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## Abstracts

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Guest Editors
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Laszlo Hegedüs: Head of the Scientific Board and a consultant for International IBSA. Chair of the Novo Nordisk committee for Non-diabetic Endocrinology.
Leonidas Duntas: Recipient of educational Grants from Merck, Berlin Chemie, and IBSA.

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Oral Presentations

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# Saturday, 4th September 2021

## Topic Highlights

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### ILLUMINATING THYROID DEVELOPMENT AND GROWTH USING THE ZEBRAFISH MODEL SYSTEM

*Meghna Shankar*[1], *Macarena Pozo Morales*[1], *Sema Elif Eski*[1], *Ines Garteizgogeascoa*[1], *Camille Perazzolo*[1], *Pierre Gillotay*[2], *Sabine Costagliola*[3], *Sumeet Singh*[1]

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In the simplest form, the vertebrate thyroid gland is an iodine accumulating organ. And it does so by utilizing a structure that is conserved among vertebrates: a follicle composed of epithelial tissue with a central lumen for storage of iodinated molecules. To elucidate the fundamental processes regulating thyroid physiology, we harness a genetically tractable vertebrate model system, the zebrafish. Zebrafish provide the advantage of transparency during early development, allowing direct visualization of thyroid morphogenesis and growth. Moreover, 84 per cent of genes known to be associated with human disease have a zebrafish counterpart, allowing modeling of human disorders.

Here, using *in vivo* live imaging, we demonstrate the zebrafish thyroid follicular cells (TFCs) are ciliated, polarized and surrounded by a network of blood and lymphatic vessels. To understand the differentiation of thyroid from progenitors, we performed single-cell RNA-Seq (scRNA-Seq) of foregut endoderm at a stage when thyroid progenitors are specified, but not yet differentiated. This allowed us to compare the molecular characteristics of thyroid progenitors with the neighboring endoderm, providing clues into regulators for thyroid specification. Using loss of function analysis, we uncover gene regulatory network underlying thyroid morphogenesis, defects which lead to congenital hypothyroidism.

Moreover, using newly generated transgenic reporters for cell-cycle, we capture replicating TFCs in their native environment. Using scRNA-Seq, we will develop an atlas of the cell-cycle in thyroid follicular cells, providing genes involved in goiter and cancer.

Finally, to understand maintenance of TFCs during homeostasis, we performed scRNA-Seq of the region in adult zebrafish encompassing the thyroid follicles. With this, we identify genes expressed in mature TFCs and the surrounding cells. Using the prolife of TFCs at cellular resolution, we identify transcriptional heterogeneity within the population, validated by knock-in reporter line; suggesting that thyrocytes exist in multiple states. Currently, we are investigating the epigenetic mechanism underlying dynamic gene expression within the mature thyrocyte population, and how they are modulated during oxidative stress and stress.

Our scRNA-Seq for thyroid progenitors and adult TFCs points to multiple genes with a yet unappreciated role in thyroid physiology. We hope that our efforts using the zebrafish to illuminate the process and underlying genetic network of thyroid development and maintenance might help identify candidate genes with a role in diseases related to the thyroid.

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### STATINS FOR GRAVES' ORBITOPATHY (STAGO): PRELIMINARY RESULTS OF A PHASE II RANDOMIZED CLINICAL TRIAL

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**Objectives:** Over the past few years a role of cholesterol and statins has emerged in Graves' Orbitopathy (GO): a protective action of statins on the development of GO was observed in two retrospective studies; an association between high cholesterol and GO was reported in a cross-sectional investigation; a worse response of GO to immunosuppressive treatment was described in patients with high cholesterol levels. Based on these observations, we conducted the present clinical trial, designed to investigate whether administration of atorvastatin improves the outcome of GO to intravenous glucocorticoids in hypercholesterolemic patients with moderate-to-severe, active GO.

**Methods:** Eighty-eight patients with moderate-to-severe, active GO and high LDL-cholesterol were randomized in a 1:1 ratio into treatment with intravenous methylprednisolone (500 mg/week for 6 weeks, 250 mg/week for 6 weeks) plus atorvastatin (20 mg/daily for 12 weeks) (statin group) or methylprednisolone alone (non-statin group). Patients were evaluated at baseline and then at 12 and 24 weeks. The primary endpoint was the overall outcome of GO at 24 weeks, assessed using a composite evaluation. The secondary endpoints were: 1) outcome of GO at 12 weeks; 2) improvement in quality of life, using a specific questionnaire (GO-QoL) at 12 and 24 weeks; 3) GO relapse at 24 weeks. Here we present the preliminary results obtained in 80/88 patients.

**Results:** At week 24, the proportion of responders in the intention-to-treat population was significantly higher in the statin group [48.7% vs. 23% in the non-statin group; P=0.01). GO relapse at 24 weeks was more frequent in non-statin group [15.3% vs 0% in the statin group; P=0.01]. A significant greater improvement in quality of life across the follow-up period was observed in the statin group compared with the non-statin group (P=0.03). No significant differences were observed with the remaining secondary outcome measure (overall GO outcome at 12 weeks). No significant difference in LDL-cholesterol levels was observed across the follow-up period between GO responders and non-responders, suggesting that the effect of atorvastatin is likely due to its anti-inflammatory actions. No severe adverse events were observed.

**Conclusions:** Atorvastatin seems to improve the outcome of moderate-to-severe, active GO in hypercholesterolemic patients. A phase III, possibly multicentric, clinical trial is needed to introduce statins in clinical practice of GO. The effect of atorvastatin seems to be independent from lowering of cholesterol, suggesting that it could possibly be employed also in patients with normal cholesterol.

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## CHARACTERIZATION OF THE CELLULAR HETEROGENEITY OF HUMAN THYROID TISSUE BY SINGLE CELL TRANSCRIPTOMICS

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Human thyroid tissue is characterized by a marked heterogeneity. This is evident at different scales ranging from intra- and inter-follicular differences in thyroid marker expression (e.g., NIS, TPO, DUOX2) and functional activity (e.g., iodide uptake and organification), respectively, to regional differences in tissue composition (e.g., nodular tissue, fibrosis, lymphoid infiltration). A major question is how tissue heterogeneity is linked to pathogenesis and progression of thyroid diseases. Afforded by recent advances of single cell omics technologies, it meanwhile became possible to comprehensively characterize the cell composition of thyroid tissue, perform connectomic analyses to deduce potential paracrine and autocrine signaling networks and delineate trajectories of cell state changes.

In this study, we began to address these questions by performing single-cell RNA-sequencing (scRNA-seq) of human adult thyroid tissue samples. For single cell profiling, we obtained fresh tissue samples from surgical material and used a droplet-based technology to obtain single cell expression data for about 8,000 cells per sample.

