf-01,Perm". The index of liquid volume of hepatical sinusoid (ILV l/m2) was determined during the examination. The arterial bloodcirculation was assessed by hepatical index (HI l/min/m2) taking in consideration arteriosinusoidal perfusion index (ASPI, conventional units). 25 almost healthy patients formed the control group.

Results: The rates of HI (0,43±0,08 l/min/m2 and 0,11±0,01 l/min/m2 respectively), ILV (1,00±0,10 l/m2 and 0,53 ±0,04 l/m2 respectively) and ASPI (0,41±0,05 conventional units and 0,20±0,01 conventional units respectively) were reliably higher in patients with thyrotoxicosis than in almost healthy patients group. The rates of HI and ASPI correlated with the thyrotropic hormone level.

Conclusion: Thyrotoxicosis is accompanied by a compensatory arterialization of hepatic sinusoids which depends on thyroidal status.
LOW TSH LEVELS AND BONE MINERAL DENSITY IN ELDERLY WOMEN AND MEN

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Hyperthyroidism in the elderly may have severe complications as well as osteoporosis and both diseases can easily be missed in patients older than 65 years.

Objectives: To study the effect of low TSH levels on the bone mineral density (BMD) and the prevalence of low bone mass in elderly women and men.

Methods: In two groups of 62 women (73.0±5.1yrs) and 16 men (70.3±4.9yrs), with low TSH blood concentrations, the BMD (g/cm²) were evaluated at the lumbar spine, hip and distal forearm, by dual X-ray absorptiometry using the Hologic QDR Discovery W densitometer. According to the T-score of the BMD obtained in at least in one of those skeletal sites, the groups were divided in normal, low bone mass or osteoporosis subgroups. No patient was previously treated for osteoporosis or low BMD. Fast blood collection was also performed to measure the TSH, FT4 and FT3 levels. BMI was calculated.

Results: In the elderly women group, osteoporosis was detected in 69.4% (n=43, which were the oldest, P=0.018), low bone mass in 24.2% (n=15) and a normal BMD just in 6.4% (n=4) of these patients. In the group of men, low bone mass was observed in 43.7% (n=7), osteoporosis in 18.8 (n=3) and the BMD was normal in 37.5% (n=6); nevertheless, the osteoporosis prevalence is similar to the detected in the general population of males. The mean hormone levels were identical among the subgroups and no relationship between the BMD measured at several regions and the hormone blood levels were detected.

Conclusions: The results of this study suggest that low TSH levels, frequently a subclinical hyperthyroidism in elderly people, may be an important risk factor to increase the bone mass loss (mainly in women), which in association with the muscle wasting and weakness, may increase the risks of falls and osteoporotic fractures.
SEVERE ADVERSE ANTITHYROID DRUGS EFFECTS - EXPERIENCE OF GARCIA’S THE ORTA ENDOCRINOLOGY DEPARTMENT

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Introduction: Antithyroid drugs (ATD) are the mainstay therapy of hyperthyroidism due Grave's disease, in Europe. Although ATD are generally safe, side effects (classified as minor or major, based on their degree of morbidity), may limit their usefulness.

Objective: Evaluate the major ATD side effects assisted in the department from 2000 to 2008. Methods: Prospective study. Data obtained from clinical charts.

Results: 32 inpatients admissions were related with uncontrolled hyperthyroidism. All patients received ATD as a primary treatment or as a preparation protocol to $^{131}$I or surgery. Six patients (18.7%) had severe ATD side effects as described in table-1.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex / Age (years)</th>
<th>Diagnosis</th>
<th>Drug Dose (mg)</th>
<th>Time lapse (days)</th>
<th>Reaction type</th>
<th>Alternative treatment</th>
<th>Evolution</th>
<th>ICU</th>
<th>Disclosure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F 61</td>
<td>Toxic Goiter</td>
<td>PTU 300</td>
<td>58</td>
<td>Agranulocitosis</td>
<td>MTZ</td>
<td>Improve</td>
<td>No</td>
<td>Controlled</td>
</tr>
<tr>
<td>2</td>
<td>F 52</td>
<td>Grave's Disease</td>
<td>PTU 400</td>
<td>270</td>
<td>Polyarteritis</td>
<td>MTZ/I</td>
<td>Improve</td>
<td>No</td>
<td>Cured</td>
</tr>
<tr>
<td>3</td>
<td>F 24</td>
<td>Grave's Disease</td>
<td>MTZ 30</td>
<td>31</td>
<td>Cholestatic liver</td>
<td>I</td>
<td>Improve</td>
<td>Yes</td>
<td>Cured</td>
</tr>
<tr>
<td>4</td>
<td>F 56</td>
<td>Grave's Disease</td>
<td>MTZ 40</td>
<td>5</td>
<td>Cholestatic liver</td>
<td>PTU</td>
<td>Vasculitis</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>5</td>
<td>F 48</td>
<td>Grave's Disease</td>
<td>PTU 150</td>
<td>105</td>
<td>Agranulocitosis</td>
<td>Thyroidectomy</td>
<td>Improve</td>
<td>No</td>
<td>Cured</td>
</tr>
<tr>
<td>6</td>
<td>F 53</td>
<td>Grave's Disease</td>
<td>MTZ 15</td>
<td>12</td>
<td>Cholestatic liver</td>
<td>PTU / Plasamaferesis /Thyroidectomy</td>
<td>Improve</td>
<td>Yes</td>
<td>Cured</td>
</tr>
</tbody>
</table>

Table-1: PTU- Propylthiouracil; MTZ - Methimazole; ICU - Intensive care unit

Cholestatic liver was the most frequent major side effect (50%). One of the patients had different reactions to MTZ and PTU. ICU was needed in three cases. Mortality rate was 16%.

Conclusion: Although described as very rare, cholestatic liver is in our casuistry the most frequently seen complication. Physicians should be aware that ATD can be associated with severe side effects with non-negligible morbidity and mortality.
EARLY OUTCOMES OF RADIOIODINE TREATMENT IN PATIENTS WITH AUTOIMMUNE AND NON-AUTOIMMUNE VARIANTS OF MULTINODULAR TOXIC GOITER

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Introduction: The aim of our study was to assess the effect of coexistence of multiple autonomously functioning nodules and Graves disease on the results of radioiodine treatment for hyperthyroidism.

Patients and methods: 163 patients: 42 with Graves disease, 42 with solitary toxic nodule and 79 (67 females, 12 males; age range 40-84yr) with multinodular toxic goiter (MNTG) were treated with radioiodine (131I) for hyperthyroidism. The last-mentioned group was analyzed. Diagnosis of MNTG was established before admittance to our department on the clinical grounds (absence of extrathyroidal signs of autoimmune disease) and laboratory investigations (including thyroid ultrasonography and scintigraphy). Irrespective of this, before radioiodine treatment serum concentration of TSH receptor antibodies (TRAb) were determined and patients were divided into 2 subgroups: A - 5 (6,3%) patient with MNTG and TRAB - positive test and B - 74 (93,7%) patients with MNTG and TRAB - negative test.

Patients have received the therapeutic dose of 131I (ranged from 6 to 31 mCi) according to the formula: A(mCi) = D(rd) X G/18 X Umax x Teff

G - thyroid (nodule) mass
Umax - maximal thyroid uptake
Teff - radioiodine effective halt-life
D - constant: 15-20rd

Results: 28 patients with MNTG were lost to follow-up. Thus, 1 year after radioiodine administration effectiveness of the therapy was evaluated in 51 patients. 41 (80,3%) of them were in euthyroid state, 8 (15,7%) had hypothyroidism and 2 (4%) had a recurrence of hyperthyroidism.

40 out of 46 (87%) patients in subgroup B had euthyroidism, 6 (13%) -hypothyroidism and anyone - hyperthyroidism; while in subgroup A the results were, respectively, 1(20%), 2 (40%) and 2 (40%).

Conclusions: Ineffective radioiodine treatment of multinodular toxic goiter may be caused by coexisting Graves disease. Serum TRAb concentration should be used in differential diagnosis between autoimmune and non-autoimmune variants of MNTG.
VARIATION OF THE HYPER-FUNCTIONAL THYROID MASS AFTER RADIOIODINE TREATMENT

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Aim: Radioiodine treatment reduces thyroid function and thyroid volume in patients with Graves disease or autonomic nodules. The goal of this study is to evaluate, with scintigraphic imaging, the degree of variation of the thyroid mass in patients who underwent more than one radioiodine treatments.

Methods: The clinical chart of 16 hyperthyroid patients, who underwent at least two radioiodine treatments between February 2004 and February 2009, were reviewed. Fourteen patients with Graves's disease and 2 with an autonomic nodule - 12 women and 4 men, age range of 23-62 yr, mean age 43.3 yr - were studied. The mean activity of the radioiodine treatments was 396±114.7MBq (10.7±3mCi), range 185-673MBq (5-18mCi). The hyper-functional thyroid mass existing one week before the treatments was estimated by using a scintigraphic method based on the i.v. administration of 185MBq (5mCi) 99mTc. Then, the mass of the functional gland was calculated before the 1st treatment and before the 2nd and, in two cases, before the 2nd and before the 3rd treatments. The variation in thyroid hyper-functional mass between radioiodine treatments was calculated.

Results: A reduction in the thyroid mass was observed after the radioiodine treatments. The mean mass before the 1st treatment was 70.7±24g (range 23-122g) and before the 2nd was 40.2±15g (range 18-77g). This corresponds to a decrease of 29.3±17g (range 5-86g) and to a mean variation of 39.4% (range 13-70.5%). No side effects have been reported.

Conclusions: We conclude that, in this group of patients, radioiodine therapy has been an effective and safety treatment not only for thyroid function reduction but also for decreasing thyroid mass of 40% of its initial value.
Objectives: Hashimoto's thyroiditis (HT) is a most common thyroid disorder. We investigated clinical and biochemical characteristics of patients examined in our outpatients' during the period 1998-2007.

Methods: 1095 patients (age at diagnosis 7-83 years, mean 41.3, 969 women). TSH levels and thyroid autoantibodies titers were recorded at diagnosis, during follow-up and on several occasions after discontinuation of therapy. Family history was also recorded.

Results: TSH levels at diagnosis were 0.3-99 mU/L (mean 6.65, median 3.5, 2.5th percentile: 0.6, 97.5th: 43.29). At diagnosis, 50.1% were euthyroid, 31.5% had subclinical hypothyroidism (SCH) and 18.1% clinical hypothyroidism (CH) (mean TSH 1.97, 6.05 and 20.96 mU/L respectively). Family history for HT was reported by 90.4% of euthyroid subjects, 82.6% and 73% of those with SCH and CH respectively (p=0.009). Thyroxine was administered to 881 patients (80.4%); it was later discontinued in 601. Of these, 469 patients (78%) were euthyroid both before and after discontinuation of treatment. Of those with an initial diagnosis of SCH or mild CH (n=132, 22%) the ΔTSH (TSH at diagnosis - TSH after the discontinuation for 6-48 months) had a range of -15.2 to -0.5 in 31% of patients, +0.5 to +2.5 in 30% while in 39% no substantial difference in TSH levels was observed. During follow up antiTPO levels increased in 27.1%, decreased in 46.3% and in 40.5% did not change. AntiTG levels increased in 20.4%, decreased in 46.3% and in 33.3% they remained stable.

Conclusions: The positive family history for HT may be one of the causes leading to early diagnosis of HT in still euthyroid subjects. The vast majority of previously euthyroid patients who discontinued thyroxine remained euthyroid, while, from those with mild thyroid dysfunction at diagnosis, 30% showed progression and 31% improvement of thyroid dysfunction after discontinuation of treatment.
Introduction: Gastric acid secretion seems to be necessary for the effective absorption of oral thyroxine. Helicobacter pylori (Hp) is the primary cause of gastritis and peptic ulcer disease. 13C-urea breath test (UBT) is a simple, non-invasive test for the detection of Hp infection.

Objective: To evaluate whether the failure in achieve a euthyroid state in patients with hypothyroidism treated with thyroxine was associated with Hp infection.

Material and methods: 16 patients with hypothyroidism who were receiving daily treatment with thyroxine (at least 1.5 µg/kg body weight) and failed to achieve target TSH levels were investigated for possible Hp infection with UBT. Variation in the TSH level was prospectively studied in 7 patients before and after eradication of Hp infection.

Results: 11 patients were Hp-positive and the other 5 patients were Hp-negative. Eradication of Hp infection was confirmed with a negative UBT performed >8 weeks after treatment (antibiotics and proton pump inhibitor). TSH level reversed to target level (euthyroid state) after eradication of Hp infection in all the 7 patients investigated, maintaining the same daily thyroxine dose in 4 patients and using a lower thyroxine dose in the other 3 patient.

Conclusions: Screening for Hp infection seems to be indicated for patients being treated with thyroxine that fail to achieve target TSH levels. In patients with Hp infection under thyroxine therapy, thyroid hormone levels have to be monitored and thyroxine doses adjusted following eradication of Hp infection.
THE SUCCESS RATE OF L-THYROXINE THERAPY IN ACHIEVING BIOCHEMICAL EUTHYROIDISM

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Background: Little is known about the success rate of levothyroxine (L-T4) treatment in providing biochemical euthyroid state in hypothyroidism.

Objective: The aim of the study was to evaluate the success rate of L-T4 treatment comparing autoimmune, postablative and postoperative hypothyroid patient groups.

Method: 316 patients, (F/M: 290/26, age: 48.65 ± 12.82 yrs) who have been treated and regularly controlled at the endocrinology outpatient unit with L-T4 treatment were analyzed. At their last visits, demographic and anthropometric features, treatment periods, applied L-T4 doses and thyroid function states of the patients were recorded. Normal TSH levels were defined as 0.4 to 4 mIU/L.

Results: 260 patients with Hashimoto’s thyroiditis, 19 patients with postablative hypothyroidism and 37 patients with postoperative hypothyroidism were included in the study. Mean daily LT4 dose and mean TSH level were 1.5 mcg/kg ± 0.56 (mean ± SD) and 4.71 mIU/L (ranging from 0.01 to 133.9), respectively. The duration of the L-T4 therapy ranged between 3 months up to 480 months and mean treatment duration was 76.64 months ± 78.47 (mean ± SD). Only 58.5 % of the patients were biochemically euthyroid. Thyroid function states of the patients with respect to type of hypothyroidism are shown in Table 1. There was no significant association between the establishment of euthyroidism and age, body weight as well as the treatment duration.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>TSH &lt; 0.4mIU/L</th>
<th>TSH= 0.4-4.0mIU/L</th>
<th>TSH &gt; 4mIU/L</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune Hypothyroidism</td>
<td>31</td>
<td>146 (56.2%)</td>
<td>83</td>
<td>260</td>
</tr>
<tr>
<td>Postablative Hypothyroidism</td>
<td>3</td>
<td>14 (73.6%)</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Postoperative Hypothyroidism</td>
<td>4</td>
<td>25 (67.5%)</td>
<td>8</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>185 (58.5%)</td>
<td>93</td>
<td>316</td>
</tr>
</tbody>
</table>

[Thyroid function states of the study subjects]

Conclusion: These results emphasize the poor success rates of establishing an euthyroid state in patients who have had LT4 replacement treatment due to different types of hypothyroidisms. The rate of establishing an euthyroid state might be improved by applying more frequent serum-TSH measurements.
PURPOSES: Neutrophil gelatinase-associated lipocalin (lipocalin-2; Lc2), which is induced in epithelial cells during inflammation, has recently been associated with coronary artery disease. Our objective was to investigate whether Lc2 levels are modified by thyroid hormones and thereby represent a cardiovascular risk factor.

Methods: 38 untreated hypothyroid patients aged 25-64 yr were recruited and stratified according to their TSH levels. 22 patients with TSH: 4-12 mU/L were defined as moderate hypothyroidism (MH), 16 patients with TSH > 12 mU/L were characterized as overt hypothyroidism (OH), while 22 euthyroid individuals with TSH from 0.4-4 mU/L formed the control group (CG). Serum levels of Lc2 were measured by Elisa. We also measured serum thyroid hormones, cholesterol, HDL and LDL-cholesterol, triglyceride, hsCRP, anti-TPO and anti-Tg and we calculated BMI and HOMA. Measurements of Lc2 levels were repeated after 3 months following LT4 treatment in 6/16 with OH and in 6/22 patients with MH.

Results: Serum Lc2 levels were measured increased up to 41% in OH as compared to MH and CG (67.2 ± 19 µg/L vs. 31.4± 11 µg/L and 27±12 µg/L, p< 0.01, respectively). The values of MH were not statistically significant different from those of CG. A correlation was found between Lc2 and TSH, hsCRP (r=0.22, p=0.041), cholesterol (r=0.21, p=0.048) and LDL-cholesterol (r=0.21, p< 0.05). In contrast, no correlation could be detected between Lc2 and thyroid hormones, BMI and HOMA. Treatment with LT4 induced a slight decrease of Lc2 levels in OH patients.

Conclusions: These results isuggest that Lc2 concentrations are increased in severe hypothyroidism and that TSH may be a modifier of neutrophil activity. A plausible mechanism may be neutrophil degranulation induced by the chronic inflammatory process in OH. However, It should be clarified whether Lc2 may constitute a link between dyslipidemia of OH and the cardiovascular disease.
ALTERATIONS OF CIRCULATING SELECTIN, INTERCELLULAR ADHESION MOLECULE-1, AND VASCULAR CELL ADHESION MOLECULE-1 IN PATIENTS WITH NON-IMMUNE THYROID DISEASE

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Cytokines, including adhesion molecules, are a family of protein mediators that are important in transducing information between various cell types, where not only thyroid cancer, but also non-immune cells may be important sources of certain cytokines.

Aim: Aim of this study was to evaluate the levels of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-selectin (sE-selectin) in patients with non-immune thyroid disease and with papillary thyroid cancer.

Materials and methods: We formed two study patient groups: 22 patients with non-toxic, non-immune nodular thyroid disease (TD) and 22 patients with papillary thyroid cancer (TC). 22 healthy subjects were selected as controls (C). In all of the cases we performed the histological examinations. All patients were without atherosclerosis, autoimmune diseases and systemic diseases. Smokers were excluded from the study. The study groups were matched for age, sex, and body mass index. sICAM-1, sVCAM-1 and sE-selectin were measured by xMAP technology (Luminex-200 analyzer).

Results: sICAM-1, sVCAM-1 and sE-selectin levels were statistically significantly elevated in TD and TC patient groups compared to the healthy subject group (TD vs. C, p< 0.05; TC vs. C, p< 0.001).

Conclusion: Our findings show that patients with non-toxic, non-immune nodular thyroid disease and with papillary thyroid cancer have significantly elevated sICAM-1, sVCAM-1 and sE-selectin levels.
Background: Appropriate scale validity has recently been documented for the thyroid-specific patient-reported outcome, ThyPRO. However, for an instrument intended for clinical application, it is also important to evaluate clinical validity, e.g. known-groups validity, where patients expected to have high scores (i.e. high impact of thyroid disease) are formally compared to patients who are expected to have low scores.

Aim: To investigate clinical known-groups validity of the Danish ThyPRO.

Methods: For each of the 13 scales, an 'expected high' and 'expected low' score group was defined a priori. The expected high score groups were: 'Goitre symptoms': patients with non-toxic goitre (n=105), 'Hyperthyroid symptoms': overt hyperthyroidism (n=70), 'Hypothyroid symptoms': overt hypothyroidism (n=20) 'Eye symptoms': TAO and NOSPECS >1 (n=16), 'Tiredness', Cognitive impairment', 'Emotional susceptibility', 'Impaired social life', 'Impaired daily life' and 'Impaired sex-life': patients with overall clinical condition rated by physicians as "very bad" (n=12), 'Anxiety': HADS-anxiety score indicating anxiety (n=146), 'Depressivity': HADS-depression score indicating depression (n=64) and 'Cosmetic complaints': patients with TAO and NOSPECS>1 and patients with goitre >150 ml (n=21). Low score groups were: 'Goitre symptoms': patients with treated autoimmune hypothyroidism (n=107), 'Hyperthyroid symptoms', 'Hypothyroid symptoms' and 'Eye symptoms': non-toxic goitre (n=161), 'Tiredness', Cognitive impairment', 'Emotional susceptibility', 'Impaired social life', 'Impaired daily life' and 'Impaired sex-life': overall clinical condition “excellent” (n=87), 'Anxiety': HADS-anxiety score indicating no anxiety (n=577), 'Depressivity': HADS-depression score indicating no depression (n=703) and 'Cosmetic complaints': autoimmune hypothyroidism and overall clinical condition “excellent” (n=22). Mean scores were compared by unpaired t-tests.

Results: For all 13 comparisons, the expected high score groups scored substantially and significantly higher (p< 0.001 for 11 scales, < 0.05 for two).

Conclusion: The ThyPRO was able to distinguish between patients clinically expected to have high vs. low impact on quality of life. We thus found support for the clinical validity of the ThyPRO questionnaire.
P186
TIME ANALYSIS OF L-THYROXINE TREATMENT ON LIPID PARAMETERS IN PATIENTS WITH VARIOUS DEGREES OF HYPOTHYROIDISM

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Objectives: There is still an ongoing debate over the efficacy of LT4 treatment on the lipoproteins in patients with hypothyroidism. Our objective was to analyze the effects of treatment over the course of time in relation to various degrees of hypothyroidism.

Methods: 94 female patients with hypothyroidism, aged 50-65yr, were stratified according to their TSH values. Gr1 (n=31) TSH: 3-5.9 mU/L; Gr2 (n=34) TSH: 6-11.9 mU/L; Gr3 (n=29) TSH >12 mU/L. Serum total cholestero (TC), HDL and LDL-cholesterol and triglyceride were measured before and at 3 and 6 months of LT4 treatment.

Results: Basal TC and LDL-C increased gradually in parallel with the rise in TSH. In Gr1 TC was reduced by 3.1% from 226±11 mg/dl to 219 ±14mg/dl, while LDL-C was decreased by 4.6% (229 ± 13 to 225 ± 12 mg/dl, n.s) at 3 months while no further decrease was found at 6 months. In Gr2 TC was decreased by 5.1% at 3 months and LDL-C by 6.7% (164±14 mg/dl to 153± 12 mg/dl). There was no effect on serum HDL-C and triglyceride concentrations at any time of the study. In Gr3 TC was reduced at 3 months by 13.8% from 311±23 mg/dl to 265±18 mg/dl, p< 0.05. Respectively, LDL was decreased by 17% (188±13 mg/dl to 149±14 mg/dl, p< 0.001). However, it should be emphasized that on an individual basis 6/34 in Gr 2 and 11/29 patients in Gr3 were hypercholesterolemic according to NCEP criteria at the end of study.

Conclusions: The results show a rapid effect of LT4 treatment on lipids within 3 months. The decrease is dependent on the degree of hypothyroidism as well as on the level of initial TC and LDL-C concentrations. Patients who remain dyslipidemic despite LT4 therapy should be individually considered for combined treatment with statins or other hypolipidemic drugs.
It is well known today that hyperhomocysteinemia is strongly associated with cardiovascular risk. There is little information about thyroid status in patients with elevated homocysteine. The aim of our study was to evaluate TSH and homocysteine (H) level in heart ischemic disease (HID) patients.

