Thyroid cancer
Oral

LONG-TERM OUTCOME IN 210 CASES OF PAPILLARY THYROID CARCINOMA (PTC) PRESENTING IN PATIENTS <21 YEARS OLD AND MANAGED AT ONE INSTITUTION DURING 1940–2005: A POSSIBLE PYRRHIC VICTORY?

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Although prognosis is excellent in childhood PTC, controversy continues regarding the aggressiveness of initial therapy. We studied 210 PTC patients <21 year old, who had initial management during 1940–2005. The patients (71% female) were aged 3–20 years (median 16 years). Mean tumor size was 2.6 cm. Ten (5%) had lung metastases at diagnosis; only 11 (6%) had incomplete tumor resection. Unilateral lobectomy (UL) was the usual procedure during the 1940s. Bilateral lobar resection (BLR) comprised 94% of primary ops performed since 1950; near-total thyroidectomy for 48%. 86% had nodes removed; 79% had initial nodal involvement. During 1950–2000 radioiodine remnant ablation (RRA) was administered to 27%. Patients were followed up to 63 years; median follow-up was 26.7 years. After complete surgical resection, PTC recurred in 28% by 20 years; in 33% by 40 years. At 20 years the recurrence rates at distant, local and regional sites were 5%, 7%, and 22%, respectively. During 1940–69 locoregional recurrence rates after UL were significantly higher than after BLR ($p<0.001$). Since 1950 RRA was performed after BLR in 31%, and did not improve recurrence rates at any site. There were no deaths from PTC within 20 postoperative years, and only 2 fatal events between 25 and 30 years, for a cause-specific mortality at 50 years of only 2%., All-causes mortality rates did not exceed expectation through 20 years, but from 30 through 50 years were significantly ($p<0.001$) higher than expected (21 vs 9.5 expected); 15/21 (71%) died from malignancy (87% non-thyroid). We conclude that survival from childhood PTC should be expected, but later death from non-thyroid malignancy is concerning. 11/14 (79%) dying of non-thyroid cancers had antecedent irradiation (I-131 or XRT/radium), which may prove to be relevant to subsequent carcinogenesis.

Thyroid cancer
Oral

COMBINED IMMUNOSTAINING WITH GALECTIN-3, FIBRONECTIN-1, CITED-1, HBME-1, CYTOKERATIN-19, PPAR-GAMMA AND NIS ANTIBODIES FOR THE DIFFERENTIAL DIAGNOSIS OF THYROID CARCINOMA

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Background: The microscopic distinction between benign and malignant thyroid lesions is often difficult because neoplastic lesions share histological features. ***Aim:*** This study was performed to evaluate the diagnostic value of Galectin-3 (Gal-3), HBME-1, cytokeratin (CK)-19, CITED-1, Fibronectin (FN)-1, PPAR-gamma and intracellular NIS (iNIS) staining in a large panel of thyroid neoplasms. Our study differed from earlier ones with regard to the identification of optimal semiquantitative cut-off levels using receiver operator curve (ROC) analysis and the use of hierarchical cluster analysis. ***Methods:*** We used tissue arrays containing normal thyroid tissue (64) and tissue from Graves disease (10), multinodular goiter (MNG, 14), follicular adenoma (FA, 12), papillary thyroid carcinoma (PTC, 53), follicular thyroid carcinoma (FTC, 13) and follicular variant of PTC (FVPTC, 11). Antibody staining was scored semiquantitatively and differential expression was analysed in 2x2 tables and with hierarchical cluster analysis. ***Results:*** In general, we found overexpression of FN-1, CITED-1, Gal-3, CK-19, HBME-1 and iNIS in malignant thyroid lesions. Gal-3, FN-1 and iNIS had the highest accuracy in the differential diagnosis of follicular lesions. A panel of Gal-3, FN-1 and iNIS, identified by hierarchical cluster analysis, had a 98% accuracy to differentiate between FA and malignant thyroid lesions. In addition, HBME-1 was found to be useful in the differentiation between FA and FVPTC (accuracy 88%). ***Conclusion:*** A combination of protein expression markers increases the diagnostic value in the differential diagnosis of thyroid neoplasms. The combination of FN-1, Gal-3 and iNIS had the best accuracy (98%), whereas HBME-1 may be useful in the differentiation of FVPTC from FA.

Thyroid cancer
Oral

BRAFV600E MUTATIONS ARE CORRELATED WITH A LOWER EXPRESSION OF BOTH NIS AND TPO MRNA EXPRESSION IN PAPILLARY THYROID CANCER (PTC)

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Several studies demonstrated a relationship between oncogene activation and expression of thyroid differentiation genes (TDG). Aim of this study was to analyze BRAF and RET/PTC1-3 alterations and their influence on the expression of TDG such as Tg, TPO, TSH-R, TTF-1, NIS. 78 PTC samples were studied. Quantitative analysis of TDG were performed by real time RT-PCR. Our results indicate that BRAF mutation ($BRAF^{V600E}$) and RET/PTC rearrangements were present in 35.8% and 19.4% of cases. In particular, 50/78 cases (64.1%) were positive for one genetic alteration and 7/78 (9%) showed 2 gene mutations. $BRAF^{V600E}$ was more frequently found in classical than in follicular variant (p=0.01). At variance the RET/PTC3 rearrangement was found to be strongly correlated with tumor size (p=0.01) and class (p=0.003). Genetic alterations were correlated with mRNA expression of TDG. mRNA expression of NIS and TPO gene was significantly lower (p=0.0001 and p=0.004) in PTC with $BRAF^{V600E}$ than in non mutated samples. By NIS-immunohistochemistry we found that the percentage of positive cells was lower (p=0.005) in PTC with $BRAF^{V600E}$ mutation (53.5% of cells) than in negative cases (72.6%). We did not observe any significant difference in the expression of the other TDG neither when compared with $BRAF^{V600E}$ or RET/PTC rearrangements. Furthermore no relationship was found between serum TSH and the expression of NIS mRNA in thyroid tumors. In conclusion our data indicate that PTCs with $BRAF^{V600E}$ showed a lower expression of both NIS and TPO mRNA which are genes involved in maintaining the differentiation features of follicular cells. At variance, no correlation was found between RET/PTC rearrangements and TDG expression. These findings suggest that the $BRAF^{V600E}$ might play a role in PTC dedifferentiation process.

Thyroid cancer
Oral

CELECOXIB MIGHT PLAY A ROLE IN MAKING MEDULLARY THYROID CANCER CELLS (TT) MORE SENSITIVE TO CHEMOTHERAPY BY REDUCING COX-2 AND MDR-1 mRNA Expression

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At present, the treatment of advanced and metastatic medullary thyroid carcinoma (MTC) is unsatisfactory. The resistance of MTC cells to conventional chemotherapy has been attributed to the over-expression of MDR-1 gene, encoding for a multidrug trasporter that actively pumps drugs out of the cells. MDR-1 is up-regulated by cyclooxygenase-2 (COX-2), and COX-2 inhibitors have been reported to block the increase of MDR-1 expression. ***Aim*** of the present study was to investigate the in vitro effects of one COX-2 inhibitor, celecoxib, on a MTC derived cell line, TT. ***Material and Methods:*** We treated TT cells with various concentrations of celecoxib for 24h, 48h, 72h and one week and then we analyzed the growth rate, the occurrence of apoptosis and inhibition of DNA synthesis, the cell cycle and cytoxicity. Besides, we analyzed the effect of celecoxib on MDR-1 and COX-2 mRNA expression, by quantitative RT-PCR. ***Results:*** We observed that celecoxib 1 and 10 microM clearly inhibited cell growth after one week. The treatment with celecoxib induced a slight variable increase of nuclear enrichment, which is a consequence of apoptosis, but it did not affect either DNA synthesis, or cell cycle and it was not cytotoxic. Celecoxib determined an initial increase of both MDR-1 and COX-2 mRNA expression, which reached a maximum after 72h, followed by a significant decrease of expression of both genes (0.5 and 0.2 folds, respectively) after one week. The discontinuation of the treatment allowed the progressive recovery of MDR-1 and COX-2 expression to initial levels. ***Conclusion:*** Our data show that celecoxib was able to inhibit TT cell growth and expression of MDR-1 and COX-2 genes. This finding suggests that celecoxib might be used as adjuvant drug in patients affected by MTC who must be treated with chemotherapy.

Thyroid cancer
Oral

EFFECTS OF SUPERACTIVE HUMAN TSH ANALOG ON THYROIDAL UPTAKE OF 18F-FLUORODEOXYGLUCOSE AND 131I-IODIDE

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Since its initial approval by the US FDA in 1998 recombinant human TSH (Thyrogen, Genzyme) has been widely used to preserve quality of life in thyroid cancer patients. Trophogen has developed novel superagonist analogs of hTSH with greatly increased potency and, under certain conditions, increased maximal efficacy. The current studies focus on the clinically relevant endpoints of radioiodide uptake and fluorodeoxyglucose (^{18}F-FDG) uptake in both rat thyroid FRTL-5 cells as well as in the human thyroid carcinoma cell line ML-1. ***Methods:*** Highly purified preparation of human TSH analog (TR-1401) produced in CHO cells was compared to rhTSH and bTSH in several in vitro assays including stimulation of cAMP production, ^{18}F-FDG and ^{131}I-iodide uptake. Cells were incubated with 0.5 MBq/mL ^{18}F-FDG for 1 h or 20 kBq/mL ^{131}I-iodide for 15 min in the presence and absence of various concentrations of rhTSH, bTSH and TR-1401 (0.6–2,400 ng/ml). $(Bu)_2$cAMP was used as cAMP enhancer and wortmannin as irreversible inhibitor of PI3-kinase. ***Results:*** TR-1401 was 40-50 fold more potent than Thyrogen in cAMP stimulation in both FRTL-5 and ML-1 cells. Interestingly, TR-1401 displayed 33-fold increase in potency and 34% increase in Vmax when ^{18}F-FDG uptake was determined in FRTL-5 cells. Such Vmax increase was not present in stimulation of radioiodide uptake (43-fold increase in potency). $(Bu)_2$cAMP mimicked the effect of hTSH and TR-1401 on ^{18}F-FDG uptake, whereas wortmannin inhibited FDG uptake by 28–35%. ***Conclusion:*** TR-1401 showed increased potency combined in selected assays with an increase in maximal efficacy suggesting that such new analogs could serve as second generation recombinant TSH for the diagnosis and treatment of thyroid carcinomas with limited number of TSH receptors and modified signaling pathways.

Thyroid cancer
Oral

1513A/C POLYMORPHISM IN THE P2X7 RECEPTOR GENE IN PAPILLARY THYROID CANCER: CORRELATION WITH THE FOLLICULAR VARIANT AND CLINICAL OUTCOME

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Extracellular nucleotides modulate several biological functions via specific receptors (P2Rs) of the X and Y subtype. The modulation of P2X7R function may be implicated in human carcinogenesis. 1513A/C polymorphism of the gene encoding for P2X7 induces loss-of-function while 489 C/T gain-of-function of the receptor. We evaluated the prevalence of these two polymorphisms in 122 patients with papillary thyroid carcinoma [PTC; 44 follicular variant (mean volume 2.4±1.8 ml); 78 classical variant (mean volume 1.5±1.6 ml)], and in 100 healthy subjects. DNA was extracted from lymphocytes and submitted to polymorphism assay by the TaqMan MGB probe technique. The minor allele frequency for 1513A/C polymorphism was similar in control subjects and patients with the classical variant of PTC (0.20 and 0.26, respectively) but significantly higher in patients with the follicular variant (1.36, p=0.005). In detail, 2% of both healthy controls and patients with PTC classical variant as compared to 6% of patients with PTC follicular variant were homozygous for the 1513C allele. Moreover, a significant correlation between PTC dimension and 1513A/C polymorphism was observed (p=0.02). According to AJCC-TNM staging criteria, the loss-of-function polymorphism was associated with an advanced disease stage (p=0.01). Our data demonstrate an increased prevalence of 1513A/C polymorphism only in patients with the follicular variant of PTC. This loss-of-function polymorphism correlated with cancer dimension and increased biological aggressiveness.

Thyroid cancer
Oral

PROPHYLACTIC CENTRAL AND LATERAL LYMPH NODE DISSECTION IN PATIENTS WITH PAPILLARY THYROID CARCINOMA

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Objectives: Prophylactic lymph node dissection in patients with papillary thyroid carcinoma (PTC) remains controversial. The purpose of this study was to re-evaluate the significance of lymphatic spread into cervical compartments in patients with PTC. ***Patients:*** We retrospectively reviewed the charts of 230 patients who had undergone thyroidectomy and a central and lateral neck dissection (modified radical neck dissection). Between 2000 and 2006, 90 patients (19 men, 71 women, mean age: 45±16 years) with PTC without lymph nodes metastasis detected on preoperative palpation and ultrasonographic examen were included**. *Results:*** Patients had total or near-total thyroidectomy and a prophylactic central and lateral lymph node dissection for PTC (pT1 n=30, pT2 n=30, pT3 n=29, pTx n=1). Forty-one patients (45.5%) had lymph nodes metastasis. Twenty-eight patients (31%) had a central and a lateral involvement. Thirteen patients (14.5%) had only a cervico-central localization. All patients free of lymph node metastasis in central compartment were also free in lateral compartment. There were no correlation between lymph node status and age, sex, tumor size and TNM staging. Temporary and permanent laryngeal nerve palsy rates were 26% and 2%. Temporary and definitive hypoparathyroidism occured in 36% and 10% of the patients. Conclusion: Regional lymph node metastasis are frequent at the time of diagnosis in patients with PTC. Prophylactic lymph node dissection might be associated with significant co-morbidities. Therefore, central neck dissection could be considered at pre or intraoperative diagnosis of PTC whereas lateral neck lymph node dissection should be performed only in patients with preoperative suspected and/or intra-operatively proven lymph node metastasis.

Thyroid cancer
Oral

INITIAL RESULTS FROM A PHASE 2 TRIAL OF AMG 706 IN PATIENTS (PTS) WITH DIFFERENTIATED THYROID CANCER (DTC)

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Purpose: To evaluate safety and efficacy of AMG 706, an oral, investigational inhibitor of angiogenesis in pts with advanced thyroid cancer (TC) stratified by DTC or medullary TC (MTC). Presented are the DTC results. ***Methods:*** Multicenter, phase 2, open-label, single-arm study. The primary endpoint was objective tumor response per modified RECIST by independent central review. Secondary DTC endpoints were duration of response and progression-free survival (PFS). Pts ≥18 years with advanced, ^{131}I-resistant TC, ECOG 0-2, and no prior vascular endothelial growth factor receptor inhibitor therapy received AMG 706 125mg QD until disease progression or unacceptable toxicity. Assessments included tumor response (q8w), pharmacokinetics (PK), and safety. ***Results:*** 93 DTC pts were enrolled and received ≥1 dose of AMG 706; 96% had prior ^{131}I therapy. With median follow-up of 32 weeks, objective response (CR or PR) rate was 12% (95% CI: 6.1, 20.2); SD, 69% (durable SD ≥24 weeks, 24%); PD, 8%. Median (95% CI) time to response was 103 (53, 161) days; median PFS was 276 (221, not estimable) days. 85% of pts were alive >8 months after therapy start. All pts had some treatment-emergent adverse events (AE): grade 3/4/5, 65/13/5% (all grade 5 were deemed treatment unrelated). Common AEs included diarrhea, 70% (grade 3, 11%); fatigue, 58% (5%); hypertension, 49% (22%); headache, 43% (4%); and hypothyroidism and/or increased TSH, 17% (0%). None of these were grade 4 or 5. 6% of pts had cholecystitis. At 125mg QD AMG 706 PK was comparable to other monotherapy data at that dose level. ***Conclusion:*** In this study of DTC pts, AMG 706 showed encouraging antitumor activity and had tolerable toxicities. Updated data will be presented.

Thyroid cancer
Oral

MiRNA EXPRESSION PROFILES IN THYROID TUMORS

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Purpose and Methods: Recently discovered small non-coding RNA molecules, known as microRNAs (miRNAs), function as negative regulators of coding gene expression. Expression of specific miRNAs varies between normal and cancer tissues and between different cancer types. We studied the expression pattern of 158 human miRNAs in 32 thyroid samples including 3 normal thyroids (NTs), 9 papillary carcinomas (PCs), 5 follicular carcinomas (FCs) of either conventional or oncocytic type (OCs), 4 follicular adenomas (FAs), 4 poorly differentiated and anaplastic carcinomas, and 2 medullary carcinomas (MCs) using RT-PCR TaqMan MicroRNA Human Panel on ABI 7500. The Bayesian Infinite Mixture Model in the gimmR package was used to perform hierarchical clustering. ***Results:*** 148 (94%) miRNAs were found expressed in thyroid tissue. In thyroid tumors, 20% of miRNAs were upregulated and 31% downregulated with fold change >2.0 as compared with NTs. Cluster analysis of 158 miRNA demonstrated different miRNAs expression signatures for MCs, HCs and the rest of follicular cell-derived tumors. Unique expression profiles were found in PCs and FCs after filtering out miRNAs whose expression significantly deviated from NTs. Several miRNAs were found to be highly (9–41-fold) and consistently overexpressed in PCs and FCs, but not in NTs and FAs. ***Conclusions:*** Our results indicate that a large number of miRNAs are differentially expressed in thyroid tumors as compared to normal tissue. Expression of some miRNAs correlated with specific tumor-initiating mutations, suggesting that miRNAs may play a role in tumorigenesis. Several miRNAs may serve as novel diagnostic markers for thyroid cancer in fine needle aspiration (FNA) and surgically removed thyroid samples.

Thyroid cancer
Oral

INITIAL RESULTS FROM A PHASE 2 TRIAL OF AMG 706 IN PATIENTS (pts) WITH MEDULLARY THYROID CANCER (MTC)

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Purpose: To evaluate safety and efficacy of AMG 706, an oral, investigational inhibitor of angiogenesis in pts with advanced thyroid cancer (TC) stratified by MTC or differentiated TC (DTC). Presented are the MTC results. ***Methods:*** Multicenter, phase 2, open-label, single-arm trial. Primary endpoint was objective tumor response (OR) per modified RECIST by independent central review. Secondary MTC endpoints were duration of response, progression-free survival (PFS), and symptom assessment (diarrhea). Pts ≥18 years with measurable, progressive or symptomatic MTC, ECOG 0-2, and no prior VEGFr inhibitor therapy received AMG 706 125mg QD until disease progression or unacceptable toxicity. Assessments included OR (q8w), pharmacokinetics (PK), safety, and patient-reported outcomes (PRO). ***Results:*** 91 MTC pts received ≥1 dose of AMG 706. With median follow-up of 32 weeks, OR (CR or PR) rate was 1% (95% CI: 0.0, 6.0); SD, 80% (durable SD ≥24 weeks, 24%); PD, 10%. Median PFS was not estimable. 78% of pts had some decline in absolute tumor size. Based on PRO, 71% of pts had diarrhea at baseline, with 47% showing improved diarrhea frequency by week 24. 99% of pts had treatment-emergent adverse events (AE): grade 3/4/5, 41/2/5% (all grade 5 after disease progression). Common AEs included diarrhea, 56% (grade 3/4/5, 12/0/0%); fatigue, 41% (5/1/0%); nausea, 30% (1/0/0%); hypertension, 26% (8/0/0%); and hypothyroidism and/or increased TSH, 35% (0/0/0%). 4% of pts had cholecystitis. AMG 706 PK data showed lower exposure in MTC pts than observed in previous monotherapy studies at 125mg QD. ***Conclusion:*** In this study of MTC pts, AMG 706 had tolerable toxicities and showed antitumor activity. Updated data will be presented.

Thyroid cancer
Oral

GENE EXPRESSION AND THE BIOLOGICAL PHENOTYPE OF PAPILLARY THYROID CARCINOMAS

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The purpose of this presentation is to correlate the molecular phenotype of papillary thyroid carcinoma (PTC) to their biological pathology. We hybridized 26 PTC on microarrays and showed that nearly 44% of the transcriptome was regulated in these tumors. We then combined our dataset with 2 published PTC microarrays studies to produce a platform- and study-independent list of PTC-associated genes. We further confirmed the mRNA regulation of 15 genes from this list by qRT-PCR. Analysis of this list with statistical tools led to several conclusions: (1) there is a change in cell population with an increased expression of genes involved in the immune response, reflecting lymphocyte infiltration in the tumor compared to the normal tissue. (2) the JNK pathway is activated by overexpression of its components. (3) the activation of ERKK1/2 by genetic alterations is supplemented by activation of the EGF but not of the IGF signaling pathway. (4) there is a downregulation of immediate early genes. (5) we observed an overexpression of many proteases in accordance with tumor remodeling, and suggested a probable role of S100 proteins and annexin A2 in this process. (6) numerous overexpressed genes favor the hypothesis of a collective migration mode of tumors cells.

Thyroid cancer
Oral

DENDRITIC CELL VACCINATION WITH HETEROCLITIC CALCITONIN PEPTIDE IN A TRANSGENIC TUMOR MOUSE MODEL FOR MEDULLARY THYROID CARCINOMA LEADS TO SPECIFIC ANTI-TUMOR IMMUNITY AND DIMINISHED TUMOR OUTGROWTH

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Previously, we and others have demonstrated that immunization with dendritic cells (DC) lead to anti-tumor immunity in human malignancies such as medullary thyroid carcinoma (MTC). The aim of the present study was to optimize the vaccination strategy in transgenic MTC (Ret/Cal) mice with a Ret-Protooncogene mutation (exon 11, codon 634). As all of these mice develop a MTC they represent an ideal animal model for the establishment of new therapy strategies. Altogether, 75 Ret/Cal mice were included into the study of whom 30 were immunized with heteroclitic salmon calcitonin-pulsed DC. Salmon calcitonin has been chosen for immunization because of their higher immunogenicity as compared to murine calcitonin. After a follow-up of 6 months tumor peptide treated Ret/Cal mice developed an antigen-specific immunity. For quantification of epitope-specific CTLs, tetramer stainings of splenic, inguinal and partumorous cervical lymhocytes were performed covering three different epitopes of salmon calcitonin peptide. In tumor peptide-treated mice an increase of calcitonin epitope-specific CTLs were already seen in spleens and inguinal lymph nodes (LN). Strikingly, in paratumorous cervical LN more than six percent of all CD8+ T cells were specific for on of three tumor epitopes tested. On immunohistochemistry, salmon calcitonin-immunized mice showed a strong tumor infiltration with CD8+ CTLs, whereas control mice did not. Importantly, salmon calcitonin-treated mice revealed a largely diminished tumor outgrowth (–74.3%) compared to non-immunized and control protein-immunized Ret/Cal mice ($p < 0.0001$). In parallel, serum calcitonin levels in calcitonin-vaccinated Ret/Cal mice were lower compared to the control group. In summary, this is the first description of a DC immunotherapy trial in a transgenic MTC mouse model. Our results clearly demonstrate that DC vaccination with hetereoclitic calcitonin peptides induces cytotoxic immunity leading to diminished tumor outgrowth. Based on these results, a new immunotherapy trial in MTC patients has now been initiated.

Thyroid cancer
Oral

IDENTIFICATION OF A CANDIDATE CHROMOSOMAL REGION USING A SNP LINKAGE PANEL SUGGESTS A SECOND LOCUS RESPONSIBLE FOR NON-RET MEN2 FAMILIES

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Background: Multiple Endocrine Neoplasia Type 2 (MEN2) is a familial cancer syndrome characterized by the development of different endocrine tumors. A clinical sub-classification of MEN2 has been established according to tumor spectrum, with medullary thyroid carcinoma (MTC) being the common hallmark of all three subgroups: MEN2A, MEN2B and familial MTC (FMTC). The gene responsible for the disease in more than 95% of cases is the RET proto-oncogene. However, for non-RET MEN2 families causal *locus* or *loci* remain unknown. ***Aim:*** The aim of the current study is to identify the gene/genes responsible for non-RET MEN2 families. ***Material and Methods:*** A Spanish family with 3 generations, 12 affected members and 3 unaffected individuals, was available for this study. The presence of germline mutations of RET were ruled out by sequence analysis of the entire gene. A haplotype study using four RET intragenic markers (rs1800858, rs1800860, rs179939, and rs1800863) was also carried out to confirm that the disease does not segregate with *RET* gene in this family. We then performed a genome wide SNP linkage assay using Illumina\'s Linkage IV Panel which included 5,860 molecular markers distributed evenly across the genome. Multipoint linkage analysis was performed along the entire length of each chromosome using the MERLIN program. ***Results:*** Preliminary analysis revealed significant evidence of linkage at one chromosomal region with a non-parametric lod score of 10.3, and a parametric lod score of 3.2 assuming a dominant model. The linked region includes 74 genes. Further details about the molecular markers and potential *locus* will be presented. ***Conclusions:*** This finding is the first step in explaining the genetic susceptibility to non-RET MEN2. The subsequent identification of the causal *locus* in this region will improve the genetic counseling of those families with currently unknown molecular basis.

Thyroid cancer
Oral

EFFECT OF LATENCY ON DIFFERENT TYPES OF THYROID CANCER POST CHERNOBYL

on behalf of the Pathology Panel and the Scientific Project Panel of the CTB
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The increase in cancer post Chernobyl has thus far been confined to papillary carcinoma (PTC). However, an apparent increase over time in follicular cancer has been identified by the Pathology Panel of the Chernobyl Tissue Bank. Our aim was to define the incidence of benign and malignant follicular lesions, as function of time since exposure (latency), in those exposed to fallout from Chernobyl as children or adolescents. The dataset comprised 1,858 cases of thyroid tumour (1,179 PTC, 585 benign follicular lesions, 63 FTC, 31 MTC). 81% of the cases were from contaminated regions and 19% were from non-contaminated regions.Thyroid surgery was performed at a median age of 6.7 +/– 5.6 yrs and at a median latency of 15 +/– 4 years after exposure (75% female, 31% Russian, 69% Ukranian). In the non-contaminated regions, the percentage and actual of number of cases within each phenotype did not vary with latency analysed as four discrete quartiles (Q1 - Q4: 4-19.5 years) since time of exposure. However, in the contaminated regions, the percentage of benign follicular lesions rose from 32% in Q1 to peak at 36% in the Q2 and then declined over time to 32% in Q3, and 27% in Q4 (p=0.007). The percentage of follicular thyroid cancers was lowest in Q2 (0.8%) and demonstrated a successive increase in Q3 (3.2%) and Q4 (5.8%: p=0.007). The percentage of MCT and PTC did not show significant variation over time in the same population. Although the main increase in thyroid tumours post Chernobyl has been of the papillary type, there is a possibility that a secondary rise in follicular cancer with a longer latency, perhaps related to the progression from adenoma to carcinoma, requires investigation. Longer-term studies will be required to determine the risk to the population of thyroid cancer post Chernobyl. Although the main increase in thyroid tumours post Chernobyl has been of the papillary type, there is a possibility that a secondary rise in follicular cancer with a longer latency, perhaps related to the progression from adenoma to carcinoma, requires investigation. Longer-term studies will be required to determine the risk to the population of thyroid cancer post Chernobyl.

Thyroid cancer
Oral

CELL ADHESION PROTEINS EXPRESSION IN PAPILLARY THYROID CANCER

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Introduction: Many cell adhesion molecules are up-regulated in papillary thyroid cancer (PTC). In our previous microarray studies we have specified several of them (Jarzab et al. 2005), with strong diagnostic accuracy of galectin-3. The aim of the present study is to verify the differences in expression cell adhesion proteins panel by tissue microarray approach. ***Material and Methods:*** Tissue microarray paraffin block with 101 spots (0.6 mm) of PTC and surrounding normal thyroid tissue (ST) was prepared from 23 patients diagnosed and operated with PTC. EnVision (biotin-free) immunochemistry was applied. 10 spots were not evaluated because of the connective tissue content, the remaining were scored for expression of galectin-3 (LGAL3), MUC1, cadherin P (CDH3), CD44v6 and carbohydrate antigen CA50. Also, the presence of endogenous biotin was visualised by LSAB+ method. Heterogeneity of reaction intensity was evaluated for all antigens. ***Results:*** The significant difference between PTC and ST was noted for LGAL3 (100%, intensive reactions in PTC versus 17%, weak in ST), CA50 (96.4% versus 37.9%), less for CD44v6 (100% versus 69.2%, weak) while CDH3 and MUC1 showed only weak or no difference. High amounts of endogenous biotin were revealed in PTC by labelled streptavidin-biotin without primary antibody. ***Conclusions:*** Our tissue microarray study does not confirm differences in protein expression of MUC1 and cadherin P on the level of protein, suggested by the clear differences seen on RNA level by DNA microarray. Simultaneously, it indicates on high expression of carbohydrate CA50 antigen and on the high content of endogenous biotin in PTC cells, in addition to the well known galectin-3 overexpression.

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Thyroid cancer
Oral

EXPRESSION OF HYPOXIA-RELATED GENES IN PAPILLARY THYROID CANCER

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Purpose: Hypoxia is a well known feature of malignant tumors, decreasing their response to radiation therapy. Until now it has not been widely studied in thyroid cancer. In the present study we analyzed the changes in expression profile of genes found to respond to hypoxia in our previous microarray-based studies of malignancy. Among them were genes participating in angiogenesis (VEGF, ADM, SPARC), apoptosis (BNIP3, , PBEF1, IER3, ANXA2, indirectly also MAP3K1), cell adhesion and migration (LGAL3, VCL, CTSB), glucose metabolism (GLUT1, HK2, ALDOC) and some others, in total 28 transcripts. ***Material and Methods:*** The analysis was carried out in 31 papillary thyroid cancers (PTCs) taken intraoperatively and respective normal surrounding thyroid tissue (ST). QPCR was carried out by Universal Probe Library probes on ABI 7900HT. Gene expression values were normalized against three-gene index, selected form 10 house-keeping transcripts by GeNorm software. ***Results:*** For 17/28 related gene the difference in gene expression between tumor and ST was significant, for 13/28 genes at $p<0.001$. All of them but VEGF showed higher expression in PTC than in ST. The highest difference in expression (on average 8.2x) was seen for LGAL3 and KDELR3 (4.4x). The overexpression was particularly visible for hypoxia-related cell adhesion and migration genes, proline hydroxylases and lysosomal degradation related genes. Contrary to our expectations, VEGF gene showed no distinct up-regulation, but other pro-angiogenic factors like adrenomedullin (ADM) could exert similar effect. Genes controlling glycolysis were inconsistently up-regulated - the overexpression of HK2 was not accompanied by GLUT1 expression change. ***Conclusions:*** Hypoxia-related cell adhesion and migration genes are up-regulated in PTC which contributes to its metastatic potential. Simultaneously, changes in apoptosis related genes indicate on its reduced apoptotic potential.

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Thyroid cancer
Oral

ROLES OF MAST CELLS AND THEIR MEDIATORS IN HUMAN THYROID CARCINOMAS

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There is compelling evidence that chronic inflammation may facilitate tumor initiation and progression. Mast cells play a central role in the regulation of allergic reactions, T-cell mediated immunity and the regulation of immune tolerance. Mast cell density is increased in a variety of human tumors where they play a role in tumor angiogenesis and lymphangiogenesis. We have investigated the possible role(s) of mast cells and their mediators in the development of human thyroid carcinoma (TC). To this end, we evaluated the density of mast cells (tryptase+ cells) in 124 thyroid carcinomas compared to normal thyroid tissues (n=14), goiters (n=10), and adenomas (n=5). We found that mast cell density was increased in 95% of papillaty TC (n=97), 90% of poorly differentiated TC (n=20), and 71% of anaplastic TC (n=7). Mast cell presence at the invasive front was correlated with capsule invasion (p=0.02) and loss of differentiation of the cancer (p=0.03). Conditioned media obtained from thyroid cancer cells induced mast cells chemotaxis in vitro. This effect was mainly mediated by vascular endothelial growth factor (VEGF-A) produced by cancer cells. VEGF-A (30–300ng/ml) was chemotactic for human mast cells in vitro. Co-injection of a human mast cell line (HMC-1) accelerated xenografts of human thyroid cancer cells (NPA87) in athymic mice. This effect was mediated by increased cancer cell proliferation, as shown by Ki67 staining. Interestingly, the pro-tumorigenic effect of mast cells was not associated with an increase in vessel numbers. Conditioned media from two mast cells lines (HMC-1 and LAD2) induced thyroid cancer cell proliferation and survival in vitro. The latter effects were mainly mediated by the cooperation of three mast cell-derived mediators, histamine and two chemokines (CXCL1/GROα and CXCL10/IP10). Our results demonstrate that mast cell density is increased in different human thyroid carcinomas. In conclusion, mast cells recruited into tumor site by VEGF-A can locally stimulate thyroid cancer cell proliferation.

Thyroid cancer
Oral

TCF/LEF FACTORS IN THYROID CELLS: INDICATIVE FOR A CONSERVED REGULATORY Wnt SIGNATURE REMINISCENT OF STEM CELL NICHES AND COLON CANCER CELLS

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Canonical Wnt activity can balance proliferation over differentiation through eliciting transcriptional responses via activating or inhibiting members of the TCF/LEF family. The balance of TCF isoforms may determine the cellular fate and may thus be altered in cancerous cells as compared to normal tissue. We asked whether they are involved in the phenotype of thyroid cancer cells which may be viewed as blocked differentiating thyroid progenitor cells. Model thyroid cancer cell lines were assayed by qPCR, proteins were stably transfected and proteins were subjected to SDS-PAGE. Relative expression of TCF isoforms varied considerably throughout the panel of studied thyroid cancer types with very high expression levels of single isoforms varying between the cell lines. This is exemplified by exceptionally high expression levels of the repressive TCF-1 in the anaplastic cell line. It bears APC mutations leading to overactivity of canoncial Wnt signalling. As they express surprisingly low levels of the Wnt target genes, cyclin D1 and surviving, overexpression of the repressive TCF1 may counteract canonical Wnt signalling in these cells and may explain the phenotype. In contrast, grossly increased TCF-3 over TCF-1 levels was characteristic for a follicular cancer cell line known to lack PTEN. After re-introduction of wild-type or mutant PTEN, TCF-3 levels decreased significantly in parallel to a marked decrease in proliferation rate. This seems not to be directly mediated via AKT; indicative GSK3ß Ser 9 phosphorylation was not altered by the transgene. ***Conclusion:*** These data suggest that TCF isoforms are important regulators in the pathogenesis of thyroid cancers. Given the central role Wnt/TCF signalling in stem cell regulation, the results are reminiscent of a Wnt signature first observed in the adult colon stem cell niche and in cancers are likely to alter their phenotype derived from it due to their common embryological origin.

Thyroid cancer
Oral

GENE ALTERATIONS IN PAPILLARY THYROID CARCINOMAS IDENTIFIED BY ARRAY CGH

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RET/PTC rearrangements are frequently found in papillary thyroid carcinomas (PTC). Recent studies have shown that ret rearrangements show a heterogeneous distribution within individual tumours, suggesting that multiple genetic alterations may be present within the same tumours. We have investigated 25 PTCs (12 PTCs from adults and 13 childhood post Chernobyl PTCs) with known RET/PTC status using 1Mb BAC array CGH. Array CGH showed DNA gains and losses whilst hierarchical cluster analysis revealed distinct groupings of tumours, one of which showed frequent genetic gains on 19 and 21 or losses on 1, 6, 9, 13, 20. In contrast the 6 RET/PTC negative cases from adults differed significantly from the other cases, harbouring more frequent losses of regions on chromosomes 7q and 22. Statistical analysis (maximum permutation t test) revealed significant differences between adult RET/PTC+ and infantile RET/PTC+ cases on chromosome 1p and between adult RET/PTC+ and RET/PTC- negative cases on chromosomes 1p, 3q, 7p, 4p, 9p, 9q, 10q, 12q, 13q and 21q. Tumour-related candidate genes within these distinctive regions are JAK1, RAB3B (1p), BCHE (3q), RBAK (7p), TXK, RHOH (4p), JAK2 (9p), DEC1, DBC1 (9q), FAS, PTEN (10q), TCF1 (12q), SLITRK1-3 (13q) and ERG (21q). Losses on chromosome 1p appear to be associated with adult PTCs whilst losses on chromosome 1q are specific for adult RET/PTC+ cases. Gain on chromosome 19 is specific for RET/PTC+ cases, losses on chromosome 19 are specific for adult RET/PTC- cases. Deletion of chromosome 13 was found only in RET/PTC+ childhood cases. FISH analysis for specific chromosomal areas has confirmed the BAC array findings. Further studies will be required to determine which of these genes cooperate with ret rearrangement to stimulate growth and which provide an alternative route to tumour growth.

Thyroid molecular and cellular biology
Oral

THE HINGE REGION OF THE TSHR – IMPORTANCE FOR TSH BINDING AND RECEPTOR ACTIVATION

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The hinge region links the extracellular hormone binding domain of the TSHR with the transmembrane domain. There is a lack of structural and functional data for this region but an involvement in receptor signaling is assumed. In vitro data provide evidence for a functional importance of the hinge region for sulfation, TSH binding and receptor activation. We recently identified residues $P_{400}D_{403}EFNP_{407}$ and K_{291} flanking the hinge region C-terminally and N-terminally, respectively, as extracellular functional switches for constitutive activity. To investigate further potential determinants for receptor activation in this region we selected conserved, hydrophilic amino acids for single alanine substitutions. All TSHR mutants were characterized by measuring cell surface expression, TSH binding, and cAMP accumulation. Most of the mutants showed a cell surface expression comparable with the wt TSHR, but a reduced TSH stimulated cAMP response. Although mutations $E_{297}A$, $E_{303}A$, and $D_{382}A$ were well expressed compared to the wt, evaluation of the specific Bmax values revealed a strong decrease in TSH binding. Using further substitutions we investigated whether the negative charge at these three positions is necessary for hormone binding. $E_{297}Q$ and $D_{382}N$ also showed a decreased hormone binding capability, whereas $E_{303}Q$ did not. Our data indicate that (i) several mainly charged amino acids of the hinge region are important for intramolecular signal transmission and (ii) apart from amino acids of the LRR also positions E_{297} and E_{303} in the N-terminal, and D_{382} in the C-terminal portion of the hinge region are most likely involved in hormone binding. Moreover, we show, that the negative charge at E_{297} and D_{382} is necessary for hormone binding, in contrast to position E_{303}.

Thyroid molecular and cellular biology
Oral

COMPARISON OF TSH AND FSH BINDING TO THEIR RESPECTIVE RECEPTORS AT MOLECULAR LEVEL

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TSH, FSH, LH and hCG have closely related structures as do their respective receptors (R). However, cross-reactivity is weak if it occurs at all, and we now describe a study of the residues involved in TSH-TSHR and FSH-FSHR interactions using the new crystal structure (2.55Å resolution) of the TSHR (amino acids 1-260) and the crystal structure (2.9Å resolution) of FSH in complex with the FSHR (amino acids 1-268). These two structures were used to construct a new comparative model of TSH bound to the TSHR. The interactions between TSH and the TSHR in the complex were then optimised. Hydrogen bonds, van der Waals interactions, electrostatic interactions between charged atoms and shape complementarity were studied in the TSH-TSHR and FSH-FSHR complexes and compared. A number of equivalent residues in the TSHR and FSHR produced similar interactions with the α chains of their respective hormones. Several TSHR and FSHR residues were identified as candidates for determining binding specificity with the β chains of the hormones and the buried ion pair between FSHβ Arg97 and FSHR Glu76 appears to stabilise the interface in the FSH-FSHR complex but is absent from the TSH-TSHR complex. The molecular basis of higher affinity binding of FSH for the FSHR compared to TSH-TSHR binding was investigated and whereas FSHR Lys179 is involved in attractive electrostatic interaction with FSHβ Asp90 and forms a hydrogen bond with FSHβ Ser89 the equivalent TSHR Asn186 is not involved in strong interactions with TSHβ. Overall, our studies allow an understanding of glycoprotein hormone specificity at the molecular level.

Thyroid molecular and cellular biology
Oral

TSH-MEDIATED EXPRESSION OF S100 Ca2+ BINDING PROTEINS AFFECTS CALCIUM-DEPENDENT SIGNAL TRANSDUCTION IN THYROID CELLS

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Recently, we reported that in FRTL-5 cells S100 calcium binding protein mRNAs are differentially regulated depending on stimulation with TSH or activating TSHR mutations. We found a strong correlation between S100 mRNA expression and a modulation of intracellular calcium signals evoked by endogenous P2Y receptor stimulation. To support the physiological relevance of our findings, we examined the calcium response characteristics and S100 mRNA and protein expression of human primary thyrocytes. Similarly to FRTL-5 cells, we found a TSH-dependent regulation of S100 mRNAs and also protein, e.g. S100A4. Primary human thyrocytes also showed a slightly decreased calcium release which correlates with S100 expression levels. To demonstrate that the calcium signals are sensitive to S100 expression, we cloned and overexpressed several S100 proteins in FRTL-5 and HEK cells and examined calcium response characteristics. We used a reporter construct which expresses DsRedExpress, a red fluorescent protein, under control of the IRES sequence. This allowed expression of the respective S100 protein and the reporter fluorescent protein in a fixed relation of about 1:1 and thereby a direct readout of the degree of S100 overexpression simultaneously to calcium response measurements. With this method we found that e.g. S100A6 decreases the calcium response occurring after ATP stimulation of endogenous P2Y receptors and increases the calcium signals elicited by repeated stimulations. It is very likely that these findings reflect the ability of S100 proteins to bind calcium and act as calcium buffering mechanism, suggesting that S100 proteins may modulate cell signalling pathways not only through protein-protein interactions, but also act as a general modulator of thyroid calcium signalling.

Thyroid molecular and cellular biology
Oral

TEMPORAL GENE EXPRESSION PROFILES DURING EXPERIMENTAL INDUCTION OF GOITER IN A NON-MAMMALIAN MODEL ORGANISM, *XENOPUS LAEVIS*

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To date, information on molecular mechanisms involved in thyroid gland function is almost completely lacking for non-mammalian vertebrates. In this study, we used the model of experimental goiter induction in *X. laevis* tadpoles to gain first insight in gene expression changes in thyroid tissue during TSH overstimulation in vivo. Tadpoles were treated for 12 days with 50 mg/L ethylenethiourea (ETU) or 20 mg/L sodium perchlorate (PER) to block thyroid hormone (TH) synthesis and thyroid tissue was sampled on treatment days 1, 3, 5, 8, and 12. Thyroid histopathology revealed colloid resorption, follicular cell hypertrophy/hyperplasia and goiter formation due to ETU and PER treatment. Using real-time PCR, we then analyzed thyroid tissue samples for temporal expression profiles of candidate genes related to TH synthesis, protein synthesis/modification/trafficking, and cell proliferation. Compared to the control group, goitrogen treatment rapidly resulted in strong upregulation of known TSH-responsive genes such as NIS and TPO. Similarly, expression of genes involved in mRNA translation and endoplasmatic reticulum stress responses was rapidly increased, whereas genes related to protein trafficking displayed a delayed upregulation. Several cell cycle genes were upregulated at early time points but showed depressed expression levels at late time points. Notably, among the genes showing differential responses to ETU versus PER treatment, first candidates were identified that could aid in the distinction of the mode of goitrogen action on the thyroid gland. This study shows that *X. laevis* offers promise as a novel model organism for the development of a non-mammalian model system to study molecular mechanisms of vertebrate thyroid function.

Thyroid molecular and cellular biology
Oral

THE INACTIVATION OF THE CONNEXIN32 Gene Causes A Reduction Of The Oncogenic POTENTIAL OF RET/PTC3 AND E7 In Mice

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Connexins (Cx), by themselves or through the formation of gap junctions, play regulatory roles on cell growth and differentiation. It was previously reported that the deletion of Cx32 (one of the Cx expressed in the thyroid) confers a growth advantage to thyroid cells subjected to TSH stimulation. In the present study, we examined the impact of the inactivation or the over-expression of the Cx32 gene on thyroid growth induced by two oncogenes, RET/PTC3 and E7 of HPV16. Transgenic mice overexpressing Cx32 exclusively in the thyroid (Cx32-T+) and Cx32 knock-out mice (Cx32-KO) were crossed with transgenic mice developping thyroid hyperplasia in response to RET/PTC3 (RC3) or E7 expression. The thyroid phenotype of the progeny was studied at 8 to 21 weeks of age. At the age of 12 weeks, thyroid of RC3 and E7 mice was, respectively, 8- and 20-fold larger than that of wild type littermates. Both RC3 and E7 mice had normal or slightly elevated serum T4 concentration. As found previouly, thyroid of Cx32-KO and Cx32-T+ mice was, respectively, comparable to and about 20% smaller than that of wild type mice. Cx32-T+/RC3 and Cx32-T+/E7 mice exhibited the same thyroid hyperplasia as RC3 and E7 mice, respectively. Unexpectedly, thyroid of Cx32-KO/RC3 and Cx32-KO/E7 mice was reduced by about 50% as compared to that of RC3 and E7 mice. Thus, thyroid hyperplasia induced by RET/PTC3 or E7 was not affected by the overexpression of Cx32 but markedly reduced in the absence of Cx32. In conclusion, Cx32, which exerts a negative control on thyroid growth stimulated by TSH and the cAMP cascade, behaves as a positive effector of thyroid growth triggered by oncogenes which act through other signalling cascades. This dual thyroid growth control by Cx32 could reflect the coexistence of two separate modes of mitogenic activation in thyrocytes.

Thyroid molecular and cellular biology
Oral

THE HIGH BASAL ACTIVITY OF THE TSHR INCREASES ITS SUSCEPTIBILITY FOR CONSTITUTIVELY ACTIVATING MUTATIONS

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The aim of the present study was to evaluate whether there is a relation between the high basal TSH receptor (TSHR) activity and the high number of pathogenic constitutively activating mutations (CAMs). Evaluation of the Sequence-Structure-Function Analysis resource [1] (www.fmp-berlin.de/ssfa), which is based on 900 published mutant phenotypes of glycoprotein hormone receptors, revealed several mutants that express decreased basal TSHR activity (constitutively inactivating mutations (CIM)). We designed double mutants combining a CIM (M572A) located in the extracellular loop 2 (ECL2) with several CAMs in different receptor regions such as in the ectodomain (S281F), in ECL1 (I486M), in transmembrane helix 3 (TMH3) (L512Q), and in TMH6 (A623V). All double mutants showed either an abolished or decreased constitutive activity (reduced to up to 50%) compared to the corresponding single CAMs. This finding indicates that the occurrence and efficacy of CAMs are strongly dependent on the basal activity of the TSHR. For the first time we provide evidence for inverse agonism of a TSHR mutation that decreases both wt-TSHR basal activity and the activity of CAMs. Furthermore, our findings suggest that the basal TSHR conformation is a trigger for constitutive activation. This is supported by the fact that compared with the homologous glycoprotein hormone receptors LHCGR and FSHR, the TSHR expresses the highest level of basal activity and is at the same time characterized by the highest number of CAMs. Taken together, the high basal TSHR activity is a prerequisite for constitutive receptor activation and might explain its susceptibility for CAMs.

[1] Kleinau G, Brehm M, Wiedemann U, Labudde D, Leser U, Krause G: Implications for molecular mechanisms of glycoprotein hormone receptors using a new sequence-structure-function analysis resource. Mol Endocrinology, 2007;21(2):574-580. Epub 2006 Nov 16.

Thyroid molecular and cellular biology
Oral

THE SYNERGISTIC CONFORMATIONAL EFFECTS OF ALL 3 EXTRACELLULAR LOOPS ARE REQUIRED FOR FULL TSH-RECEPTOR ACTIVATION

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The aim of the present study was to evaluate further pieces of the molecular puzzle to understand the extracellular activation mechanism of the thyroid stimulating hormone receptor (TSHR). Since several constitutive active mutations (CAMs) are located in all three extracellular loops (ECLs 1-3), it is very likely that the TSHR conformation is constrained at distinct but diverse locations in the extracellular region. We hypothesized that for full receptor activation upon extracellular TSH binding the TSHR transfers its molecular activation via multiple extracellular signal initiations. Therefore, we combined known CAMs in all three ECLs (ECL1: I486A, ECL2: I568V, ECL3: V656F) in double and triple mutants to test whether additive effects on increased basal cAMP activity exist. Indeed, the level of basal signaling is significantly increased in all multiple substituted receptors compared to the single CAMs. Surprisingly, the basal activity of mutant combinations of ECL1/ECL2 and ECL1/ECL3 is not only additive, but also synergistic. Moreover, the triple mutant I486A/I568V/V656F, comprising CAMs of all three ECLs, leads even to a 55-fold increase of ligand independent activation compared to basal activity of wt-TSHR. We conclude from our findings that destabilization of the wt-TSHR and/or stabilization of a receptor conformation towards the fully activated state requires multiple signal initiations that are transferred via all three extracellular loops. These events result in multiple conformational shifts with synergistic effects and very likely represent multiple regions for extracellular signal propagation towards the transmembrane region for full receptor activation.

Thyroid molecular and cellular biology
Oral

FETAL CELL MICROCHIMERISM IN PAPILLARY THYROID CANCER

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Fetal cells enter the maternal circulation during pregnancy and can persist in the maternal blood or tissues for decades, creating a physiological microchimerism. Fetal microchimeric cells have been documented in women with autoimmune and non-autoimmune diseases. Aim of the present study was to investigate the possible association between fetal cell microchimerism (FCM) and papillary thyroid cancer (PTC). An ELISA-PCR for the sex-determining region on the Y chromosome, has been used to detect fetal male cells in PTC samples. Results were always confirmed by fluorescence in situ hybridisation (FISH) with X and Y chromosomes-targeted probes. Thyroid tumor tissues were obtained from women who had at least a male child before the diagnosis of PTC (group 1, n=41) and from women with female offspring or nulliparous, with no history of abortion, transfusion or organ transplant (group 2, n=21). The latter group was used as negative control, while the DNAs obtained from the tumoral or normal tissue of 6 men were used as positive controls. FCM was demonstrated in 7/41 women of group 1 and in none of group 2. The normal thyroid tissue controlateral to the 7 PTCs with FCM, was always negative. Interestingly, in patients positive for FCM the delivery of a male neonate occurred even many years (range 7–40) before thyroidectomy. No correlation between the presence of FCM and the tumor staging at diagnosis or the outcome was found. In conclusion, the presence of fetal cell microchimerism, deriving from fetomaternal cell trafficking, has been demonstrated for the first time in PTC. Fetal precursor cells might play a role in carcinogenesis, inducing alterations of the immune system, or they might differentiate to repair the affected tissue, as a response to carcinogenesis. To clarify this issue immuno-FISH analyses are in progress to identify if microchimeric cells have a hematopoietic origin or whether they have differentiated into thyroid tissue.

Thyroid molecular and cellular biology
Oral

GHRELIN INHIBITS in vitro THYROID CELL FUNCTION

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Ghrelin is a bioactive peptide from the upper gastrointestinal tract, which is secreted during fasting and especially immediately before a meal. The most recognized action of ghrelin is to increase appetite through stimulation of Agouti related peptide (AgRP) and neuropeptide Y (NPY) neurons in the arcuate nucleus of the hypothalamus. However, ghrelin has a pronounced effect on body weight, which cannot be explained by its effect on food intake. Thus, for example the adipocyte appears to be another important target for ghrelin as it decreases lipolysis either through a direct effect on the adipocytes or via the effect of ghrelin on the autonomous nervous system. Still, the effect of ghrelin on lean body mass cannot be explained through these mechanisms. Since the ghrelin receptor is in fact highly expressed in the thyroid gland we hypothesise that ghrelin could be a physiological co-regulator of thyroid function. Administration of ghrelin (10^{-7} M) to monolayer cultured human thyroid cells isolated from para-nodular tissue decreased TSH stimulated thyroglobulin release by 77% ±18. (Ghrelin treated cells 351 (2,021–3,627) µg/L, median (range); control cells 3,455 (1,135–5,890) µg/L, n=5). Ghrelin also decreased TSH stimulated cAMP release from the primary thyroid cell cultures. (Ghrelin treated cells 185 (124–258) nmol/L; control cells 213 (133–293) nmol/L, n=5). RT-PCR experiments showed that ghrelin also decreased the mRNA expression of thyroglobulin, the TSH receptor, the sodium-iodide symporter and thyroid peroxidase in these cells. Ghrelin had no influence on interleukin-6 expression in the cells. It is concluded that these studies add the thyroid gland as a potential target through which ghrelin may act to preserve energy by a decrease in thyroid function during starvation.

Thyroid molecular and cellular biology
Oral

THE FORKHEAD BOX TRANSCRIPTION FACTOR FOXA2 IS EXCLUDED FROM THE MEDIAN THYROID ANLAGE AND THE ULTIMOBRANCHIAL BODIES IN THE ANTERIOR ENDODERM BUT IS AN EARLY MARKER OF C-CELL PROGENITORS

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We recently found that deletion of Sonic hedgehog (Shh) or the T-box transcription factor Tbx1 causes a similar thyroid phenotype, mimicing thyroid hemiagenesis. In view of the fact that Shh is required for Foxa2 expression in the pharyngeal endoderm and Foxa2 is necessary for Tbx1 transcription activity in the same tissue, this raises the possibility that Foxa2 might work as a signaling intermediate in thyroid organogenesis. Conditional knock-out studies suggest that Foxa2 exerts distinct developmental effects in the definitive foregut endoderm that at least partly appear to be cell lineage specific. A previous in situ hybridization study has suggested that hepatocyte nuclear factor-3beta (HGF-3β, the former name of Foxa2) mRNA is transiently expressed, approximately between E10-14, in the developing mouse thyroid. However, in this early work the developing thyroid was not clearly identified by thyroid markers. As a first step to elucidate a potential role of Foxa2 protein expression in thyroid development, we examined in mouse embryos by confocal immunofluorescence microscopy the distribution pattern of Foxa2 in the midline thyroid primordium and ultimobranchial bodies. From E9 and onwards these structures gradually develop to the complete formation of the gland. Surprisingly, we find that Foxa2 is specifically excluded from the midline thyroid anlage and the ultimobranchial bodies as they start to bud, but is present in the neighbouring endoderm. However, later on Foxa2 expression identifies an expanding subpopulation of presumptive C-cell precursors within the early ultimobranchial epithelium, which maintains Foxa2 expression during late development and adulthood when they differentiate into calcitonin-producing C-cells. Our current work aims at elucidating whether repression of Foxa2 in the thyroid placode is of functional importance for early steps of thyroid development. Our results thus revise the current view of Foxa2 and thyroid development and identifies Foxa2 as an early molecular marker of the C-cell lineage in the thyroid.

Thyroid hormone metabolism and action
Oral

WHAT IS THE ROLE OF Pit1 IN THE NEGATIVE REGULATION OF TSHβ GENE BY T3 AND ITS RECEPTORS?

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Although the negative regulation of TSHβ by T3 and its receptors (TR) is most important to the thyroid hormone homeostasis, its molecular mechanism has been unknown. Previously we have clarified (1) TSHβ gene is activated in CV1 cells by expressing Pit1 and GATA2 and suppressed by T3/TR, (2) unliganded TR cannot activate the TSHβ gene without Pit1 and GATA2, (3) although the intact DNA binding domain is essential, TR binding is unnecessary, (4) when the sequence downstream to the GATA binding sites was deleted, GATA2 alone could activate the reporter gene, suggesting that GATA2 is an activator of TSHβ gene, (5) T3-dependent recruitment of TRAP220 to TR is important to mediate the suppression (Biochem J 2004, Mol Endocrinol 2007). GATA2 is an activator of the TSHβ gene, then what is Pit1 doing? We studied the Pit1's function in T3/TR-mediated inhibition of the TSHβ gene. When we deleted the sequence between –82 and –52, the basal activity of TSHβ promoter enhanced dramatically, suggesting the existence of suppressor(s) against GATA2. We designated this sequence as a suppressor region (SR). The suppressive effect of SR was independent on its orientation and the distance from TATA box. Pit1 interacted with the Zn finger domain of GATA2 and counteracted SR-inhibitory effects on GATA2. Pit1 overexpressed also crippled the T3/TR-mediated inhibition of TSHβ promoter. Mutant Pit1s identified in patients with combined pituitary hormone deficiency did not work. Cooperative function of Pit1/GATA2 was strictly determined by the positions of their binding sites in the TSHβ promoter. Finally, minimal sequence for SR was mapped to the 9 bp-sequence of -82/-73. In conclusion, Pit1 stabilizes the function of GATA2 to activate the TSHβ gene by protecting it from the SR- and T3/TR-meadiated inhibitions.