K-means clustering revealed six major cell types and cluster annotation based on cell type-specific markers identified thyroid follicular cells (TFC), endothelial cells (EC), smooth muscle cells, fibroblasts, macrophages and immune cells. Unbiased clustering then identified 13 transcriptionally distinct cell clusters and revealed a marked heterogeneity in the composition of the thyroid parenchyma (4 TFC subtypes) and the thyroid vascular bed (4 subtypes of arterial capillary EC). Expression patterns of marker genes identified all 4 TFC subtypes as *bona fide* differentiated TFC given the co-expression of key thyroid transcription factors (*NKX2-1, PAX8, FOXE1*) and differentiation markers (*TSHR, TPO* and *CTSB*). However, TFC subtypes differed with respect to expression of genes involved in iodide handling, hydrogen peroxide production, intracellular signaling pathways, cell cycling and oxidative stress responses. The concept of angiofollicular units states that local reciprocal TFC-EC interactions integrate iodide availability, neuroendocrine stimulation and oxidative stress signals into adaptive mechanisms to ensure normal thyroid function and maintain thyroid tissue homeostasis. Interrogation of single cell data for cellular communication networks between TFC and EC confirmed a prominent role of VEGF signaling but also identified novel TFC subtype-specific signaling interactions with EC and other cell types.

Results of this study provoke a number of new testable hypotheses and identified prospects and caveats of the current analysis pipeline paving the way for generation of a reference cell atlas of healthy and diseased human thyroid tissue based on single nucleus and spatial gene expression analyses.

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## THE PRESENCE OF A WHOLE TUMOR CAPSULE IDENTIFIES A SUBGROUP OF INDOLENT CASES NOT ONLY AMONG THE FOLLICULAR VARIANT BUT ALSO IN CLASSICAL VARIANT OF PAPILLARY THYROID CANCER

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**Background:** In 2016, after the demonstration of an indolent course, the follicular variant of papillary thyroid cancer with a whole capsule was renamed as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and not more considered a malignant entity. Recently, we demonstrated that the presence of a whole tumor capsule was an independent prognostic factor of clinical remission both in follicular (FVPTC) and classical variant (CVPTC). However, these are retrospective data with patients treated by surgery and 131I-ablation therapy, a therapeutic strategy that nowadays is limited only to intermediate and high risk PTC. The aim of this study was to demonstrate, in a prospective study, that the presence of a whole tumor capsule in CVPTC could have a good prognostic role as in NIFTPs

**Methods:** we have prospectively collected data of patients with a histology of CVPTC and a whole tumor capsule (En-CVPTC). In parallel, data of NIFTP cases were also collected. In both cases the tumor was analyzed according to the criteria used for the NIFTP. All patients performed a clinical and biochemical evaluation within 6 months from surgery and then every 6-12 months. The DNA and RNA of a sub-group of cases were sequenced on the ION S5 platform.

**Results:** From January 2018 to December 2019, 100 En-CVPTC and 148 NIFTP were collected. A similar age and gender distribution were found between the two groups. A significantly higher rate of NIFTP patients was submitted to lobectomy (p=0.0003). No differences in pathological features were found between NIFTP and En-CVPTC except for the tumor size, significantly larger in NIFTP [22±16mm *vs* 8±11mm, p<0.001]. After a median follow-up of 15 months all NIFTP and En-CVPTC patients had an excellent response. We identified 27 case-control pairs matched for age, gender and tumor size: 12/27(44.4%) NIFTP and 9/27 (33.3%)En-CVPTC harbored one or more genetic alteration. Nine/27(33.3%) and one/27(3.7%) NIFTP harbored RAS and rare BRAF mutation, respectively. Two/27(7.4%) and 2/27(7.4%) EnCVPTC harbored RAS and rare-BRAF mutation, respectively. No gene fusions targeted in our panel were detected in both groups.

**Conclusions:** with this study we demonstrated that, in a short term follow-up, En-CVPTCs, prospectively collected and treated conservatively, showed a favorable outcome similar to that of NIFTPs. The molecular landscape showed a similar low-risk molecular profile in both NIFTP and EnCVPTC. If a longer follow-up of these cases will confirm these findings, we could consider En-CVPTC as a "pre-malignant lesion" as like as NIFTP.


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## Oral Session 1

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### ADIPOSITY AND SMOKING SHOW OPPOSITE ASSOCIATIONS WITH MATERNAL TSH IN EARLY PREGNANCY, BUT SIMILARLY INCREASE THE T3/T4-RATIO: EVIDENCE FROM 5,529 DANISH PREGNANT WOMEN

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**Objectives:** A higher activity of the deiodinase type 2 (DIO2) has been proposed in overweight/obese and smoking pregnant women as reflected by a higher T3/T4-ratio. Pregnancy is associated with physiological alterations in deiodinase activity and in binding proteins, and we speculated how maternal body mass index (BMI) and smoking status associate with different measures of maternal thyroid function in the early pregnancy.

**Method:** Thyroid function tests (TSH, total T4 (TT4), total T3 (TT3), free T4 (fT4), and free T3 (fT3)) were performed in stored blood samples (median pregnancy week 10) from 6,282 women in the North Denmark Region Pregnancy Cohort. Biochemical measurements were performed using an automatic immunoassay (Roche Diagnostics). Results were linked to nationwide registers and live-birth pregnancies with available information on maternal pre-pregnancy BMI and smoking status and in which the woman did not receive medical treatment for thyroid disease were included. The association between log-transformed TSH, fT3/fT4-ratio and TT3/TT4-ratio, and categories of maternal BMI and smoking were assessed using linear regression and reported as adjusted β (aβ) with 95% confidence interval (95% CI). The adjusted model included maternal BMI, smoking, gestational week of blood sampling, age, parity, origin, and diabetes.

**Results:** Altogether 5,529 pregnant women were included in the study, and 56% were normal weight (BMI 18.5-24.9 kg/m$^2$), whereas 10% were smoking in the pregnancy (Table). Higher maternal BMI associated with higher TSH, whereas maternal smoking was associated with lower TSH in the early pregnancy (Table). Considering different measures of the T3/T4-ratio, both higher BMI and smoking associated with a higher ratio (Table). Adding to this, lower maternal BMI associated with a lower ratio (Table).

**Conclusion:** In a large cohort of Danish pregnant women, adiposity and smoking showed opposite alterations in maternal TSH. On the other hand, both conditions associated with a higher T3/T4-ratio in early pregnancy, which may reflect deiodinase activity.