**Methods:** 73 HID patients participated in our study. Coronarography was performed for all of them. We considered age, smoking status, diabetes mellitus (DM) for all patients. TSH and homocysteine level were measured by immunoassay methods.

**Results:** In group of high H. level 30.6% of patients had trunkal stenosis of left coronary artery and 44.4% had multivessel damage, when in group of normal H. level only 13.9% of them had trunkal stenosis and 27.8% multivessel damage (p=0.01; p=0.02). Hyperhomocysteinemia was associated with smoking status: in group with high H. there was 64.7% of smokers and in group of normal H. only 44.2% (p=0.04). DM was in 21.9% of cases in high H. group and 8.6% in normal H. group (p=0.01). TSH was higher in high H. group (6.91+ 2.05 IU/l) and normal in normal H. group (3.59+0.59 IU/l) (p=0.05).

**Conclusion:** Hyperhomocysteinemia in HID patients was associated with severe damage of coronary arteries, DM, smoking and mild thyroid failure.
Introduction: Sunitinib is a new tyrosine kinase inhibitor with antitumor and antiangiogenic effects used in the treatment of renal cell carcinoma (RCC). Several studies have identified unexpected rates of thyroid dysfunction with sunitinib treatment.

Objectives: The aim of this study was to determine the incidence hypothyroidism in RCC patients receiving sunitinib at the Portuguese Oncology Institute of Oporto.

Methods: A retrospective study of all patients with RCC treated with sunitinib at our Institute was undertaken, from July 2007 to February 2009. TSH and T4 levels were recorded at baseline and during the follow-up, according to the drug cycle scheme.

Results: We evaluated a total of 12 patients, 8 men and 4 women; Six (50%) developed elevated TSH levels after a median of two months (minimum 1, maximum 17). Mean baseline TSH value was 1.08mU/L and mean TSH after sunitinib was 17.6mU/L. Both men and women were equally affected.

Conclusions: This study confirms the high incidence of hypothyroidism in sunitinib treated patients. Close monitoring of thyroid function is mandatory every three months to insure and improve the quality of life of these patients.
NEUROPSYCHIATRIC FUNCTION AND QUALITY OF LIFE INDEX IN SUBCLINIAL HYPOTHYROID STATUS ELDERLY SUBJECTS

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Background: Subclinical hypothyroidism (SCH) is a prevalent condition among the elderly. Even though overt thyroid dysfunction was known to be related to depression, cognitive dysfunction and the quality of life, the association between SCH and neuropsychiatric (NP) function remains controversial.

Methods: To investigate the relationship between SCH and NP parameters and quality of life in elderly, total 1,000 elderly individuals aged 65 or over, who were living in Seongnam City, were randomly selected and then contacted by letter and telephone and asked to participate in the Korean Longitudinal Study on Health and Aging (KLoSHA) between September 2005 and September 2006. KLoSHA was designed as a population-based prospective cohort study of Korean elderly people. Practice registers asked about current or recent treatment for thyroid disease. Serum free T4, T3, thyroid stimulating hormone and thyroid peroxidase antibodies was measured. All the subjects were evaluated with a standardized clinical interview and depression and cognitive dysfunction was assessed using mini international NP interview (MINI) version 5.0, depressive symptom checklist (DSC) of CERAD and 17-item Hamilton depression scale, GDS-K and CES-D. The quality of life of subjects was assessed using SF-36.

Results: 754 subjects (79.9%, M:F 327:427 ) of subjects had euthyroid and 145 subjects (15.3%, M:F 73:91) were SCH. There was no difference of depression, cognitive dysfunction and quality of life between euthyroid and SCH subjects.

Conclusions: However, SCH is a prevalent disease in older ages, screening test for NP related disease would not be essential for asymptomatic SCH from our results.
Objectives: Primary biliary cirrhosis (PBC) is presumably autoimmune disorder, characterized by progressive destruction of small intra-hepatic bile ducts and gradual development of biliary cirrhosis. Autoimmune thyroid disease seems to be associated with PBC, but the data in the literature are scarce. The aim of the study was to evaluate the prevalence of concomitant thyroid diseases in our group of PBC patients.

Methods: We investigated 94 patients with PBC (93 females and 1 male), aged between 37 and 86 years (mean, 62.4 ± 11.2), who have been regularly evaluated and treated for PBC between 2.3 and 21.4 years (mean, 9.3 ± 4.9). TSH, thyroid hormones and thyroid antibodies were determined, including thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb), and TSH receptor antibodies. Thyroid structure and the presence of thyroid nodules were evaluated using ultrasound with 7.5 MHz linear transducer. In some patients with thyroid nodules scintigraphy with Tc99m was additionally performed.

Results: Thyroid disease was confirmed in 62 (66%) patients with PBC, while only in 32 (34%) normal thyroid was found. Hashimoto’s thyroiditis was diagnosed in 31 patients (33%), among them 17 (55%) were treated for hypothyroidism. 22 (23%) were TPOAb positive and 17 (18%) were TgAb positive. Among euthyroid patients 14 patients (15%) presented with multinodular goitre, 8 patients (9%) with solitary nodule and 1 patient (1%) with thyroid cyst. 5 patients (5%) were treated for hypothyroidism after thyroidectomy. In 3 patients (3%) hyperthyroidism due to thyroid autonomy was diagnosed and therefore they were treated with radioiodine.

Conclusions: Our results indicate that more than two thirds of our PBC patients have thyroid disease, most frequently Hashimoto’s thyroiditis with prevalence of 33%. According to a very high prevalence of concomitant thyroid diseases we believe that in patients with PBC periodical testing of thyroid function would be strongly recommended.
POLYCYSTIC OVARY SYNDROME DOES NOT CONTRIBUTE TO THYROID DISEASE
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Objective: It has been demonstrated that autoimmune thyroid disease is more frequent in PCOS patients than normals in a few studies. The aim of this study was to determine the frequency of autoimmune thyroid disease in our study group.

Methods: Fifty-seven patients with PCOS, aged 16-36 years and 67 -age and body mass index-matched women, aged 16-37 years, were recruited to the study. PCOS was defined according to Rotterdam Criteria. Thyroid ultrasound was performed for both patients and controls.

Results: Patients with PCOS had elevated LH/FSH ratio (1.6±0.9 vs 1.2±0.8, P=0.016), testosterone (0.7±0.3 vs 0.5±0.2 ng/mL, P=0), DHEAS ( 287.9±123.9 vs 240.5±101 ug/dl, P=0.028) and low progesterone (1.9±2.2 vs 5.9±5.6 ng/mL, P=0). Thyroperoxidase antibodies (43.4±91.5 vs 31.4±91.4 U/l, P=0.48) and TSH (2.2±1.3 vs 2.2±1.7 uIU/mL, P=0.9) levels did not differ between groups statistically as well as thyroglobulin antibodies (73.3±151.5 vs 36.8±75.1 U/l, P=0.09); however thyroglobulin antibodies were found higher than controls in PCOS patients. Elevated thyroperoxidase or thyroglobulin antibodies were found in 13 of 57 patients (22.8%) and 11 of 67 controls (16.4%; P=0.8). The frequency of subclinical hypothyroidism was higher in control group (1.8% vs 10.4%, P=0.05). Thyroid volumes were similar in both groups (PCOS and controls; 12.6±5.2 vs 12.5±4.5 ml, P=0.9). The frequency of hypoechoic areas in patients with PCOS was 30.4% and 26.9% in controls. Soliter nodule was present in 3 of PCOS patients (5.3%) and in 2 of controls (2.9%; P=0.5). Nodular disease accompanying hypoechogetic thyroid parenchyma was present in 5 of PCOS patients (8.8%) and in 3 of controls (4.5%; P=0.3).

Conclusion: PCOS did not contributed to any thyroid disease in our population. In our opinion, the results of the present study can be attributed to the high prevalence of thyroid disease in our country. Furthermore, thyroid disease are endemic in this region of our country.
Objectives: Incidentally found thyroid lesions are frequently detected in patients undergoing 18F-FDG PET/CT. The aim of this study was to investigate the prevalence of incidentally found thyroid lesions in patients undergoing 18F-FDG PET/CT and determine the risk for thyroid cancer.

Methods: 18F-FDG PET/CT was performed on 3,379 patients for evaluation of suspected or known cancer or cancer screening. Medical records related to the 18F-FDG PET/CT findings, US findings, FNA, operative method and pathology were reviewed retrospectively.

Results: Two hundred eighty five patients (8.4%) were identified to have FDG uptake on 18F-FDG PET/CT. 99 patients with focal or diffuse FDG uptake underwent further evaluation. The cancer risk of incidentally found thyroid lesions on 18F-FDG PET/CT was 23.2% (22/99) and the cancer risks associated with focal and diffuse FDG uptake were 30.9% and 6.4%. There was a significant difference in the SUVmax between the benign and malignant nodules. There was a significant correlation between the SUVmax and the size of the cancer.

Conclusions: The results of this study suggest that incidentally found thyroid lesions by 18F-FDG PET/CT, especially a focal FDG uptake and a high SUV, have a high risk of thyroid malignancy. Further diagnostic work-up is needed in these cases.
Aim of the study: Static elastography of thyroid nodules has become an additional tool for the characterization of thyroid nodules. It relies the assessment of the nodule elasticity in comparison to that of the surrounding normal tissue. The aim of the study was to assess the feasibility of quantitative strain elastography and to evaluate several indices for the characterization of thyroid nodules.

Methods: 57 patients (53 women and 4 men) were referred for the elastography of thyroid nodules. Each nodule was studied using B-mode imaging and color Doppler US with a linear transducer (L12-5) on an IU 22 (Philips Ultrasound, Bothell, WA, USA). The cineloops were saved in raw data format for quantification using QLAB software (Philips Ultrasound). The elastography index was calculated by positioning a Region-of-Interest upon the nodule and the surrounding thyroid. The differential strain rate was calculated as the ratio between the nodule and the normal tissue. Cytologic correlation was obtained in all cases using needle aspiration. In addition, histologic confirmation of cancer was obtained in case of positive cytologic findings (3 papillary cancers, one medullary, one follicular). Benign cytology was observed in 52 nodules: 38 colloid adenomas and follicular neoplasms, 5 cysts and 9 nodular thyroiditis.

Results: Thyroiditis nodules exhibited a high strain rate (0.216), in the same range than the normal tissue (0.233). The five cancers exhibited the lowest elasticity (0.052) without overlap with benign nodules (0.096) and cysts (0.106) (p< 0.01)

Conclusions: Real time quantitative elastography is a feasible technique. Relative strain rate of variation across compression and relaxation showed significant differences between cancers and benign nodules and appeared to be the best discriminant criteria. Larger series will allow to determine optimal cut-off values.
THE ABLATION RATE IN PATIENTS WHO UNDERWENT PRE-ABLATION 131-RADIOIODINE DIAGNOSTIC SCAN PRIOR TO RADIOIODINE REMNANT ABLATION FOR WELL DIFFERENTIATED THYROID CARCINOMA FOLLOWING THYROIDECTOMY. THE STUNNING EFFECT OF 131-RADIOIODINE: A MYTH OR REALITY?

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Objectives: The role of pre-ablation whole body diagnostic scan (DxWBS) using 131-radioiodine (131I) performed prior to radioiodine remnant ablation following thyroidectomy for well differentiated thyroid carcinoma remains controversial due to the concern of stunning effect. This study evaluated the success rate in radioiodine ablation in patients who had pre-ablation scans prior to their radioiodine therapy.

Methods: We evaluated 329 patients who underwent pre-ablation 131I scan prior to radioiodine ablation following thyroidectomy for quantitative assessment of thyroid remnant. All patients were given 30-40 MBq of 131I seven days prior to admission for radioiodine ablation. The pre-ablation DxWBS was performed 24 hours following 131I administration. All patients went on low iodine diet 3 weeks before DxWBS and stopped their thyroid medication (4 weeks for T4 & 2 weeks for T3). Patients who could not cope with hypothyroid symptoms were offered thyrogen (Rh-TSH) for their post ablation scans.

Results: The median ablative dose of 131I administered was 3527 MBq. 292 patients underwent DxWBS following radioiodine ablation. 206 patients had their thyroid hormone withdrawn and 86 had thyrogen. 224 and 68 patients had DxWBS at >6 months and 3-6 months respectively. The DxWBS in 283 (97%) patients showed no significant uptake and 9 (3%) patients had >0.1% uptake in the neck. Thyroglobulin (Tg) and thyroglobulin antibodies (TgAb) levels of 273 patients were available for analysis. 245 patients had Tg ≤ 2ng/ml and 25 had detectable TgAb. 26 patients had Tg > 2 ng/ml and 2 had detectable TgAb. 220 out of 246 patients (89.4%) had Tg ≤ 2ng/ml without detectable TgAb.

Conclusions: The ablation rate in patients who had pre-ablation 131I scan is high and comparable to other published series without pre-ablation 131I scan. The theoretical concern regarding stunning effect using low dose 30-40MBq of 131I could be disputed based on this clinical data.
Medullary thyroid carcinoma (MTC) usually presents as advanced disease often with distant metastases. Persistently elevated levels of calcitonin (CT) or its increase postoperatively is indicative of residual or recurrent disease. Finding the source of CT is a challenge as conventional imaging methods usually fail. Preliminary reports of 18F-FDG-PET use in MTC patients showed encouraging results.

**Objectives:** We report the initial experience with FDG-PET imaging for suspected recurrent or persistent MTC at our institution.

**Methods:** Consecutive patients who underwent FDG-PET imaging for suspected recurrent or residual MTC over a period of 4 years were identified and their data reviewed.

**Results:** Eighteen patients (8F, 10M) were identified, with a mean age of 44.6±12.7 years. At diagnosis, 27.8% had stage II, 55.6% had stage III and 16.7%, stage IV disease (TNM stages by AJCC). RET mutation was present in 3 (MEN2A). Time between diagnosis and PET-FDG varied from 3 months to 15.5 years. Three patients underwent FDG-PET although they had normal values of CT. In these, two scans were negative and one was suspicious (not confirmed by clinical follow-up). In the other fifteen patients, CT levels ranged from 52 to 19702 pg/ml (median 252; nr< 19). Nine scans were positive (CT 52-19702 pg/ml, median 2379). After removal of residual tumour or metastases, histopathology confirmed initial suspicion in 100%. Two scans were reported as suspicious: one underwent surgery but histopathologic findings were benign, the other was missed for follow-up. Four scans were negative (CT 77-375 pg/ml, median 96). These patients remained without treatment, without evidence of disease.

**Conclusion:** In our findings, FDG-PET is a valuable procedure for the imagiologic detection of occult residual or metastatic MTC disease. It seems useless in routine post-operative evaluation in the absence of elevated CT.
THE VALUE OF 18F-FDG PET/CT, 111IN-OCTREOTIDE AND 99MTC MIBI SC TO DETECT RECURRENCE AND METASTATIC FOCI IN DIFFERENTIATED THYROID CANCER (DTC) Pts WITH HIGH TG AND/OR ANTITG(ATG) AND NORMAL 131I WBS

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This prospective study was to evaluate the success of F-18 FDG, In-111 octreotide and Tc-99m MIBI sc for detecting the metastatic foci in pts with high Tg and/or Atg while normal 131I WBS follow up RAI ablation therapy.

Material-methods: 50 pts with DTC (37 F, 13 M, median age 50.06±13.1) were included to the study. All of them were operated on before RAI therapy. While their diagnostic 131I WBS in follow up period were normal, Tg and/or Atg were high. They were categorised in 3 groups according to Tg-Atg. (group 1 both high Tg and Atg, group 2 high Tg and normal Atg, group 3 normal Tg and high Atg). 99mTc MIBI 45/50 and 111In octreotide sc was performed in 24/50 pts, PET/CT was performed in 48/50 pts and 26/48 were under suppression while 22/48 had L-thyroxin off. 21 pts were operated on due to pathological findings in PET/CT and the histopathological confirmation was made to them.

Results: The success rate of MIBI and octreotide for detecting the recurrence were similar while as PET/CT was significantly successful. The histopathological findings after the second surgery due to recurrence were concordant with primary histopathological results. Biopsy and/or operation was performed in 21 pts with FDG(+) scans. The histopathological results were accepted as gold standard and the sensitivity of 99mTc-MIBI, 111In octreotide and 18F-FDG PET/CT were %26.7, %44.4, %100 respectively.

Conclusion: The possibility of metastatic foci must be evaluated not only high Tg but also high Atg in DTC patients with normal 131I WBS. 99mTc-MIBI and 111In octreotide sc results were similar to detect recurrence and/or metastatic foci. PET/CT was more successful than other sc. No any differences was observed in on suppression and off L-thyroxin in PET/CT. FDG uptake was seen in differentiated tumor foci regarding to pathological results.
Objective: With the development of ultrasound (US) technology and increased clinical application, detection rates of thyroid nodules have increased. Current leading diagnostic method for thyroid nodules is fine needle aspiration biopsy (FNAB). However there is no clear guidelines for selecting nodules that will undergo FNAB.

In this study we aimed to assess CDUS findings in thyroid nodules, and to establish CDUS criteria that can be used for benign-malignant nodule differentiation.

Methods: Nodules which underwent US guided FNAB or which were scheduled for thyroidectomy were evaluated by color Doppler US (CDUS). One hundred and forty six patients (123 women, 23 men, age range: 21-83) and 200 nodules were included in this study. FNAB was performed in 103 patients. Forty three patients underwent thyroidectomy. The vascularization pattern, flow spectrum pattern, maximum velocity (cm/sec), minimum velocity (cm/sec), maximum/minimum velocity ratio (S/D), pulsatility index (PI), resistivity index (RI), acceleration time (sec) and acceleration (cm/sec²) were assessed in the CDUS evaluation. Nodules were divided into 2 groups as malignant and benign nodules according to their cytologic and histopathologic results. There were 156 benign nodules and 12 malignant nodules. Fisher’s exact test and chi square analysis were applied to the final data and student-T-test and Mann-Whitney U test were used to compare means between the 2 groups (p< 0.05).

Results: In malignant nodules vasularisation pattern, flow spectrum pattern, S/D, PI, RI and acceleration values were significantly different than for benign nodules (p< 0.05).

Discussion: There’s a limited number of studies which have evaluated CDUS findings in thyroid nodules. Because there are multiple nodules in a patient, follow up is difficult and there’s no criteria for estimating the malignant potential of a nodule. We believe that CDUS evaluation, performed in addition to US can be a useful tool for selecting nodules that will undergo FNAB.
IMPORTANCE OF THYROID COLOR-DOPPLER ULTRASONOGRAPHY IN THE DIFFERENTIAL DIAGNOSIS OF AMIODARONE-ASSOCIATED HYPERTHYROIDISM

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This work is aimed at evaluating the importance of color-doppler ultrasonography (US) in the differential diagnosis of type I and type II amiodarone-induced hyperthyroidism. Six consecutive patients (4 men and 2 women), aged 44 to 64 years, presented severe hyperthyroidism during treatment with amiodarone. They were taking amiodarone for more than 6 months up to 3 years, and complained of tremor, diaphoresis, palpitations and weight loss. Four patients, including one with resistance to thyroid hormone (RTH) had a non tender enlargement of the thyroid gland with multiple palpable nodules. A diffuse tender goitre was found in two patients. Amiodarone was discontinued and they were started on methimazole (15 to 30 mg daily). Initial values of thyroid function were: FT3 10.1±5.8 (normal range: 2.8-4.8); FT4 5.5±3.8 (0.9-1.8); TSH < 0.01. Antibodies against thyroglobulin were positive in two patients; thyroperoxidase and TSH receptor autoantibodies were negative. The ratio of FT3:FT4 was significantly lower than in patients with Graves disease (2.25±0.8; n=6 vs 4.14±1.9; n=26; t test; p=0.0025), what seems to be ascribed to amiodarone inhibition of type 1 deiodinase. After 12 weeks of treatment, 4 patients became euthyroid, but the other 2 remained in hyperthyroidism. The RTH patient had thyroid hormone levels above normal range but with normal TSH. In hyperthyroid patients, color-doppler US revealed an almost absence of thyroid vascularisation as opposed to highly vascularised glands found in euthyroid patients. The two hyperthyroid patients were started on prednisolone (20 mg/day), and euthyroidism was restored in less than four weeks. In conclusion, thyroid color-doppler US seems to be an useful tool in the differential diagnosis of the two types of amiodarone-induced hyperthyroidism. Type II may benefit from a short term course of corticotherapy given the inflammatory basis of the disorder. Amiodarone can induce hyperthyroidism in RTH patients.
P199
THE ROLE OF TC-99M MIBI SCINTIGRAPHY FOR THE EVALUATION OF SOLITARY THYROID NODULES: WHICH ONES ARE BENIGN?
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Technetium-99m-methoxyisobutylisonitrile (MIBI) is a myocardial imaging agent which also accumulates in the thyroid gland. The aim of this study was to find out which thyroid nodules retain MIBI and whether preoperative evaluation of malignancy is possible.

Methods: Over a period of 12 months, 17 patients (17 women, 1 man; aged 28 - 65 yr) selected from a group with solitary thyroid nodules were studied. All patients underwent prior 99mTc-pertechnetate thyroid scanning. Single injection, dual-phase (30 min and 2 hr) thyroid scintigraphy with 99mTc-MIBI was also performed on all patients who had a single cold nodule (n=14), single hot nodule (n=4). MIBI scans were considered positive if there was tracer retention involving the thyroid nodule on the delayed 120-min image compared to the early 30-min image. Sonographic examination (7 MHz transducer) and fine-needle aspiration biopsy, guided by ultrasonography, was also done on each patient. In the following days and weeks 11 patients underwent surgery.

Results: Five and 11 patients had microcalcification and central vascularisation in the thyroid nodule respectively. There were 12 patients with MIBI retention on the delayed images. 8 of them underwent surgery. Histopathological diagnoses revealed a total of 4 thyroid carcinomas which were all MIBI positive. Out of 8 patients who were MIBI positive and underwent surgery, 4 patients had follicular adenoma. Six nodules did not have MIBI retention. They were all biopsied with no evidence of malignant disease. 6 month follow-up on these nodules did not demonstrate any evidence of enlargement or malignant characteristics on USG.

Conclusion: These results indicate that although MIBI accumulation and retention in the thyroid nodule is not tumor specific, absence of Tc-99m MIBI retention is likely to indicate benign disease.
Endoscopic thyroidectomy has become now a useful option of surgical methods for benign thyroid disease. Now, we report the cases of Grave’s disease with endoscopic thyroid surgery in our institute. Fourteen patients with Grave’s disease are included in this study. They received endoscope assisted thyroid resection from November, 2006 to February, 2009. The weights of extracted thyroid gland ranged from 28g to 122g. Now, we usually perform Video Assisted Neck Surgery using an anterior neck-skin lifting method for thyroid diseases. In this method, we preserve all anterior cervical muscles with 3 cm skin incision on anterior chest wall under the subclavicular line using our original wire hook retractor. This wire hook retractor brings the excellent endoscopic view and working space in the narrow neck space without the skin incision of the neck area. Most of the patients who have thyroidectomy have failed in pharmacotherapy, so we consider that total thyroidectomy or near-total thyroidectomy is more feasible than subtotal thyroidectomy for avoiding the recurrence in these patients. Our experience showed that the patients with large thyroid gland over 100g had resulted in excessive bleeding and extending operation time. For the patients with the thyroid gland less than 100g, endoscopic neck surgery with a better cosmetic appearance is a useful surgical treatment for the Grave’s disease.
WONDERING ABOUT THE IDEAL TIME FOR THE POST-THERAPY ¹³¹I WHOLE-BODY SCAN

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Objectives: The post-therapy ¹³¹I whole-body scan is the better study in staging differentiated thyroid carcinoma, but the ideal time to perform it is not consensual. We aimed to compare the scans obtained at the second and seventh days after therapy, to consider doing only the first study, at the moment of patient discharge.