Thyroid hormone metabolism and action
Oral

MICE DEFICIENT IN THE THYROID HORMONE TRANSPORTER MCT8 Exhibit Tissue-Specific Abnormalities In Thyroid Hormone Metabolism

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Purpose: Inactivating mutations in the gene coding for the thyroid hormone transporter MCT8 are associated with a severe form of psychomotor retardation and imbalanced thyroid hormone serum levels. In order to elucidate the pathophysiological mechanisms of the syndrome we determined the tissue-specific consequences of MCT8 deficiency by analyzing thyroid hormone metabolism in brain, liver and kidney of MCT8 null mice. ***Methods:*** For that purpose, serum and tissue T3 and T4 levels, iodothyronine deiodinase actvities and expression levels of T3-regulated gene products were determined. Uptake of radiolabeled T3 and T4 into the brain, liver and kidney were assessed. ***Results:*** In line with the clinical observations, MCT8 deficiency in mice leads to highly increased serum T3 and decreased serum T4 levels. Analysis of T3 and T4 tissue content and determination of deiodinase activities in the brain revealed a hypothyroid situation whereas the liver and kidney of MCT8 null mice were found to be in a rather hyperthyroid state. Most strikingly, uptake of T3 but not of T4 into the brain was diminished in MCT8 null mice indicating a pivotal role of MCT8 in facilitating the entry of T3 into the CNS. In contrast, hepatic uptake of T3 and T4 was not affected by the absence of MCT8 most likely due to the presence of other thyroid hormone transporters in the liver. Surprisingly, transport of T3 and T4 into the kidney of MCT8 null mice was elevated suggesting that MCT8 might also contribute to the efflux of thyroid hormones under normal conditions. In summary, absence of MCT8 leads not only to abnormal thyroid hormone levels in the circulation but also results in tissue-specific impairment of thyroid hormone transport processes.

Thyroid hormone metabolism and action
Oral

EXPRESSION OF DEIODINASES D1, D2 AND D3 mRNA IN HEARTS OF AMIODARONE AND DRONEDARONE TREATED RATS

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Background: Deiodinases are important in the regulation of the intracellular concentration of thyroid hormone (T3). It is know that the potent antiarrhythmic drug amiodarone (AM), has an inhibitory effect on the binding of T3 to the TRα1 and TRβ1 whereas dronedarone (Dron) only has an inhibitory effect on TRα1. In this study we investigate the effect of AM and Dron on D1, D2 and D3 mRNA expression in the heart. ***Objective and Methods:*** Three groups of male rats received either water, Dron or AM orally daily for two weeks. Plasma T3, T4 and TSH were measured. In the right atrium (RA) of the heart mRNA concentrations were measured using real time PCR; values are corrected for GAPDH. Results are expressed as mean ± sd, differences between groups were evaluated using t test (*p<0.01;**p<0.05;***p=0.05). ***Results:*** In AM treated rats T3 was lower (C 1.21±0.13; Dron 1.09±0.19; AM 0.78±0.11* nmol/L), and T4 (C 72±10; Dron 68±14; AM 150±21* nmol/L) and TSH (C 2.11±0.80; Dron 1.45±0.75; AM 6.70±3.39* ng/ml) were higher compared to control rats. D1 mRNA was not detectable in RA of the heart in all groups. D2 mRNA in RA expression did not change after AM or Dron treatment. However D3 mRNA expression in RA of the heart was lower in both Dron and AM treated rats compared to controls (C 1.94±0.31; Dron 1.29±0.27**; AM 0.99±0.41*** arbitrary units, Dron vs AM NS). ***Conclusion:*** Dronedarone and amiodarone treatment in rats resulted in a decrease of D3 mRNA in right atrium of the heart. It is hypothesized that the fall in D3 mRNA (and thereby less inactivation of thyroid hormone) serves to counteract the local hypothyroid-condition in the heart induced by amiodarone and dronedarone.

Thyroid hormone metabolism and action
Oral

MECHANISMS INVOLVED IN LOSS OF FUNCTION CAUSED BY MUTATIONS IN THYROID HORMONE TRANSPORTER MCT8

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Background: Loss of function mutations in MCT8 lead to severe psychomotor retardation and elevated serum T3 levels in affected males. Phenotypical characteristics correlate with genotype: patients with mutations L434W, L568P and S194F walk independently or develop some rudimental speech; milestones that patients with other mutations do not reach. Residual thyroid hormone transport might contribute to this genotype–phenotype correlation. We aimed to determine the mechanisms involved in (incomplete) loss of MCT8 function. ***Methods:*** Expression, location and function of MCT8 mutants V235M, L434W, S448X, L568P, S194F, insI189 and delF230 were investigated by QT-PCR, immunoblotting, BrAcT3 affinity-labelling, immunocytochemistry and uptake and metabolism of T3 and T4 in transfected JEG3 and COS1 cells. ***Results:*** Thyroid hormone uptake and metabolism studies showed complete loss of function for most MCT8 mutants. S194F and L568P showed ~25%, and L434W ~35% of wild-type MCT8 activity in vitro. QT-PCR did not show significant differences in mRNA expression between WT and mutant MCT8. Whereas non-functional mutants V235M, insI189 and delF230 are located mostly in the cytoplasm, mutants with residual function are expressed at the plasma membrane. S194F and L434W show high protein expression, but low affinity for BrAcT3; L568P shows low expression, but relatively high affinity. ***Discussion:*** Mechanisms involved in the loss of function caused by mutations in MCT8 include reduced protein expression, impaired trafficking to the plasma membrane and reduced substrate affinity. We demonstrate that mutants L434W, L568P and S194F have significant residual transport capacity. This might contribute to more advanced psychomotor development observed in patients with these mutations.

Thyroid hormone metabolism and action
Oral

THE INACTIVATION OF THE THYROID HORMONE RECEPTOR β IN THE THYROID LEADS TO A NEW FUNCTIONAL STEADY-STATE OF THE GLAND PROBABLY DEPENDENT ON THE DOWN-REGULATED EXPRESSION OF IODOTHYRONINE DEIODINASES

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Thyroid epithelial cells express functional thyroid hormone receptors (TR) but no precise role has been assigned to either TRα or TRβ in the thyroid gland. In this study, we analyzed the impact of the inactivation of the TRβ gene in the mouse thyroid. Mice expressing the Cre recombinase selectively in the thyroid, named Thyr-Cre (obtained by targeting a single copy of a Tg promoter-Cre construct to the HPRT locus) were crossed with mice carrying floxed TRβ alleles. Molecular analyses showed that floxed TRβ allele(s) underwent a complete recombination in the thyroid of mice expressing the Cre transgene. Mice with the thyroid-targeted inactivation of TRβ (Thyr-TRβ-/-) were characterized by a 30% decrease in thyroid weight as compared to wild type littermates. Morphological examinations of the thyroid of Thyr-TRβ-/- mice did not evidence any alteration of the structure or size of thyroid follicles. The decrease in thyroid weight was associated with a decrease in serum TSH concentration and a small, not statistically significant, increase in serum T4 concentration. Serum T3 was unchanged. The functional activity of the thyroid assessed by measurements of the expression level of thyroid-specific genes appeared reduced in Thyr-TRβ-/- mice. NIS, TPO, DIO1 and DIO 2 mRNA levels were 2–3 times lower in Thyr-TRβ-/- than in control mice. In conclusion, mice with a thyroid-targeted inactivation of TRβ exhibit an original clinical picture associating signs of hyperthyroidism and a reduction of the size and activity of the gland. The maintenance of a normal or slightly elevated T4 production, despite a thyroid hypoplasia and a reduction of functional activity, would result from a decrease in intrathyroidal deiodination of T4 linked to the down-regulated expression of iodothyronine deiodinases.

Thyroid hormone metabolism and action
Oral

hOATP1B1: AN IMPORTANT FACTOR IN HEPATIC THYROID HORMONE TRANSPORT AND METABOLISM

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Introduction: Organic anion transporting polypeptides (OATPs) facilitate cellular uptake of many ligands. Human (h) OATP1B1, expressed in hepatocytes, is reportedly capable of T4 and T3 transport. Recent studies have shown that polymorphisms in hOATP1B1 have an effect on the pharmacokinetics of various drugs. Therefore, we analyzed if hOATP1B1 indeed facilitates thyroid hormone (TH) transport and metabolism, and if the hOATP1B1-Val174Ala polymorphism causes changes in TH transport and serum TH parameters. ***Methods:*** Iodothyronine uptake and metabolism was studied in cells transiently transfected with either hOATP1B1-Val^{174} or -Ala^{174} together with CRYM, an intracellular TH-binding protein, or with the iodothyronine deiodinase D1. Furthermore, 155 healthy blood donors were genotyped for the OATP1B1-Val174Ala polymorphism and related to serum TH parameters. ***Results:*** Transfection of COS1 cells with hOATP1B1 strongly induced uptake of rT3 and, in particular, T4 sulfate (T4S), but not of T3 or T4. hOATP1B1 also produced large increases in intracellular rT3 and T4S metabolism by D1. Induction of rT3 and T4S uptake and metabolism was ~30% lower for the Ala^{174} than for the Val^{174} variant.The in vitro data were supported by in vivo association analysis: carriers of the Ala^{174} allele had 13% higher rT3 levels ($p=0.009$), 11% lower T3/rT3 ratio ($p=0.03$). Furthermore, carriers had 25% higher T4S levels than non-carriers ($p<0.001$). ***Conclusion:*** These data suggest that hOATP1B1 strongly induces uptake and metabolism of rT3 and T4S, but not of T3 and T4. The hOATP1B1-Val174Ala polymorphism lowers rT3 and T4S clearance, resulting in an increase in serum rT3 and T4S. Together, these data suggest an important role for hOATP1B1 in hepatic transport and metabolism of TH.

Thyroid hormone metabolism and action
Oral

SPECIFIC INTERACTION OF IODOTHYRONAMINES WITH THE MCT8 THYROID HORMONE TRANSPORTER

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We previously identified MCT8 as an important thyroid hormone (TH) transporter, mutations of which cause severe psychomotor retardation. Recently, we found that human (h) MCT10, which has 49% amino acid identity with hMCT8, is also a very active TH transporter. In view of the brain-associated pathology in MCT8 patients, we investigated the possible competition for T3 transport by hMCT8 and hMCT10 using various neurotransmitters, aromatic amino acids, iodothyronines, and iodothyronamines such as 3-iodothyronamine (T1AM), a TH metabolite with neurotransmitter-like actions in brain. COS1 cells, transiently transfected with hMCT8 or hMCT10 were incubated for 5 min with 1 nM [125I]T3 without or with increasing concentrations of competitor. Competition for T3 uptake by hMCT8 decreased in the order: T3AM ~ T1AM ~ T3 > 3,5T2AM > 3,5-T2 >> 3-T1. In contrast to the high potency of T1AM, T1 was completely inactive. Competition for T3 uptake by hMCT10 decreased in the order T3 ~ 3,5T2 > T1 >> T3AM ~3,5T2AM ~ T1AM. No competition for T3 transport by hMCT8 or hMCT10 was found with up to 100 μM of neurotransmitters like dopamine, serotonin and GABA, and of the aromatic amino acids Phe, Tyr and Trp. We conclude that although hMCT8 and hMCT10 are closely related and both transport T3, T3 uptake by hMCT10 is predominantly inhibited by iodothyronines and hMCT8 by iodothyronamines, particularly T1AM. This interaction with T1AM is perhaps important for the pathogenesis of the psychomotor retardation in patients with MCT8 mutations.

Thyroid hormone metabolism and action
Oral

SCREENING POTENTIAL ENDOCRINE DISRUPTORS OF T3-DEPENDENT TRANSCRIPTIONAL REGULATION

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Endocrine disruptors (ED) are exogenous compounds of natural or synthetic origin which interfere with the endocrine system resulting in endocrine dysregulation. With respect to the thyroid hormone (TH) system ED might act via modes of action affecting TH biosynthesis, TH distribution, cellular uptake of TH, TH activation/inactivation, and target gene regulation by TH. A novel reporter gene assay was established in 96-well cell culture microplates as a tool to screen test substances for their ability to interfere with TH regulated gene transcription. This assay utilizes the human hepatocarcinoma cell line HepG2 transiently cotransfected with a T3-reporter construct and T3 receptor expression plasmids. This highly sensitive assay allows determination of TH concentrations as low as 10-10 M and is characterized by a wide dynamic range covering an up to 100-fold induction by 10-7 M T3 relative to solvent control. Using the synthetic T3 agonist GC-1 and the T3 antagonist NH-3, respectively, we validated the suitability of our assay for screening transcriptional effects of potential ED stimulating or inhibiting the TH system. Analysis of several ED test compounds of human relevance revealed significant effects on the T3-dependent reporter only at higher concentrations (1 micromolar or above). For example the UV-screen benzophenone-3 frequently used in commercial cosmetic products, acted as an agonist and induced reporter gene expression 2-fold at a concentration of 1 µM. In contrast 4-nonylphenol which is used in the production of detergents, plastics, tensides and softeners, exhibited mixed agonistic and antagonistic effects at this concentration. Whether these effects are of concern for human health or wildlife needs to be clarified by determination of in vivo concentrations of ED in relevant target tissues during development or in the adult organism.

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Thyroid autoimmunity
Oral

CRYSTAL STRUCTURE OF THE TSH Receptor Bound To A Thyroid Stimulating Autoantibody

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A stable complex of the TSH receptor (amino acids 1-260; TSHR260) bound to the thyroid stimulating human monoclonal autoantibody M22 was purified to homogeneity, deglycosylated, crystallised and the structure determined by X-ray diffraction (2.55Å resolution). TSHR260 formed a curved helical tube and M22 Fab clasped the receptor's concave surface at 90° to the tube length axis. The receptor-antibody interface buried in the complex was large (2500Å^2) with an extensive network of hydrogen bonds and salt bridges (n=22) as well as polar and hydrophobic bonding. Both the heavy and light chains of M22 contributed to the interaction with the receptor with 14 hydrogen bonds and salt bridges formed by the heavy chain and 7 by the light chain. There was essentially no movement of the atoms of M22 residues between receptor bound and unbound M22. Mutation of residues in the TSHR and/or in M22 which showed strong interactions in the complex had the expected effect on M22 receptor binding and stimulating activity. Consequently binding arrangements present in the crystal structure reflect interactions of M22 with full length functionally active TSHR. The positioning of M22 on the TSHR surface was remarkably similar to the positioning of FSH on the FSHR surface and consequently the TSHR binding features of M22 and of TSH are very similar. Overall, our high resolution crystal structure shows details of the interactions between the TSHR and M22 at the molecular level and provides foundations for developing new strategies to understand and control glycoprotein hormone receptor activation TSHR autoimmunity.

Thyroid autoimmunity
Oral

A HIGH AFFINITY TSH RECEPTOR BLOCKING TYPE HUMAN MONOCLONAL AUTOANTIBODY

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Peripheral blood lymphocytes from a patient with high levels of blocking type TSH receptor (TSHR) autoantibodies (TRAb) were immortalised by infection with Epstein Barr virus. Cells in TRAb positive cultures were fused with the K6H6/B5 cell line and cloned four times to obtain a single clone producing the monoclonal TRAb 5C9 (IgG1/kappa). 5C9 IgG bound the TSHR with high affinity ($4x10^{10}$ L/mol) and low concentrations (<1µg/mL) of 5C9 IgG inhibited TSHR binding by labelled TSH or by labelled thyroid stimulating human monoclonal antibody M22. In addition, patient serum TRAbs (stimulating or blocking type) inhibited labelled 5C9 IgG binding to the TSHR. 5C9 showed no ability to stimulate cyclic AMP production in TSHR transfected CHO cells but acted as a blocking type TRAb and inhibited the ability of TSH, M22, thyroid stimulating mouse MAbs and patient serum TRAb to stimulate cyclic AMP production. As such 5C9 has the characteristics of patient serum TRAb with blocking (ie antagonist) activity. In addition, 5C9 IgG reduced basal levels of cyclic AMP production in CHO cells expressing wild type TSHR and TSHRs containing activating mutations. 5C9 $F(ab')_2$ and Fab preparations were active antagonists. Analysis of the effect of different TSHR amino acid mutations on 5C9 biological activity indicated that TSHR Lys129 and Lys183 are important for 5C9's actions. This is the first description of a human monoclonal autoantibody with the characteristics of patient serum blocking type TRAbs. 5C9 will be valuable in developing our understanding of the relationship between TSHR binding by TRAbs and subsequent biological effects and such studies may lead to new therapeutic strategies to control TSHR activity.

Thyroid autoimmunity
Oral

THE EFFECT OF RITUXIMAB IN THYROID-ASSOCIATED OPHTHALMOPATHY MAY BE DEPENDENT ON REDUCED ANTIGEN B-CELL PRESENTATION IN THE ORBIT

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In this study we analyzed the changes of peripheral lymphocytes after RTX therapy (1000 mg twice at a 2-week interval) in 10 patients with Graves' disease, 8 of whom had associated ophthalmopathy (TAO). In all patients we studied lymphocytic populations at baseline and monthly for up to 2 years. 9/10 patients showed total CD20+ B-cell depletion after the first infusion while 1 patient persistence of <5% CD19+CD5+ lymphocytes. In 8/10 patients B cell depletion lasted 4–6 months, in 2 patients 2 and 10 months, respectively. Return of CD20+ cells until about 50% of baseline levels was observed at 18 months and in 4 patients at 26 months. No change of T lymphocytes was observed. Concurrently with B-cells depletion in 8/10 patients we found a reduction of DR+CD20+ cells of about 70%. In 7/10 patients we found also a reduction of DR+CD3+ cells of about 40%, in in 2 of 8 and 17% and no reduction in the patient with persistence of CD19+. We have reported clinical improvement of TAO after treatment which did not correlate with CD20+ cell changes. The patient with persistent CD19+5+ cells, even after re-treatment with RTX (1000 mg), did not deplete and showed only transient improvement of severe TAO. After B cell return (mean 16 weeks), DR+CD3+ cells showed an increase at about 50 weeks and normalisation at about 75 weeks. Evidence of CD19+5+ in the orbital tissues was observed after orbital decompression. In conclusion, TAO patients treated with RTX show long term reduced CD20+ B-cells. This is associated to a consistent disease improvement and lack of relapse. The presence of reduced DR+CD3+ cells at the time of B-cell depletion suggests that the effect of RTX therapy on TAO might be due to reduced antigen presentation and consequent T cell activation in the orbital tissues. Persistence of CD19+5+ lymphocytes after RTX seems to be associated to a not completely satisfactory therapeutic response.

Thyroid autoimmunity
Oral

SELENOMETHIONINE ADMINISTRATION MAY ATTENUATE PROINFLAMMATION IN DE QUERVAIN'S SUBACUTE THYROIDITIS

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Although De Quervain's subacute thyroiditis (DeQ) is presumed to be caused by viral infection, the etiology remains uncertain. The disease is characterized by clinical and laboratory signs of inflammation. The trace element selenium (Se) possesses anti-inflammatory properties probably by minimizing the effects of inflammatory mediators and reducing apoptosis of thyroid follicular cells. This study was designed to investigate whether administration of Selenium (Se) combined with ASS may alleviate the symptomatology and thus reduce the course of DeQ. ***Methods:*** 21 patients (17 female, 4 male) with mean age of 49+–9years and diagnosed DeQ were randomly assigned in two groups. Gr I (n=11) received ASS 1,500 mg/day and Gr II (n=10) was treated with 1,500 mg ASS plus Se in the form of selenomethionine (SeMe) 200 μg/day. Se and serum TSH, FT4, ESR and CRP were measured and thyroid US examination was performed prior to and 2 months after treatment. ***Results:*** After 8 weeks treatment attenuation of thyroid inflammation, defined by the decrease of CRP and ESR levels as well as by considerable amelioration of the echogeneicity map in US, was achieved in Gr I. 2/11 patients who received reduced ASS dosage relapsed. In Gr II almost total restitution of thyroid function and elimination of symptomatology and normal levels of CRP (–21% vs.GrI) levels were achieved in only 6–7 weeks. No relapse was registered. Se levels were found to be 55+–9μg/l at the lower end of the scale and increased to 77+–12 after treatment. ***Discussion:*** Our data indicate that the intake of Se combined with ASS is associated with lower CRP concentration, thus accelerating thyroid recovery by influencing the inflammatory process underlying the pathology of DeQ. It remains to be established whether SeMe may reduce the dosage of ASS or even that of steroids in severe forms of DeQ.

Thyroid autoimmunity
Oral

INDUCTION OF HYALURONAN SYNTHASE 2 AND 3 mRNA IN DIFFERENTIATED HUMAN ORBITAL FIBROBLASTS BY GRAVES IgG BUT NOT BY CONTROL IgG

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Introduction: Differentiation of orbital fibroblasts (OF) into adipocytes, under simultaneous upregulation of the TSH receptor, plays an important role in the pathogenesis of Graves' ophthalmopathy (GO). OF may produce excessive amounts of hyaluronan, which is synthesized by three types of hyaluronan synthases (HAS1; HAS2; HAS3). ***Aim:*** The aim of the study was to evaluate the effects of immunoglobulins of patients with Graves disease (GD-IgG) or controls (c-IgG) on the mRNA expression of the HAS1, HAS2 and HAS3 genes in OF before and after adipocytic differentiation. ***Methods:*** Human OF, obtained from patients with severe, but inactive GO during orbital decompression surgery, were cultured either in standard medium or in differentiation medium. Differentiation was evaluated using Oil-Red-O staining. Both the undifferentiated and the differentiated OF were stimulated with c-IgG (100 ng/ml) or GD-IgG (100 ng/ml) for 48h. HAS1, HAS2 and HAS3 mRNA expression were measured using RT-PCR. ***Results:*** GD-IgG and c-IgG had no effect on HAS mRNA expression in undifferentiated OF. In differentiated OF, however, HAS2 and HAS3 mRNA expression increased in response to GD-IgG ($p<0.01$), but not to c-IgG. ***Conclusion:*** GD-IgG increase hyaluronan synthase gene expression in OF. This may contribute to excessive glycosaminoglycan production in Graves' ophthalmopathy.

Clinical thyroidology
Oral

NUMBER OR URINE SAMPLES NEEDED TO ESTIMATE THE IODINE INTAKE IN A POPULATION AND IN AN INDIVIDUAL

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Objective*:* The iodine intake level in a population is determined in cross-sectional studies. Urinary iodine varies considerably and the number of samples needed to determine population and individual iodine excretion level is unsettled. ***Design:*** A longitudinal study of sixteen healthy free-living men from an area of mild to moderate iodine deficiency. ***Methods:*** Iodine and creatinine concentrations were measured in spot urine samples collected monthly for thirteen months. Variance was used to calculate the number of urine samples needed to determine the iodine excretion level for iodine concentration and for 24h iodine excretion estimated from age and gender specific creatinine excretions.
Results: Participant mean urinary iodine excretion varied from 30 to 87 µg/L (31 to 91 µg/24h). Sample iodine varied from 10 to 260 µg/L (20 to 161 µg/24h). Population SD and average individual SD were larger for crude urinary iodine than for estimated 24h iodine excretion (32 vs 26 and 29 vs 21; Bartlett's test, $p < 0.01$) as was CV% (44.0 % vs 37.5 %). The number of spot urine samples needed to estimate the iodine level in a population with 95% confidence within ±10% was about 125 (100 when using estimated 24h iodine excretions), within ±5% was about 500 (400), and within ±2% was about 3,000 (2,400). Within ±20%/±10% in an individual required 12/146 (7/46) urine samples.
Conclusions: Estimating population iodine excretion requires 100–500 spot urine samples for each group or subgroup in a homogenous population. Less than 10 urine samples in an individual may be misleading.

Clinical thyroidology
Oral

CONSEQUENCES OF THE NATIONAL ACADEMY OF CLINICAL BIOCHEMISTRY (NACB) PROPOSAL FOR SERUM THYROTHROPIN (TSH) REFERENCE INTERVALS

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Background: In 2003 NACB proposed a reference interval of 0.5 to 2.5 mU/L for TSH. Several studies of diurnal variation of TSH show a decrease from early morning to noon of about 30%, but only few studies of population based reference intervals state the time of the blood sampling. Moreover, there is no relationship between method bias and upper reference limit in the literature. Additionally, reference limits and clinical decision limits are often mixed up. ***Aim:*** To investigate the consequences of the NACB proposal for the TSH reference interval in relation to the well known effect of the diurnal variation of TSH. ***Methods:*** To simulate different reference distributions of TSH (morning and afternoon), and estimate the percentages of healthy individuals classified outside the limits, given application of the NACB limits. ***Results****:* The decrease in TSH of 30 % from morning to noon was confirmed by measurements in 20 healthy individuals, so we used our published log-Gaussian reference interval at 8 to 9 AM of 0.58 to 4.1mU/L and a calculated interval at 3 PM of 0.41 to 2.8 mU/L for the simulations. If applying the NACB limits to our morning reference interval, 16 % of healthy individuals would exceed the upper limit, and 1.2 % would fall below the lower limit. Repeating the procedure for the afternoon interval the percentages would be 4.5 and 6.2, respectively. ***Discussion and Conclusion:*** The proposed NACB reference limits for TSH neither fits our or the majority of published reference intervals, independent of the sampling time. If used as decision limits, the NACB interval may be relevant for early clinical detection of thyroid diseases, but the consequence will be a significant number of additional investigations of healthy individuals. One could consider time-related reference intervals.

Clinical thyroidology
Oral

DO WHO GUIDELINES FOR NEONATAL BLOOD TSH ACCURATELY REFLECT POPULATION IODINE STATUS?

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While the primary purpose of neonatal TSH screening is the detection of congenital hypothyroidism, other applications such as the distribution of neonatal blood TSH in a population have been used as an index of dietary iodine status. Arising from these studies the WHO/UNICEF/ICCIDD has recommended that a frequency of < 3% of blood TSH > 5.0 mIU/L is indicative of adequate dietary iodine supply. The present study was prompted by the recent finding of declining urinary iodine excretion in Irish pregnant women mirroring reports from other developed countries. The distribution of neonatal blood TSH was used to investigate any consequences of this decline. Analysis of the distribution of findings from 73,019 neonatal blood TSH values assessed over the years 1995–2006 during winter (January N= 35,079) and summer (August N= 37,940) months, selected in view of the known seasonal variation in dietary iodine intake in the Irish population, showed a consistent and highly significant move towards higher values ($p<0.001$). In view of the observed decline in maternal UI it is surprising that neonatal TSH levels were not even higher. Despite this trend the proportion of TSH > 5.0mU/L remaining relatively constant (2.35–2.83%) and would not by convention be indicative of iodine deficiency. Whether such minor alterations in TSH reflect a suboptimal supply of thyroid hormones to the developing brain is unknown, but in view of the possible consequences of even a minor degree of hypothyroxinaemia for neuropsychological development and subsequent IQ, they emphasise the need to utilise readily available neonatal TSH data and not to rely solely on an arbitrary cut- off of the % of TSH values > 5.0 mIU/L especially in areas considered iodine replete.

Clinical thyroidology
Oral

NEONATAL SCREENING PROGRAM FOR CONGENITAL HYPOTHYROIDISM: A 7-YEAR EXPERIENCE WITH LOW TSH CUT-OFF LEVELS

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Congenital Hypothyroidism (CH) is the most frequent neonatal endocrine disease. In economically advanced countries, its prevalence is currently reported to be of about 1:3000 newborns, with a dominant proportion of dysgenetic defects (about 80%). The introduction of ultra-sensitive and accurate TSH assays have prompted a revaluation of normal TSH ranges and cutoff limit for diagnosis of primary hypothyroidism. In this report, we focus on the impact generated by the introduction of lower blood TSH (b-TSH) cutoff values for newborn recall. During 1999–2005, 629.042 newborns were screened in this Center with b-TSH cutoffs of 12mU/L in 1999–2002 and 10mU/L in 2003–2005. The recall rates after the 1st b-TSH determinations increased from 0.57 to 1.06% consistent with the possible existence of a relevant number of false negative (FN) results with previous screening strategies. The prevalence of confirmed CH after serum TSH determination varied between 1:2.500 newborns in 2001 and 1:1.024 in 2005. The positive newborns presently on L-T4 treatment are 417 with 55 athyreosis (13%), 88 ectopies (21%) and 274 with gland in situ (66%). Children with b-TSH ranging 10,0–19,99 mU/L are 186 (45% of positive cases) and 7% are dysgenetic forms. The prevalence of transient forms of CH in a representative sample of these latter patients was 17%, thus indicating that FN cases were unexpectedly elevated using b-TSH cut-off values >20 mU/L. In conclusion, the prevalence of CH in our newborn population appears to be the double than previously thought. The majority of such defects are due to mild thyroid dysfunction associated with gland in situ. These findings prompt future studies aimed to investigate the impact of unrecognized neonatal mild hypothyroidism.

Clinical thyroidology
Oral

L-TIROXINE (L-T4) REQUIREMENT IN PATIENTS WITH AUTOIMMUNE HYPOTHYROIDISM AND PARIETAL CELL ANTIBODIES (PCA)

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Purpose: Impaired gastric acid secretion due to H.Pylori infection reduces l-T4 absorption. Based on frequent association of PCA (another cause of impaired gastric secretion) and autoimmune thyroiditis (AT) we aimed to ascertain whether this association may be a cause of increased l-T4 needs in hypothyroid patients. ***Methods:*** We studied 697 patients with AT receiving l-T4 for hypothyroidism. We selected patients (n=413) with normal serum TSH concentration (0.3–3.3μU/ml) on l-T4 and we screened them for serum PCA. ***Results:*** 273/413 (66%) patients were PCA negative (PCA-) and 140/413 (34%) were PCA positive (PCA+). Two groups not differ for duration of hypothyroidism, serum TSH before therapy (23.9±36.76 μU/ml in PCA- and 19.3±21.7 μU/ml in PCA+), basal thyroid volume (10.8±6.4 ml in PCA- and 11.3±86 ml in PCA+), age and sex. Mean±SD serum TSH at the time of study was similar (1.49±0.77 μU/ml in PCA- and 1.57±0.75 μU/ml in PCA+). The daily requirement of l-T4/kg was significantly higher in PCA+ (1.21±0.43 μg/kg/die) compared to PCA- patient (1.09±0.39) (p=0.002). The significant increase in daily dose of l-T4 of PCA+ patients was maintained when H.pylori infection was excluded. Gastric endoscopy was performed in 51 PCA+ patients: the daily requirement of l-T4 was significantly higher in 33 patients with gastric atrophy compared to 18 with normal gastric mucosa (1.42±0.48 vs 1.16±0.32μg/kg/die) (p=0.02). Thus, autoimmune gastritis is an additional factor influencing daily requirement of l-T4 in patients with autoimmune hypothyroidism. Screening for PCA should be performed in any patient with unexplained increase of l-T4 requirement.

Clinical thyroidology
Oral

PATIENTS AND CLINICIANS OFFER COMPLEMENTARY PERSPECTIVES ON WHAT IS RELEVANT WHEN EVALUATING QUALITY OF LIFE IN THYROID PATIENTS

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Purpose: To investigate whether clinicians and patients differ in their evaluation of what is relevant when measuring quality of life of thyroid patients. ***Methods:*** Fifteen thyroid experts and 80 thyroid outpatients (14 with non-toxic goitre, 12 nodular toxic goitre, 21 Graves´ disease, 17 thyroid associated ophthalmopathy (TAO) and 16 primary hypothyroidism) were interviewed, using semi-structured interviews. The relevance of 138 thyroid-related issues was rated separately for each of the 5 diagnoses. Patients´ rating of importance was combined with prevalence of the issue in question to calculate a mean relevance rank for each patient category. Experts rated the relevance directly. Patient and expert relevance ratings were compared using nonparametric correlation. To characterize the perspectives in greater detail, the 15 issues considered most relevant by the patients were compared to the 15 issues considered most relevant by the clinicians. ***Results:*** The Spearman correlations between patient and expert ratings ranged 0.35 (goitre) to 0.69 (Graves´). About half of the 15 issues considered most relevant by the patients were also among the 15 most relevant to clinicians. Generally, the issues deemed highly relevant only by clinicians were symptoms and problems characteristic of the diagnosis in question. The issues deemed highly relevant only by patients were broader, less specific issues such as emotional susceptibility and nervousness and general physical symptoms. ***Conclusion:*** Patients and clinicians offer complementary perspectives on what is relevant for quality of life-evaluation of thyroid patients. The inclusion of experts' contribution to the issue generation process may increase the specificity of a resulting questionnaire.

Clinical thyroidology
Oral

NEEDLE-FREE DELIVERY OF LIDOCAINE FOR REDUCING THE PAIN ASSOCIATED WITH THE FINE-NEEDLE ASPIRATION BIOPSY OF THYROID NODULES: TIME SAVING AND EFFICACIOUS PROCEDURE

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Fine-needle aspiration biopsy (FNAB) is a mandatory procedure in evaluation of thyroid nodules. However, it is sometimes perceived as a painful procedure by the patients. Efficacy of the needle-free injection of local anesthesia for reducing the pain associated with other cutaneous procedures that involve needle insertion was previously reported. In this double-blind, placebo-controlled clinical trial, we evaluated the effectiveness of a needle-free injection of lidocaine in achieving satisfactory pain control in patients undergoing FNAB of thyroid nodules. Patients were allocated to receive either lidocaine administered by needle-free injection system (n=55) or placebo (isotonic saline) (n= 52) 2 to 3 minutes before FNAB. A series of 4 aspirations of each nodule was performed. The patients rated pain associated with the procedure according to a 100-mm visual analog scale (VAS), an 11-point numeric rating scale (NRS), and a 4-category verbal rating scale (VRS). The 2 groups studied were similar with respect to age, sex, thyroid volume, nodule size, and nodule site. When the effectiveness of lidocaine was compared with that of placebo, the mean VAS score was 11.4 ± 13.6 mm versus 38.2 ± 35.5 mm ($p<0.0001$) and the mean NRS score was 1.4 ± 1.5 points versus 3.9 ± 2.6 points ($p< 0.0001$), respectively. The absolute numbers according to VRS score in each group was also significantly different ($p<.0001$). The percentage of patients with “no pain” or “mild pain” in the lidocaine group (90.9%) was significantly higher than that in the placebo group (44.2%) ($p <0.0001$). Less than 10% of the patients in lidocaine group experienced moderate pain and none experienced severe pain. No adverse treatment-related effects were observed. To our knowledge, this is the first study demonstrating that the needle-free delivery of lidocaine is an effective, useful, and noninvasive method of providing local anesthesia for the FNAB of thyroid nodules.

Clinical thyroidology
Oral

THE DEGREE OF HYPERTHYRODISM DETERMINES PERSISTENTLY REDUCED WHITE MATTER GLUTAMINE IN TREATED GRAVES' DISEASE

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Patients with Graves' disease often have neurophychiatric and cognitive complaints, whether these symptoms disappear after treatment remains disputed. This prospective study reports magnetic resonance spectroscopy (MRS) from 27 patients with newly diagnosed Graves' disease before and after antithyroid medical treatment. Short echo time proton MR spectroscopy data was acquired from occipital and frontal grey matter and from parieto-occipital white matter. The sampled MR spectra, 85 from occipital grey matter, 84 from parieto-occipital white matterand 50 from frontal grey matter fulfilled all the technical acceptance criteria, and the quality of the spectra in between groups was not significantly different as judged by full width of half minimum and by the signal to noise ratio. Previous studies in acute Graves' disease showed reduced choline and myo-inositol compared to healthy volunteers. These findings were confirmed, and the abnormalities were reversible. Acute phase measures of total choline in parieto-occipital white matter and myo-inositol in occipital grey matter correlated significantly with impaired thyroid function. The glutamate in occipital gray matter decreased from the acute phase to the follow-up ($p<0.05$), and initial triiodothyronine correlated significantly with glutamine in occipital grey matter of the treated patients ($r=–0.52$, $p<0.01$). Glutamine was and remained significantly reduced in parieto-occipital white matter of Graves' disease patients. The persistent reduction of glutamine in white matter combined with decreasing glutamate in occipital grey matter and the correlation with severity of the initial disease indicate, that there is a persistent and possibly progressive disturbance of the glutamate glutamine cycling in Graves' disease.

Thyroid cancer
Poster

IS RADIOIODINE ABLATION NECESSARY IN SMALL (≤ 1 cm) Papillary And Follicular Thyroid CARCINOMAS?

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Background: Papillary thyroid microcarcinomas have an excellent prognosis. No radioiodine ablation is necessary in patients with papillary microcarcinomas and limited surgical resection. It was the aim to compare the risk-profile of patients with papillary and follicular thyroid carcinomas ≤ 1 cm. ***Patients and Methods:*** 1,484 patients with thyroid cancer were treated at our department of nuclear medicine between 1995 and 2005. For the 352 patients with papillary and follicular carcinomas ≤ 1 cm a comparative correlation of extrathyroidal growth, lymph node spread, distant metastasis and survival was performed. The mean follow-up time was 6 years. ***Results:*** 330 patients had small papillary and 22 follicular thyroid carcinomas ≤ 1 cm. High-dose radioiodine therapy was performed in 77% and 100% of patients with small papillary and follicular carcinomas, respectively. Multifocality (13% vs. 5%), extrathyroidal growth (12% vs. 0%), lymph node spread (13% vs. 5%) and distant metastasis (2% vs. 0%) were more common in patients with small papillary than follicular tumors. ***Conclusion:*** Small follicular thyroid carcinomas ≤ 1 cm have a lower prevalence of lymph node and distant metastases as compared to papillary microcarcinomas. The indication of radioiodine ablation in small papillary and follicular carcinomas should be reconsidered in case of limited surgery.

Thyroid cancer
Poster

ROLES OF 18F-FDG PET AND OTHER DIAGNOSTIC TOOLS IN THE MANAGEMENT OF METASTATIC THYROID CARCINOMA PATIENTS

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The management of thyroid carcinoma patients with suspected of recurrent disease by different diagnostic modalities and some discordance between them presents considerable problems. The purpose of this study is to determine the roles of FDG-PET and other conventional diagnostic modalities, such as thyroglobulin (Tg), thyroglobulin antibody (Tg-Ab), I-131 WBS, US, CT and MRI in the management of these patients. ***Methods:*** Forty thyroid carcinoma patients who underwent FGD-PET studies are included in this study. Whole-body images were acquired 1 h after intravenous injection of 370 MBq (10 mCi) FDG using a PET scanner. Results of FDG-PET studies were evaluated by histolopatholgy, CT or MRI, and follow-up of Tg levels in 2 years clinical follow up period. ***Results:*** Sixteen patients had positive findings on PET, which were proven to be true-positive in 15 patients, whereas 1 patient had false-positive finding. 24 patients had negative scans. These were true-negative findings in 12 patients, whereas the remaining 12 patients had false-negative results, 6 of them had positive I-131 WBS. Cervical US was the only test which determined cervical lymph node metastases in two patients with TSH-stimulated Tg <0.2ng/ml. Tg levels of all true negative cases were less than 5 ng/ml. FDG- PET revealed metastatic foci in two patients with very high TgAb levels and negative I-131 WBS. ***Conclusions:*** FDG-PET is a valuable diagnostic tool in patients with differentiated thyroid cancer who present with increased Tg levels of >5 ng/ml and negative ^{131}I scans. Elevated TgAb levels are also an indicator of recurrent disease and FDG-PET is also very useful in these patients. US is the most sensitive method to detect local recurrence and lymph node metastases at an early stage and should be performed all routine check-ups.

Thyroid cancer
Poster

IDENTIFICATION OF LOW PENETRANCE GENES ASSOCIATED TO PAPILLARY THYROID CARCINOMA BY MEANS OF HIGH THROUGHPUT GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

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Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy (80%). It is a neoplasia arising from follicular thyroid cells and behaves as a complex disease where the individual susceptibility is determined by multiple common genetic variants located in different 'Low Penetrance Genes' (LPG). Our aim was to identify some of these variants by a case-control study, which included over 400 PTC patients *versus* 500 representative Spanish healthy controls. We selected a total of 97 candidate genes to analyze. 77 of them were selected because their relation to thyroid metabolism itself. 20 genes were additionally selected because they were found to be differentially expressed by using cDNA arrays when compared PTC with normal tissue, follicular thyroid carcinomas (FTC) and anaplastic or poorly differentiated thyroid carcinomas. The 97 genes selected were well characterized by 768 single nucleotide polymorphisms (SNPs). The SNPs were chosen through different SNPs databases (NCBI, Ensembl and HapMap project) and *in silico* tools (Pupa Suite) to include Tag SNPs and putative functional SNPs (specially the non-synonymous ones) when possible. Then, we carried out a high throughput genotyping assay using the *Illumina Sentrix Array* platform. Association tests were performed on single SNPs, haplotypes (considering linkage disequilibrium blocks defined by HapMap Project) and dyplotypes to define susceptibility PTC loci.
These results will be presented.

Thyroid cancer
Poster

P53, P21 And Bcl-2 PROTEINS EXPRESSION AND CLINICAL SIGNIFICANCE IN PAPILLARY THYROID CARCINOMA

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Background: There were some investigations about the role of p53, p21 and bcl-2 protein expressions of tumorigenesis in thyroid cancer. It had been debated that these protein expressions were associated with aggressive features of thyroid papillary carcinoma. We studied about these protein expressions and their clinical significances in thyroid papillary carcinoma. ***Methods:*** We selected 49 patients with thyroid papillary carcinoma who had been operated in our hospital for last 10 years. Immunohistochemical stains for p53, p21 and bcl-2 were done by use of paraffin embedded tissues which were keeping in our hospital. We analyzed the results of immunohistochemical stains for p53, p21 and bcl-2 and the patients age, sex, multifocality, both lobe invasion, extrathyroidal invasion, cervical lymph node invasion, distant metastasis and clinical outcomes. ***Results:*** Immunohistochemical stain for p53 was positive in 10 patients (20%), p21 was positive in 36 patients (73%) and bcl-2 was positive in 18 patients (39%). P53 and bcl-2 expressions were not associated with clinical parameters. Tumor multifocality and extrathyroidal invasion were significantly higher in p21 positive group (both $p < 0.05$). ***Conclusion:*** This study showed p21 protein expression was associated with tumor multifocality and extrathyroidal invasion in thyroid papillary carcinoma. Immunohistochemical stains for p21 may be used as the parameters for tumor aggressiveness in thyroid papillary carcinoma.

Thyroid cancer
Poster

RET PROTO-ONCOGENE MUTATIONS AND POLYMORPHISMS IN FRENCH MEDULLARY THYROID CARCINOMAS PATIENTS

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Purpose*:** Germline mutations in the RET (REarranged during Transfection) proto-oncogene are responsible for inherited forms of medullary thyroid carcinoma (MTC) : MEN2A, MEN2B and FMTC (familial medullary thyroid carcinoma). However the majority of MTC have no associated family history neither indications that might suggest hereditary cause, they are classified as apparently sporadic medullary thyroid carcinoma (SMTC). The aim of this study was to characterize the RET mutations in MEN2A, FMTC and SMTC cases originating from the region Champagne-Ardenne of France. ***Methods: Genomic DNA from 86 MTC patients (1 MEN2A, 6 FMTC, 79 apparently SMTC) were analysed. Exons 8, 10, 11, 13, 14, 15 and 16 of the RET gene were analysed by DHPLC and DNA sequencing. ***Results:*** Germline mutations (8) were identified : C634Y in MEN2A, C609F, E768D, L790F, V804M (2) in FMTC and S891A, V804M in 2 SMTC. No mutation was found in a FMTC. Any polymorphism was detected in 20.8% SMTC. The S836S polymorphism was observed in 5.2% SMTC and in 3.6% normal controls. This slightly overrepresentation in SMTC has been reported in other studies. In our study this polymorphism was associated with L769L polymorphism. These both polymorphisms were too observed in the patient apparently SMTC and carrier of V804M mutation. The frequencies for three other polymorphisms (G691S, L769L, S904S) and their association are presented. ***Conclusion:*** The present study of the RET gene in a French population allowed the identification of 2 FMTC among 79 apparently SMTC.

Thyroid cancer
Poster

NEW INSIGHT IN mRNA AND PROTEIN EXPRESSION OF GLUCOSE TRANSPORTERS (GLUT) IN HUMAN THYROID CARCINOMA CELL LINES

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18FDG-PET is based on cell capability to take up glucose. Increment in expression of the glucose transporters (GLUT), especially GLUT1 has been observed in thyroid tumors with poor prognosis although we have few data on expression of other GLUTs in thyroid carcinoma cell lines. In this study, we evaluated expression and functionality of several GLUT isoforms in five human thyroid carcinoma cell lines: ARO and FRO (anaplastic carcinoma), NPA (poorly-differentiated papillary carcinoma), WRO (follicular carcinoma), TT (medullary carcinoma). Expression of GLUTs mRNA was assessed by conventional and Real-time RT-PCR, we performed cell ^{3}H-DG uptake and we studied GLUT1 and GLUT3 proteins. From our results, cell lines showed a wide range of GLUT expression levels by conventional RT-PCR although, by Real-time quantitative RT-PCR, only GLUT1 and GLUT3 showed significant levels of mRNA expression. Cell lines were then studied for ^{3}H-DG uptake displaying different uptake rates (NPA>WRO>ARO>FRO>TT). Evaluation of GLUT1 and 3 proteins by Western blot analysis, demonstrated that GLUT1 protein was only present in anaplastic carcinoma cell lines (FRO, ARO) while GLUT3 was present in NPA and WRO (follicular and poorly-differentiated papillary carcinoma) and in ARO (anaplastic carcinoma). For the first time, expression and functionality of different glucose transporters was studied on five thyroid carcinoma cell lines; we found mRNA expression of different GLUT isoforms, mainly GLUT1 and GLUT3; like in previous data, expression of GLUT1 protein can justify glucose uptake in anaplastic carcinoma cell lines while uptake levels measured in differentiated forms can be due to expression of GLUT3 which could raise importance in intermediary stages of thyroid tumor de-differentiation

Thyroid cancer
Poster

APOPTOSIS AND PROLIFERATION RELATED MOLECULES (Bcl-2, Bax, p53, PCNA) In Papillary Microcarcinoma vs. PAPILLARY CARCINOMA OF THE THYROID

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The relationship between clinical papillary thyroid carcinoma (PTC) and papillary microcarcinoma (PMC), concerning their biological behaviour and consequent clinical management is still unanswered. On the other hand, it is known that tumour growth and aggressiveness are determined by the ratio between anti- and pro-apoptotic molecules and its balance to proliferative activity of neoplastic cell. In order to gain better insight into the differences in biological behavior between PMC and PTC we examined immunohistochemically apoptosis-related molecules: Bcl-2 (antiapoptotic), Bax and p53 (proapoptotic) and proliferation related marker (PCNA) in 34 archival cases of PMC and 52 cases of PTC. Expression of Bcl-2 and Bax was found in most PMCs and PTCs. The average Bcl-2 staining score did not differ significantly between PMC and PTC ($p>0.05$), but the average Bax score was significantly lower in PMC ($p<0.05$). Therefore, Bcl/Bax ratio was significantly higher in PMC vs. PTC ($p<0.05$). Similar expression of p53 was found in PMC and PTC, without correlation with clinical data, but it was associated with high Bax expression ($p<0.05$) in these cases in both groups. Nonmalignant tissue expressed only BCl-2, but not p53 or Bax. PCNA expression was significantly lower ($p<0.05$) in PMC vs. PTC and positively correlated with tumour size ($p<0.05$). Higher Bcl-2 /Bax ratio and lower proliferative activity in PMC suggest the differences in the balance between apoptosis and proliferation in PMC vs. PTC. The presence of p53 and Bax in PMC (which are not expressed in normal thyroid tissue) indicates the malignant potential of PMC, so this lesion should be treated with a caution.

Thyroid cancer
Poster

THYROID PEROXIDASE AND GALECTIN-3 IMMUNOSTAINING IN DIFFERENTIATED THYROID CARCINOMA: DIAGNOSTIC USEFULNESS AND CLINICOPATHOLOGICAL CORRELATION

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Various molecular markers which may contribute to an accurate diagnosis of thyroid neoplasia are currently being studied. The use of immunohistochemical markers thyroperoxidase (TPO), a specific thyroid protein, and galectin-3, a promising molecular marker of thyroid malignancy, has been suggested as a contribution to differential diagnosis of thyroid neoplasia. Our aim was to study the usefulness of combined expression of TPO and galectin-3 (gal-3) in the diagnosis of differentiated thyroid carcinoma and to correlate immunostaining of these markers with the clinical/pathological features of patients with thyroid cancer. We analysed by immunohistochemistry the expression of TPO and gal-3 in a series of 194 thyroid tumours (28 FTA, 19 FTC, 149 PTC) and paired peritumoral tissues. Underexpression of TPO and expression of gal-3 were observed in a majority of the carcinomas. Combination of high TPO expression and gal-3 absence was specific (81.7%) for nonmalignant tissues. The sensitivity for diagnosis of differentiated thyroid carcinoma was 85.5% for TPO and 81.9% for gal-3. The combination of gal-3 and TPO increased the sensitivity up to 94%. Age (>45 years), nodal involvement, extrathyroidal invasion and high TNM stage in PTC patients were related to TPO absence and high gal-3 expression ($p<0.05$). In FTC patients only the extent of invasiveness was associated with gal-3 positivity. Combined immunohistochemistry for the molecular markers: gal-3 and TPO, might be useful in the diagnosis of malignant thyroid tumours, particularly in selected cases in which these two markers are less useful individually. In addition, these tumour markers may indicate more or less aggressive biological behaviour of differentiated thyroid carcinomas.

Thyroid cancer
Poster

IS IT NECESSARY TO PERFORM THYROID FINE NEEDLE ASPIRATION TO INFRACENTIMETRIC THYROID NODULES?

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Purpose: Management of infracentimetric thyroid nodules (ICN) are controversial. We evaluated to rate and risk of malignancy in ICN. ***Material and Methods:*** 426 patients underwent ultrasonography guided fine needle aspiration biopsy (US-FNAB) in evaluation of total 520 nodules. Number of ICN's were 247, (group 1) and number of supracentrimetric nodules (SCN) were 273 (group 2). The indication of surgery included: malign and suspicious cytology. The cytological findings were divided in to four groups as: benign, malignant, suspicious, inadequate. ***Results:*** The rate of malignancy in ICN was 4.9% and SCN was %1.5. The suspicious cytogical results were four hurthle cell neoplazm and 16 follicular neoplazm. Among the patients who have had operation for suspicious cytology; 6 of them had papillary carcinoma but others were normal pathology. 16 of the patients who had malign cytology had also reported as papillary carcinoma. after operation. Inadequate cytology in ICN's were 16.6% and SCN's were 22.3%. All patients with inadequate cytology were advised to return for a repeat US-FNAB. Central microcalcification, hypoechogenity and long diameter/short diameter<1.5cm were associated with malignancy and found to be statistically significant in group one ($p<0.05$). In group 2 patients only hypoechogenity was found to be associated with malignancy ($p<0.05$).Overall, solitary nodules and anteroposterior diameter/transverse diameter ≥1 were not associated with increased malignancy ($p>0.05$).**Conclusion:** Malignancy in ICN's were not very rare in our results. So we strongly recommend US-FNAB to every nodules including ultrasonografic malignancy findings.

Thyroid cancer
Poster

ABSENCE OF LATE RECURRENCES IN PATIENTS WITH A NEGATIVE FIRST FOLLOW-UP AFTER I-131 ABLATION FOR DIFFERENTIATED THYROID CARCINOMA

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Objectives: In the past, large retrospective studies have shown that recurrences will frequently occur more than five years after initial treatment of differentiated thyroid carcinoma. The objective of this study was to study the recurrence rate and -time in patients with differentiated thyroid carcinoma who underwent total thyroidectomy, received I-131 ablation, had negative TSH-stimulated Tg-levels and a negative I-131 whole body scan (WBS)at the first follow-up after ablation. ***Methods:*** Retrospective data from 3 hospitals from 1980 (1 hospital) or 1990 (2 hospitals) onward on initial treatment and follow-up of patients who received I-131 ablation for differentiated thyroid carcinoma were pooled. Ablation dosages varied from 1110 to 5550 MBq. Patients were followed with Tg-measurement and periodic I-131 whole body scintigraphy at various intervals. ***Results:*** 456 patients were included**.** 10 patients (2.2%) developed a recurrence after an average interval of 32 months (range: 14-58 months) following administration of the ablative dosage of I-131. Overall disease-free survival at both 5 and 10 years was 95.9%. Recurrence was first discovered by Tg-measurement during suppression in 5 patients, by TSH-stimulated Tg-measurement in 4 patients, and by detection of a pathologic lymph node in 1 patient. ***Conclusion:*** In differentiated thyroid carcinoma patients who had a negative first follow-up I-131 WBS and negative concurrent TSH-stimulated Tg-levels after I-131 ablation, recurrence is a rare event, and in our populations never occurs more than 5 years after initial treatment. Beyond the first follow-up after ablation, I-131 WBS seems to have no additional diagnostic value.

Thyroid cancer
Poster

ARSENIC TRIOXIDE HAS MULTIPLE CELLULAR TARGETS IN THYROID CANCER CELL LINES

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Prompted by the fact that arsenic trioxide (ATO) is an effective second-line treatment in retinoic acid refractive promyelocytic leukemia we addressed the question if ATO could also be effective in re-differentiation treatment of thyroid carcinoma. Cell lines derived from follicular and papillary thyroid carcinomas were investigated for 3H-thymidine and 125I-iodide uptake. To characterize the action of ATO in the cells the following parameters were investigated: apoptosis by hypodiploic cells, tumor metabolism by 3H-glucose-uptake, GLUT-1 and transketolase-like-1 protein (TKTL-1) expression, interaction with the AKT-pathway by co-incubation with Akt-inhibitors, and expression of p-Akt and expression of thyroid-specific proteins (sodium-iodide symporter and pendrin). ATO decreased thymidine and increased iodide uptake/cell. ATO appeared to act neither by interaction with the AKT-pathway nor by changes in the expression of sodium-iodide symporter and pendrin protein. Upon treatment with ATO apoptosis was increased, and TKTL-1 expression and glucose uptake reduced. TKTL-1 expression level was not related to the efficacy of ATO. GLUT-1 was expressed in all cell lines but the expression level was not changed upon treatment with ATO. ATO appears not to possess a uniform mode of action in thyroid carcinoma cell lines. In the cell lines investigated in this study, important targets of action were apoptosis, TKTL-1 and glucose uptake. ATO may represent an alternative re-differentiation treatment for certain thyroid carcinomas.

Thyroid cancer
Poster

EMPIRICAL 131I DOSE IN PATIENTS WITH THYROID CANCER WITH HIGH THYROGLOBULIN AND NEGATIVE DIAGNOSTIC WHOLE BODY SCAN

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Previous studies have shown a high rate of visualization of uptake and a decrease in serum thyroglobulin (Tg) after therapeutic doses of ^{131}I in well-differentiated thyroid cancer (DTC) patients with elevated Tg levels but negative diagnostic ^{131}I whole body scan (WBS). We evaluated the effect of ^{131}I in patients with elevated Tg levels but negative diagnostic WBS in 55 patients, 37 with papillary & 18 with follicular cancer, who underwent total or near-total thyroidectomy & remnant ablation and developed recurrent disease during the follow-up period. Tg levels ranged from 22-600ng/ml. All patients received ^{131}I therapy. Post ^{131}I WBS were still negative in 15 patients with papillary & 2 with follicular cancer. In the remaining 38 patients the post-treatment WBS was positive: a) out of 22 patients with papillary cancer, 6 presented uptake in the lungs, 3 in bones, 2 in the mediastinum & 11 had local recurrence or lymph nodes; 2) out of 16 patients with follicular cancer lung metastases were revealed in 8, lung/bone in 3, mediastinum in 3 & local recurrence in 2. Twelve months after ^{131}I therapy in all patients Tg levels were measured off thyroxine: a) in papillary were undetectable in 3 patients with positive & in 4 with negative post ^{131}I WBS, in further 6 with positive & in 4 with negative post ^{131}I WBS were reduced >50% of the pretreatment values and remained high in 20 patients; b) in follicular cancer, in only 1 patient were reduced >50% of the pretreatment value. In conclusion ^{131}I treatment in these patients has a therapeutic effect especially in papillary cancer (at least for palliation) during short-term observation and can disclose previously undiagnosed lesions (in some DTC patients).