**Table 1.** (for Abstract 14)

| | | TSH | | fT3/fT4 | | TT3/TT4 | |
|---|---|---|---|---|---|---|---|
| | n | aβ | 95% CI | aβ | 95% CI | aβ | 95% CI |
| **BMI (kg/m$^2$)** | | | | | | | |
| < 18.5 | 216 | -0.093 | -0.222; 0.036 | -0.028 | -0.045; -0.010 | -0.038 | -0.058; -0.018 |
| 18.5-24.9 | 3,096 | Ref. | | Ref. | | Ref. | |
| 25.0-29.9 | 1,350 | 0.065 | 0.005; 0.125 | 0.048 | 0.040; 0.056 | 0.054 | 0.045; 0.063 |
| ≥ 30.0 | 867 | 0.216 | 0.144; 0.288 | 0.079 | 0.070; 0.089 | 0.096 | 0.085; 0.107 |
| **Smoking** | | | | | | | |
| No | 4,958 | Ref. | | Ref. | | Ref. | |
| Yes | 571 | -0.132 | -0.214; -0.050 | 0.072 | 0.061; 0.083 | 0.097 | 0.085; 0.110 |

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### PRE- AND POST-OPERATIVE CALCITONIN VALUE AS A PREDICTIVE FACTOR OF DISEASE SPECIFIC MORTALITY IN SPORADIC MEDULLARY THYROID CARCINOMA

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**Introduction:** Medullary thyroid carcinoma (MTC) is a rare tumor, it originates from the C cells producing calcitonin (CT) and can occur in sporadic or hereditary form. The initial treatment is represented by total



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thyroidectomy associated with central compartment lymph nodes dissection and possible extension to the latero-cervical compartment. CT is the main marker and play a key role in the pre- and post-operative evaluation of MTC patients. However, less is known about CT values able to identify those patients who showed a higher risk of disease specific mortality (DSM). The aim of the present study is to evaluate several predictive factors of DSM in a large series of sporadic MTCs.

**Patients and Methods:** We evaluated 538 consecutive patients surgically treated for sporadic MTC, from 2000 to 2018, and followed at the Endocrine Unit of the University Hospital of Pisa. Demographic, pathologic and clinical data were collected and pre (preOP-CT) and post-operative (postOP-CT) basal CT values were investigated and correlated with DSM.

**Results:** After a median follow-up of 91.5 months (IQR 47-149), 60/538 (11.5%) died for MTC.

ROC curve analysis indicated best cut-off of preOP-CT >288 pg/ml (sensitivity 87%; specificity 62.5%) and postOP-CT >31 pg/ml (sensitivity 96.7%; specificity 75,3%) in identifying patients who died for the disease.

At univariate analysis, factors significantly correlated with death were the male gender, tumor dimension > 4 cm, presence of lymph node (pN1) and/or distant metastasis (M1) at the diagnosis, multifocality, minimal extrathyroidal extension (mETE), initial staging, preOP-CT >288 pg/ml and postOP-CT >31 pg/ml. At multivariate analysis, statistical significance persisted only for staging and postOP-CT.

**Conclusions:** In our study a significant improvement in the survival of sporadic MTC patients, compared to the previous studies, was observed. A more advanced staging at the time of diagnosis has been confirmed as a negative prognostic factor, highlighting the importance of an early diagnosis for improving DSM. Threshold of pre- and post-OP basal CT values associated to DSM were identified. Both, PreOP-CT >288 pg/ml and postOP-CT >31 pg/ml significantly correlated with DSM, however this association was stronger for postOP-CT.

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## ULTRASOUND FEATURES AND ROLE OF RISK STRATIFICATION SYSTEMS IN IDENTIFYING MEDULLARY THYROID CARCINOMA

*Carla Gambale*[1], *Antonio Matrone*[2], *Alessandro Prete*[3], *Rossella Elisei*[4], *Paolo Vitti*[5]

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**Objectives:** In the last years, several scientific societies designed ultrasound risk stratification systems (RSS) of thyroid nodules, to enhance the interobserver reproducibility of descriptions, to simplify communication of the results and to help physicians in their clinical practice in the selection of nodules to submit to Fine-Needle Aspiration Cytology (FNAC). However, RSS have been developed against papillary thyroid carcinoma, and the knowledge about their role in identifying medullary thyroid carcinoma (MTC) are unclear. The aims of this study are to describe the ultrasound features of MTC and to evaluate the performance of RSS in identifying MTC.

**Methods:** We retrospectively evaluated pre-operative data of 152 consecutive patients treated for sporadic or hereditary MTC, from January 2014 to September 2020. Each patient performed pre-operative clinical, biochemical and ultrasound assessment at the Endocrine Unit of the University Hospital of Pisa. Ultrasound features of each MTC were collected and classified according to the five main RSS. Moreover, we evaluated the indications of the five main RSS in performing FNAC.

**Results:** Median MTC dimension on ultrasound was 1.3 cm. Most of the nodules showed solid composition (92.8%), hypoechoic pattern (82.9%) and regular margins (67.1%). About half of the nodules (55.3%) showed the presence of calcifications, but only a subgroup had microcalcifications (17.7%). A minority of the nodules (10.5%) showed a "taller than wide" shape. Only 7.9% of all MTC showed the simultaneous presence of 4 US features suggestive for malignancy (solid composition, hypoechogenicity, irregular margins and microcalcification). A percentage varying from 45.4 to 47.4% of MTC were correctly classified as high-risk suspicious category, according to the different RSS. Moreover, the indication to perform FNAC according to the 5 RSS was present in only 48.7-63.8% of all MTC.

**Conclusions:** In our series of 152 cases, no single or associated ultrasound features are strongly suggestive for MTC. The 5 main RSS correctly identify less than 50% of MTC at ultrasound and do not suggest of performing FNAC in about half of the cases, leading to a delay in the diagnosis with a consequent unknown impact on their clinical management.

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## THE EFFECT OF THYROID PEROXIDASE ANTIBODIES AND THYROGLOBULIN ANTIBODIES ON THYROID FUNCTION IN PREGNANT WOMEN – AN INDIVIDUAL PARTICIPANT DATA META-ANALYSIS WITHIN THE CONSORTIUM ON THYROID IN PREGNANCY

*Sofie Bliddal*[1], *Arash Derakhshan*[2], *Julie Lyng Forman*[3], *Yi Xiao*[4], *Robin Peeters*[5], *Tim Korevaar*[6], *Ulla Feldt-Rasmussen*[7]

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**Objectives:** Thyroid autoimmunity in pregnant women is associated with altered maternal thyroid function and adverse obstetric outcomes. However, a distinction between thyroid peroxidase autoantibodies (TPOAbs) and thyroglobulin autoantibodies (TgAbs) is rarely applied, and most studies focus on TPOAbs as assessed by a dichotomous negative-positive distinction. The present study aimed at determining the effect of TPOAbs and TgAbs, respectively and interdependently, on maternal thyroid function.

**Methods:** An individual participant data meta-analysis of 20 cohorts included in the Consortium on Thyroid in Pregnancy. Women with spontaneous singleton pregnancies and no history of thyroid disease were included. The associations of TPOAbs and TgAbs with cohort-specific z-scores of TSH, FT3, FT4, TT3, TT4 and T3:T4 were evaluated in a linear mixed model assuming a linear effect of the log-transformed antibody concentration when above the manufacturer's cut-off. Effects of TPOAbs and TgAbs were evaluated with and without mutual adjustment. Analyses were repeated with and without adjustment for covariates; age, gestational age, BMI, smoking, foetal sex, and parity.