Methods: We analysed ¹³¹I whole-body scans from 2006-01-02 to 2008-03-31 after therapeutic ablation (group A: 113 patients, 27M/86F, 51±15 years, 59 of whom having low risk disease) and after other therapies (group B: 51 patients, 16M/35F, 56±20 years).

The scans of second and seven days were compared as to the presence, number and intensity of lesions.

We used a Siemens® E-Cam Dual Head gamma camera with high energy collimators.

Results: The ¹³¹I uptake results were as follows:

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<tr>
<th>Group</th>
<th>Number of patients</th>
<th>Thyroid bed</th>
<th>Cervical lymph nodes</th>
<th>Mediastinal lymph nodes</th>
<th>Lungs</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>59 (low risk)</td>
<td>Only in thyroid bed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>51</td>
<td>9,8%</td>
<td>17,6%</td>
<td>21,6%</td>
<td>33,3%</td>
<td>2,0%</td>
</tr>
</tbody>
</table>

[Results of radioiodine uptake (% of patients)]

In group A, the second and seventh day scans of low risk patients were identical; in the others, the second day scan was better in 15/54 patients (5 for presence, 8 for number and 2 for intensity of lesions) and the seventh day images were better in 3/54 (1 for presence, 1 for number and 1 for intensity of lesions). Moreover, 2/51 group B patients had better second day (1 for presence and 1 for number of lesions) and 12/51 better seventh day scans (7 for presence, 3 for number and 2 for intensity of lesions).

Conclusions: The ¹³¹I whole-body scan may be done only in the second day after ablative therapy in low risk patients. Otherwise, it is useful to do both exams, owing to their complementarity.
The diagnosis of thyroid tumor is usually made in the process of investigating a thyroid nodule with clinical examination, ultrasonography and fine-needle aspiration cytology (FNAC). The follow-up is mainly based on iodine scans and serum thyroglogulin measurement. The aim of this present study was to investigate the role and efficacy of 18F-FDG PET in the differential diagnosis of doubtful thyroid nodules.

101 cases with thyroid tumor were included in this study. It contained 51 of papillary carcinoma and 50 of follicular tumors.

FDG-PET disclosed almost all tumors in thyroid gland with hot spots. It had a tendency to show a higher sugar uptake value (SUV) in follicular tumors than in papillary carcinoma. In papillary carcinoma, otherwise, FDG-PET could reveal the primary microcarcinoma with 8mm but not detect the intra-thyroidal metastatic portion with 4mm. Although it is unlikely, the question whether FDG PET is able to give a better differentiation between benign and malignant tumours in the preoperative assessment of thyroid nodules is not answered up to now. However that may be, FDG-PET/CT is a powerful and useful tool for assessing patients with thyroid tumors.
The aim of this prospective study was to assess the efficacy of low-dose 1-131 ablation for the postoperative ablation of low-risk thyroid cancer patients.

**Patients & methods:** 41 patients (4m, 37f, mean age: 46.9±23.8) were enrolled to the study. All had stage I or II thyroid cancer. After total thyroidectomy when serum TSH was > 30 ng/ml, patients underwent Tc99m-thyroid scintigraphy, I-131 uptake (IU) and thyroid ultrasonography (tUSG) for the assessment of the residual thyroid tissue to be ablated. After patient-based radiation exposure calculation with Becker’s formula, 20 mCi 1-131 was given if 300 Gy remnant radiation, which is the minimum dose required for successful ablation was achieved. The ablation efficacy of 20 mCi 1-131 study group was compared to that of control group which consisted of 20 patients with diagnosis of stage I and II thyroid cancer who received standard 100 mCi ablation dose.

**Results:** Patients were re-evaluated at 6 months after ablation with 1-131 whole body scan (WBS), tUSG and serum thyroglobulin (Tg) levels. Patients with Anti-Tg levels> 20ng/ml were not included to the final analysis. Patients were divided into 3 groups; Group A consisted of patients who had no residual 1-131 uptake on WBS with serum Tg levels < 0.2 ng/ml, Group B consisted of patients with minimal (< 0.5%) RAIU with Tg< 0.2 ng/ml, and group C consisted of patients with minimal (< 0.5%) IU uptake on WB scan with Tg< 10 ng/ml. Based on these criteria, ablation success rate is 20%, 80%, 100% for the low and 25%, 80%, and 100% for high dose groups respectively. No significant difference was observed for the success of ablation between patient who received low and high I-131 dose.

**Conclusion:** Low-dose 1-131 ablation on an outpatient setting is an efficient therapeutic option for the low-risk thyroid cancer patients.
Objectives: To assess the efficiency of J-131 whole body scan (WBS) with diagnostic dose was compared with thyroglobulin (Tg) values in the post operative follow-up of patients with differentiated thyroid carcinoma.

Material and methods: Sixty two operated patients, 49 female (36 ± 18 yrs) and 13 male (30 ±16 yrs) with well-differentiated thyroid carcinoma (43 papillary, 19 follicular) were scanned. The patients were imaged after administering a diagnostic J-131 dose of 111 - 260 MBq. Images were diagnosed for the presence and the intensity of thyroid lesions. J-131 WBS was performed for the first time after total thyroidectomy and radioiodine ablation of thyroid remnant, after that once a year under hypothyroid conditions (TSH > 30 U/ml). In the interim we performed Tg measurement and high-resolution ultrasound of the neck. The sensitivity, specificity and accuracy of Tg levels for diagnosis of tumor residue or metastases were calculated and compared with results obtained by diagnostic J-131 WBS.

Results: From 54 consecutive patients who had complete remission, tumor recurrence or metastases were detected: 5/54 (9.2%) only by J-131 WBS; in 3/54 (5.5%) only by Tg measurement; in 7/54 (11,1%) by both methods. The sensitivity, specificity and accuracy of diagnostic J-131 WBS were 77%, 100% and 83% respectively, where as for the Tg levels determination were 81%, 61% and 73%. If results of J-131 WBS and Tg were taken into consideration, sensitivity reached 95%, specificity 100% and accuracy 98%.

Conclusions: The management and follow up of patients with differentiated thyroid carcinoma diagnostic J-131 WBS is essential since Tg measurement alone can not replace J-131. Using both J-131 WBS and Tg as diagnostic tools in search for metastases and recurrence of the disease gives much better accuracy.
Introduction: The effectiveness of high-dose RAI treatment is controversial in pts with high Tg and/or Anti-Tg levels and negative I-131 wbs.

Material-method: 51 pts with DTC were evaluated. After thyroidectomy, pts had an ablative dose of RAI. All pts had negative I-131 wbs and elevated Tg and/or Anti-Tg levels. Imaging modalities (USG, CT and 18F-FDG PET/CT) were performed in all pts. 28/51 pts (20F, 8M age range 22-70) with metastatic and/or recurrent disease underwent surgery before RAI and metastases were confirmed with histopathology. We evaluated other 23/51 pts (4 M, 19F mean age 27-76). During the follow-up each patient had at least one of the other diagnostic imaging techniques to confirm metastasis and/or recurrence suspicion. In this group, surgery was not performed because of patient-related reasons and RAI was given on the basis of elevated Tg and/or Anti-Tg levels. After 5-36 months, Tg and Anti-Tg were measured and pre-posttherapy levels were compared.

Results: 5/23 pts had high Anti-Tg (group I), 2/23 pts had both increased Tg and Anti-Tg (group II) and 16 had only high Tg levels (group III). In group I lesions were detected with other imaging modalities while 2 pts were evaluated as normal. Empiric I-131 therapy was given to all pts and pathologic uptake was observed in 3 pts with positive findings and 1 pt with negative findings. Tg levels was decreased at least 20% in all pts in 6-36 months follow-up. In group II, although all pts had positive lesions in other imaging modalities, one of them had positive I-131 posttherapeutic wbs. After the empiric I-131 treatment, in 11/16 pts Tg levels decreased more than 30%, in 1/16 pt Tg levels was not changed. On the other hand in 4/16 Tg levels were increased.

Conclusion: In pts with increased Tg and/or Anti-Tg levels and negative I-131 wbs, high doses of RAI may improve the uptake of I-131 in micrometastatic foci. A decrease in Tg and/or Anti-Tg can be evaluated with other diagnostic work-up as response to therapy.
rhTSH has been widely accepted in thyroid remnant ablation and monitoring of patients with differentiated thyroid cancer (DTC). It has been also used as preparation for 131-I therapy of advanced DTC, however, there are only few data on repeated rhTSH treatments. The aim of this retrospective study was to evaluate results and safety of this therapy.

**Patients and methods:** From more than 378 rhTSH assisted radiiodine therapies, 44 patients with radioiodine avid metastatic disease and at least three consecutive rhTSH assisted therapies were selected. 214 (median 4) rhTSH therapies were performed in those 44 patients. All patients had radioiodine uptake in metastases as confirmed in wholebody scintigraphy. rhTSH administered as standard schedule was followed by 3.7 to 5.5 GBq of 131-I. Treatments were repeated in 4-6 month intervals. TSH and thyroglobulin (Tg) were assessed on day 1st, 3rd and 6th.

**Results:** Stimulated serum TSH level rose from 0.1 mU/L to >or=25 mU/L (median 160 mU/L) and there were no statistically significant differences in peak TSH level between courses. There was a statistically significant increase in Tg after rhTSH (2140 day1 v. 5042 ng/ml day6; p< 0.05). After first two cycles of rhTSH aided 131-I therapy there was a statistically significant decreases in stimulated Tg concentration level on day 6 (1121 v. 240 ng; p< 0.05). However, in 6 out of 28 (21%) patients during subsequent treatments Tg started to increase. In 7 (16%) partial remission was diagnosed in radiological examinations. Median time to progression was 40 months.

Therapy was well tolerated, there were no grade 3 or 4 toxicities. The most frequent side-effect was increase in metastatic bone pain.

**Conclusions:** Repeated rhTSH assisted radiiodine therapies are well tolerated and save. Adequate TSH stimulation can be obtained in all patients and results in Tg response and radiologically confirmed tumour regressions.
Background: Traditionally, withdrawal of thyroid hormone is used to attain the increase in serum TSH concentrations in preparing patients for diagnostic procedures, thyroid remnant ablation and treatment of patients with differentiated thyroid cancer (DTC). Recombinant human TSH (rhTSH) was developed to provide TSH stimulation without withdrawal of thyroid hormone and the associated morbidity.

Objective: To present our clinical experience of four years with rhTSH.

Methods: We assessed the results of 189 patients to whom rhTSH was administered for various reasons, mostly for diagnostic evaluation, between January 2005 and December 2008. They were 85.2% female and 14.8% male, with a mean age of 51.4 ± 13.4 years (18-85). Administration of 0.9 mg of thyrotropin alpha (Thyrogen ®), by the intramuscular route, was performed in two consecutive days and serum TSH and thyroglobulin (Tg) assays were performed 72 hours after last injection. Some patients had also TSH measurement at day 3.

Results: In the fifth day after the first administration of rhTSH, serum TSH was 20.3 ± 15.8 µIU/ml (1.95-100). Peak serum TSH at day 3 was 96.5 ± 15.4 µIU/ml. The value of serum Tg was 27.7 ± 169.2 ng/ml (≤0.2-1905 ng/ml), 52.2% with Tg values of less than 0.2 ng/ml and 27.4% above 2 ng/ml. Anti-thyroglobulin levels were 43.2 ± 92 UI/ml (0.2-500). No clinical relevant side effects were noticed.

Conclusions: Levothyroxine withdrawal frequently causes clinical hypothyroidism, with cognitive impairment, emotional dysfunction, physical discomfort, impaired quality of life, inability to work and health risks in patients who are elderly, frail or have concomitant illnesses. These issues can be avoided with rhTSH administration. This is a safe, effective and very useful diagnostic tool in the long-term management of patients with DTC.
POSSIBILITY OF THYROID REMNANT ABLATION BY FRACTIONATED DOSING FOR THE OUTPATIENTS - THE PRESENT STATE IN JAPAN

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Objectives: Total thyroidectomy followed by remnant ablation with 131I is the initial therapy indicated for most patients with differentiated thyroid carcinoma. Patients administered a somewhat larger radioactive iodine may be required hospitalization for 48-72 hrs or so, depending on the dose administered. But now in Japan, there are so few institutions for the hospitalization that many of the post-operative patients are left unablated. However, work from India has suggested that activities as low as 30mCi may work just as well as the 100-150mCi with about 80% success rate. So we tried to administer radioactive iodine to outpatients for remnant ablation in following two methods.

Methods:
(a) Fractionated dosing: 481MBq and 436MBq radioactive iodine is administered on the first and the third day. Because, in Japan, maximum dose allowed to the outpatients is 500MBq.
(b) Single dosing: 1110MBq radioactive iodine is administered on the first day. Candidates, who are able to be isolated from the household members for 7 days, are selected strictly.
All the radiation exposure to household members from patients dosed 30mCi was proved below the dose limit for the public exposure by ICPR (5.0 mSv). The uptake of the radioactive iodine (RAIU) on the fifth day was quantificated and compared.

Results: RAIU of 8 patients by fractionated dosing were superior to 12 by single dosing because of stunning effect in case of fractionated dosing. But in 4 of 12 cases of fractionated dosing, RAIU of the second administration on the third day was not so influenced by the first administration and good enough.

Conclusions: The incidence of stunning varies depending on patient selection, criteria used, time interval after the first dosing, and iodine avidity of the target lesions. Further investigation of these factors might reach a more appropriate fractionated dosing of remnant ablation for the outpatients in Japan.
P35 Thyroid Cancer 6

P209
CLINICAL BEHAVIOR AND OUTCOME OF PAPILLARY THYROID CANCERS SMALLER THAN 1.5 CM IN DIAMETER

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Objective: Investigate predictors of morbidity of papillary thyroid cancers smaller than 1.5cm.

Patients and methods: We studied 118 patients with small papillary cancers. 69 patients had surgery due to FNAC results- nonincidental carcinoma and 47 patients diagnosed after a surgery for a benign thyroid disease- incidental carcinoma. 50% patients received I131 ablative therapy and 11% patients had I131 therapeutic doses.

Results: Mean age at diagnosis was 49.1 years, 12 males and 106 females, average follow up was 6.3 years. At presentation 87 cases with tumours ≤10mm (group I) and 31 cases with tumours > 10mm and ≤15mm (group II). The histopathological characteristics over all were: 35% multicentric, 6.7% extrathyroidal, 10% lymph node involvement and 0.8% distant metastases. In group I: 35% multicentric, 3.4% extrathyroidal, 5.7% lymph node involvement and no distance metastases. In group II: 36% multicentric, 16% extra thyroidal, 23% lymph node involvement and one patient with distant metastases. Between the two groups there was a significant difference in lymph node involvement (p = 0.014) and extrathyroidal extension (p = 0.029). In the nonincidental tumours 39% multicentric, 10% extra thyroidal, 10% lymph node involvement, 1.4% distant metastases and in the incidental tumours 28% multicentric, 2.1% extrathyroidal, 2% lymph node involvement and 0% distant metastases. In comparison between incidental and nonincidental carcinomas only lymph node involvement was significant (p = 0.027). Currently 5 patients (4.2%) still have active disease, 2 in group I and 3 in group II with no significant difference (p = 0.079) between them. All patients with nonincidental tumours are cured. Mortality rate due to small papillary cancer was zero.

Conclusions: Good prognosis of small papillary cancers. Large cancers (1.1-1.5cm) have higher prevalence of signs of aggressiveness but this doesn't seem to have a worse outcome. Nonincidental carcinomas have also more negative prognostic factors.
Introduction: The Thyroid Cancer Consultation Group, comprising specialists of endocrinology, surgery, pathology and nuclear medicine was created in 2006 with the aim of standardizing the initial treatment and the follow-up of patients with thyroid carcinoma at our institution.

Objectives: To present aspects of the series of patients seen in our consultation during the period 2006 to 2008.

Methods: Retrospective analysis of clinical data of patients presented to the consultation group of thyroid cancer. Statistical analysis was done with SPSS 16.0 for Windows.

Results: During this period 526 patients were evaluated, 443 women and 83 men with a mean age of 51.8±14.7 and 54.8±13.8 years old respectively. One hundred and sixty-nine patients were aged ≤ 45 years (32.1%) and 357 were aged > 45 years (67.9%). Of these patients, 392 were submitted to total thyroidectomy, 122 to a lobectomy, 68 to totalization of thyroidectomy and 5 to other type of surgery. The most commonly observed histologies were classic papillary cancer in 282 (51.9%), follicular variant of papillary cancer in 180 (33.1%), oncocytic variant of papillary cancer in 50 (9.2%) and follicular cancer with minimal invasion in 10 (1.8%). According to the TNM classification, 408 tumors (69.5%) were ≤ 2cm and of these 259 were microcarcinomas (≤ 1cm); 92 had tumor size between 2 and 4 cm (15.7%) and 43 were above 4 cm (7.3%).

Conclusions: This series illustrates the high prevalence of well differentiated thyroid tumors mainly due to the presence of small tumours (69.5% were ≤ 2 cm) which can be explained by the widespread use of imaging methods and increased detection of subclinical tumours. We also verified that the vast majority of tumors are variants of the papillary type (96%), the follicular type is uncommon (1.8%) and the other types are much more rare.
Introduction: Poorly differentiated (PDTC) and anaplastic thyroid carcinoma (ATC) comprise a small subset of aggressive thyroid tumors (about 10% and 2%, respectively) that are associated with a poor prognosis. The peak incidence occurs in the 6th to 7th decade of life. PDTC may represent intermediate entities in the progression of well differentiated thyroid carcinoma to ATC.

Clinical cases: In our institution from 2006 to 2008 we operated on 526 patients with thyroid cancer and we report 6 cases (1.14%), 2 males and 4 females, 3 diagnosed with PDTC and 3 with ATC. Mean age at diagnosis was 65 years (55-73) for PDTC and 72 years (61-83) for ATC. Most patients presented with local compressive symptoms including dyspnea (2), dysphagia (2), dysphonia (3), and/or a rapidly growing neck mass (3) with cervical pain (1). One PDTC was diagnosed after routine thoracic imaging. Median mass diameter at diagnosis for PDTC was 5.7 cm (5-6) and for ATC was 3.2 cm (2.7-4). At presentation lymph node involvement was documented in two ATC and systemic metastases (pulmonary, bone) occurred in two PDTC and two ATC. Total thyroidectomy was feasible in two PDTC and one ATC and maximal debulking surgery in one ATC. The remainder were irressecable. Radiotherapy after surgery was done in one ATC. Five patients died, with survival of 2 to 19 months for PDTC and 1 to 5 months for ATC. One patient with PDTC is alive 2 years after diagnosis.

Conclusion: This series illustrates that PDTC and ATC are two of the most aggressive solid cancers known to affect humans. In these patients ATC is smaller but much more aggressive than PDTC at diagnosis. Unfortunately, conventional therapy is rarely curative. New therapeutic strategies based on molecular approaches are desirable, such as vascular and growth factor-targeted therapies or tumor suppressor gene induction.
THE CHARACTERISTICS AND PATTERN OF RECURRENCE FOLLOWING THYROIDECTOMY AND RADIOIODINE FOR WELL-DIFFERENTIATED THYROID CARCINOMA

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Objectives: To evaluate the pattern of recurrences in patients with well differentiated thyroid carcinoma following surgery and radioiodine therapy.

Methods: We evaluated all the patients who had recurrent thyroid carcinoma between June 2004 and February 2009. Data on initial surgery, radioiodine therapy, and subsequent treatment detail were collected.

Results: Twenty-one patients (11 males; 10 females) developed recurrent thyroid cancer. The age ranged between 21-78 years. Eighteen patients had papillary carcinoma and three had follicular carcinoma. Median duration from initial thyroid surgery to diagnosis of recurrence was 25 months (range: 4 -171 months). In the initial thyroid surgery, 4 patients had subtotal thyroidectomies (19%), 17 patients had total thyroidectomies (81%) of which 6 had lateral neck dissections and 3 had central neck dissections. All patients had postoperative radioiodine therapy. 12(57%) presented with raised thyroglobulin, 6 with palpable neck lumps and 3 with both raised thyroglobulin and neck lumps. There were 14 lateral neck, 4 central neck and 6 thyroid bed recurrences. There were 8 lateral neck recurrences in 12 patients who did not have any neck dissection at presentation. All 3 patients who had central neck nodal disease had lateral neck recurrences.

Conclusions: Most recurrences were detected through raised thyroglobulin. Majority of recurrences were in lateral neck compartment and this was the case in two-third of cases who did not have any neck dissection at Initial surgery. Initial central compartment nodal metastasis may be predictive for lateral neck recurrences.
Background: DTC is generally associated with a long-term survival. However, DTC prognosis is greatly affected by DMs, which occur in 10-15% of cases. In these patients cause-specific survival rates are approximately 40%, 30% and 25%, after 5, 10 and 15 years, respectively. DMs usually develop during the first years of follow-up but may also represent the first sign of thyroid cancer.

Aims: To evaluate whether DMs occurring as the first sign of the disease may affect patient prognosis, we carried out a retrospective analysis in a consecutive series of DTC patients with DMs.

Patients and methods: Over 3,000 patients were referred to our thyroid clinic for DTC in the period 1981-2008. 208 patients had DMs and in 34 DMs were the first sign of the disease (group A). In the remaining 174 patients DMs occurred during follow-up (group B).

Results: Age and gender were not different in the two groups. The relative prevalence of PTC was 41.1% and 58.9% in group A and B, respectively. FTC patients were 58.9% in group A and 34.5% in group B. In both groups DMs more frequently localized at lungs. Metastases not involving the lung were more frequent in group A than in group B (29.4% vs. 6.9%, respectively, \( P=0.0001 \)). When the lung was involved, other metastatic sites coexisted in 87.5% of cases in group A and in 48.2% in group B (\( P=0.0003 \)). After a mean follow-up of 108.6±93 months (range 5.7-524.3), 47.0% patients from group A and 22.4% patients from group B had died for the disease (\( P=0.0029 \)).

Conclusions: When DM is the first sign of disease, DTC prognosis is worse than in patients who develop DMs during follow-up. An increased proportion of FTC, of multiple DMs and DMs not involving the lung are observed in these patients.
**PRESENCE OF RET POLYMORPHISMS IN PATIENTS AFFECTED BY MEDULLARY THYROID CARCINOMA**

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**Objectives:** Single-nucleotide polymorphisms of RET gene (RETpol) -including those located in exons 11, 13, 14, 15- have been described in the general population and in patients affected by medullary thyroid carcinoma (MTC). The presence of RETpol was evaluated in patients affected by MTC and in healthy first-degree-relatives of patients with MTC (FDR-M) to assess possible association of polymorphisms with clinical outcome.