Thyroid cancer
Poster

THE SECOND FOLLICULAR THYROID CARCINOMA PRESENTING AS A HOT THYROID NODULE WITH A SOMATIC I486F TSH-RECEPTOR (TSHR) GENE MUTATION

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Up to date four cases of follicular carcinomas presenting as a hot nodule with a TSHR mutation have been published. Here we report a further patient with a follicular thyroid carcinoma presenting as a hot nodule with a somatic TSHR mutation. A 62 years old patient was admitted to the department of pulmanory disease with the symptoms of dyspnea, cough, weakness, nervousness and palpitation. Multiple lung metastases were detected on CT. Histology obtained by bronchoscopy showed multiple lung metastases of a follicular thyroid cancer. High free T4 and free T3 and low TSH levels showed hyperthyroidism. Scintigraphy showed a large hot nodule in the thyroid gland with the suppression of the surrounding thyroid tissue. Total thyroidectomy was performed. Histology confirmed capsule and vascular invasion and the diagnosis of follicular carcinoma. Postoperatively the patient received ^{131}I for remnant ablation. DNA was extracted from fresh frozen tissue samples of the hot follicular thyroid carcinoma. Exons 9-10 of TSHR were screened by denaturating gradient gel electrophoresis (DGGE) and subsequent DNA sequencing. A TSHR gene mutation I486F was detected in exon 10 of the TSHR by sequencing the DNA obtained from purification of the DGGE band from the polyacrylamid gel. This mutation has previously been detected in a hot nodule and in a hot follicular thyroid carcinoma and has been shown to be constitutively activating. We present the 5th case of follicular thyroid carcinoma presenting as a hot thyroid nodule with a somatic TSHR gene mutation and the second case of a hot follicular carcinoma with the I486F mutation. Three of the five cases of hot follicular carcinomas with TSHR mutation presented with metastasis.

Thyroid cancer
Poster

DENDRITIC CELL EXPRESSION CD 83 IN SOLITARY THYROID NODULE

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Background: Thyroid carcinoma typically present as a dominant solitary thyroid nodule. Fine-needle aspiration biopsy (FNAB) is considered by most to be valuable in the evaluation of palpable thyroid nodules . At present, effort is directed to find additional markers of malignancy that may prove to be a useful adjunct to FNAB. ***Aim:*** To establish the role of dendritic cell (DC) expression as a complementary marker to fine needle aspiration biopsy in the diagnosis of solitary thyroid nodule. ***Study Design:*** As CD83 is expressed on mature dendritic Cells, CD83 +DC was studied by immunohistochemistry in 30 specimens from solitary thyroid nodules removed during surgery. They were compared to CD 83 + DC in surrounding tissue from the same patients. ***Results:*** CD83+DC was significantly higher in papillary carcinoma14.287±3.76 compared to normal tissue and to follicular carcinoma. DC expression was very low in medullary carcinoma 0.023±0.03 as compared to normal tissue and other cases of benign and malignant adenoma. The sensitivity of CD83+DC was 85 % and the specificity was 82%. ***Conclusion:*** CD 83 + DC expression in papillary carcinoma may help in its differential diagnosis from follicular carcinoma and other papillary variants. The very low DC expression in medullay carcinoma could help in the diagnosis of such cases in addition to fine needle aspiration biopsy. Dendritic cell expression potentially represents a new marker technique to improve the sensitivity and specificity in evaluating solitary thyroid nodule.

Thyroid cancer
Poster

IDENTIFICATION OF TWO NOVEL RET PROTO-ONCOGENE VARIANTS IN SPORADIC MEDULLARY THYROID CARCINOMA

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Medullary Thyroid Carcinoma (MTC) is a malignant tumour arising from calcitonin secreting parafollicular "C" cells. It can be either sporadic or inherited. The sporadic form corresponds to 75–90 % of cases. Contrasting with the high frequency of germline mutations of the proto-oncogene *RET* observed in patients harbouring the familial form, the frequency of somatic mutations observed in those with the sporadic form varies, from 23% to 69%, depending on the series. We aimed to comprehensively characterize *RET* somatic mutations in a series of 52 apparently sporadic MTC by direct sequencing of PCR products of exons 5, 8, 10–16. To definitively establish the somatic origin of a sequencing variant, germinal DNA was also studied whenever a variant was identified in tumour DNA. So far, we have already screened 34 tumours and the study of the remaining 18 tumours is ongoing. Results are summarized in the following table:

Affected exon	Type of alteration
Exon 10 (9.5%)	TGC620CGC (1 patient)
	TGC620TCC (1 patient)
Exon 11 (24%)	TGC630CGC (2 patients)
	TGC630GGC (1 patient)
	TGC634CGC (1 patient)
	2061del18 + TGC634TGT (1 patient)
Exon 15 (9.5%)	GTA882GTT + GCT883TTT (1 patient)
	GCT883TTT (1 patient)
Exon 16 (57%)	ATG918ACG (12 patients)

Mutations were present only in tumour DNA.

Conclusions: Somatic mutations were found in 62% of patients. In accordance to previous studies, the most frequent mutation was ATG918ACG. However, in more than 40% of cases the mutation was found elsewhere. Two novel mutations were identified: a heterozygous point mutation at codon 630 (TGC/GGC) and a deletion of 18 bp at exon 11 associated, in the same allele, with a silent nucleotide substitution at codon 634.

Thyroid cancer
Poster

REGULATION AND CHARACTERISATION OF CASPASE3 IN THYROID TUMOURS

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Caspase3 (C3) acts downstream of the apoptosome and activates several proteins being responsible for the processing of cell death. Disturbances in apoptosis are implicated in tumorigenesis in several tissues. The aim of this study was to define the role and potential regulatory mechanisms of C*3* in benign thyroid nodules and thyroid cancer. RT-PCR for *C3* and its regulators *bim* and *caspase3s (C3s)* was performed in 87 specimen, including toxic thyroid nodules (TTN), Graves' disease thyroids (GD), benign cold thyroid nodules (CTN), normal thyroid tissues and thyroid cancers (FTC, PTC PDTC and ATC). In addition, C*3* and activated *C3* protein expression was studied by immunohistochemistry in 109 benign and malignant thyroid pathologies and normal thyroids. (I) The mRNA level of *C3s* was significantly increased in FTCs, while mRNA expression of *C3* did not differ between FTCs and benign thyroid nodules. (II) Upregulation of *C3* mRNA was found in PTCs and GD. (III) *Bim* mRNA levels did not differ significantly between malignant and benign thyroid samples. (IV) *C3* protein expression was similar in thyroid malignancies and benign thyroid tissues. (V) In contrast, increased expression of activated C*3* protein was found in carcinomas compared to benign thyroid nodules, whereby activated *C3*showed a tumour specific distribution with nuclear staining in benign samples and exclusive cytosolic staining in FTCs and PTCs.The mRNA upregulation of the antiapoptotic splice variant *C3s* together with a lack of nuclear accumulation of activated *C3* in FTCs suggest a block in apoptosis and may involve altered nuclear membrane degradation leading to cytosolic protein retention. Since the mRNA of *C3s* was upregulated in FTCs, *caspase3s* may serve as a new specific thyroid tumour marker.

Thyroid cancer
Poster

PREOPERATIVE LOCATION OF METASTATIC CERVICAL LYMPH NODES WITH Tg-FNAB AND PATENT BLUE INJECTION FOR PATIENTS WITH RECURRENT DIFFERENTIATED THYROID CARCINOMA (RDTC): USEFULNESS FOR SURGICAL CURE?

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Purpose: The high reliability of thyroglobulin measurement in fine-needle aspiration biopsy (Tg-FNAB) to detect metastatic cervical lymph nodes in patients with RDTC is well established. Ultrasound guided injection of patent blue within pathologic node can improve its per-operative location. For patients with RDTC, node surgery consist in neck dissection or 'Berry picking', according to the extent of surgical lymph node at the initial surgery. The aim of this study was to evaluate the impact of these procedures on complete remission. ***Patients and Methods:*** We measured thyroglobulin (Tg) by IRMA in the needle wash-out of FNAB in 13 differentiated cancer patients (11 papillary, 2 follicular) suspected of persistence or recurrence disease on clinical criteria (2), ultrasound criteria (5) or elevated Tg (6). Whole body scan was negative. Tg-FNAB higher than serum Tg level was considered as positive. Neck dissection was performed in 5 patients who had initial surgery without neck dissection; Berry picking was performed in 8 patients who had previously neck dissection. Patent blue was injected within positive nodes under US control in 11 patients. ***Results:*** FNAB was malignant in 9, non diagnosed in 2 and unperformed in 2 patients. Tg-FNAB was positive in all 13 patients. Among the 5 patients with neck dissection, 3 were cured with normal clinical and sonography exams, undetectable stimulated serum Tg but 2 remained with persistent disease. Among the 8 patients with Berry picking, 5 were cured but 3 remained with elevated stimulated serum Tg. ***Conclusion:*** Tg-FNAB improved FNAB sensitivity to100%. Preoperative location with Tg-FNAB and patent blue helped to surgical procedure but don't predict surgery cure.

Thyroid cancer
Poster

THE POTENTIAL DIAGNOSTIC AND PROGNOSTIC ROLE OF F-18-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY IN THE INITIAL EVALUATION OF DIFFERENTIATED THYROID CANCER

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Context: The role of F-18-fluorodeoxyglucose positron emission tomography (PET) in the initial evaluation of differentiated thyroid cancer (DTC) has not been studied. ***Objectives:*** To compare PET with diagnostic radioiodine whole body scanning (DxWBS) in newly diagnosed DTC patients (pt), hypothesizing that PET is more likely to disclose cervical lymph node (CLN) and distant metastatses. We also assessed the prognostic value of PET at the initial evaluation of DTC. ***Methods:*** DxWBS and PET scanning were performed in 26 pt who had thyroidectomy but have not yet received radioiodine-131 and the 26 pairs of scans were compared. ***Results:*** Of the 26 PET scans, 18 scans (69.2%) were positive while 8 scans (30.8%) were negative. A total of 40 foci were seen in the 18 positive PET scans (14 foci thyroid bed, 15 foci in CLN, 1 focus in the lung, 1 focus in the bone, 7 foci in the axillae, and 2 foci in the breast). The corresponding DxWBS showed a total of 47 foci. In contrast to the uptake on PET which showed 26 foci (65%) outside the thyroid bed, 45 foci (95.7%) of uptake on DxWBS were in the thyroid bed while only 2 foci (4.3%) were in CLN and no focus was seen outside the neck area (p=0.00). Seven of the 8 negative PET scans were in stage 1 while all pt with disease higher than stage 1 had positive scans. Over a median follow-up period of 30 months (10–48), 7 of 8 pt (87.5%) with negative PET scans were in remission compared with only 8 of 18 pt (44.4%) with positive PET scans (p 0.045). ***Conclusions:*** PET is frequently positive at the initial evaluation of DTC. In contrast to DxWBS which reveals uptake mostly in the thyroid bed, PET is more likely to reveal CLN or distant metastases. There is a correlation between PET positivity, the TNM stage and the long-term outcome.

Thyroid cancer
Poster

COMBINED ANALYSIS OF GALECTIN-3 AND BRAFV600E IMPROVES THE ACCURACY OF FINE-NEEDLE ASPIRATION BIOPSY WITH INDETERMINATE CITOLOGY

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The sensitivity and specificity of fine-needle aspiration biopsy (FNAB) are high. However, 5-15% of results are uncertain. Among the most promising markers of malignancy, galectin-3 (Gal-3) is higly sensitive but less specific, while the oncogene $BRAF^{V600E}$ has poor sensitivity and high specificity. ***Aim:*** It was to determine wether the combined analysis of Gal-3 and $BRAF^{V600E}$ in thyroid aspirates could improve the diagnosis in FNAB with indeterminate cytological findings. ***Patients and Methods:*** 243 surgical thyroid tissues and 94 thyroid aspirates with indeterminate findings, including benign lesions, papillary (PTC) and follicular (FTC) carcinomas were analyzed for the presence of Gal-3 by immunohistochemistry and for $BRAF^{V600E}$ by mutant allele-specific PCR amplification (MASA). ***Results:*** Gal-3 expression was present in 33.8% benign nodular goiters, in 91.9% PTC and in 75.0% FTC. $BRAF^{V600E}$ was not detected in benign lesions, as well as in FTC, while was found in 42.9% PTC. No correlation was found between BRAF mutation and Gal-3 expression. 47 consecutive FNAB with indeterminate results, were analyzed for the presence of the two markers. 23 nodules resulted benign lesions at histology, none of witch displayed $BRAF^{V600E}$, 26.1% were positive for Gal-3 and 73.9% were negative for both markers. 20 nodules resulted PTC and four FTC at histology. Of these 24 carcinomas, 37.5% resulted positive for $BRAF^{V600E}$, 70.8% for Gal-3 and 91.7% for one or both the markers. ***Conclusions:*** The sensitivity, specificity and accuracy for the presence Gal-3 and/or $BRAF^{V600E}$ were 91.7%, 73.9% and 83.0% respectively, significantly higher than those obtained for Gal-3 alone (70.8%, 73.9% and 72.3% respectively). We propose this method to improve accuracy of FNAB with indeterminate cytologic findings.

Thyroid cancer
Poster

IS A 2nd RH-TSH STIMULATION TEST (2-3 YEARS AFTER THE FIRST ASSESSMENT) NEEDED IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA (DTC) WHO HAVE UNDETECTABLE BASAL SERUM THYROGLOBULIN (TG) LEVELS?

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Purpose: Follow-up of DTC consists of basal/rhTSH-stimulated serum Tg and neck-ultrasound (US) with/without 131-I WBS, 6-8 months after thyroidectomy and ablation. At this time, most patients (~80%) are in remission (negative neck-US and rhTSH-Tg). Whether and when these patients need further rhTSH-Tg is controversial. ***Methods:*** To answer this question, 87 AbTg-negative DTC patients underwent a 2nd rhTSH-Tg and neck-US, 2-3 years after 1st evaluation. At the time of 1st evaluation, 68/87 patients (~78%; Group A) had undetectable rhTSH-Tg. All but one had negative US and were in remission likely. Only 1 patient had evidence of disease at neck-US and received therapy. 19/87 patients (~22%; Group B) had positive rhTSH-Tg (>1 ng/ml), with no evidence of disease in 12 and evidence of disease (positive neck-US or other imaging) in 7. ***Results:*** All patients with no evidence of disease (67 of Group A and 12 of Group B) were reevaluated 2-3 years later with rhTSH-Tg and neck-US. In Group A all patients had undetectable basal serum Tg which remained undetectable after rhTSH in 66/67 (~98%) while became >1 ng/ml in 1 patient (1.5%) who had positive neck-US. Also in Group B basal serum Tg was undetectable in all 12 patients and remained undetectable in 2 patients with negative neck-US, while converted to >1 ng/ml in 7. Compared to the 1st rhTSH-Tg, stimulated-Tg decreased in 2 patients, increased in 3 and remained stable in 2. Three other patient are awaiting their follow-up. ***Conclusion:*** The 2nd rhTSH-Tg was informative in most patients of Group B but not in Group A where the only patient with local recurrence was diagnosed by neck-US. Thus, rhTSH-Tg should be repeated only in patients who have positive 1st rhTSH-Tg and who continue to display undetectable basal serum Tg 2–3 years later.

Thyroid cancer
Poster

PREOPERATIVE DIAGNOSIS OF PAPILLARY THYROID CARCINOMA BY DETECTION OF B-RAFT1799A MUTATION ON FINE NEEDLE ASPIRATION BIOPSY SPECIMENS

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The prevalence of B-RAF^{T1799A} mutation in thyroid tumours has been reported to be 20-80% and specifically associated with papillary thyroid carcinoma (PTC). B-RAF^{T1799A} mutation can be detected on fine-needle aspiration biopsy (FNAB) specimens and has been proposed as an useful diagnosis tool to improve the accuracy of FNAB in the evaluation of thyroid tumours. ***Aim:*** Evaluate the usefulness of B-RAF^{T1799A} mutation status in the preoperative diagnosis of PTC. ***Materials and Methods:*** FNAB thyroid smears from 262 patients considered for thyroid surgery were analyzed for the presence of the B-RAF^{T1799A} mutation by genomic DNA extraction and polymerase chain reaction (PCR)-restriction fragment polymorphism (RFLP) analysis. DNA sequencing was performed in positive samples to confirm the presence of the B-RAF^{T1799A} mutation. ***Results:*** Cytological diagnosis was suspicious of PTC in two patients, and B-RAF^{T1799A} mutation was positive in both. Histopathology after thyroid surgery of these patients confirmed the diagnosis of PTC. In 260 cases, the cytological diagnosis was negative for PTC. Five of these patients were positive for the presence of the B-RAF^{T1799A} mutation. Two patients underwent thyroidectomy and PTC was confirmed histopahologically. The other three patients are included in our Hospital's protocol for surgery. ***Conclusion:*** The determination of the presence of B-RAF^{T1799A} mutation in FNAB has shown to be an useful tool to predict the diagnosis of PTC in specimens not detected by cytological analysis. This findings place the B-RAF gene as a powerful biomolecular marker for assessing the nature of a thyroid nodule, and a potential application in the preoperative protocol.

Thyroid cancer
Poster

DIARRHEA BURDEN IN PATIENTS (pts) WITH MEDULLARY THYROID CANCER (MTC): IMPACT OF AMG 706 ON DIARRHEA SYMPTOMS (Sx) - INTERIM RESULTS

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Purpose: To describe the burden of MTC-related diarrhea and impact on diarrhea sx of AMG 706, an oral, investigational inhibitor of angiogenesis with antitumor activity, in pts with MTC. ***Methods:*** 91 pts with locally advanced or metastatic MTC enrolled in an ongoing Ph2, single-arm study of AMG 706. A newly developed, 11-item MTC Related Sx Questionnaire (MTCRSQ) was administered at baseline (BL) and q8w until wk 48 (or withdrawal) to assess diarrhea frequency (number of episodes/day), severity, and sx interference (sleep, work, etc). Descriptive analyses of BL to wk 8, 16, and 24 (last value carried forward imputation method, no adjustments for multiple comparisons) included pts completing BL and ≥1 MTCRSQ assessment (n=78; MTC cohort) and pts reporting ≥1 episode of diarrhea/day at BL (n=55; diarrhea subgroup). ***Results:*** In the MTC cohort there were no changes, based on MTCRSQ scores, between BL and wk 24 in diarrhea frequency (mean [range] 3.9 [0–15] vs 3.9 [0–20]), severity (mean [SE] 56.7 [4.5] vs 53.3 [4.4] on a 0-100 scale; 100=least severe), and sx interference (mean [SE] 74.3 [3.2] vs 71.7 [2.9] on a 0-100 scale; 100=no interference). In the diarrhea subgroup, there was a decrease from BL to wk 16 and wk 24, respectively, in diarrhea frequency (mean [range] 5.2 [1–15] vs 4.0 [0-15], $p<0.05$; or 4.5 [0–20] at wk 24) and severity (mean [SE] 42.7 [4.6] vs 56.4 [4.7], $p<0.05$; or 52.7 [4.6] at wk 24). Sx interference was unchanged (mean [SE] 66.0 [3.7] vs 70.4 [3.2]; or 68.8 [3.4] at wk 24). ***Conclusion:*** Interim results suggest that MTC pts in this Ph2 study who had diarrhea at BL showed improvement in diarrhea frequency and severity in the first 16 wks of AMG 706 therapy. Updated data will be presented.

Thyroid cancer
Poster

EXACERBATION OF POSTSURGICAL HYPOTHYROIDISM DURING TREATMENT OF ADVANCED DIFFERENTIATED (DTC) OR MEDULLARY (MTC) THYROID CARCINOMA WITH AMG 706

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Purpose: To investigate the effect of AMG 706, an oral, investigational angiogenesis inhibitor, on hypothyroidism (previously documented with other class molecules) in a phase 2 safety/efficacy study in patients (pts) with advanced DTC or MTC and postsurgical hypothyroidism. ***Methods:*** Multicenter, open-label, single-arm study. Eligible patients (DTC n=93; MTC n=91) received AMG 706 125mg QD until disease progression or unacceptable toxicity. AM thyroid function was tested prior to the first dose and q4w while on treatment. Sera were assayed by EIA for TSH (reference range 0.32–5.00 mU/L; interassay CV 7.69% at 0.62 mU/L) and free T4 (reference range 0.7–1.9 ng/dL; interassay CV 3.94% at 1.28 ng/dL). ***Results:*** For the DTC cohort, the baseline (BL) geometric mean TSH (95% CI) was 0.11 mU/L (0.08–0.15); mean fT4 was 1.57 ng/dL (1.48–1.65). TSH peaked at 0.84 mU/L (0.51–1.38) ($p<0.0001$ compared with BL) at a mean 13.1 wks on treatment, while fT4 fell to 1.29 ng/dL (1.20–1.38). TSH rose >10-fold in 43% of DTC pts and >100-fold in 15%. Of 69 DTC pts with low BL TSH, 28% had a peak TSH within and 22% above the reference range. For the MTC cohort, the BL geometric mean TSH was 0.69 mU/L (0.48–1.00); mean fT4 was 1.25 ng/dL (1.18–1.31). TSH peaked at 5.88 mU/L (4.36–7.95) ($p<0.0001$ compared with BL) at a mean 13.5 wks on treatment, while fT4 fell to 1.06 ng/dL (1.02–1.11). TSH rose >10-fold in 48% of MTC pts and >100-fold in 9%. Of 76 MTC pts with BL TSH below or within reference range, 61% had a peak TSH above that range. ***Conclusion:*** In this study, AMG 706 therapy was associated with marked exacerbation of postsurgical hypothyroidism in DTC and MTC pts, suggesting close TSH monitoring and thyroxine dose changes to maintain appropriate TSH levels in these pts.

Thyroid cancer
Poster

THE ROLE OF DIAGNOSTIC IODINE 123 Whole Body Scan (WBS) IN DIFFERENTIATED THYROID CANCER

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Purpose: Differentiated thyroid cancer is treated by thyroidectomy and in selected cases ^{131}I. Some treat with ^{131}I without a diagnostic scan, others use ^{131}I in doses ranging from 37–370 MBq. That raises concern of stunning and low sensitivity. We evaluated ^{123}I (WBS). ***Patients and Methods:*** ^{123}I (74MBq) was administered to 132 patients (99 women average age 41 years and 33 men average age 53 years). All patients had been treated by thyroidectomy and 57 with ^{131}I. ***Results:*** 87 had positive ^{123}I scans and 85 were treated with ^{131}I. 75 (88%) had similar post therapy scans (PTS). In 7 (8%) the PTS was more abnormal and the stage was changed in 2. One showed new bone uptake and one hepatic, however, follow-up scan and Tg have been negative. ^{123}I showed more in 3 (4%). The remaining 2 with very high uptake were referred for completion of thyroidectomy. 31 had negative scans and were not treated. 28 had low stimulated Tg. 29 have no recurrence, 2 were lost to follow-up. Seven had negative ^{123}I scans but high Tg (mean 117 ng/ml average TSH 64mIU/l)) and were treated with a mean of 5.4 GBq ^{131}I. Six had negative PTS and one showed faint uptake in the thyroid bed. No patient has achieved an undetectable TG. Six had negative scans and high Tg (mean 305 ng/ml, average TSH 80 mIU/ml) and were treated by operation after PET or PET/CT and ultrasound identified a lesion. One other patient with a negative ^{123}I scan and Tg of >16,000 ng/ml) was treated by chemotherapy. ***Conclusion:*** A positive ^{123}I WBS has a high sensitivity compared to PTS. A negative ^{123}I scan has a moderate specificity, 29 of 43 (67%) patients followed. A negative ^{123}I scan predicts a negative PTS and implies ^{131}I treatment would not be of value.

Thyroid cancer
Poster

THERAPEUTIC IMPACT OF 124I POSITRON EMISSION TOMOGRAPHY DOSIMETRY IN ADVANCED DIFFERENTIATED THYROID CANCER

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Purpose: This study evaluated the impact of ^{124}I positron emission tomography (PET) dosimetry on post-primary surgery therapy in radioiodine-naïve patients with advanced differentiated thyroid cancer (DTC). ***Material and Methods:*** In each of 28 thyroidectomized patients with high-risk DTC, we gave 23-50 MBq of ^{124}I as an oral capsule and performed PET dosimetry to calculate the individualized therapeutic ^{131}I activity that would, insofar as possible, achieve a radioiodine dose ?100 Gy to all metastases without exceeding 2 Gy to the blood (a surrogate for bone marrow toxicity). We did so by determining the absorbed lesion dose per GBq of administered ^{131}I activity (LDpA) based on serial PET and the critical blood activity (CBA) based on serial blood and whole-body radiation counting. We compared the actual dosimetry-based interventions with standard empirical protocol. ***Results:*** 25 patients had a total of 126 iodine-positive metastases. Eighteen (72%) of the 25 had only iodine-avid metastases, while seven had both iodine-avid and -non-avid metastases. In two patients (7%) none of the metastases could have been treated with a sufficient radiation dose. Relative to the empirical protocol, ^{124}I PET dosimetry findings changed management in 7 (25%) patients e.g. applying activities > 11 GBq ^{131}I. Further changes included implementation of hematological back-up in a patient found to be at risk of life-threatening marrow toxicity, and early multimodal therapy in 9 (32%) patients. ***Conclusion:*** ^{124}I PET dosimetry is a useful routine procedure in advanced DTC and may allow safer or more effective radioiodine activities and earlier multimodal interventions than do standard empirical protocols.

Thyroid cancer
Poster

SIMILARITIES AND DIFFERENCES IN DIFFERENTIATED THYROID CANCER GUIDELINES

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Background: Diagnostics and treatment of thyroid cancer has changed. These changes were inspired by results of studies and meta-analyses. As these results are all available it is puzzling why recently published guidelines still show striking differences. Although advances have been made, lack of evidence still maintain the existence of controversy. But also “global evidence” can be and in fact is interpreted in various ways. British,European,American and Dutch guidelines elucidate the differences in items that are founded on evidence as well as in recommandations based on expert opinion. Especially expert opinion recommendations illustrate local preferences. ***Results:*** Comparison reveals remarkable differences: 1Diagnostic procedures: examination of incidentalomas and calcitonin are not routinely advised in the British and Dutch guidelines 2Initial treatment reveals discrepancies in risk stratification, extension of surgery and application of ablation 3Follow up protocols differ, in frequency of control, interpretation of thyroglobulin and indication for radioiodine retreatment 4Organization of care:In the European and American guidelines the multidisciplinary team is not defined. The necessary skills and experience of surgeons are specifically mentioned in the Dutch. ***Implications****:* Although thyroidcancer has a good prognosis aspects of quality of life are important to find a balance between optimal follow-up strategies and minimal physical/psychological burden. Also cost-effectiveness have to be taken into account. To minimize these differences clinical trials have to be performed and possibly an institution of an international expert panel could support this initiative.

Thyroid cancer
Poster

THE FOLLOW-UP OF PATIENTS WITH DIFFERENTIATED THYROID CANCER AND UNDETECTABLE Tg AND TgAb DURING ABLATION

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Context: An undetectable thyroglobulin (Tg) at the time of ablation therapy without the presence of antibodies means a clinical dilemma. The clinical outcome of this specific group is not clear as is the follow-up strategy. ***Objective:*** This retrospective study describes the role of serum Tg in relation to tumor characteristics in the prediction of recurrence in patients with differentiated thyroid cancer (DTC) with negative Tg at the time of ablation. ***Design:*** Between 1989 and 2006, 94 of 346 (27%) consecutive patients with DTC had undetectable Tg at the time of ^{131}I ablation, and were included in this evaluation. The group of 94 patients consisted of 15 males and 79 females with age ranging from 16 to 89 years. Median follow-up was eight (range 1–17) years. All medical records and follow-up parameters of the 94 patients were evaluated for the occurrence of recurrent disease. In patients with recurrent disease hematoxylin-eosine-stained slides of the primary tumors and/or metastatic lesions were also reviewed for histologic features including immunostains for Tg. ***Results:*** Eight of 94 (8,5%) patients developed recurrent disease during follow-up: in the course if the disease two patients showed Tg positivity, three showed TgAb positivity and the other three showed persistently undetectable Tg and TgAb. Patients who developed Tg and/or TgAb positivity during follow-up had a significant shorter disease-free survival period compared to patients with persistently undetectable Tg and TgAb ($p<0.006$). Histological features and Tg immunostaining were not able to predict the recurrent status. ***Conclusions:*** Tg and TgAb negativity at the time of ablation is not a predictive determinant for future recurrent status.

Thyroid cancer
Poster

EVALUATION OF A CENTRAL AND LATERAL LYMPH NODE DISSECTION (CLLND) SURGICAL PROTOCOL IN THYROID DIFFERENTIATED CANCER (DTC) FROM A REGIONAL TUMOR REGISTRY

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Purpose: The aim of this study was to evaluate first if an established surgical protocol may be well observed, second the quality, the safety and the efficiency of total thyroidectomy with CLLND in the treatment DTC. ***Methods:*** In 1993 the regional work group established the surgical protocols of DTC treatment.When total thyroidectomy is performed in patients with DTC over 10 mm, radical lymph node dissection has to be carried out when lymph nodes metastases are evident either clinically or by sonography. For patients without evidence of lymphadenopaty, routine ipsilateral level VI, IV, Vc nodes dissection is performed. From the data base of 142 patients treated for DTC between 1995 and 2000, from a prospective tumour Registry, we studied: Number of lymph nodes per dissection, hypoparathyroidism and recurrent laryngeal nerve (RLN) injuries and local events (mean follow-up of 8.7 years). ***Results:***. The DTC diagnosis is made before surgery by cytological examination for 65 patients and during surgery for 77 patients. 132 patients have CLLND. The proportion of patients with lymph node metastasis is 47.7%. CLLND (>6 nodes) is a good quality for 80% of patients. 13 (10%) patients have permanent hypoparathyroidism and 12 (9%) have permanent RLN injury. 7 (5%) patients recurred and have repeat operation. 10 patients have no CLLND, none have complication but 3 (30%) are reoperated for nodes metastasis. 117 /142 patients (83%) are cured with normal clinical and sonography exams and Tg level undectable under stimulation at the end of the study. ***Conclusion:*** The surgical protocol is well observed for 93% of patients. Despite a high level of hypoparathyroidism and RLN injuries we conclude that total thyroidectomy with CLLND allow to lower lymph nodes recurrences when the DTC diagnosis was made before or during surgery.

Thyroid cancer
Poster

OXIDATIVE DNA DAMAGE AND REPAIR IN THYROID TUMOURS

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Oxidative stress could be an important mechanism in the pathogenesis of thyroid diseases. We performed an IHC study to address the extent of 8-hydroxy-2`-deoxyguanine (8oxoG) formation, a key indicator of oxidative DNA base damage, in benign and malignant thyroid nodules. In addition, we studied the expression of OGG-1, MYH and NTH, enzymes responsible for repair of oxidative DNA damage. Ten toxic thyroid nodules (TTN), 14 cold benign thyroid nodules (CTN), their respective normal surrounding tissues (NT), 20 follicular (FTC), 18 papillary (PTC), 2 anaplastic (ATC), 5 medullary thyroid cancers (MTC) and 7 Graves` disease (GD) samples were investigated.
Thyroid cancers showed a strong, morphology-specific subcellular 8oxoG staining pattern. In PTC cytoplasmatic labelling prevailed. In contrast, prominent nuclear staining occurred in FTC. Comparison of differentially functioning benign nodules showed at least 2x increase in cytoplasmatic labelling in CTN compared to NT, while no difference was observed for TTN, their NT and GD. OGG-1, MYH and NTH mRNA expression was detected in all thyroid tissues with a significant downregulation of all 3 repair enzymes in FTC. In PTC we also observed downregulation but this failed to reach significance.
The present study suggests increased oxidative stress as a feature of benign and malignant thyroid nodules. Distinct subcellular localisation of 8oxoG labelling in these pathologies may reflect different degrees of mitochondrial or nuclear oxidative DNA damage, which, if accompanied by defective DNA repair, is known to increase risk for mutational events. This may propagate e.g. cell proliferation and dedifferentiation and could thus lead to thyroid cell transformation.

Thyroid cancer
Poster

ANTIPROLIFERATIVE EFFECT OF TYPE I PROTEIN KINASE A ACTIVATION IN POORLY DIFFERENTIATED THYROID CANCER

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cAMP-dependent protein kinase A (PKA) exists as two different isozymes, type I and II, depending upon the specific regulatory subunits. We previously demonstrated an anti-proliferative effect of specific PKAI stimulation with selective phosphodiesterases-resistant cAMP analog pair in human thyroid carcinoma cell lines. In this study we tested the efficacy of 8-Cl-cAMP, a potential anticancer agent. 8-Cl-cAMP is a stimulator of PKA but its antitumoral action involves undefined non-PKA mechanisms probably involving its degradation products. We compared proliferation data obtained with increasing concentration (1–400 μM) of 8-Cl-cAMP and PKAI analog pairs in de-differentiated papillary (ARO, NPA) and follicular (WRO) thyroid carcinoma cells. We used FRTL-5, a TSH-dependent thyroid differentiated cell line, as control. All these cell lines express PKAI regulatory subunits. In FRTL-5, selective PKAI activation had no proliferation effect and 8-Cl-cAMP caused a limited mitogenic effect only at the maximal concentration, thus excluding a toxic action by these compounds. In WRO, NPA and ARO cells, 8-Cl-cAMP had a potent inhibitory effect on growth (55–75% reduction) with an IC_{50} ranging 2–15 μM. Selective PKAI stimulation had similar antiproliferative effects only in BRAF-mutated ARO and NPA, but with a higher IC50 (>37 μM). A pro-apoptotic effect was associated only with 8-Cl-cAMP treatment. In conclusion, 8-Cl-cAMP has potent anti-tumoral effects on thyroid cancer cells that are independent of the histotype and oncogenes that are involved in cell transformation. These data support the view that 8-Cl-cAMP action involves non-PKAI mechanisms. 8-Cl-cAMP may have a therapeutic potential for the treatment of poorly differentiated thyroid cancer in vivo.

Thyroid cancer
Poster

EFFECTS OF 1,25(OH)2D3 And Analog WY1112 ON PROLIFERATION AND DIFFERENTIATION OF FRO CELLS

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Introduction: Dedifferentiated thyroid cancer fails to respond to standard treatment. The development of new treatments inhibiting proliferation and/or inducing redifferentiation is crucial to improve the prognosis. ***Purpose:*** To investigate the effects of $1,25(OH)_2D_3$, the active form of vitamin D, and WY1112, a vitamin D-analog, on proliferation and differentiation in the FRO cell line, a poorly differentiated human follicular cell line. ***Results:*** Proliferation (3H-thymidine incorporation) was reduced up to 40% compared to vehicle-treated cells after 72 hrs of incubation with $1,25(OH)_2D_3$ (10^{-6}M). In the presence of WY1112, a comparable antiproliferative effect was observed at 10^{-8}M. Absolute cell counts declined 50% after 3 days of incubation with $1,25(OH)_2D_3$ and up to to 65% after 5 days. An additive effect was seen when $1,25(OH)_2D_3$ was combined with paclitaxel. Cell cycle analysis (FACS) showed that $1,25(OH)_2D_3$ increased the percentage FRO cells in the G0-G1 phase (60% to 72%) and decreased the percentage of cells in the G_2-G_m phase (8% to 5%) and in the S-phase (32% to 23%). WY1112 shows similar effects at lower concentrations. On mRNA level, $1,25(OH)_2D_3$ increased p21 expression and decreased expression of cyclinD1, Ki67 and E2F1. In vieuw of redifferentiation, we observed that $1,25(OH)_2D_3$ increased the mRNA expression of NIS and TPO. No effect was seen for TSH-receptor expression. Currently, the uptake of radioactive iodine is being studied in FRO cells after incubation with $1,25(OH)_2D_3$.***Conclusion:*** $1,25(OH)_2D_3$ is promising as an antiproliferative and redifferentiating agent in the treatment of dedifferentiated thyroid cancer, where standard therapie fails, alone or in combination with paclitaxel.

Thyroid cancer
Poster

IS THERE A HIGH-RISK GROUP OF PATIENTS WITH LYMPH NODE METASTASIS FROM WELL DIFFERENTIATED THYROID CANCER?

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Well differentiated thyroid cancer (WDTC) frequently metastasizes to cervical lymph nodes (N1). However, the clinical significance of N1 is uncertain. The purpose of our study was to try to isolate a high-risk group for long-term, cause specific survival and recurrence rates among N1 WDTC. ***Patients and Methods:*** Patients who had total thyroidectomy and radioiodine ablation for WDTC with initial N1 in our institution from 1978 to 2006 were studied. The following data were reviewed: age, gender, pathology (type and size, multifocality, tumour extension beyond the thyroid capsule, distal metastasis) and cancer-related death. ***Results:*** 302 patients with N1 WDTC were studied; 23% had a recurrence in a median delay of 1.6 year. No differences between recurrent and nonrecurrent N1 WDTC patients were observed for age, gender, thyroid function, multifocality, extrathyroidal extension of the tumor and distal metastasis. Patients with follicular thyroid carcinoma (40.9%) presented a higher recurrence rate than those with papillary carcinoma (21.4%, p=0.036). The tumor size was also a risk factor since 10.4% microcarcinoma developed a recurrence vs. 27% carcinoma over 10 mm in diameter (p=0.0026). Cancers that involved both thyroid lobes 30% vs. 19% carcinoma limited to one thyroid lobe (p=0.029) and vascular invasion 33% vs. 19% without vascular invasion (p=0.016) had a higher rate of recurrence. The cancer-related death rate was higher in patients who developed a recurrence 11.6% than in patients without recurrence 4.3% (p=0.024). **Conclusion:** A group of patients with N1 WDTC are at high risk of recurrence, especially patients with follicular carcinoma, multicentric, bilateral, and large tumors, and tumors with vascular invasion. This group should have an intensive follow-up to detect and treat recurrences at an early stage.

Thyroid cancer
Poster

FasL (APO-1L/CD95L) IMMUNOREACTIVITY IN PAPILLARY MICROCARCINOMA OF THE THYROID

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Introduction: Fas ligand (FasL) is a transmembrane protein of tumor necrosis factor family and induces apoptosis by binding to and activating the Fas (APO-1/CD95) receptor. Fas and FasL expression has been described in thyroid carcinoma, the presence of FasL in papillary microcarcinoma lesions of the thyroid is unclear. It was aimed to investigate the presence of FasL immunohistochemically in papillary microcarcinoma (PMC) of the thyroid. ***Materials and Methods:*** We evaluate the FasL immunoreactivity in patients with PMC of the thyroid. For this purpose, paraffin sections of thyroid specimens obtained from 47 papillary thyroid carcinoma consecutive patients with at least 5 yr follow-up were stained using antibody to FasL. ***Results:*** There were 15 patients with <5 mm, 19 patients with 6–10 mm and 13 patients with 10–15 mm according to tumor size. The percentage of FasL immunreactivity and the mean of intensity were significantly higher in lesions of PMC of the thyroid than peripheral thyroid tissue (26.8±37.1% and 1.23 ± 1.25 vs. 1.1±3.1% and 0.28±0.77, respectively; $p<0.001$). There were no differences in the percent and the intensity of FasL immunreaction according to age (below vs above at 45 years), to tumor size (> 10 mm vs <10mm), to the presence of tumor capsule and tumor invasion, invasion of thyroid capsule, peripheral tissue invasion, vascular invasion and multicentricity ($p>0.05$). However, FasL positivity was significantly higher in patients above 45 years old (19/26) versus below 45 years (6/21) (in respectively 73.1 % and 28.6 %, $p=0.002$). ***Conclusions:*** In the present preliminary study, we have shown that FasL is present in PMC of the thyroid with increased immunoreactivity above 45 years.

Thyroid cancer
Poster

FOLLOW-UP OF DIFFERENTIATED THYROID CANCER PATIENTS WHO UNDERWENT RADIOIODINE ABLATION OF POSTSURGICAL THYROID REMNANTS AFTER RECOMBINANT HUMAN THYROTROPIN (rhTSH) Or Thyroid Hormone Withdrawal

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We demonstrated equivalent thyroid remnant ablation rates in two groups of postoperative thyroid cancer patients prepared for administration of 3.7GBq of ^{131}I either with rhTSH, euthyroid group (EU) n=33, or after withholding L-T4 therapy, hypothyroid group (HYPO) n=30. We compared the outcome of the EU and HYPO patients 3–4 years after ^{131}I administration. Sixty-one of 63 patients were eligible for enrollment in this follow-up study. Of the 61 contacted patients, 51 (28 EU, 23 HYPO) participated (median follow-up 3.7 yrs). All but 3 patients underwent rhTSH administration with standard procedures and serum samples were collected for (thyroglobulin) Tg and anti-Tg measurement. A ^{131}I whole body scan (WBS) was performed in 43 patients and evaluated by 3 independent readers. Successful ablation was defined according to criteria used in the ablation study. According to the "uptake <0.1% in thyroid bed" criterion, 100% of patients (43/43) remained ablated. When the "no visible uptake" criterion was used, 5 patients (4 EU, 1 HYPO) had visible uptake (rate of ablation: 84% EU *vs* 94% HYPO). Forty-five patients could be evaluated for stimulated-Tg, and all but 2 (1 EU, 1 HYPO) had a stimulated Tg<2ng/ml (low Tg: 95% EU *vs* 96% HYPO). Seven (3 EU, 4 HYPO) patients received additional treatment (surgery or ^{131}I) between the two studies based on detectable Tg or evidence of local disease by neck ultrasound or other imaging. At the time of this study, all patients had a negative rhTSH-stimulated WBS and 5/7 (2 EU, 3 HYPO) had stimulated Tg<2 ng/ml. In conclusion after a median follow-up of 3.7 yrs, thyroid cancer patients prepared for postoperative remnant ablation with rhTSH and HYPO had comparable remnant ablation and disease recurrence rates.

Thyroid cancer
Poster

PRIMARY CELL CULTURES OBTAINED BY FINE-NEEDLE ASPIRATION FROM ANAPLASTIC THYROID CANCERS: RESULTS OF CHEMOSENSITIVITY TESTS

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Purpose: Anaplastic thyroid cancer (ATC) is often unoperable and chemotherapy and radiotherapy are the main treatments. The sensitivity of ATC from different subjects to chemotherapy and radiotherapy is widely variable. The possibility to test the sensitivity of ?primary ATC cell cultures? (ANA) from each subject to antiblastics could permit to increase the effectiveness of treatments. Until now ANA were obtained from surgical biopsies. The possibility to develop ANA from fine needle aspiration (FNA) could permit to avoid unnecessary surgical procedures. ***Methods and Results:*** Primary cell cultures were obtained by FNA (FNA-ANA) from 4 patients with ATC and the chemosensitivity of FNA-ANA to antiblastics was evaluated. FNA-ANA from the ATC patients were cultured in RPMI 1640 and propagated in Coon's medium. The chemosensitivity was evaluated by the inhibition of the proliferation of FNA-ANA (analyzing the number of viable cells by the cleavage of tetrazolium salts), in presence of increasing concentration of four different antiblastics: bleomycin, cisplatin, gemcitabine and etoposide. Antiblastics significantly inhibited (>50%) FNA-ANA proliferation. Another ANA culture was obtained from a biopsy specimen, for each patient; the results for the chemosensitivity tests were quite similar to that obtained with FNA-ANA. ***Conclusions:*** Our study demonstrates the possibility to obtain FNA-ANA, and opens the way to the use of FNA-ANA to test the chemosensitivity to different antiblastics (and possibly the radiosensitivity) in each patient, avoiding unnecessary surgical procedures and the administration of inactive chemotherapeutics.

Thyroid cancer
Poster

ADDITIVE ANTIPROLIFERATIVE ACTIVITY OF PparGAMMA AGONISTS AND ANTIBLASTICS IN PRIMARY CULTURED ANAPLASTIC HUMAN THYROID CANCER CELLS

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Purpose: Agonists (TZD) of peroxisome proliferator-activated receptor-gamma (PPARg) induce apoptosis and exert antiproliferative effects on continuous anaplastic carcinoma cell lines. Until now, no study has evaluated the possible antiproliferative effect of TZD in "primary cultured anaplastic carcinoma cells" (ANA), that could be very useful to identify patients responsive to therapy, and no comparison has been reported with the antiproliferative effect of antiblastics, or about a possible additive antiproliferative effect. ***Methods:*** The proliferation of ANA cells was evaluated by: 1) cell counting; 2) a proliferation assay (WST-1). ANA were incubated with increasing concentrations of two different PPARg agonists (rosiglitazone, pioglitazone), or four different antiblastics (bleomycin, cisplatin, gemcitabine and etoposide), or the combination of PPARg and antiblastics. ***Results:*** The results of WST-1 assay, mean of 7 different experiments, showed a reduction of proliferation by TZD with respect to the control both with rosiglitazone (20 and 30 mcM; respectively, 20% and 28%; $p<0.001$) or with pioglitazone (20 and 30 mcM; respectively, 20 and 24%; $p<0.001$). Bleomycin, cisplatin, etoposide and gemcitabine significantly (>50%) inhibited ANA proliferations. An additive antiproliferative effect ($p<0.05$) was observed with rosiglitazone 30 mcM in combination with bleomycin or with etoposide, and with pioglitazone 30 mcM in combination with bleomycin. Cells counting confirmed the above mentioned results. Conclusions: TZD exert an antiproliferative effect in ANA in vitro; an antiproliferative effect of antiblastics was observed, too; TZD in combination with antiblastics have additive antiproliferative effect in human anaplastic thyroid cancer.

Thyroid cancer
Poster

CALCITONIN MEASUREMENT IN WASH-OUT FLUID FROM FINE NEEDLE ASPIRATION OF NECK MASSES IN PATIENTS WITH PRIMARY AND METASTATIC MEDULLARY THYROID CARCINOMA

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Objective: To evaluate the usefulness of calcitonin (CT) assay in fine needle aspiration biopsy (CT-FNAB) wash-out fluid alone or combined to cytology in the pre-surgical study of medullary thyroid carcinoma (MTC) patients with thyroid nodules and of suspicious neck MTC recurrences/metastases. ***Subjects and Methods:*** Thirthy-six ultrasound (US)-guided FNAB were performed in neck masses from 23 patients with borderline or high basal and pentagastrin (PG) stimulated serum CT. Cytology and CT-FNAB were performed on a total of 18 thyroid nodules (TN) and 3 neck lymph nodes (LN) from 12 patients examined before thyroidectomy and on 6 suspicious local recurrences (LR) and 9 LN from 9 totally thyroidectomized MTC patients. On the basis of CT-FNAB values found in 15 non-MTC lesions, CT-FNAB >36 pg/ml was considered as indicative of MTC. ***Results:*** All the 21 positive CT-FNAB lesions (10 TN, 6 LN and 5 LR), 13 with positive cytology, were confirmed as MTC at histology. Among the 15 negative CT-FNAB suspicious masses (8 TN, 6 LN and 1 LR) 5 displayed a benign lesion at histology. The remaining 10 cases, all with benign cytology, were not operated and no evidence of MTC has been detected at follow-up. CT-FNAB reached 100% of sensitivity and specificity for MTC, while cytology displayed 61.9% of sensitivity and 80% of specificity when calculated in the operated lesions. ***Conclusions:*** US-guided CT-FNAB resulted the best tool to identify primary MTC and local recurrences/node metastases in MTC operated subjects with much more sensitivity and specificity than cytology. This may have important implications in the management of MTC patients.

Thyroid cancer
Poster

THE ROLE OF RET GENOTYPES AS MODIFIER LOCI FOR SPORADIC MEDULLARY THYROID CANCER

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Data from different cohorts reporting a higher prevalence of germline single nucleotide polymorphisms (SNPs) of the *RET* gene in patients with sporadic medullary thyroid cancer (sMTC) with respect to controls are highly discrepant, probably due to the number of patients enrolled and/or to the genetic variability among different populations. In the present study, a large panel of *RET* SNPs, including the intronic variant IVS1-126G>T and 5 exonic variants has been tested in the largest cohort reported to date, 140 sMTC patients, and in an age- and gender-matched control group.Fifteen different haplotypes and 38 different genotypes (comprising pairs of *RET* haplotypes) were generated in sMTC patients and controls, and their association with the risk to develop the disease and with the clinical characteristics of the patients were evaluated by stepwise logistic regression and Chi-square analyses. Interestingly, 6 genotypes resulted unique to sMTC patients and 15 to controls. Haplotype I and genotypes AB, BB and BE, all including the variant of intron 1, were significantly associated with sMTC patients or with a higher tumor aggressiveness. No statistical significant differences in the prevalence and distribution of the *RET* variants considered separately were noted between sMTC and controls. In particular, the reported correlation between the only non-synonimous *RET* variant G691S and sMTC was not confirmed in the present cohort. Consistently, NIH3T3 cells transfected with the Ret9-G691S mutant did not show an increase neither in transforming activity nor in tyrosine-phosphorylation, in contrast with a possible role in *RET* activation. In conclusion, *RET* genotypes including the variant IVS1-126G®T in intron 1 were associated with the risk to develop sMTC or to a more aggressive behaviour. Further studies are warranted to elucidate whether if this *RET* genotype per se could play a role in the genesis of sMTC or whether this variant is in linkage disequilibrium with another susceptibility gene.

Thyroid cancer
Poster

IMPACT OF 18F-FLUORO-2-DEOXY-D-GLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG-PET) ON PATIENTS WITH METASTATIC MEDULLARY THYROID CANCER

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Background: Despite the availability of numerous imaging modalities, localization of metastatic disease in patients with MTC who has elevated serum calcitonin (CT) levels is often problematic. The aim of the study was to determine the usefulness of FDG-PET in localizing MTC metastases in patients with elevated serum CT levels. ***Methods:*** Patient and lesion based results were compared with the findings obtained from neck ultrasonography, FNAB (USG), thorax computed tomography (CT), abdominal USG, bone scintigraphy (BS), FDG-PET and histopathology when available. 11 MTC patients with a mean follow up period of 103 (28–264) months were enrolled.

Results:

Case no	Neck USG	Thorax CT	Abd. USG	FDG-PET	Calcitonin pg/mL	FNAB Cytology	histopathology
1	Cervical LN	(-)	(-)	Cervical LN	698	(-)	MTC met
2	(-)	Aorticopulmonary LN	(-)	Aorticopulmonary LN	1564	_	MTC met
3	(-)	Nodule on the left lung	(-)	Nodule on the left lung	529	_	metastatic gut adenocarcinom
4	(-)	(-)	(-)	(-)	722	_	_
5	(-)	(-)	(-)	(-)	117	_	_
6	(-)	(-)	(-)	(-)	137	_	_
7	(-)	(-)	(-)	(-)	597	_	_
8	Cervical LN	(-)	(-)	Cervical LN	113	(-)	(-)
9	Cervical LN	(-)	(-)	Cervical LN	553	(-)	(-)
10	Cervical LN	(-)	(-)	(-)	650	(-)	Waiting for surgery
11	Cervical LN	(-)	(-)	(-)	618	(-)	Waiting for surgery

Conclusion: FDG-PET seemed to help in two patients (no 1–2) with false negative FNABs, to confirm the results of other imaging modalities and strengthen the indication for surgery. It detected a secondary malignancy in one patient (no 3). Localization studies were not successful in four patients(no 4–7). False positive results were reported in two patients (no 8–9). Two FDG-PET(-) patients are waiting for surgery, decided upon strong sonographical and clinical suspicion (no 10–11). FDG-PET is useful for some metastatic MTC patients, in terms of surgical localization, strengthen indication and may even detect secondary malignancies, however false positive results are not rare, in our hands up to now.

Thyroid cancer
Poster

EPIDEMIOLOGICAL EVIDENCE FOR A LINK BETWEEN DENTAL X-RAYS AND THYROID CANCER

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Context: An increasing incidence of thyroid cancer has been reported in many countries over the last few decades. Much of this increase may be due to increased ascertainment, but there may be other contributing factors. The thyroid gland is highly susceptible to radiation carcinogenesis. Dental radiography, which is a common source of radiation exposure in the general population, is often overlooked as a source of radiation to the gland and may be associated with the risk of thyroid cancer. An increased risk of thyroid cancer has been reported in dentists and dental assistants and exposure to dental X-rays have been associated with an increased risk of meningiomas and salivary tumours. ***Objective:*** To investigate the relationship between dental X-rays and risk of thyroid cancer. ***Design:*** Population based case-control study. ***Setting:*** National Cancer Registry, Kuwait Cancer Control Centre, Kuwait (population 2.8 million). ***Methods:*** 313 patients with thyroid cancer; 313 control subjects individually matched to each thyroid cancer patient for age, gender, nationality, and district of residence. Information on dental X-rays and other relevant exposures was collected through a personal interview with the cases and controls, and the data were recorded in a structured questionnaire. ***Main Outcome Measures:*** Risk of thyroid cancer associated with exposure to dental X-rays. ***Results:*** There was an approximately 2-fold significantly increased risk of thyroid cancer in individuals who were exposed to dental X-rays (OR=2.1; 95% CI: 1.4–3.1) (p= 0.001). There was also a statistically significant dose-response relationship which showed an increasing trend in risk with increasing number of dental X-rays (p-trend <0.0001). This relationship remained after controlling for sociodemographic factors (gender, nationality, level of education), number of live births, or age at diagnosis. ***Conclusions:*** These data support the hypothesis that exposure to dental X-rays is associated with an increased risk of thyroid cancer.

Thyroid cancer
Poster

INHIBITION OF THYROID CARCINOMA CELLS BY THE MULTI-KINASE INHIBITOR SORAFENIB[1] IS INDEPENDENT OF THE PRESENCE OF BRAF MUTATIONS

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Purpose: A subset of thyroid carcinomas undergo dedifferentiation and lose the ability to concentrate radioiodine. Therapeutic options for these patients are rare and alternative treatment strategies are needed. Among the most promising new agents for these patients are protein kinase inhibitors like the BRAF- and multi-targeted kinase inhibitor sorafenib. ***Methods:*** In order to test the efficacy of sorafenib we analyzed growth inhibition and induction of apoptosis in thyroid carcinoma cell lines (anaplastic, follicular and papillary). The presence of activating BRAF mutations was investigated by direct sequencing. Flow cytometric cell cycle analyses and western blot analyses with phospho-specific antibodies were performed to investigate the mechanism of sorafenib action. ***Results:*** The anaplastic cell line SW1736 as well as the papillary cell lines BHT101 and BCPAP harboured a BRAF V599E mutation, whereas C643, HTh7, HTh74, HTh83, FTC133, FTC236, FTC238, ML1 and TT2609 cells had only wild type allels. Proliferation assay revealed a significant growth inhibition with 1 to 2 µM sorafenib in all cell lines examined. Western blot analyses depicted a rapid inhibition of the MEK/Map-kinase signalling pathway within 5 minutes by sorafenib. Cell cycle analyses after 24 h treatment showed a sorafenib-dependent induction of apoptosis, while in remaining living cells G0/G1 peak was diminished and the portion of cells in S phase was increased. ***Conclusion:*** The efficacy of the BRAF- and multi-targeted kinase inhibitor sorafenib on thyroid carcinoma cells in vitro is independent on the presence of activating BRAF-mutations and may thus point to a new therapeutic option for dedifferentiated follicular and papillary thyroid cancer.

[1] Sorafenib was kindly provided by Bayer HealthCare.

Thyroid cancer
Poster

OUTCOME OF DIFFERENTIATED THYROID CANCER DIAGNOSED IN PREGNANT WOMEN

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Thyroid cancer is very frequent among tumors diagnosed during pregnancy. It is still debated if it could have a poorer prognosis due to the thyrotropic action of human chorionic gonadotropin. Aim of the present study was to compare the clinical outcome in the following 3 groups of patients affected with differentiated thyroid cancer (DTC): group 1 (Gr.1): 12 women, with tumor diagnosis during pregnancy, submitted to thyroidectomy during pregnancy or in the first year after delivery; group 2 (Gr.2): 33 women with diagnosis at least 1 year after the delivery; group 3 (Gr.3): 49 women with diagnosis and treatment before pregnancy or nulliparous. The 3 groups were matched for age, treatment, histology and follow-up. All patients of group 1 were treated with total thyroidectomy and radiometabolic treatment. No significant differences in tumor size, capsular invasion and/or distant metastases were observed between the 3 groups. At variance, a high prevalence of lymph-nodal involvement was found in patients of Gr. 1. Indeed, the 9/12 patients submitted to lymph-node dissection showed at histology a high number of metastatic lymph-nodes. Remission or persistence of disease were defined by basal or rhTSH stimulated thyroglobulin (Tg) in the absence of anti-Tg antibodies, and/or of Total Body Scan. Patients with DTC treated during pregnancy showed a higher frequency of persistence/relapse of disease (n=2 regional metastases, n=2 distant metastases and n= 5 elevated Tg levels) with respect to patients of the other 2 groups (Gr. 1 *vs* Gr. 2: P=0.0035; Gr.1 *vs* Gr. 3: P=0.0057; Gr1 *vs* Gr. 2+3, P=0.018; Gr.2 *vs* Gr.3: P=NS). In conclusion, present data show that thyroid cancer diagnosed during pregnancy is associated with a high lymph-nodal involvement and has a poorer prognosis with respect to tumors developed in the non gravidic period thus suggesting the need for an aggressive treatment by thyroidectomy during the second trimester and radiometabolic therapy soon after delivery.