**Results:** In total, 51,512 women were included of whom 51,456 had data on TPOAbs and 27,912 on TgAbs. Antibody-positivity was found in 11.1%

**Table 1.** Effect of thyroid autoantibodies on TSH and FT4 z-scores (for Abstract 80)

| Exposure (log2) | Outcome (adjusted z-scores) | n | Coefficient | SE | 95%CIL | 95%CIR | p-value | Adjusted p-value |
|---|---|---|---|---|---|---|---|---|
| TPOAb without adjusting for TGAb | TSH | 45,968 | 0.13 | 0.01 | 0.11 | 0.16 | <0.000 | <0.000 |
| TGAb without adjusting for TPOAb | TSH | 26,634 | 0.09 | 0.02 | 0.05 | 0.13 | <0.000 | <0.000 |
| TPOAb adjusting for TGAb | TSH | 26,591 | 0.09 | 0.03 | 0.04 | 0.14 | <0.000 | <0.000 |
| TGAb adjusting for TPOAb | TSH | 26,591 | 0.05 | 0.03 | 0.00 | 0.11 | 0.051 | 0.204 |
| TPOAb without adjusting for TGAb | FT4 | 46,106 | -0.07 | 0.01 | -0.09 | -0.04 | <0.000 | <0.000 |
| TGAb without adjusting for TPOAb | FT4 | 26,721 | -0.08 | 0.03 | -0.14 | -0.02 | 0.011 | 0.264 |
| TPOAb adjusting for TGAb | FT4 | 26,682 | -0.04 | 0.02 | -0.08 | 0.01 | 0.092 | 1.00 |
| TGAb adjusting for TPOAb | FT4 | 26,682 | -0.06 | 0.03 | -0.12 | -0.002 | 0.044 | 1.00 |

for TPOAbs and 8.6% for TgAbs, respectively. There was a highly significant dose-response effect with increasing TPOAb concentrations being associated with increasing TSH concentrations both with and without adjustment for TgAbs (table 1). TgAbs also had a significant effect on TSH concentrations, however, not when adjusting for TPOAbs (table 1). FT4 concentrations decreased slightly with increasing TPOAb concentration, which lost significance when adjusting for TgAbs. FT3:FT4 increased significantly with increasing TPOAbs concentration both with and without adjustment for TgAbs. After adjustment for multiple comparisons, increasing TgAb concentration had no association with any other outcome. Adjusting for demographic characteristics had little effect on any of the results and none on the conclusions reached.

**Conclusions:** In this individual participant data meta-analysis of 51,512 pregnant women we demonstrated a highly significant increase in TSH with increasing TPOAbs, which was unaltered after adjustments for TgAbs and demographic characteristics. Although an effect of TgAbs was found, this was not significant when controlling for TPOAbs. The findings support the current practice of using TPOAbs in initial laboratory testing and as exclusion criterion in establishment of pregnancy-specific reference ranges without adding TgAb measurements.

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## ASSOCIATION OF PER- AND POLYFLUOROALKYL SUBSTANCES WITH THYROID SYSTEM PARAMETERS DURING PREGNANCY

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**Objectives:** To investigate the association of exposure to per- and polyfluoroalkyl substances (PFAS) during early pregnancy with markers of the maternal thyroid system.

**Methods:** Serum concentrations of seven PFAS as well as thyroid stimulating hormone (TSH), free and total thyroxine (FT4 and TT4), free and total triiodothyronine (FT3 and TT3) and thyroid antibodies were measured in pregnant women in early pregnancy in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study, a population-based prospective cohort. Outcomes were absolute concentrations of TSH and thyroid hormones, the FT4/FT3 or TT4/TT3 ratios as markers of peripheral T4 metabolism, the TSH/FT4 ratio as a marker of the negative feedback loop and TT4/FT4 or TT3/FT3 ratios as markers of the binding of thyroid hormones to binding proteins.

**Results:** After exclusions, the study population comprised 2,008 women median (95% range) gestational age of 10 (6-14) week. There was no association of PFAS with TSH. Higher PFNA, PFDA, PFHpA and PFOA were associated with a higher FT4 (largest effect estimate for PFDA: β [95% CI]: 0.27 [0.10 to 0.45], P=0.002). A higher PFUnDA, but no other PFAS, was associated with a lower FT3 (β [95% CI]: -0.05 [-0.09 to -0.01], P=0.005). For total thyroid hormones, a higher PFUnDA was associated with lower TT4 (β [95% CI]: -1.58 [-3.07 to -0.09]) and there was an inverted U-shaped association of PFOS with TT4 (P=0.03). Higher PFDA, PFUnDA, PFHpA and were associated with a lower TT3. Except for PFNA, PFHxS and PFOA, higher PFAS concentrations were associated with a higher FT4/FT3 ratio. Moreover, except for PFOA, higher PFAS concentrations were also associated with a higher TT4/TT3 ratio. There was no association of PFAS with the TSH/FT4 ratio. A higher PFDA, PFUnDA or PFHpA was associated with a lower TT4/FT4 ratio. Moreover, except for PFHxS and PFOA, higher PFAS were associated with lower TT3/FT3 ratio.

**Conclusions:** Maternal exposure to PFAS during early pregnancy is associated with higher absolute concentrations of FT4 while the peripheral T4 metabolism might be lower based on the higher ratio of T4/T3. Furthermore, there was evidence that exposure to PFAS might decrease the binding of thyroid hormones to the transporter proteins. These findings translate results from experimental studies suggesting that exposure to PFAS may interfere with the thyroid system during pregnancy. Further experimental studies should take into account the combination of experimental and human evidences to better understand the potential underlying mechanisms of thyroid disruption by PFAS in humans.

# Oral Session 2

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## LONG-TERM EFFICACY OF TRIIODOTHYRONINE ANALOGUE TRIAC IN CHILDREN AND ADULTS WITH MONOCARBOXYLATE TRANSPORTER 8 DEFICIENCY: AN INTERNATIONAL, REAL-LIFE COHORT STUDY

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**Background:** MCT8 deficiency is a rare disorder caused by mutations in the thyroid hormone transporter MCT8, comprising severe neurodevelopmental delay and high serum T3 concentrations resulting in thyrotoxicosis in peripheral tissues. This predisposes to substantial morbidity and mortality. Recently, we reported the results of an international trial, in which biochemical and clinical outcomes improved in patients who were treated with Triac for 12 months. However, long-term follow-up data of such patients treated with Triac are lacking, particularly in young children. We investigated the long-term efficacy of Triac therapy in a worldwide cohort of patients with MCT8 deficiency.