**Methods:** 104 subjects were studied. They performed laboratory tests (calcitonin, CEA, NSE) and instrumental investigations: neck/abdomen ultrasonography, chest/vertebral column radiography, total body TC/RMN, \(^{131}\)I-MIBG/\(^{111}\)In-Pentetreotide scintigraphy, \(^{18}\)F-FDG positron emission tomography. RET exons containing mutations/polymorphisms of interest were amplified by a PCR-restriction fragment-length polymorphism assay and exons were sequenced.

**Results:** Out of 104 subjects, 63 were affected by MTC and 41 were FDR-M. 18 patients had RET mutations: Cys634Trp, Cys634Arg, Val804Met and Met918Tre. 70/104 subjects were screened for RETpol: G691S(exon 11), L769L(exon 13), S863S(exon 14), and S904S(exon 15). Fifty-eight of these 70 subjects evidenced RETpol; they were divided into 2 groups: group 1 consisted of 25 patients with MTC, group 2 of 33 FDR-M.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Ex13</th>
<th>Ex14</th>
<th>Ex11,13</th>
<th>Ex11,14</th>
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<th>Ex13,14</th>
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<tbody>
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<td>Mut+</td>
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<td>1pt</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>Mut-</td>
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<td>-</td>
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<td>1pt</td>
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<td>2pts</td>
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<td>5pts</td>
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</tbody>
</table>

Ten patients of group 1 had locoregional, mediastinal, pulmonary and bone metastases. 18 subjects of group 2 had multinodular goitre and 2 patients chronic thyroiditis. All MTC patients without RETpol showed no metastases; 4 FDR-M without RETpol had no thyroid disease.

**Conclusions:** Our results confirm the important role of early genetic diagnosis of MTC. In this study, the presence of RETpol is associated with a worse clinical outcome. Therefore, the presence of RETpol may be of help in the evaluation of both MTC patients and FDR-M subjects.

**Key words:** RET, polymorphisms, medullary thyroid carcinoma
MOLECULAR ALTERATIONS IN A FOLLICULAR VARIANT OF PAPILLARY THYROID CARCINOMA IN STRUMA OVARII

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Introduction: Struma ovarii (SO) is a rare monodermal form of ovarian teratoma in which more than 50% of the tumour is composed by thyroid tissue. The histologic appearance of SO mimics those of several thyroid lesions (from benign to malignant), being considered as malignant SO (MSO) whenever presenting morphologic features of papillary thyroid carcinoma (PTC) or of follicular thyroid carcinoma (FTC). The diagnosis of PTC and FTC in SO is however very difficult in most cases. A few cases of SO have been characterized regarding the genetic alterations detected in well-differentiated thyroid tumours (BRAF, NRAS, RET/PTC). So far, no RAS mutations and/or PAX8-PPARγ rearrangement in SO cases have been reported, despite their prevalence in thyroid tumours with follicular architecture.

Objective: To perform the histological and molecular characterization of an unusual case of SO displaying the morphological appearance of follicular variant of PTC (FVPTC).

Material and methods: A right ovarian cyst from a 38-year-old female was studied macroscopically, histologically and immunocytochemically leading to the tentative diagnosis of FVPTC. PCR/direct sequencing for H-RAS, N-RAS and BRAF genes was done, as well as FISH for RET/PTC and PAX8-PPARγ rearrangements.

Results: The histological examination showed a tumour with a follicular growth pattern, composed of cells with large, roundish and clear nuclei with evenly distributed coarse chromatin. A tentative diagnosis of MSO with FVPTC phenotype was made. The molecular study reveals a Q61R mutation in NRAS and a PAX8-PPARγ rearrangement.

Conclusion: The detection of the aforementioned molecular alterations supports the diagnosis of a MSO with a FVPTC phenotype. We suggest the search of molecular alterations should be performed in SO cases raising difficult differential diagnostic problems. The coexistence of the NRAS mutation and the PAX8-PPARγ rearrangement in this FVPTC arising in a SO fits with results obtained in primary thyroid carcinomas with this same histotype.
Thyroid hormone resistance (RTH) corresponds to a rare syndrome where high levels of thyroid hormones are found with inappropriate secretion of thyroid stimulating hormone (TSH). This syndrome is highly associated with mutations in the thyroid hormone beta-receptor (THRβ) where several mutations have been described. THRβ somatic mutations have been described in RTH, but association of germline or somatic THRβ mutations with malignant thyroid tumours has not been reported to date as far we are aware.

**Objectives:** Clinical and molecular characterization of a patient with RTH presenting thyroid nodules.

**Methods:** Thyroid function tests. Direct sequencing of THRβ exons 7, 8, 9 and 10 using DNA extracted from peripheral blood. Direct sequencing of BRAF exon 15, H and NRAS exon 2 in DNA extracted from paraffin-embedded tumour.

**Results:** The patient was a 19 year-old female, referred to the Endocrinology Dept due to the presence of thyroid nodules. The cytology revealed a follicular tumour. Due to the high levels of T3 and T4 with measurable TSH, and absence of any pituitary tumour in RMN, RTH was suspected. The molecular study revealed a germline mutation at codon 320 on exon 9 of THRβ gene, confirming the diagnosis of RTH syndrome. The patient was referred for total thyroidectomy. Histological examination showed, besides two follicular adenomas, a 4 mm papillary microcarcinoma. The patient was submitted to radioiodine treatment but a few months later showed several lymph node metastasis from the papillary carcinoma. BRAF V600E mutation was found in the papillary microcarcinoma and in the metastasis. No RAS mutations were detected.

**Conclusion:** We describe a patient with RTH presenting a papillary microcarcinoma with an unusual aggressive course. A BRAF mutation, detected in the primary and the metastatic tumour, together with the increased TSH stimulation in a RTH background, may serve as (co)drivers of malignant transformation and aggressiveness.
MEDULLARY THYROID CARCINOMA AT YOUNG AGE - IMPORTANCE OF GENETIC TESTING

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Introduction: About 20% of all medullary thyroid carcinoma (MTC) are genetic in origin (45% of this heritable fraction is attributable to MEN 2A). The likelihood of a RET germline mutation with apparently sporadic MTC is 1 to 7%. This probability is higher at young age and/or in multifocal tumors.

Case report: We present a kindred whose index case was a 22-year-old caucasian female, with multinodular goiter and a diagnosis of MTC by FNAB. She was admitted to our hospital and submitted to thyroidectomy with lymph node dissection (TwLND), after exclusion of pheochromocytoma (PHEO) and hyperparathyroidism (HPT). Histopathologic findings confirmed the diagnosis of multifocal MTC. Although initially she didn’t know, later the patient reported family history of thyroid cancer and sudden death. Genetic testing showed a RET mutation in exon 11-C634R. Screening of elements of the family identified 4 carriers and enabled the diagnosis of the following MEN 2A manifestations:

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>Gender</th>
<th>MTC</th>
<th>PHEO</th>
<th>HPT</th>
<th>Cutaneous lichen amyloidosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Index</td>
<td>22</td>
<td>female</td>
<td>√</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>TwLND</td>
</tr>
<tr>
<td>2-Mother</td>
<td>42</td>
<td>female</td>
<td>√</td>
<td>Bilateral</td>
<td>-</td>
<td>√</td>
<td>TwLND, bilateral adrenalectomy</td>
</tr>
<tr>
<td>3-Brother</td>
<td>20</td>
<td>male</td>
<td>√</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>TwLND</td>
</tr>
<tr>
<td>4-Uncle</td>
<td>40</td>
<td>male</td>
<td>√</td>
<td>Bilateral</td>
<td>-</td>
<td>-</td>
<td>TwLND, bilateral adrenalectomy</td>
</tr>
<tr>
<td>5-Cousin</td>
<td>26</td>
<td>female</td>
<td>√</td>
<td>Left</td>
<td>√</td>
<td>-</td>
<td>TwLND, left adrenalectomy, Parathyroidectomy</td>
</tr>
</tbody>
</table>

Conclusion: This work underline the importance of genetic testing in MTC. In MEN 2A, there is a relevant genotype-phenotype correlation. The identified mutation is associated with cutaneous lichen amyloidosis and with a high risk for MTC, PHEO and HPT in childhood. Carriers with this mutation should have prophylactic thyroidectomy before the age of 5 years (or even before 2 years), whereby periodic screening and genetic counselling are essential.
Introduction: Considering the number of patients with differentiated thyroid carcinoma and their long survival, it became relevant to define how to make the surveillance of these patients. Tg measurement after TSH stimulation with rhTSH and cervical ultra-sound (US) are the main tests in the follow-up of low risk thyroid cancer patients. US is sensitive to detect lymph node enlargement and, if suspicious, it should be followed by fine needle aspiration (FNA) for cytology and assessment of Tg in the needle washout (FNA-Tg), because the material for cytology is often inadequate. In our department, this practice has been used in the last two years, with good results.

Clinical case: A 58 year woman, submitted to total thyroid ablation in 2006, after cytology of a lymph node metastases, with a “papillary thyroid cancer, follicular variant of columnar cells… invasive…” She was submitted to I131 and the whole body scan revealed cervical fixation. Later the US showed suspicious lymph nodes. Cytology: “metastases…” FNA-Tg - 225 ng/ml. TG after rhTSH undetectable (with undetectable Tg-Ab). She was submitted to lymph node excision in 2008 and the diagnosis was confirmed. Recently the Tg remains undetectable but the US revealed lymph nodes metastasis on the other side. She went to surgery once more.

Discussion: In this case we have confirmed the advantages of FNAC and FNA-Tg in the diagnosis of lymph node metastases in patients with differentiated thyroid carcinoma. This procedure is useful even in patients with undetectable serum Tg or in the presence of TgAb. We can conclude that Tg after rh-TSH should be evaluated together with FNAC and FNA-Tg, in the presence of any suspicious lymph nodes in the US.
Introduction: The well-differentiated thyroid carcinomas are poorly aggressive tumors and thus determine a long survival. By contrast, the anaplastic thyroid carcinoma is one of the most lethal tumors, with very short survival, ranging between 3 and 7 months. The anaplastic transformation of differentiated thyroid carcinomas is described classically in follicular carcinomas, being very rare in papillary carcinomas.

Case report: Female, 71 years, without relevant personal or family history. Admitted in the emergency department after a two-month history of dysphagia, odynophagia, dysphonia, cervical pain, dyspnea and orthopnea. At physical examination the patient showed a cervical mass of hard consistency, painless, fixed, without palpable cervical or supraclavicular adenopathies. Thyroid function was normal. The chest x-ray showed tracheal deviation and enlargement of the right mediastinum. The indirect laryngoscopy was normal. A cervicothoracic CT scan revealed a lesion with neoplastic characteristics, contiguous with the right thyroid lobe, extensive to the superior mediastinum, conditioning deformity of trachea and esophagus, by compression and probable invasion. Fine needle aspiration biopsy revealed carcinoma. Total thyroidectomy was performed with maintenance of residual tumor. The histological examination revealed anaplastic transformation of a 4-cm papillary thyroid carcinoma with extrathyroidal extension. The patient was readmitted 4 days later due to worsening of dyspnea secondary to lymphangitic carcinomatosis. Death occurred at the 20th hospitalization day.

Conclusion: This paradigmatic case of evolution of anaplastic carcinoma confirms the extreme difficulty and importance of controlling both local and advanced disease whenever possible. It is unequivocally shown the anaplastic transformation of a papillary carcinoma. Despite of the rarity of this transformation, this event when occurs, has devastating consequences, which emphasizes the importance of an early diagnosis and treatment of the differentiated thyroid carcinoma.
**Objective:** The aim of the present study was to investigate the diagnostic value of a combined strategy of 18F-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) with pentagastrin stimulation to identify metastases of MTC in patients with elevated calcitonin (CT) postoperatively.

**Methods:** We performed two FDG-PET scanning to three patients, who had postoperative basal CT levels over 100 pg/mL, with pentagastrin stimulation initially and without stimulation 15 days later. The patients were then sent to surgery for the detected lesions.

**Results:** There was no significant difference between baseline and pentagastrin stimulated FDG-PET uptakes and the SUVmax values (Table 1).

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
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<tbody>
<tr>
<td>Baseline CT pg/mL (0-10)</td>
<td>124.7</td>
<td>331.4</td>
</tr>
<tr>
<td>Stimulated max CT pg/mL</td>
<td>243.8</td>
<td>990.9</td>
</tr>
<tr>
<td>CEA ng/mL (0-3.4)</td>
<td>5.97</td>
<td>5.61</td>
</tr>
<tr>
<td>Baseline FDG-PET Lesion (SUVmax)</td>
<td>Right deep cervical LAP (early:4.8, late:5.71) Left sup. jugular and deep cervical LAP (early:4.7, late:4.3)</td>
<td>Bilateral paramandibular and submental LAP (early:3.9, late:4.6) Left ant. cervical LAP (early:2.5, late:4.5)</td>
</tr>
<tr>
<td>Pentagastrin Stimulated FDG-PET Lesion (SUVmax)</td>
<td>Right deep cervical LAP (early:5.3, late:5.3) Left sup jugular and deep cervical LAP (early:4.2, late:4.7)</td>
<td>Bilateral paramandibular and submental LAP (early:2.47, late:4.64) Left ant cervical LAP (erken:3.72, late:3.85)</td>
</tr>
<tr>
<td>Neck USG</td>
<td>Right level III, left level III LAP</td>
<td>Left level III and IV LAP</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Waiting for result</td>
<td>Waiting for result</td>
</tr>
</tbody>
</table>

[Basal and stimulated CT, CEA, FDG-PET of 3 patient]

**Conclusion:** FDG-PET combined with pentagastrin stimulation was not superior to FDG-PET alone for the localisation of MTC metastases for these three cases. However histopathological results of two of the three patients are yet to be taken.
A CASE OF THYROID MYOSARCOMA IN A 14 YEARS OLD GIRL

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Introduction: Thyroid myosarcoma is exceedingly rare and with a very poor prognosis. To our knowledge none of the reported cases are children or adolescent.

Case report: We present a case of primary thyroid myosarcoma in a 14 years old girl. On June 2003 she noticed a left thyroid nodule measuring 3x4 cm on ultrasound; 2 weeks later, she complained of dysphonia, dyspnoea and disphagia and had a stony mass measuring on ultrasound 6x8 cm. On July the 18th, she was submitted to total thyroidectomy with bilateral central neck dissection, in Egas Moniz Hospital. A huge thyroid mass with macroscopic invasion of trachea, oesophagus and lymph nodes was found. Microscopically, the tumour showed a highly cellular proliferation with fusocellular appearance, infiltrating the thyroid and perithyroid tissues. The neoplastic cells did not express epithelial markers, thyroglobulin, calcitonin or TTF1. Other endocrine and neuroendocrine markers were also negative. There was a diffuse staining with desmin, HHF35 and smooth muscle actin. MyoD1 and myogenin were focally positive. The diagnosis of a high grade myosarcoma was done. In the Department of Paediatrics of Portuguese Cancer Institute she was submitted to chemotherapy according to the protocol 935B MMT95, followed by external radiotherapy to the neck with a total dose of 50Gy. Complete remission was achieved and in the last follow up on February 2009, with 19 years old, there was no evidence of disease. Treatment with thyroxine and calcitriol is required.

Conclusion: Only a few cases of primary thyroid myosarcoma have been reported in the literature, all of then occurring in adults with a dramatic evolution. Here we report the unique case of a 14 years old girl with an unexpected five and a half years disease free survival.
P222
CLINICAL AND PDS GENE ANALYSIS IN A FAMILY WITH PENDRED’S SYNDROME ASSOCIATED WITH THYROID PAPILLARY CANCER
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Objective: Pendred’s syndrome is an autosomal recessive disorder characterized by sensorineural deafness, euthyroid or hypothyroid diffuse goitre and a positive perchlorate test and it is caused by PDS (7q13) gene mutation (SLC26A4). In the course of Pendred’s syndrome thyroid cancer was observed, particularly the follicular type. No PDS gene mutation characteristic for developing thyroid cancer in these patients was described. We present clinical and molecular genetics studies in a family with Pendred’s syndrome, in which one affected individual developed papillary thyroid cancer.

Methods: Two out of five children were affected and displayed the classic Pendred’s syndrome triad. DNA was isolated from whole blood. All exons and exon-intron boundaries of the PDS/SLC26A4 gene were amplified by PCR and sequenced.

Results: Because of an enlarged goitre, patients were operated on and histopathological findings showed papillary thyroid cancer in a boy and multinodular goitre in a girl. The PDS gene analysis in this family revealed a transition of G to A in the splice donor site of intron 8 (IVS8+1G>A). The two affected individuals were homozygous for this mutation, whereas both parents and one unaffected daughter were heterozygous for it. The remaining two unaffected individuals have no PDS gene mutation.

Conclusions: The question remains whether the thyroid should be operated on in patients with Pendred’s syndrome coexisting with thyroid solitary nodule or multinodular goitre because of the possibility of malignancy and whether the specific PDS mutation exists, which may predispose to the development of thyroid cancer.
Anaplastic transformation may occur in primary tumors but also in metastatic lymph nodes. Our 63-years-old patient underwent total thyroidectomy because of T4N0M0 papillary carcinoma of the left lobe. The tumor infiltrated strap muscles and was fixed to the esophagus and hypopharynx. Lymph nodes were not enlarged. Surgical procedure was declared as R1 resection. Histopathology revealed classical moderately differentiated papillary thyroid carcinoma. Tumor diameter was 3x1.2 cm, with extensive transcapsular infiltration and lymphatic invasion. Tumor was in anterior, and close to posterior and cranial surgical margins. Postoperative ablation of thyroid remnant with radioiodine was performed, followed by external radiotherapy with a Co^{60} unit and two opposed fields. The radiation field included the entire neck up to the level of the mastoid process, bilateral supraclavicular and infraclavicular regions, and the superior mediastinum. Total target dose was 48.6 Gy. After one year, radioiodine thyroid test revealed no accumulation. The patient was on suppressive thyroid hormone therapy thereafter. Clinical exam and US investigation 84 months after surgery did not show local recurrence or suspicious lymph nodes. However, 86 months after surgical procedure, the patient observed rapid tumor growth in the left submandibular and mastoid region. FNAB was performed and anaplastic thyroid carcinoma was verified by cytology. According to US investigation the tumor encased internal and external carotid artery. Two courses of chemotherapy were applied without any effect. The patient succumbed because of locoregional and distant disease three months after diagnosis. Probably, anaplastic carcinoma appeared de novo in a lymph node at the margin of the radiotherapy field in a preexistent metastatic differentiated papillary carcinoma. The message of this case report is that in the patient with advanced well differentiated thyroid carcinoma a modified radical neck dissection should be performed also in order to prevent the dedifferentiation of tumor in a lymph node metastasis.
P224
METASTATIC BONE: FIRST PRESENTATION OF THYROID FOLLICULAR CARCINOMA (THREE CASES)
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Introduction: Follicular thyroid carcinoma is a well-differentiated tumour. It may spread to other organs. This is due to angioinvasion. Lymphatic involvement is also possible. At diagnosis, 10-15% patients have distant metastasis to lung and bone. Some have been evaluated initially for pulmonary or osteoarticular symptoms. We report three cases of metastatic bone disease as the first presentation of thyroid follicular carcinoma.

Case 1: A 47 years female patient presented a huge mass on 7th right rib. Biopsy: metastatic thyroid carcinoma. Thyroid ultrasound showed a 1.5 cm nodule on right lobe. Total thyroidectomy with lymph node dissection was performed (histology follicular carcinoma) and thoracic mass was removed (T2N1M1). She was submitted on 131-Iodine (total dose: 930 mCi) and external radiotherapy. Last evaluation: bone metastasis (dorsal and cervical vertebrae); thyroglobulin 31 ng/mL with TSH < 0.09.

Case 2: A 47 years male patient had a pain on the left arm. X-ray: lytic lesion on left umero. Surgery was performed (histology: metastatic lesion of follicular carcinoma). Thyroid ultrasound showed multinodular goiter. Total thyroidectomy was done (histology: follicular carcinoma). T2bNxM1. During follow-up pulmonary metastasis were detected and surgery was done. He went on four treatments with 131-Iodine (total dose: 614 mCi). Last evaluation: pulmonary metastasis; thyroglobulin 41 ng/mL with TSH 0.051.

Case 3: A 71 years male patient pain on the left leg. X-ray: lytic lesion on iliac bone. Biopsy: metastases of thyroid follicular carcinoma. Thyroid ultrasound showed micro nodules. Total thyroidectomy was done (histology: follicular carcinoma with 7mm). T1NxM1. He went on several treatments with 131-Iodine (total dose: 994 mCi). Last evaluation: mielodisplasic syndrome.

Conclusions: Follicular thyroid carcinoma with metastasis is associated with poor prognosis. Bone metastases may be the first presentation of thyroid follicular carcinoma. These lesions must be distinguished with primary bone tumours.
INTEGRATED \textsuperscript{18}F-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY / COMPUTED TOMOGRAPHY THYROID INCIDENTALOMA: CASE REPORT AND LITERATURE REVIEW

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Objective: To present a case of thyroid \textsuperscript{18}F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (FDG-PET/CT) incidentaloma; to discuss the clinical relevance of FDG-PET and FDG-PET/CT thyroid incidentalomas, the role of these imaging techniques in preoperative work-up of cytologically \textit{Indeterminate} thyroid nodules and their role in preoperative diagnosis and staging of thyroid cancer.

Methods: PubMed was used for literature research. Search words: thyroid FDG-PET/CT and FDG-PET.

Case: 56 years female on breast cancer follow-up program with a suspicious finding in her lung. FDG-PET/CT was performed showing no FDG fixation in the lung, but intense focal fixation in thyroid right lobe and diffuse thyroid background fixation were observed. A firm nodule measuring 1.5cm in the right lobe was palpable and confirmed on ultrasound, with no cervical adenopathy detected. Fine needle aspiration biopsy result was “Suspicious for Papillary Cancer”. She underwent near total thyroidectomy plus central compartment lymph node dissection. Histopathological diagnoses: multifocal thyroid papillary cancer with main lesion on the right lobe measuring 1.5cm, background Hashimoto's Thyroiditis and 4 positive lymph nodes (pT1N1Mx). She was sent to radiodine ablation therapy.

Discussion: FDG-PET incidentalomas prevalence approaches 2%; 14% to 47% of these lesions are malignant\textsuperscript{1}. Reported FDG-PET/CT incidentaloma prevalence approaches 4%, with 36.7% being malignant\textsuperscript{2}. Use of Standard Uptake Values (SUVs) for malignancy risk prediction and the use of FDG-PET for preoperative assessment of cytologically \textit{Indeterminate} thyroid nodules are controversial issues\textsuperscript{1-5}. Thyroid papillary microcarcinomas SUVs correlate with tumor size but not with extrathyroidal extension\textsuperscript{6}. Thyroid cancer preoperative evaluation of cervical lymph nodes with FDG-PET/CT overall sensitivity, specificity and diagnostic accuracy has been reported as 30.4%, 96.2% and 87.9%, respectively, offering no advantage over CT or ultrasound\textsuperscript{7}.

Conclusion: This case illustrates the high malignancy risk of FDG-PET/CT incidentalomas but this imaging technique was unable to fulfil precise preoperative staging.
Objective: Describe a case of a patient diagnosed with papillary thyroid carcinoma by retropharyngeal tumor secondary to infiltration.