Thyroid cancer
Poster

HOW MOLECULAR EXPLORATION OF 11 CLASSES OF FOLLICULAR THYROID LESIONS IMPROVES THE CLASSIFICATION OF TUMOUR OF UNCERTAIN MALIGNANT POTENTIAL

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Purpose: The different follicular thyroid tumours are often difficult to distinguish on histology, particularly when less invasive types are involved. Microarray experiments were used successfully to find markers of the main classes of tumours. However, markers were not defined to simultaneously all the thyroid lesions. It questioned their biological specificity and thus their ability to classify tumours of uncertain malignant potential (T-UMP). ***Methods:*** We determined the gene expression profiles of more than 90% of the different types of follicular thyroid lesions by using pangenomic cDNA microarrays. We had 166 samples of thyroid tissues which represented all the well-defined classes of thyroid pathologies, from benign disorders to malignant tumours and also T-UMP. We defined accurate automated classifiers by comparing 4 algorithms with univariate forward selection procedures. ***Results:*** Gene expression data contributed substantially to the pathological classification by showing significant class similarities and dissimilarities. Follicular adenoma subtypes should be either gathered or separated from each other. Oncocytic tumours showed strong divergent profiles from other follicular or papillary lesions. We showed that selected profiles lead to powerful predictions and can be used to reclassify T-UMP into benign or malignant tumours. The molecular classification of 12 classes of thyroid tissues is complementary to the WHO pathological classification. An alternative classification should be set by considering observed class similarities. Finally, dedicated microarrays may be proposed on fine-needle aspiration samples to improve clinical diagnosis and to classify follicular lesions of uncertain malignant potential.

Thyroid cancer
Poster

ANTHROPOMETRIC FACTORS IN THE RISK OF THYROID CARCINOMA IN FRENCH POLYNESIA : A POPULATION-BASED CASE-CONTROL STUDY

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A case-control study on thyroid cancer was conducted in French Polynesia, where thyroid cancer incidence is very high. 195 females and 24 males with differentiated thyroid carcinoma diagnosed before the age of 56, between 1981 and 2004 were matched on birth date with 315 female and 44 male controls randomly selected from the registry of births. Face to face interviews conducted from 2002 to 2004 collected ethnic group, weight and height before diagnosis, menstrual and reproductive factors and smoking habits. Quartiles of weight (≤54, 55–60, 61–72, ≥73kg), height (<1.60, 1.60–1.63, 1.64–1.67, ≥1.68m) and body mass index (BMI) (≤20.5, 20.5–23, 23–28, >28 kg/m²) were calculated among females and the highest quartile was compared to the lowest, while medians were used among males. Odds ratios (OR) derived from conditional logistic regression were adjusted for ethnic group, educational level, smoking, number of births, menopausal status, interviewer, history of head and neck radiographic examination and height where necessary. Women in the highest quartiles of weight, height and BMI had a 2.8, 2.1 and 2.3-fold increased risk of thyroid cancer respectively.Men with a weight over 72 kg had a 5.5-fold increased risk of thyroid cancer. Those who had a height over 1.71m had a 3.6 increased risk, and those with a BMI over 23.5 kg/m² had a 26-fold increased risk compared to those under the median.Our results are in agreement with other studies and show that obesity and elevated height are risk factors of differentiated thyroid cancer among women and men in French Polynesia.

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Thyroid cancer
Poster

GENE EXPRESSION INDUCED BY BRAF ONCOGENE MUTATION IN PAPILLARY THYROID CANCER

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Purpose: Giordano et al. (2005) have indicated on the distinct differences in gene expression profile of papillary thyroid carcinoma (PTC) related to the presence of BRAF mutations and this observation was confirmed in our own analysis. To validate the differences in gene expression related to the presence of BRAF mutation we use real time QPCR. ***Methods:*** We performed a meta-analysis of joined sets of 39 our papillary thyroid carcinoma cases: and 51 PTC cases analyzed by Giordano et al., based on Bayesian limma method. The verification of the selected genes was carried out on an independent group of PTCs by QPCR normalized to 3 control genes selected by GeNorm. ***Results:*** So far 3 genes selected from the microarray analysis were validated, PGF, a placental growth factor, related to VEGF, PHLDA1, an evolutionarily conserved proline-histidine rich nuclear protein showing pleckstrin homology-like domain and TM7SF4, a transmembrane molecule preferentially expressed in dendritic cells. PGF and PHLDA1 exhibited a significantly reduced expression in PTCs harbouring BRAF V600E mutation ($p<0.01$) and TM7SF4 showed higher expression in these PTC cases ($p<0.001$). ***Conclusions:*** The obtained results indicate on different properties of BRAF-induced papillary thyroid cancers in comparison to other cases. The role of PGF is still unknown, the diminished expression of PHLDA1 may contribute to IGF-1 induced apoptosis while TM7SF4 may take part in antigen presentation by dendritic cells, thus, influence the immune response to PTC.

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Thyroid cancer
Poster

THE ANALYSIS OF TRANSCRIPTOME OF MEDULLARY THYROID CARCINOMA: SEARCH FOR LINEAGE-SPECIFIC MARKERS

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Introduction: The analysis of medullary thyroid carcinoma (MTC) transcriptome by gene expression profiling encounters difficulties related to the lack of comparable normal tissue. Para-follicular C cells are embedded in thyroid parenchyma and are not easily accessible to RNA isolation in quantities sufficient for analysis. To omit this difficulty we present other, bioinformatical approach to specify genes characteristic for this histotype. We compare gene expression pattern of MTC to other types of thyroid tumors and normal samples to obtain transcripts specific only for MTC and discard overall cancer-related genes and genes characteristic for normal thyroid contamination. To verify the selected genes we apply quantitative real-time PCR analysis. ***Material and Methods:*** Microarray analysis was carried out by HG-U133A arrays (Affymetrix) in 40 MTC samples: 20 tumors and 20 corresponding normal tissue and 70 different thyroid tumor and normal samples. An independent set of 17 MTC with paired normal tissues and 17 PTC samples was used for QPCR validation. ***Results:*** Up to now, 8 genes were analyzed : EEF1A2, GRP, NEFL1, SCG2, SCG3, SST, TFF1, TFF3. All analyzed transcripts were overexpressed in MTC samples, when compared to PTC and normal thyroid. The difference between PTC and MTC was statistically significant ($p<0,01$) for all analyzed transcripts. The significant differences in expression were also seen in comparison between MTC and corresponding normal thyroid tissue for all the genes, with the exception of TFF3. ***Conclusion:*** We present a gene expression signature of medullary thyroid cancer, selected by the degree of differences in RNA level, not by previous knowledge on its origin, and useful for further analysis of transcriptome of MTC.

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Thyroid cancer
Poster

INCIDENCE OF DISTANT METASTASIS IN PAPILLARY CARCINOMA THYROID AND THE ROLE OF IODINE ABLATION

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Background.The incidence of distant metastasis in papillary carcinoma thyroid is not defined in Asian population. ***Method:*** We reviewed patients with papillary carcinoma thyroid for the past 30 years. ***Results:*** 1,538 patients had carcinoma thyroid, papillary carcinoma were 1,228. 21 had bone ,49 had lung, 4 had brain parenchymal metastases .They are diagnosed by iodine uptake scan ,xray[16 ,32% were positive for mets in lungs] CT brain, Bronchoscopy. The male female ratio was 3:1. More than 70% were of 40 to 60 years of age.Total thyroidectomy done in all except four .26% had neck dissection.Pathological variants were insular,follicular' microcarcinoma. six had received radiotherapy to neck,three to spine Metastases were ribs pelvis vertebra skull shoulder humerus femur parietal lobe corpuscallosum bronchial mucosa pyriformfossa. 6 had bone and lung lesions. The ablation dose ranged from 90 to 900mcu. Patients with rib[the commenest] and 28 with lung secondaries had complete remission The size of the tumor did not correlate. Response to ablation was poor in four with bone and thirteen with lung metastasis Brain lesions were static in spite of negative uptake . Moderate rise in thyroglobulin [TG] at the time of diagnosis, had better response than with very high level of serum TG. ***Follow Up:*** Patients with successful ablation have been followed up to six years five non responders were alive at three years two patients died. ten patients lost follow up. ***Conclusion:*** The overall distant metastasis in papillary carcinoma thyroid is 6%. Iodine ablation has definite role in distant metastasis. The initial higher value of serum thyroglobulin can be a predictor of poor response to ablation. Brain parenchymal lesions can be static for a reasonable period of time.

Thyroid cancer
Poster

VALIDATION OF POTENTIAL MOLECULAR MARKERS OF PAPILLARY THYROID CARCINOMA BY QUANTITATIVE REAL-TIME PCR

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Introduction: In our previous DNA microarray profiling studies we specified a multigene classifier of papillary thyroid carcinoma, which included some novel transcriptps, not analyzed until now in the context of PTC biology. The aim of this study was to validate them by quantitative real-time PCR on an independent set of PTC samples. We included such new genes as EVA1, CDH3, KCJN2, LRP4, Q-PCT GALE (up-regulated) and HGD, TACSTD2 (down-regulated) and compared them to the well known PTC markers, which were confirmed by our microarray analysis (DPP4, FN1, KRT19, MET, RXRG, ADORA1, up-regulated, and TFF3, down-regulated). ***Material and Methods:*** Total RNA was isolated from 31 paired normal and PTC samples by Chomczynski-Sacchi method. 0.5 mg of RNA was used in the reverse transcription reaction. cDNA was further used in Q-PCR reaction. The expression was measured by Universal Probe Library LNA probes (Roche) and was normalized by the index obtained from 3 reference genes (UBE2D2, HADHA, EIF3S10) by GeNorm software. ***Results:*** All analyzed genes except TACSTD2 were highly significantly differentiating tumor and normal samples. When the diagnostic efficiency of these genes was assessed by ROC (relative operating characteristic) analysis, we found out that for overexpressed markers, the area under ROC curve (AUC) was higher than 0.9 for all of them and for down-regulated ones, this criterion was met for TFF3 and HGD. We trained the multigene classifier on the obtained data and currently we are performing its validation on independent set of samples. ***Conclusions:*** Our data supply a next step of validation of PTC markers, before the required verification on routine diagnostic material obtained by fine needle biopsy of benign and malignant thyroid nodules.

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Thyroid cancer
Poster

THE EXPRESSION OF PTEN TUMOR SUPPRESSOR IS DOWN REGULATED BY THE DOMINANT NEGATIVE p73 ISOFORM ΔNp73 IN THYROID CANCER CELLS

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Down regulation of PTEN tumor suppressor and the consequent Akt hyperphosphorylation is a common feature of thyroid carcinomas and may contribute to thyroid tumor progression. However, the mechanisms underlying the reduced PTEN expression in thyroid cancer are still poorly understood. Since ΔNp73, a dominant negative component of the p53 family proteins is selectively expressed in malignant thyroid cells and may play a role in thyroid tumor progression. We investigated whether ΔNp73 may promote thyroid cancer progression by down regulating PTEN expression. Indeed, western blot analysis revealed that in p73 positive thyroid cancer cells the inhibition of ΔNp73 expression by siRNA results in PTEN protein up regulation. In contrast, transient transfection of ΔNp73 in thyroid cancer cells result in PTEN down regulation. These results were confirmed also in p73 negative thyroid caner cells stably transfected with ecdysone inducible ΔNp73. Incubation of these cells with ecdysone resulted in decreased PTEN expression, increased Akt phosphorylation by western blot, increased cell proliferation and resistance to apoptotic stimuli by FACS analysis. This effect of ΔNp73 in thyroid cancer cells was PTEN/Akt dependent as it was completely abolished by the PI3-Kinase inhibitor LY294002. Moreover, luciferase assays indicated that ΔNp73 was able to reduce PTEN promoter activity. These results were confirmed by real time PCR experiments indicating that in thyroid cancer cells ΔNp73 is able to reduce PTEN transcript levels. Taken together these results indicate that ΔNp73 expression may be responsible for PTEN down regulation in thyroid cancer. PTEN down regulation, therefore, may be one mechanism responsible for ΔNp73 effect on thyroid tumor progression.

Thyroid cancer
Poster

DIETARY PATTERNS AS A RISK FACTORS OF DIFFERENTIATED THYROID CANCER

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Nutritional factors are known as an important factor in development of various metabolic diseases. The history of nodular or diffuse goitre is closely connected with risk of thyroid cancer. Iodine is the main factor of the thyroid gland function so the researches are focused on iodine intake. ***Aim:*** To assess if the dietary patterns could be the risk factors of differentiated thyroid cancer (DTC) in the endemic goitre area. ***Materials and Methods:*** The case-control study based on questionnaire included information of dietary patterns was performed in 284 patients: 30 males (mean age 58,4±13,7 years), and 188 females (mean age 52,1 ±13,8 years), and 345 controls: 58 males and 287 females randomly selected and adjusted by age and gender to the group of DTC. Nutritional products enclosed in the questionnaire: starchy foods, meat, dairy products, vegetables, fruits, and beverages were analysed. ***Results:*** Consumption of vegetables, fruits, saltwater fish and cottage cheese was significant lower in patients with DTC than in controls, quite contrary to starchy foods - especially white bread. ***Conclusions:*** Dietary patterns could play role in modifying the risk of DTC. Rich in vegetables and fruit diet could be an important protective factor.

Thyroid cancer
Poster

SU11248, AN ORAL MULTI-KINASE INHIBITOR, SHOWS ANTIPROLIFERATIVE AND ANTITUMORAL ACTIVITY IN A HUMAN MEDULLARY THYROID CARCINOMA MODEL

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Purpose: Medullary thyroid carcinoma (MTC) are rare cancers of the thyroid that secrete calcitonin (TCT) and, in most cases, involve activating mutations in the proto-oncogene RET. MTC prognosis remains pejorative as metastases, the principal cause of death, remain insensitive to conventional chemotherapies. Thus, development of new drugs constitutes a major challenge in order to improve treatment and cure of patients. Among targeted therapies, SU11248, a multitargeted tyrosine kinase inhibitor (ITK), constitutes a potent inhibitor of RET. In the present study, we used the TT cell line, derived from a human MTC and bearing a RET C634W activating mutation, to investigate both in vitro and in vivo effects of SU11248. ***Methods:*** SU11248 was synthesized according to previously published procedures. PP2 was purchased from Biaffin GmbH & Co KG. Human TT cells were grown in RPMI 12% SVF in the presence or absence of ITKs (0–1,000 nM) over a 15 days period. Cell viability and cell proliferation were assessed by MTT and BrdU assays, respectively. TCT gene expression and release in medium were measured by Q RT-PCR and ELISA, respectively. Antitumoral activity was evaluated on TT tumor xenografts in athymic nu/nu mice. Tumor volumes were measured by ultrasonography over a two-week oral gavage period (80 mg/kg/d). ***Results:*** SU11248 and PP2 inhibited TT cell viability with IC_{50} ~200 nM and ~900 nM, respectively. SU11248 showed antiproliferative effects at 200 nM ($p<0.001$). At this concentration, a three-fold decrease of TCT release as well as decreased gene expression, were observed after a 6-day exposure. In mice bearing xenografted TT tumors, a two-week in vivo treatment by SU11248 at a 80 mg/kg/d resulted in a three-fold reduction of tumor volume. Together these data demonstrate that SU11248 displays both antiproliferative and antitumoral activities on MTC cells, indicating that it could be an effective treatment in metastatic patients.

Thyroid cancer
Poster

THE [99mTc-EDDA/HYNIC]OCTREOTATE SCINTIGRAPHY IN THE DIAGNOSIS OF MEDULLARY THYROID CANCER

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Medullary Thyroid Cancer (MTC) is a rare histological type of thyroid cancer. Both the primary tumour and the metastases of the MTC are ^{131}I scintigraphically-negative. ***Aim:*** To evaluate the possibility of [^{99m}Tc-EDDA/HYNIC]octreotate use in the imaging in patients with MTC. ***Material and Methods:*** [^{99m}Tc-EDDA/HYNIC] octreotate (740 MBq) scintigraphy was performed in 14 patients: All our patients were referred to the study with a confirmed MTC diagnosis. 1 patient was before thyroidectomy, 3 patients were in the remission after surgery with low calcitonin level and in 10 patients there was diagnosed recurrent or persistent disease with hypercalcitoninaemia. Other imaging techniques: US, spiral CT, MR were also used in individual cases. ***Results:*** Metastases were found in 7, and the primary tumour was detected in one case. Three patients post radical thyroidectomy with elevated calcitonin levels and abnormal pentagastrin tests had negative scans. In two of them metastases were detected in CT and MR imaging. One patient with low calcitonin level was false positive. ***Conclusions:*** The scintigraphy using [^{99m}Tc-EDDA/HYNIC] octreotate seems to be useful in the diagnosis of MTC. Histological confirmation of the presence of somatostatin receptors in MTC tissue may be helpful in planning radionuclide therapy.

Thyroid cancer
Poster

INCIDENCE OF DIFFERENTIATED THYROID CANCER IN THE SELECTED AREAS IN POLAND (1990–2005)

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Aim: To evaluate the incidence rate (IR) and histotype of the differentiated thyroid cancer (DTC) in the selected areas with varying iodine deficiency. ***Methods:*** The study was carried out in Krakow (endemic goiter area with 1.99 million mixed rural and urban population), Gliwice (moderate iodine deficiency area mostly industrial with 4.89 million inhabitants) and Olsztyn (slight iodine deficiency area, mainly rural with 0.77 million inhabitants).The DTC registries were developed using regional cancer registries, hospitals and pathological departments data. Histological evaluation was carried out according to ICD-10 and WHO classification. The IR of thyroid cancer was calculated as a number of a new diagnosed cases per year and per 100,000 habitants. Statistical analysis was assessed using Statistica software. ***Results:*** From 1990 to 2005 IR of DTC increased in Krakow, Olsztyn and Gliwice from 2.0 , 1.34 and 0.7 to 6.8 , 6.0 and 3.0 in 2000 and to 6.0 , 8.3 and 4.0 in 2005 respectively. In the youngest (0–20 years) age group no significant increase of IR was observed. Over 80% of patients represented group of age over 40 years with F/M ratio 5,8. Between 1990 and 2005 papillary/ follicullary thyroid cancer ratio (P/F) significantly increased especially in Krakow and Olsztyn regions: from 1.0 and 3.0 to 6.0 and 10. The dynamics of increase of the DTC incidence markedly diminished after 1999. ***Conclusion:*** an increase in IR of differentiated thyroid cancer in the study area was caused mainly by the suspension of iodine prophylaxis in 1980 and was diminished by the introduction of an obligatory model of iodine prophylaxis in 1996. It was modified in terms of histotype and dynamics of increase by exposure to ionizing radiation after the Chernobyl accident in 1986.A very specific group at risk on the population level were women aged 20–40 years in the reproductive age exposed to iodine deficiency and ionizing radiation.

Thyroid cancer
Poster

CASE REPORT: ULTRASOUND-GUIDED PERCUTANEOUS LASER ABLATION TREATMENT IN INOPERABLE AGRESSIVE COURSE ANAPLASTIC THYROID CARCINOMA: THE INTRODUCTION OF A NOVEL ALTERNATIVE PALLIATIVE THEPAPY-SECOND EXPERIENCE IN THE LITERATURE

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Introduction: Anaplastic thyroid carcinoma (ATC) is one of the most aggressive human malignancies with a mean survival of only 6 months. Different theurapeutic options are combined frequently in the treatment. ATC management has two goals: prevention of local complications by the primary tumor and palliation from distant metastatic disease. US-guided percutaneous laser ablation(PLA) treatment is a new alternative treatment method of the thyroid gland. To our knowledge, this is the second experience of PLA procedure in palliative treatment of ATC in the literature. ***Case Report:*** A 85-year-old male patient with a history of painful tumoural enlargement on neck, difficulty in swallowing was admitted to our clinic. Computed tomography scan of the neck revealed a heterogenous mass originating from the thyroid gland and surrounding the trachea both anteriorly and posteriorly. A fine needle aspiration biopsy of the thyroid mass suggested anaplastic thyroid carcinoma type 2. The patient was accepted as inoperable. External hyperfractioned radiotherapy to thyroid region was applied in palliative treatment. Due to decompression of the trachea, tracheostomy was suggested but the patient declined. Total volume of the gland measured by US was 82.89 ml. But in the computed tomography of the thorax in the 3 dimensional axis the total volume of the gland was more than 182 ml. The patient was treated with an output power of 5W for 300 seconds for each lobe of the gland. The total energy delivered was 9000J in 6 sessions. Although symptomatic improvement was achieved, on 15th day of postPLA period he deteriorated gradually and died due to massive venous thromboembolism. ***Conclusion:*** US-guided PLA is a new, minimally invasive technique that has proven to be safe for tumour palliation in patients with advanced cancers. It can be tried in palliative treatment in patients with aggressive course of ATC who also need tracheostomy in clinical follow-up.

Thyroid cancer
Poster

THE EFFECT OF HIGH DOSE RADIOACTIVE IODINE ON PARATHYROID FUNCTION IN PATIENTS WITH THYROID MALIGNANCY

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Aim: There is some reports about developing hypoparathyroidism after radioactive iodine (RAI) therapy in patients with thyroid carcinoma. The aim of current study was to investigate whether RAI treatment have any effect on parameters related to parathyroid functions, in a relatively long period. ***Material and Methods:*** The study included a group of thyroid cancer patients (n: 19; male/female: 8/11; age 46.5±13.2 years) who have no problem about parathyroid functions. Some biochemical parameters in relation with parathyroids were evaluated at baseline and after RAI treatment in a one year period, on four time points (1st, 3rd, 6th and 12th months). These parameters were plasma calcium (Ca), phosphate (P), creatinine (Cr), alkaline phosphatase (ALP) and parathyroid hormon (PTH), urinary Ca, P and cAMP concentrations, and TMP/GFR. ***Results:*** In the study period, there was significant changes in PTH (p= 0.040) and urinary P levels (p=0.027) (ANOVA). The PTH measurements from baseline to 12th month visit were 37.13±16.67 pg/ml, 40.16±17.23 pg/ml, 35.22±14.99 pg/ml, 27.51±13.66 pg/ml and 42.56±14.78 pg/ml respectively. In Post Hoc analysis, the levels on 6th month were significantly lower than 1st (p=0.014) and 12th (p=0.004) month. On the other hand, urinary P levels were 69.55±33.55 mg/dl, 49.26±32.28 mg/dl, 48.41±29.08 mg/dl, 39.23±22.54 mg/dl and 55.17±24.71 mg/dl at baseline, 1st, 3rd, 6th and 12th months of follow up. The Post Hoc analysis showed that basal urinary P levels were higher than 1st, 3rd and 6th months' levels (respectively p=0.032, p=0.026 and p=0.002). However, TMP/GFR ratios which is a more reliable test about P excretion than urinary P levels, were showed no difference during the study period. In addition, there was no statistical significancy with ANOVA for plasma Ca, P, Cr, ALP, urinary Ca and cAMP concentrations. ***Conclusion:*** The study indicated a transient decline in PTH levels on 6th month after high dose RAI therapy.

Thyroid cancer
Poster

INCIDENCE OF DIFFERENTIATED THYROID CANCER IN THE SELECTED AREAS IN POLAND (1990–2005)

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Aim: To evaluate the incidence rate (IR), and histotype of the differentiated thyroid cancer in the selected areas with varying iodine deficiency. ***Methods:*** The study was carried out in three areas: Krakow, (Carpathian endemic goiter area with 1.99 million mixed rural and urban population), Gliwice (Upper Silesia-moderate iodine deficiency area mostly industrial with 4.89 million inhabitants) and Olsztyn (slight iodine deficiency area, mainly rural with 0.77 million inhabitants).The thyroid cancer registries in areas of investigation were developed on the basis of regional cancer registries, hospitals and pathological departments data. Histological evaluation of differentiated thyroid cancer was carried out according to ICD-10 and WHO classification. The incidence rate (IR) of thyroid cancer was calculated as a number of the new diagnosed cases per calendar year and per 100.000 habitants. Statistical analysis was assessed using computer Statistica software. ***Results:*** In period of the time from 1990 to 2005 IR of differentiated thyroid cancer increased in Krakow, Olsztyn and Gliwice from 2.0, 1.34, and 0.7 to 6.8, 6.0 and 3.0 in 2000, and to 6.0, 8.3 and 4,.0 in 2005 respectively. In the youngest (0–20 years) age group no significant increase of IR was observed. Over 80% of patients represented group of age over 40 years with F/M ratio 5.8. Between 1990 and 2005 papillary/ follicullary thyroid cancer ratio (P/F) significantly increased especially in Krakow and Olsztyn regions : from 1.0 and 3.0 to 6.0 and 10.0 respectively. The dynamics of increase of the thyroid cancer incidence markedly diminished after 1999. ***Conclusion:*** An increase in IR of differentiated thyroid cancer in the study area was caused mainly by the suspension of iodine prophylaxis in 1980 in Poland and was diminished with increasing of P/F ratio by the introduction of an obligatory model of iodine prophylaxis in 1996/1997. It was modified in terms of histotype and dynamics of increase by exposure to ionizing radiation after the Chernobyl accident in 1986.A very specific group at risk on the population level were women aged 20–40 years in the reproductive age exposed to iodine deficiency and ionizing radiation.

Thyroid cancer
Poster

MIB-1 PROLIFERATION INDEX IN THYROID TUMORS' FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) : A PROGNOSTIC TOOL ?

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Introduction: Fine needle aspiration cytology (FNAC) is a safe method in thyroid tumor diagnosis. It is often hard though to differentiate between benign and malignant follicular tumors. Our goal in this study was to investigate if the analysis of the proliferation index MIB-1 can be useful in discriminating follicular adenoma from follicular cancer. Moreover, we studied the potential of the same index in providing useful prognostic information in patients with papillary thyroid cancer preoperatively. ***Methods:*** Preoperative MIB-1 index analysis was carried out on 448 FNAC samples from thyroid, of which 341 were tumor samples. MIB-1 index was calculated as percentage of stained tumor cells and statistical analysis (Kruskal-Wallis, Mann-WhitneyU, Chi2, Log-Rank test and Cox regression) was applied to check its relation with diagnosis and/or prognosis. ***Results:*** MIB-1 index varied between 0 and 100% with a median value of 50% for anaplastic cancer, 2% for papillary and follicular cancer and 1% for follicular adenoma. There was a tendency towards higher MIB-1 values in follicular cancer than follicular adenoma, but the difference did not prove to be statistically significant. As far as the papillary type of cancer is concerned, our study shows that a MIB-1 value greater than 5% or a tumor size larger than 4cm constitute reliable and independent prognostic factors. ***Conclusion:*** In previous studies, MIB-1 index calculated on paraffin-embedded tissue from thyroid cancer was shown to be of high prognostic significance, especially for values greater than 3% or less than 1%. This was not the case with follicular tumors. In this study we verified the finding that MIB-1 index, this time in FNAC, is not a powerful diagnostic tool among follicular tumors. Nevertheless, we proved that this index provides important prognostic information in cases of papillary thyroid cancer and should, therefore, not be excluded from the clinical work-up of patients with a thyroid lesion.

Thyroid cancer
Poster

THYROID CARCINOMA CLASSIFICATION AND OUTCOME ACCORDING TO THE V AND VI EDITIONS OF THE AJCC/UICC STAGING SYSTEM

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TNM classification VI edition for thyroid cancer has introduced important changes in the classes of risk as compared to the V edition. Whether this last edition performs better than the previous one is still unclear. We compared thyroid tumor classification and outcome according to TNM-V and TNM-VI editions. All thyroid carcinomas diagnosed in years 2002-2005 (2,056 cases) were evaluated according TNM-V and VI. Patients followed at our Center for Thyroid Cancer (635 cases) were evaluated also for disease persistence (follow-up range 6.0-61.8 months, median 27.3). Tumors classified T1 and T3 increased in TNM–VI as compared with TNM-V (from 49.9% to 67.6% and from 3.4% to 16.2%, respectively) while tumors in classes T2 and T4 decreased (from 29.3% to 11.9% and from 14.4% to 1.3%, respectively). Class T4 included only 27/2,056 tumors (mainly anaplastic cancers). Significant heterogeneity was present in T1 tumors: the larger ones (11-20 mm, 26.1%) more frequently presented lymph-node metastases (p=<0.0001) and multiple foci (p=0.0001) in comparison to T1 tumors ≤10 mm. When T class was considered, T1 and T2 tumors had a similar outcome in TNM-VI (p=0.231) but a different outcome in TNM-V (p=0.0005). In contrast, T2 and T3 tumors had different outcome in TNM-VI (p=0.013) but similar outcome in TNM-V (p=0.429). When staging was considered, outcome of stage 2 did not significantly differ from stage 3 tumors in both TNM editions. Moreover, outcome of stage 1 vs. stage 2 tumors was not significantly different in both TNM editions, although TNM-V performed slightly better (p=0.601 vs. p=0.072, respectively). Conclusions: Approximately 70% of thyroid carcinomas fall in the T1 class of TNM-VI, which is rather heterogeneous. TNM-VI staging does not appear to perform better than the previous edition as far as tumor persistence is taken into account.

Thyroid cancer
Poster

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. The aim of our study was to evaluate the clinical significance of endogenous TSH-stimulated serum Tg measurement determined before ^{131}I ablation. We retrospectively analysed the clinical records of 547 patients followed in our department. Patients with aggressive variants of thyroid carcinoma or positive anti-Tg antibodies were excluded. An endogenous TSH-stimulated (TSH above 20 µIU/mL) Tg measurement was obtained before ^{131}I ablative therapy. Serum thyroglobulin was assayed by an immunoradiometric assay with a functional sensitivity of 0.9 ng/mL. Disease stage was defined according to the UICC classification (6th edition). All patients had undergone a total or near-total thyroidectomy. Periodic follow-up included clinical and ultrasound examination, ^{131}I whole body scintigraphy and serum Tg measurements. One hundred seventy-four patients (142 women and 32 men) were included in the analysis. Mean age at diagnosis was 46.4 ± 14.8 years (range, 9–82 years). One hundred sixty-two patients had papillary carcinoma and 12 had follicular carcinoma. One hundred twenty-eight patients were stage I or II, 33 were stage III and 13 were stage IV. One hundred forty-four patients had no evidence of persistent or recurrent thyroid cancer during follow-up. None of the patients with a Tg below 1.0 ng/mL had persistent disease or developed a local recurrence or distant metastases. A Tg of at least 50.0 ng/mL was always associated with persistent disease. We conclude that an undetectable endogenous TSH-stimulated Tg determined before radioiodine ablation is highly predictive of absence of disease and a high value predicts the persistence or recurrence of the thyroid cancer.

Thyroid cancer
Poster

CLINICAL EXPERIENCE WITH RECOMBINANT HUMAN THYROTROPIN (RHTSH) IN THE FOLLOW-UP OF DIFFERENTIATED THYROID CARCINOMA

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Introduction: Differentiated thyroid cancer (DTC) may recur years after initial treatment. Follow-up of patients with DTC is long term. Use of rhTSH is indicated as adjunctive diagnostic tool for serum thyroglobulin (Tg) testing, with or without radioiodine whole-body scan, in the follow-up of patients with DTC. Maximum TSH stimulation of both normal residual or neoplastic thyroid cells has classically been accomplished by temporary thyroid hormone withdrawal (THW). rhTSH has been developed to facilitate monitoring for thyroid carcinoma recurrence or persistence without the morbidity of hypothyroidism. ***Objectives:*** The purpose of this work is to present our clinical experience with, and to identify the optimal conditions for the use of, rhTSH (Thyrogen®) in the management of patients with DTC. ***Material and Methods:*** The study involved 54 patients with DTC evaluated between 2005-2006 in our outpatient clinic. rhTSH was administered in two consecutive daily (approximately 24 h apart) IM injections of 0.9 mg of thyrotropin-alfa (Thyrogen®). Blood was drawn for TSH and Tg measurements, 72 hours after the second injection of rhTSH. ***Results:*** Among the 54 patients, 83,3% (n=45) females and 16,7% (n=9) males, mean age was 50.58 ± 11.81 (25 – 83) years. rhTSH protocol was followed. Seric TSH mean was 20.88 ± 14.62 µUI/ml (4.05 – 72.41). The serum Tg mean was 3.32 ± 7.64 ng/ml (0.2 – 36.6). 53,7% (n=29) of the patients had Tg < 0,5 ng/ml and 27,7% (n=15) had values higher than 2 ng/ml. There were no notification of side effects. ***Conclusions:*** The use of rhTSH adds a new dimension to long-term follow-up of DTC. It avoids putting patients through hypothyroidism symptoms, and offers the opportunity to follow some patients with rhTSH-stimulated serum Tg levels without performing I131 whole-body scans. rhTSH administration is a safe and effective mean of stimulating radioiodine uptake and serum Tg levels in patients undergoing evaluation for DTC persistence and recurrence.

Thyroid molecular and cellular biology
Poster

IODINE DEFICIENCY ACTIVATES ANTIOXIDANT GENES AND CAUSES DNA DAMAGE IN THE THYROID GLAND OF RATS AND MICE

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Because thyroid nodules are frequent in areas with iodine deficiency the aim of this study was to characterise molecular events during iodine deficiency that could explain mutagenesis and nodule formation. We therefore studied gene expression of catalytic enzymes prominent for H_2O_2 detoxification and antioxidative defence, quantified DNA oxidation and damage as well as spontaneous mutation rates (SMR) in mice and rats fed an iodine controlled diet. Antioxidative enzymes such as superoxide dismutase 3, glutathion peroxidase 4 and the peroxiredoxins 3 and 5 showed increased mRNA expression, which indicates increased radical burden that could be the cause of additional oxidized base adducts found in thyroidal genomic DNA in our experiments of iodine deficiency. Furthermore, the uracil content of thyroid DNA was significantly higher in the iodine deficient compared to the control group. While SMR is very high in the normal thyroid gland it is not changed in experimental iodine deficiency. Our data suggest that iodine restriction causes oxidative stress and DNA modifications. A higher uracil content of the thyroid DNA could be a precondition for C→T transitions often detected as somatic mutations in nodular thyroid tissue. However, the absence of increased SMR would argue for more efficient DNA repair in response to iodine restriction.

Thyroid molecular and cellular biology
Poster

THE EFFECT OF FREEZING AND LONG-TERM STORAGE ON SERUM THYROTROPIN, THYROID HORMONES AND AUTOANTIBODIES

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Background: Validity and stability of samples before and after long-term storage is essential in utilizing samples from biobanks for scientific and clinical purposes. The data concerning the stability of thyroid hormones and antibodies in frozen and stored serum is scarce. In this study we evaluated the effect of A) bench-lag time, B) freezing and C) storage on serum concentrations of thyroid hormones and antibodies (TSH, fT4, fT3, TPO-Ab and TG-Ab). ***Methods*****:** TSH, fT4, fT3, TPO-Ab and TG-Ab concentrations were analysed in samples (n=695) from population-based serum bank, Finnish Maternity Cohort (FMC). A) The effect of bench-lag time (BLT) (0, 1, 3 and 6 days) was studied from fresh sera (n=8). B) The effect of freezing was evaluated by comparing frozen samples (n=50) with fresh serum samples (n=50). C) 645 samples stored 0–23 years in –25°C were analyzed to evaluate the effect of storage time. ***Results*****:** Freezing or storage up to 23 years did not have an effect on TSH concentrations. fT4 concentrations tended to increase but storage time explained only 5.5% of its variation. Storage did not affect fT3 concentrations and only a marginal increase was seen due to freezing. Freezing did not affect TPO-Ab or TG-Ab concentrations unless 14 years storage time was exceeded, when the median concentrations increased and the variations were wider in both parameters. Storage time explained 19.7% and 19.4% of variation in TPO-Ab and TG-Ab concentrations, respectively. BLT up to six days did not have any effect on the parameters. ***Conclusions:*** TSH, fT4 and fT3 can be reliably measured from samples with a storage time up to 23 years. Thyroid autoantibodies were stabile if storage time was less than 14 years. Comparison of TPO-Ab and TG-Ab with different storage times must be done cautiously if storage time exceeds 14 years.

Thyroid molecular and cellular biology
Poster

AMYLOID PRECURSOR EXPRESSION AND REGULATION IN HUMAN THYROID PATHOLOGIES

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Purpose: Beside its role in Alzheimer´s disease pathogenesis, recent data provide evidence, that the amyloid precursor protein (APP) is also involved in cell proliferation, tumorigenesis and signal transduction. Using a proteomic approach we have shown before, that APP, the APP-binding protein and the APP-p3-component are highly expressed in benign cold thyroid nodules. In this work we investigated the expression and regulation of APP in differentially functioning thyroid pathologies. ***Methods:*** Real-time PCR for *APP* and its isoforms APP*770, 751 and 695* was performed in a series of 105 thyroid specimen, including toxic thyroid nodules (TTN), Graves' disease thyroids (GD), benign cold thyroid nodules (CTN), normal thyroid tissues and thyroid cancers (FTC, PTC, ATC). In addition, the protein expression of the APP endo- and ecto-domain was studied by immunohistochemistry in 48 FA, 51 FTC, 11 PTC, 2 ATC and 5 MTC. ***Results:*** We demonstrate that (I) the expression of the endo- and ectodomain of APP is higher in malignant thyroid tissues and thyroid nodules compared to normal thyroid tissues; (II) in contrast to the cytoplasmatic localization of the endodomain, the expression of the ectodomain is targeted to the cell membrane. However, this observation was only made for malignant thyroid nodules. (III) Accordingly, thyroid carcinomas show evidence for a significantly increased APP-mediated signalling as demonstrated by the higher mRNA expression of APP-scaffold proteins JIP1, Shc A and Fe65. (IV) The expression of APP and secretion of sAPP is dependent on the thyroid-stimulating hormone (TSH) and insulin and (V) is regulated by crosstalks of the MAPK and PI3K-pathway. ***Conclusion:*** These data propose the amyloid precursor protein as a novel player in thyroid tumorigenesis.

Thyroid molecular and cellular biology
Poster

COOPERATION OF THE TRANSMEMBRANE HELICES IN THE PROCESS OF TSHR ACTIVATION

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It is known that activation of GPCRs requires movements of the transmembrane helices (TMHs) to induce a fully activated receptor. In contrast to ligand stimulation constitutively activating mutations (CAMs) induce a partially activated receptor. This suggest that more than one residue participates in the activation process. To investigate this hypothesis we combined CAMs of the TMHs to achieve a further increase of basal activity. We chose a CAM from each TMH of the TSHR (TMH1:G431S; TMH2:M453T; TMH3:L512Q; TMH5:Y601N; TMH6:A623V, TMH7:N674D) and combined them with every other mutation listed above. Mutants were characterized for cell surface expression, cAMP accumulation and basal cAMP production by linear regression analysis. Single mutants were expressed in a range of 46–106% and double mutants showed a surface expression of 12–75% (wt=100%). Linear regression analyses demonstrated an increase in basal activity (17–40fold wt=1) compared to the single substitutions (4–10fold) for all of the characterized double mutants except one combination. Combination of TMH6/7 showed 40fold constitutive activity, whereas the combination TMH3/6 showed only a basal value of 7fold compared to the single substitutions L512Q (4.5fold) and A623V (5fold). Synergistic effects by combining CAMs suggest that multiple conformational changes and coorporation of all TMHs are most likely necessary to achieve a complete receptor activation beyond the partial activation by single CAMs.

Thyroid molecular and cellular biology
Poster

THE POTENTIAL OF ASTATINE-211 For NIS-MEDIATED RADIONUCLIDE THERAPY IN PROSTATE CANCER in vitro AND in vivo

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We reported recently the induction of selective iodide uptake activity in prostate cancer cells (LNCaP) by PSA promoter-directed sodium iodide symporter (NIS) expression that allowed a significant therapeutic effect of I-131. However, although in some non-thyroidal tumors therapeutic efficacy may be limited by rapid iodide efflux due to lack of iodide organification. In the current study, we therefore studied the potential of At-211, as an alternative radionuclide, also transported by NIS. At-211 represents a high energy (E=5.86 MeV) alpha particle emitter with a physical half-life of 7.2 h. We demonstrated that LNCaP cells stably expressing NIS under the control of the PSA promoter (NP-1) concentrated At-211 in a perchlorate-sensitive manner, which was not observed in the NIS-negative control cell line P-1. While only 5% of the P-1 cells were killed by a 30 min exposure to At-211, the selective killing effect of At-211 was more than 95% in NP-1 cells as shown in an in vitro clonogenic assay. After establishment of NP-1 cell xenografts in athymic nude mice, animals were administered 1 MBq At-211 followed by imaging of tumoral At-211 uptake using a gamma camera. While P-1 tumors showed no radionuclide uptake, NP-1 tumors accumulated 8% of the total At-211 dose with an effective half-life of approximately 12 h. Considering a tumor mass of 1 gram the absorbed dose to the tumor was calculated to be 1,580 mGy per injected MBq. After a single i.p. application of 1 MBq At-211, NP-1 tumors showed a significant tumor volume reduction of up to 82%, compared with P-1 tumors and saline-treated tumors. In conclusion, a dramatic therapeutic effect of At-211 has been demonstrated in prostate cancer after induction of tumor-specific radionuclide uptake activity by PSA promoter-directed NIS expression in vitro and in vivo.

Thyroid molecular and cellular biology
Poster

IMAGE-GUIDED RADIOIODINE THERAPY OF MELANOMA FOLLOWING TUMOR-SPECIFIC SODIUM IODIDE SYMPORTER (NIS) Gene Transfer

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In its early stages malignant melanoma, which reveals an increasing incidence in western populations, can be cured by surgical resection, but once it has progressed to the metastatic stage it does not respond to current therapies. Therefore, the development of novel treatment strategies, such as gene therapy, is urgently needed. To investigate an alternative approach, we examined the feasibility of radioiodine therapy of melanoma following human sodium iodide symporter (NIS) gene transfer using a melanoma-specific tyrosinase-promoter construct of 870 bp to target NIS expression to melanoma cells. For this purpose, a human melanoma cell line (Lu1204) was stably transfected with hNIS cDNA under the control of the tumor-specific tyrosinase-promoter. The stably transfected Lu1204 cell line showed a 71-fold increase in iodide accumulation. Tumor-specific NIS expression was confirmed on mRNA and protein level by Northern blot and Western blot analysis, respectively. In addition, immunostaining of NIS-transfected melanoma cells revealed hNIS-specific immunoreactivity, which was primarily membrane-associated. In an in vitro clonogenic assay approximately 75% of NIS-transfected Lu1205 cells were killed by a 7h exposure to 29.6 MBq I-131, while approximately 95% of control cells survived after incubation with I-131. In conclusion, a highly significant therapeutic effect of I-131 has been demonstrated in melanoma cells following induction of tumor-specific iodide uptake activity by tyrosinase-promoter-directed NIS expression. This study demonstrates the potential of NIS as a therapeutic gene allowing radioiodine therapy of melanoma following tumor-specific NIS gene transfer and therefore offers an innovative strategy for melanoma therapy.

Thyroid molecular and cellular biology
Poster

NO CORRELATION OF TSH RECEPTOR (TSHR) MUTATION'S in vitro ACTIVITY WITH THE CLINICAL COURSE OF PATIENTS WITH SPORADIC NON-AUTOIMMUNE HYPERTHYROIDISM

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Up to date, 12 patients with non-autoimmune hyperthyroidism (NAH) caused by sporadic germline mutations in the TSHR gene were reported. Nearly all case reports discussed possible correlations of TSHR mutation's in vitro activity (IVA) with clinical course (CC). Therefore, we analyzed the case reports in a systematic review and the investigation of TSHR mutation's IVA in selected cases. Recently, linear regression analysis (LRA) of constitutive activity as a function of TSHR expression determined by ^{125}I-bTSH binding or FACS analysis compared to the wt TSHR was described as a more reliable way of characterizing the IVA of a constitutively activating TSHR mutation. Therefore, we determined the LRA for all sporadic germline mutations which had not previously been reported. We systematically evaluated all case reports of sporadic NAH for evidence of a possible correlation of the CC with the IVA of the mutated TSHR.The LRAs were determined for M453T (5.19±0.8), L512Q (4.48±0.7), F631L (45.9±9.4), T632I (14.5±2.7), D633Y (16.4±6.4).In most of the cases, an elevated LRA was correlated with prematurity, craniosynostosis and postsurgical relapsing hyperthyroidism (HT); but patients with very high LRA didn't show any of these clinical signs. We found no correlation of an increased LRA with fetal onset of HT, low birth weight, advanced bone age, mental retardation and a long period of HT.Moreover, the comparison of the CCs of patients harboring the same mutation (M453T, S505N) showed no correlation.Considering the different diagnostic circumstances, therapeutic strategies and the limitations of a systematic analysis of case reports we found no clear evidence for correlation of the TSHR mutation's IVA with the CC of the patients.

Thyroid molecular and cellular biology
Poster

QUANTIFICATION OF SOMATOSTATIN-RECEPTOR EXPRESSION IN THE THYROID GLAND BY IN-111-PENTETREOTIDE SCINTIGRAPHY AND Ga-68-Dotatoc PET AND ASSOCIATION WITH THYROID PATHOLOGIES

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In-111-pentetreotide scintigraphy (octreoscan) as well as Ga-68-Dotatoc PET are based on somatostatin analogue peptides binding to somatostatin-receptors (SSTRs). SSTR expression could be of relevance for the pathophysiology of several thyroid diseases. We systematically quantified all octreoscans for one year and eight months as well as all PET scans with Ga-68-Dotatoc for nine months for their thyroid uptake. The relative uptake of In-111-pentetreotide in the thyroid gland was measured 24 hours after its application in 71 consecutive patients undergoing octreoscan. Octreoscan planar images were analysed by ROI technique. An upper mediastinal region was chosen for calculation of target to background ratios (TB-ratios). The relative uptake of Ga-68-Dotatoc in the thyroid was measured one hour after its application in 56 consecutive patients undergoing Ga-68-Dotatoc PET. PET-scans were analysed by ROIs in two coronal slices with a thickness of one centimeter. TB-ratio calculation was done as described. TSH, fT3, fT4 and anti-TPO-antibodies were measured, thyroid ultrasound was performed and the patient's history was recorded. Patients without thyroid pathology show a significantly higher density of SSTRs in their thyroid region as compared to patients after thyreoidectomy as measured by both methods ($p=0.019$ and $p<0.01$ respectively). Compared to normal thyroid glands the density of SSTRs in goiters with or without thyroid nodules is significantly increased ($p=0.005$ and $p=0.008$ respectively). The markedly increased density of SSTRs determined by octreoscan for the two patients with a hot nodule and one patient with toxic multinodular goiter is in line with previous reports.

Thyroid molecular and cellular biology
Poster

THE COMPARISON OF DIFFERENT DIAGNOSTIC METHODS IN ANALYSIS OF THYROID TUMORS IN CHILDREN: NEOPLASTIC MARKERS IN PREOPERATIVE MATERIAL vs. POSTOPERATIVE HISTOPATHOLOGICAL MATERIAL

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Diagnostic difficulties in detection of thyroid carcinoma preoperatively still exist in patients with thyroid nodules and therefore the adequate management is problematic to set up. The strong neoplastic markers are desperatly needed. The aim of the study was to analyse the reliability of markers in preoparative material from biopsy aspirates, in relation to final postoperative histopathological result. Expression analysis of selected markers [galectin-3 (GAL-3), HBME-1, cytokeratin 19 (CK19)] in preoperative biopsy aspirates (n=32) was compared with immunohistochemical (IH) and in situ RT-PCR analysis of postoperative material (nonneoplastic noninflammatory, nonneoplastic inflammatory, adenoma, adenoma with undetermined prognosis and carcinoma). Isolated total RNA from aspirates was used as a template in RT-PCR reaction for expression of GAL-3 and CK19. A complementary analysis (IH and in situ RT-PCR) was performed on tissues from paraffin-embedded blocks. A large number of preoperative samples was false-positive for GAL-3 expression, in both nonneoplastic and neoplastic tumors. Such a reaction was observed also in thyrocytes transformed to oxyphilic cells and in oncocytes from Hashimoto's thyroiditis (IH). The similar phenomenon was observed in adenoma with oxyphilic transformation. These results prove the low usefulness of GAL-3 as a differentaial marker of thyroid neoplasm in contrast to antibodies against HBME-1 and CK19. The latter results were in agreement with RT-PCR analysis in preoperative material. CK-19 was more specific, directed to neoplastic process. The results from RT-PCR in situ were less attractive because of costs and difficulties in interpretation. None of the analysed markers was enough sensitive and specific for the reliable diagnostic protocol.

Thyroid molecular and cellular biology
Poster

FUNCTIONAL AND MOLECULAR CHARACTERIZATION OF WILD TYPE PENDRIN AND SOME MUTANTS FOUND IN THE SPANISH POPULATION

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Pendrin (*PDS*) is a glycosylated transmembrane protein mainly expressed in the thyroid, inner ear and kidney, where it acts as a chloride/anion exchanger. Mutations in the *PDS* gene (*SLC26A4*) cause non-syndromic and syndromic deafness; particularly, Pendred syndrome is an autosomal recessive disease characterized by sensorineural hearing loss, inner ear malformations and impaired iodide organification, which may lead to goiter. We recently set up a fast fluorometric method, exploiting the EYFP (Enhanced Yellow Fluorescent Protein) properties, for functionally scrutinize the iodide/chloride exchange activity of wild-type pendrin and its mutant forms of clinical interest. By this method, we confirmed that pendrin is able to work as an iodide/chloride exchanger, when iodide is present in high concentration (135 mM) in the extracellular solution. We also measured the transport activity of several pendrin mutants, most of them new, found in the Spanish population in individuals affected by Pendred syndrome or non-syndromic deafness with Enlarged Vestibular Aqueduct (EVA); we could discriminate between non-functional, partially functional mutants and polymorphisms. We also assessed the subcellular localization of some mutants by means of western blot experiments (anti-pendrin antibody kindly provided by Prof. Bernard Rousset); we found, for instance, that PDS R409H is able to reach the plasma membrane (in agreement with its reduced, but significant ion transport capability), though protein abundance at the endoplasmic reticulum and plasma membrane levels is reduced with respect to wild type pendrin. In conclusion, the functional and molecular characterization of pendrin mutants of clinical interest could help to elucidate the mechanisms underlying the Pendred syndrome and other diseases.

Thyroid molecular and cellular biology
Poster

IMMUNOHISTOCHEMICAL MARKERS ARE USEFUL TO PREDICT THE RISK OF LYMPH NODE METASTASIS IN PAPILLARY THYROID CARCINOMA

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Several immunohistochemical markers have been used to aid in the diagnosis of papillary thyroid carcinoma (PTC). They have never been used to predict the risk of lymph node metastasis from PTC. The purpose of our study was to evaluate these immunohistochemical markers as predictive factors between PTC and lymph node metastasis. ***Patients and Methods:*** The study cohort consisted of 44 well differentiated PTC including 29 with lymph node metastasis (N1 PTC) and 15 without lymph node metastasis (N0 PTC), embedded in a microarray and immunostained with antibodies to HBME-1, CK 19, Galectin-3, TPO, and c-erbB-2. ***Results:*** All N1 PTC and 9 N0 PTC (61%) showed reactivity for HBME-1 (p=0.0007). With c-erbB-2, 5 of 24 N1 PTC (21%) and none N0 PTC exhibited diffuse reactivity (p=0.021). Expression of other markers (CK 19, Galectin-3, and TPO) did not show any specificity or sensitivity for lymph node metastasis. Twenty-one (88%) N1 PTC and 10 (43%) N0 PTC had more than two markers that showed reactivity (p=0.0015). Various combinations between the different markers were tested; the more significant were Galectin-3+CK19 (N0 PTC 26% vs. N1 PTC 0%; p=0.007), and HBME-1+CK19+C-erbB-2+Galectin-3 (N0 PTC 0% vs. N1 PTC 17%; p=0.047). ***Conclusion:*** Our study shows that immunohistochemical markers are useful in the diagnosis of extension of PTC to the lymph nodes. HBME-1, which has proved its utility as a positive marker for PTC may be also the most useful marker for lymph node metastasis. Our results suggest also that if more than 2 markers show reactivity, the risk to have lymph node metastasis is high.

Thyroid molecular and cellular biology
Poster

PROGNOSTIC SIGNIFICANCE OF MICROSATELLITE INSTABILITY AND IMMUNOHISTOCHEMICAL ANALYSIS IN PAPILLARY THYROID CARCINOMA WITH LYMPH NODE METASTASIS

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Lymph node involvement (N1) is frequent in papillary thyroid carcinoma (PTC). The purpose of our study was to estimate the risk of recurrence of N1 PTC with immunohistochemical markers routinely used for diagnosis of malignancy and microsatellite instability (MSI). ***Methods:*** Eighteen patients with PTC and synchronous lymph node metastasis were studied. They were treated by total thyroidectomy with modified radical neck dissection and radioiodine therapy. Genomic DNA isolated from thyroid, lymph node tissue and blood samples (matched control) was amplified by polymerase chain reaction on twelve microsatellites: *D2S115, D2S123, D2S399, D11S912, Ret, TSHR, THRA1, NR21, BAT-26, BAT-25, NR24*, and *Mono 27*. Thyroid tissue samples (tumoral and nontumoral) were also evaluated for the expression of HBME-1, Galectin-3, CK-19, TPO and c-erbB-2, using a DakoCytomation Autostainer-Plus®. A univariate analysis was performed to detect a correlation between MSI, immunophenotypic signature, and cancer recurrence. ***Results:*** There were 13 women and 5 men with a median age of 47.5 years (range: 27–87 years). There were 6 pT1, 2 pT2, 10 pT3 (2002 TNM classification). HBME-1 was positive in 12 cases (66%), Galectin-3 in 13 (72%), CK-19 in 14 (77%), TPO in 0, and c-erbB-2 in 1 (5%). Eight tumors (44%) presented a MSI-positive status. *THRA1* displayed the highest level of MSI at 28%. MSI occurred in *D2S399* at a frequency of 22%, *NR21* at 11%, *D2S115, D2S123* and *Ret* at 5%, with no occurrence (0%) at the nearby *D11S912, TSHR, BAT-26, BAT-25, NR24* or *Mono 27* loci. The median follow-up was 58 months after thyroidectomy. Ten patients (56%) developed a locoregional recurrence. No correlation was found between immunophenotypic status and recurrence. Recurrence occurred in 4 MSI-positive (50%) and 6 MSI-negative (60%, p=0.67). ***Conclusion:*** Immunohistochemical techniques and MSI status do not allow predicting the risk of recurrence in PTC patients with synchronous lymph node metastasis.

Thyroid molecular and cellular biology
Poster

ROLE OF PAX8 IN THE MIGRATORY/INVASIVE BEHAVIOUR OF THYROID CANCER CELL LINES

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Introduction and Purpose: Paired box-containing transcription factors play fundamental roles in tissue development and cell differentiation, regulating also cell proliferation, migration and survival. Among these factors, Pax8 is critical for the maintenance of the thyroid differentiated phenotype through the expression of tissue-specific genes, NIS, TPO and Tg. Moreover, Pax8 expression decrease or is lost in follicular thyroid carcinomas as well as in oncogene-transformed thyroid cells. Since the dedifferentiation process is associated with an increased aggressiveness in thyroid transformation, our purpose was to relate Pax8 status with thyroid cancer invasiveness. ***Results:*** Q-RT-PCR and Western blot studies for Pax8 were performed in order to estimate its expression levels in cells derived from follicular (WRO), papilar (NPA and TPC1) and anaplastic (FRO and ARO) thyroid tumours, using as positive and negative controls differentiated PCCl3 cells and medullary TT cells, respectively. The results obtained showed that Pax8 expression was depleted in NPA and ARO cell lines. Moreover, the proliferation (MTT assay) and invasiveness studies, including wound healing and Matrigel invasion assays, confirmed increased malignant properties for these Pax8 null cell lines. Altogether these data suggest a relationship between the lost of Pax8 expression and a mechanism of invasiveness, despite that these data do not demonstrate a cause-effect. In order to prove that Pax8 is the responsible of the effects observed, we have generated a Pax8-containing adenovirus in order to check this malignant behaviour by over-expressing this gene in Pax8-null cells (NPA and ARO). ***Conclusions:*** Our preliminary results suggest a relevant role of Pax8 in the malignant behaviour of thyroid tumours and future studies will be directed to screen putative migration-related targets.