**Methods:** We investigated the efficacy of Triac treatment in 68 patients with MCT8 deficiency in 19 countries. Triac dose was titrated according a predefined dose-escalation scheme aiming to normalize serum T3 concentrations (target 1.4-2.5 nmol/L). Thyroid function tests, body weight, heart rate and biochemical markers reflecting thyroid hormone action in peripheral tissues (SHBG, creatine kinase, creatinine) were measured at baseline and during control visits.

**Findings:** 68 patients with a median baseline age of 4.4 years (range 6 months–66 years) were treated, including 24 patients aged 0-2.5 years. They were treated during 181 patient years; follow-up time was >5 years in 12 patients and 2-5 years in 26 patients. Median dose was 38 µg/kg/day (range 15-105 µg/kg/day).

Mean serum T3 concentrations decreased from 4.6 to 1.7 nmol/L (normal 1.4–2.5 nmol/L). Body weight-for-age improved compared to treatment-naïve historical controls (0.67 SD increase). Heart rate-for-age improved from 1.56 to 0.96 SD. SHBG and creatinine concentrations improved from 247 to 213 nmol/L (normal 40-140 nmol/L) and from 32 to 39 µmol/L (normal 31-68 µmol/L), respectively. No drug-related severe adverse events were reported.

**Interpretation:** Triac is a safe treatment resulting in sustainable improvements of the severe thyrotoxic state in paediatric and adult patients with MCT8 deficiency.

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## THYROID HORMONE TRANSPORT IMPACTS MULTIPLE STAGES OF GABAERGIC INTERNEURON DEVELOPMENT

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Cortical interneuron neurogenesis is a strictly regulated process that depends on the presence of proper thyroid hormone (TH) signalling. A hypothyroid situation in the developing rodent cerebral cortex culminates in a reduced number of inhibitory GABAergic interneurons positive for the Calcium binding protein Parvalbumin (PV). Such a scenario is also observed in patients lacking the highly specific TH transporter MCT8 (Allan Herndon Dudley syndrome, AHDS). These patients present a severe psychomotor retardation that is most likely due to an impaired TH transport across the blood-brain barrier and/or in neural cells during critical stages of neuronal differentiation. This phenotype is replicated in Mct8/Organic anion transporting polypeptide (Oatp1c1) double knockout (dko) mice, an established mouse model of AHDS. Here, we addressed the question if the generation of cortical PV positive interneurons is subject to cell-autonomous action of Mct8 and Oatp1c1. To this end, we compared Mct8/Oatp1c1 dko mice to conditional knockout animals in which the expression of both TH transporters is abolished in neuronal progenitor cells that give rise to PV positive interneurons. We analysed interneuron composition in the early postnatal somatosensory cortex by immunohistochemistry and found a transient delay in the appearance of PV positive interneurons in the conditional knockout model while cell numbers remained permanently reduced in Mct8/Oatp1c1 dko mice. Using fluorescence in situ hybridisation on E12.5 embryonic brains, we detected reduced expression of components of the sonic hedgehog signalling in Mct8/Oatp1c1 dko embryos, but not in conditional TH transporter deficient mice. Moreover, we revealed that both TH transporters exhibit a spatially distinct expression pattern at brain barriers already at E12.5. In sum, our results point to the existence of multiple mechanisms that depend on proper TH transport during the generation, migration and maturation of cortical interneurons.

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## THE EFFECT OF THYROID HORMONE RECEPTOR TRUNCATING MUTANTS ON GENE TRANSCRIPTION IN NEURONAL CELLS

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**Introduction:** Thyroid hormone receptor (TR) α1 is the predominant TR isoform in the brain and plays a vital role in neurodevelopment. Mutations in TRα1 that reduce or abolish T3 binding to the receptor are the cause of resistance to thyroid hormone alpha (RTHα), characterized by motor and cognitive impairment and high T3/T4 ratio in blood. The severity of the neurological phenotype of RTHα patients does not always correlate with the degree of T3 binding impairment of mutant receptors. However, the mechanism underlying this phenotypic variation is unclear.

**Objective:** To understand the relationship between TRα1 mutants and phenotypic variation, we determine the differences in gene regulation by TRα1-C380fsx387 (severe neurocognitive phenotype) and -F397fsx406 (mild neurocognitive phenotype) in human-derived neuronal (SHSY5Y) cells.

**Methods:** We analyzed gene expression in cells that expressed wild-type (WT) or mutant (C380fsx387 and F397fsx406) TRα1. RNA was extracted from SHSY5Y cells stably expressing FLAG-HA-tagged (FH) WT or mutant TRα1 after 6 hours stimulation with vehicle or 10 nM T3. Triplicate experiments were analyzed by RNA sequencing (Illumina HiSeq2500). Reads were matched against the human reference genome (GRCh38 version). Gene expression values were called using HTseq-count (version 0.9.1) and differential gene expression was analyzed by R.

**Results:** In the presence of WT TRα1, the expression of genes was significantly changed by 10 nM T3. In contrast, cells expressing the mutant receptors lacked any significant T3-induced gene expression. Unstimulated gene expression was also different in the cells expressing mutant versus WT receptors. This difference was more pronounced in FHTRα1-C380fsx387 than in -F397fsx406 expressing cells, indicating a differential effect of these mutants on baseline gene expression. 721 unstimulated genes were highly differentially expressed between FHTRα1-C380fsx387 and -F397fsx406 cell lines (at least a 4-fold difference expression). Half of these were significantly enriched for at least one gene ontology (GO) terms, mostly related to the physiology of neurons. Many genes that were dysregulated explicitly by FHTRα1-C380fsx387 but not -F397fsx406 compared to both WT are involved in the nervous system development and neuronal migration.

**Conclusions:** The transcriptomes regulated by two truncating mutants are widely different. Unstimulated gene expression controlled by the TRα1-C380fsx387 mutant is more different from WT than that controlled by the TRα1-F397fsx406 mutant. This involves many genes that have a vital role in neuronal development and may explain the more severe neurological phenotype found in the patient carrying the C380fsx387-TRα1 mutation.

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## NOVEL THYROID HORMONE TRANSPORTERS IN THE SOLUTE CARRIER 22 FAMILY

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**Objectives:** Thyroid hormone transporters represent a critical first step in governing intracellular thyroid hormone regulation. Several TH transporters belonging to different transporter families have been identified, but are insufficient to explain all the transport processes. We hypothesized that candidates for novel TH transporters reside in the SLC22 family consisting of organic cation (OCT) and anion (OAT) transporters, as several members have substrates in common with members of the OATP family, which also contains several TH transporters. Therefore, we screened the entire SLC22 family for TH transporters.