Methods: A 46 year old male was referred from the Otorhinolaryngology consult to our department with the post-operative diagnosis of papillary thyroid carcinoma. With 6 months history of upper airway dyspnea and dysphagia to solids. Physical Examination: Right side adenopathies, not painful on palpation. No goitre. Laryngoscopy: Hypertrophy of right posterior pharyngeal wall, epiglottis with rotation. Glottis was permeable. Mobile vocal cords. Pyriform sinus free. Cervical and thoracic TC: Right retropharyngeal and internal jugular adenopathic conglomerates. Subcentimetrethral mediastinal lymph nodes. Nasopharyngeal MRI: Right retropharyngeal paracentral mass rejecting adjacent structures. FNA of cervical lymph node: Lymph node with metastases of papillary thyroid carcinoma.

Results: Surgical treatment is decided. Transcervical excision was performed in right parapharyngeal tumor, sacrificing laryngeal pedicle and right superior laryngeal nerve, and total thyroidectomy with cervical emptying of areas: bilateral VI, right II (A and B) and V. Tracheostomy. Pathological diagnosis: Thyroid: Papillary thyroid microcarcinoma (7mm) located in the right upper pole, partially encapsulated and behind the capsule without infiltrators. Right cervical lymphadenopathy: multiple lymph node metastases of papillary carcinoma. Retropharyngeal mass: Extensive infiltration of papillary carcinoma. Six weeks after surgery is given an ablative dose of I131.

Conclusions: Approximately 0.5% of all head-and-neck tumors present in the parapharyngeal space (PPS). The neoplastic processes, which may involve the PPS, include primary PPS tumors, the direct extension of tumors from regions adjacent to the PPS and regional or distant metastases to the PPS. Thyroid papillary carcinoma presenting as a pharyngeal mass is a rare clinical occurrence and has only been reported sporadically.
INSULAR CARCINOMA OF THE THYROID GLAND: TWO CASES WITH UNUSUAL LONG SURVIVAL

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Introduction: Insular thyroid carcinoma has an aggressive behaviour, with high levels of local recurrence and distant metastases. It represents 3 to 7% of all thyroid carcinomas. Patients usually exhibit an advanced disease stage. Comparative studies revealed lower survival rates in comparison to differentiated carcinomas.

Case report 1: In 1988, a 52 years male presented a right thyroid mass, without other signs of thyroid disease. On ultrasonography, the mass compressed the trachea and on radionuclide imaging it was a “cold” nodule. The patient was submitted to total thyroidectomy (histology: insular thyroid carcinoma), and 131-Iodine therapy. Eight years later, he showed iodine fixation on radionuclide imaging, being submitted to another iodine dose. In 2007, multiple metastases in the brain, lungs and lymph nodes were diagnosed. Last evaluation, under 175µg of levothyroxine: thyroglobulin 7.8ng/mL, TSH 0.17µUI/mL (0.4-4) and FT4 1.9ng/dL (0.8-1.9).

Case report 2: In January 2001, a 70 years old man was observed in our Department with a cervical mass with two years of evolution. The ultrasonography revealed “…multiple cervical bilateral nodules…” Fine needle aspiration: “folicular tumour”. The patient was submitted to total thyroidectomy and neck dissection. (histology: insular thyroid carcinoma - T3N0?N1Mx). Eight months later, he developed a thoracic mass, which was excised (“…pulmonary metastases” - T4N1M1). Two other surgeries were performed due to thyroid and ganglionar recurrences, and two years later the patient had multiple pulmonary metastases. During follow-up, he was submitted to five treatments with radioactive Iodine (total dose 941 mCi). Last evaluation, under 125µg of levothyroxine: thyroglobulin 64ng/mL, TSH 0.72µUI/mL (0.4-4) and FT4 1.5ng/dL (0.8-1.9).

Conclusions: Insular thyroid carcinomas are associated with poor prognosis and short life expectancy, with some patients surviving less than one year. The authors present these cases because, in spite of diffuse dissemination of the disease, patients have an uncommonly long survival.
Angiosarcoma is a very rare entity of soft tissue neoplasm with an aggressive and a destructive biological behaviour. Thyroid angiosarcoma is usually reported in Alpine regions, with only exceptionally rare cases arising in non-mountainous areas. In the Alpine regions it constitutes 2-10% of all malignant thyroid tumors. We report a case of a thyroid non-alpine angiosarcoma in a 71 years old female with a 10 years old multinodular goiter. The cervical mass underwent rapid growth in the last year, convincing her doctor to send her for surgical treatment. A 15 cm mass was found on right side of the neck, invading adjacent tissues and displacing the trachea without obvious invasion. Fine needle aspiration cytology showed “carcinoma”. Lung metastasis were present. Although difficult, total thyroidectomy was possible with resection of an esophageal implant. Post-operatively, she had respiratory failure that eventually recovered, but, on 39th post-operative day, the patient died of violent hemoptyses, probably due to invasion by mediastinal metastasis. Pathological findings are discussed.
FLOW CYTOMETRY COMBINED WITH FINE NEEDLE ASPIRATION BIOPSY IS ESSENTIAL FOR DIAGNOSIS OF A RAPIDLY GROWING THYROID GLAND

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Background: Thyroid lymphomas are uncommon neoplasms accounting for less than 5% of all thyroid malignancies. They are usually originated from a thyroid gland with Hashimoto’s thyroditis. Prognosis of thyroid lymphoma is excellent. However, the diagnosis may be delayed if the evaluation is performed according to a standard management of a thyroid nodule.

Case report: In October 2008, a 83-years old woman presented with dysphagia, diffuse neck swelling and hoarseness for a few months duration. She was not known to have any thyroid disease. Neck examination demonstrated a firm, enlarged thyroid gland. Thyroid ultrasound showed diffusely enlarged a severely heterogeneous gland (thyroid volume was 33,33 ml). Anti-thyroid peroxidase antibody was high, while thyroid function tests were consistent with euthyroid sick syndrome (Table 1). Cytological examination of fine needle aspiration biopsy (FNA) demonstrated florid lymphoid phase of Hashimoto’s thyroiditis. A second FNA was performed and the cytological examination was concordant with the first. However, accompanied with the clinical picture, lymphoma could not be excluded. Therefore, the third FNA was performed. Cytological examination in combination with flow cytometry suggested B cell Non-Hodgkin lymphoma infiltration, with CD 20, CD 79, and CD 10 positivity on most of the B cells. The tumor has shrunk following three courses of chemotherapy, a regimen including Rituximab.

Conclusion: In a case of Hashimoto’s thyroiditis with a rapidly growing thyroid gland, FNA samples should also be assessed by flow cytometry for discriminating the normal lymphocytes from the malignant lymphoma cells, in order not to cause a delay in diagnosis.

<table>
<thead>
<tr>
<th>Laboratory values of the patient at presentation</th>
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<tbody>
<tr>
<td>TSH (mIU/ml, 0,3-4,5)</td>
</tr>
<tr>
<td>Free T3 (pmol/l, 3-6,8)</td>
</tr>
<tr>
<td>Free T4 (pmol/l, 10-22)</td>
</tr>
<tr>
<td>Anti-thyroid peroxidase antibody (IU/ml, 5-34)</td>
</tr>
<tr>
<td>Anti-thyroglobulin antibody (IU/ml, 10-115)</td>
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</table>
Efficacy of Plasmapheresis in Thyroid Storm with Persistent Ventricular Tachycardia

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Introduction: Thyroid storm is a rare medical emergency with mortality between 20-30%. Persistent VT is infrequent. Therapeutic management consists of a multidrug approach. Plasmapheresis has been reported as a last option when clinical deterioration persists.

Case report: A 43-year-old man, with a history of insomnia, emotional lability and anxiety for three months complained of palpitations, malaise and limb edema for a week, associated with fever in the last three days. The patient was febrile, with sinus tachycardia of 130 bpm, diminished lung sounds, neutrophilic leukocytosis, elevated CRP and ProBNP and chest X-ray with bilateral pleural effusion. Transthoracic echocardiogram revealed a normal left ventricular ejection fraction and moderate pericardial effusion without tamponade. A diagnosis of myopericarditis was made and medication with salicylates was started. 12 hours later he developed an arrhythmic storm in ventricular tachycardia and was admitted to Cardiac ICU, in need of inotropic support and invasive ventilation. Echocardiogram revealed depressed cardiac function, dilated right ventricle and thrombus in inferior vena cava suggestive of pulmonary embolism. Previously he already had hyperthyroidism but after the salicylates were administered, FT3 increased dramatically. Although several external cardioversions were performed and therapy started with tenecteplase and heparin, propylthiouracil, Lugol’s solution, cholestyramine and corticoids, he remained in persistent ventricular tachycardia. As last resource, albumin plasmapheresis was performed, after which he converted to sinus rhythm. FT3 decreased from >120 pg/ml to 12 pg/ml in 12 hours. The patient was discharged on day 16. One month later he is asymptomatic, euthyroid with propylthiouracil therapy and in sinus rhythm with propanolol. Trab’s were elevated and thyroid echography revealed an enlarged gland consistent with Grave’s disease.

Conclusion: This case is illustrative of a Grave’s disease presenting as an arrhythmic storm in thyroid crisis probably due to the administration of salicylates to a patient already in hyperthyroidism. Plasmapheresis was key point to terminate the persistent ventricular tachycardia.
P231
MOTHER’S TRAB’S AND BABY’S LAB’S - A CASE OF FETAL GOITER
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Introduction: Graves’ disease (GD) complicates about 0.2% of pregnancies and 1% of neonates born to women with GD develop clinical hyperthyroidism. During pregnancy, both antibodies and antithyroid drugs (ATD) cross the placenta and affect the fetal thyroid. Hyperthyroidism is caused by anti-TSH receptor antibodies (TRAbs) that can act on the fetal thyroid during the second half of pregnancy. Few cases of maternal TRAbs-related fetal hyper or hypothyroidism have been reported. Neonatal hyperthyroidism, usually apparent by the 10th day, should be considered an emergency and promptly treated to prevent newborn damage. This case illustrates some of the problems in GD management during pregnancy.

Case report: A 23 year-old woman with GD diagnosed at 19th gestational week (GW), with TRAb’s 137 U/L (0-1.8), was referred to our Institution on the 36th GW for voluminous fetal goiter with tracheal compression suspected on ultrasound (US), and poor maternal thyroid function control despite propylthiouracil (300mg/day) introduction at diagnosis. US was repeated in our Hospital, suggesting hypothyroidism, and intra-amniotic levothyroxin was administered. Fetal NMR did not confirm tracheal compression, and a planned in utero tracheal intubation was withheld. A normal male baby was delivered by scheduled C-section performed at 38th GW. Umbilical TSH levels were 81.5µU/mL. At day 6, the baby developed hyperthyroidism, requiring methimazole treatment until the 48th day of life.

Conclusion: ATD in GD during pregnancy should be balanced to control both maternal and fetal thyroid. It can induce fetal hypothyroidism, and transplacental TRAb passage can cause hyperthyroidism. Close following of maternal T4 and TSH levels, TRAb assay and fetal ultrasonography (including the thyroid) are recommended for therapy guidance. Early diagnosis is the key to successful management. Close teamwork among endocrinologists, obstetricians, radiologists and pediatricians is essential to ensure normal fetal thyroid function.
The patient was a 22-year-old female who started to show exophthalmoses, excessive appetite and a lack of ability to concentrate when she was 10 years old and was diagnosed with Graves' disease at a local clinic based on laboratory findings (TSH < 0.1µIU/ml, FT4 7.0 ng/dl, TRAb 50.3%). Treatment with methimazole (MMI) (15mg/day) was initiated on August 23rd, 1996 and her thyroid function became normal. She was then treated with MMI 5-30mg/day and levothyroxine sodium hydrate 25-75µg/day without any side effects as a pediatrics outpatient at our hospital until 2007. In the middle of December 2007, she showed palpitation, weight loss, and insomnia and laboratory findings suggested worsening of Graves' disease (TSH < 0.03, FT4 6.4, FT3 20.0). Treatment with MMI 30mg/day improved her thyroid function (TSH < 0.03, FT4 1.0, FT3 3.5 on February 19th) and MMI was reduced to 20mg/day. On March 21st, 2008, white blood cell (WBC) count suddenly decreased to 1,380 and neutrophile level was also very low (13.9%), suggesting neutropenia and she entered to our hospital as an emergency case. Although neutropenia due to the side effects of MMI usually starts within several months after initiation of the therapy, we terminated MMI and began treatment with granulocyte-colony stimulating factor (GCSF), antibiotics and propylthiouracil (PTU) 200mg/day (divided in two doses). By March 29th, WBC levels recovered to 3,920 without any serious infection. Drug lymphocyte stimulation test (DLST) for MMI showed 1008cpm (S.I. 327%) making it obvious that MMI was related to the neutropenia. In view of long-term prognosis, subtotal thyroidectomy was performed. After surgery, thyroid function stabilized and she was observed without medication. Although neutropenia induced by long-term treatment with MMI is very rare, this case suggests that it is necessary to consider the possible occurrence of neutropenia induced by antithyroid drugs, even more than 10 years after initiation.
Hypercalcemia caused by thyrotoxicosis is generally mild, but rarely - severe hypercalcemia occurs and causes symptoms (anorexia, nausea, vomiting, polyuria, confusion) that may obscure the diagnosis of hyperthyroidism. The most common cause of symptomatic hypercalcemia in the setting of hyperthyroidism is concomitant primary hyperparathyroidism. We describe two cases of symptomatic hypercalcemia as a consequence of thyrotoxicosis alone.

A 48-year-old woman was referred to our unit with a 2 months history of clinical features of thyrotoxicosis, associated with nausea, vomiting, anorexia and confusion. In addition to laboratory confirmation of thyrotoxicosis (high FT4, high T3 and suppressed TSH), a severe hypercalcemia (14.6 - 13.7 mg/dl, with normal serum albumin) associated with a suppressed serum PTH was found; phosphatemia was normal and alkaline phosphatase was increased. Due to severe hypercalcemia, we used higher doses of methimazole and metoprolol, together with normal saline IV, furosemide and IV glucocorticoids (hydrocortisone hemisuccinate). After 6 weeks of therapy, serum calcium became normal after achieving euthyroidism.

A 49-year-old man was admitted in our unit with a 4 months history of clinical features suggesting Graves disease, associated with polyuria and muscle weakness. One month before admission an anterior mediastinal mass (4.6/2.2 cm, found by CT scan) was operated in a thoracic surgery clinic and a thymic hyperplasia was diagnosed postoperatively. Laboratory tests revealed high FT4, high T3, suppressed TSH, a high serum TRAb value and hypercalcemia (13.2 - 12.4 mg/dl, with normal serum albumin), normal phosphatemia and serum PTH in the lower normal range. In addition to methimazole and metoprolol, therapy of symptomatic hypercalcemia consisted of normal saline IV, furosemide and pamidronate, being followed by persistently normal serum calcium.

Screening for hypercalcemia is required in the patients with newly diagnosed hyperthyroidism. Thyrotoxic patients with calcium levels greater than 12 mg/ml should be immediately and aggressively treated.
We present an extremely rare and didactic case of thyrotoxicosis factitia, a form of Munchausen syndrome.

A 29-year-old woman presented in 2007 with clinical signs of thyrotoxicosis. Initial investigation confirmed the disease. After one year treatment with antithyroid drugs, which was unsuccessful, she underwent total thyroidectomy. Clinical symptoms of thyrotoxicosis persisted after the operation. Her symptoms remained unchanged 5 months after the operation, despite the administration of high doses of antithyroid drugs. At that time she had high FT4, FT3 levels with normal TSI antibodies and low thyroglobulin levels. Chest MRI revealed a 4x2 cm soft tissue mass in the anterior mediastinum. 131-I whole body scan after stimulation with Thyrogen demonstrated pathological uptake in the latter. An ultrasound of uterus and ovaries was normal. A therapeutic dose of 40 mCi 131-I after stimulation with Thyrogen was administered, but without any clinical improvement. Three months after the administration of 131-I, and without her physician's approval, she underwent surgical excision of the anterior mediastinal mass, which proved to be hyperplastic thymus. Six months after she presented to our clinic with all the symptoms of thyrotoxicosis. FT4, FT3 were elevated, with suppressed TSH levels. Anti Tg, anti TPO and TSI antibodies were undetectable with very low levels of thyroglobulin and extremely high levels of urine iodine (1150 µg/24h, NR< 150). A whole body scan with 131-I revealed no uptake.

All the above tests are indicative of a case of excess thyroid hormone ingestion, which the patient strongly denied. She was strongly advised to seek a psychiatric opinion.
GO is clinically relevant in 45% of unselected patients with Graves' disease if eyelid changes are included. Spontaneous retro-ocular pain is a validated sign of active GO and can be relieved with anti-inflammatory drugs and treatment of dysthyroidism. Exacerbations of ocular signs might hint relapse of the disease. We report an unusual case of a premenopausal, smoking female patient who suffered from Graves' disease with a moderate GO since 2 years. The hyperthyroidism was treated by antithyroid drugs for two years and 8mCi of radio-iodine with improvement of eye signs. As the hyperthyroidism relapsed, she complained of asymmetric oppressive and intense pain on and behind the globe, exacerbated on attempted any eye movement. Eye examination was characterized by a proptosis of 23 and 24mm /119mm, eyelid swelling, watering and restriction of elevation of both eyes, no visual dysfunctions. Serum TSH-receptor autoantibody (TRab) level was > 200 IU/l (nl: < 15) Despite restoration of euthyroidism and improvement of inflammatory eye signs, the retro-ocular pain, particularly in the left side persisted and an orbital Magnetic Resonance Imaging (MRI) study was performed. The MRI showed a 1.5 cm-diameter infiltrative pituitary tumor with osteolysis of the turcica sella. Further investigations excluded local malignancies: metastases, plasmocytoma or chordoma. Prolactin concentrations were high at 197µg/l (nl< 20). Biopsy of the tumor confirmed the diagnosis of prolactinoma. The patient was treated with cabergoline, an agonist dopaminergic drug and the pain disappeared after 2 months. The patient could stop the anti-inflammatory drugs after 6 months. A MRI performed after 1 year of treatment showed a significant shrinkage of the pituitary tumor. This exceptional case of an association between painful GO and a prolactinoma with osteolysis of sella turcica illustrates the need for complementary check-up and close interdisciplinary cooperation, when unusual clinical symptoms of GO are persisting.
APLASIA CUTIS ASSOCIATED WITH PROPYLTHIOURACIL TREATMENT FOR GRAVES DISEASE

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Background: Aplasia cutis is a well circumscribed skin defect typically on the vertex of the skull which appears at birth. The cause is unknown but an association with methimazole or carbimazole is well described. To date, of the 24 reports of an association in the literature, no case has been described in neonates exposed to propylthiouracil.

Objective: We report a neonate with aplasia cutis whose mother became pregnant whilst taking carbimazole and was subsequently transferred to propylthiouracil.

Case: A 34 year old lady presented with florid signs of thyrotoxicosis (FT4 70pmol/l, FT3 17pmol/l TSH< 0.01 mU/l) and strongly positive TSH-Receptor antibody 9.7 [0-0.4 /l) confirming Graves disease. She was treated with carbimazole with good effect. Six months into her course of treatment, when taking carbimazole 15 mg daily, she conceived her first pregnancy. Her carbimazole was reduced to 10mg daily and continued to 36 weeks gestation when propylthiouracil 50mg bd was substituted. She continued on this treatment for the rest of the pregnancy and for a full 18 months in total. She had an uncomplicated delivery of a healthy baby boy. He was born with a 2cm skin defect of aplasia cutis congenita on the skull. Over the next few months, the skin lesion gradually healed leaving an alopecia scar.

Discussion: Whether the association with antithyroid treatment is a causal one is controversial but even if so, the risk of aplasia cutis is very low. Our patient had a small scalp lesion that healed rapidly without needing surgical treatment. The child had been exposed to carbimazole early in pregnancy and propylthiouracil in the last few weeks. If propylthiouracil is considered not to cause this complication, then clinicians should start treatment with it as soon as pregnancy is confirmed rather than wait until the last trimester.
THYREOTOXICOSIS AND CONCOMITANT TAKO-TSUBO CARDIOMYOPATHY IN A PATIENT WITH CHRONIC HEPATITIS C: AN UNUSUAL CLINICAL ASSOCIATION

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Background: Thyroid dysfunction has been described as an extrahepatic manifestation of chronic HCV infection, and this disorder can be precipitated or exacerbated by interferon-alpha, especially in women. Hyperthyroidism induced by IFN-alpha could correspond to the first phase of silent thyroiditis, to Graves’ disease or to the succession of both ('triphasic' thyrotoxicosis).

Case report: We report the case of a 47 year old women with chronic active hepatitis C who had been treated with pegylated-IFN-alpha (180 µg/week) and ribavirin (1000 mg daily) for 7 month, with no personal history of cardiovascular or thyroid disease, admitted to ICU for acute retrosternal pain, V1-V6 ST segment elevation with increased troponin (2 ng/ml), suggesting STEMI. Transient disturbance of cardiac kinetics was present on echocardiography, with initial left ventricular apical akinesia and septal hipokinesia, which regressed in 4 weeks. The clinical picture associated with normal epicardial coronaries at angiography and the absence of late enhancement on cardiac MRI enabled us to diagnose a stress-related acute reversible ventricular apical dysfunction. She was leucopenic and developed concomitant thyroiditis with thyreotoxicosis (TSH=0.000 µU/ml, FT4=2.41 ng/dl, ATPO=3.8 UI/ml, TSHRAb negative and low RAIU= 3%). She received supportive therapy for her cardiomiopathy and glucocorticoids, beta-blockers, anxiolitics for the transient thyreotoxic phase of thyroiditis. In 3 months she developed transient hypothyroidism (TSH=32.16 → 29.13 → 5.17 µU/ml) and no thyroid dysfunction ever since.

Conclusions: Rigorous diagnostic procedures and prospective monitoring of patients with chronic active hepatitis C treated with IFN alpha are necessary to avoid a false diagnosis and inappropriate therapy.
Severe flaccid quadriplegia during the recovery phase of the illness, although rare, and gastrointestinal bleeding are two important complications of thyrotoxic crisis. We report two such cases.

**Case 1:** A 46-year-old emaciated woman was admitted because of thyrotoxic crisis induced by aspiration pneumonia and heart failure requiring mechanical ventilation. Her progress was complicated by hematemesis due to gastric ulceration which was treated endoscopically. Perforation of the ileum occurred on the 20th day and histopathological examination of the resected ileum demonstrated microthrombi in the perforated area, probably associated with disseminated intravascular coagulation. She was weaned off the ventilator on the 50th day, but severe flaccid quadriplegia developed requiring six months rehabilitation before complete recovery of muscle strength.

**Case 2:** A 47-year-old woman was admitted because of thyrotoxic crisis associated with severe heart failure and atrial fibrillation. She developed cardiogenic shock and marked jaundice, requiring mechanical ventilation, percutaneous cardiopulmonary support and plasma exchange. On the 4th day, a hemorrhagic duodenal ulcer associated with melena was treated endoscopically. Severe flaccid quadriplegia became apparent soon after complete removal of sedation and weaning off the ventilator on the 15th day. The paralysis resolved completely after three months of rehabilitation. In both cases, Graves' hyperthyroidism was treated with antithyroid medications, inorganic iodide and glucocorticoids.