Thyroid molecular and cellular biology
Poster

PROLIFERATION OF THYROID PAPILLARY CARCINOMAS IS DEPENDENT BY Ca2+-CALMODULIN DEPENDENT KINASE II

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RET/PTC, TRK or activating mutations of Ras, are present in thyroid papillary cancers (PTC) and all lead to Raf-1/MEK/ERK cascade activation. The Ca^{2+}-calmodulin dependent kinase-isoform II (CaMKII) regulates Ras-induced Raf-1 activation in thyroid cells. ***Aim:*** Determine the role of CaMKII in the signal transduction leading to cell proliferation in PTC cells. ***Methods:*** We investigated expression, activation, signal transduction and role of CaMKII in primary cultures from normal thyroids, PTC, and in TPC-1 cell line. CaMKII was inhibited by a specific cell-permeant peptide and adenovirus carrying dominant-negative CaMKII. RasN17, lovastatin and wortmannin were used to inhibit p21Ras and p21-activated kinase (PAK). CaMKII activation was measured by Western blot and in vitro kinase assay. ***Results:*** CaMKII was expressed in any cell type. In normal cells CaMKII and ERK were in the inactive form at starvation and were activated by serum. In all cancer cells CaMKII and ERK were active also at starvation. CaMKII cDNA was not mutated. Calmodulin, phospholipase C (PLC) or CaMKII inhibition de-activated CaMKII in cancer cells, demonstrating that constitutive CaMKII activation was induced by up-stream phospholipase C-dependent factors. RasN17, lovastatin and wortmannin did not affected CaMKII phosphorylation, demonstrating that CaMKII activation is independent by Ras and PAK. CaMKII inhibitors arrested cancer cell proliferation in culture. ***Conclusions:*** Thyroid papillary carcinomas harbor an active calcium/calmodulin-, PLC-dependent signal inducing constitutive activated CaMKII. This permissive state allows up-stream signals to activate the Raf-1/MEK/ERK pathway leading to uncontrolled cell proliferation and is a site of pharmacological intervention.

Thyroid molecular and cellular biology
Poster

TOWARDS A PHYSIOLOGICAL TEC CULTURE SYSTEM - WILL SERUM-FREE MEDIUM LEAD THE WAY?

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Cell culture allows researching differentiated cellular functions under conditions mimicking the in vivo biology as well as the pathophysiology of the thyroid gland. Interpretable results require the preservation of physiologically functioning cells. Human thyroid epithelial cells (TECs) are often cultured in Ham's F12 medium (F12), but F12 has never been optimised for culture of TECs. F12 both contains serum and hormones in the supraphysiological range, probably influencing physiological functions and hampering the extrapolation of results to clinical conditions. We evaluated a physiologically composed serum-free medium (MPM-LK) with respect to the preservation of TEC differentiated functions in primary and secondary monolayer cultures. Representative parameters were evaluated by immunostaining and light microscopy (morphology and intracellular thyroglobulin (Tg) content) and by fluorescence-based measurement of H_2O_2 and cathepsin B in a microplate system. TEC differentiated function was influenced by media: Cell adhesion and cell number was lower in MPM-LK than in F12. In primary cultures without TSH, H_2O_2 production was lower in MPM-LK than in F12, whereas cathepsin B activity and intracellular Tg content was higher in MPM-LK. TSH influence on TEC differentiated also differed between media: In primary cultures, TSH increased Tg content and decreased H_2O_2 production in F12 only, but decreased cathepsin B activity in both media. Levels of all measured parameters and their response to TSH were altered by passage. In sum, culture conditions influence TEC functions and complicate data use. Comparison of results obtained in primary and secondary cultures should be performed with care. Even though doubt remains as to the best markers of physiological TEC function in culture, our results indicate the possibility of establishing culture conditions more optimal for the preservation of physiological TEC function than the usually employed secondary cultures in F12.

Thyroid molecular and cellular biology
Poster

AN in vivo STUDY OF THE DIRECT RESPONSE OF RECOMBINANT HUMAN TSH IN SERUM LEVELS OF ADIPOCYTOKINES

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Background: Extrathyroidal TSH receptors are suggested to have a functional capacity. Moreover, the expression of TSH receptors increases in Grave's ophtalmopathy, which originates from an increased volume of the orbital content. **Aim**: To explore the effect of rhTSH on extrathyroidal receptors in human adipose tissue. ***Method:*** RhTSH (0.9 mg) was administrated intramuscularly to 18 adults. Six patients had treated central hypothyroidism (CH group), six had untreated CH (newCH group), six had pituitary insufficiency but were considered TSH-sufficient (nonCH group). Six healthy matched controls underwent the same study-protocol. In the CH group, levothyroxine was replaced with triiodothyronine five weeks prior to stimulation with the treatment withdrawn the week before the rhTSH administration. Serum levels of leptin, adiponectin, resistin, IL-6 and TNFα were analysed at baseline, 24 and 48 *hours after 0.9 mg injection.* ***Results:*** A TSH induced effect was revealed in adiponectin (mean 9.3±1.2 to 9.9±1.4 µg/L, p=0.008) and resistin (mean 12.8±0.7 to 11.6±0.6 µg/L, p=0.008) when all subjects were studied as one group. Moreover, adiponectin was reduced in hypothyroidism (CH group 5.7±1.7 µg/L vs controls 12.8±3.8 µg/L), p=0.037, and IL-6/kg body weight decreased (0.057±0.04 to 0.032±0.04 ng/L/kg, p=0.028) when euthyroid individuals became hyperthyroid after stimulation with rhTSH. ***Conclusion:*** Changes in adiponectin and resistin occurred in response to stimulation with rhTSH, which indicate that adipose tissue respond directly to TSH in vivo. This could be an important feature in endocrine (Grave's) ophtalmopathy.

Thyroid molecular and cellular biology
Poster

NM41, A CYSTINE-KNOT PROTEIN SECRETED FROM THYROID CELLS, IS EXPRESSED EARLY IN MOUSE AND HUMAN DEVELOPMENT

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“Cystine-knots” are proteins with a conserved pattern of cysteines that form disulfide bonds and three-dimensional loop-structures with the appearance of a knot. Many are “morphogens” expressed early in development, they are secreted as dimers and trigger signals through specific membrane receptors in an autocrine/paracrine way. We identified NM41 as a cDNA preferentially expressed in human thyroid, but also present in other tissues, like lung. NM41 protein displays a pattern of cysteines overlapping the consensus for “cystine-knots”, and a signal peptide for secretion. To identify the role and mechanism of action of NM41, we studied the presence of typical properties of cystine-knots in this novel protein.Treating lysates from cells stably expressing human NM41-myc recombinant protein with the crosslinker disuccinimidil suberate (DSS), we show that NM41 heterodimerizes with a partner protein of approximately 70 KDa. NM41-myc is also secreted out of cells, since a protein band of its molecular weight is detected in concentrated conditional medium of HEK293 cells transfected with the corresponding plasmid.Finally, we explored the timing of expression of NM41 by RT-PCR. Presence of NM41 was first confirmed in mouse and human adult thyroid and in lung tissues. Then, using 11.5 days whole mouse embryo cDNA and human lung samples from spontaneous abortions at 13.5 and 15 weeks gestation, we show that NM41 is present early in mouse development as well as in human fetal lung. These findings identify the main functional features of the “cystine-knots” in NM41. Since thyroid and lung share many early expressed developmental genes, NM41 shapes as a candidate gene for thyroidal (and lung) embryonic defects. So far, only transcription factors (NKX2.1, PAX8, FOXE1) but no “morphogens”, the true effectors of organ development, have been involved in the pathogenesis of human thyroid dysgenesis.

Thyroid molecular and cellular biology
Poster

THE EXPRESSION OF ALTERNATIVELY SPLICED 5'-UTR mRNA VARIANTS OF HUMAN THYROID HORMONE RECEPTOR BETA 1 (THRB1) IS DEPENDENT ON THE TISSUE TYPE AND ABERRANT IN CLEAR CELL RENAL CELL CARCINOMA (ccRCC)

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Thyroid hormones are important for all human tissues and play essential role in development, growth and proliferation acting via triiodothyronine (T3) dependent nuclear receptors (THRA and THRB). Most of the effects of thyroid hormone occur at the level of cell-cycle and metabolism-dependent gene expression regulation through the interactions of nuclear thyroid hormone receptors and DNA regulatory regions called thyroid hormone response elements (TRE). The aim of our study was to investigate the meaning of alternatively spliced 5'-untranslated regions (5'-UTRs) of THRB1 mRNAs in clear cell renal cell carcinoma (ccRCC) when compared to their controls (the contralateral pole of the same kidney not infiltrated by cancer). We have demonstrated that 5'-UTR of THRB1 mRNAs in other tissues are expressed in different patterns dependent on tissue type. Moreover, analyzing the expression profile of THRB1 5'-UTR mRNAs by semiquantitative real time PCR we have observed that mRNA levels in ccRCC samples and their controls were significantly lowered in cancer tissues which was accompanied by alteration in the ratios of different 5'-UTR alternatively spliced variants. It has been shown before that the length and the sequence of 5'-UTRs may have an impact on lowering the gene expression by inhibition of protein translation. The change we have observed in expression profile of THRB1 5'-UTR mRNA variants could be possible explanation for poor correlation between mRNA and protein level showed by many previous reports regarding ccRCC. Our results indicate as well that protein synthesis may be regulated at the pretranslational level by 5'-UTR length- and sequence- dependent manner.

Thyroid molecular and cellular biology
Poster

DECREASING SODIUM IODINE TRANSPORTER EXPRESSION AND IODINE UPTAKE IN FRTL-5 AFTER LOW DOSE IRRADIATION WITH 131I IS CAUSED BY A REVERSIBLE CELL CYCLE ARREST

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Stunning in thyrology is defined as reduced therapeutical radioiodine uptake after diagnostic 131I application in patients with differentiated thyroid cancer .Recently we demonstrated that FRTL-5 cells are an useful system to investigate changes in radioiodine uptake after 131I irradiation. This “stunning” effect with low dose irradiation was pronounced, NIS-associated and suggests a compatibility to radioiodine uptake in vivo. The current investigation focus on a possible cell cycle arrest of FRTL-5 cells caused by DNA damage after low dose 131I administration. ***Method:*** TSH-stimulated FRTL-5 cells were incubated during their period of growth with 131I (100 kBq/ml medium). Time-dependent 131I uptake and viability of FRTL-5 cells was assessed at 2–168 h after radioiodine application. Cells were collected and stored frozen. By means of WESTERN blotting of the cell homogenates of 2–168 h G1/S and G2/M cell cycle checkpoints were investigated using mAb against phosphorylated cdc2, chk1, chk2, rb and p53. ***Results:*** G1/S cell cycle arrest was observed after 48 h of 131I application, while G2/M arrest appeared after 96 h. Cell cycle arrest coincided with reduced radioiodine uptake and decrease of NIS mRNA. ***Conclusions:*** The “stunning” effect in FRTL-5 with low dose irradiation appears to be the cause of DNA damage by e- induced free radicals. The resulting cell cycle arrest and transcription block of NIS may be reversed by DNA repair.

Thyroid molecular and cellular biology
Poster

BRAF-INDUCED DEDIFFERENTIATION AND INVASIVENESS IS MEDIATED BY AN AUTOCRINE LOOP INVOLVING TGF-BETA IN THYROID CANCER

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Deregulation of TGF-beta has been implicated in the pathogenesis of a variety of diseases, including cancer and fibrosis. The role of TGF-beta in cancer biology and cellular signalling is complex and it is believed that cellular context is a crucial determinant of the ultimate outcome of TGF-beta signalling in both normal and tumour cells. RAS has been reported previously to inhibit TGF-beta signalling in epithelial cells via phosphorylation of the linker region of Smads, which prevents their translocation to the nucleus. However, we have previously shown that in thyroid cells BRAF not only does not interfere with TGF-beta-mediated Smad signalling but seems to induce by itself phosphorylation and nuclear translocation of endogenous R-Smads, enhancing Smad-dependent transcriptional activity. We also demonstrated that this cooperativity was involved in BRAF-induced dedifferentiation, namely NIS expression. Using an inducible form of BRAF in PCCl3 cells we have further demonstrated that NIS repression is dependent on the operation of an autocrine loop involving TGF-beta, whose secretion was induced by BRAF. Subsequently, inhibition of TGF-beta receptor by either a neutralizing TFG-beta monoclonal antibody or a small-molecule TGF-beta receptor I kinase inhibitor clearly reinduced NIS functional activity in thyroid cancer cells. In addition, preliminary results show that BRAF-induced invasiveness can also be abrogated by blocking TGF-beta signalling using these two inhibitors. In view of this dual role of TGF-beta in dedifferentiation and invasiveness during thyroid carcinogenesis, we believe that antagonizing TGF-beta signalling can be a new and promising targeted therapy for thyroid cancer.

Thyroid molecular and cellular biology
Poster

RELATIONSHIP BETWEEN SUSCEPTIBILITY FACTORS INHERITANCE TO GRAVES DISEASE AND PATIENTS? OUTCOME

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Cytochrome P4501A1(CYP1A1) gene encodes for the enzyme aryl hydrocarbon hydroxylase, plays a key role in phase I metabolism of polycyclic aromatic hydrocarbons. The glutathione S-transferase (GST) system consists of a large multigenic group of detoxifying enzymes whose phase II activity, catalyzing the conjugation of toxic and mutagenic compounds with glutathione, is essential for cell protection and related to smoking. We aimed to evaluate the influence of polymorphisms in both phases I and II genes encoding, respectively, CYP1A1m1 and GSTM1, GSTT1 and GSTP1 enzymes in Graves\' disease (GD) susceptibility and patients? outcome. Using a PCR that included beta-globin gene as a DNA quality control to study GSTM1 and GSTT1 and a PCR-RFLP approach to study GSTP1 and CYP1A1m1 polymorphisms, 374 GD patients (163 with ophthalmopathy) and 494 paired controls were studied. GSTM1 and GSTT1 genotypes were equally distributed in cases and controls. However, GSTP1 variants (GSTP1v) were more frequent in GD patients (GSTP1 Ile/Ile= 40.1%, Ile/Val= 48.3%, Val/Val= 11.4%) than in controls (GSTP1 Ile/Ile= 59.3%, Ile/Val = 31.5%, Val/Val = 9.1% - $p < 0.0001$). Also, CYP1A1 variants (CYP1A1v) were over-represented in GD patients (wild-type= 44.6%, heterozygous= 52.6%, homozygous= 2.6%) compared to controls (wild-type= 62.4%, heterozygous= 34.6%, homozygous= 2.8% - $p= 0.0015$). The risk for GD in a subject with GSTPv genotype, after adjusting for gender, age and ethnic group was increased more than 2 times (OR= 2,177; 95% CI= 1,655–2,863) and for CYP1A1v was increased in 2 times (OR= 2,063; 95% CI= 1,322–3,219). There was no association between clinical features, including ophthalmopathy, or treatment outcome and the GST or CYP genotypes. We conclude that the inheritance of phase I CYP gene variants and phase II GSTP variants represent a susceptibility factor to GD but do not influence its response to treatment.

Thyroid molecular and cellular biology
Poster

INFLUENCE OF IFN-GAMMA AND IODIDE EXCESS ON HLA DR EXPRESSION AND THYROGLOBULIN PRODUCTION IN HUMAN PRIMARY THYROID CELLS

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HLA class II molecules are heterodimeric, polymorphic transmembrane glycoproteins expressed on cells of the immune system and on the affected cells of autoimmunity. A formerly sequestred or modified antigen must be bound to MHC class II molecules and carried with them to the cell surface in order to be presented to T cells and to trigger autoimmune process. Very low doses of cytokines produced in the site of chronic inflammation, could induce intracellular structure damage and consequently thyroid cell apoptosis. Also, the inhibitory effect of iodide on thyroid cell proliferation and thyroglobulin production in vivo and in cultured thyroid cells has been previously reported. The aim of this study was to examine the influence of iodide excess with low doses of IFN-gamma on HLA DR expression and thyroglobulin production in human primary thyroid cells. In medium with six hormone mixture a low percentage of primary human thyrocytes express HLA DR molecules. IFN-gamma alone induced HLA DR expression in doses from 2U/ml, whereas KI alone did not induce HLA DR expression even when doses over 10 mM were used for stimulation. However, a significant decrease in HLA DR positive cells was obtained when doses over 10 μM of KI were combined with low dose IFN-gamma. The growth curve did not reveal any effect on the cell proliferation, nor the signs of apoptosis or necrosis were detected. Thyroglobulin (Tg) level augmented when TSH was added in aggregates of thyroid cells, but its production was inhibited with doses over 10 μM of KI alone. The combination of KI with low dose IFN-gamma inhibited Tg secretion significantly more, but less than IFN-gamma alone. The results from this study show that low as well as high dose of IFN-gamma may induce HLA DR expression and inhibit Tg production in human primary thyroid cells. However, low dose IFN-gamma and excess KI in combination had inhibitory effect on HLA DR expression while Tg production was less decreased in comparison with IFN-gamma alone. These results suggest a protective role of small iodine excess on the IFN-gamma induced cellular function and immune profile changes of human thyroid cells in culture.

Thyroid molecular and cellular biology
Poster

THE Na+/I- SYMPORTER (NIS) TRANSPORTS TWO OF ITS SUBSTRATES, I- AND ClO4-, WITH DIFFERENT STOICHIOMETRIES

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The sodium/iodide transporter (NIS) mediates active I- uptake in thyroid, lactating breast, salivary gland, and stomach epithelial cells. NIS-mediated I- transport is electrogenic with a 2:1 Na+:I- stoichiometry, i.e., when NIS activity is measured electrophysiologically in NIS-expressing oocytes, inward positive currents are recorded upon addition of I- or other anions that are NIS substrates. However, no currents are detected when perchlorate (ClO4-), a competitive inhibitor of NIS is used. This suggests that ClO4- either blocks NIS function without being transported, is translocated with a 1:1 stochiometry, or is transported at an extremely slow rate. The possible impact of environmental ClO4- exposure on the thyroid function of adults and nursing newborns is widely debated. We report a thorough analysis of NIS-mediated ClO4- transport in vivo and in vitro. When lactating rats received ClO4-, both mothers and suckling pups exhibited a ~50% decrease in thyroidal I- uptake relative to controls. For in vitro studies, we used a polarized MDCK epithelial monolayer setup in which NIS is expressed on only one side. Simultaneous addition of I- and perchlorate markedly slowed NIS translocation of I- to the the opposite side, as compared to the control with I- alone, because perchlorate was translocated first. Hill plot analysis of NIS-mediated Na+-dependent perrhenate transport revealed that perrhenate, an analogue of ClO4- is transported with a 1:1 stoichiometry, explaining the absence of electrical currents observed with perrhenate also. Taken together, these observations provide novel mechanistic information on NIS, i.e., that NIS catalyzes substrate transport with different coupling ratios. In addition, that perchlorate is unequivocally transported by NIS and therefore actively concentrated in the milk, suggests that ClO4- water contamination may be more serious than previously thought, particularly for the most susceptible population, pregnant and lactating women and nursing newborns.

Thyroid molecular and cellular biology
Poster

APPROACHES IN ELUCIDATING THE DISULPHIDE BOND PARTNER OF CYSTEINE 283 IN THE EXTRACELLULAR DOMAIN OF THE HUMAN THYROTROPIN RECEPTOR

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The ectodomain segment containing the highly conserved SHCC motif in the human thyrotropin receptor has been identified as an important region in intramolecular signaling. Cys283 or its putative disulphide bond is crucial for maintaining receptor's TSH binding ability. This study adopted several approaches to identify the partner of this critical residue: 1) by mutating potential Cys partner on the B subunit individually, and 2) by cumulatively mutating consecutive Cys residues downstream of the SHCC motif. For each mutant receptor, relative specific binding (R.S.B.) was calculated as a measure of TSH-binding ability after normalization with receptor surface expression. The first approach led to severe R.S.B. reduction and failed receptor trafficking, a likely result of altered receptor conformation from illegitimate disulphide bridge formation. The second approach managed to narrow candidate partners down to Cys at codon 398 and 408. Cys 398 was the more likely bond partner because tempering with this amino acid resulted in greater loss of TSH binding compared to Cys 408. Leaving Cys 283 and 398 as the only Cys pair in the C-terminus could alone support 40% of ligand binding capacity. Substitution of disulphide bond at these sites by direct bridging with either electrostatic salt bridge through arginine/glutamic acid coupling or bi-histidine and nickel ligation did not restore R.S.B values which suffered from loss of critical Cys283.

Thyroid hormone metabolism and action
Poster

DIFFERENTIAL REGULATION OF DIO2 AND DIO3 DURING METAMORPHOSIS IN SENEGAL SOLE (SOLEA SENEGALENSIS): DAILY AND DEVELOPMENTAL CHANGES

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It is generally accepted that thyroid hormones (TH) lead metamorphosis in flatfish as well as in anuran amphibian. However, the respective roles of Dio2 and Dio3 iodothyronine deiodinases, two crucial enzymes for anurans metamorphosis remain unknown in fish.The ***aim*** of this work was to investigate the regulation of Dio2 and Dio3 during metamorphosis of Senegale sole, a flat-fish species and the daily variations of these deiodinases. ***Methods:*** We sampled pre-, early-, medial- and late-metamorphic sole larvae at different times of the day. The expression of both enzymes was studied by real time-PCR, and T3 and T4 concentrations were measured by radioimmunoassay. Finally, D2 activity was measured using 125I-T4 (2nM) as substrate.***Results:*** The main morphological changes which take place during sole metamorphosis are that the left eye migrates on the right side, being the left side of the body facing to the bottom. These changes were associated to a significant increase of both TH (2–6-fold depending on daytime) at the middle-late metamorphosis. The expression of *dio2* was slightly higher during metamorphosis than in premetamorphic larvae. However, Dio2 activity increased at a higher degree during sole development (from ~20 to ~80 fmol/h/mg prot). Sole *dio3* was up-regulated (2–3-fold) at the middle-metamorphosis stage and down regulated at late metamorphic stages. Besides, *dio3* mRNA abundance was higher at the end than at the beginning of the day. ***Conclusion:*** Changes in Dio2 and Dio3 expression during sole development support a role of both enzymes in flatfish metamorphosis. Daily variations in *dio3* mRNA suggest a role of D3 in transducing environmental information during sole metamorphosis. Whether these enzymes regulate TH actions in a temporal and tissue specific way in fish remains to be investigated.

Thyroid hormone metabolism and action
Poster

RESULTS OF IODIC SECURITY MONITORING OF BYELORUSSIAN CHILDREN

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The urgency of problem of liquidation of iodic deficiency in Belarus is based on lack of average iodic (Median Urinary Iodine (UI) 44.5 mkg/l), established as a result of the first national research (1997–1999). The purpose: to estimate iodic security in Belarus after active, five years, preventive actions based on universal and constant use of iodated salt. Method: 1304 children (9–14 years), living in 12 settlements were surveyed. UI (Sandell-Kolthoff reaction) and ultrasonic sizes of thyroid gland have been estimated during the research.Results: The median of UI in Belarus has made 179.2 mkg/l (UI has increased from 44.5 up to 179.2 mkg/l), including: The level of UI less than 20 mkg/l has been stated with 0.2%; 20–49.9 mkg/l - with 1,0 %, and 50–99.9 mkg/l - with 11.3 surveyed children. Excretion iodine with urine more than 100 mkg/l has been registered with 86 % of surveyed children. The frequency of diagnosing the goiter remained high enough and has made 12,8% that testifies about persistent a goiter. The reverse dependence between the prevalence of goiter and iodic security hasn't been stated: the frequency of prevalence of goiter has been decreased in some areas which influenced national parameter – the prevalence of goiter has decreased from 17.2 to 12.8%, though has not achieved minimum values characterizing the absence of iodic deficiency. However the increase of frequency of goiter registration in Gomel area (less in Minsk area) was stated and cause of studying other reasons which may cause goitrogenic effect. Thus, the carried out research testify the efficiency of strategy of liquidation of iodic deficiency based on a large-scale use of iodated salt.

Thyroid hormone metabolism and action
Poster

ERRONEOUS REGULATION OF E2F1 BY TRIIODOTHYRONINE AND ITS NUCLEAR RECEPTORS: A POTENTIAL IMPACT ON OVEREXPRESSION OF E2F1 IN CLEAR CELL RENAL CELL CARCINOMA

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T3 co-regulates cell proliferation mostly by the regulation of transcription of the genes involved in this process. *E2F1* encodes one of the key regulators of proliferation. Unliganded TR bound to *E2F1*'s negative TRE activates expression of this gene, while in the presence of T3 the activity of *E2F1* decreases. ***The aim of the work*** was assessment the regulation of *E2F1* promotor by wild type and mutant TRs in the presence of different amounts of T3, the expression level of TRs and their main function (the disturbance of TR binding to DNA and to T3), the expression level of E2F1 (mRNA and protein) in ccRCC. ***Materials:*** Tissues samples from 34 ccRCC, 34 peritumoral respective controls and 15 cancer-free kidney. We use real-time PCR, EMSA, radioligand binding assay, western blot, cell culture and transcription regulation assay. ***We showed*** that in the presence of unliganded wild type TR's, *E2F1* promoter fragment is activated, while in the presence of T3 the transcription is repressed in T3 dose-dependent manner. However, in the presence of a dominant-negative 23TRα1 mutant *E2F1* promoter activity was approximately 3-fold higher than in the presence of wild type TRs both in the presence and in the absence of T3. The amount of TRs is increased in ccRCC and the disturbance of TR binding to DNA and to T3 is present. A significant overexpression of E2F1 on mRNA and protein levels was found in ccRCCs. The mean E2F1 mRNA and protein levels correlated significantly in both control types and in ccRCCs. No correlation was observed between the amount of TRs, or their DNA or T3 binding activity and E2F1 mRNA amount. ***Our results*** indicate that all T3-TR alterations observed in ccRCC tissues might contribute to the increased *E2F1* activity and, possibly, to activation of E2F1-downstream genes regulating cell cycle progress.

Thyroid hormone metabolism and action
Poster

A CASE OF THYROID HORMONE RESISTANCE SYNDROME IN A NEWBORN

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A total 122 different TRb gene mutations have been identified so far, with 46 occurring in more than one family. We present another family with thyroid hormone resistance syndrome. A newborn who was fourth sibling of a 35 year old mother was presented with exophthalmus, tachycardia, hyperbilirunemia and polycytemia 46 hours after delivery. His serum TSH and FT4 levels were markedly elevated (TSH :15.73mIU/l; normal range 0.5-4.2 mIU/ml, and FT_4: 100 pmol/l; normal range 12-22 pmol/L). Mother of the index patient had underwent to thyroidectomy for TMNG 8 years ago. Postoperatively her FT4 and TSH levels had remained elevated and pituitary microadenoma had been found on MRI finding. Thus a diagnosis of TSHoma had been suspected and octreotid LAR 20 mg had been administrated in another outpatient clinic. The patient had not followed regularly for the last 3 years. Similar laboratory findings were detected in mother's blood samples (TSH :5.30U/l; FT3: 14.96;FT_4: 36.8 pmol/l). TRH stimulation test showed exaggerated response of TSH to TRH (5, 28.62 and 30.29 pmol/L at 0, 15 and 30 minutes, respectively). On the basis of these findings, thyroid hormone resistance syndrome has been suspected. For the precise diagnosis, genetic testing was carried out on the index patient, two siblings, his mother and father and his two aunts . Exon 9 and 10 of the TRb-1 were amplified by PCR and possible candidate mutations screened by using direct DNA sequencing. In conclusion, in this presented case and his mother, a well known mutation (A317T) was detected on exon 9 of the TRb-1 gene and also it is shown that the thyroid hormone resistance has an autosomal dominant inheritance pattern in this family.

Thyroid hormone metabolism and action
Poster

MUSCLE D2 mRNA EXPRESSION INCREASES DURING NTI; NO RELATION WITH SERUM T3 LEVELS

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Introduction: It has been postulated that decreased muscle D2 expression might contribute to the decrease in serum T_3 observed during nonthyroidal illness (NTI). The D2 promoter however contains NFκB responsive elements which means that proinflammatory cytokines induced by NTI may increase D2 gene expression. ***Aim:*** To evaluate muscle D2 mRNA expression in several animal models of NTI which differ in the production of proinflammatory cytokines during the acute phase response.
Materials and Methods: Serum T_3 and muscle D2 mRNA were measured in three experimental mouse models of NTI:

- LPS administration: t=0, 4, 8 and 24h, saline-treated controls
- *Streptococcus Pneumoniae* infection: t=48h, saline-treated controls
- Turpentine induced abscess in the hindlimb: t=1, 2 and 5 days, saline-treated, pair fed controls.

Results: LPS administration and turpentine injection resulted in increased muscle D2 mRNA expression ($p<0.01$ for hindlimb-muscle in LPS and forelimb-muscle in turpentine), while serum T_3 levels decreased compared to baseline. In contrast; *S. Pneumoniae* infection decreased hindlimb-muscle D2 mRNA after 48h compared to control mice ($p<0.01$), while serum T_3 levels were not different between both groups. ***Conclusion:*** Muscle D2 mRNA expression increased after LPS administration and turpentine injection, but decreased after *S.pneumoniae* infection. These differences might be due to differences in the production of proinflammatory cytokines during the acute phase response and the severity of illness. Mice recover after LPS administration and turpentine injection, while mice die after *S. pneumoniae* infection. The observed changes in muscle D2 mRNA expression were not related to the observed changes in serum T_3 in these animal models.

Thyroid hormone metabolism and action
Poster

RISK FACTORS OF PERMANENT HYPOPARATHYROIDISM AFTER THYROIDECTOMY

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Postoperative hypocalcemia is a common complication after total thyroidectomy. It is usually transient but permanent hypoparathyroidism is of particular concern. The purpose of this study was to identify risk factors in the development of permanent hypoparathyroidism. ***Patients:*** From January 1991 to September 2006 all patients who underwent a total had serum calcium analysis on postoperative day 1 (POD1). Another sample was taken on POD2 in case of hypocalcemia. Hypocalcemia was defined where <1.9mmol/L and in symptomatic patients with calcemia between 1.9 and 2.0 mmol/L. Vitamin D was added to calcium supplementation if calcemia was <1.6 mmol/L. Hypoparathyroidism was permanent if hypocalcemia persisted beyond 6 months. ***Results:*** 7040 patients had a total thyroidectomy. The overall hypocalcemia rate was 11%. Permanent hypoparathyroidism occurred in 2.5%. Age, sex, extent of thyroidectomy, weight of the gland and parathyroid hormone levels after surgery were not significant risk factors for permanent hypocalcemia. This complication was more frequent in completion total thyroidectomy, in case of malignancy, when a lymph node dissection was performed and if lymph nodes were metastatic. POD1 calcemia was 1.86 in patients who developed a permanent hypocalcemia and 1.84 in patients with transient hypocalcemia (p=0.24). On POD2, calcemia dropped to in the permanent group and slightly rose to 1.87 in the transient group (p=0.0011). When early vitamin D administration was required during hospitalization, the risk to develop permanent hypoparathyroidism was high. ***Conclusion:*** Risk factors for permanent hypoparathyroidism can be determined in hypocalcemic patients after total or near-total thyroidectomy. Evolution of calcemia between POD1 and POD2 is mandatory.

Thyroid hormone metabolism and action
Poster

TRANSIENT AND CONTINUED FETAL/NEONATAL HYPOTHYROIDISM AFFECTS EPIDIDYMIS, LEYDIG CELL AND SERTOLI CELL DEVELOPMENT

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Previously the effects of neonatal hypothyroidism on testes development have been investigated using propyl-thiouracil (PTU) treatment. These studies showed that PTU induced hypothyroidism delayed testes development. Recently it has been demonstrated that PTU influences Leydig cell function and possibly development directly, making it difficult to interpret the former data. In the present study a mild form of hypothyroidism was induced already during fetal development. Dams were fed an iodide-poor diet to which perchlorate was added to deplete endogenous iodide stores, or received a control diet. The hypothyroid diet was continued up to 0, 7, 14, 28, 35 of age, or continued through life (continuous hypothyroidism). Pups were sacrificed between days 16 and 85 after birth. No significant differences in testes development were found if treatment was discontinued at the age of 0 or 7 days. Transient hypothyroidism (14 or 28 days) resulted in a decrease in body and testes weight of the pups (up to 70%). Leydig cell proliferation, as identified by BrdU and 3ß-HSD labelling, was increased by transient and absolute hypothyroidism from day 35 onwards. Plasma testosterone levels were significantly elevated from day 21 to day 42 post partum. In contrast to the controls, tubular lumen formation was significantly delayed in the testes of both transient and continuous hypothyroid rats. 5α-reductase type 2 expression was decreased in the epididymis in the continuous hypothyroid group from day 28 onward. The data suggest that (transient) hypothyroidism over an age of 7 days influences both testis and epididymis maturation. Most strikingly however was the capacity of the continuous hypothyroid animals to reproduce at an age of 85 days although their body weight was reduced by 60–70%.

Thyroid hormone metabolism and action
Poster

THIOCYANATE OVERLOAD DECREASES IODINE SUPPLY IN IODINE-DEFICIENT BREAST-FED INFANTS

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Background: Na/I symporter in mammary gland (Tazebay *Nat Med* 2000) could mitigate Iod deficiency in breast-fed babies (Laurberg *Thyroid* 2002) & is blocked by thiocyanate (SCN) exposure in Danish smoking mothers reducing breast-milk Iod (Laurberg *JCEM* 2004). ***Methods:*** Data mining (1980), Iod-deficient & SCN exposed population, Congo(R.D.) ***Results:*** Iodised oil during pregnancy corrects severe Iod deficiency of babies (optimal breast-milk Iod around 110 µg/day, urine concentration around 30 µg/dL) (In *Dietary reference intakes…* . 2000. Washington, DC). Elevated serum TSH is more frequent in babies than in breast-feeding mothers of untreated group. Breast-milk Iod was reduced in mothers of untreated group with a urine SCN concentration >P50 (2,1 mg/dL). No such effect was observed in treated group. ***Conclusion:*** The detrimental interaction of severe Iod deficiency and SCN exposure on breast-milk Iod can be prevented by iodised oil to the mother during pregnancy.

Iodine nutritional status at Karawa, Northern Congo (R.D.) (1980). Geom. means (-/+sd). Mothers ± 1 mL iodised oil IM during pregnancy. t test or X2.

	Mother	**Baby**	**Prob**
Untr. Urine Iodine (µg/dL)	3,63(1,00-13,21)(58)	2,29(0,42-12,54)(50)	0,002
Untr. Blood TSH>=20 mU/L	12/104	33/92	5,30E-05
Tr. Urine Iodine (µg/dL)	26,06(9,95-68,31)(73)	15,36(3,46-68,23)(66)	2,40E-06
Tr. Blood TSH>=20 mU/L	0/105	4/93	NS
Maternal Urine SCN	**<= 2,1 mg/dL**	**>2,1 mg/dL**	
Untr. Breast-milk Iodine	2,89(1,07-7,80)(26)	1,62(0,74-3,54)(32)	0,015
Tr. Breast-milk Iodine	9,45(3,05-29,27)(39)	7,85(2,76-22,31)(32)	NS

Thyroid hormone metabolism and action
Poster

TYPE 3 DEIODINASE EXPRESSION IN INFLAMMATORY SPINAL CORD LESIONS IN RAT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Introduction: Axonal injury and neuron damage followed by remyelination failure occur within inflammatory lesions in the CNS of patients with multiple sclerosis (MS). Thyroid hormone is known to activate oligodendrocyte precursor cells (OPC) involved in remyelination. In addition, thyroid hormone improves and accelerates remyelination during experimental autoimmune encephalomyelitis (EAE), an experimental animal model of MS. We have recently shown that infiltrating inflammatory cells express type 3 deiodinase (D3), which catalyzes the inactivation of thyroid hormone, during chemical and bacterial inflammation. ***Aim:*** We tested the hypothesis that D3 expression occurs in inflammatory lesions during EAE. ***Materials and Methods:*** We studied D3 expression at the protein level by means of immunohistochemistry in the spinal cord of rats during various stages of relapsing EAE. Double immunostaining was performed to identify D3-expressing cells. ***Results:*** EAE inflammatory lesions were characterized by infiltrates of competent immune cells around the vessels in the white matter. These cells showed abundant D3 immunoreactivity while spinal cord without any infiltrate showed only sparse D3 positive cells. Double labelling indicated that ED1+ macrophages represent a minority of cells expressing D3 in inflammatory lesions. Morphological analysis indicated granulocytes as major cell type expressing D3. ***Conclusion:*** Inflammatory cells of infiltrates present in the spinal cord of rats developing EAE express D3 protein, suggesting local degradation of thyroid hormone. Decreased bioavailability of thyroid hormone in MS lesions may contribute to the pathogenesis of remyelination failure.

Thyroid hormone metabolism and action
Poster

CHARACTERIZATION OF POLYMORPHISMS IN THE THYROID HORMONE RECEPTOR BETA (THRB) GENE AND ESTIMATION OF THEIR INFLUENCE ON THYROID HORMONE PARAMETERS USING A LARGE TWIN COHORT

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Background: Thyroid hormone (TH) action is primarily mediated through nuclear TH receptors (THR). THRβ, encoded by the *THRB* gene (3p24.2), is involved in TH feedback regulation. TH levels show large inter-individual variation, whereas the set-point for each individual is within a narrow range, suggesting genetic influence. Previously, single nucleotide polymorphisms (SNPs) in the TSH receptor, the deiodinases, and TH transporters have been associated with serum TSH and TH levels. In this study we investigate the relationship between SNPs in the *THRB* gene and TH parameters. ***Methods:*** In DNA from 50 randomly selected subejcts SNPs were identified by sequencing all *THRB* exons and flanking regions. SNPs of interest were genotyped in two populations (156 healthy blood donors and 1,078 healthy Danish twins) by TaqMan assays and related to TH parameters. Heritability estimates were calculated using the Mx programme. ***Results:*** By sequencing, several SNPs were identified, of which three with allele frequencies of >5% were selected for analysis in blood donors and twins. Two of these were in high linkage disequilibrium with a R^2-value of 0.82, consisting of a synonymous SNP and an intronic SNP 63 bases upstream of exon 9. A third SNP was located 9 bases downstream of exon 9. No association between the SNPs and TH parameters were found in the blood donors. In the much larger twin population, the SNP located 9 bases downstream of exon 9 was associated with higher TSH levels (wild type: 1.70 ± 0.94; carriers: 1.86 ± 0.93 mU/l [mean ± SD], p=0.008), explaining 0.84% of the variation in serum TSH. ***Conclusions:*** In Danish twins an intronic SNP in the *THRB* gene is significantly associated with serum TSH, suggesting that genetic variation in the *THRB* gene influence TH feedback regulation.

Thyroid hormone metabolism and action
Poster

ORGAN-SPECIFIC EFFECTS OF 3,5-DIIODO-L-THYRONINE ON FATTY ACID UPTAKE AND RELEASE IN HIGH-FAT DIET-FED RATS THROUGH DIFFERENTIAL REGULATION OF AMP-ACTIVATED PROTEIN KINASE

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When administered to rats receiving a high-fat diet (HFD), 3,5-diiodo-L-thyronine (T2) prevents body weight-gain and fat accumulation. It induces an increase in hepatic fatty acid (FA) oxidation concomitant to their less efficient utilization. The effects of T2 on fatty acid oxidation (liver) and their release (white adipose tissue: WAT) involve the AMP-activated protein kinase (AMPK), but the course of T2-mediated modulation of AMPK phosphorylation in these tissues is unknown. Twelve groups of rats were used, either receiving a 1-day, 1-week, 2-week or 4-week HFD, with or without a daily i.p. injection of T2 (25 µg/100 g BW), or a standard diet for the corresponding periods (N). After 1 day, 1 week and 2 weeks, liver AMPK phosphorylation was decreased in both HFD and HFD-T2-treated rats with respect to normally fed (N) controls whereas after 4 weeks the HFD-T2-treated rats showed a marked increase in liver AMPK phosphorylation with respect to N controls. AMPK phosphorylation in the HFD-T2-treated rats was correlated with the hepatic lipid content which increased upto 2 weeks of treatment but was almost absent after 4 weeks. In WAT, after 1 day AMPK phosphorylation was increased, but from 1 to 4 weeks, HFD-T2-treated rats showed a marked decrease in AMPK phosphorylation with respect to HFD controls and to N rats. Phosphorylated AMPK is known to inhibit WAT lipolysis through Ser-565 phosphorylation of hormone sensitive lipase (HSL), blocking phosphorylation (and activation of HSL) by protein kinase A (PKA) at position Ser-563. Indeed, the T2-induced reduction of AMPK phosphorylation was associated with an increased lipolysis in WAT, evaluated by measuring the release of glycerol from adipocytes freshly isolated from abdominal WAT. In conclusion, treatment with T2 results in increased FA utilization (liver) and release (WAT), through differential, transient regulation of AMPK phosphorylation.

Thyroid hormone metabolism and action
Poster

THE IMPACT OF THE C785T POLYMORPHISM IN THE DEIODINASE TYPE 1 GENE (DIO1) ON THE INTER-INDIVIDUAL VARIATION IN SERUM THYROID PARAMETERS

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Introduction: Thyroid hormone (TH) levels show substantial inter-individual variation. About 65% of this variation is due to genetic factors, whereas 35% is accounted for by environmental factors. Different polymorphisms in TH pathway genes have been identified. The contribution of these polymorphisms to the inter-individual variation is yet unknown. The C785T polymorphism in DIO1 has previously been associated with serum TH parameters in a healthy population. Here, we estimated the proportion of TH heritability that is accounted for by this polymorphism. ***Methods:*** Genotypes of the DIO1-C785T polymorphism were determined by Taqman assay in 1192 healthy Danish twins and associated with serum TH parameters using regression analysis. The effect of the polymorphism on the heritability estimates was quantified by incorporating the genotype information into structural equation modelling using the Mx program. ***Results:*** The DIO-C785T was in a dose dependent manner associated with higher serum rT3 (wildtype: 0.35±0.004; heterozygote: 0.37±0.005; and homozygote: 0.40±0.010 nmol/l (mean±SE); $p<0.001$) as well as higher serum FT4 ($P=0.005$). In addition, carriers of the DIO-785T allele had a lower T3/T4 ratio ($p<0.001$) and T3/rT3 ratio ($P<0.001$) and a higher rT3/T4 ratio ($p<0.001$). Controlling for this polymorphism the heritability estimate for serum rT3 was reduced from 65.5% to 64.8%. Furthermore, the DIO1-C785T explained 0.86% of the variation in serum FT4 levels. No significant associations between the polymorphism and serum TSH, FT3, T4 and T3 were found. ***Conclusion:*** The DIO1-C785T polymorphism is consistently and significantly associated with serum rT3 and FT4. The proportion of inter-individual variation of rT3 and FT4 that is accounted for by this genetic marker is limited.

Thyroid hormone metabolism and action
Poster

3,5-DIIODO-L-tHYRONINE-INDUCED PROTEOMIC CHANGES IN LIVER MITOCHONDRIA FROM HIGH FAT-FED RATS: EVIDENCES FROM TWO-DIMENSIONAL ELECTROPHORESIS

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The liver supports processes involved in energy and substrate metabolism. It participates in the control of blood glucose levels modulating the balance between glycogenolysis and gluconeogenesis and is involved in fat oxidation and storage being the major site of fatty acid synthesis in presence of excess dietary cardohydrates, amino acids or ketone bodies. Diet-induced obesity, commonly associated with diseases such as type 2 diabetes, hypertension, heart failure or cancer, can also lead to steatosis, which is correlated to alterations of the mitochondrial function. 3,5-diiodothyronine (T2), a naturally occurring iodothyronine, has been shown to be biologically active. It is able in affecting energy metabolism and mitochondrial efficiency. Recently, it has been reported that long term treatment with T2 powerfully reduces adiposity in rats fed high fat diet stimulating hepatic fatty acids oxidation and increasing mitochondrial uncoupling, leading to a less efficient utilization of lipid substrate reducing body weight-gain and liver steatosis. However, how the mitochondrial phenotype responds to T2-treatment remains unknown. Today, proteomic tools allow the study of proteoms and their qualitative and quantitative changes under various conditions. Therefore, we combined two-dimensional gel electrophoresis (2D-E), and mass spectrometry to analyzed liver mitochondrial proteome from standard diet-fed control rats (N), high-fat diet-(HFD) fed rats and T2-long term (30 days) treated HDF rats (HFD+T2). The identification of differentially expressed (two-fold changes and $p< 0.05$) proteins (from 2D-E maps) among the experimental groups, allowed us to obtain an integrated view of the phenotypic/ metabolic adaptation (oxidative stress, lipid metabolism, urea cycling and respiratory chain activity) occurring in liver mitochondrial proteome during HFD and steatosis and after T2- treatment. Interestingly, T2-treatment counteracted several changes induced by HFD. The identified proteins represent novel starting points for elucidating the mechanisms of T2 action on liver mitochondrial metabolism.

Thyroid hormone metabolism and action
Poster

HIGH INDUCTION OF TYPE III DEIODINASE (D3) EXPRESSION AFTER PARTIAL HEPATECTOMY IN THE REGENERATING MOUSE AND RAT LIVER

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Background: The deiodinases type I (D1) and type II (D2) catalyze the activation of T4 to T3, whereas type III deiodinase (D3) catalyzes the inactivation of T3 and T4. D3 plays a key role in controlling thyroid hormone bioavailability. It is highly expressed during fetal development, but also in other processes in which proliferation occurs, e.g. in vascular tumors. Since tissue regeneration is dependent on cellular proliferation and often associated with activation of fetal genes, we evaluated deiodinase activities and mRNA expression in rat and mouse liver after partial hepatectomy (PH). ***Methods:*** Mice and rat underwent PH by removing 70% of the liver. Liver tissues were collected at PH (t=0) and at different time periods after PH; the same region of the liver was isolated from sham-treated animals. Tissues were analyzed for deiodinase activity (HPLC analysis) and mRNA expression (RT-PCR). ***Results:*** In rat, D3 activity was 9-fold increased at 20h after PH (t=20) and 3-fold at t=48; also D3 mRNA expression peaked at t=20. In mice, D3 activity was 40-fold increased at t=36 (and 5-fold at t=12; 8-fold at t=24; 15-fold at t=48; 7-fold at t=72). Furthermore, BrdU incorporation (measure for cell proliferation) peaked at t=24 in rat and at t=36 in mice. No significant effect on D1 activity or mRNA expression was found upon PH. D2 activity was absent in all tissues. ***Conclusion:*** The high induction of hepatic D3 expression after PH suggests that D3 is important in the modulation of thyroid hormone levels in regenerating liver, in which proliferation takes place and thus probably low T3 is needed. Serum and tissue thyroid hormone levels will be measured to study the impact of the high D3 activity on systemic and local thyroid status.

Thyroid hormone metabolism and action
Poster

DIFFERENCES IN T3 UPTAKE AND METABOLISM IN FIBROBLASTS OF MCT8 PATIENTS REFLECT PHENOTYPIC VARIABILITY

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MCT8 facilitates T3 uptake in various cell types, notably neurons in the CNS. The MCT8 gene is located on the X-chromosome. Mutations are associated with severe psychomotor retardation and high serum T3 levels in affected males. We studied consequences of mutations in cultured skin fibroblasts of previously reported patients P1 (del267–360), P2 (2.4 kb deletion), P3 (G564R), a novel patient P4 (delF501) and the heterozygous mothers of P3 (M3) and P4 (M4). Although all patients have severe psychomotor retardation, the phenotype of P4 appears milder, characterized by less severe hypotonia and better motor skills. Initial T3 uptake rate in fibroblasts was 24% of control in P1, 21% in P2, 26% in P3, 41% in P4, 53% in M3 and 66% M4. Increasing the time of incubation, net uptake of T3 relatively increased in P4 and his mother compared with control and other mutant fibroblasts, suggesting an impaired T3 efflux. This was confirmed in transfected cells by direct measurement of T3 efflux. Relative to controls, type 3 deiodinase (D3) activity was decreased in fibroblasts of P1, P2, P3, M3 and M4. In contrast, D3 activity was increased 4.9-fold in P3. We conclude that mutations in MCT8 result in decreased T3 uptake in skin fibroblasts. The milder phenotype of P4 may be correlated with less impaired T3 influx by the delF501 mutant compared with the other patients. The greater defect in T3 efflux vs influx of delF501 may even lead to an increased intracellular T3 as confirmed by the increased expression of D3, which is under positive control of T3. Therefore, MCT8 mutations may be associated with either a decreased or an increased intracellular T3.

Thyroid hormone metabolism and action
Poster

THE RELATIONSHIP BETWEEN DIURNAL VARIATION OF TSH AND THYROID BLOOD FLOW WITH DOPPLER ULTRASONOGRAPHY IN HEALTHY ADULTS

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Thyroid stimulating hormone (TSH) shows pulsatile and circadian variation. The aim of present study was to determine the presence of a correlation between the Doppler parameters of thyroid blood flow and TSH levels in the morning, late afternoon and at midnight in healthy adults. Thirty individuals were included in the study. They were between 24 and 54 years of age. There were 15 female (50%) and 15 male (50%). The mean age 32.20 ± 7.39 years and the mean BMI was 24.70 ± 3.80 kg/m^2. Blood samples for measuring free T3, free T4, and TSH levels were taken at 08.00, 16.00 and 24.00 o'clock. Simultaneously, the dimensions of the thyroid gland were measured and thyroid volumes were calculated with conventional gray scale ultrasound. Subsequently the peak systolic velocity (PSV) and resistance index (RI) of the inferior thyroid artery were measured with Doppler ultrasound. TSH levels started to increase in the early morning and reached the highest levels at the midnight. Difference of the TSH levels during this period was statistically significant($p<0.001$). Average thyroid volumes were significantly higher at 24.00 h compared to 08.00 h and 16.00 h. Differences between these thyroid volumes were all statistically significant ($p<0.001$). The variations of the PSV were evaluated at the same times. Average blood flow was higher significantly at 24.00 h compared to 08.00 h and 16.00 h($p<0.01$ and $p<0.05$). Mean RI values measured at 24.00 h were lower than those measured at 08.00 h and 16.00 h. Difference between these average values were evaluated as significant statistically ($p<0.001$ and $p<0.05$). Although thyroid volume and TSH levels increase at midnight compared to 08.00 h and 16.00 h there's no significant correlation between these parameters($p>0.05$). This is the first study that shows blood flow variations of thyroid gland due to diurnal variations of TSH levels. Doppler ultrasound is important in the evaluation of the thyroid pathologies and it becomes increasingly popular. We think that diurnal blood flow of the thyroid gland must be evaluated with Doppler ultrasound in TSH dependent thyroid pathologies or in autoimmune diseases affecting the TSH receptors.

Thyroid hormone metabolism and action
Poster

THE ASSOCIATION BETWEEN SELENIUM AND THYROID FUNCTION TEST

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Purpose: The thyroid contains more selenium per gram of tissue than any organ and selenium like iodine, is essential for appropriate thyroid hormone synthesis , activation and metabolism. selenium is mainly present in the follicular or thyrocyte compartments. This would appear reasonable , because thyroid follicles continuosly produce H2O2 for thyroid hormone synthesis throughout life. H2O2 is essential for the TPO enzyme in the process of iodide oxidation. In the human thyroid gland, the H2O2 generating system is under the control of TSH through the stimulation of the phospholipase PIP2-IP3-Ca2 cascade. The cytotoxic effect of H2O2 on thyroid cells induced caspase 3-dependent apoptosis that occurs at H2O2 concentrations that are sufficient to induce necrosis.. Therefore in thyrocyte need a very potent antioxidative defens system aginst H2O2 and reactive oxygen intermediates drived thereof. ***Methods:*** We performed a descriptive, observational, cross-sectional study in the setting of endocrinology clinic. We studied 150 subjects were selected that none of them had other known endocrine diseases. To evaluate the thyroid function, thyroid stimulating hormone (TSH), thyroxine (T4), (T3),(T3RU), FTI levels were measured in the serum of patients. Two arbitrary serum-TSH threshold levels (TSH < 1.0 and > 4.0 mU/L) were introduced in order to classify, respectively, hyperthyroidism and hypothyroidism, as well as euthyroid conditions (1.0 < TSH < 4.0 mU/L. Serum selenium levels determine by Graphite Furnace Atomic Absorption. ***Results:*** There was a positive correlation among serum selenium levels and T3 (r= 0.53, p= 0.01) T4 (r= 0.32, p=0.01) FTI (r= 0.29/ p= 0.01) but correlation between selenium and TSH was negative (r= –0.46, p=0.01) and the serumic selenium levels in hyperthyroids patients were lower than other groups. To conclude. Serum selenium correlates with thyroid hormones, and might play a role in thyroid enlargement & thyroid dysfunction. Our finding suggest that selenium as an antioxidant trace element protect against thyroid dysfunction.

Thyroid hormone metabolism and action
Poster

3, 5-DIIODOTHYRONINE (T2) INDUCES CALCIUM OSCILLATIONS AND NO RELEASE IN GH3 CELLS

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T2, a naturally occurring metabolite of T3, not only exerts rapid, short-term effects on energy metabolism in vivo via mechanisms involving mitochondrial pathways but also exerts effects on deiodinase I activity in pituitary-derived GH3 cells in vitro. GH3 cells display an oscillatory pattern of intracellular calcium ($[Ca^{2+}]_i$) either spontaneous (oscillating cells) or induced by several receptor agonists (quiescent cells) that modulates a variety of cellular functions. In addition, endogenous nitric oxide (NO) plays a permissive role in the occurrence of $[Ca^{2+}]_i$ oscillations by inducing both extracellular calcium influx and intracellular release from IP3- and ryanodine-sensitive stores. Aim of this study was to test the effects of T2 on $[Ca^{2+}]_i$ and NO production in GH3 cells. Interestingly, the exposure to T2 (10nM) induced an appearance of $[Ca^{2+}]_i$ oscillations in quiescent cells and an increase of their frequency in oscillating cells, as evaluated by single cell FURA-2 microfluorimetry. In addition when GH3 cells were exposed to T2 (10 nM, 20 min) an increase of intracellular NO, measured with the specific intracellular dye DAF-2 DA, occurred. IP3-sensitive stores depletion induced by ATP (100 μM) and the SERCA inhibitor thapsigargin (1 μM) did not prevent both $[Ca^{2+}]_i$ increase and NO production induced by T2. By contrast, the pre-treatment with the mitochondrial protonophore FCCP (250 nM) completely prevented both the increase of $[Ca^{2+}]_i$ and NO production induced by T2. Collectively, our results suggest that :1.T2 induces $[Ca^{2+}]_i$ oscillations in quiescent GH3 cells; 2. T2-triggered oscillations are linked to NO production; 3. T2 -triggered oscillations are dependent on the modulation of mitochondrial activity.

Thyroid hormone metabolism and action
Poster

SULFUR- AND SELENIUM-CONTAINING DERIVATIVES OF MMI AND PTU ON TYPE I 5′-DEIODINASE AND THYROPEROXIDASE

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Thiourea compounds such as methimazole (MMI) and 6-propyl-2-thiouracil (PTU) are widely used antithyroid therapeutics in the treatment of hyperthyroidism. While MMI and PTU effectively inhibit thyroperoxidase (TPO), high concentrations of PTU additionally block type I 5′-deiodinase (5'DI). However, these drugs may cause various side effects after long term administration. Therefore, alternatives might be beneficial. We tested novel derivatives of MMI and PTU for their in vitro effects on 5′DI and TPO. Among them were 5-methyl-2-thiouracil (C1), 6-benzyl-2-thiouracil (C2), 5-carboethoxy-2-thiouracil (C3) and Se-methimazole, the selenium-containing analogue of MMI (C4). Effects on 5′DI were analyzed in crude membrane extracts prepared from the human hepatocarcinoma cell line HepG2 using 3,3′,5-triiodothyronine (reverse T3) as substrate and dithiothreitol as cofactor. Inhibition of TPO was tested using digitonin extracts of membrane preparations of the human thyroid carcinoma cell line FTC-238 stably transfected with an expression plasmid coding for human TPO. TPO activity was determined with guaiacol as substrate and H_2O_2 as cosubstrate. The PTU-derivatives C1, C2 and C3 inhibited 5′DI with IC50 values of 0.7, 0.1 and 1.7 µM, respectively. PTU had an IC50 value of 1.7 µM in our assay. C1, C2 and C4 inhibited TPO with IC50 values of 55, 121 and 31 µM, respectively, as compared to 9 µM for MMI and 125 µM for PTU. In summary, C1 and C2 were more efficient inhibitors of 5′DI than PTU. Although C1 and C4 are less efficient inhibitors of TPO than MMI, they are more potent than PTU. The exchange of selenium for sulfur in C4 does not enhance the inhibitory effect of the compound as compared to MMI. It will be of interest to test the inhibitory potency of the substances in vivo along with their possible side effects.-
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Thyroid autoimmunity
Poster

BIOLOGICAL VARIATION OF THYROID AUTOANTIBODIES AS A TOOL FOR FUTURE MONITORING OF PATIENTS

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Purpose: It has been shown that the level of autoantibodies against thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) in combination with s-thyrotropin (TSH) is predictive of future thyroid disease. Due to the poor sensitivity of assays for measurements of these antibodies, the possibility of following early stages of increasing antibody concentrations has not hitherto been possible. Novel sensitive analytical methods, however, provide the first tool for investigation of these early increases. The next tool needed, is estimates of biological variation in healthy individuals. ***Material:*** Twenty-four healthy women (23 to 46 years) were investigated by blood sampling between 7.30 and 11.00 twice a week for a month. ***Methods:*** The antibodies TPOAb and TgAb were determined on AutoDELFIA, Perkin Elmer/Wallac. Biological variations were estimated from a three-level analysis of variance. ***Results:*** Nine women had antibodies above the upper reference limits of the laboratory. There was no systematic variation in the autoantibodies during the menstrual cycle. The biological variation (without analytical variation) was 11.3 % for TPOAb and 8.5 % for TgAb, which both are higher than for total serum-IgG of 3.0 %. Analytical variation was approximately 10 % for both antibody assays. ***Discussion:*** We hypothesize that increases in antibody concentrations – also within the reference intervals - will precede the changes in serum TSH and in clinical symptoms as well. ***Conclusions:*** It is possible to measure TPOAb and TgAb in all samples with the AutoDELFIA. The estimates of biological variations allow interpretation of monitoring data from patients monitored during early stages of thyroid diseases.