**Materials and Methods:** Uptake of the iodothyronines 3, 3'-T2, rT3, T3, T4, and the sulfated iodothyronines T3S and T4S was tested in COS1 cells transfected with expression constructs for SLC22 proteins. Plasma membrane expression of the SLC22 proteins was verified by cell surface biotinylation assays. In addition, the specificity and efficacy of the pharmacological inhibitors lesinurad and probenecid for these positive TH transporters was determined.

**Results:** We first tested 25 mouse (m) SLC22 proteins for TH uptake and found that most members of the OAT-clade (mSlc22a8, mSlc22a19, mSlc22a26-30) were capable of T3 and/or T4 transport. Next we generated a phylogenetic tree of the mSlc22 and human (h) SLC22 proteins. 8 hSLC22s that grouped with mSLC22s that had tested positive were selected for TH uptake measurements. Of these, hSLC22A8, hSLC22A9, hSLC22A11 and hSLC22A24 were capable of T3 and/or T4 transport. Especially hSLC22A11 showed robust (3-fold of empty vector transfected cells) and specific uptake of T4. In addition, some members, most notably mouse and human SLC22A8 and hSLC22A9, showed strong uptake of T3S and T4S. Particularly hSLC22A8 and 22A9 induced T3S uptake by 10-fold and 4-fold respectively. Finally, the zebra fish SLC22A6/8 orthologues drOatx and drSlc22a6I also transported virtually all substrates tested. Lesinurad and probenecid inhibited TH or THS transport by most SLC22 proteins, both with IC50 values for mouse and human SLC22A8 in the low micromolar range.

**Conclusions:** Members of the OAT clade of the SLC22 family constitute a novel but evolutionary conserved group of transporters for sulfated and non-sulfated iodothyronines. Future studies will have to reveal the relevance of these transporters in TH homeostasis.



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## A COMBINING THYROID RISK SCORE (TRS) FOR NODULES WITH INDETERMINATE CYTOLOGY

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**Background:** Cytology is the gold standard method for the differential diagnosis of thyroid nodules, though 25–30% of them are classified as indeterminate and in some cases surgery is required for a definitive diagnosis.

**Aim:** We aimed to set up a 'thyroid risk score' (TRS) to increase the diagnostic accuracy in patients with indeterminate cytology and to apply it to a validation series.

**Methods:** The pre-surgical TRS derived from the sum of the scores assigned at cytology, namely EU-TIRADS classification, nodule measurement, and molecular characterization (24 different genetic alterations, including point mutations and gene fusions, analysed by our customized assay PTC-MA assay). We prospectively tested 136 indeterminate thyroid nodules for the model evaluation, while further 34 patients have been enrolled to date for the model validation.

**Results:** 66/136 analyzed nodules underwent surgery and 20/66 (30.3%) were malignant. The risk of malignancy (ROM) increased paralleling the score: in the category >4 and ≤ 6 (low suspicion), >6 ≤ 8 (intermediate suspicion), and >8 (high suspicion) ROM was 10, 47 and 100%, respectively. ROC curves selected the score >6.5 as the best threshold to differentiate between malignant and benign nodules ($P < 0.001$). The TRS > 6.5 had a better performance than the single parameters evaluated separately, with an accuracy of 77% and 82% upon inclusion of noninvasive follicular thyroid neoplasm with papillary-like nuclear features among malignant or benign cases, respectively. In the new series, 10/34 nodules with TRS>6 will undergo surgery in order to confirm TRS cut-off.

**Conclusions:** In conclusion, for the first time, we generated and applied a score combining a cost-effective molecular assay with already validated tools, harboring different specificities and sensitivities. The combination of different parameters reduced the number of false negatives inherent to each classification system. The TRS > 6.5 was highly suggestive for malignancy and retained a high accuracy in the identification of patients to be submitted to surgery. A proper role of the TRS can be also predicted in the evaluation of large nodules routed to surgery in most cases. Indeed, in the era of mini-invasive procedures, a low TRS could favor the possibility to submit older patients and cases with co-morbidities to these techniques. The validation series will give more insights into the actual reliability of our TRS cut-off.

# Tuesday, 7th September 2021

## Young Investigator Session / Clinical and Translational

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## VARIATION IN THE CLASSIFICATION OF MATERNAL THYROID FUNCTION IN EARLY PREGNANCY WITH REPEATED BLOOD SAMPLING: A STUDY OF 1,466 INDIVIDUAL CASES IN THE NORTH DENMARK REGION PREGNANCY COHORT

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**Objective:** The assessment of maternal thyroid function in early pregnancy is debated and controversies exist in terms of who and how to test. The recommendation regarding thyroid function testing is that method- and pregnancy-specific reference ranges preferably should be established and used. We speculated how repeated blood sampling in the early pregnancy would influence the classification of maternal thyroid function if method- and pregnancy week-specific reference ranges were used.

**Method:** We identified singleton pregnancies from the North Denmark Region Pregnancy Cohort in which the pregnant woman had a blood sample drawn twice in the early pregnancy as part of nationwide prenatal screening for chromosomal anomalies. Each blood sample was used for the measurement of TSH (ADVIA Centaur, Siemens Healthineers). Method- and pregnancy week-specific reference ranges were previously established within the cohort and used for classification of maternal thyroid function. Results were linked to Danish nationwide registers and women who received current treatment for thyroid disease in the pregnancy were not included.

**Results:** Altogether 1,466 pregnancies were included in the study. The first blood sample was drawn in median pregnancy week 8 (interquartile range (IQR): 7-8) and the second blood sample in median week 12 (IQR: 11-13). When pregnancy week-specific reference ranges were used, a total of 89 women had TSH above the upper reference limit in the first blood sample, and 49% of these women (n=44) similarly had high TSH in the second sample. A total of 47 women had TSH below the lower reference limit in the first blood sample, and 40% of these (n=19) similarly had low TSH in the second sample. The agreement in the classification of maternal TSH was dependent on the TSH-level, thus, if maternal TSH was above 6.0 mIU/L or below 0.1 mIU/L in the first blood sample, the classification of high and low TSH, respectively, was consistent between the first and the second blood sample in minimum 80% of the cases.


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**Conclusion:** In a large cohort, the classification of maternal thyroid function in the early pregnancy was not consistent across repeated individual samples when pregnancy week-specific reference ranges were used. The inconsistent classification reminds clinicians to consider variation in TSH and results emphasize a focus on the severity of thyroid function abnormalities in pregnant women.

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## NEW PROPOSAL FOR A HISTOLOGICAL GRADING OF MEDULLARY THYROID CARCINOMA: INSIGHTS FROM A LARGE SERIES OF SPORADIC CASES

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**Introduction:** Medullary thyroid carcinoma (MTC) is a rare neuroendocrine cancer, originating from thyroid c-cells. Although many other neuroendocrine tumors show a grading system recognized by the World Health Organization, a grading system for MTC is still lacking. We performed an extensive pathological and clinical review of 331 MTCs to evaluate their histologic features and building a grading system to identify those MTC with a worst prognosis.