**Discussion & conclusion:** Critical illness polyneuromyopathy is an axonal neuropathy and myopathy which develops during the treatment of critically ill patients. Although thorough electrophysiological examinations were not performed, nerve conduction velocities were normal in both cases. Critical illness polyneuromyopathy, glucocorticoid therapy, prolonged immobilization, thyrotoxic myopathy and/or severe emaciation are possible causes for the transient severe flaccid quadriplegia. When treating patients with thyrotoxic crisis, potentially serious gastrointestinal bleeding and chronic neuromuscular complications should be kept in mind.
Introduction: Resistance to thyroid hormone (RTH) is a rare autosomal dominant disorder, characterized by reduced end-organ responsiveness to thyroid hormone, usually caused by mutations at the beta thyroid hormone receptor (THRβ). High serum free thyroxine (FT4) and free triiodothyronine (FT3) and non-suppressed levels of thyroid-stimulating hormone (TSH) are essential for diagnosis. Clinical phenotype is heterogeneous. Differential diagnosis includes TSH-secreting pituitary adenoma and hyperthyroidism.

Objectives: To study a family with different clinical and molecular presentation of the disease.

Methods: The study was performed in five family members and included measurements of FT3, FT4, TSH, anti-thyroperoxidase antibodies (ATPAb), anti-thyroglobulin antibodies (ATGAb), anti-thyrotropin receptor antibodies (ATRAb); thyrotropin releasing hormone (TRH) test, cervical ultrasound, pituitary magnetic resonance (MR) and molecular study of THRβ.

Results: Only two members of the family were clinical and biochemical positive for RTH, the father and the daughter. In daughter: FT3 5.0 pg/ml (1.8-4.2 pg/ml), FT4 2.6 ng/dl (0.8-1.9 ng/dl), TSH 5.1 uUI/ml (0.4-4 uUI/ml), ATPAb 27 UI/ml (< 40 UI/ml), ATGAb < 20 UI/ml (< 40 UI/ml), ATRAb 2.4 U/l (< 9 U/l). In father: FT3 22 pg/ml (1.8-4.2 pg/ml), FT4 1.9 ng/dl (0.8-1.9 ng/dl), TSH 1.2 uUI/ml (0.4-4 uUI/ml), ATPAb 11 UI/ml (< 40 UI/ml), ATGAb 20 UI/ml (< 40 UI/ml), ATRAb 1.7 U/l (< 9 U/l). Both had TRH test positive for RTH. Ultrasound detected a simple goiter only in the daughter and MR showed a pituitary microadenoma on both. The THRβ gene molecular study detected a germinal mutation Arg383Cys (CGC→TGC) on exon 10 in the daughter and an intronic polymorphism IVS9+9G→A in the father. Both are medicated with βblockers for adrenergic symptoms.

Conclusions: This family with RTH has a clinical heterogeneous phenotype. Both present pituitary microadenoma, setting diagnostic and therapeutic challenges. The genetic study at the moment doesn ´t support the autosomal dominant transmission.
REMISSION OF PERSISTENT INFLAMMATORY SYNDROME IN A PATIENT WITH GRAVES’ DISEASE AND PAPILLARY CARCINOMA AFTER ANTITHYROID DRUG TREATMENT

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Objectives: To report an unusual association between Graves’ disease and papillary thyroid carcinoma, presenting with persistent inflammatory syndrome.

Methods: A 53 years old man, resident in an iodine sufficient area, was referred by a rheumatologist for persistent fever, arthralgia, weight loss, excessive sweating. Serum levels of TSH, T3, T4 were measured by immunoradiometric assays.

Results: Physical exam showed I degree goiter (WHO classification) with a 1.5 cm nodule in the right lobe. Positive inflammation markers were noticed: ESR= 120 mm/h, C reactive protein (76.01 mg/L); rheumatoid factor was high normal (16.61 IU/L), anti dsDNA, anti SS-A (Ro) antibodies were negative. Microbiological tests revealed no bacterial infection. HIV was negative. Mild anemia (Hb= 9.2 g/dL, Ht= 26.9%), mild leucopenia (3300/mm3) were present. Immune electrophoresis showed no abnormalities. Laboratory test revealed thyrotoxicosis (TSH < 0.03 mIU/L, T3= 324 ng/dL, T4= 12.5 mcg/dL); TSHR Ab were positive (5 IU/L); thyroid ultrasound revealed a 20.33 mL goiter with hypoechoic nodule 1.6/1.2 cm with multiple calcifications in the right thyroid lobe, found to be “cold” on scinti scan. Fine needle biopsy aspiration from the thyroid nodule revealed proliferative risk, suggesting a follicular variant of a papillary thyroid carcinoma. Antithyroid therapy was started (Thiamazole 30 mg/day), with clinical improvement in 6 weeks: disappearance of fever and arthralgias, weight gain. Parallel to thyroid hormone decrease (T3= 210 to 134 ng/dL; T4= 9.7 to 4.9 mcg/dL), Hb increased (11.3 to 14.1 g/dL) and ESR decreased (55 to 37 mm/h). After achieving euthyroidism, total thyroidectomy was performed. Histological exam revealed a 2.2/1.8 cm (PT3) diffuse sclerosing variant of papillary carcinoma; the rest of the thyroid parenchyma showed hyper functioning aspect with medium epithelial retraction due to antithyroid drugs.

Conclusion: Hyperthyroidism should be considered in patients with persistent febrile syndrome when infectious and autoimmune etiologies were excluded.
Introduction: Hyperthyroidism is a condition sometimes associated with liver dysfunction. Usually these disturbances include elevation of liver transaminases, alkaline phosphatase or bilirubin. Cholestatic jaundice is a rare manifestation in hyperthyroidism. Alteration of cholestatic jaundice caused by introduction of methimazole in hyperthyroidism treatment is an extremely rare complication with typical clinical presentation. A slow recovery after the initial outbreak of hyperbilirubinemia is the reason for opinions that support the involvement of the immune system in etiopathogenesis of such adverse response.

Case report: P.H. male, 78 years old, was diagnosed as hyperthyroid after two months of symptoms caused by adrenergic stimulation and hypermetabolism. He had a diffuse toxic goiter and initially slight hyperbilirubinemia (25) was noticed. Soon after the introduction of methimazole the patient developed jaundice, the concentration of bilirubin was significantly elevated up to 131 (predominantly direct 85). In the same time concentration of the liver transaminases, \( \gamma \text{GT} \), and ALP were normal. No other reasons for hyperbilirubinemia were previously detected. Furthermore, our diagnosis was confirmed after typically slow and steady normalization of bilirubin levels after methimazole was disconnected. In the meantime the patient had a spontaneous remission of hyperthyroidism.

Conclusion: Cholestatic jaundice is a rare complication of methimazole treatment, possibly involving the immune system, that requires change in therapeutic approach.
We report a case of hyperthyroidism due to TSH secreting pituitary adenoma in a 24-year-old man. The patient was clinically and laboratory thyrotoxic (T4 = 220 nmol/L, FT4 = 63 pmol/L, FT3 = 12.1 pmol/L, TSH - 4.1-5.1 mU/ml). After thyrostatic therapy the thyroid hormonal values decreased: T4 = 180 nmol/L, FT4 = 24.7 pmol/L, but TSH was elevated: 25.3-42.8 mU/ml. Computerized tomography (CT) scan and magnetic resonance (MRI) reveal a pituitary tumor. Elevated alpha TSH-subunit levels confirmed the diagnosis. Being operated the patient became euthyroid.

This case we report as a rare form of hyperthyroidism, first reported case in Macedonia. Also, this case demonstrates the needs for determination of TSH levels in all cases of clinical hyperthyroidism.
Context: Central congenital hypothyroidism (CH) is mostly combined with other pituitary hormone deficiencies, but could also appear as an isolated defect of pituitary function. Mutations of the TSH beta-subunit gene is one of the causes of Isolated CH but only thirty-four families with such mutations are reported worldwide. The diagnosis is usually delayed because the TSH serum levels are not elevated leading to a negative result in the newborn TSH screening. In addition, symptoms and signs of thyroid insufficiency might be interpreted as those of other pituitary hormone deficiencies.

Objectives: To investigate two sisters from one consanguineous family affected by growth retardation and hypoglycemia.

Methods: Pituitary functions, including somatotropic, thyreotropic, and adrenal ones were analyzed. Mutation characterization for the beta-TSH gene was performed by PCR amplification followed by direct sequencing.

Results: Biological analysis revealed low levels of T4 and T3, and low basal TSH levels whereas others deficiencies in anterior pituitary functions were finally excluded. Some subtle clinical features in these two siblings were retrospectively found to be compatible with hypothyroidism but diagnosis and treatment were delayed until 10 months. Molecular analysis identified a homozygous mutation in exon 3 of the beta-TSH resulting in a premature stop at codon 49 (Q49X), inherited from heterozygous parents. This mutation was already reported in other four families from different ethnic origin suggesting a hot spot mutation but common ancestry cannot be excluded.

Conclusion: Our observation outlines the problem of the undiagnosed CH by neonatal screening and the clinical features that could mimic GH deficiency. Additionally, our molecular finding supports the theory that Q49X mutation may be a common cause of isolated TSH deficiency.
FAILURE OF RENAL ALLOGRAFT ASSOCIATED WITH LEVOTHYROXINE WITHDRAWAL BEFORE RADIOIODINE EXPOSURE - A CASE REPORT

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Background: rhTSH stimulation is advocated, in our country, as an expensive measure that should be used in specific situations such as pituitary insufficiency, cardiac, pulmonary or psychiatric diseases, which may be uncompensated by hypothyroidism. Clinical hypothyroidism is associated with a decrease in cardiac output, renal blood flow and glomerular filtration rate, leading to impaired renal excretion of water.

Case report: We report a 65 year-old woman with terminal chronic renal insufficiency of unknown aetiology that was submitted to renal transplantation in February 2002. As comorbidities she had arterial hypertension since 1988, Diabetes Mellitus diagnosed in 2004, obstructive pulmonary disease, and cardiac structural changes such as mild left auricular dilatation, concentric left ventricular hypertrophy and degenerative valvular heart disease. She had been submitted to total thyroidectomy in October 2008. The histological examination revealed it was a follicular variant of a papillary carcinoma. She began suppressive therapy with levothyroxine. In January 2009 she started levothyroxine withdrawal in order to be submitted to ablative radiodine therapy. Three weeks and a half later she was sent to the urgency room because she had developed dyspnoea, orthopnoea, cough, dysuria, and fever. It was noted hypotension, peripheral oedema, and weight gain (9 Kg). Laboratory examination showed a slightly elevation of inflammatory markers, bacteriuria, piuria and deterioration of the renal allograft function. She was admitted to the renal transplant unit where she started levothyroxine and haemodialysis. Echocardiogram showed pericardial effusion. There was a favorable clinical evolution of the hypervolemic state and of the pericardial effusion in 3 weeks, although there was not a recovery of the allograft function to date.

Conclusions: The authors consider it is important to call attention to the benefits that stem from the use of rhTSH, which can prevent complications such as those we found in this clinical case.
HYPOTHYROIDISM PRESENTING WITH XANTHODERMA

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Introduction: Clinical presentation of hypothyroidism can be varied. Thyroid hormone is essential for normal activity of various enzyme systems.

Case report: A 5 year old girl presented with growth failure. O/E she was pale, had a lemon yellow tinge to her skin (without yellowish discoloration of sclera). She also had dry skin and hair loss. Her height was < 3rd centile for her age. There was no history suggestive excessive dietary intake of Vitamin A. Thyroid function test revealed low fT4 (4 pmol/l) and TSH >150 mU/L and anti-TPO antibody was strongly positive (>1290) consistent with hypothyroidism (auto-immune aetiology). Liver function test was normal. Toral serum cholesterol was also elevated (10.4 mmol/L) Bone age was delayed. (Bone age: 2.1 years, chronological age: 5.2 years) The other investigations were as follows: Alpha-Carotene level 44mcg (14-60), Beta-Carotene level 1358 mcg (92-312), Vitamin A level: 1.5 mcmol/L (1-2.8).

A diagnosis of hypercarotenaemia secondary to hypothyroidism was made and she was started on levothyroxine replacement therapy. Her xanthoderma reversed within weeks of levothyroxine therapy. Her growth velocity improved dramatically on starting levothyroxine therapy.

Discussion: Hypothyroidism may rarely present with xanthoderma which is thought to be due to reduced conversion of carotenoids to retinol. Often this leads to Vitamin A deficiency which may lead to dry scaly skin. High concentrations of carotene are also found in patients with a history of excessive intake of carotene rich foods and also in patients with severe hyperlipidaemia. Correction of hypothyroidism is known to cause reversal of hypercarotenaemia. Awareness Clinicians need to be aware of this rare clinical presentation of hypothyroidism.
Background: Hashimoto’s Thyroiditis (HT) is an autoimmune disease with an incidence between 1-4 patients/1000 inhabitants. There are rare cases of neuropathy associated, with immune mechanisms involved.

Case report: We describe the case of a male, with 25 years old, previously healthy, that went to the emergency room with complaints of a not floating diplopia. We identified a right eye hypo-exotropia with ipsilateral ptosis; he couldn’t achieve neither convergence nor the bilateral horizontal conjugate movement. The pupils were normal, as both ocular fundus. Brain MRI and angio-CT didn’t reveal lesions. CSF cytochemical and microbiological studies were normal. There were no oligoclonal bands, although a serum polyclonal hypergammapathy was observed. He was immune to rubella, HSV, EBV and VZV, but not to toxoplasmosis and CMV. Syphilis screening was negative, like the search for HIV and Borrelia antibodies. The study of thyroid function revealed an elevated TSH (10.8 µUI/l), with normal fT4 and fT3. Titers of anti-thyroglobulin and anti-peroxidase antibodies were elevated (315 UI/ml and >1000 U/ml, respectively). TRabs were negative and the patient began on levothyroxine. Two weeks later, limited adduction to the left was observed, maintaining right deficits. Anti-AchR titers were negative, as anti-Hu, anti-Ri, anti-Yo, anti-GM1, anti-GM2, anti-GM3, anti-GQ1b, ANA, ENA, ANCA, anticardiolipin, anti-β2-glycoprotein-1 and antiphosphatidilserin. A CT and an orbital MRI were performed, which kept not revealing lesions. He started on intravenous immunoglobulins (IgIV), 0.4 g/kg/day, during 5 days and prednisolone, 1 mg/kg. Near two years after the second cycle of IgIV and without corticosteroids, he has no neurological deficits.

Discussion/conclusion: After exclusion of the main causes for mobility eye commitment, the association with HT seems possible. The response to the immunosuppressive treatment is another argument favoring an autoimmune etiology.
Introduction: Pretibial dermopathy or myxedema is a rare form of extra-thyroidal manifestation of Graves’ disease and is less frequent in Hashimoto’s thyroiditis. The pathogenesis, although unknown, seems to be autoimmune.

Objective: Rare association with Hashimoto’s thyroiditis.

Results: A 65-year-old male patient, with laboratory hypothyroidism and previous use of amiodarone for 12 years. Thyroid assessment: TSH= 47uU/ml, anti-TPO = 381 UI/ml. The patient received 50 ug/day of LT4. Five years later, patient presented thickening and hyperemia of legs and hands, ocular irritation, proptosis and lagophthalmus in the left eye. Laboratory assessment: TSH = 3.8 uU/ml, T3=87 ng/dl, T4=6.1 ug/dl, T4L=0.6 ng/dl, anti-TPO=427 U/ml, TRAb=90%; thyroid volume by US was 24cm³. Histopathological assessment: compatible with myxedema in the affected areas. CT of the orbits showed optical nerve involvement caused by the thickening of the retro-ocular muscle. Prednisone (80 mg/day, 90 days) was introduced with marked improvement of the ocular alteration and dermopathy.

Discussion: The pretibial myxedema, together with the ophthalmopathy, comprises an extra-thyroidal manifestation of Graves’ disease. Rare cases have both manifestations in the presence of Hashimoto’s thyroiditis. Similarly to what occurs in the ophthalmopathy, TSH receptors in the conjunctive tissue might be the antigens responsible for the immune process. This patient presented high titers of TRAb (90%), probably blockers. The clinical hypothyroidism recognition in patients treated with amiodarone is not always easy to achieve. The TSH levels must be determined before the medication, in order to rule out a previous thyroid dysfunction, before attributing it to the direct cytotoxic effect or the effect of the drug on the immune system. Patients with thyroid auto-antibodies are more prone to developing the deficiency. There is some evidence that amiodarone interferes specifically with T cells and immunization against thyrocytes.
Introduction: Anti-TNFα agents are used to treat various autoimmune diseases. Thyroid diseases are rarely described in association with these drugs. We report here a case of subacute thyroiditis in a patient treated with etanercept for rheumatoid arthritis.

Case report: A 68 year-old woman was treated by prednisolone, methotrexate and etanercept (50 mg every week by subcutaneous injection) since 2002 for rheumatoid arthritis evolving since 1976. In 2008, she developed hyperthyroidism (FT4 : 60.9 pmol/L (12-22), FT3 : 14.9 pmol/l (3.1-6.8) and suppressed TSH) with a large and hard irregular goitre. TSH-receptor and thyroperoxydase antibodies were negative, in contrast, with positive thyroglobulin antibodies. Thyroglobulin was 114.2 ng/mL and there was no iodine excess. Ultrasonography showed a large heterogeneous goitre and no uptake was seen on scinti-scan. Fine needle aspiration cytology revealed a subacute thyroiditis (multinuclear giant cells and lymphoid cells infiltrate). The treatment with Carbimazole was withdrawn after 10 days because of FT4 decrease. Three weeks later, Levothyroxin was introduced because of hypothyroidism. The goitre had shrunk and softened.

Discussion: TNFα plays an important role in T-cell mediated immunity. Some adverse events are described with anti-TNFα (infection, cancer, lymphoma, lupus, vasculitis, psoriasis, interstitial lung disease, antiphospholipid syndrome). To our knowledge, 3 cases of thyroid disease including 1 subacute thyroiditis have been reported with etanercept. We report, here, the second case of subacute thyroiditis during treatment with anti-TNFα agent. In this case the goitre was painless but the corticosteroids and etanercept, which are anti-inflammatory agents, may have reduced the pain.

Conclusion: With anti-TNFα, thyroid dysfunctions seem to be uncommon but should be screened for.
Introduction: Intrathyroidal parathyroid adenoma is an infrequent lesion which occurs by abnormal embryonic migration of the parathyroid glands, resulting in anomalous locations and being frequently overlooked as a cause of surgical failure in primary hyperparathyroidism.

Case report: The authors present the case of a 73 year old man, with nephrolithiasis, referred to the endocrinology clinics for a plunging goiter with associated compressive symptoms and normal thyroid function. The thyroid ecography revealed an enlarged left lobe occupied by a solid heterogeneous nodule with 50x37mm, having a substernal component confirmed by CT scan. Fine needle aspiration cytology (FNA) was diagnostic of a benign nodule. Additional workup revealed normal thyroglobulin and peroxidase antibodies but an abnormal calcium metabolism (serum calcium 13 mg/dL; PTHi 1136 pg/mL; 24h urinary calcium of 252mg for a creatinine clearance of 47,32mL/min) with renal calculi and osteoporosis. After a negative Sestamibi scan the Magnetic Resonance Imaging (MRI) showed a right lobe homogeneous nodule with 10 mm and a left lobe heterogeneous nodule with 45 mm, without any other thyroid, cervical or mediastinal lesions. Each of this nodules were considered to be consistent with parathyroid adenomas in the right clinical setting. Taking into account the plunging goiter with compressive symptoms and the primary hyperparathyroidism diagnosis, the patient was proposed for total thyroidectomy and cervical bilateral exploration, held without unexpected complications or other findings. The pathology analysis was consistent with an intrathyroidal left lobe parathyroid adenoma with 50 mm. The patient is under treatment with calcium, calcitriol, ibandronic acid and levotiroxin with excellent clinical and analytical response.

Conclusions: The localization of a parathyroid adenoma in a patient with goiter is challenging and surgical treatment as to be tailored to the presenting intraoperative findings. This case is reported for its unusual presentation in which the preoperative MRI was crucial.
Replacement therapy with thyroid hormone is essential for the treatment of hypothyroidism. The authors present the case of a 40 years old woman with iatrogenic hypothyroidism refractory to L-T4 therapy. Four months after administration of I\textsuperscript{131} for the treatment of Graves’ disease, the patient presented hypothyroidism (TSH = 129 uUI / ml (N = 0.4-4.0); (FT4) = 0.39 ng / dl (N = 0.8 -1.9); FT3 = 1.0 pg / ml (N = 1.8-4.2)) and began oral replacement therapy with L-T4. Even with gradual dose increase, up to 400ug of L-T4 and 150ug of L-T3 daily, there was no response. The patient took no other medication and the upper and lower gastrointestinal tract endoscopy, urinary D-xylose test, stool cultures, small-bowel contrast radiology, abdominal ultrasound and extended laboratory tests, excluded disorders of absorption and other digestive tract diseases. The absence of FT4 rise in serum after oral supervised intake of L-T4 was confirmed. The patient's mother showed a normal response in the T3 suppression test and the other family members had normal thyroid function tests. The most likely causes of TSH elevation in patients treated with L-T4 (disorders of absorption, poor treatment compliance, drug interactions, thyroid hormone resistance syndrome) seemed to be excluded. Parenteral therapy was initiated to improve clinical signs and symptoms. Despite a partial response, with periods of normalization of thyroid hormones levels, it was not possible to sustainably normalize TSH levels, nor to establish clinical euthyroidism. Currently, the patient is medicated with L-T4 intramuscular, 0.5 mg, 2 times per week and oral L-T4, 1.2 mg per day and presents asthenia, macroglossia and myxedema. This seems to be a rare abnormal response to pharmacological preparations of thyroid hormone but, the underlying pathological mechanism remains unclear. We are particularly concerned about the long term risks of sustained hypothyroidism.
HYPOTHYROIDISM: A PITFALL IN THE MANAGEMENT OF EXTRAPYRAMIDAL DISORDERS

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Background: Although it is commonly recognised that thyroid diseases can simulate extrapyramidal disorders, a review of the causes of parkinsonism shows that they are briefly reported. The development of hypothyroidism in a patient with Parkinson's disease can go undetected, since the course of both diseases can involve similar features.

Case report: We describe the case of a woman, 80 years old, that came to the Emergency Room (ER) with complaints of hands, feet and eyelids oedema, with near 2 years of evolution, but recent aggravation, tremor, fatigue and constipation. Medical history was relevant for Parkinson's disease (since 2007), cardiac insufficiency, hypercholestherolemia and anemia and was under treatment with levodopa 150mg/day, propanolol 10mg/day, folic acid 1mg/day, ferrous sulphate 90mg/day, lansoprazol 30mg/day, furosemide 60mg/day, sinvastatine 20mg/day and acetylsalicylic acid 150mg/day; five months before, she was also observed in the ER because of cognitive dysfunction and behavioural abnormalities and a diagnosis of dementia was made. Physical examination revealed a pill-rolling resting tremor of moderate amplitude and low frequency, involving preferentially the right upper extremity, hypomimia, dry skin, cold extremities, bilateral eyelid, hands and pretibial oedema. Blood analysis revealed a normocytic/normochromic anemia. Electrocardiogram showed signs of a low voltage sinusal bradycardia (52 bpm); chest radiogram was normal. Thyroid analyses were requested and the results confirmed a hypothyroidism: TSH=67.3µUI/ml, fT4< 0.3ng/dl and fT3=1.1pg/ml. Patient was discharged home under treatment with levothyroxine increasing doses. One year after this ER episode, on external consultation, we observed a marked clinical (tremor, oedemas, lethargy and constipation) and analytical (TSH=7.38µUI/ml) improvement.