Thyroid autoimmunity
Poster

LOW CONCENTRATIONS OF THYROID PEROXIDASE ANTIBODIES (TPOAb). REAL ANTIBODIES OR ANALYTICAL NOISE?

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Background: In the literature on thyroid peroxidase antibodies (TPOAb) there is a huge variation in reference limits and decision points. Some authors as well as manufacturers of reagents claim to be able to measure TPOAb in all individuals whereas others are unable to obtain this sensitivity of their assays. ***Purpose:*** To demonstrate that the TPOAb concentrations measured in healthy individuals by our method (AutoDelfia, Perkin-Elmer/Wallac), are indeed TPOAb and not just analytical noise. ***Material and Methods:*** Experiment 1: Recovery of the WHO66/387 standard of TPOAb dissolved in various protein matrixes: A) Bovine serum albumin (BSA) and B) BSA + 8 g/L IgG, isolated from a pool of sera from patients with M-components. Experiment 2: TPOAb were removed by sequential extraction from three genuine patient serum samples at different levels of TPOAb (16, 64 and 298 kIU/L) and residual concentrations were estimated. ***Results:*** Experiment 1: For the TPOAb concentration 0.5 to 10 kIU/L the recovery was 118% in the BSA and 112% in the IgG. Experiment 2: In the three patient samples with TPOAb concentrations, the remaining concentration after extraction varied from 0.1 to 0.7 kIU/L corresponding to a residual of 0.16 to 0.56% of the original TPOAb concentration. Thus the residual concentration which is considered ‘analytical noise’, is below the detection limit of 1.0 kIU/L – and below the the lower reference interval limit of 2.1 kIU/L. ***Discussion:*** Each IgG M-component is a monoclonal antibody against an arbitrary unknown antigen. The concentration of all other natural antibodies including TPOAb is very low. ***Conclusion:*** It is possible to measure TPOAb in all samples from healthy individuals with the AutoDELFIA.

Thyroid autoimmunity
Poster

REFERENCE INTERVALS FOR ANTI-THYROID PEROXIDASE ANTIBODIES BASED ON NATIONAL ACADEMY OF CLINICAL BIOCHEMISTRY CRITERIA AND THYROID ULTRASONOGRAPHY

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Background: The aim of our study was to establish the reference intervals for anti-thyroid peroxidase antibodies (TPOAbs) in the Bulgarian population based on National Academy of Clinical Biochemistry (NACB) criteria and the results of thyroid ultrasonography. ***Material and Methods:*** We investigated a representative group of 2,415 subjects – 1,348 females, 1,067 males, mean age 47.7 years (18–94 ys) from 6 different regions of the country. They were asked to fill a questionnaire on lifestyle and medical history, serum TSH (0.39–4.2 μIU/ml) and TPOAbs (<12 IU/ml) were measured and thyroid ultrasonography was performed with units of Aloka and Esaote (7.5–10 MHz, Color Doppler). A disease-free group of 2,249 subjects was selected by excluding those at risk for thyroid disease. ***Results:*** We selected a referent group of 130 healthy males ≤ 37 years of age with no goiter or hypoechogenic structure as assessed by ultrasonography, without any personal or family history of thyroid or nonthyroid autoimmune disease and TSH between 0.5 and 2 mIU/L. The subsequent reference interval (97.5th percentile) for TPOAbs was 28.4 kIU/L. In the disease-free group 14.1 % had increased TPOAbs, 18.5 % females and 8.9 % males. TPOAbs immunoreactivity increased with age. Subjects with TSH in normal range had increased TPOAbs in 11.8 %, those with TSH < 0.39 mIU/L and TSH > 4.2 mIU/L had increased TPOAbs respectively in 9.6 % and 70.5 %. ***Conclusions:*** The prevalence of increased TPOAbs in Bulgarian population is the same as in other European countries and depends on age and sex. TPOAbs immunoreactivity is the most frequent cause of hypothyroidism.

Thyroid autoimmunity
Poster

TOO EARLY TO DISMISS YERSINIA ENTEROCOLITICA INFECTION IN THE AETIOLOGY OF GRAVES DISEASE. EVIDENCE FROM A TWIN CASE-CONTROL STUDY

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Yersinia enterocolitica (YE) infection has long been implicated in the pathogenesis of Graves disease (GD). The association between YE and GD could, however, also be due to genetic or environmental factors affecting the development of both YE infection and GD. This potential confounding can be minimized through investigation of twin pairs discordant for GD. Whether YE infection is associated with the development of TSH receptor antibodies (TSHRAb) in healthy individuals is unknown. ***Aim:*** To examine the impact of YE infection on 1) development of GD and 2) presence of TSHRAb in healthy euthyroid subjects. ***Design and Participants:*** A twin case-control study of 36 twin pairs discordant for GD and 16 pairs discordant for TSHRAb.***Methods:*** IgA and IgG antibodies to virulence-associated outer Yersinia membrane proteins (YOP) were measured in all the twins. ***Results:*** The prevalence of YOP antibodies was higher in twins with GD than in their healthy co-twins (YOP IgA, 53% vs 28%, p = 0.031 and YOP IgG, 56% vs 33%, p = 0.058). The odds ratio (95% limits) for the association between GD and YOP IgA and IgG was 5.5 (1.2–24.8), p = 0.027 and 5.0 (1.1–22.8), p = 0.037, respectively. In contrast, euthyroid twins with TSHRAb had a lower frequency of YOP antibodies compared with their co-twins (YOP IgA, 38% vs 56%, p = 0.288 and YOP IgG, 44% vs 63%, p = 0.288). The odds ratio between TSHRAb and YOP IgA and IgG was 0.4 (0.1–2.1), p = 0.273 for both antibodies. ***Conclusion:*** Within discordant twin pairs, twins with GD have an increased risk of YE infection compared to their healthy co-twins, while this was not the case for the presence of TSHRAb. This indicates a possible role of YE in the aetiology of overt GD but not in the development og TSHRAb per se.

Thyroid autoimmunity
Poster

INCREASE OF CXCL10 IN COCULTURES OF GRAVES' DISEASE THYROCYTES AND AUTOLOGOUS LYMPHOCYTES

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Introduction: CXCL10 is a chemokine that has been recently reported as a marker of inflammation in autoimmune diseases. ***Objective:*** To determine CXCL10 production during coculture of thyrocytes and autologous lymphocytes in patients with Graves'disease (GD). ***Patients and Methods:*** Thyrocytes and lymphocytes were obtained from 10 patients with GD. Thyrocytes were cultured for 24h with or without 1 or 10 U/mL interferon gamma (IFN-γ). They were also cocultured for 24h with peripheral blood (PB) lymphocytes or with intrathyroidal (IT) lymphocytes after mechanical isolation (MIT) or enzymatic dispersion (DIT). CXCL10 and IFN-γ concentrations were determined in culture medium by ELISA, using kits from R&D Systems or Beckman-Coulter for CXCL10 or IFN-γ, respectively. Thyroid cell survival was assessed with the 3-(4,5 dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide (MTT) assay. ***Results:*** The addition of 10 U/mL IFN-γ induced a rise in the CXCL10 secretion into the culture medium. Coculture with autologous lymphocytes increased the CXCL10 production whatever the lymphocyte origin. When thyrocytes were cocultured with IT lymphocytes (MIT or DIT), the CXCL10 levels and IFN-γ concentrations were higher than those obtained with PB lymphocytes. Stimulation of CXCL10 secretion and IFN-γ production was maximal in the presence of MIT lymphocytes. ***Conclusion:*** Lymphocytes induced an increase of CXCL10 concentrations when cocultured with autologous Graves' thyrocytes. The IFN-γ - induced CXCL10 secretion is maximal with MIT lymphocytes. The mechanisms involved in this stimulation remain to be defined.

Thyroid autoimmunity
Poster

DIFFERENTIAL EXPRESSION OF FAS ON PERIPHERAL T- LYMPHOCYTES FROM PATIENTS WITH GRAVES' DISEASE AND HASHIMOTO'S THYROIDITIS

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Purpose: Fas/Fas Ligand (FasL) apoptosis has a key role in the pathogenesis and clinical expression of autoimmune thyroid disease (AITD). Fas, FasL expression in intrathyroidal T lymphocytes and thyrocytes is regulated in a manner leading to thyrocyte apoptosis in Hashimoto's thyroiditis (HT) or lymphocyte apoptosis in Graves' disease (GD). We examined whether Fas and FasL are differentially expressed on peripheral lymphocytes in AITD. ***Methods:*** Participants were 30 patients with HT in subclinical hypothyroidism, 15 with untreated hypothyroid HT, 13 with untreated hyperthyroid GD and 20 healthy subjects. Fas, FasL expression on CD4, CD8 lymphocytes was evaluated using two and three color flow cytometry on FACScan and the appropriate software (CELL Quest, Becton Dickinson). ***Results:*** The proportion of Fas expressing CD4 T cells was increased in GD (64.14±14.18), HT (61.13±15.13) and subclinical hypothyroidism (61.42±13.02) compared to controls (49.93±7.78). The proportion of Fas expressing CD8 T cells was also increased in HT and subclinical hypothyroidism compared to controls. Mean fluorescence intensity (MFI) of Fas on CD4 cells, reflecting number of Fas antigens per cell, was decreased in patients with GD (89.92±12.47) compared to subclinical hypothyroidism (124.7±47.55), HT (117.74±46.33) and controls (117.5±15.32). MFI of Fas was also decreased in CD8 cells of GD patients compared to all groups. FasL expression was below 2%. ***Conclusion:*** Increased proportion of Fas expressing CD4 and CD8 T cells in HT and GD may indicate activation of the Fas pathway in AITD. Reduced intensity of Fas expression on T cells in GD may reflect the different degree of apoptosis compared to HT.

Thyroid autoimmunity
Poster

PRESENCE OF SERUM ANTI-THYROID ANTIBODIES (TAb) REPRESENTS A NEW PREDICTIVE PARAMETER IN HIGH AGGRESSIVE BREAST CANCER

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Purpose: A high incidence of TAb has been found in patients with breast cancer (BC). Aim of this study was to evaluate the prognostic value of TAb in aggressive BC. ***Methods:***The study group included 47 consecutive women (age: 53.1 ± 10.1 yr, mean ± SD) submitted to radical mastectomy for high malignancy degree ductal infiltrating carcinoma. All patients were evaluated for thyroid disorders after breast surgery and before any anti-tumoral adjuvant therapy. After surgery all patients were submitted to the same protocol of adjuvant chemo-hormonal therapy. ***Results:*** 31/47 (65.9%) patients were alive (survivors group: SG) and 16/ 47 (34.1%) were dead (deaths group: DG), five years after BC diagnosis. The overall prevalence of TAb was 15/47 (31.9%): 14/31 (45.1%) in SG and 1/16 (6.2%) in DG (p=0.008). Five years mortality was 15/32 (46.9%) in TAb- and 1/15 (6.7%) in TAb+ patients (p= 0.01). Eight out of 47 (17.0%) patients had Hashimoto's thyroiditis and 7 of them (87.5%) were in SG. Estrogen Receptor (ER) was measured in 43/47 (91.5%) BC specimens. ER was detected in 19/30 (63.0%) patients in SG and 3/13 (23.1%) in DG (P= 0.01). Five year mortality was 10/21 (47.6%) in ER- and 3/22 (13.6%) in ER+ patients (p= 0.008). According to the Proportional Hazard regression model the absence of ER expression (O.R. 6.54; p=0.006) and absence of TAb (O.R. 9.37; p=0.03) were related to a higher mortality rate. TAb were detected in 8/21 (38.1%) ER- and in 7/22 (31.8%) ER+ patients; no relation was found between ER expression and TAb positivity (p=NS). ***Conclusion:*** Patients with RE+ and TAb+ have a better prognosis. The absence of a significant relationship between these two parameters suggests an independent prognostic role of TAb in high malignancy degree BC.

Thyroid autoimmunity
Poster

COMPARATIVE ANALYSIS OF BLOOD REDOX PARAMETERS BY THYROID FUNCTION OF PATIENTS WITH AUTOIMMUNNE THYROID DISEASES

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Purpose: To clarify influence of thyroid metabolic status on blood redox parameters, were performed a complex estimation of patients with autoimmune diseases (ATD). ***Methods:*** 50 patients with ATD have been studied: 16 - with hyperthyroidism; 20 - euthyroidism, and 14 - hypothyroidism. 10 healthy subjects were selected as a control group. Thyroid metabolic status by ELISA, and blood redox parameters by EPR have been investigated. ***Results:*** The results of correlation analysis in whole group shown that ceruloplasmin (CP) inversely correlated with T4 ($r= -0.3718$, $p=0.008$); Fe^{3+}-transferrin (Fe^{3+}-T) inversely correlated with TSH ($r=-0.4447$, $p=0.001$); methemoglobin directly correlated with TG-ab ($r=0.3431$, $p=0.015$); lipid peroxyradicals directly correlated with TSH ($r=0.3548$, $p=0.011$) and TPO-ab ($r=0.2976$, $p=0.036$). In patients with hyperthyroidism has been only observed inverse correlation between CP and thyroid volume ($r=-0.5177$, $p<0.001$); in patients with euthyroidism only inverse correlation between CP and FT4 ($r=-0.4970$, $p<0.001$). But in patients with hypothyroidism inverse correlation between Fe^{3+}-T and TSH ($r=-0.6501$, $p<0.001$) and direct correlation between nitric oxide and FT4 ($r=0.5657$, $p<0.001$), and lipid peroxyradicals and FT4 ($r=0.5620$, $p<0.001$) have been established. Thus, according to obtained results oxidative stress occurs at ATD in spite of thyroid status. However, it is aggravated in case of hypothyroidism. The association of the thyroid function and REDOX-system should be considered in the treatment of ATD.

Thyroid autoimmunity
Poster

COMPARATIVE PHENOTYPE ANALYSIS OF INTRATHYROID AND PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS WITH AUTOIMMUNE THYROID DISORDERS

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Objectives: To compare differentiating markers of peripheral blood lymphocytes and lymphocyte infiltrating thyroid gland in patients with autoimmune thyroid diseases: Graves\' disease (GD) and Hashimoto\'s thyroiditis (HT). ***Materials:*** The study included 17 patients with GD and 5 with HT. Peripheral lymphoid cells expressing respective markers (CD 3, 4, 5, 8, 16, 19, 38, 23, 25, 95) were determined (number and percent) on a flow cytometer. Lymphocytes infiltrating thyroid tissue were examined for the presence of CD4+ and CD8+ markers by immunohistochemical assays. ***Results:*** We observed a slight percentage rise in CD4+ lymphocytes (T-helpers/regulators) in patients with GD; they remained in the normal range in HT patients. Percentage of CD8+ (T-suppressors) in GD was significantly smaller than in HT (p=0.011). Immunohistological studies showed that in the majority of patients with GD (88%), over 60% of lymphoid cells expressed CD4+ and only 30% had CD8+ marker. In HT, 30% of intrathyroid lymphocytes expressed CD4+ and 60% CD8+ differentiating antigen. Given autoimmune nature of either pathology, we determined activation markers of lymphoid cells in peripheral blood. Percentage of CD38+ lymphocytes (marker of activated lymphocytes) was significantly enhanced in HT and especially in GD. Activated B-lymphocytes (CD23+) and (CD5+/CD19+) B1 lymphoid cells characteristic of autoimmune processes were higher in GD suggesting predominantly B-cell activation in this pathology. The number of lymphocytes expressing apoptosis marker (CD95+) was higher in TH than in GD and correlated with the prevalence of programmed death of thyrocytes in TH. ***Conclusions:*** GD is characterized by higher B-cell activation and impaired apoptosis of peripheral blood lymphocytes compared with TH.

Thyroid autoimmunity
Poster

ALTERATIONS IN STIMULATORY TSH RECEPTOR AUTOANTIBODIES AFTER B-LYMPHOCYTE DEPLETION WITH RITUXIMAB IN GRAVES´ DISEASE

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Purpose: Rituximab (RTX) is a B-lymphocyte depleting agent. We recently reported that RTX was effective in inducing sustained remission in a subset of Graves´ disease (GD) patients, although no RTX-specific effect was observed on overall anti-thyrotropin receptor antibody (TRAb) levels. Here we assess the effect of RTX therapy on stimulatory TRAb activity (TSAbs). ***Methods:*** Sera from GD patients treated with methimazole+RTX (+RTX, n=8) or methimazole alone (-RTX, n=6) were drawn on day 1, day 8 or day 22 [ie. immediately prior to the 1st, 2nd and 4th (final) weekly RTX infusion]. Sera were incubated with cultures of TSH-R expressing CHO cells. Changes in serum TSAb activity, measured as cAMP production in the culture supernatant (Rao et al, Endocrinology 2003), were assessed using non-parametric statistics. ***Results:*** At day 22, overall TRAb levels had decreased to 81% baseline levels in both the +RTX and -RTX treated patients. However in +RTX patients, serum TSAb activity decreased to 45% (range: 13–62) of baseline levels (p=0.03); whereas in -RTX patients, serum TSAb activity was unchanged at 99% (range: 47–369) of baseline levels (p=0.50). Accordingly at day 22, the ratio of cAMP production / TRAb concentration decreased significantly in +RTX patients to 31% of baseline levels (p=0.03), whereas no change was observed in –RTX patients. ***Conclusion:*** The results point towards a differential rapid decrease in TSAb activity following RTX therapy. Changes in overall TRAb levels did not differ between groups. Whether this specific decrease depends upon humoral factors (e.g. changes in cytokine levels) or upon cellular factors (such as effects on particular plasma-cell subsets, or on abrogated B-cell antigen-presentation) remains to be determined.

Thyroid autoimmunity
Poster

ANTICLASTOGENIC EFFECT OF GINKGO BILOBA EXTRACT (EGb 761) IN GRAVES DISEASE PATIENTS RECEIVING RADIOIODINE THERAPY

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Chromosomal damage, as assessed by clastogenic factors (CFs) and micronuclei (MN) appearance, following 131I therapy of Graves' disease has been reported. We evaluated the effect of Ginkgo biloba extracts (EGb 761) treatment on time-course of CFs and MN appearance in lymphocytes from patients with Graves' disease after 131I therapy, in a double blind, placebo-controlled study. In the placebo group, MN increased early ($p<0.001$) after 131I, peaking at 21th day ($p=0.0003$) and declining thereafter. In EGb 761-treated patients MN increased early ($p<0.05$), returning to baseline value thereafter. Therefore, mean MN increment was significantly higher in the placebo group than in EGb 761-treated patients ($p<0.01$). Moreover, an early ($p<0.01$) and sustained ($p=0.002$) MN increase induced by CFs was observed in the placebo group. Conversely, in EGb 761-treated patients MN increase induced by CFs never reached the statistical significance; therefore, the mean of the MN increments was significantly lower than in placebo ($p<0.05$). A positive correlation between MN maximum increment and the bone marrow dose was observed in the placebo group only ($p=0.03$). No significant difference was observed in clinical outcome. In conclusion, EGb 761 treatment neutralized genotoxic damage induced by 131I therapy, without affecting the clinical outcome. Although 131I therapy is essentially safe, our data encourage for the use of Gingko Biloba extracts to prevent possible harmful genetic effects, particularly when treating children or young adults.

Thyroid autoimmunity
Poster

ASSOCIATION OF THE FUNCTIONAL NFKB1 PROMOTER POLYMORPHISM WITH GRAVES' DISEASE IN POLISH POPULATION

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Purpose: Genes encoding NF-κB related proteins, which are major transcription regulators of immune responses, are interesting candidates for association studies in autoimmune disorders. The aim of the present study was to investigate an association of a functional insertion/deletion polymorphism (-94ins/delATTG) located in the promoter region of the *NFKB1* gene (encoding p105/p50 NF-κB isoforms) with susceptibility to and clinical phenotype of Graves' disease (GD) in Polish population. ***Methods:*** Initially 388 patients with GD and 688 healthy controls, recruited in Warsaw, were genotyped for the *NFKB1* promoter polymorphism by the polymerase chain reaction restriction fragment length polymorphism method. Results of the study were replicated in another polish cohort (from Gliwice) which consisted of 198 patients and 194 controls. ***Results:*** In the Warsaw group, we observed a significantly increased frequency of genotypes possessing the polymorphic (-94del) allele in patients with GD compared to healthy controls (70.1% *vs*. 63.2%; p=0.02; OR=1.36; 95%CI:1.04-1.78). A replication study performed in another Polish cohort (from Gliwice) confirmed observed results: in GD patients the polymorphic genotypes were found more frequently compared to healthy subjects (74.3% *vs*. 63.9%; p=0.03; OR=1.63; 95%CI:1.06–2.51). In both groups no correlation was found between the investigated polymorphism and the clinical phenotype of GD: age of the disease onset, severity of eye changes, gender, positive family history or smoking habits. ***Conclusions:*** Our results suggest that the functional insertion/deletion polymorphism of the *NFKB1* gene confers susceptibility to GD in Polish population, however it does not seem to be associated with the clinical phenotype of GD.

Thyroid autoimmunity
Poster

THE PRO12Ala PPARgamma GENE POLYMORPHISM IS ASSOCIATED WITH LESS SEVERE AND LESS ACTIVE GRAVES' ORBITOPATHY (GO)

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Introduction – Aim: The PPAR gamma transcription factor, is involved in both adipogenesis and inflammation, which have been implicated in the pathogenesis of GO. The aim of this study was to explore the possibility that the Pro[12]Ala polymorphism of the PPARγ gene, associated with a modified transcriptional activity, might be affecting the severity of GO. ***Patients and Methods:*** We studied two cohorts of patients with Graves' disease: Group 1 comprised 160 patients of Dutch ethnic origin with GO, who attended the outpatients' clinic, dept of Endocrinology and Orbital Center of the Academic Medical Centre, Amsterdam. Group 2 comprised 106 unselected patients with Graves' disease of Greek ethnic origin, 42 of whom also had GO. CAS score, exophthalmometry measurements, lid oedema etc were recorded. Autoantibody levels were measured. ***Results:*** Allele frequency was 11.5%. There was no difference in the distribution of the polymorphism between Graves'disease patients with and without GO or between groups 1 & 2. Among group 1 patients proptosis was significantly lower in Pro[12]Ala carriers (20.1±3.3 vs 22.1±3.1, p=0.003, t test). PPARγ polymorphism carriers had lower TRab levels (mean rank 61.8 vs 83.2, p=0.015) and lower CAS score (available in 110 pts) (mean rank 38.9 vs 55.4, p=0.022, M-W test). The frequency of the polymorphism decreased with increasing CAS score (p=0.023 linear by linear association). Multivariate analysis (step) showed that the association of either proptosis or CAS score with the PPARγ gene variant remained significant when age, smoking and TSHRab levels were taken into account (p=0.01). ***Conclusions:*** The distribution of the Pro[12]Ala PPARγ gene polymorphism is equally present in patients with Graves' disease with or without GO. Among patients with GO this polymorphism is associated with less severe and less active disease.

Thyroid autoimmunity
Poster

RADIOIODINE THERAPY OF THE MARINE-LENHART-SYNDROM

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Aim: Marine-Lenhart Syndome (MLS) was defined as Graves`disease with autonomously functioning nodules. Currently there exist no data about the actual incidence of this desease and the results of radioiodine therapy. ***Methods:*** In a prospective trial 1110 patients with initial diagnosed hyperthyreoidism were evaluated. Physical examination, interview, ultrasound, scintigraphy and determination of T3, T4, TSH and antibodies were carried out in all patients. All patients were followed up at least one year after radioiodine treatment. ***Results:*** MLS was diagnosed in 37 patients (incience 3.3%). Following treatment with radioioidine 10 patients had normal serum TSH levels. Hypothyreoidism was present in 14 patients and hyperthyreoidism persisted in 4 patients. A newly diagnosed Orbitopathy was not observed after radioiodine treatment. ***Conclusion:*** The actual incidence of MLS was 3,3 % in patients with newly diagnosed hyperthyreoidism. Treatment with radioiodine offers the chance of euthyreoidism but includes a significant probability for a second therapy.

Thyroid autoimmunity
Poster

HASHIMOTO'S THYROIDITIS AND THYROID CANCER: RESULTS OF A ECHOGRAPHIC, CYTOLOGICAL AND HISTOLOGICAL STUDY

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Objective: The association between lymphocytic thyroiditis or Hashimoto's thyroiditis (HT) and papillary thyroid carcinoma (PTC) has been suggested by pathological studies, but it is difficult to prove due to the contrasting results obtained with different methodological approaches. Aim of this study was to evaluate this association comparing several diagnostic parameters. ***Patients and Methods:*** In a total of 207 patients thyroidectomized for thyroid nodules, we retrospectively compared: a) cytology and histology, b) presence of anti-thyroid autoantibodies (ATA), thyroid hypoecogenicity at ultrasound examination (USH) and lymphocytic thyroid infiltration (LTI), as parameters of HT. Cytology were classified as: benign (class II); mild increased risk (class III) and suspect or malignant (class IV) nodules. Histology were indicated as: follicular adenoma (FA), follicular carcinoma (FC) and PTC. ***Results*****:** When presence/absence of HT parameters were considered, a significantly increased prevalence of class IV *vs* class II and III cytology (51.2% *vs* 35%) and PTC *vs* FA (44.5% *vs* 26.7%) in ATA positive nodules were found ($p<0.05$). A significantly increased prevalence of PTC *vs* FA (54.8% *vs* 35.6%, $p<0.05$) and an high prevalence of class IV *vs* class II and III cytology (57% *vs* 44.6%) were also observed in LTI positive nodules. These results were also confirmed with the highest ATA titres and diffuse LTI pattern were considered. Although the presence of USH correlated with ATA and LTI positivity, no association between USH and cytological and histological results were found. ***Conclusions:*** Our study confirmed a significant association between PTC and TH, in particular with ATA and LTI positivity, providing evidence for a true association instead a secondary autoimmune reaction to PTC.

Thyroid autoimmunity
Poster

INTERFERON-GAMMA-INDUCIBLE ALPHA-CHEMOKINES CXCL9 AND CXCL11 EXPRESSION IN THYROCYTES, FIBROBLASTS AND PREADIPOCYTES FROM PATIENTS WITH GRAVES? OPHTHALMOPATHY: MODULATION BY PIOGLITAZONE AND ROSIGLITAZONE

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CXC alpha-chemokines play an important role in the initial phases of autoimmune thyroid disorders. Serum CXC alpha-chemokine CXCL10 is high in patients with active Graves? ophthalmopathy (GO). Human thyrocytes, orbital fibroblasts, and preadipocytes in primary culture from GO patients produce large amounts of CXCL10 when stimulated by IFN-gamma and TNF-alpha; furthermore, PPAR-gamma agonists (TZD), rosiglitazone and pioglitazone, dose-dependently suppressed IFN-gamma+TNF-alpha-induced CXCL10 release. Here, the effects of IFN-gamma and TNF-alpha stimulation and of increasing concentrations of TZD (pioglitazone or rosiglitazone; 0.1 mcM–10 mcM) on alpha-chemokines CXCL9 and CXCL11 secretion in primary cultures of thyrocytes, retrobulbar fibroblasts and preadipocytes from GO patients were tested. In primary cultures of thyrocytes, fibroblasts and preadipocytes from patients with GO, CXCL9 and CXCL11 were undetectable in the supernatant. IFN-gamma dose-dependently induced CXCL9 and CXCL11 release, whereas TNF-alpha alone, had no effect. However, the combination of TNF-alpha and IFN-gamma had a significant synergistic effect on CXCL9 and CXCL11 secretion. Treatment of cultures of thyrocytes, fibroblasts or preadipocytes with rosiglitazone or pioglitazone, added at the time of IFN-gamma and TNF-alpha stimulation, dose-dependently inhibited CXCL9 and CXCL11 release. TZD alone had no effect and did not affect cell viability or total protein content in thyrocytes, fibroblasts and preadipocytes. TZD did not cause significant adipogenic changes (as judged by Oil Red O staining) in either cell type after a 24-h period of incubation. In conclusion, in GO: 1) thyrocytes and retrobulbar cell types participate in the self-perpetuation of inflammation by releasing CXCL9 and CXCL11 chemokines under the influence of cytokines; 2) PPAR-gamma activation plays an inhibitory role in this process.

Thyroid autoimmunity
Poster

DIRECT ASSAY OF TSH RECEPTOR AUTOANTIBODIES CAUSING GRAVES' DISEASE CORRELATES WITH THE CLINICAL DIAGNOSIS CLOSER THAN ASSAYS BASED ON TSH DISPLACEMENT

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Hyperthyroidism in Graves' disease is caused by stimulating TSH receptor (TSHR) autoantibodies (sTRAb). Current assays quantify the pool of all TSHR autoantibodies (Ab) including blocking Ab. We developed the first in vitro assay for direct detection of sTRAb suitable for routine clinical diagnosis. A bridge assay is performed on microplates (MP) using recombinant chimeric hTSHR-rLH/CG receptors (chim-hTSHR) where the epitope for blocking Ab is replaced. Chim-hTSHR is anchored to MP by a C-term directed antibody. One arm of the sTRAb binds to the anchored chim-hTSHR, the second arm bridges to chim-hTSHR fused with alkaline phosphatase. Applying chemiluminescent substrate sTRAb were quantified using a luminometer (CentroLIA LB 961). The range of detection was established using WHO 90/672 showing between-run precision (lots and days) of CV <20 % from 0.3–50.0 IU/L at within-run CV <10 %. The power of bridge assay to distinguish between sera positive or negative for sTRAb related to clinical diagnosis was tested via ROC plot analysis to be excellent. Compared to TSH displacement assay bridge assay demonstrated a higher diagnostic accuracy. Clinical diagnosis expresses the degree of hyperthyroidism evaluated by clinical (e.g. tachycardia) and laboratory parameters (T3, T4, TSH) and dosis of anti-thyroid drugs in treated patients. For several patient sera with negative clinical diagnosis and positive result in TSH displacement assay the result in sTRAb bridge assay was negative according to clinical diagnosis. In one case the patient has to be treated with thyroxine to reach euthyroid metabolism giving proof that high value in the TSH displacement assay is caused by blocking TSHR autoantibodies. In addition, for other patients with positive sTRAb result and negative TSH displacement value the existence of sTRAb is confirmed by positive clinical diagnosis. Further improvement by bridge assay is an explicit diagnosis instead of grey zone value for several patient sera.

Thyroid autoimmunity
Poster

PROGRESSION OF THYROID-ASSOCIATED OPHTHALMOPATHY (TAO) IN RELATION TO CLINICAL ACTIVITY AND SERUM TSH-RECEPTOR ANTIBODIES

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Aim of this prospective study was to analyze the relationship between the inflammatory activity and the progression of thyroid-associated ophthalmopathy (TAO). In addition to the clinical activity score (CAS) we have studied the predicting value of serum TRAb, reported to be associated to active disease and independent risk factors for severity. Serum TRAb have been associated to hyperthyroidism and the clinical features of TAO at onset, but not to eye disease duration or progression. Fifty-one patients with TAO were studied prospectively. In order to calculate the CAS, all patients were evaluated in basal condition and 1–4 months after the first examination. All but one patients were followed for 4–29 months. Progression of TAO was assessed based on worsening of severity, of the CAS value or the decision to submit patients to treatment. ANN was employed for statistical analysis. Overall, 10 patients (20%) had a CAS³3. The CAS was significantly and inversely correlated with the duration of thyroid disease (p=0.02), but not of TAO. Twenty-one patients showed disease progression at 1–20 months of follow-up. All 10 patients with CAS³3 persistent at 2–6 months from the first patient's examination always showed disease progression. Among the 40 patients with CAS<3 progression at follow-up was observed in 11 (27.5%). A significant positive correlation was found between serum TRAb levels and the CAS values (p=0.028). Positive (>1.5 U/L) TRAb were detected in all patients with a CAS³3 and in 28/40 with a CAS<3. Serum TRAb levels were also found to be significantly higher in patients with CAS³3, when compared to those with CAS<3 (p=0.004). Furthermore, ANN showed that all patients with a CAS³3 and 9/11 of those with a CAS<3 had detectable serum TRAb and progression of the disease, for an overall predictive power of about 90% of patients. In the present study, we have shown that the progression of TAO is best predicted by a CAS³3 persisting 2–6 months from initial assessment. TAO may also progress in about 25–30% of patients who present with a milder disease activity (CAS<3), and can be predicted based on the presence of serum TRAb and statistical analysis by ANN.

Thyroid autoimmunity
Poster

PRIMARY SPONTANEOUS AUTOIMMUNE HYPOTHYROIDISM IS OFTEN DIVIDED INTO PRIMARY ATROPHIC HYPOTHYROIDISM AND HASHIMOTO´S HYPERTROPHIC HYPOTHYROIDISM

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Aim: To investigate thyroid volume in patients with primary spontaneous autoimmune hypothyroidism. ***Methods:*** In a population-based study, a computer-based system identified patients (n=685) with incident overt hypothyroidism (high TSH, low total T4 or free T4) in Denmark in a four year period. Among 578 patients classified as having spontaneous hypothyroidism, 247 subjects were invited to participate in an extensive investigation program. 144 patients + matched controls from the population (1:4) attended the program including ultrasound measurement of thyroid volume, and thyroid auto-antibody measurement (TPOAb, TgAb, TRAb). ***Results:*** Patients with newly diagnosed spontaneous autoimmune hypothyroidism had a median thyroid volume of 12.0 ml (controls 13.5 ml, p=0.001, Mann-Whitney). Log thyroid volume showed a perfect Gaussian distribution in both males (n=25) and females (n=119) with no bimodal pattern as often suggested, but dispersion of volumes was larger in patients than in controls (p<0.001, Levene´s test on log-transformed values). When three groups of patients with different thyroid volumes were compared (lower-25%/middle-50%/higher-25% volumes; median thyroid volumes 4.7/11.5/24.1 ml), patients with large thyroid volumes had more antibodies (median TPOAb 1,540/4,137/7,058 kU/l; median TgAb 72/125/1,195 kU/l), and thyroid function was lower in patients with small volume (median T4 21.0/45.0/36.7 nmol/l; median TSH 81.0/44.7/55.6 mU/l). No difference was observed in age, duration of symptoms or prevalence of TRAb (14.7/9.9/8.3 %). Findings were confirmed in multivariate *models.* ***Conclusion:*** In primary autoimmune hypothyroidism log thyroid volume follows a normal distribution. Cases with thyroid atrophy and goitre are only extremes within this distribution, and they do not represent separate disorders.

Thyroid autoimmunity
Poster

PATIENTS WITH EUTHYROID AND PRIMARILY HYPOTHYROID STATUS HAVE LOW TRAB LEVELS AND DEVELOP A TENDENTIOUS MILDER AND SIGNIFICANTLY MORE ASYMMETRIC GRAVES' OPHTHALMOPATHY

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Objective: To evaluate the clinical symptoms of euthyroid and primarily hypothyroid patients (eu/hypo) with Graves' ophthalmopathy (GO). **Patients and Methods:** 143 primarily hyperthyroid (hyper), 28 euthyroid (eu) and 11 primarily hypothyroid (hypo) consecutive patients with Graves' ophthalmopathy (GO) were seen within the first 6–12 months after the onset of GO and were followed for at least 12 month. All patients received standard thyroid and antiinflammatory treatment. Severity of GO was evaluated 12 months after GO onset. All patients were classified into mild or severe GO according to their CAS and NOSPECS scores at 12 months after GO onset. ***Results:*** Among the eu/hypo patients were 20 (51%) with mild and 19 (49%) with severe GO in comparison to 56 (39%) with mild GO and 87 (61%) with severe GO among the hyper patients (n.s.,p=0.17). The difference in the NOSPECS score at 12 months after GO onset was significant (4.1 versus 4.9; p=0.002), but not the difference in the CAS score. TRAb levels 6 months after GO onset were higher in hyper patients (mean 12.8 IU/l) than in eu/hypo patients (3.2 IU/l), (p=0.02) and in both groups significantly higher in patients with severe GO in comparison to mild GO. Eu/hypo patients developed significantly more asymmetry in the Hertel measurements than hyper patients (side difference: 1.9mm versus 1.0mm; p=0.01). A side difference of ≥3mm occurred in 23% of the eu/hypo and in only 4.8% of the hyper patients. ***Conclusion:*** The knowledge about the asymmetry is especially important for the euthyroid patients to consider GO in the differential diagnosis of unilateral proptosis. Like in primarily hyperthyroid patients higher TRAb levels are associated with severe GO stages.

Thyroid autoimmunity
Poster

AUTOIMMUNITY AND IODURIA AMONG A PREGNANT WOMEN POPULATION

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Introduction: The relationship between autoimmunity and iodine supplementation is controversial. ***Aims:*** 1. To determine the antiperoxidase antibody (TPO) prevalence among pregnant women (PW) from the Pyrenees (mountain area) and from the coast area. 2. To show the possible relationship between TPO prevalence and ioduria. ***Material and Method:*** A transversal study was setup including all consecutive pregnant women of the 4 hospitals of the Catalan Pyrenees and Hospital of Mataró. TPO was measured by an immunoanalitic assay 1-Bayer (positive >60 iu/mL) (TPO+) and ioduria by the Benotti method. ***Results:*** 266 women in the first trimester (1T) of gestation participated in the study (139 from the Pyrenees and 127 from the coast). Median of ioduria was 209 mcg/L among PW from Pyrenees area and 142 mcg/L among PW from coast area. Prevalence of TPO+ in the whole group in 1T was 17.6% (CI 95%: 13.1–22.3%): 11.8% (CI 95%:6.2–17.4%) among coast PW and 23% (IC95%:16–30%) among Pyrenees PW (p=0.012). TPO were analyzed in 72 PW in the third trimester of gestation (3T). 21% of them were TPO+ in 1T and 9.7% in 3T (p=0.008). By areas, 31.4% in the Pyrenees and 11.1% in the coast (p=0.03) in 1T and 8.6% and 10.8% respectively (NS) in 3T were TPO+. No correlation between TPO and ioduria was observed at 1T and 3T. 36 (50%) of PW studied in 3T, were supplemented with iodine (150 mcg/day). 7(19.4%) of them were TPO+ in 1T and 4(11.1%) in 3T. No case with TPO– in 1T was changed to TPO+ in 3T after iodine supplementation. ***Conclusions:*** Although TPO+ prevalence was higher in Pyrenees (population with a better ioduria), neither in 1T nor in 3T the autoimmunity was associated with the urinary iodine. TPO+ prevalence among PW of Pyrenees area decrease during gestation. Neither iodine supplementation nor better ioduria was related with an autoimmunity increase.

Thyroid autoimmunity
Poster

CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 GENE A/G (49) POLYMORPHISM IS RELATED TO ATROPHIC THYROIDITIS IN TURKISH COMMUNITY

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Polymorphic changes in the gene coding for the cytotoxic T-lymphocyte antigen-4 (CTLA-4) molecule are known to be effective in development of Autoimmune Thyroiditis (AIT). We investigated the A/G polymorphism at 49th position in exon 1 of CTLA-4 gene and frequency of it in various clinical forms of the disease, in patients with AIT. 100 cases, which have been diagnosed with AIT due to clinical, laboratory, ultrasonographic and scintigraphic evaluations, and 100 healthy volunteers were included in the study. When the frequencies of the AA, AG and GG alleles in patients followed up with the diagnosis of Hashimoto's Thyroiditis (HT) were compared to the control group, the difference was not found to be statistically significant. On the other hand, while no difference was found when the frequencies of the A/A and A/G alleles in patients followed up with the diagnosis of Atrophic Thyroiditis (AT) were compared to those of the control group; the frequency of the G/G allele in patients with AT was found to be significantly higher (p= 0.045, OR= 3.225). In conclusion, the frequency of G/G allele at 49th position in exon 1 of CTLA-4 gene is a risk factor for the development of AT in Turkish patients.

Thyroid autoimmunity
Poster

AUTOIMMUNE THYROID DISEASE, THYROID ULTRASONOGRAPHY FINDINGS AND THEIR RELATION WITH ANTI-GAD IN PATIENTS WITH TYPE1 DIABETES MELLITUS

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Purpose: To determine the frequency of anti-TPO, anti-thyroglobulin (TG) and glutamic acid decarboxylase autoantibodies (anti-GAD) in Turkish patients with type-1 diabetes mellitus (DM) and compare the frequency of anti-TPO and anti-TG in the presence or absence of anti-GAD. ***Material and Method:*** We included 56 males and 48 females type-1 DM patients and age, gender, matched 31 males and 27 females as control. All patiens and control goups were performed TSH, FT4, FT3, anti TPO, Anti TG, Anti GAD and thyroid ultrasonography. ***Results:*** There was no difference between groups in terms of TSH and FT4 levels but FT3 levels were statistically significantly higher in the control group. There were significant differences between groups in terms of anti-GAD and anti-TPO, but not anti TG. There were difference for thyroid volume between groups. Total thyroid volume was not correlated with anti-TPO, anti-TG, anti-GAD, TSH, and gender. Positive anti-GAD was detected in 30.8% (n=32) in Type-1 DM patients. In patients with positive anti-GAD, rate of positive anti-TPO and Anti TG were 37.5%, 18.8% respectively. In patients with normal levels of anti-GAD, rate of positive anti-TPO and Anti TG were 9.7% and 2.8% respectively (p=0.005). In patients with positive anti-GAD, rate of positive anti-TPO and anti-TG was 15.6%. Rate of positive anti-TPO and anti-TG was 1.4% in normal levels of anti-GAD (p=0.004). ***Conclusion:*** The present study demostrated that rate of thyroid antibodies (TA) and TSH dysfunction was high in patients with type-1 DM. TA of patients with positive anti-GAD was apparently higher when compared with negative anti-GAD. Thus patients with positive anti-GAD should be followed more frequently and more carefully in terms of autoimmune thyroid disease.

Thyroid autoimmunity
Poster

IS EUTHYROID PATIENT WITH HASHIMOTO'S THYROIDITIS REALLY EUTHYROID?

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Purpose: Patients with Hashimoto's thyroiditis usually requires life-long L-thyroxine (L-T4) treatment. In this study we aimed to evaluate tissue hypothyroidism by using TSH response to TRH test in euthyroid Hashimoto patients. ***Method:*** Forty one patients with Hashimoto's thyroiditis and 15 healthy individuals who admitted to Gazi University Hospital Endocrinology department between January 2004–June 2004 were included in the study. Patients whose TSH levels were not between 0.35–0.5 μIU/ml were excluded from the study. TRH (400 μg) stimulation test was applied to each patient. Patients' demographic properties were recorded as well as their autoantibody levels. There were 41 patients in the study group (38 females and 3 males) and mean age was 39.7±13.4 where as there were 15 patients on the control group (13 females and 2 males) and the mean age was 42.2±13.6. Statistical analyses were done by using SPSS 11.5 package programme and the data were evaluated by Whitney U and Pearson correlation tests. p value under 0.05 was accepted as statistically meaningful. ***Results:*** In our study, thyroid autoantibodies and mean TSH values at 0, 20, 60 minutes after TRH stimulation were significantly different between study groups and control groups. However no difference was detected in means of free T3 (fT3) and free T4 (fT4) levels between groups. There was statistically significant correlation between anti-microsomal antigen antibodies (MsAb) values and TSH levels at 0 and 20 minutes [r=0.28 (p=0.03); r=0.41 (p=0.002) respectively]. Moreover there was also statistically significant correlation between anti-thyroglobulin antibodies (TgAb) and TSH levels at 0 minute [r=0.39 (p=0.003)]. ***Conclusion:*** In patients with Hashimoto's thyroiditis, high basal levels of TSH are more sensitive than all other routine tests as a sign of insufficiency. In addition to this, exaggerated TSH response to TRH test is more useful in detecting insufficiency in asymptomatic Hashimoto patients. In euthyroid patients with Hashimoto's thyroiditis, exaggerated TSH response is a sign of decreased thyroid reserve (tissue hypothyroidism, preclinical hypothyroidism, compensated hypothyroidism).

Thyroid autoimmunity
Poster

PRE-TRANSPLANT SERUM FT3 LEVELS IN KIDNEY GRAFT RECIPIENTS ARE USEFUL FOR IDENTIFYING PATIENTS WITH HIGHER RISK FOR GRAFT FAILURE

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End stage renal disease (ESRD) is a condition associated to thyroid disturbances both in function and morphology. Recent studies demonstrated that serum FT3 levels are negatively correlated with serum markers of inflammation and endothelial activation in patients with ESRD. However, no previous study evaluated serum thyroid function parameters in relation to kidney graft outcome. Serum FT3, FT4 and TSH levels were measured before transplantation in 196 kidney graft recipients. The graft survival rate at 5 years for all patients was 92.3%. Kidney graft recipients with normally functioning grafts showed similar serum pre-transplant thyroid parameters than patients who experienced graft failure. Life time analysis, were performed after stratification of patients according to pre-transplant serum FT3 levels < or > 3.1 pmol/L. A significant different 5-yr death-censored graft survival rate (93.9% *vs.* 76.5% for patients with normal or low FT3 levels, respectively; $p<0.01$) and similar survival rate (death of patients with functioning grafts) (21.1% vs. 5.9%; $p=0.288$) were observed. No similar feature was found for FT4 and TSH, suggesting that the effect is not related to hypothyroidism but rather dependent upon inappropriately low FT3 levels. Among patients displaying FT3 $<10^{th}$ centile, those experiencing graft loss had significantly higher FT4/FT3 ratio as compared to patients with normal functioning grafts (6.8±1.9 vs. 4.8±1.0; $p<0.05$). Pre-transplant serum FT3 levels were similar in patients who experienced early acute rejections as compared to non-rejector patients. The results of this study demonstrate that among patients with ESRD undergoing kidney transplantation, those displaying lower pre-transplant serum FT3 levels are at higher risk for subsequent graft failure. The demonstration of a predictive value of serum FT3 levels for graft survival suggests that measurement of pretransplant serum FT3 levels might represent a clinically useful parameter to identify patients with increased risk for graft failure.

Clinical thyroidology
Poster

SUBCLINICAL HYPOTHYROIDISM: DOES TRH TEST HELP TO NARROW CURRENT REFERENCE RANGES?

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TSH cut off levels that define subclinical hypothyroidism are still largely debated. TSH values greater than 2.5 mUI/l have been proposed to diagnose subclinical hypothyroidism.As TRH test has been used to define overt hypothyroidism, we employed it to predict subclinical hypothyroidism in a group of 143 patients. Group A (n=42, 2≤TSH≤2.5 mUI/l) and Group B (n=68, 2.5<TSH<4.6mUI/l) were compared to Group C (n=33, 0.5<TSH<2) age and sex matched controls. All patients were controlled for TSH/TRH stimulated values at 20 min, FT3, FT4, TPOAb values, presence of thyroid nodules and volume, echogenicity suggesting thyroiditis, familial thyroiditis and antidepressant use. Significant differences ($p<0.0001$) were found in basal TSH (A:2.2±0.15, B:3.3±0.6, C:1.24±0.4mUI/L), TSH peak >20 mUI/L (A: 47.6%, B:58.8%, C:3%), TPOAb positivity (A: 10%, B:25%, C:6%), echogenic thyroiditis (A: 19%, B:48%, C21%) and familial thyroiditis (A:26%, B:28%, C:0%) between the three groups. Patients with positive TRH test (TSH peak >20 mUI/L) were mostly women with higher baseline TSH, TPOAb positivity, smaller thyroid volume and thyroiditis family history. In multivariate analysis, the probability to predict subclinical hypothyroidism based on TRH positive test was higher in women (OR 0.05, $p<0.001$) with a higher TSH (OR 7.84, $p<0.0001$) and lower FT4 levels (OR=0.64, $p=0.0006$), with a concordance of 89%. Subclinical hypothyroidism should be considered when basal TSH > 2.5 mUI/L or when TSH > 2mUI/L and TRH test is positive. The test should be adressed to a group of patients with baseline TSH values in the upper normal range (2– 2.5 mUI/L) and additional biological and/or clinical hypothyroid features.

Clinical thyroidology
Poster

REDUCED THYROID VOLUME AND NODULARITY IN DYSLIPIDEMIC PATIENTS ON STATIN TREATMENT

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Background: Little information is available concerning the possible antiproliferative effects of HMG-CoA reductase inhibitors (statins) observed in few studies on thyroid cell lines in vitro, and no clinical study had the clear purpose of specifically investigating the effects of these drugs on thyroid gland. We have hypothesized that the antiproliferative effects of statins observed in thyroid cell lines in vitro may have a clinical counterpart that could be detected by investigating the prevalence and size of thyroid nodules in patients on long-term treatment with statins, as compared to untreated controls. ***Methods:*** We retrospectively evaluated 135 dyslipidemic patients receiving statin therapy continuously for at least 5 years and 137 controls. All the subjects were submitted to ultrasound investigation of the thyroid gland. ***Results:*** Subjects treated with statins showed markedly lower prevalence of thyroid nodules (36.3 vs. 67.9%, $p<0.001$), as well as reduced number and smaller total volume of lesions, as compared to the control group. A logistic regression analysis, taking into account age, sex, risk factors for the development of thyroid nodules and concomitant drug treatment revealed that treatment with statins remained the only important predictor of the presence of thyroid nodules [odds ratio (95% confidence limits): 0.312 (0.156–0.625), $p<0.001$] besides risk factors. ***Conclusions:*** Our data could provide the first evidence of the ability of HMG-CoA reductase inhibitors to reduce the prevalence, number and volume of thyroid nodules, and are consistent with an antiproliferative and/or pro-apoptotic effect of long-term statin treatment on thyroid cells, in vivo.

Clinical thyroidology
Poster

IMPACT OF THE OVARIAN HYPERSTIMULATION SYNDROME ON THYROID FUNCTION: A CASE REPORT

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Background*:* The ovarian hyperstimulation syndrome (OHSS) is a complication of controlled ovarian hyperstimulation (COH) used for assisted reproduction. OHSS can vary from increased ovaries over abdominal effusion to collapse and leads to high estradiol levels (E_2: ≥5.000 ng/L) in early pregnancy. ***Case Report:*** In an infertile woman, with known autoimmune hypothyroidism thyroid function (serum TSH and FT_4; ref: 0.3–4.2 mIU/L and 9–17 ng/L, respectively) was followed before and after COH. Under treatment with 125 µg thyroxine, TSH was 3.5 mIU/L and FT_4 11.1 ng/L. Before starting COH, she was advised to increase thyroxine to 150 µg/day. During COH, serum E_2 levels increased to 5.549 ng/L, which was accompanied by a moderate degree of OHSS, but with severe changes in TSH and FT_4 (41.5 mIU/L and 7.7 ng/L, respectively). When pregnancy was confirmed (serum hCG: 1.140 IU/L), the thyroxine dosage was immediately increased to 200 µg/day. Two weeks later, TSH was 13 mIU/L and FT_4 13 ng/L, with a normalised serum TSH at 6 weeks of gestation (serum hCG: 35.340 IU/L and E_2: 3.900 ng/L). ***Discussion:*** The patient was worried about the initial results and instead of increasing the dosage of thyroxine, she asked an urgent abortion, because of what she read on the impact of hypothyroidism on the pregnancy outcome. In the literature, there is no evidence to advice abortion in such a case and after the increment in thyroxine dosage, the increasing serum hCG and decreasing E_2; thyroid function normalised and the pregnancy remained uneventful. ***Conclusion:*** OHSS has a strong impact on thyroid function, especially when TAI is associated and thyroid function should be determined in infertile women and followed-up/treated when necessary.

Clinical thyroidology
Poster

THYROTOXIC HYPOKALEMIC PERIODIC PARALYSIS IN A CAUCASIAN POPULATION: NOT TOO MUCH, NOT TOO LOW – THREE NEW CASE REPORTS AND META-ANALYSIS OF 38 TURKISH CASES

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Objective: Thyrotoxic hypokalemic periodic paralysis (THPP) is rarely seen in the Caucasian populations. In this study, we aimed to make a meta-analysis of THPP in a Caucasian population which is the first analysis of a homogenous Caucasoid group. ***Subjects:*** A total of 40 Turkish cases were found to be ailed with THPP which of three were new cases and were assigned as index cases. Two cases weren't included to the meta-analysis for having lacking data. ***Results:*** THPP was diagnosed at the first attack in 10 cases and the first attack group had statistically significant shorter complete recovery time ($p<0.01$). Hypokalemia was screened in majority of the cases while there were two normokalemic cases. When we grouped the cases according to the potassium (K) level, the group with K levels under 2.5 meq/L had statistically longer amelioration time than cases with equal or more than 2.5 meq/L. We grouped the cases according to using intravenous or oral K. Mean amelioration time of the intravenous group was 6.8±3.6 hours and the oral group was 13.1±7.6. Mean complete recovery time of the intravenous group was 29.4±16.2 hours and the oral group was 52.8±18.0. The intravenous group had shorter amelioration time and complete recovery time than the oral group which were statistically significant ($p<0.05$ for each). ***Conclusions:*** THPP may be seen between the Caucasians and recognising in the first attack may affect the recovery time positively. The degree of hypokalemia seems to have a significant effect on recovery time and initial low K level may lead to decay in patient's health much more than mild or near normal levels. We suggest intravenous K instead of oral K for the amelioration time and complete recovery time may be shortened by this way.

Clinical thyroidology
Poster

SERUM-TSH AND SERUM-TPO-AB OSCILLATE IN PARALLEL AND HIGH URINARY IODINE EXCRETION (UIE) PREDICTS SUBSEQUENT THYROID FAILURE. A ONE YEAR STUDY OF PATIENTS WITH UNTREATED SUBCLINICAL HYPOTHYROIDISM (SH)

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Background: Cross-sectional population studies have shown association between high s-TSH and high s-TPO-Ab levels. Both high s-TSH and presence of TPO-Ab are associated with risk of developing overt hypothyroidism in SH patients. Short term variation in s-TSH and s-TPO-Ab in individual patients with untreated SH is largely unknown, and the interaction with iodine intake remains to be studied. Aim: With a repeated measure design, during a year with monthly measurements, we investigated the mutual correlation of s-TSH, s-TPO-Ab and UIE in patient with untreated SH. ***Methods:*** 21 patients (2 men) without former thyroid disease, with TSH between 5–12mU/l and normal s-T4 at two occasions were enrolled. Subsequently, 13 monthly measurements of s-TSH, fT4, s-TPO and UIE were performed. Within-person correlations and correlations with no or various time displacements between variables were calculated. ***Results:*** Overall mean levels of s-TSH, fT4, s-TPO-Ab and UIE were (median/interquartile range): 6.5/4.9–8.0 mU/l, 13.7/12.6–14.5 pmol/l, 1,756/760–1,0528 kU/l and 102/82–147 micg/24h. During the study s-TSH significantly increased in five patients, 16 had unchanged s-TSH, whereas none improved. From clinical and biochemical inclusion data, it was not possible to predict the later increase in TSH. In individual patients (measurements, n=13) a highly significant correlation between s-TSH and s-TPOAb were found ($r=0.37$, $p<0.0001$). This correlation was also significant between s-TSH and UIE ($r=0.14$, $p=0.034$). Time displaced correlations showed best correlation between s-TSH and s-TPO-Ab obtained at the same time point, whereas UIE correlated best to s-TSH and s-TPO one month later. ***Conclusion:*** During a year, 24% of SH patients deteriorated in thyroid function, while none improved. At inclusion it was not possible to predict who would increase in s-TSH. S-TPOAb and s-TSH showed high degree of parallel variation, whereas high UIE predicted high s-TSH and s-TPOAb one month later.