**Method:** Pathological and clinical features of 331 patients with sporadic MTC, surgically treated at the Endocrine Surgery Unit and followed at the Endocrine Unit of the University Hospital of Pisa, from 2000 to 2018, were retrospectively collected. We evaluated the MTC histopathologic variants (classical, clear cell, follicular, oncocytic, papillary, small cell and spindle cell), grade of fibrosis (absent, "low-grade" defined as below 33% and "high-grade" as higher than 33%), presence of coagulative necrosis, mitosis ("rare" as less than 5 and "frequent" as more/equal than 5 mitosis per 10 hpf), lymphovascular and perineural invasion.

**Results:** Patients were followed for a median time of 7.6 years. The MTC variants were distributed as follows: 207/331 (62.5%) classical variant, 66/331 (19.9%) spindle cell variant and 58/331 (17.4%) other variants with a frequency <5% each. A wide group of MTC had absent or low-grade fibrosis (226/331, 68.7%), conversely high-grade fibrosis was present in 103/331 patients (31.3%). Frequent mitosis and coagulative necrosis were present in 8.2% and 6.4%, respectively. Lymphovascular invasion was present in 36.2% of patients, whereas perineural invasion in 5.2%. Kaplan-Meier analysis showed that only high-grade fibrosis, lymphovascular and perineural invasion had a worse impact on disease specific survival. Accordingly, we proposed the following three-tiered grading system including grade of fibrosis, lymphovascular and perineural invasion: low-grade in case of the absence of all of them, intermediate-grade in case of the presence of one of them, and high-grade in case of the presence of at least 2 of them. Kaplan-Meier analysis confirmed that MTC specific survival was significantly associated to the grading system proposed, being the worst in high-grade category.

**Conclusions:** MTC is a well-differentiated thyroid tumor, however, several peculiar histologic features can be associated with a worse prognosis. In our large MTC series, high percentage of fibrosis and presence of lymphovascular and perineural invasion were correlated with death. The three-tiered grading system we proposed, was able to identify the MTCs with the worst prognosis. Further analysis should be performed to correlate this grading system with stage and genotype.

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## ASSOCIATION OF MATERNAL THYROID FUNCTION DURING PREGNANCY WITH OFFSPRING RESPIRATORY FUNCTION AND ASTHMA IN CHILDHOOD

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**Objective:** Suboptimal respiratory function and asthma considerably impact quality of life in childhood, but often the underlying cause remains unknown. Thyroid hormone is an important regulator of (fetal) lung development. Suboptimal fetal thyroid hormone availability due to maternal thyroid dysfunction in early pregnancy may therefore affect respiratory function during childhood.

**Methods:** Serum TSH, FT4 and TPOAbs were measured during early pregnancy in 3,208 women, participating in a population-based prospective cohort. Offspring lung outcomes such as FEV1, FEV1/FVC and FEF75 were measured by spirometry, and data on asthma symptoms and diagnosis at an age of 9 was collected. After exclusion of TPOAb positive women, thyroid disease entities were defined according to population-based reference ranges. We investigated the association of maternal thyroid function during pregnancy with offspring respiratory function and asthma by using multivariable logistic and linear regression models adjusting for maternal age, parity, smoking during pregnancy, ethnicity, educational level, pre-pregnancy BMI and child sex.

**Results:** There was a negative association of TSH with FVC (β±SE: -0.03±0.01, *P*=0.04) but there was no association of TSH or FT4 with other offspring respiratory function measurements. As compared to euthyroid women, hypothyroxinemia was associated with a lower FEV and FEV/FVC (β±SE: -0.2±0.11, P=0.05 and β±SE: -0.2±0.11, *P*=0.04, respectively).

There was an inverted U-shaped association of FT4, but not TSH, with asthma (*P*=0.16) and hypothyroxinemia and hyperthyroidism were associated with a 1.8-fold (95%CI: 0.8-4.2) and a 2.2-fold (95%CI: 0.5-9.8) higher risk, respectively. However, none of these results reached statistical significance due to limited statistical power.

**Conclusion:** Maternal hypothyroxinemia is associated with lower offspring FEV and FEV/FVC. While the results of this study are supported by experimental evidence and could be explained by fetal programming, further clinical studies with more statistical power are required to determine whether maternal thyroid function is a determinant of offspring respiratory function and/or asthma.

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## CLINICOPATHOLOGICAL CHARACTERISTICS OF A COHORT OF PATIENTS WITH RADIOIODINE-REFRACTORY THYROID CANCER (RAIR-TC): WHO ARE THE PATIENTS THAT WE CHOOSE TO TREAT WITH TYROSINE KINASE INHIBITORS (TKI)?

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**Objective:** The most challenging issue in RAIR-TC is to early identify patients with slow progressive disease who require surveillance only, respect to those who would better benefit from a systemic therapy. The present study aims to characterize these two types of RAIR-TC patients.

**Methods:** We retrospectively evaluated the clinicopathological characteristics of 279 consecutive RAIR-TC patients, referred to our attention from 2016 to 2019. 99 received indication to start TKI (Group A), while 180 were maintained under active surveillance (Group B). Clinical examination, serum markers measurements and neck ultrasound were performed every 6-12 months. Disease staging was assessed by total-body Computed Tomography and/or other imaging exams. Disease progression was defined according to RECIST 1.1. Definition criteria of RAIR-TC and the indication to start TKI therapy were determined according to 2016 ATA guidelines.

**Results:** Group A started TKI therapy after a mean time of 8.3±6.6 years from initial diagnosis and 3.5±3.4 years from diagnosis of radioiodine refractoriness (d-RAIR). At first diagnosis, Group A patients had a larger primary tumor (4.1±2.2cm vs 2.8±1.8cm; $p<0.001$) with a more frequent macroscopic extrathyroidal extension (ETE) (19.1% vs 7.8%; $p=0.027$), aggressive histotype (FTC 32.7% vs 14%; PDTC 10.2% vs 6.7%; $p<0.001$), T3 (42.3% vs 27.5%; $p<0.001$) and T4 (21.8% vs 5.6%; $p<0.001$) classification. Group A showed a higher prevalence of distant metastasis (DM) (35.8% vs 14.3%; $p<0.001$), and a higher ATA risk of recurrence (high-risk 75.3% vs 39.8%; $p<0.001$). At d-RAIR, DM were detected in 80.8% of Group A and 45.5% of Group B ($p<0.001$), while a disease limited to lymphnodes only in 57.1% of Group A and in 12.9% of Group B ($p<0.001$). The mean time from d-RAIR to the first progression (FP) was shorter in Group A [median 16.5 months (0.13-136) vs 34.9 months (3.1-196.9); $p<0.001$]. FP diagnosis within 25 months from d-RAIR was associated to a 23-fold higher risk to initiate TKI treatment (HR 23.1; 95%IC 13.2 to 40.3; $p<0.001$).