Discussion/conclusion: The hormone replacement therapy showed to be someway effective on improving the extrapyramidal symptoms and the remaining clinical constellation. It is very wise to suspect hypothyroidism in patients with Parkinson's disease and these observations stress the possible role played by thyroid hormones in dopaminergic metabolism.
A CASE OF RESISTANCE TO THYROID HORMONE (RTH)
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Introduction: Hyperthyroxinemia and a nonsuppressed TSH are the RTH hallmarks. RTH syndrome has two forms: generalized (GRTH) and pituitary resistance to thyroid hormones (PRTH). The absence of thyrotoxic symptoms suggests GRTH. The variability in clinical manifestations results from severity of hormonal resistance, effectiveness of compensatory mechanism, presence of modulating genetic factors and effects of prior therapy.

Objective: To report a rare case of thyroid hormone resistance (RTH) emphasising its diagnostic and therapeutic challenges.

Methods: We describe clinical and biochemical results in a patient with hyperthyroidism due to RTH, and review the related literature.

Case report / results: A 27-year old woman, with past history of subtotal thyroidectomy due to diffuse goiter, presents with a recurrence of goiter, elevation of serum levels FT4 and FT3 and non-suppressed TSH. She is clinically euthyroid. TBG, anti-TPO and anti-TG were within normal range. Response to TRH test was normal. During oral administration of L-T3, TSH concentrations were not suppressed. Genetic study is still pending. These data suggest partial thyroid hormone resistance at pituitary and peripheral tissues. The goiter is well tolerated and the patient is euthyroid due to compensation of high levels of TSH, so no treatment was initiated.

Discussion/ conclusions: RTH should be considered in all hyperthyroxinemic patients who are clinically euthyroid. Inappropriate thyroid ablation generally results in hypothyroidism and puts the patients in risk of thyrotrhaphy hyperplasia and of need for supraphysiologic thyroxine replacement. Additionally, its recognition allows appropriate genetic counseling and prenatal diagnosis, minimizing morbidity in future generations. Although 15% of subjects present non-TR RTH of unknown cause, mutations interfering with the three major steps required for TH action on target tissues have been identified (TR-beta, MCT8 and SBP2). Each mutation is associated with a distinctive pattern of heritage and of TH concentration.
CONGENITAL HYPOTHYROIDISM WITH THYROID ECTOPY - CASE REPORT
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Introduction: Congenital hypothyroidism can occur due to an anatomic defect in the gland, an inborn error of thyroid metabolism, or iodine deficiency. Congenital hypothyroidism occurs in approximately 1:4000 newborns, is the most common treatable cause of mental retardation. The thyroid gland develops from the buccopharyngeal between 4 to 10 weeks of gestation. Errors in the formation or migration of thyroid tissue can result in thyroid aplasia, dysplasia, or ectopy. One possible cause of congenital hypothyroidism is the ectopy of thyroid (lingual or sublingual thyroid gland). Thyroid scanning with technetium - 99m may be useful in defining the cause of hypothyroidism, allowing the demonstration of the absence of thyroid gland and the presence of an ectopic thyroid. Ultrasonography may be a reasonable alternative. Thyroid hormone replacement and medical monitoring are required for life.

Case report: We report a case of a 53 years-old female patient, with known hypothyroidism since childhood. She was treated with levothyroxine with very poor compliance. She had delays in areas such as language reading, comprehension and arithmetic. She has short stature (1,44 m) although her parents have normal stature, and has a history of delay menarche (19 years-old). Thyroid scanning with technetium - 99m showed no radionuclide uptake in the thyroid anatomic position and also demonstrated the presence of ectopic thyroid tissue in sublingual region. Ultrasonography confirmed the presence of an ectopic gland with 15 x 10 x 14 mm of dimensions. A fine needle aspiration cytology was made which confirmed the diagnosis and excluded malignancy.

Conclusion: We present this case to remember the relevance of an early diagnosis, an adequate treatment, compliance, and follow up to prevent the complications of the disease. Nowadays screening programs are implemented in several countries (in Portugal since eighties), and this clinical situations are being prevented.
HYPERCAPNIC COMA: A LIFE-THREATENING PRESENTATION OF SUBSTERNAL GOITER

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Nearly 5-19% of the patients undergoing thyroid surgery present with substernal goiter. Among all, the substernal location poses the most unique challenges to the surgeon. Slow growing during years, these goiters can reach critical sizes and severe compressing symptoms. Dyspnea, stridor, dysphagia, voice hoarseness or vascular compression symptoms are common and rarely urgent airway intubation will have to be performed.

We report a case of a 65 year old female with intrathoracic goiter with more than 30 years of evolution presenting with severe respiratory acidosis (pH: 6.987; pCO2: 168.6) requiring immediate mechanical ventilation. The chest CT scan showed a massive goiter extending to the tracheal bifurcation and a total thyroidectomy through a cervical approach was then performed.

This particular case illustrates a rare but potentially lethal complication of a benign disease that should always be kept in the surgeon's mind.
Objectives: Describe thyroid dysfunction in a patient with chronic HCV infection as a side effect of interferon based treatment. Hepatitis C is the main cause of chronic hepatic disease and important hepatic and extrahepatic complications. Some of these patients develop thyroid disorders related to the viral infection and induced by interferon/ribavirin, especially if they have previous positivity for anti-thyroid antibodies. We'll describe a clinical case of a 49 year old caucasian woman infected with HCV during a blood transfusion. This woman had 4c/4d genotype, interferon/ribavirin double therapy indication during a 48 week period.

Methods: Follow-up the described patient, analyzing and describing the HCV infection evolution, and associating her clinical condition with thyroid dysfunction as a possible side effect of interferon based treatment after 48 weeks, apporting clinical evolution data, laboratorial analysis, hepatic tests and thyroid function studies.

Results: Approximately after the 18th treatment week, laboratorial tests showed an hepatic enzyme normalization, anemia, thrombocytopenia, lymphopenia, high level of thyroid antibodies, painless thyroiditis with a decreased thyroid function.

Conclusions: In this clinical case it's possible to associate the high titer of autoantibodies and the interferon based treatment to thyroid dysfunction. The hypothyroidism can be a direct result of IFN-alpha associated to the viral activity.
RhTSH has become the standard of care in patients with differentiated thyroid cancer. We present this case to illustrate an insufficient elevation of serum TSH after 2 or 3 rhTSH injections in a female morbid obese woman with a BMI of 51. After total thyroidectomy due to a papillary thyroid cancer, thyroid remnant ablation with 50 mCi $^{131}$I was indicated after two 0.9 mg rhTSH injections. Serum TSH level, measured on day three after the first injection, was 27 mUI/ml. One year later, a new radioiodine dose was indicated. In this opportunity, we decided to administer three 0.9 mg rhTSH doses. The serum TSH, thyroglobulin (Tg) results and protocol were as follows:

**Day 1**: TSH 0.03 mUI/ml + first 0.9 rhTSH injection;
**Day 2**: TSH = 36.5 mUI/ml;
**Day 3**: second 0.9 rhTSH injection;
**Day 5**: TSH = 35.8 mUI/ml + third 0.9 mg rhTSH injection;
**Day 6**: TSH = 42.2 mUI/ml and
**Day 8**: TSH = 39.2, Tg 0.2 ng/ml with negative Tg–Ab.

These results probably indicate that the obese woman was not getting intramuscular injections, so the rhTSH was injected into the fat and it is possible that the absorption is lower than after injection into the muscle. Even with that elevation of serum TSH, it was possible to observe a beneficial effect of rhTSH–aided ablation.

In conclusion, in morbid obese patients, it would be recommendable to administer the rhTSH injections in the quadriceps or in the deltoid muscles to assure the correct drug absorption.
IODINE INTAKE AND PROXIMITY TO THE SEA

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Communities residing in close proximity to the sea were traditionally thought to be iodine replete due to ready availability of marine foodstuffs and use in farming of seaweed fertiliser. However in more developed countries foodstuffs are widely accessed with little or no difference between coastal and inland areas. The aim of this study was to compare urinary iodine (UI) excretion in schoolchildren residing on the Atlantic coast of Ireland (Carna, Co Galway; N= 46) with those residing in an inland area (Mullingar, Co Westmeath; N= 86)). Urinary iodine was measured using the multiplate ammonium persulphate digestion method with findings reported as µg L⁻¹ urine. The median UI in Carna was 145 µg/L compared to that of 92 µg/L in Mullingar. Surprisingly the number of UI suggestive of iodine deficiency (< 50 µg/L) was equivalent in both areas (12.0% v 12.5% respectively). However the distribution of lower values (< 100 µg/L) was 30% in Carna compared to 57% in Mullingar (p< 0.01). Lower (< 100µg/L) values predominated in boys attending school in Mullingar (62%) v 16% in Carna; p< 0.01) The findings, while not identifying significant iodine deficiency in either study area, do indicate a continuing degree of iodine insufficiency in the inland area. As dietary intake is unlikely to differ significantly between the study areas, both of whom are ethnically and socioeconomically similar, the differences observed may reflect iodine intake by respiration in children residing and playing, more vigorously in the case of boys, in an area with atmospheric iodine concentration enhanced by adjacent large seaweed beds.
DETRIMENTAL EFFECTS OF IODINE DEFICIENCY ON THE THYROID IN OLD AGE. A 10-YEAR FOLLOW-UP
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Objectives: The influence of long term iodine deficiency in old age needs to be detailed. We previously found a high occurrence of thyroid disorders among 67 year olds living in the iodine deficient community of Randers in Jutland, Denmark. We now aimed to assess the importance of continuous iodine deficiency for the thyroid function and disorders in old age.

Methods: Blood and spot urine samples had been collected and a questionnaire filled in by 423 Randers dwellers aged 67 years at the first data collection, and the 301 still living in Randers when aged 77 years were invited for repeated procedures in a 10-year follow-up study prior to the initiation of the Danish iodine supplementation programme.

Results: Participation rate was 57.5% (173; men/women: 63/110). Urinary iodine content (median;25-75%) was similar at first (42;29-71 micr.g/L) and second (54;34-95 micr.g/L) investigation (p=0.47). TSH differed by more than 1 i.e. in 23.4% of participants. In the lower/upper tertile of the normal range 15.4%/27.3% crossed the lower/upper limit of the reference range. The reverse occurred in 19.2%/27.2% of those categorised as hyperthyroid/hypothyroid when aged 67 years. The occurrence of hyper-/eu-/hypothyroidism and goitre at ages 67 and 77 years were 9.9/73.8/3.8/12.5 % and 17.3/54.9/8.1/19.7% respectively. More had thyroid disorders at the age of 77 than at 67 years (p < 0.001) due to increase in both hyperthyroidism (p=0.01) and hypothyroidism (p=0.03). Of the 136 individuals who were euthyroid at age 67 years, 19 (14%) were hyperthyroid and 7 (5%) were hypothyroid after 10 years. Iodine deficient Randers dwellers were more prone to develop hyper- than hypothyroidism (p=0.05) continuing well into old age.

In conclusion urinary iodine was unaltered after 10 years. TSH varied around the reference range limits. Thyroid disorders are common in old age, and continue to develop with advancing age in iodine deficiency.
Salt iodization is the current strategy to control iodine deficiency. Most European countries reduce salt consumption to prevent hypertension. So, the use of iodized vegetable oil as a complement to iodized salt has been investigated.

**Objective**: To test the efficacy and safety on iodine status of a sunflower oil fortified with iodine in a population consuming iodized salt, using two different iodine fortificants.

**Method**: The study was carried out among school aged children, iodine replete. They were divided into two groups A (n=32) and B (n=34) who received daily 1 ml of a sunflower oil fortified with 60 µg/ml of iodine mixed with the diet over a period of 30 days. The iodine fortificant was Lipiodol for group A and Brassiodol for group B. The total amount of iodine provided by iodized salt and vegetable oils represented 2/3 RDA. Samples for urinary iodine (UI) measurement were collected (three times/week) a month before, during and after the study.

**Results**: Median urinary iodine (mUI) values increased significantly (p< 0.01) during the study from baseline in both groups A, from 140 µg/l to 192.5 µg/l, and B, from 131.3 µg/l to 195µg/l. No values were above 300 µg/l. In group A, mUI values came back to the baseline values within 2 months, but in group B, the clearance was slower with values still significantly above the baseline values at 4 months (167,5 µg/l, p< 0,01 ).

**Conclusions**: Iodized sunflower oil improves significantly iodine status, the efficacy is similar whether Lipiodol or Brassiodol are used and mUI remains within the range of safety in spite of the concurrent iodized salt consumption. Given the slower UI clearance with Brassiodiol, Lipiodol seems to be more appropriate for a long term administration. Iodized sunflower oil appears to be an effective complement to salt to prevent IDD.
Introduction: Guinea-Bissau (West Africa) is the third poorest country in the world, with a young growing population. Since the 1950's, many districts of the hinterland are known to have endemic goitre but the current iodine deficiency status is unknown.

Objectives: To evaluate for the first time the iodine deficiency status in school-children of Bolama district, an island of the Bijagos archipelago, with an estimate population of 6,000 people.

Methods: Random morning spot urine samples were collected in school-children population between 6 and 12-yr old (n=52). Urine iodine concentration (UIC) was obtained using a modified Sandell-Kolthoff method.

Results: The mean UIC was 47.0 ± 23.2 mcg/L (mean ± standard deviation). Ninety-two percent had UIC inferior to 100 mcg/L and 75% had less than 50 mcg/L. Only 3 children (6%) presented goitre with either grade 1A or 1B. There was no correlation between different malnutrition parameters (weight-height, weight-age, height-age and body mass index-age indexes) and the level of iodine urinary excretion.

Conclusions: This was the first study in Bolama district, an area located away from the goitrogenic regions previously identified in Guinea-Bissau. Despite the proximity to the sea, these results suggest that there is a moderate to severe iodine deficiency. An urgent national iodine deficiency survey with implementation of a salt iodisation programme is needed.
A NEW QUANTITATIVE METHOD FOR MEASURING URINARY IODINE

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Aims: Iodine, an essential element for thyroid function, is important for normal growth and development of the brain and body.
Since urinary iodine concentration directly reflect dietary iodine intake, the measurement of urinary iodine is one of the most common method for biochemically assessing the iodine status of a population.
The aim of this study is the comparison between two methods for assessing urinary iodine concentration: Method A is a semi-quantitative in-house method recommended by the ICCIDD for epidemiological studies; Method B is a new quantitative assay, designed by Bioclone Australia.

Methods: Urinary iodine concentration was determined in urine samples, by the two methods. Both assays are an improvement of the Sandell-Kolthoff reaction, in which the urine is first digested with ammonium persulfate and the iodine is then determined from the catalytic reduction of ceric ammonium sulfate in the presence of arsenious acid. In Method A (semi-quantitative) the colour change is recognised visually, while in Method B (quantitative) a photometric detection is used.

Results: The results show an agreement of 94% between the two methods.

Conclusions: This preliminary study suggests an equivalence in the results obtained by both methods. Method A is suitable for its proposal and Method B can be used when a quantitative assay is indicated.
Objective: To estimate time course of endemic goiter against of iodine preventive maintenance (1998 - 2008) in West Siberia Russia. To estimate a role of latent deficiency of iron as goiter's factor.

Materials and methods: In total 21177 children of 6-12 years, with use of the unified criteria of estimation IDD (urinary iodine (UI), occurrence of the goiter on ultrasonic results) were surveyed. The neonatal thyrotropin was researched under the program of TSH screening (n=273803). In addition serum ferritin (SF), soluble receptors (TfR) measurements were determined by method ELISA.

Results: By results of epidemiological researches of 1995 in West-Siberian areas a heavy degree of endemic goiter defined. Since 1996 year in region iodine preventive maintenance was started. It is chosen food salt as the carrier of iodine.

In 2006 year the level of a median UI had made 130 µg/l , in 2008 year -127 µg/l . For ten years occurrence of the children's goiter (WHO, 2003) had decreased from 40% to 19%. Decrease in frequency of birth of children with neonatal TSH more than 5mU/l from 38,1% in 1995 year to 16,7% in 2006 (p < 0,05) is marked. It is established, that for children with the goiter lower progress at school (r = - 0,693, p < 0,05) is determined. The frequency of latent deficiency of iron at children by level SF < 15 µg/l in the midst of the goiter has made 25%. Between volume of thyroid glands at children and SF correlation dependence are detected (r = - 0,14 is determined; p = 0,002).

Conclusions: The association between serum ferritin and volumes of thyroid glands is caused by a combination of two significant conditions for a population: heavy endemic goiter and often latent deficiency of iron.
**Background**: Zinc is essential for many biochemical processes and cell proliferation. Thyroid functions influence zinc metabolism, and zinc deficiency also affects TRH synthesis and plasma TSH and thyroid hormone levels.

**Aim**: To evaluate the association between zinc levels and thyroid volumes, thyroid hormone and autoantibody levels of normal people and the patients with Hashimoto's disease and nodular goitre.

**Methods**: We evaluated 61 people (20 men, 41 women) who have not under medical treatment, and did not have surgery or radio-iodine treatment before. Nineteen patients had nodular goitre, twenty-four with Hashimoto's disease, and 18 with normal thyroid. Their thyroid volumes were calculated by ultrasonographic measurements, using the formula "lengthXwidthXdepthXpi/6". Their urine iodine levels were measured to exclude the iodine deficiency. Serum free T4 and T3, TSH, anti-thyroglobulin and anti-thyroid peroxidase levels were measured.

**Results**: The mean TSH level of these 61 patients was 2.53 ± 2.66 mIU/mL and the mean zinc level was 68.33 ± 10.64 mcg/dL. Evaluation of the whole 61 people by correlation analysis revealed that zinc levels (p=0.02) and urinary iodine levels (p=0.002) were associated with TSH levels. In normal people, zinc levels were associated with free T3 levels (p=0.031), but in nodular goitre group zinc showed no association with thyroid tests or volume (p>0.05), only urinary iodine levels were related with TSH levels (p=0.02). Also in patients with Hashimoto's thyroiditis zinc had no association (p>0.05). None of the patients showed iodine deficiency according to urinary iodine measurements.

**Conclusion**: Our data suggests zinc levels may have importance in thyroid hormone levels of normal people without iodine deficiency, but not in Hashimoto's thyroiditis patients. In nodular goitre patients iodine status of patients is still important in this region.
P43 Iodine Deficiency and Goiter 2

P264
PREDICTION OF HIGH URINARY IODINE EXCRETION BY URINARY IODINE EXCRETION FIVE YEARS AGO

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Objective: Urinary iodine excretion levels are used to determine the state of iodine supply in populations. Large intra-individual variations of iodine excretion levels mainly arise from iodine ingestion. Currently, it has not been investigated whether there is an association between different urinary iodine excretion measurements and whether such an association remains after adjustment for nutritional habits. Thus, the aim of the present study was to investigate the relation between iodine-creatinine-ratio (ICR) at two measure points 5 years apart.

Design and methods: Data of 2659 individuals from SHIP with complete follow-up and without known thyroid disorders at baseline or follow-up were analyzed. Urinary iodine concentrations were measured by a photometric procedure with Sandell and Kolthoff reaction. Creatinine was analyzed on the basis of the Jaffé reaction. ICR was treated as a continuous and a categorical variable (cut-offs: 100 µg/g and 300 µg/g). ANCOVA and Poisson regressions were used to associate baseline with follow-up ICR.

Results: Median ICR decreased moderately from 133 µg/g to 129 µg/g between baseline and follow-up. After adjustment for age, sex, weight, nutrition habits, educational level and time of blood collection, baseline ICR was significantly associated with follow-up ICR (p=0.018). Particularly, baseline ICR > 300 µg/g was related to an ICR > 300 µg/g at follow-up (relative risk [RR]: 2.67; 95%-confidence-interval [95%-CI]: 1.83, 3.90; p< 0.001). The association was stronger in males (RR: 5.72; 95%-CI: 3.10, 10.56; p< 0.001) than in females (RR: 1.94; 95%-CI: 1.24, 3.05; p=0.004).

Conclusion: The present study demonstrates that, after adjustment for nutritional habits, high ICR is predicted by high ICR five years ago. The mechanisms underlying this relation are currently not clear. Genetic and non-hereditary disorders of renal iodine reabsorption might represent a hypothetical explanation, but also residual confounding cannot unequivocally be ruled out.
Thyroid diseases are the most prevailing concomitant pathology in patients with primary parathyroidism (PPT). It's possibly due to genetic mutations in polypotential embryonic cell originating a neural crest.

226 patients with PPT were operated on in our Institute within the period of 1987 to 2009. According to histologic investigation, parathyroid adenoma was verified in 124 patients, parathyroid hyperplasia - in 84, and parathyroid carcinoma - in 17. Together with the surgical intervention on parathyroid glands, the patients underwent either thyroid incision biopsy or thyroid surgery because of the surgical thyroid diseases revealed.

At the preoperative stage, there were significant difficulties in topic diagnosis noted in cases of associated thyroid and parathyroid diseases because of the similar echotomographic images of both thyroid tumors with metastases in paratracheal lymphatic nodes and typically located parathyroid tumors. A pathologic parathyroid gland at intrathyroid location is undistinguishable from the pathologic thyroid gland itself. Parathyroid adenoma was associated in 20 patients with diffuse colloid goiter, in 18 patients - with nodal colloid goiter, and in 6 - with follicular thyroid adenoma. Parathyroid hyperplasia was associated in 16 patients with diffuse colloid goiter, in 14 - with nodal colloid goiter, in 11 - with follicular adenoma, in 4 - with papillary carcinoma, and in 1 patient - with medullar carcinoma. In 2 of 17 patients with verified parathyroid carcinoma, PPT was associated with nodal colloid goiter, and in 1 patient - with papillary carcinoma; in 1 other observation, parathyroid carcinoma was located in mediastinum and associated with follicular adenoma, and in another 1, parathyroid carcinoma was located in the thyroid gland and associated with papillary carcinoma.

Our experience confirms a considerable incidence (55.8%) of PPT association with different thyroid diseases which should be taken into account during topic diagnosis and surgical treatment of PPT.
Objective: Thyroid hemiagenesis (TH) is a rare congenital abnormality of the thyroid gland, characterized by the absence of one lobe. The true prevalence of this congenital abnormality is not known because the absence of one thyroid lobe usually does not cause clinical symptoms by itself. This study aims to identify the frequency of thyroid hemiagenesis and associated diseases in outpatients referring to our clinic for the first time.