Clinical thyroidology
Poster

TO TREAT OR NOT TO TREAT EUTHYROID AUTOIMMUNE DISORDER DURING PREGNANCY

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Background: Subclinical autoimmune hypothyroidism during pregnancy is associated with increased risk of miscarriage and has deleterious effect on fetal development. However, systematic prenatal screening of thyroid function and treatment of euthyroid autoimmune disorder remain controversial. **Methods.** We retrospectively revised 784 files of pregnant women, systematically screened for thyroid function and antithyroid antibodies, to determine the thyroid function of negative and positive antiperoxydase antibodies (TPO-Ab) pregnant women, and to analyse the possibility to adopt a strategy for treatment with levothyroxin (LT4). ***Results:*** From the 75 positive TPO-Ab, 42 patients received LT4 treatment during the pregnancy. Even though the great variation of the TSH serum levels, the mean TSH level was significantly higher in women with positive TPO-Ab (3 mIU/L versus 1 mIU/L). No significant difference was observed in the incidence of obstetrical complications between TPO-Ab positive and negative populations. ***Conclusions:*** Our study provides informations about the normal TSH and free Thyroxin serum levels for the Belgian pregnant women. We propose to treat with 50 µg/day of L T4, pregnant woman with positive TPO-Ab, unless the TSH level is lower than 1 mIU/L.

Clinical thyroidology
Poster

ERECTILE DYSFUNCTION IN STAGES OF THYROID ALTERATION: A DETAILED INVESTIGATION

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Erectile dysfunction (ED) is a common male disability associated with aging and numerous diseases. Connection between ED and thyroid disturbances has gone largely unstudied. ***Aim:*** to assess the impact of hyper- and hypothyroidism on male sexual health. We distributed the Sexual Health Inventory (SHIM), a five-item questionnaire, which focuses on a man's ability to attain and then maintain an erection, to 71 men, 27 hyper- and 44 hypothyroid (54.7±14.8 yrs) and similar number of normal matched controls. FT4, TSH and TRAb's were measured in hyper- while FT4, TSH, TPOAb's and TGAb's in all hypothyroid patients and controls. About 1/3 of the patients and controls were taking medications mainly beta-blockers, statins or diuretics. After the questionnaire was completed, treatment was started either with antithyroid drugs or thyroxine. A year after the initiation of treatment and when all the patients were euthyroid the same questionnaire was distributed to all patients and they were asked to respond. Fifty-six (78.9%) men had a SHIM score of 21 or less, indicating a diagnosis of ED, while only 24 (33.8%) from the controls ($p<0.0001$). Twenty-one of 56 (37.5%) patients with SHIM score of 21 or less had severe ED with a score ≤10, while only 6 (25%) from the 24 controls ($p<0.01$). From the 56 patients 19 had hyperthyroidism, while 37 were hypothyroid (NS). Significant negative correlation was found between SHIM and TSH. No other correlation was found. After treatment a significant improvement was observed. Only 20 from the patients' group had a SHIM score of 21 or less and from those only 7 had severe ED (SHIM score<than 10). ***Conclusion:*** ED is common in men with thyroid disturbances and no specific treatment is needed.

Clinical thyroidology
Poster

PAPILLON INITIATIVE 2006: THYROID PALPATION vs. SONOGRAPHY

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In Germany, only 1/3 of family physicians has access to thyroid sonography, so the diagnostic validity of thyroid palpation vs. sonography has been tested by the Papillon Initiative 2006. Between April 24th and 30th 2006, 80 physicians participated in a prospective epidemiological survey (among them 41 family practitioners and 37 internists). They examined 865 patients by both palpation and ultrasonography of the thyroid. The diagnostic validity of thyroid palpation is compared statistically to ultrasonography as “golden standard”. The sample consists of 626 women (mean age 50.0 ± 16.5 years) and 239 males (mean age 51.9 ± 15.3 years). In the subset of patients (n=567) without any thyroid pretreatment, 38.7 % of women and 34.5 % of males presented with goitre according to the reference levels for Germany (18 ml for women, 25 ml for males). Comparing palpation to sonography with respect to the detection of goitre, concordance of both methods has been seen in 86.1 % with a sensitivity of 76.9 % and a specificity of 91.6 % for palpation. With respect to the detection of thyroid nodules (defined as lesion with a diameter of ≥ 5 mm, concordance between palpation and sonography has been observed in 71.8 % with a sensitivity of 46.7 % and a specificity of 88.6 % for palpation. Nodules with a diameter of more than 20 ml could be detected with a sensitivity of 77.0 %. Enlarged thyroids can be detected by palpation in 77 % of the patients. With the same sensitivity of 77 %, nodules of a diameter ≥ 20 mm can be palpated. The sensitivity of palpation for detection of smaller nodules with less than 50 % is not sufficient. Nevertheless physicians should be trained to palpate the thyroid since palpation at least allows detecting goitre and/or larger nodules.

Clinical thyroidology
Poster

QT DISPERSION IN SUBCLINICAL HYPOTHYROIDISM

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Increased QTd is an electrocardiographic parameter shown to associate with malignant ventricular arrythmias and sudden death. It has been detected to relate with thyroid stimulating hormone (TSH) levels in overt hypothyroidism. We aimed to investigate the corrected QT dispersion (QTdc) of women with subclinical hypothyroidism (SH), and determine the changes following normalization of TSH levels. Fifty eight women with naive SH due to autoimmune thyroiditis and 54 age-sex matched healthy controls with normal serum TSH levels were included following the exclusion of any factor that might interfere with cardiac conductibility. Electrocardiographic measurements were performed with a magnifier from the point where Q-wave started and T-wave returned to iso-electrical line. Bazzet Formula was used to calculate QTdc. L-thyroxine 1–2 µcg/kg/day was administered to the subgroup who had serum TSH higher than 10µIU/ml. Mean QTdc interval of the study group (n=58) was significantly longer than the controls; 0.10±0.03 vs 0.076±0.03 seconds (sec), (p=0.000). It was also longer in *subgroup a* (5>TSH>10, n=36); 0.098±0.03 sec and *subgroup b* (TSH>10, n=22); 0.12±0.03 sec, (p=0.001, p=0.000, respectively). In *subgroup b*, following normalization of TSH levels for at least three months, mean post-treatment QTdc value was found to be similar with that of the control group; 0.075±0.04 vs 0.076±0.03 sec, (p>0.05). In conclusion, we detected prolonged QTdc among SH cases. The prolongation remained significant within the whole group, as well as within the subgroups. Achieving suggested TSH levels accompanied with normalization of QTdc intervals among cases whose TSH were over 10µIU/ml.

Clinical thyroidology
Poster

IS SELF-REPORTED THYROID DYSFUNCTION AN OBSTETRICAL RISK FACTOR?

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Purpose: To evaluate obstetric history and pregnancy outcome in women with self-reported thyroid dysfunction in population-based cohort. ***Methods:*** Prospective Northern Finland 1986 Birth Cohort (n=9247) has been followed by questionnaires and clinical examinations. Data on thyroid dysfunction was available from questionnaire during pregnancy and hospital registers. Women with and without self-reported thyroid dysfunction were compared using Students t or Fishers exact tests. ***Results:*** 120 women self-reported thyroid dysfunction in questionnaire. Of them, 41% had thyroidectomy, 29% had autoimmune-based dysfunction, 15% had no entry of thyroid disease on hospital registers and 15% had other thyroid diseases. Women with self-reported thyroid dysfunction were older (mean age 30.9 vs. 28.2 years, $p<0.001$), heavier (mean weight 65.0 kg vs. 61.7 kg at first visit, $p<0.001$ and 76.5 kg vs. 73.2 kg at last visit, $p<0.001$) and fewer were nulliparous (24.2% vs. 34%, $p<0.001$). They had more miscarriages (28.4% vs. 19.5%, $p=0.009$) and premature births (11.7% vs. 5%, $p=0.005$) in earlier obstetrics history, but not more low-weight infants, fetal or neonatal deaths or infertility treatments. No significant differences in current pregnancy complications like preterm delivery, low-weight infants, fetal deaths, malformations, breech presentation and cesarean section were seen between the groups. Women with self-reported thyroid dysfunction had higher rates of placental abruption (2.5% vs. 0.5%, $p=0.02$). ***Conclusion:*** Women with self-reported thyroid dysfunction have less favorable obstetric history. In this birth cohort there were no more pregnancy complications in current pregnancy except for elevated risk for placental abruption.

Clinical thyroidology
Poster

CAN AUTONOMOUSLY FUNCTIONING NODULES CAUSE TACHYCARDIA IN PATIENTS WITH NORMAL TSH SERUM LEVELS?

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Aim: To evaluate whether a solitary autonomous functioning thyroid adenoma (AFTA) can cause tachycardia in patients with normal serum TSH levels. ***Methods:*** 31 patients with AFTA and normal TSH-levels (range 0.4–2.6 µU/ml, mean 1.0 µU/ml, normal: 0.3–4.0 µU/ml) who complained about tachycardia as leading symptom were prospectively included. AFTA was confirmed by ultrasound and scintigraphy under exogenous TSH-suppression. All patients underwent I-131 therapy under exogenous TSH-suppression. Between 400 and 2000 MBq I-131 (200 Gy / thyroid) was orally administered. All patients were interviewed regarding local or hyperthyreotic symptoms prior, 2, 4 and 12 months following I-131 therapy. In addition, thyroid scintigraphy and ultrasonography were repeated 4 and 12 months after therapy. ***Results:*** The post-therapy scintigraphy was absolutely normal without residual autonomous regions in 30 of the 31 (96.8%) patients. On ultrasonography, the volume of the AFTAs showed a mean reduction of 67% (SD±24%). Absence or improvement of the leading symptom tachycardia was reported by 27 (87.1%) or 3 (9.7%) patients, respectively. Tachycardia persisted without improvement in only 1 (3.2%) patient. Twelve months after therapy, 3 patients had slightly increased TSH-levels (4.1, 4.5 and 6.7µU/ml) while TSH remained normal (range 0.4–3.8 µU/ml, mean 2.2 µU/ml) in the other 28 patients. ***Conclusion:*** The results of our study indicate that autonomously functioning thyroid nodules can cause intractable tachycardia even when the serum TSH level is within normal limits. In these patients I-131 therapy was related with a 97% cure rate and a low prevalence of hypothyreosis when complete TSH (< 0.01 µU/ml) suppression is provided as described above.

Clinical thyroidology
Poster

THE EFFICIENCY OF INDIVIDUALIZE FORMULA OF RADIOIODINE DOSE CALCULATION FOR THYROTOXICOSIS TREATMENT (CLINICAL POINT OF VIEW)

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Aim: To assess the efficiency of radioiodine calculated dose for treatment of thyreotoxicosis caused by Graves' disease (GD) from clinical point of view. ***Methods:*** 50 patients with GD (44 female and 6 male), mean age 45,4±13.0 years received radioiodine treatment (RIT). Before 131-I therapy euthyroidizm in all patients were achieved by antithyroid medication. Antithyroid medication was stopped 10 days before RIT. The target dose of 131-I was calculated according to thyroid volume (TV) and 13-I uptake in 24 hours. ***Results:*** The median dose of radioiodine was 11.5 mCi. The median of TV before RIT was 30 ml. The median of TV was 11.6 ml and TV reduction was 67.4% in 6 months in patients received 131-I dose less than 0.3 mCi per 1 ml of TV (n=12). The median of TV was 8.3 ml and TV reduction was 71% in 6 months in patients received 131-I dose more than 0,3 mCi per 1 ml of TV (n=38). The high relapse of thyrotoxicosis (33.3 %) was observed when dose of 131-I was less than 0.3 mCi/ml and reduced up to 13.2% if greater activity was apply (p=0.2). Hypothyreosis was achieved in 25% patients received less than 0.3 mCi/ml 131-I and in 63.2% patients received more than 0.3 mCi/ml (p=0.04). ***Conclusion:*** The radioiodine dose less than 0.3 mCi/ml significantly reduce efficiency of radioiodine treatment of thyrotoxicosis. Thus, it is unreasonable prescribe dose of radioiodine less then 0.3 mCu/ml in case of formula calculation

Clinical thyroidology
Poster

IODINE INTAKE IN PORTUGUESE PREGNANT WOMEN. PRELIMINARY RESULTS

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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. Preliminary results of this on going study from 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); 2214 urines from 10 maternity hospitals were analysed. Urinary iodide-A fast colorimetric method (Gnat et al, Clin Chem 2003) is being used. ***Statistical Methods:*** Central methods and proportional comparison tests. ***Global Results:*** Median urinary iodide concentration was 86.83µg/L, being 22.9% below 50µg/L.17.6% had values above 150µg/L. ***Results by Hospital:*** Median urinary iodine varied from 68 to 124µg/L; 13.9% to 29.6% of women had values below 50µg/L and 9.8 to 34% had values above 150 µg/L. ***Conclusions:*** Although these results are preliminary they point out to an inadequate iodine intake in pregnant women assisted in most hospitals. Taking into account these preliminary results, the on going study needs to be complete and 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.

Clinical thyroidology
Poster

RANDOMIZED PROSPECTIVE STUDY COMPARING A SINGLE RADIO-IODINE DOSE AND A SINGLE LASER THERAPY SESSION IN AUTONOMOUSLY FUNCTIONING THYROID NODULES

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Objective: To compare efficacy of interstitial laser photocoagulation (ILP) with radioiodine in hot thyroid nodules. ***Design:*** 30 consecutive outpatients with subclinical or mild hyperthyroidism and a scintigraphically solitary hot nodule with extraglandular suppression were randomized to either one ILP session or one radioiodine (^{131}I) dose. ***Methods:*** ILP was performed under continuous ultrasound (US)-guidance and with an output power of 2.5–3.5 W. ^{131}I was given as a single dose based on thyroid volume and a 24-h thyroid ^{131}I uptake. Thyroid function and nodule volume were evaluated at inclusion and one, three and six months after treatment. ***Results:*** Normalisation of serum TSH was achieved in seven of fourteen patients in the ILP group and in all fifteen patients in the ^{131}I group ($p=0.0025$). In the ILP group, mean thyroid nodule volume reduction was 44%±5% (SEM), ($p<0.001$), and in the ^{131}I group 47%±8%($p<0.001$), within six months, without between-group difference ($p=0.73$). The mean reduction of total thyroid volume was 7%±5% in the ILP group ($p=0.20$), and 26%±8% ($p=0.006$) in the ^{131}I group ($p=0.06$ between-group). Two patients in the ^{131}I group developed hypothyroidism but no major side effects were seen. ***Conclusions:*** This first randomized study, comparing ILP with standard therapy, demonstrates that ILP and ^{131}I therapy approximately halves thyroid nodule volume within 6 months, but in contrast to ^{131}I, extranodular thyroid volume is unaffected by ILP and no patient developed hypothyroidism. Using the present design, ILP seems inferior to ^{131}I therapy in normalization of serum TSH. The potential value of ILP as a nonsurgical alternative to 131 I needs further investigation

Clinical thyroidology
Poster

FREQUENCY AND CHARACTERISTICS OF TBII-SERONEGATIVE PATIENTS WITH GRAVES' HYPERTHYROIDISM

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Introduction: Graves' hyperthyroidism is caused by thyrotropin-binding inhibitory immunoglobulin (TBII) but for unknown reason there exists a subset of patients without detectable TBII in serum despite a homogeneous uptake on thyroid scintigraphy. ***Aim:*** To evaluate the frequency of undetectable TBII in Graves' hyperthyroidism and to compare the characteristics of TBII-negative with TBII-positive patients. ***Methods:*** A prospective multicenter observational study in 241 untreated patients with a first episode of Graves' hyperthyroidism diagnosed by biochemical thyrotoxicosis with diffuse homogeneous uptake on thyroid scintigraphy was performed. Serum TBII was measured using a second generation assay (DYNOtest TRAKhuman, from B.R.A.H.M.S. Diagnostica Berlin, detection limit 1 U/l). ***Results:*** Serum TBII was positive (>2 U/l) in 231 patients (95.8%) and negative in 10 patients (4.2%) of whom 5 had TBII < 1 U/l. In the whole group TBII was positively related to FT3index and FT4index. TBII-negative patients related to TBII-positive patients had lower FT3 and FT4 indices ($p = 0.06$), a lower prevalence of smoking (22.2% vs. 37.5%, $p = 0.02$) and a lower prevalence of ophthalmopathy (0% vs. 19.4%, $p < 0.01$). There were no differences between both groups in age, sex, BMI, clinical thyrotoxic score, goiter size, duration of symptoms or family history of thyroid disease. ***Conclusion:*** TBII seronegativity exists in 4.2% of untreated Graves' hyperthyroid patients. These subjects have biochemically less severe thyrotoxicosis, no ophthalmopathy and are less often smokers than TBII-positive (> 2 U/l) patients.

Clinical thyroidology
Poster

MANAGEMENT OF THYROID NODULES IN NORTH-EASTERN BULGARIA: CYTOHISTOLOGICAL CORRELATION

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Objective: Nodular thyroid disease is found in approximately 5% of the population with palpation and is almost ten-fold more frequent at ultrasound screening. Fine-needle aspiration biopsy (FNAB) currently is the main diagnostic procedure for distinguishing benign from malignant nodules. The aim of this study was to assess the accuracy of FNABs in our hospital by comparing the cytological and histological diagnosis. ***Methods:*** We performed a prospective study including 321 patients with thyroid nodules admitted to “St. Marina” Hospital from January 2004 to December 2006. We evaluated clinical and US data, TSH, FT3, FT4, anti-TPO, cytological and histological findings. ***Results:*** FNABs were performed in all 321 cases, 290 of them women and 31 men; mean age 52.9 years. Cytological evaluation considered 67.3% of FNABs as benign, 15.6% as suspicious, 4.7% as malignant and 12.4% as inadequate sample. 88 of the patients underwent thyroid surgery. The comparison between cytological and histological findings showed that 99.1% of cytologically benign nodules were histologically confirmed, as well as 100% of the malignant lesions. 89.5% of the cases classified as suspicious on FNAB turned out to be benign on histology and 10.5% were malignant. The discrepant cases were 2 false-negative results, which had a cytological diagnosis of nodular hyperplasia and turned out to be a medullary carcinoma. Our results showed a sensitivity of 88.2% and a specifity of 100%. ***Conclusion:*** The incidence of histologically proven thyroid cancer among all nodules is 4.7%, whereas among the suspicious FNABs it is 10.5%, which confirms the need for surgery in the latter group. Thyroid FNAB is an accurate and cost-effective preoperative tool for selection of patients who would benefit from surgery.

Clinical thyroidology
Poster

THYROID INVOLVEMENT IN A PATIENT WITH CHANARIN-DORFMAN SYNDROME

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Chanarin-Dorfman syndrome (CDS) is a rare condition caused by an autosomal-recessive inherited disorders of neutral lipid metabolism with accumulation of no-membrane enclosed intra-cytoplasmic vacuoles. Clinical expression depends on organs involvement. Skin manifestation (including non-bullous congenital ichthyosiform erythroderma) are the most common symptoms but most patients show skeletal myopathy and fat liver. We describe the case of a 41-year old man with CDS and nodular thyroid disease unexpectedly identified at neck ultrasound. CDS's diagnosis was carried out by muscular biopsy, typical blood smears leukocytes and genetic analysis. Fine needle biopsy (FNB) of thyroid nodules suggested clear cells neoplasia. The patient had a marked hypotrophy of neck, shoulder and arms muscles. Laboratory exams were normal except increased serum creatinine kinase and CK-MB. Serum thyroid hormones and TSH were normal, negative anti-thyroid antibodies and calcitonin. Thyroid ultrasound confirmed a slight multinodular enlargement of the gland with faint hypo-echogenic pattern. Neck CT scan detected an increased thyroid volume and hypo-dense areas with an inhomogeneous uptake of iodate contrast medium. FNB of thyroid nodules and extra-nodular tissue showed adipocyte-like clear cells with no nuclear atypia: cytoplasm was PAS negative and May-Grunwald-Giemsa staining showed a typical pattern of intra-cytoplasmic lipid accumulation. Immunocytochemistry for thyroglobulin was positive while CD10 expression was negative. The presence of neutral lipid was demonstrated by oil red-O method.Thyroid involvement has never been reported in this syndrome. This thyroid cytological pattern in patients with CDS should be taken in account for differential diagnosis of thyroid clear cells neoplasia.

Clinical thyroidology
Poster

EFFECT OF T4-T3 COMBINATION THERAPY vs. T4 MONOTHERAPY IN PATIENTS WITH HYPOTHYROIDISM. A DOUBLE BLIND RANDOMIZED CROSS-OVER STUDY

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Purpose: To compare the effect of T4-T3 combination therapy versus T4 monotherapy in patients with autoimmune hypothyroidism, on stable T4-substitution therapy ***Study Design:*** Double blind, randomized cross-over design. In 59 patients (55 women; aged 46 yrs (range 19–76)); TSH at diagnosis 68 mU/L (20–370); period of stable euthyroidism on T4-substitution 12 months (6–180)), 50 microgram of the usual T4 dose were replaced with a tablet containing either 20 microgram of T3 or 50 microgram of T4 for 12 weeks, followed by cross-over for another period of 12 weeks. S-TSH (data on T4 and T3 measurements were blinded) was measured after 4 weeks, and the T4 dose was regulated if needed, intending unaltered TSH levels despite changed medication. Evaluation: blood tests, weight and tests of quality of life (QOL) and depression at base line and after the 2 treatment periods. At the end of the study the patients were asked which treatment period they preferred. ***Results:*** 28/59 patients described reduced QOL at base line despite relevant treatment

(median and range)	at base line	T4+ T3 (20 ug)	only T4
TSH (mU/L)	1.104 (0.100–5.600)	0.756 (0.020–7.930)	0.990 (0.010–5.400) NS
T4 dose (ug/day)	128 (50–200)	75 (25–164)	125 (25–175) NS
Becks Depression Index (min 0, max 63)	10 (0–25)	4 (0–25)*	7 (0–25)*
Preferred treatment		49%	15% p=0.002 Therapeutic gain 34% (95% CI 13–54)

* = p<0.05.

Conclusion: Combination therapy with T4 and T3 was superior to monotherapy with T4 *when* monitoring TSH levels carefully and obtaining equal levels in the treatment groups,

Clinical thyroidology
Poster

EFFECTS OF IMPROVED IODINE SUPPLEMENTATION ON THE THYROID. RESULTS OF AN EPIDEMIOLOGICAL STUDY

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Topic title: clinical thyroidology Aim of study: to evaluate effects of increased iodine intake on thyroid status (volume, function, autoimmunity). Methods: epidemiological survey of randomly selected group of persons (age 18–65 years - total number 1,716) was performed at five years interval after realization of improved program of iodine supplementation. Clinical evaluation, thyroid ultrasonography, TSH, FT4, TPO and/or tyreoglobulin antibodies were evaluated. Results: ioduria: mean increased from 138.1 to 178.5 ug/l and median from 112.5 to 119.2 ug/l ($p < 0.001$) distribution of individual values tends to supranormal values (over 300 ug/L - $p < 0.001$) The similar tendency was observed for distribution of categories of ioduria according to WHO criteria ($p <0.001$) thyroid volume was evaluated separately for men and for women, a significant decrease of volume and/or of thyroid volume to body surface ratio was proved for women only thyroid function: no significant changes of TSH were recorded, fT4 increased from 14.78 to 15.79 pmol/l (medians, $p < 0.001$) thyroid autoimmunity: prevalence of pozitive titres of TPO (over 20.0 IU/ml) and of tyreoglobulin antibodies (over 150.0 IU/ml) increased significantly in women ($p <0.001$), but no in men Summary: increased iodine intake in a randomly selected group of persons led during five years to the following changes: increase of ioduria, changes of its individual distribution, decrease of thyroid volume, increase of FT4, increase of pozitivity of thyroid autoantibodies

Clinical thyroidology
Poster

THE MAJORITY OF DANISH NON-TOXIC GOITRE PATIENTS ARE INELIGIBLE FOR LEVOTHYROXINE SUPPRESSIVE THERAPY

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Background: Levothyroxine therapy (L-T4), aimed at shrinking thyroid nodules is controversial. According to recent guidelines (AACE/AME) it should be avoided in most cases. Despite evidence of long-term side effects due to TSH-suppression, questionnaire surveys indicate that L-T4 therapy is still widely used. ***Aim:*** To determine retrospectively, in consecutive non-toxic goitre (NTG) patients, the proportion eligible for L-T4 therapy. ***Subjects and Methods:*** During 1997–2001, 825 consecutive patients were referred to our endocrine unit on suspicion of NTG. Each patient was evaluated with fine needle biopsy, scintigraphy and ultrasound. 72 subjects had no thyroid disease and 19 had a solitary thyroid cyst. Thus, the study group comprised 734 patients (619 women and 115 men; median age 47 years, range 11–90). Based on evidence and guidelines from AACE/AME we defined conditions where L-T4 therapy is considered contraindicated or relatively contraindicated. These included: Postmenopausal women, men older than 60 years, goitre volume above 100 ml, intrathoracic goitre, osteoporosis, cardiovascular disease, clinically suspicious lesions, inadequate cytology, previous L-T4 therapy, s-calcitonin above the normal range, TSH level <0.3 mIU/L, TSH level <1.0 mIU/L in postmenopausal women or men > 60 years, a dominant thyroid cyst. ***Results:*** 66 % of patients had one or more exclusion criteria. In diffuse goitre (n=37) 40%, in uninodular goitre (n=307) 55 % and in multinodular goitre (n=390) 76% were ineligible for L-T4 therapy. Main ineligibility reasons were: age (n=291), TSH-level (n=173), goitre volume (n=114), intrathoracic component (n=105) and clinical suspicion (n= 70). ***Conclusion:*** In consecutive Danish NTG patients, at least 2 of 3 were ineligible for L-T4 suppressive therapy.

Clinical thyroidology
Poster

THE OPTIMAL SUPPLEMENTARY DOSE OF IODINE DURING PREGNANCY IN CONDITION OF ESTABLISHED IODINE PROPHYLACTIC

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Pregnancy is the period of increased demand for iodine. The recommended dose of iodine supplemented to pregnant women in Poland is 200 µg but it was established during mild iodine deficiency. The goal of the study was to reassess that recommendation 10 years after institution of obligatory iodine prophylactic. The study included 80 healthy pregnant women which were examined trice: between 6th and 12th week, between 33rd and 36th week and 11 to 14 weeks after the delivery. In each timepoint ultrasound examination was performed and TSH, FT3, FT4, anti-TPO, anti-TG and ioduria were assessed. After the first examination all women were randomly divided into 3 groups receiving 100, 150 or 200 µg of iodine, respectively. At the beginning of pregnancy mean ioduria was 103.2 µg I/L of urine (with median value 80.0) and mean volume of the thyroid gland was 13.7 mL. Thyroid volume above 18 mL was found in slightly above 10% of women with maximum value of 27 mL. There were no significant differences in the thyroid volume during the course of pregnancy and shortly after the delivery irrespectively to administered dose of iodine. However, a tendency to the increase of thyroid volume was observed in the group receiving the lowest dose. No significant increase in titres of antithyroid antibodies was observed. Our observations suggest that the recommended dose of iodine supplemented (additionally to iodized salt) during pregnancy in Poland should be 150 µg daily.

Clinical thyroidology
Poster

REMISSION AFTER POTASSIUM IODIDE THERAPY IN PATIENTS WITH GRAVES' HYPERTHYROIDISM SHOWING THIONAMIDE-INDUCED SIDE EFFECT

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Purpose: High prevalence of side effects of the thionamide drugs prompted us to reevaluate the effectiveness of potassium iodide (KI) therapy in the treatment of Graves' hyperthyroidism. ***Materials and Methods:*** There were 31 patients (male 4, female 27, aged 24–73 years) who showed various side effects of thionamides (MMI or PTU) such as leukocytopenia or urticaria. Thionamide drugs were discontinued and KI (10–100mg/day) was administered. The patients were followed for more than 5 years (5–22 years). ***Results:*** Side effects of thionamide drugs disappeared and none of the patients showed side effects of KI. Three patients remained thyrotoxic even with high dosage KI (400mg/day) and 3 other patients showed relapse of hyperthyroidism during the clinical course. They were successfully treated by radioactive iodine or surgery and then became hypothyroid requiring life-long replacement therapy (poor control group). Thirteen patients remained euthyroid for 6 to 22 years with continued KI therapy (20–200mg/day) (good control group). Twelve other patients eventually had a remission of Graves' disease after 5–11 years KI therapy (remission group) with decrease in TSH Binding Inhibitor Immunoglobulin activity (42 ± 22 to 3.3±5.8%) or estimated thyroid weight (34 ± 13 to 14 ± 5g). ***Discussion and Conclusion:*** KI therapy was effective to keep the patient euthyroid in more than 50% of the patients with Graves' disease who showed thionamide-induced side effects and even remission could be expected after keeping continuous KI therapy.

Clinical thyroidology
Poster

FIRST TRIMESTER REFERENCE RANGES FOR THYROID HORMONES: SIMILARITIES AND DIFFERENCES BETWEEN WALES AND TURIN, ITALY

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The Controlled Antenatal Screening Study, a prospective randomised study of child IQ in hypothyroid mothers (as defined by TSH or FT4) has recruited 22,000 women from Wales (UK) [70%] and Turin [30%]. Measurement of FT4 and TSH were taken in 13,554 Welsh women at 8–15 completed weeks of pregnancy (n = 144 week 8 to 4,761 women at week 12) and 5,483 Turin women at 11–13 completed weeks (1,993 week 11, 3,296 week 12, 194 week 13). We used regression analysis, separately for Wales and Turin, to quantify the relationship between both FT4 and TSH with gestational age measured in days. To account for these relationships, FT4 and TSH values were expressed as multiples of the median (MoM). We then estimated 95% reference ranges for FT4 and TSH. Median Wales FT4 ranged from 14.6 pmol/L at 8 weeks to 13.3 at 15 weeks and decreased by 1.2% [95% CI 1.1–1.4%] per week. Median TSH increased from 0.96 mU/L to 1.26 mU/L from 9 to 15 weeks at 3.4% per week [CI 2.8–4.1%]. Corresponding FT4 and TSH values for Turin women between 11 to 13 weeks were 12.2 pmol/L FT4 decreasing to 11.7 pmol/L at 2.6% per week [CI 1.7–3.5%] and 1.02 mU/L TSH increasing to 1.18 mU/L at 13 weeks at 9.1% per week [CI 4.2–14.3%]. The reasons for the difference in rate of change of FT4 and TSH between Wales and Turin are not clear but may be due to differences in gestational age calculation or physiological differences. The 95% reference ranges for Wales were 0.79–1.28 MoM for FT4 and 0.06–3.16 MoM for TSH. The ranges for Turin were similar: 0.79–1.32 MoM for FT4 and 0.04 MoM for TSH. We conclude that MoMs are useful to compare analyte ranges for screening purposes.

Clinical thyroidology
Poster

EFFECTS OF LEVOTHYROXINE REPOSITION ON INSULIN SENSITIVITY, LEPTIN LEVELS AND FAT MASS IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM

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To evaluate the effects of LT4 reposition on serum leptin levels, insulin sensitivity and amounts of fat mass (FM) in women with Subclinical Hypothyroidism (SH) we did a randomized clinical trial including 33 patients (mean TSH of 7.35 ± 2.4 µUI/ml) in two study groups. One group received levothyroxine (LT4) to restore euthyroidism and another was submitted to clinical observation (OBS). Determinations of leptin, lipid profile, insulin and glucose levels were perfomed. HOMA-IR index was calculated to assess insulin sensitivity. Bioelectrical impedance was used to determine fat mass (kg) and percentual of FM (%FM) as to calculate Fat Mass Index (FMI). After one year, nineteen LT4 and fifteen OBS were reevaluated. The randomization generated groups that had similar clinical and laboratorial parameters, like age, BMI, waist circumference, %FM, FMI, HOMA-IR and lipid profile. The leptin levels decreased with LT4 (–21.8 ± 18.2 ng/ml; p<0.001) but also decreased without medication (–17.7 ± 19.3ng/ml), and the comparison between the effects in the two groups did not demonstrate statistical difference (p=0.627). The comparison of the mean modifications in the HOMA-IR were also not different between LT4 (–0.018 ±1.3) and OBS (+0.570 ± 1.2), (p=0.544). In the two groups (LT4 vs OBS, respectively) there were no modifications in BMI (–0,7 ±2,4 vs +0.6 ± 1.8; p=0.863), %FM (+4.4 ± 8.4 vs +4.0 ±7.0, p=0.889), FMI (+0.4 ±3.7 vs +0.3±4.8; p=0.968) and waist (+1.3 ±8.8 vs +0.3 ±6.1;0.939). The treatment of SH did not lead to any improvement in insulin sensitivity nor to any change in amount of FM or leptin levels.

Clinical thyroidology
Poster

THYROID HORMONES IN PREGNANCY: INTRA-INDIVIDUAL VARIABILITY AND CORRELATION TO FETAL GROWTH

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The purpose of the study was to investigate the intra-individual variability of thyroid hormones during pregnancy, and to estimate the influence of thyroid function on fetal growth. 133 pregnant women without known thyroid disease had 7 (1–12) blood samples and 3 (1–8) ultrasound examinations (for estimations of fetal weight) performed during gestation. Thyroid stimulating hormone (TSH); thyroxine (T4); free T4; triiodothyronine (T3); free T3; and antibodies against thyroglobulin (anti-TG) and thyroid peroxidase (anti-TPO) were determined by immunoassays. Z-scores for hormone levels were calculated for five weeks intervals throughout gestation. Fetal growth rate in the third trimester was calculated from individual linear regression analyses. The serum concentrations of thyroid hormones changed during gestation as expected. There was a large inter-individual variation of thyroid hormone levels, whereas individual z-scores showed little variability throughout gestation. Anti-TG was present in 41 (31%), and anti-TPO in 28 (21%) women. The thyroid variables showed largely similar results whether auto-antibodies were present or not. Mean free T3 and z-scores of free T3, but not the other thyroid parameters, were correlated with fetal growth rate ($r = 0.305$, $p = 0.001$; $r = 0.317$, $p = 0.001$, respectively). There were no correlations between maternal thyroid parameters and fetal size at birth. In conclusion, individual thyroid hormone levels showed little variability during pregnancy compared to inter-individual variation. Maternal serum concentration of free T3 correlated positively with fetal growth rate in third trimester. Assessment of the individual change in thyroid function variables seems superior to gestation based reference intervals.

Clinical thyroidology
Poster

SIGNIFICANT INTERACTION BETWEEN FAMILIAL PREDISPOSITION AND ENVIRONMENTAL EXPOSURE IN GOITRE ETIOLOGY

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It is often assumed that interactions between genetic background and environmental exposure play important roles in goitre etiology. Demonstrations of such interactions have, however, not previously been published. We analysed data from 4,360 subjects from the DanThyr studies. Subjects were asked about thyroid disease among first degree relatives and categorised with or without predisposition (+disp/-disp). Thyroid ultrasonography with measurement of thyroid volume was performed. Iodine excretion was estimated from age- and sex adjusted iodine / creatinine ratio in spot urine samples. Data were analysed in regression models with control for possible confounding. Iodine excretion and familial occurrence of thyroid disease were both significant determinants of thyroid volume ($p<0.001$). However, significant interactions were found between iodine excretion and familial occurrence ($p=0.005$).With very low iodine excretion (< 50 µg/day), no difference in thyroid volume was found between +/-disp. With slightly low iodine excretion of 50–150 µg/day marked differences were found with thyroid volume 20.9 ml +disp and 15.0 ml -disp ($p<0.001$). With normal or high iodine excretion no difference was found +/– disp. Likewise, risk of thyroid enlargement was only significantly different between +/– disp in the group with slightly low iodine excretion (Odds Ratio 2.9 +disp vs. 1.3 -disp compared to normal iodine excretion), but not with normal or very low iodine excretion. We demonstrate statistically significant interactions between iodine excretion and familial occurrence of thyroid disease in the association with thyroid volume. In other words: slightly low iodine intake is only a risk factor for goitre when combined with familial predisposition.

Clinical thyroidology
Poster

GROUNDS FOR THYROID VOLUME CHANGES DURING PREGNANCY AND AFTER DELIVERY IN AN IODINE SUFFICIENT AREA

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Purpose: Even in iodine sufficient areas an increase of thyroid volume during pregnancy was reported. Our aim was to determine grounds for thyroid volume changes during pregnancy and after delivery in such an area. ***Patients and Methods:*** We followed 191 healthy pregnant women with mean age 30.9 ± 4.3 years in the first trimester (period I), 171/191 women in the third trimester (period II), 143/191 women three months after delivery (period III) and 87/191 women twelve months after delivery (period IV). All were negative for thyroid autoantibodies. TSH was measured in periods I, II and III, urinary iodine in periods II, III and IV, thyroid volume was measured by ultrasound in periods I, II, III and IV, and peak systolic velocity (PSV) by colour flow Doppler sonography in all women in periods III and IV and in some in period II using a 7.5 MHz linear transducer. ***Results:*** TSH concentration was significantly higher in period II when compared with periods I and III ($p=0.002$, $p=0.0001$, respectively). Urinary iodine concentrations in different periods were not significantly different. Mean thyroid volume in period II (11.1 ± 3.0 mL) was significantly greater than in period I (8.8 ± 2.5 mL, $p<0.00001$), in period III (8.5 ± 2.9 mL, $p<0.00001$) and in period IV (7.6 ± 2.3 mL, $p<0.00001$). Thyroid volume in period I was significantly greater than in period IV ($p=0.0004$). Similarly, PSV in period II (13.4 ± 2.1 cm/s) was significantly higher than in period III (8.6 ± 1.4 cm/s, $p<0.00001$) and in period IV (7.9 ± 1.3 cm/s, $p<0.00001$). PSV in period III was significantly higher than in period IV ($p=0.0005$). ***Conclusion:*** We found that increased intrathyroidal blood flow during pregnancy may contribute to the observed enlargement of thyroid gland.

Clinical thyroidology
Poster

BclI POLYMORPHISM IN THE GLUCOCORTICOID RECEPTOR GENE IN PATIENTS WITH GRAVES OPHTHALMOPATHY TREATED WITH GLUCOCORTICOIDS

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Background: Most action of glucocorticoids are mediated by the glycocorticoid receptor (GR). The interindividual response to glucocorticoids can be influenced by polymorphism in the gene coding for the GR. ***Objective:*** The aim of this study was to evaluate association of BclI polymorphism with outcome of glucocorticoid treatment in patients with Graves ophthalmopathy (GO). ***Methods:*** Study group consisted of 20 patients with severe GO treated with 2 doses of 500mg of methylprednisolon during 3 days followed by oral prednisone 40mg/d tapered to 10mg/d in 4 weeks. The treatment lasted for 6 months. The characterization of the BclI polymorphism was performed by PCR with restriction fragment length polymorphism (RFLP) analysis. To characterize the underlying base substitution of the BclI polymorphism, we directly sequenced the PCR products. ***Results:*** Nine patients had no glucocorticoid gene receptor polymorphism, ten patients were heterozygous for BclI and one was homozygous. After 6 months, 9 of 11 patients (82%) with polymorphism had a treatment response compared with 6 of 9 patients (67%) with no polymorphism. Ten of 11 patients (91%) with polymorphism improved CAS for ≥ 2 points and only 6 of 9 (67%) patients without polymorphism. Reduction in proptosis was also greater in the group with polymorphism (p=0.08) as well as improvement in visual acuity (p=0.09). ***Conclusion:*** These results showing positive therapeutic response of glucocorticoids in patients with BclI are promising and requires further investigation on larger group of patients.

Clinical thyroidology
Poster

EFFICACY OF DIFFERENT MINIMALLY INVASIVE TREATMENT METHODS IN PATIENTS WITH AUTONOMOUSLY FUNCTIONING THYROID NODULES - RESULTS OF CLINICAL APPLICATION

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Purpose: Comparison of efficacy of different minimally invasive treatment methods in patients with autonomously functioning thyroid nodules (AFTN). ***Materials:*** Following methods of treatment were used at patients with AFTN: percutaneous ethanol injection (PEI) - 50 patients, laser thermal ablation - LTA (26 patients); combined application of PEI and LTA (12 patients); radiofrequency ablation (RFA) - 12 patients, combined application of RFA and PEI (3 patients). The age of patients varied from 38 to 74 years. Maximal nodules' size - 22 up to 48 mm. ***Methods:*** 95% alcohol solution was used for PEI. LTA was carried out with diode laser (wave length 970 nanometers, power 2 W, optical fiber diameter 400 micrometers, time 6–12 minutes). RFA was carried out by means of generator RITA MEDICAL 1500X, providing destruction focus of 2–5cm. The target temperature during RFA was 105 Celcium degrees, the time of exposition - 3 minutes. PEI was carried out on an outpatient basis, laser destruction and RFA - on an inpatient basis. ***Results:*** Normalization of thyroid hormones rate was achieved at 64.3% of patients after PEI; at 50% of patients after LTA, at 91.7% of patients when RFA was used. Combined application of LTA and PEI led to positive results at 87.5% patients, in case of RFA and PEI - at 3 out of 3 patients. No major complications occured. ***Conclusions:*** RFA and combined ablation methods are the most effective in treatment of patients with AFTN. These methods allow to eliminate thyrotoxicosis at overwhelming majority of patients.

Clinical thyroidology
Poster

CLINICAL EVALUATION OF TRUE-CUT BIOPSY AND GALECTIN-3 DETECTION IN PATIENTS WITH FOLLICULAR NEOPLASMS

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Objectives: The purpose of the study is evaluation validity of large needle biopsy and detection of galectin-3 in patients with follicular neoplasms. ***Methods:*** Large needle biopsy under ultrasound control was used in 40 patients with cytological diagnosis “follicular neoplasm”after fine needle aspiration biopsy. Then histological evaluation was performed followed by immunohistochemical staining with antibodies to galectin-3 and thyroperoxidase. Data were analysed using the Chi square test. A p value of less than 0.05 was considered to be statistically significant. ***Results:*** Preoperative histologic examination provided 78.95 % of truenegative results, 10.53 % of truepositive results, 5.26 % of falsenegative results, no falsepositive results and 5.26 % of noninformative material. The sensitivity of the method was 66.67 %, specificity −100 %, positive predictive value −100 %, negative predictive value −93.75 %, accuracy −94.44 %. There were 85.71 % of truenegative results, 14.29 % of truepositive results and no falsepositive or falsenegative, or noninformative results, when evaluating only immunohistochemical staining. The combination of histological evaluation together with immunohistochemical staining proved to be the most accurate and allowed to differentiate all cases of “follicular neoplasms” into benign and malignant groups preoperatively. According to our findings we developed an algorythm of preoperative diagnostics and type of treatment of cytologically unclear “follicular neoplasms”.

Clinical thyroidology
Poster

APPLICATION OF ULTRASOUND DOPPLEROGRAPHY AT PATIENTS WITH THYROTOXICOSIS SYNDROME

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Objectives: to evaluate different variants of color Doppler imaging (CDI) and power Doppler mapping (PDM) at patients with thyrotoxicosis. ***Materials and Methods:*** 102 patients with thyrotoxicosis were investigated, 58 with Grave`s disease, 10 with recurrent thyrotoxicosis after subtotal thyroidectomy, 34 with thyroid toxic adenomas. Mean age was 46.3+/–12.3 years. Functional autonomy of thyroid nodules was determined by means of scintigraphy with Tc99. Ultrasound of thyroid gland was carried out with Doppler bloodflow examination of upper thyroid artery, CDI and PDM. Peak systolic blood flow speed (Vps), peak diastolic blood flow speed (Ved), pulse index (PI) and Resistance index (RI) were measured. ***Results:*** All patients with Grave`s disease and recurrent thyrotoxicosis had increased levels of Vps up to 45.7±6.2 cm/sec. Patients with toxic nodular goiter had increased Vps upto 34.5±4.95 cm/sec, and it was higher than Vps at the contralateral side (p-level 0.029). There were no statistically significant differences of other speed characteristics (Ved, PI, RI) compared with normal values. 96.5% of patients with recurrent thyrotoxicosis and 100% with Grave`s disease had tissue hypervascularization. 91.1% of patients with autonomously functioning nodules had mixed type of vascularization, which correlated with high accumulation of Tc99 (p-level 0.025). ***Conclusions:*** Dopplerography examination of bloodflow in the upper thyroid arteries may be recommended as an additive method of investigation of immunogenic and nonimmunogenic thyrotoxicosis at surgical patients.

Clinical thyroidology
Poster

IODINE DEFICIENCY AND THYROID GLAND VOLUME CORRELATION ASSESSMENT IN SCHOOL-AGED CHILDREN IN SOME AREAS OF LENINGRAD REGION OF RUSSIA

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Purpose: Screening examination of school-aged children from different communities of six areas of Leningrad region to assess the iodine defency in the frame of “The ThyroBus Project” carried out by the North-West District Medical Centre of Ministry of Public Health of Russia. ***Methods:*** According to the suggestions of the World Health Organization in order to estimate the iodine sufficiency selected sampling units in clusters of 45 schoolchildren aged 7 to 10 were examined. In those clusters urinary iodine and thyroid volume were determined by ultrasonography. ***Results:*** Out of 21 communities of Leningrad region involved in the study the iodine deficiency hadn’t been determined only in 8 of them (5 of which are located in the same area), while in keeping with urinary iodine data the light iodine deficiency had been assessed in the vast majority of the selected communities of other areas. The level of enlargement of thyroid size had been matched with median iodine defency. ***Conclusions:*** The studies have demonstrated that the iodine defency is characterized by kind of mosaic allocation which requires a one-off approach to solving the problem of organizing prophylaxis in every particular community. Prevalence of thyroid goitre doesn’t correlate with the concept of the iodine deficiency allocation in different communities, which calls for further studies of other goitrogenic factors, supposedly anthropogenic ones.

Clinical thyroidology
Poster

TAO WITH THYROIDITIS OF HASHIMOTO COMBINED WITH ACROMEGALY

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Interactive session thyroid cases: Endocrine ophthalmopathy TAO with thyroiditis of Hashimoto combined with acromegaly. In May 1999 a 42-year old male caucasian has underwent transnasal hypophysectomy due to tumor. A postoperative SCAN in 2002 reveals remaining tumor tissue. After oral glucose toleranse test Groth hormon is 7.63 mU/l(normal range 1.0–6.0); iGF 1–2.1 (normal 1.0–2.1). Prolactin- 111.1(59–619pmol/l) ACTH- 28 pg/ml (7.9–66.1). Treatment is carried out with 50 mkr Levo-thyroxin daily and 2 tablets Dostinex per week. Follows subsequent hospitalisation due to new complaints - the patiet experienced oozing, light-irritation, a feeling of foreigh body, headache for 2 months. Painful,oppressive feeling behind the globe. Physical examination revealed: bilateral periorbital oedema, chemosis, swollen caruncle, positive Graefe symptom on the right side without impaired function. The thyroid gland had an enlargement of 1-b degree; heart rate -72, blood pressure -110/70. CAS severity-5/10. Results of US of throid gland-thyroiditis of Hashimoto. TSH2.1 mU/l (0.35–5.0), FT4-14.1 pmol/l (9.15–23) anti TPO- Abs- 42 (<12 IU/l) SCAN of the hypophysis - a soft tissue lesion (dimensions 19/9)was detected in the right half of sella turcica, The hypophysal infundibulum was pushed aside from the median line; the upper pole of the lesion projected to the insular cystern; there was no compression and dislocation of the third ventricle and the frontal cornua of lateral ventricles. SCAN of the orbits fusiform thickening of medial oculomotos muscles. The changes of the right orbit and the manifested right exophthalm made us consider two possibilities in the differential diagnosis: dorsal destruction of of sella turcica from the invasion of the tumor process or a combination of TAO with Thyroiditis of Hashimoto. We discussed 111 DTPA-D -Ph octreotide scintigraphy ih the pre-treatment evaluation of TAO. Inclusion of somatostatin analogues would be a relevant therapeutic approach in case early stage of TAO combined with acromegaly is confirmed.

Clinical thyroidology
Poster

CLINICAL APPLICATIONS OF THE SECOND GENERATION TBII ASSAYS USING RECOMBINANT HUMAN TSH RECEPTOR IN GRAVES' DISEASE

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Background: Detection of autoantibodies to the TSH receptor in Graves' disease (GD) has found widespread use in clinical routine and has been performed mostly by commercial radioactive receptor assays measuring TSH binding inhibitory activity with porcine TSH receptors as ligand (pTBII). To increase the sensitivity of the assays many groups have tried to replace the porcine source of TSH receptor by human recombinant antigen (hTBII). In this study we evaluated the clinical applications of second generation TBII assays for the diagnosis of GD by comparing pTBII and hTBII. ***Methods:*** Sera from 40 patients with newly diagnosed or relapsing GD without or with less than 4 weeks of treatment with antithyroid drugs and 40 patients with Hashimoto's thyroiditis were examined. TBII was also measured in sera from 50 healthy controls. TBII was measured by using both the conventional porcine TBII assays (pTBII) and the human recombinant TBII assays (hTBII). ***Results:*** The cut-off values of pTBII and hTBII assays, defined as two standard deviations from the mean of healthy controls, were 4.0 IU/L and 1.0 IU/L respectively. Sensitivity was 100% (40/40) for hTBII compared to 82.5% (33/40) for pTBII in diagnosing GD. Specificity was 100% (50/50) for hTBII compared to 92% (46/50) for pTBII. Of 40 patients with Hashimoto's thyroiditis, 7 patients had positive TBII titers using hTBII and 4 patients had positive TBII titers using pTBII. ***Conclusions:*** These data show that the human recombinant TSH receptor assays are more sensitive and specific methods for the diagnosis of GD compared to the conventional porcine TBII assays. We consider that this new TBII assays should be used widely instead of conventional assays as first line diagnostic markers for GD.

Clinical thyroidology
Poster

IMPACT OF PREGNANCY ON PREVALENCE OF GOITRE AND NODULAR THYROID DISEASE IN WOMEN LIVING IN A REGION WITH BORDERLINE IODINE DEFICIENCY

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An interplay of genetic, epigenetic and environmental factors contributes to thyroid disease. In a cross-sectional study we aimed to determine the actual influence of parity on goitre and nodular thyroid disease (NTD) in women living in a region with borderline iodine deficiency. Thyroid ultrasonography (7.5MHz; Merck Thyromobil) was performed by the same investigator in 731 women living in Thuringia and Saxony. Furthermore, age and BMI were documented and all probands were asked about the number of previous pregnancies, family history of thyroid disease and past or present smoking. Goitre prevalence was 17.6%. Solitary thyroid nodules were detected in 21.2%, multiple nodules in 24.6% of the study population. Age was positively correlated with goitre prevalence and NTD (due to multiple but not solitary nodules). No association was found between parity (n=396; 87% women) and goitre prevalence. In contrast, a significant increase in both solitary (22.2%) and multinodular thyroid disease (23.7%) was observed in women with at least one pregnancy compared to nullipara (11.9% and 16.9%, respectively). BMI in women with goitre (27.3kg/m2) was significantly higher than in women without (24.8kg/m2). In addition, a significant correlation was detected between BMI and presence of multinodular disease (26.5kg/m2). 24.5% of women with goitre reported a positive family history for thyroid disease, as opposed to 15% of women with normal size thyroid gland. Neither goitre nor NTD were associated with a history of smoking. NTD and/or goitre are present in up to 50% of women in an area with borderline iodine deficiency. Besides age, BMI and family history, parity is positively correlated with presence of NTD, whereas smoking was not associated with goitre/NTD.

Clinical thyroidology
Poster

CHOICE OF 131I ACTIVITY FOR RETREATMENT AFTER THE FIRST UNSUCCESSFUL THERAPY IN PATIENTS WITH GRAVES' DISEASE

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Persistent hyperthyroidism is not uncommon in patients with Graves' disease (GD) treated with ^{131}I and is usually treated by the second ^{131}I dose. However, there is no consensus, as to the activity of the second ^{131}I dose i.e., if it should be higher or smaller than the first one. This analysis was undertaken to determine the effect of various ^{131}I retreatment doses in patients with Graves' hyperthyroidism on the therapy outcome. ***Materials and Methods:*** Ninety patients (18 ♂, 72 ♀, age 21-74 years) treated, at least twice with ^{131}I for GD were retrospectively analysed. The diagnosis of GD was based on clinical examination, supported by thyroid scintigraphy, ultrasound and measurement of antithyroid antibodies. Persistent hyperthyroidism was diagnosed when either concentration of free thyroid hormones was elevated and/or TSH was suppressed after 6 months from ^{131}I therapy. Hypothyroidism was diagnosed when TSH concentration was elevated, either at that time-point or earlier, in patients, who required permanent levothyroxine replacement. All the other patients were classified as euthyroid. Before each therapy, the thyroid gland size was estimated by ultrasound and thyroid iodine uptake was measured after 24h. The delivered ^{131}I activity per gram of tissue was calculated retrospectively by the formula - administered activity x thyroid uptake / thyroid gland mass.

Results:

Results of 2nd therapy	No. of patients	Activity per gram of tissue - median [µCi/g]		p
		1st therapy	2nd therapy	
Hypothyroidism	69	185	332	0.00005
Euthyroidism	13	153	283	0.05
Hyperthyroidism	8	199	201	0.95

Conclusions: The second dose of ^{131}I, calculated as the activity per gram of thyroid tissue, should be greater than the first one in patients, retreated after 6 months for GD.

Clinical thyroidology
Poster

TOTAL THYROID VOLUME OF SCHOOLAGE CHILDREN IN ENDEMIC WATERBORNE FLUOROSIS AND MILD IODINE DEFICIENCY AREA IN TURKEY

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Endemic fluorosis caused by ingestion of large amounts of fluoride either through water or rarely from foods. In our city, Isparta which is located in southwest part of Turkey, flouride content of natural drinking water in South side of it, is high so fluorosis is endemic. At the same time this region is one of endemic mild iodine deficiency area. Iodine prophylaxis programme was began at 1999.
There are some reports based on casual observations that fluoride is goitrogenic in man.
We studied a total of 559 schoolage children (287 girls and 272 boys) of 10–15 years of age in two areas, characterized by ingestion of high fluoride drinking water (group 1, n:261) and normal fluoride drinking water(group 2, n:298). Ultrasonography was used to evaluate thyroid volumes. Iodine and floride in spot urine were measured. Body surface area (BSA) also calculated from height and weight of children. There was no significant correlation between urinary iodine and fluoride excreation and TTV. Total thyroid volume (TTV), height and BSA were increased with age. So, we calculate a ratio of TTV (ml) per BSA (m^2) (TV/SA) for each children. TV/SA is significantly high in group1(mean: 6.94±2.14) than group 2 (mean: 6.48±1.54). Mean urinary iodine excreation was 95.94±35.94 µg/L and it was decreased with age. Fiftyseven % of children had iodine deficiency and 50% of them had mild form of it. Children with teeth discoloration and/or had urinary fluoride exreation more than 0.6 mg/L named as severe fluoride exposure but no correlation was found between this group and TTV and TV/SA. In conclusion; we did not find direct correlation between urinary fluoride excreation or fluorosis and thyoid volume in schoolage children.

Clinical thyroidology
Poster

THYROID HORMONE LEVELS IN UNTREATED PATIENTS WITH GRAVES' DISEASE CORRELATE WITH THE PEAK SYSTOLIC VELOCITY

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Purpose: An increase of thyroid blood flow has been observed in untreated patients with Graves\' disease (GD), but there is less data on the association of intrathyroidal blood velocity with the degree of hyperthyroidism and autoimmune reactivity. ***Patients and Methods:*** We evaluated 84 newly diagnosed hyperthyroid patients with GD, 77 females and 7 males, aged between 20 and 77 years (mean, 38.6 ± 11.3). Thyroid hormones, thyroglobulin antibodies, thyroid peroxidase antibodies and TSH receptor antibodies were determined. Thyroid volume was measured and colour flow Doppler sonography (CFDS) was performed using a 7.5 MHZ linear transducer. CFDS pattern was estimated and peak systolic velocity (PSV) at the level of intrathyroid arteries was measured and expressed as a mean of five measurements. ***Results:*** In 38 patients (45.2%) we estimated the CFDS pattern III, in 44 patients (52.4%) pattern II and in 2 patients (2.3%) pattern I. A significant correlation between PSV and both free T_4 and free T_3 concentrations was established (p=0.002 and p=0.00004, respectively). PSV also significantly correlated with thyroid volume (p=0.01), while a negative correlation with the duration of symptoms characteristic for hyperthyroidism was found (p=0.04). There was no association of PSV with neither of thyroid autoantibodies. ***Conclusion:*** Our results indicate that the degree of PSV in untreated GD patients depends on the severity of hyperthyroidism. Thus, CFDS may enable early recognition of more hyperthyroid patients with GD.

Clinical thyroidology
Poster

NON INVASIVE ABLATION OF A TOXIC NODULE BY HIFU (HIGH INTENSITY FOCUSED ULTRASOUND)

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Surgery and radioiodine therapy are the established methods for the definitive therapy of toxic nodules but a few patients are not eligible for these treatments. High intensity focused ultrasound (HIFU) is a new non-invasive technology which allows elective tissue destruction by thermal effect. A 26 year old male presented with weight loss, muscle weakness and general asthenia for the last three months. Biology showed TSH 0.02 mU/L, normal FT4 and FT3, and negative TRAK assay and TPO antibodies. Thyroid ultrasonography showed a 9 x 8 mm isthmic nodule. On scintigraphy, the nodule appeared as hyperfunctionning with near total extinction of the gland, but displayed a low iodine uptake of 2%. Daily iodine excretion was normal (155 µg/24). Since a radioiodine treatment was thus impossible, and to avoid thyroid surgery, the patient was offered to enroll in a clinical HIFU investigation. After lidocaine local anesthesia, the patient had a HIFU treatment of 15 minutes with a total of 4 kJ of therapeutic acoustic energy delivered under ultrasound real time imaging control. HIFU session was well tolerated. At day 14, the nodule had become cystic, hypoechoic, FT4 and FT3 levels remained normal. At 3 months, TSH reached normal value (1.91 mU/L) and ultrasonography showed a hypoechoic 3–4 mm residual nodule. The patient will be followed for an additional 9 months. HIFU treatment resulted in a satisfactory clinical and biological response, and may be an effective and safe alternative to radioiodine or surgery in selected patients.

Clinical thyroidology
Poster

A THREE-YEAR FOLLOW-UP OF THE RADIOACTIVE (131I) TREATMENT OF MULTINODULAR GOITER PRECEDED BY 0.1MG OF RECOMBINANT HUMAN TSH

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This study aimed to evaluate the long-term effects of out-patient radioiodine (RAI) treatment preceded by rhTSH in relatively large multinodular goiters (MNG) patients (185±168mL). Forty two patients were included in this study. All of them had lived in moderate iodine-deficient rural areas. Presently, patients had evidence of excessive nutritional iodine intake (urinary iodine 308±130µgI/L). All were advised to follow a low iodine diet. Three patients with clinical hyperthyroidism were treated with methimazole. After 120days urinary iodine declined to 165±90µgI/L ($p<0.001$). Thyroid function tests were obtained at baseline and after out-patient administration of 30mCi of RAI (1.11GBq) preceded by 0.1mg of rhTSH, 24, 48, 96, 168h and at 6months intervals for 36months. Thyroid volume was estimated by computed tomography. A complete cardiologic evaluation was obtained before and during the first two weeks after RAI. As expected, there was a surge of thyroid hormones (serum Free T4 2.94mg/dL, Total T3 640mg/dL) in the first week of RAIdose, declining to normal values within 30days. Serum TG also rose to 732 ± 351ng/mL. There was few clinical signs of hyperthyroidism (half of the patients were on beta-blockers). Cardiac evaluation did not indicate major changes as compared with baseline. There was a continuous and significative reduction of the MNG volume reaching a mean of 75.2% of the baseline in 36months. Late-onset hypothyroidism was confirmed in 21.4% of the patients.We concluded that MNG stimulation with 0.1mg rhTSH followed by 30mCi of RAI resulted in continuous reduction of the MNG volume. The patients could be treated in an out-patient mode avoiding the costs of hospitalization. The surge of hyperthyroidism after RAI did not altered heart function. This modality of MNG treatment with RAI+rhTSH is cost-effective with acceptable low risks for the patients.

Clinical thyroidology
Poster

RADIOIODINE THERAPY IN PATIENTS WITH GRAVES' DISEASE AND TOXIC MULTINODULAR GOITER IN A SCOTTISH POPULATION- COMPARISION OF EFFICACY OF TWO DIFFERENT DOSES

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Introduction: Different doses of radio-iodine therapy have been used for treatment of hyperthyroidism. Few studies have compared the difference of efficacy between different doses of radioiodine. ***Methodology:*** We retrospectively (approximately 10 year study) compared the efficacy of radio-iodine therapy in the management of patients with Graves's disease and toxic multinodular goiter. Patients received either 375 MBq or 550 MBq of radio-iodine. We used development of hypothyroidism following a single dose of radio-iodine therapy as gold standard. ***Results:*** 381 patients we studied. The results are summarized in the following tables:

Diagnosis	No.	mean age	females	males	received medication	previous H/O surgery
Graves	311	63.4	270	41	253	12
Toxic MNG	70	77.4	67	3	48	2

Radio-iodine therapy in Graves'

Dose (m bq)	No. of pts	response	% success
375	127	106	83.5
550	184	165	89.67

Radio-Iodine therapy in toxic Mng

Dose (M Bq)	no. of pts	response	% success
375	20	16	80
550	50	40	80

Conclusions: Both lower (375 MBq) and higher (550 MBq) doses of radio-iodine are highly successful modality of treatment for patients of hyperthyroidism. Patients of Graves disease had a better response to radio-iodine therapy than patients of toxic MNG. However statistically there was no difference in outcome with regards to the two different doses of radio-iodine therapy.

Clinical thyroidology
Poster

NATURAL HISTORY OF SPONTANEOUS SUBCLINICAL HYPERTHYROIDISM. A.PROSPECTIVE AND OBSERVATIONAL STUDY

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In order to analyze the natural course of spontaneous subclinical hyperthyroidism (SCHyper) and the potential risks factors involved on the progression to overt hyperthyroidism (OHyper) a prospective and observational cohort study have been carried out. Sixty-one consecutive outpatients (55 female, 6 male), mean age 55.5 ± 13.8 yr (23–83 yr) with persistent (> 3 months) spontaneous SCHyper (serum TSH < 0.45 mU/L, normal FT_4 and T_3) and no previous history of thyroid disease were followed by a mean period of 43.4 ± 30 months (6–147 months) with repeated determinations of TSH, FT_4 and T_3. Eight patients (13.1%) progressed to OHyper, 14 (22.9%) normalized their TSH levels and 39 (63.9%) remained on SCHyper. The overall progression-rate to OHyper was 36 cases per 1,000 patient-year and the time to progress to OHyper was significantly higher compared to time to normalization of TSH (42 ± 20.2 *vs*. 22.3 ± 15.9 months, p = 0.01). Initial TSH concentration was lower (0.04 ± 0.04 *vs*. 0.16 ± 0.1, p < 0.05) and initial thyroid weight (measured by ultrasound) was higher (69.9 ± 55.9 *vs*. 25.9 ± 4.8, p < 0.01) in patients who progressed to OHyper in comparison with euthyroid patients. Kaplan-Meier analysis showed that thyroid weight (TW), low than 60 g (p = 0.01) and low T3 concentration at baseline were related to normalization of TSH (p = 0.01). Using a regression analysis, any of the factors as age, FT_4, T_3, and TPOAb were significantly related to progression to OHyper. Further, TW (p<0.001), thyroid autonomy (p < 0.01) and serum high T3 concentration at baseline (p = 0.01) were related to persistence of SCHyper. All these data indicate that most of patients remained in SCHyper or would progress to OHyper. At the baseline very low serum TSH concentration, elevated serum T3 levels, high TW and thyroid autonomy were the predicting factors for both persistence of SCHyper and progression to OHyper.

Clinical thyroidology
Poster

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF THYROID HEMIAGENESIS: ULTRASOUND SCREENING IN PATIENTS WITH THYROID DISEASE AND NORMAL POPULATION

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Thyroid hemiagenesis (TH) is a rare form of thyroid dysgenesis, in which one thyroid lobe fails to develop. The detection of TH is usually an incidental finding during the evaluation of other thyroid disorders. The true prevalence is estimated to be 0.05–0.2% in normal population. We evaluated 3,241 adult patients sonographied for various thyroid diseases in our thyroid outpatient clinic and found eight cases with thyroid hemiagenesis (0.25%). All were unrelated individuals. Only one of eight hemiagenesis cases occurred in a male patient (M/F ratio 1:7). TH was due to the agenesis of the left lobe in all cases. The isthmus was visualized in four of eight patients (50%). The underlying thyroid diseases were Hashimoto's thyroiditis (n= 4), euthyroid multinodulated goiter (n= 2), and toxic adenoma (n= 1). One subject has no underlying thyroid disease. We also retrospectively analyzed the results of two large sonographical surveys, performed in 1997–1999 and 2002 for the iodine status of school age children (n= 4,772) and thyroid disorders of adult subjects (n=2,935) in Turkey and Ankara respectively. The absence of the left lobe was detected in only two cases (M/F 1:1), indicating a true prevalence of thyroid hemiagenesis of 0.025% in severe to moderately iodine deficient areas. TH is rarely identified during the evaluation of the underlying thyroid disease. Similar to previously reported observations, this study confirms that TH is almost always is due to left lobe defect. The higher prevalence among females in our out-patients clinic is a possible consequence of the fact that thyroid diseases are more common in women. Our community based data is in accordance with previous community-based in terms of male to female ratio and prevalence.

Clinical thyroidology
Poster

GRAVES' DISEASE IN PREGNANCY COMPLICATED WITH FETAL GOITROUS HYPOTHYROIDISM – SUCCESSFUL IN UTERO TREATMENT WITH L-THYROXINE

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Since the first report of in utero treatment of fetal goitrous hypothyroidism by intraamniotic injection of L-thyroxine 19 cases have been presented in the English literature, 8 (42%) had maternal hyperthyroidism and 11 (58%) of the cases were due to dyshormogenesis of the fetal thyroid gland or maternally derived goitrogen. We report 3 cases within the last 3 years of intra-amniotic thyroxine treatment of fetal goiter and hypothyroidism caused by overtreatment with antithyroid drugs of pregnant women with Graves' disease. The 3 cases all had Graves' disease and were between week 24 and 32 when fetal goiter and polyhydramnios were demonstrated at ultrasound. In a female foetus also bilateral ovarian cysts were found. The mothers were treated with a daily dose of carbimazole 17.5, 20 mg and propylthiouracil 400 mg. TSH were in upper normal range but free T4 between 5.8 and 8.3 pmo/L (7–20). Cord blood TSH levels were elevated (8.3–35.4 mU/L, range), (normal 3.8–10.8 mU/L) and low T4. The daily dose of antithyroid drug was extensively reduced or paused and L-thyroxine was given intra-amnioticly 100–150 mg weekly. The fetal goiters were gradually reduced in volume and the volume of amniotic fluid likewise. The babies were without visible goiters at delivery. Fetal goitrous hypothyroidism causes intrauterine hyperextension of the fetal neck resulting in obstruction of the esophagus and hydrops and of the trachea with risk of asphyxia and death at delivery. Thus, prenatal diagnosis and treatment are essential. In women treated with antithyroid drug frequent measurement of peripheral thyroid hormones with appropriate adjustment of dosage of the antithyroid drug keeping peripheral thyroid hormones in the high upper normal range, and fetal ultrasound should be performed to look for presence of goiter, evidence of fetal thyroid hypothyroidism, hydrops and growth restriction. Also, the findings of fetal ovarian cysts should lead to investigation for fetal hypothyroidism.

Clinical thyroidology
Poster

SERUM ANTI-THYROID ANTIBODIES ARE NOT ASSOCIATED TO CYTOLOGICAL DIAGNOSIS OF PAPILLARY THYROID CANCER

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A possible association between thyroid autoimmunity and papillary thyroid cancer (PTC) has been reported, but not yet proven. Aim of this work was to evaluate a possible relation between the presence of anti-thyroid antibodies (TAb) and a cytological diagnosis of PTC. Between 2000 and 2004, 15,542 patients underwent to fine needle aspiration biopsy (FNAB) of thyroid nodules and simultaneous measurement of anti-thyroglobulin (TgAb) and anti-thyroperoxidase (TPOAb) antibodies. Patients included in the study were those with benign thyroid nodules (n=1,0541, BTN group) or with a cytological diagnosis of PTC (n=559, PTC group). The prevalence of TAb was 36.0% in BTN group and 33.6% in PTC group (p=ns), with TgAb detected respectively in 26.0% and 22.4% (p=0.05) and TPOAb in 23.6% and 23.1% (p=ns). In patients with multi-nodular goiter (MNG), TAb were present in 36.3% of subjects of BTN group and 30.7% of patients of PTC group (p =0.07), TgAb in 26.8% and 20.2% (p=0.02) and TPOAb in 23.1% and 19.0% (p=ns). In patients with single or isolated thyroid nodules, these percentages were respectively 31.1 % vs 31.8% (p=ns) for TAb, 21.7 % vs 21.5%.(p=ns) for TgAb and 20.9 % vs 23.1% (p=ns) for TPOAb. The prevalence of TAb in females was 38.3% in BTN group vs 35.0% in PTC group (p=ns) and in males 25.5 % in BTN group vs 29.9 % in PTC group (p=ns). In female, the prevalence of TgAb was higher, even though not statistically significant, in BTN group than in PTC group (respectively 27.8% vs 23.6%, p= 0.055). According to the multiple regression model, TgAb and TPOAb were not significantly more frequent in PTC group. In conclusion TAb are not associated to an higher risk of PTC, but TgAb are more frequent in BTN group, mainly in females and in patients with a clinical diagnosis of MNG.

Clinical thyroidology
Poster

CHROMOSOMIAL DISORDERS AND GRAVES' DISEASE: TWO CASE REPORTS

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About 15–20% of adults with Down\'s syndrome have autoimmune hypothyroidism. Among patients with Down\'s syndrome, prevalence of Graves disease (1–2%) may not be increased compared with the general population. About 15% of patients with Turner\'s syndrome have autoimmune hypothyroidism.Until 2006, only 15 cases of Graves disease have been reported among patients with Turner\'s syndrome. We present 2 patients with chromosomial disorders (Down\'s syndrome, Turner\'s syndrome) with Graves disease without clinical Graves ophthalmopathy. Clinical picture of thyrotoxicosis was oligosymptomatic in the patient with Down\'s syndrome, probably because it represents a syndrome of accelerated aging.Before treatment, TRAb was positive in both patients.Both were pretreated with ATD before radioiodine therapy; three radioiodine doses were required in the patient with Down\'s syndrome and a single radioiodine dose was sufficient in the case with Turner\'s syndrome.In our patient with Turner\'s syndrome, ATD therapy should be delayed 6 months due to liver cytolysis syndrome.Both of them developed postradioiodine hypothyroidism : after 8 months in the patient with Turner\'s syndrome and after 11 years in the case with Down\'s syndrome.Associated vitiligo was recorded in the patient with Down\'s syndrome.Adults with these two chromosomial disorders should be screened annually for autoimmune thyroid dysfunction.

Clinical thyroidology
Poster

LONG TERM EFFECTS OF IODINE PROPHYLAXIS IN POLAND

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On the basis of studies carried out in years 1989–1995 Poland was assessed as a region of moderate iodine deficiency, except the seaside (mild deficiency) and Hel Peninsula (replete), with accompanying goiter endemia. The madatory model of iodine prophylaxis based on house salt iodization (30±10mg KI/kg) led in a very short period to a quicker than expected decrease in goiter prevalence and resulted in Poland being classified as a territory with sufficient iodine supplementation on the population level according to ICCIDD criteria. The aim of the study carried out in years 1999–2000 and 2003–2005 with the help of a mobile ambulance “Thyromobil” provided by Merck KGaA, Darmstadt, Germany, was to evaluate changes in goitre prevalence in a stable phase of iodine prophylaxis**,** following rapid drop in goiter prevalence just after its implementation in 1997. We examined 6-12 aged children, 251 in 1999–2000 and 364 in 2003–2005 from the same schools localized in the area of former moderate deficiency in upland region (Krakow) and mountain region (Rychwald) as well as in iodine replete area (Hel Peninsula). Thyroid volume was determined by ultrasound, iodine concentration in the morning urine sample with use of Sandell-Kolthoff method. Goitre prevalence decreased from 3.6% in 1999–2000 to 1.6% in 2003–2005 in the examined group. In the subgroup of youngest children aged 6–8 a drop in the mediana of thyroid volume from 4.9 ml to 4.4 ml was observed. Unexpectedly, these changes were accompanied with lowering of ioduria – mediana decreased from 108.0 µg/l in 1999–2000 to 84.7 µg/l in 2003–2005. In Hel Peninsula goiter prevalence did not change (1.4%); in Krakow it dropped from 4.8% to 0%, in Rychwald it increased slightly from 3.1% to 3.8%. We conclude that iodine prophylaxis effects in sustained goiter prevalence reduction in Poland. Recently observed diminishment in ioduria in the face of constant salt iodization is probably related to a drop in consumption of iodine from other sources (milk, fish) and indicates the need for seeking new solutions in iodine prophylaxis.

Clinical thyroidology
Poster

COMPARISON OF PALPATION GUIDED FINE NEEDLE ASPIRATION BIOPSY WITH ULTRASOUND GUIDED FINE NEEDLE ASPIRATION BIOPSY IN THE EVALUATION OF BIG THYROID NODULES

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Thyroid fine needle aspiration biopsy (FNAB) is a diagnostic method for the evaluation of nodular thyroid disease. Although FNAB is reliable, simple method, the diagnostic value may be restricted by inadequate and suspicious material and false negative results. In our study, we aimed to compare prospectively palpation guided fine needle aspiration biopsy (PGFNAB) to ultrasound guided fine needle aspiration biopsy (UGFNAB) and to establish inadequate ratios and cost effectiveness of two different methods in big nodules (biggest nodule diameter more than 25 mm). In the study two methods were performed to the same nodule and all aspirations were performed by the same operator. Cytological evaluations were performed by the same cytologist so subjective error were tried to be decreased. We had a similar study in little nodules (biggest nodule diameter between 10–25 mm) and found UGFNAB was preferable in the little nodules. Current study is the first comparative prospective study in big nodules. A total of 97 nodules in 76 subjects were evaluated and 17 of them were operated. Mean biggest nodule diameter was 33.4±8.6 mm. Inadequate material ratio for PGFNAB was 5.15% and for UGFNAB was 6.18% and there was no statistically significant difference ($p>0.05$). False negative results were 11.76% for each method when micropapillary carcinoma was included to malign histology. False positive ratios were again same (11.76%) when follicular neoplasm was counted in suspicious category. In conclusion, against to small thyroid nodules, our study shows that there is no difference between PGFNAB and UGFNAB for getting adequate material and having more accurate cytological evaluation in big thyroid nodules.

Clinical thyroidology
Poster

THE THYROID ISTHMUS DOES NOT CONTAIN C-CELLS

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Background and Purpose: It was shown by autopsy-studies, that C-cells are distributed along the vertical axes of thyroid lobes, but probably not within the isthmus. This had to be systematically investigated in surgical patients, with and without C-cell disease, with respect to implications for surgical strategies. ***Patients and Methods:*** A) The isthmi of 75 patients, 41 of them operated on for C-cell-pathology (29 with C-cell carcinoma, 12 with c-cell hyperplasia) and 34 for pathology of the follicular cells, were investigated by immunhistochemistry for Calcitonin, Chromogranin A and CGRP. B) Consequently, in patients who underwent isthmus preserving total bilobectomy (IPTB) for pathologic, but mild hypercalcitoninemia (stimulated Calcitonin up to 300 pg/ml) the border-zones towards the remaining isthmus were investigated for Calcitonin (111 patients, 222 border-zones). ***Results:*** A) None of the removed isthmi contained Calcitonin-positive cells, except in one case with very advanced C-cell carcinoma (pT4, N1b, M1) in MEN IIb. Positive cells for Chromogranin A and CGRP were present in 62 % and 45 % of isthmi, respectively. B)None of the border-zones towards isthmus contained Calcitonin-positive cells. ***Conclusions:*** The isthmus of the thyroid gland does contain neuroendocrine cells, but no C-cells, neather in patients with disease of the follicular cells nor in patients with C-cell disease. Exceptionally, advanced C-cell carcinoma, arising from the lateral lobe, can infiltrate the isthmus region. The border-zone between isthmus and thyroid lobes can be defined reliably by the experienced surgeon. Isthmus preserving strategies for patients with early C-cell disease (hyerplasia and, probably, microcarcinoma) can serve as a better alternative to total thyroidectomy.

Clinical thyroidology
Poster

DOES AMIODARONE MODIFY PROINFLAMATORY CYTOKINES' PATTERN IN THYROTOXICOSIS?

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Background: Proinflamatory cytokines C-reactive proteine (CRP) and interleukin-6 (IL-6) are increased both in common hyperthyroidism and in amiodarone-induced thyrotoxicosis (AIT). Increased CRP and IL-6 levels are associated with an increased risk for atrial fibrillation. ***Aim:*** To assess amiodarone's influence on CRP and IL-6 levels in AIT and the relationship between proinflamatory cytokines and atrial fibrillation. ***Subjects and Methods:*** 20 patients treated with amiodarone (7M/13F), aged 60.7±2.2 years, 16 hyperthyroid patients (4M/12F), aged 58.5±4.1 years, without significant Graves ophthalmopathy, and 5 women with euthyroid endemic goiter, aged 62.2±5.9 years, were evaluated. AIT was present in 8 patients. TSH, total T_3, total T_4, free T_4 were measured by microenzymatic immunoassay. CRP and IL-6 were measured by chemiluminescent immunometric assays. ***Results:*** CRP was increased in all types of hyperthyroidism (78±25.9 mg/dL) as compared to eu/hypothyroidism (47.5±8.7 mg/dL). There was a similar increase in CRP in AIT (n=8 pts, 72.3±22.8 mg/dL) and in common hyperthyroidism (n=16 pts, 80.9±37.7 mg/dL), p=ns. Mean IL-6 levels were normal both in AIT (4.5±1.4 pg/mL) and in common hyperthyroidism (6±1.5 pg/mL). In 3 patients with AIT who showed recurrence of arrhythmia, CRP levels were higher (81.1±47.1 mg/dL) than in patients without atrial fibrillation (66.9±27.8 mg/dL), but their IL-6 levels were similar (4.4±2.4 pg/mL *vs.* 4.5±1.9 pg/mL). The 5 patients with common hyperthyroidism and atrial fibrillation showed higher CRP (174.5±110.2 mg/dL) and IL-6 (11.1±3.8 pg/mL) as compared to 11 patients with common hyperthyroidism without atrial fibrillation (38.3±17.8 mg/dL for CRP and 3.7±0.6 pg/mL for IL-6). ***Conclusion:*** Increased CRP levels reveal a proinflamatory status in all types of hyperthyroidism; amiodarone did not significantly modify this status; apart from increased thyroid hormones levels, increased CRP in hyperthyroidism could lead to atrial fibrillation.

Clinical thyroidology
Poster

TSH VALUES DISTRIBUTION IN A RANDOMISED POPULATION FROM A ITALIAN TOWN

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A randomised population of 631 subjects belonged to a town (Guardiagrele of 9970 inhabitants),located in Abruzzo, a Central Italian region was considered in the contest of FATA(Functional Autoimmune Thyroid in Abruzzo) project. The population was homogeneous for environmental factors, nutritional and iodine intake(mild deficient iodine area), of both genders , subdivided in the same percentage, ranged from 20 to 85 years.
A blood sample was drawn and an assay of TSH (commercial immunofluorescent assay) was done and an unvaried statistical analysis carried out.

Mean	2,2144	
Median	1,8350	
Std. Deviation	1,72928	
Variance	2,990	
Skewness	3,299	
Kurtosis	19,571	
Range	18,39	
Minimum	,01	
Maximum	18,40	
Interquartile Range	1,57	
Percentiles	5	,5388
	25	1,1800
	75	2,7500
	95	5,2120

The table shows the difference between TSH mean and median , referred to a wide dispersion of TSH values, that influences the mean calculation and to a particular curve shape. In fact TSH values distribution cannot fit to a normal distribution, as the figure represent. The real curve shows a maximal peak around values of 1.8 mUI/ml (like a median value) with a hump on the right, ranging from 2.5 and 5 mUI/ml (therefore our mean is 2.2). The difference between range and IQR demonstrates a great dispersion of observed values, according with the high SD. Even shape indexes are elevated: high and positive skewness shows the histogram has a long right tail, while the high kurtosis means a sharp graphic in correspondence of physiologic TSH values. This behaviour is in part explained by the presence of subjects with high anthytirod antibodies in the TSH range of 2.5 and 5 on right side of the curve.

Clinical thyroidology
Poster

THYROID PARAMETERS IN PREGNANT WOMEN WITH LOW IODINE UPTAKE

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The ongoing program of iodine enforcement by introduction of mandatory salt iodization in Romania in all districts improved the iodine uptake in schoolchildren group (in 2006, UIC - 127.5 µg/l) but not in pregnant women (in 2005, UIC - 55±48.78 µg/l). The present study looks for the consequences of this level of iodine deficiency in thyroid function in mother at term and in fetus. We involved in our study pregnant women at term from 5 districts (459 subjects). In all subjects we determined urinary iodine concentration(UIC) TSH and Thyroglobulin (TGL). In two distrticts (S,C) we determined TSH and TLG also in cordon blood (S - 25 subjects, C - 58 subjects). The measurement of UIC was done using the Sandell and Kolthoff method and for TSH and TGL the elecsys method was used. Results: in studied pregnant women at term median urinary iodine concentration was 82.87 µg/l, std. dev. 7.66 and percentile 10: 3.09, percentile 97: 30.75. The median TSH value was 1.80 µU/ml. 85 subjects had TSH value >2.5 µIU/ml and 16 subjects had TSH value > 4.2 µIU/ml. The median TLG value was 18.81 ng/ml and in range: 0.48–434.5 ng/ml. In mothers from C district, the median TSH value was 2.85 µIU/ml and std. dev. was 1.26; in cordon blood median TSH value was 12.48 µIU/ml and std. dev. was 7.23. The TLG values in cordon serum were median value 71.4 ng/ml and std. dev. 50.95. In mothers from S district, the median TSH value was 2.78 µIU/ml and std. dev. 4.29; in cordon blood median TSH value was 4.83 and std. dev. was 5.12. The TLG values in cordon serum were median value 57.25 ng/ml and std.dev. 60. ***Conclusions:*** we observed in studied groups normal TSH values in pregnant women at term at UIC 85.87 µg/l.

Clinical thyroidology
Poster

DETAILED AGE-DEPENDENT REFERENCE INTERVALS FOR TSH, THYROID HORMONES, AND THYROID ANTIBODIES ARE REQUIRED FOR ASSESSING THYROID FUNCTION IN PAEDIATRICS

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Background: The diagnostic specificity and sensitivity of the thyroid tests is affected by the validity of reference intervals. The purpose of our study was to establish reference intervals for thyroid tests in children and adolescents using a statistically sufficient number of subjects for each included age-dependent subgroup and to identify factors that may modulate the limits of these intervals. ***Methods:*** TSH, FT3, FT4, T3, T4, t-uptake, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb) and thyroglobulin (Tg) levels were determined in serum or EDTA plasma by the Elecsys system (Roche). Samples of 1,004 children and adolescents were measured within the study. The age ranged from the newborn period (cord blood) up to 20 years.Patients with known endocrine disorders or severe non-endocrine diseases that may have influenced the thyroid function were excluded. ***Results:*** A distinct overall age-dependent decrease of analyte levels was found for all parameters investigated. However, the course of both, TPO- and Tg-antibody levels demonstrated two peaks, one within the first year of life and one during puberty. Moreover, puberty leads to a timely increase of FT3 levels. Results of T4, TPO-Ab, Tg-Ab and t-uptake were significantly higher in girls (n=459) compared to boys (n=545). ***Conclusions:*** The strong dependence on age and sex for all thyroid parameters requires the use of validated reference intervals for subgroups as an obligatory tool for assessing thyroid function in children and adolescents. In summary, our results may contribute to an improved efficacy in the diagnostics of thyroid diseases in paediatrics.

Clinical thyroidology
Poster

ASSOCIATION OF THYROID VOLUME WITH NUTRITIONAL HABITS IN SOUTHWESTERN ALBANIA

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Introduction – Aim: Environmental factors are believed to be involved in the development of goiter, including dietary habits. A high number of goitrous patients was identified among patients attending the general medicine outpatients', in a mountainous region in Albania. The aim of our study was to identify possible associations of thyroid enlargement with nutritional factors. ***Methods:*** 112 consecutive patients (mean age 52.8±12.1, 104 female) attending the outpatients of the General Military Hospital of Gyrocaster, Southwestern Albania, who either reported thyroxine use (n=27) or were suspected to have thyroid disease based on symptoms and physical examination were studied. The type and frequency of food consumption was recorded; thyroid ultrasound was performed. ***Results:*** 51% of patients had thyroid volume above median (median volume: 20.35ml). Thyroid volume correlated negatively with the frequency of lamb and goat meat and vegetables consumption ($p=0.01$). Mean thyroid volume was significantly lower in those eating lamb or goat >1 times a week (21.4±13.3 vs 31.9±23, $p<0.01$). All consumed food was home produced. Multivariate analysis showed that the association of thyroid volume with lamb meat consumption was independent of sex, educational status or occupation ($p=0.004$). After those on thyroxine were excluded, 43.5% had TSH <0.3 microU/ml. In this group, log TSH correlated negatively with thyroid volume and fT4 levels ($p=0.008$), suggesting the presence of autonomy (TSHRab were found positive in only two subjects). ***Conclusions:*** Nutritional factors appear to be involved in the development of goiter in Southwestern Albania. The higher consumption of lamb and goat meat and vegetables, all non-industrialised, appears to be protective. One cannot exclude the possibility that this finding reflects better socioeconomic status, although this was not identified. There is a high degree of unrecognized subclinical hyperthyroidism probably duo to thyroid autonomy.

Clinical thyroidology
Poster

CLINICAL AND ULTRASONOGRAPHIC DILEMMA: ARE WHAT WE PALPATE ON THYROID EXAMINATION AND WHAT WE SEE ON ULTRASONOGRAPHY THE SAME?

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The purpose of this study was to compare findings of thyroid examination with findings of ultrasonography in patient attended to thyroid outpatient clinic and to determine the limits of examination and ultrasonography in general practice. We evaluated 6,835 patients who attended to our center between January 2006–December 2006, whose thyroid ultrasonographies were requested after their first examination and thyroid function tests. Thyroid palpation and ultrasound examination were performed to 1,157 patients who were included in study. The size of thyroid gland was determined by inspection and palpation using the WHO criteria 1960 by two clinicians, and patients were examined ultrasonographically by two experienced ultrasonographers. Our results suggest that (1) palpation and ultrasonography findings are 86–92.5 % correlated in the assessment of thyroid size in areas with endemic iodine deficiency, (2) if no nodule was detected in clinical examination of thyroid gland; this result is 76.7–77.4 % correct, and sensitivity of palpation in detecting the absence of nodule is 87.3–89 %, (3) compared to ultrasonography, sensitivity of palpation in detecting thyroid nodules is 56.1–56.7 %, (4) if a single nodule is detected by palpation, there are 44.3–50.4 % accompanying one or more nodules, (5) when one or more nodules are detected by palpation, even though there is no nodule on ultrasonography, 87.4–91.2 % of these cases have thyroiditis, and (6) consistency of palpation and ultrasonography in detecting thyroid nodules is 30–38 %. In conclusion, all thyroid examinations should certainly be completed by thyroid ultrasonography.

Clinical thyroidology
Poster

ETHANOL INSTILLATION OF ADENOMA OF THE THYROID GLAND – A FIFTEEN-YEAR EXPERIENCE AND FOLLOW-UP

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In a prospective study, 268 patients were treated with percutaneous ethanol injection (PEIT). The study group consisted of 177 women and 91 men. 120 patients had a heterogeneous goiter with a struma multinodosa. 155 patients were suffering from toxic adenomas and 119 from pretoxic adenomas. 165 had a solitary adenoma and 80 had ≥ 2 autonomous areas. Multi-focal autonomy was present in 23 patients. The nodule volume was 0.5–91 ml with an average of 10 ml. The number of sessions was 1–8 for each patient, with an average of 4, using an average injection of 0.6 ml 96% ethanol per ml of nodule volume at each session. In 50 % of the patients with toxic adenomas, complete success was achieved, with elimination of the autonomous area and an increase in the thyroid stimulating hormone (TSH) basal levels. At least partial success was achieved in 86 % and euthyroidisme was produced without further thyrostatic medication. Complete success was achieved in 72 % of the cases of pretoxic adenoma. The final success of the therapy was dependent on the initial size and number of the adenomas. An average reduction of 48 % in adenoma size was achieved at the end of the therapy. ***Conclusion:*** Sonographically-controlled PEI represents an effective, low-risk, low-cost, out-patient process for the treatment of focal autonomy.

Clinical thyroidology
Poster

EFFICACY AND SAFETY OF RADIOFREQUENCY THERMAL ABLATION IN THE TREATMENT OF THYROID NODULES WITH PRESSURE SYMPTOMS IN ELDERLY PATIENTS

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Purpose: Surgery and radioiodine therapy are the main therapeutic approach in patients suffering from thyroid nodules (TNs) with pressure symptoms. Surgical and post-surgical complications and financial costs of thyroid surgery and radioiodine therapy, however, are increased in elderly patients. Alternatively, radiofrequency thermal ablation (RTA) is potentially a new, relatively simple and cost-effective instrument to manage compressive TNs. The objective of this study is to investigate efficacy and safety of RTA in the treatment of compressive TNs in elderly patients followed-up for 12 months. ***Methods:*** Thirty-nine elderly patients (aged >65 years) with cytologically benign compressive TNs were prospectively enrolled in the study; 27 of them had non-toxic goiter, 12 had toxic or pre-toxic goiter. Thyroid surgery was contraindicated in 22 patients and refused by 17. RTA was performed by using a RITA © Starburst needle inserted under ultrasonographic real time guide. ***Results:*** During the 12 month follow-up, all TNs showed a significant decrease after RTA. Mean TN volume decreased from 24.3±2.6 to 6.4±1.6 ml ($p<0.001$) with a mean percent decrease of 78.6±2.5% 12 months after RTA. Compressive symptoms improved in all cases and disappeared in 82%. The treatment was well tolerated by all patients. ***Conclusions:*** RTA was a safe, effective and simple procedure for the treatment of symptomatic TNs in elderly patients. The results of this pilot study show that RTA is a valid tool to manage compressive TNs in those subjects for whom conventional treatments are contraindicated or refused.

Clinical thyroidology
Poster

THYROID FINE NEEDLE ASPIRATION BIOPSY: IS LOCAL ANESTHESIA REQUIRED?

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Purpose: Fine needle aspiration biopsy of the thyroid nodules (TFNAB) is the gold standard in the differential diagnosis of the thyroid nodules. In general, no analgesia is needed before this procedure. However, it is generally believed that the patients may be more comfortable if it is performed under local anesthetics. In this study we examined the impact of the use of local anesthetics on patient's levels of discomfort during TFNAB. ***Methods:*** Fifty patients with nodular goiter were enrolled in this study. Patients were randomized into two groups: Group 1 patients (24 females and 1 males, mean age 43.57±12.19 years) were not given, and Group 2 patients (19 females and 6 males, mean age 50±12.01 years) were given local anesthesia (EMLA® 5% cream, 1 g containing 25 mg Lidocaine and 25 mg Prilocaine) approximately 1 hour before TFNAB. Each nodule received punction by 3 times at minimum or 5 times at maximum. All patients were asked to mark the pain they felt during the TFNAB on the visual analog scale. ***Results:*** The pain scores during TFNAB were 31.92±26.07 mm and 21.84±22.15 mm in Group 1 (non-local anesthesia-receiving group) and in Group 2 (local anesthesia-receiving group), respectively. There was no significant difference between the groups ($p>0.05$). ***Conclusion:*** Local anesthesia before TFNAB provides no benefit. Moreover in clinical practice it increases the overall workload and prolonges the time of the procedure.

Clinical thyroidology
Poster

THYROID FUNCTION IN FABRY DISEASE BEFORE AND AFTER ENZYME REPLACEMENT THERAPY

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Purpose: In a previous study we highlighted that patients with Fabry disease (FD), a genetic disorder caused by lysosomal α-galactosidase-A enzyme deficiency and characterized by a systemic accumulation of globotriaosylceramide, present high prevalence of subclinical hypothyroidism***.*** Thyroid dysfunction was independent by age, gender, α-galactosidase-A activity, heart and renal failure. The pathogenic mechanism was not related to anti-thyroid autoimmunity and may be dependent by intra-thyroid lipid accumulation, as previously demonstrated. In this study, it was investigated whether thyroid function recovers in FD after long-term enzyme replacement therapy (ERT). ***Methods:*** Study population included 14 FD patients (7 females, 7 males, aged 23–65 yrs). All subjects were evaluated before and three years after the beginning of ERT with rh-α-galactosidase-A at a dose of 1 mg/kg/BW every 2 weeks. ***Results:*** TSH levels were higher before than after ERT (1.8±0.2 vs 1.3±0.2 mU/l, $p<0.01$) while fT3 and fT4 levels were normal at baseline and unchanged after ERT. Before ERT, TSH levels were >3 mU/l in 2 patients who had a hypoechoic ultasonographic pattern but negative serum antithyroid antibodies. As a whole, anti-Tg and/or anti-TPO titres were positive in 2 patients. At the thyroid ultrasonography, a nodular goiter was found in 14% while a mild hypoechoic pattern was found in 71% of cases. After ERT, no FD patient showed TSH levels above 3 mU/l. As compared to baseline, the rate of autoimmunity was unchanged and the rate of hypoechoic ultrasonographic thyroid pattern was mildly decreased (43%). ***Conclusions:*** In patients with FD, the tendency to develop primary hypothyroidism seems to be reverted by long-term ERT. It remains to be evaluated if the recovery of thyroid function during ERT is followed by an improvement of life quality and fertility also affecting FD patients. A screening of thyroid function is mandatory in all FD patients.

Clinical thyroidology
Poster

ASSESSMENT OF DISEASE ACTIVITY IN GRAVES' OPHTHALMOPATHY BY ORBITAL ULTRASONOGRAPHY, TSH-RECEPTOR STIMULATING IMMUNOGLOBULINS AND CLINICAL PARAMETERS

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Purpose: To study the correlation of orbital sonography findings with TSH-receptor stimulating immunoglobulins (TSI) and Clinical Activity Score (CAS), in the management of patients with Graves' Ophthalmopathy (GO). ***Materials and Methods:*** 28 patients (19 women and 9 men) with GO, aged 30–65 years (mean 48.8 years) and 20 healthy controls of similar age underwent an orbital ultrasonography (ATL Philips 5000). All patients were euthyroid for at least 2 months and none had diabetes mellitus or hypertension. According to CAS, 16 patients (57.1%) had active Graves' Ophthalmopathy (AGO) and 12 patients (42.8%) inactive Graves' Ophthalmopathy (IGO). Three of the patients had undergone orbital surgery and one received external radiotherapy. Thyroid peroxidase antibodies (TPO) were measured with chemiluminesence and TSI with RIA. In all cases, extraocular muscle and optic nerve-sheath complex diameter, intramuscular reflectivity and echogenity, as well as the presence of retrobulbar fluid collection were assessed. By using Color Doppler Ultrasound, peak systolic velocity (PSV), end diastolic velocity (EDV) and the resistive index (RI) were measured in the ophthalmic (OA) and central retinal artery (CRA). ***Results:*** Extraocular muscle enlargement was noticed in all patients with AGO and in 5 patients with IGO. Diffusely increased intramuscular reflectivity was observed in 10 (62.5%) and retrobulbar fluid collection was noticed in 11 (68.7%) patients with AGO. Increased PSV and EDV in the OA and CRA were observed in 14 patients with AGO (87.5%) and none of the patients with IGO. Disease activity was significantly positively correlated with PSV and EDV ($p<0.001$), extraocular muscle enlargement ($p<0.001$), fluid collection ($p<0.001$), intramuscular reflectivity ($p<0.04$) and TSI ($p<0.001$) and negatively correlated with the disease duration ($p<0.04$). ***Conclusion:*** Orbital ultrasonography in combination with CAS and TSI is an easy, safe and effective tool for distinguishing patients with inflammatory AGO who may benefit by immunosuppression.

Clinical thyroidology
Poster

THE UTILITY OF ULTRASOUND-GUIDED FINE-NEEDLE ASPIRATION IN THYROID NODULES MANAGEMENT

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Background and Aims: It is reported that the diagnostic accuracy of fine needle aspiration (FNA) of thyroid nodules is increased with ultrasound guidance (UG-FNA), especially in the case of non-palpable nodules. However, the use of UG-FNA in everyday practice is still controversial because of absence of large studies as well as unawareness of the natural history of well-differentiated microcarcinomas of thyroid gland. The purpose of this study was to evaluate the role of UG-FNA in the management of thyroid nodules. ***Methods:*** We retrospectively studied 960 patients (690 women and 270 men) aged 25–70 years (mean age 50 years) in whom a UG-FNA was performed. The nodules size was between 0.5–5.4 cm (mean 2.9cm). 228 of the above patients underwent thyroidectomy and a comparison between histological and cytological results was made. **Results:** The cytological analysis revealed non-malignant lesions in 720/960 (75%), primary thyroid carcinoma in 31/960 (3.2%) (22/31 papillary, 5/31 follicular, 3/31 medullary and 1/31 anaplastic), metastatic carcinoma in 6/960 (0.7%), suspicious lesions in 75/960 (7.8%) and non-diagnostic in 128/960 (13.3%) of the cases. In the 228 cases that underwent thyroidectomy a comparison of cytological and histological results was made. UG-FNA findings were true positive in 34, false positive in 3, true negative in 187 and false negative in 4 patients. From the 75/960 cases with suspicious results only two were papillary carcinomas. The calculated sensitivity and specificity of UG-FNA was 89.5% and 98.4% respectively. **Conclusion:** UG-FNA is a fast, economic and effective interventional method for the evaluation and management of thyroid nodules in every day practice.

Clinical thyroidology
Poster

THE THYROID GLAND VOLUME, IN YOUNG CHILDREN AND ADOLESCENTS LIVING IN SEVERE IODINE DEFICIENCY OR IODINE REPLETE AREAS, CORRELATES STRONGLY WITH FREE FAT BODY MASS

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In adults living in iodine replete areas the thyroid gland volume depends on somatometric parameters such as the Body Surface Area (BSA), the Free Fat Body Mass (FFBM) and body weight. Aim: To examine whether the above correlations apply to pediatric or pubertal populations living in severe iodine deficiency or iodine replete areas. Materials and methods: 575 children aged 6–17 years old from 11 different regions of Uzbekistan were studied. 161 boys and 216 girls underwent body weight and height measurements, FFBM and fat mass (FM) estimation, thyroid volume measurement by ultrasound, and bone age estimation by ultrasound. Urine Iodine excretion (UIE) was measured in a morning sample. Two subgroups were identified: Group A (n=62, 22 boys – 40 girls) with severe iodine deficiency (UIE<25 μg/L) and Group B (n=136, 54 boys – 82 girls) with normal UIE (>100 μg/L).
Results:
1. Uzbekistan is characterized by mild iodine deficiency and endemic goiter:

	Median UIE (mcg/L) (n=575)	Prevalence of goiter (%) (n=369)
Total	76	19
Boys	61	23
Girls	80	16

2. In group A, the thyroid volume, irrespective of gender, was significantly correlated with BSA (r=0.696), FFBM (r=0.696), age (0.648), BMI (r=0.603) and FM (r=0.6). In girls, the correlation with FFBM was stronger (r=0.71) and in a multiple regression analysis including FFBM, FM, and BMI, the FFBM was proved to be the most important parameter (r^2=0.476). 3. In group B, the thyroid volume irrespective of gender, was significantly correlated with BSA (r=0.716), age (r=0.658), FFBM (r=0.652), BMI (r=0.527) and FM (r=0.52). In a multiple regression analysis including FFBM, FM, and BMI, the FFBM was proved the most important parameter (r^2=0.426). 4. No difference was found between chronological and bone age. Conclusions: In children and adolescents, both living in severe iodine deficiency or iodine replete areas, the FFBM is of the most important parameter determining the thyroid volume. Uzbekistan is characterized by mild iodine deficiency and endemic goiter.

Clinical thyroidology
Poster

INCREASED RISK OF CARDIOVASCULAR EVENTS IN SUBCLINICAL HYPERTHYROIDISM

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Purpose: Untreated overt hyperthyroidism is known to predispose the patient to cardiovascular diseases. The aim of the study was to assess the risk of cardiovascular events in subclinical hyperthyroidism, which has been debated. ***Method:*** A population-based prospective study of 609 participants aged 51 to 91 years from a community in Copenhagen, Denmark, provided blood and urinary samples and was examined between September 1998 and January 2000. All the participants had normal left ventricular ejection fraction (LVEF > 50%) estimated by echocardiography, and they were without heart or renal failure. The follow-up period was up to 5 years (to December, 2003), and the local ethical committee approved the study. ***Results:*** 549 (90.1%) was euthyroid (TSH 0.4–4.0 mU/L), 34 (5.6%) had TSH > 4.0 mU/L and 26 (4.3%) had TSH < 0.4 mU/L. Three participants were overt hypothyroid and 1 overt hyperthyroid. After excluding the group with TSH > 4.0 mU/L, 86 participants died and 59 had first major cardiovascular event during follow-up. In the subclinical hyperthyroid group, the mean value of TSH was 0.2 mU/L (range < 0.0–0.4 mU/L). The incidence of major cardiovascular events incl. cardiovascular death were increased among the subclinical hyperthyroid HR 2.28 (95 % CI 1.05–4.96, p = 0.037), and TSH < 0.4 mU/L was independently associated with the risk of stroke HR 3.28 (95 % CI 1.11–9.64, p = 0.031), even after adjusting for sex, age and atrial fibrillation. ***Conclusion:*** Subclinical hyperthyroidism is a risk factor for developing major cardiovascular events including cardiovascular death, especially stroke, in a group of 575 nonhospitalized individuals with TSH 4.0 mU/L, aged 51 to 91 years. On that perspective, the condition subclinical hyperthyroidism properly might be regarded as a disease to be treated instead of a condition to be observed.

Clinical thyroidology
Poster

SERIOUS SIDE-EFFECTS OF INTRAVENOUS GLUCOCORTICOID TREATMENT IN SEVERE ENDOCRINE OPHTHALMOPATHY

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Endocrine ophthalmopathy (EO) is an autoimmune disorder, the treatment of which is a challenge to clinical thyroidologists. No specific immunosuppressive therapy exists, and at the moment the best response is achieved with glucocorticoid-based immunosuppressive treatments. It has been documented that intravenous (iv) administration of high pulse steroids are more effective and better tolerated than oral steroids. However, using very high doses of iv methylprednisolone (cumulated dose above 10 g) has been reported to include a risk of damage on liver cells, and four fatal cases of liver failure associated with iv steroid in patients with EO have been seen. We report two cases with cardiovascular events and one case with a cerebrovascular event in relation to iv methylprednisolone. The routine high pulse steroid therapy was 1 g daily in 5 ongoing days. A 71 years old woman with atrial fibrillation received an uncomplicated course of 5 g pulse steroid in June 03, but died in Dec 03 at day 3 of the second pulse therapy after 3 g of steroid. The cause of death was a coronary and a pulmonary thrombosis. A 76 year old woman without known heart disease received in Sept 03 5 g of steroids and in Aug 04 the second pulse therapy 2.5 g of steroids before she developed a coronary thrombosis. A 24 year old woman received a 5 g pulse therapy and developed 2 days after completion a fatal cerebral thrombosis. The reported serious side-effects were seen after cumulative doses af iv methylprednisolone of between 5 and 8 g. ***Discussion:*** Should the routine treatment be modified to lower doses and with intervals? What is the optimal iv steroid pulse therapy in terms of both efficacy and safety?

Clinical thyroidology
Poster

AN UNUSUAL ASSOCIATION OF GRAVES' DISEASE WITH HYPOPITUITARISM

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It is well know that autoimmune thyroid diseases may be associated with several other diseases of this type involving other endocrine glands and other organs. The more frequent combination implicates Hashimoto\'s thyroiditis, whereas Graves\' disease is less involved. We have recently observed 2 cases of Graves\' disease in which, once the hyperthyroidism was treated and the patients were euthyroid, symptoms of partial or total hypopytuitarism were detected and confirmed by biochemical investigation. The first case was a 52-year old man who had received 2 treatments of ^{131}I. 2 months after the last treatment he developed hypothyroidism. In spite of adequate thyroid replacement he complained of extreme fatigue, loss of weight and hairs and a decrease of the libido. During all this period the TSH remained always <0.001mUi/L. The investigation revealed a low IFG-1, FSH, LH, testosterone, cortisol, but normal prolactin. The MRI showed a small increase in the size of the pituitary without any clear evidence of an adenoma. The second patient was a 49-year old woman who had received a single treatment of ^{131}I and also developed a hypothyroidism (TSH 7.6 mUi/L) 2 months after. She also, in spite of adequate treatment (TSH 1.94 mUi/L), complained of fatigue and continued to lose weight during the following 6 months. Investigation of the pituitary function revealed a deficit of the pituitary-adrenal axis with a plasma cortisol of 44 nmol/L and an ACTH of <1.1pmol/L. HGH and prolactin were also low, whereas FSH and LH were high since the patient had an artificial menopause. MRI did not show any anomaly of the pituitary. In these patients the symptoms of hypopituitarism were masked by the presence of Graves\' disease. While we do not have yet definite prove, we suspect that a lympocitic hypophysitis could be the cause of the hypopituitarism.

Clinical thyroidology
Poster

EVALUATION OF NEONATAL THYROID FUNCTION AND ANTHROPOMORPHIC PARAMETERS IN NEWBORNS FROM MOTHERS AFFECTED BY THYROID DISEASES

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We performed a prospective study of thyroid function and anthropometric parameters in newborns from women affected by thyroid diseases. Aim of the study was to verify if maternal thyroid diseases and their pharmacological treatment affect neonatal thyroid function and anthropometric parameters. 90 newborns from mothers affected by thyroid diseases (75 with chronic autoimmune thyroiditis, 59 of whom under LT4 replacement therapy, and 15 with nodular goiter, 11 of whom under suppressive LT4 treatment) were enrolled in the study. We evaluated gestational age, mode of delivery, Apgar index (at the first and fifth minute), neonatal weight, length, cranial and thoracic circumferences at the time of birth. Serum FT4, FT3, TSH values and AbTg, AbTPO, TRAb titles at the third day of life were also measured. Data were analyzed by Chi-square test, Student t test and simple linear regression. None of the newborns of this study was affected by congenital hypothyroidism.We found a significant correlation among gestational age and weight, length, cranial and thoracic circumferences of the newborns. Furthermore, a significant direct correlation between serum FT4 and FT3 in comparison with gestational age (p value = 0.0126 and 0.0054 respectively) was found. When newborns from women with autoimmune thyroid diseases (n=75) were compared with newborns from women affected by nodular goiter (n=15), no significant differences in neonatal thyroid function nor in anthropometric parameters were found. Finally, all the anthropometric parameters and neonatal thyroid function did not show any difference between newborns from mothers affected by ATD and treated with LT4 (n = 59) and newborns from non-treated mothers with ATD (n = 16).In conclusion, serum FT4 and FT3 levels at the third day of life are directly correlated with gestational age. LT4 replacement treatment doesn’t interfere with neonatal thyroid function and anthropometric parameters.

Clinical thyroidology
Poster

CORONARY FLOW RESERVE (CFR) IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM

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Background: Treatment of mild subclinical hypothyroidism (Shypo) is debated. It has been demonstrated that patients with Shypo, even at the very early stage of disease, have diastolic dysfunction, endothelial dysfunction and increased central arterial stiffness (CAS), which are important risk factors for cardiovascular disease. However, conflicting results are reported on cardiovascular mortality and the risk of atherosclerosis and coronary disease in patients with Shypo. ***Purpose:*** Our aim was to evaluate coronary microvascular circulation and endothelial function of epicardial coronary arteries by the measurement of coronary flow reserve (CFR) at rest and in response to cold pressure test, (CPT), a non-invasive sympathetic stimulus that allows the assessment of the functional integrity of the coronary vascular endothelium in subjects with mild Shypo. ***Methods:*** We studied 10 patients (mean age 44.4±14.7) with persistent mild Shypo (TSH 5.2±2.18μU/ml, with normal levels of FT3 and FT4) due to Hashimoto thyroiditis. Ten euthyroid control subjects, matched for age, sex and body mass index (BMI) were selected as a control group. Individuals with cardiovascular disease, diabetes mellitus, chronic renal disease, hypertension and cancer were excluded from the study. In all patients coronary diastolic peak flow velocities were measured at baseline and after CPT. ***Results:*** No significant differences were observed among two groups in baseline CFR. However, CFR after CPT in patients with mild Shypo was impaired compared to control group. We observed a direct correlation between CFR and TSH parameters. ***Conclusion:*** In our study, we found an impairment of CFR after CPT in patients with Shypo in comparison to control group. Endothelial dysfunction could be responsible for this result.

Clinical thyroidology
Poster

PREPARATION TO RADIOIODINE TREATMENT WITH RECOMBINANT THYROTROPIN PREVENTS FAST GROWTH OF UNDISCLOSED REGIONAL THYROID CANCER METASTASES

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Background: Papillary thyroid carcinoma (PTC) in patient with history of Chernobyl irradiation (especially in children) demonstrates high risk of regional metastases. Even after radical thyroidectomy and neck dissection new clinically apparent metastases can appear during period of hypothyroidism along preparation to radioiodine treatment (RIT). ***Purpose:*** We studied possibility to diminish risk of fast growth of unrecognized small neck metastases after operation for PTC using preparation to RIT with recombinant human thyrotropin (rh-TSH). ***Methods:*** Among more than 1500 patients with PTC operated during last 10 years we observed 197 cases of T2-4N1bM0 (1-st group) which have been underwent RIT using conventional thyroxine withdrawal regimen of preparation during 6 weeks after radical surgery that included total thyroidectomy, central and modified radical neck dissection. During 2003–2006 years 12 patients with irradiation history, same extension of PTC and operation have been prepared to RIT with two consequent doses of rh-TSH (Thyrogen, Genzyme), followed by 100–150 mCi 131-iodine (2nd group). ***Results:*** In 29 patients (14.7%) of the 1st group (23 of them were children or adolescents in 1986) clinically apparent lymphatic node metastases were diagnosed by palpation or ultrasound just before taking isotope or after scanning. All of them were reoperated. None of metastases were recognized by before or during initial surgery. So we supposed that such concealed micrometastases grew up quickly under long stimulation by elevated TSH. In contrast, none of 12 patients of 2nd group have demonstrated new clinically significant metastases within 6–18 months after operation. One additional operation has been performed to complete clearance of the neck from new iodine-negative metastases in 1 patient 18 months after initial surgery and RIT. Our data suggest major role of rh-TSH to diminish risk of fast growth of micrometastases of advanced PTC during preparation to RIT.

Clinical thyroidology
Poster

THE AUTOIMMUNE HYPERTHYROIDISM OF GRAVES' DISEASE (GD) IS STRICTLY CORRELATED TO THE HELICOBACTER PYLORI (HP) COLONIZATION OF THE STOMACH

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Usually the GD, an autoimmune pathology of the thyroid, is often responsible of the appearance of the hyperthyroidism in the general population. On the contrary, the colonization/infection from HP has a worldwide diffusion and CagA positive are considered such as the more aggressive bacteria. Previous studies have evidenced as the autoimmune pathology of the thyroid, such as the Hashimoto's Thyroiditis, was associated to an increased positivity, tested with several methodical, of HP positivity. ***Materials and Methods:*** We investigated 35 hyperthyroid patients with GD and 35 subjects such as control group (medium age 38 ± 2years). Criteria of inclusion in the protocol of the study: a negative anamnesis or the lack of symptoms of other diseases or drug treatment. The HP presence was investigated on a fresh stool sample using an immunoassay amplification technology test (IDEIA Hp StAR, DakoCytomation). The CagA antibodies were investigated with a RADIM test. ***Results:*** The positivity of HP was 72% in the GD group versus 25% in the control group. These results were statistical significative (X2 test, $p< 0.01$). The age, smoke habitude or the presence of ophtalmopathy did not constitute a specific risk factor. The CagA-positivity was 89% in the GD group versus 53% in the control. ***Conclusion:*** The autoimmune hyperthyroidism of the GD seems to be an important factor of risk for the CagA-positive HP colonization of the gastric mucosa. The lack of a specific symptomatology, such as pyrosis, did not suggest a real complicated infection. Further studies will be useful in order to understand if the HP could be involved in the pathogenesis autoimmune of the GD.