Materials and methods: 6242 outpatients who referred for the first time were examined during the period between January 2007 and March 2009 at our thyroid clinic. For patients with TH, demographic data, family history of thyroid disorders, drugs administered, thyroid function parameters, thyroid autoantibodies and thyroid sonography and Tc-pertechnetate scintiscan were carried out. Fine-needle aspiration biopsy was performed in cold nodules. Thyroid ultrasonography was performed in first-degree relatives of patients with thyroid hemiagenesis.

Results: We identified 10 cases of TH out of 6242 outpatients with various thyroid disorders, 8 women and 2 men (ratio 4:1), age 21-63 years, indicating a prevalence of 0.16%. Associated thyroid diseases in these patients were 1 nodular Graves' disease, 1 Hashimoto's thyroiditis, 4 euthyroid nodular goiters, 2 euthyroid multinodular goiters. Two patients had no underlying thyroid disease. Family screen of patients with TH were negative. Seven patients had no lobe on the left (70%) and 3 had no right lobe (30%). The isthmus was present only in 3 patients (30%). Nine patients were clinically asymptomatic. But, the patient with nodular Graves' disease presented thyrotoxicosis.

Conclusions: We conclude that a diagnosis of hemiagenesis should always be considered when unilateral thyroid enlargement is encountered. Patients with TH should be carefully followed-up, because of the possible development of simple or nodular goiter, hypothyroidism and Graves's disease.
THYROID NODULES MANAGEMENT IN AMBULATORY SURGERY: RETROSPECTIVE ANALYSIS OF 122 CONSECUTIVE CASES

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Background: Thyroid surgery is increasingly performed in the outpatient setting with good results regarding safety, morbidity and postoperative patient rehabilitation.

Objectives: To characterize the cohort of patients submitted to partial thyroidectomy at the Ambulatory Surgery Unit in 2005-2008 and to identify preoperative clinical and tumor characteristics that could predict malignancy.

Methods: We studied 122 consecutive patients (17 men) submitted to partial thyroidectomy in the outpatient setting. The cohort was characterized for age, gender, family history of thyroid disease, presence of hypo/hyperthyroidism, anti-thyroid antibodies, compressive symptoms, nodule size, suspicious ultrasound findings, previous fine needle aspiration (FNA) diagnosis, histological diagnosis, postoperative complications and need for thyroidectomy completeness. We correlated histological diagnosis (benign vs. malignant) with preoperative clinical and ultrasound characteristics and FNA result.

Results: The cohort was characterized by mean age=46,9±12,6yrs, mean nodule size=4,1±1,2cm, compressive symptoms in 31,6% and recent nodule enlargement in 6%. No one had family history of thyroid malignancy. Laboratory assessment showed hypothyroidism in 1,7%, hyperthyroidism in 13,3% and positive anti-thyroid antibodies in 13,3%. Ultrasonography evidenced microcalcifications in 4,3% and lymphadenopathies in 2,6%. FNA diagnosis was benign in 79,5%, indeterminate/suspicious in 14,5% (17 patients) and repeatedly inadequate in 6% (7 patients). The histological diagnosis was nodular hyperplasia in 57%, follicular adenoma in 21,5%, Hürthle-cell adenoma in 4,1%, lymphocytic thyroiditis in 0,8%, follicular carcinoma in 4,1% (5 patients) and papillary carcinoma in 12,4% (15 patients). The prevalence of postoperative complications was 6,6% (transitory dysphonia and local hematoma). Seventeen thyroidectomy completeness were performed, with contralateral microfoci of papillary carcinoma found in 2 cases. No statistically significant difference was found, between benign and malignant histological diagnosis, for pre-operative clinical, ultrasound and FNA findings.

Conclusions: Thyroid ambulatory surgery is safe and accessible. Prediction of malignancy based on isolated clinical and ultrasound characteristics or FNA diagnosis (non malignant) is not reliable.
Aim of the work: To study the advisability of the operative treatment of the nodular goiter.


Results: 136 patients having nodular goiter from 1 month to 27 years since it was diagnosed were examined. Ages of the patients: 36 - 77 (50,9+1,3) years; women - 107 (78,7%), men - 29 (21,3%). A number of the nodes - from 1 (44 patients) to 2-6 (92 patients), the duration of the observance before the operation - 0,4-12 years. Patients were send for the operative treatment in the following cases: with big goiters in size, rapid progress of nodes, when thyroid carcinoma was diagnosed through fine-needle aspiration biopsy. A number of the patients with the differentiated thyroid carcinoma (HDTC) was in 2003 - 7 among 19 (36,8%, all - papillary cancer (PC); 2004 - 3 among 27 (11,1%, all - •C); 2005 - 4 among 29 (13,6%, 2 PC, 2 follicular cancer (FC)); 2006 - 6 among 26 (23%, 4 PC, 2 FC); 2007 - 13 among 35 (37,1%, 9 PC, 4 FC). In total within 5 years - 33 patients with HDTC (23,9%). The follicular adenomas (FA) were amounted for 4, 9, 11, 6, 14 - totally 44 (32,4%). The micro-macro-follicular goiter (MMFG) - 57 (41,9%), colloid goiter (CG) - 56 (41,2%), lymphoid infiltration (LI) - 34 (25%), cysts - 11 (8%), calcifications - 8 (5,9%), toxic goiter - 3 (2,2%). 81 among 136 patients (59,6%) had overlapped pathology: PC+FA+CG, PC+MMFG+LI, FC+FA+cyst+CG, PC+FA, PC+FA+LI.

Conclusions: Taking into account the high percentage of diagnosis of the HDTC and FA as far as nodular goiters are concerned and also the raising of the overlapped pathology the patients with nodes goiters are probably to be sent for the operative treatment.
ONCOMORBIDITY IN THE TREATMENT OF THE NODULAR GOITER
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Aim of the work: To study the advisability of the operative treatment of the nodular goiter (NG) taking into accounts the oncomorbidity.


Results: 136 patients having nodular goiter from 1 month to 27 years since it was diagnosed were examined. The ages of the patients: 36 - 77 (50.9±1.3) years; women - 107 (78.7%), men - 29 (21.3%). A number of the nodes - from 1 (44 patients) to 2-6 (92 patients), the duration of the observance before the operation - 0,4-12 years. The patients were send for the operative treatment in the following cases: with big goiters in size, rapid progress of nodes, when thyroid carcinoma was diagnosed through fine-needle aspiration biopsy. A number of the patients with the differentiated thyroid carcinoma (HDTC) was in 2003 - 7 among 19 (36.8%, all - papillary cancer (PC); 2004 - 3 among 27 (11.1%, all - •C); 2005 - 4 among 29 (13.6%, 2 PC, 2 follicular cancer (FC); 2006 - 6 among 26 (23%, 4 PC, 2 FC); 2007 - 13 among 35 (37.1%, 9 PC, 4 FC). In total within 5 years - 33 patients with HDTC (23.9%). The follicular adenomas (FA) were amounted for 4, 9, 11, 6, 14 - totally 44 (32.4%). The micro-macro-follicular goiter (MMFG) - 57 (41.9%), colloid goiter (CG) - 56 (41.2%), lymphoid infiltration (LI) - 34 (25%), cysts - 11 (8%), calcifications - 8 (5.9%), toxic goiter - 3 (2.2%). 81 among 136 patients (59.6%) had overlapped pathology: PC+FA+CG, PC+MMFG+LI, FC+FA+cyst+CG, PC+FA, PC+FA+LI.

Conclusions: Taking into account the high percentage of diagnosis of the HDTC and FA as far as nodular goiters are concerned and also the raising of the overlapped pathology the patients with nodes goiters are probably to be sent for the operative treatment.
Purpose: To assess the value of parathyroid hormone (PTH) assay in fine needle aspiration biopsy wash out fluid (FNA-PTH) to localize hyperfunctioning parathyroids (HP), in primary hyperparathyroidism (pHPT) associated to nodular thyroid diseases.

Subjects and methods: A total of 58 lesions suspect for HP from 40 patients with pHPT associated to multinodular goiter (MNG) and/or Hashimoto's thyroiditis (HT) were considered. Sixteen HP (form 16 patients) unequivocally localized by sestaMIBI scintigraphy (MIBI) and neck ultrasound (US) (group I), were compared to 42 suspect lesions (from 24 patients) displaying uncertain parathyroid localization (group II). In all cases US, MIBI, FNA-PTH and FNA-cytology and were performed. FNA-PTH was considered indicative for HP when it exceeded the value of 103 pg/ml (i.e. three times the maximal level found in not-HP lesions).

Results: MIBI correctly identified all HP in group I patients, while a lower diagnostic accuracy (sensitivity 70%, specificity 25%) was observed in group II, due to the presence of both MIBI negative HP (more frequently observed in HT [6/9, 66.7%] vs MNG [6/31, 19.3%], p< 0.02) and MIBI positive thyroid nodules. In contrast, FNA-PTH correctly identified all HP (38 adenomas, 2 hyperplastic parathyroid, and 1 carcinoma) in both groups of patients, with 100% sensitivity and specificity. FNA-cytology, although 100% specific, showed low sensitivity (53.7%), with no differences between the two groups.

Conclusions: In pHPT coexistent thyroid pathology (particularly HT) may cause variable degree of mismatch between MIBI and US resulting in difficult HP localization. In these cases FNA-PTH resulted an accurate and safe tool to localize HP. However, due to its reported potential risks, this procedure may be advised only to patients with uncertain HP localization.
Aim: To estimate retrospectively the informative value of fine needle aspiration rebiopsy (FNAB) in regard to ultrasound characteristics of thyroid nodules.

Methods and materials: 4552 patients with thyroid nodules underwent ultrasound examination in the regimes of B and Color Doppler with FNAB, followed by cytological examination. In 261 cases (5.7%) the material was estimated as uninformative. In 215 cases (82.4%) rebiopsies were carried out. The informative material was received in 191 (88.8%) patients after the first rebiopsy, in 23 patients (10.7%) - after the second rebiopsy. In one case (0.5%) the adequate material was gained after the third rebiopsy. In the cases of rebiopsy the nodule size, echogenecity, Halo, cystic degeneration, calcificates, and vascularization character were evaluated.

Results: At the rebiopsy a colloid nodule was observed in 130 cases (69.0%), follicular tumor (FT) - in 32 cases (16.7%), Hashimoto's thyroiditis - in 22 cases (11.5%), and papillary carcinoma - in 6 cases (2.8%). In comparison with the FT frequency at the primary FNAB, which is on average 8.9 % of thyroid nodule biopsies, we have found a reliable increase of FT frequency at the rebiopsy - 15.7%. At the material examination, depending on echographic nodule characteristics, reliable differences were observed according to the character of intraperinodular vascularization (73.3%) in FT, hypogenecity predominance in FT groups (70.0%), and papillary carcinomas (83.4%). Cystic degeneration was observed in the colloid nodule group considerably more often (32.3%) than in FT group (16.7%) and was absent in the groups of papillary carcinomas and Hashimoto's thyroiditis.

Conclusions:
1. A higher FT frequency at the rebiopsy of thyroid nodules shows the necessity of repeated FNAB.
2. Nodule hypogenecity intraperinodular vascularization can be regarded as predictive ultrasound signs of follicular neoplasm.
Objective: Efficacy of eutectic mixture of local anesthetics (EMLA) cream and the needle-free injection of local anesthesia for reducing the pain associated with fine-needle aspiration biopsy (FNAB) of thyroid nodules was previously reported. However, direct comparison of the analgesic efficacy for both methods has not been established yet. The aim of this study was to compare the analgesic efficacy of EMLA cream and needle-free injection of lidocaine for FNAB-associated pain.

Materials and methods: A total of 138 patients having their first ultrasonography-guided thyroid nodule biopsy were allocated to receive either EMLA cream (n=68) or needle-free injection of lidocaine (n=70) 1 hour and a few minutes, respectively, before FNAB of thyroid nodules. Four needle passes for biopsy of each nodule was performed. Patients rated pain associated with the procedure according to a 100-mm visual analog scale (VAS), an 11-point numeric rating scale (NRS), and 4-category verbal rating scale (VRS).

Results: When the EMLA group was compared with the lidocaine group, there were no significant differences with respect to age, sex, thyroid volume, nodule size, or nodule site. Significant differences were noted in the pain ratings of those 2 groups according to all 3 pain scales. When the effectiveness of EMLA was compared with that of needle-free injection of lidocaine, the mean VAS score was 23.4 ± 20.5 mm versus 12.7 ± 15.5 mm ($P = .001$) and the mean NRS score was 2.8 ± 2.1 points versus 1.6 ± 1.7 points ($P < .001$). The absolute numbers according to VRS score in each group was also significantly different ($P = .001$).

Conclusions: Needle-free injection of lidocaine provides more effective and faster analgesia than EMLA cream application during the FNAB.
CONTRIBUTION OF ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION CYTOLOGY TO DIAGNOSIS AND MANAGEMENT OF THYROID NODULES. RETROSPECTIVE ANALYSIS OF 617 CONSECUTIVE CASES

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Aims: To determine the role of US-FNAC in the diagnosis and management of thyroid nodules and the impact of US-FNAC in the decrease rate of surgery and surgical option in clinically suspected nodules.

Methods: We studied clinical histories of 617 patients (66 men) with solitary or multinodular goitre submitted to US-FNAC (one year). Clinical presentation, non-invasive investigations including hormone assays, ultrasonography and isotope scan procedures were compared with US-FNAC diagnosis in all cases and with histological diagnosis in the 65 cases (10.5%) submitted to surgery. Clinical management was decided upon combining all the above investigations. The relative contribution of US-FNAC was considered: essential, additional, non-contributory, misleading.

Results: US-FNAC diagnosis included: 324 (52.5%) benign colloid goitre, 106 (17.2%) benign cystic goitre, 31 (5%) thyroiditis, 21 (3.4%) indeterminate/suspicious and inadequate in 135 (21.9%). Thyroidectomy in 42 cases and Lobectomy in 23 cases. The histological diagnosis was nodular hyperplasia 49.3% (32), follicular adenoma 18.5% (12), lymphocytic thyroiditis 15.4% (10), follicular carcinoma 3% (2) papillary carcinoma 12.3% (8) and medular carcinoma 1.5% (1). When compared with clinical diagnosis, US-FNAC represent an improvement on the diagnosis of benign colloid/cystic goitre (78.8% v 88.8%) and thyroiditis (5% v 6.7%). Decreased clinically suspicious lesions in which 21 neoplasms were diagnosed (17% v 4.5%). Six neoplasms were confirmed histologically. 8 false negative (1.2%) in clinically suspicious lesions (benign adenomas in histology).

Conclusions: US-FNAC was essential in the diagnosis and management of 25.7% patients (benign lesions and neoplasms), additional in 52.7% patients (benign lesions and thyroiditis), non-contributory in 21.2% (specimens inadequate), and misleading in 1.2% (false negative). The management of thyroid nodules as influenced by US-FNAC is best exemplified in the low rate of surgical intervention (10.5%). Surgery was avoided mainly in benign lesions, thyroiditis and lymphoma. US-FNAC diagnosis may be contributory in solitary nodules with option for ambulatory surgery.
FOLLICULAR TUMOUR IN THE SETTING OF MULTINODULAR GOITRE (MNG) OR AS A SINGLE NODULE: THE SAME DISEASE ENTITY?

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Introduction: The diagnosis of malignancy of a follicular tumour (10 to 20% in most series) is only defined by the histological examination of the surgical specimen.

Objectives: Analyse our current rate of malignancy and evaluate if the number of thyroid nodules interferes with the histological result.

Methods: We performed a retrospective analysis of 200 patients admitted for follicular thyroid tumours between 2006 and 2008 and analysed the histological results according to the number of thyroid nodules diagnosed pre-operatively.

Results: 200 patients (171 women/29 men) were included with a mean age of 51 years (22 - 78 years). 140 patients (121 women/19 men) had multiple nodules and 60 patients (51 women/9 men) had a single thyroid nodule on the pre-operative ultrasonography scan (US). In the multiple nodule setting, the histological results were: 75 nodular goitres, 32 papillary carcinomas, 8 papillary microcarcinomas, 16 follicular adenomas, 5 lymphocytic nodular thyroiditis, 2 follicular tumours of uncertain malignant potential and 2 follicular carcinomas with minimal invasion. In the single nodule setting, the histological results were: 12 nodular goitres, 23 papillary carcinomas, 4 papillary microcarcinomas, 17 follicular adenomas, 3 lymphocytic thyroiditis and a cystic necrotic lesion.

Conclusions: In our series, the malignancy rate was higher than that described in the literature, in the multiple nodule (30%) and single nodule (45%) setting. The distinction between MNG / single nodule must not guide our conduct when the diagnosis of follicular tumour has been made, despite the higher rate of malignancy found in the thyroid glands with single nodules (p = 0.04). Since 97% of the diagnosed malignant tumours were papillary carcinomas, investments in the diagnostic accuracy of cytology should be made.
Introduction: Benign and malignant thyroid nodules are usually diagnosed by Fine-needle Aspiration cytology (FNA). Malignancy is confirmed at histology in the majority of the cases, yet only 25-40% of the indeterminate cases (Follicular tumor and Hürthle-cell neoplasm) are confirmed to be malignant.

Objectives: To evaluate the accuracy of FNA in Indeterminate and malignant thyroid nodules in Hospital Fernando Fonseca, in the last 2 years.

Methods: Retrospective analysis of all FNA performed between 2006 and 2008, comparing Histology from patients operated for Indeterminate or Malignant thyroid tumor diagnosed on FNA cytology.

Results: From 558 FNA performed between 2006 and 2008, 336 (60%) were benign, 17 (3%) were indeterminate (11 Follicular tumor, 6 Hürthle-cell tumors), 15 were malignant (13 Papillary Carcinoma, 1 Metastasis, 1 Undetermined malignancy) and 5 patients (1%) were suspicious of Papillary Carcinoma. The patients with malignant FNA were all confirmed at histology as being malignant. There were 40% malignant cases from the 5 patients that had a Suspicious FNA. And among the 17 patients with indeterminate FNA 30% were malignant.

Conclusions: A strong correlation between cytological and histological diagnosis was observed. The percentage of carcinomas diagnosed among patients with indeterminate FNA is equivalent to that observed in the literature and confirms the utmost importance of a timely surgical procedure.
The aim of screening was to determine the prevalence of unsuspected thyroid disease among adults in Varna region.

Methods: 1115 individuals (619 women and 496 men), mean age 44.2±13.3 years, without history of thyroid disease, answered a questionnaire and were clinically examined. Serum TSH and anti-TPO were evaluated and in cases with abnormal TSH, FT4 and FT3 were also measured. Thyroid ultrasonography was performed with Fukuda UF-750XT (9 MHz, Color Doppler).

Results: 91.8% of subjects had normal TSH, 2.1% decreased and 6.1% elevated TSH. Hypothyroidism was overt in 1.6% of men and 2.3% of women and subclinical in 2.4% of males and 5.5% of females. 688 persons had normal thyroid structure and size, with mean thyroid volume 11.6 ±2.8 ml in females and in 15.3±3.8 ml in males. The same group had mean TSH 1.84±0.96 mUI/l. Discrete inhomogeneity with mean TSH 2.43±1.25 mUI/l was found in 34 subjects, mean age 62.7 years. We considered these changes as age-related. Autoimmune thyroiditis was newly diagnosed in 125 patients (11.2%), with mean TSH 5.87 mUI/l and anti-TPO 404.8 UI/l. 3/4 of them are women, above 45 years of age, with family history for thyroid disease. Thyroid nodules were present in 21.5% of all subjects - solitary in 140 patients and multiple in 96 cases. FNAB was performed in 85 cases revealing 4 papillary carcinomas and one suspicious lesion, which turned out to be follicular variant of papillary cancer on histology.

Conclusions: Thyroid abnormalities are frequent, especially in women and elderly. Concerning thyroid nodules, ultrasonography screening is a key to detect and select them for FNAB and surgery. Common thyroid disease is autoimmune thyroiditis, frequently associated with subclinical hypothyroidism. Healthy individuals with normal thyroid ultrasonography have TSH< 2.0 mUI/l, which figures out the target of treatment for hypothyroidism, especially in pregnancy and in patients with cardiovascular risk.
CYTOLOGICAL AND HISTOLOGICAL FINDINGS OF NON-NEOPLASTIC NON-TOXIC RECURRENT NODULAR GOITER
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Objective: To evaluate incidence of malignancy in recurrent nodular goiters of patients who were operated for benign thyroid diseases.

Methods: Between January 2008 and January 2009, 114 patients with recurrent nodular goiters who were operated for benign causes enrolled in this study. The cytological results of 158 nodules and histological results of 11 cases who went to operation were examined.

Results: There were 114 patients with recurrent nodular goiter; 106 were female and 8 were male. The mean age was 49.2 ± 12.1. The mean operation time was 16.5 ± 7.5 years. The operation indication was multinodular goiter for all patients. The operation was subtotal thyroidectomy for 101 of them and lobectomy for 13. The number of patients having Levothyroxine replacement was 47. The fine needle aspiration biopsy was done to 158 nodules. 19 patients recommended for surgery according to TFNAB results which were 12 suspicious, 2 malignant and 5 with hurle cell neoplasm. One with suspicious cytology and one with hurle cell neoplasm were smaller than 1 cm and two with malignancy cytology were greater than 1 cm in diameter. 12 of patients accepted operation but one of them couldn't be operated because of vocal cord paralysis. A diagnosis of papillary carcinoma histologically confirmed in 4 of them. The incidence of malignancy was found to be % 3.4 for total patients with recurrent nodular goiter.

Conclusion: Our data show that there was considerable amount of malignancy in recurrent nodular goiters. As conservative surgery of thyroid is followed by recurrence in 2 to 70% of cases in 8-20 years, the treatment approach should be total thyroidectomy to avoid recurrence and malignancy especially in iodine deficient region.
Background: Thyroid nodules are a common clinical problem. Fine needle aspiration biopsy (FNAB) plays a crucial role in the diagnosis of thyroid nodules and enables the number of surgical procedures to be reduced.

Objective: Review our Hospital’s experience with fine-needle aspiration biopsies of the thyroid, during the last year and assess whether FNAB is a good diagnostic procedure for identification of malignant thyroid nodules.

Methods: Clinical data, cytology and histopathology results were retrospectively analyzed on all patients who underwent thyroid FNAB in our Hospital, between January and December of 2008.

Results: The study population consisted of 225 FNAB in 212 patients, 29 men and 183 women, aged 15 to 98 years (average 57.6 ± 14.4 years). The lesion size ranged from 0.9 to 7 cm (2.6 ± 1.14 cm average). The lesion type was solitary nodule in 70 patients and multiple nodules in 142 patients (98 of which were multinodular goiters). The diagnoses included 25 unsatisfactory cases (11%), 26 samples negative for malignant cells (11.5%), 100 colloid nodules (44%), 24 cysts (11%), 17 thyroiditis (7.5%), 15 follicular lesions (7%), 6 oxyphilic lesions (3%), and 12 malignant tumors (5%). 33 FNAB had subsequent surgical excision. 12 malignancies on FNAB (100%) were correctly diagnosed. On 15 follicular lesions on cytology, 11 were adenomas, 2 were nodular hyperplasia and 2 were micropapillary carcinomas, on excision.

Discussion: As expected, only a small fraction of all thyroid nodules harbor malignant disease. Our data confirm the high sensitivity and specificity of thyroid FNAB in the diagnosis of thyroid cancer.