**Conclusion:** Among patients with RAIR-TC, those with a primary tumor with aggressive pathological characteristic (large size, ETE, aggressive histotype), a more advanced disease since diagnosis (DM, higher ATA risk) and FP within 25 months from d-RAIR were more frequently addressed to TKI therapy. Taking into account these clinicopathological features, the most appropriate follow-up can be tailored for each patient, avoiding sometimes too strict monitoring.

# Young Investigator Session / Basic

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## BONE DEVELOPMENT IN MICE REQUIRES CANONICAL TRA ACTION

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**Background:** Thyroid hormone (TH) action is mediated by thyroid hormone receptors (TRs) α and β. Both TRs can either regulate gene expression by binding TH-responsive elements on the DNA (canonical/type 1 signaling) or activate cellular signaling pathways (noncanonical/type 3 signaling). Bone is a major TH target and noncanonical TRα and TRβ effects on bone development have recently been suggested. We therefore studied bones of mouse models that distinguish between canonical and noncanonical TR signaling.

**Material & Methods:** Growth of wild type (WT) mice and mice with either TR knockout (TRαKO, TRβKO) or selective loss of canonical action (TRαGS, TRβGS) was recorded until P112. Femurs and caudal vertebrae were then analysed with Faxitron X-ray microradiography (bone mineral content [BMC] and bone length) and high resolution micro-computed tomography (cortical and trabecular thickness, bone mineral density [BMD]). 3-point-bend testing to destruction with an Instron loading frame and 100 N load cell revealed yield load, maximal load and fracture load.

**Results:** Longitudinal growth of TRαKO and TRαGS mice was equally delayed compared to WT mice and normalised after postnatal week 8. Accordingly, height of caudal vertebrae did not differ between the genotypes at P112. But the trabecular bone extended further in TRαKO and TRαGS femurs than in WT femurs (39% and 41% vs. 33% of whole femur length). Trabecular number and connectivity density were increased in both mutants, while trabecular spacing was reduced. Trabecular thickness was similar in all three groups, as were cortical parameters and BMD. Thus, bones of TRαKO and TRαGS mice contained a dense and more extensive network of trabecular bone than WT mice, while the trabeculae were normally shaped. No difference was found in yield or fracture load for all three groups. After T3 treatment, the structural differences between TRα mutants and WT bones were similar to those seen in untreated samples.

Growth of TRβKO and TRβGS was not different from that in WT mice. T4 serum concentration was elevated in both mouse models due to abolished negative feedback in the HPT axis. BMC was equally reduced in TRβKO and TRβGS femurs with thinner cortical bone. There were no differences in trabecular parameters.

**Conclusion:** Adult mice with only noncanonical TRα action showed the TRα knockout phenotype. We found no evidence for noncanonical TRβ action. These results demonstrate that TH effects in bone are predominantly mediated by canonical TRα action.


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## 3-IODOTHYRONAMINE (T1AM), A THYROID HORMONE METABOLITE, WORKS AS A PRO-OXIDANT IN BROWN ADIPOCYTES

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**Objectives:** 3-iodothyronamine (T1AM) is among the by-products of thyroid hormone (TH) metabolism endowed of pharmacological activities resulting from the interaction with different targets and from its pharmacokinetic features. In this respect, T1AM undergoes towards oxidative deamination by mitochondrial monoamine oxidase type B (MAO-B) and semicarbazide-sensitive amine oxidase activities (SSAO) with the generation of the 3-iodothyroacetic acid and $H_2O_2$, a source of reactive oxygen species (ROS).

We previously reported that 6 days exposure of cultured brown adipocytes (BAs) to 20 nM T1AM (M+T1AM) shifted cell metabolism towards catabolic conditions, i.e. lower ATP levels and higher basal lipolysis than cells not exposed to T1AM (M cells). Considering that metabolic shift and the high expression of SSAO in BAs are conditions favoring ROS production, we hypothesized M+T1AM cells had signs of oxidative damages.

To this aim we investigated whether M+T1AM, in respect of M cells, showed any sign of oxidative damage including lipoperoxidation, increase of free Fe++ cell levels, sirtuin-1 (SIRT-1) expression/activity, by evaluating its downstream substrates, gene transcription.

**Methods:** BAs isolated from the stromal fraction of rat brown adipose tissue were exposed to an adipogenic medium containing insulin in the absence (M) or in the presence of 20 nM T1AM (M+T1AM) for 6 days.

At the end of the treatment, Lipoperoxidation and the free $Fe^{++}$ levels, were measured spectrophotometrically. SSAO, MAO-B, SIRT-1 expression/activity, including p53 acetylation (acetyl-p53), PGC-1α, histone methylation and acetylation (H3K9me3 and H3K9ac respectively), MAO-A activity were measured by Western-blot analysis, real-time PCR and fluorimetrically.

**Results:** M+T1AM cells showed higher lipid peroxidation and free $Fe^{++}$ levels, lower SIRT-1, higher acetyl-p53 than M cells. In respect of SIRT-1 activity on gene expression, M+T1AM cells showed higher H3K9ac and lower H3K9me3 and MAO-A levels in respect of M cells. Furthermore, while MAO-B levels were below the limit of detection in BAs, the expression of SSAO in M+T1AM cells was 50% of that found in M cells.

**Conclusions:** This is the first report indicating T1AM is a pro-oxidant amine moving Fe++ from intracellular stores and inducing lipid peroxidation. This increase of the pro-oxidant cell status associates with the reduction of the antioxidant SIRT-1 and its transcriptional activity. Overall, ROS production, likely from SSAO deamination, may represent a novel signaling mechanism for T1AM effects.

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## LACK OF CANONICAL THYROID HORMONE RECEPTOR ALPHA SIGNALING IS CARDIOPROTECTIVE

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Thyroid hormones (TH) are crucial for normal cardiac function and orchestrate multiple cardiac effects, mainly via the thyroid hormone receptor (TR) α. In myocardial infarction, the precise role of TH status and TRα signaling still requires clarification. To understand how TH impact on myocardial ischemia/reperfusion (IR) injury, we used isolated pressure constant perfused hearts of control, hypothyroid (hypo) and hyperthyroid (hyper) mice and studied infarct size, cardiac function and cardioprotective signaling pathways with matched heart rate by electrical atrial stimulation and matched left ventricular developed pressure (LVDP) in hypothyroid hearts by epinephrine addition. Canonical and noncanonical TRα signaling was investigated using hearts of mice lacking TRα ($TR\alpha^0$), hyperthyroid $TR\alpha^0$ mice ($TR\alpha^0$hyper), mice with a mutation in the TRα DNA-binding domain ($TR\alpha^{GS}$) and respective wildtypes (WT). Compared to controls, hypothyroidism was cardioprotective with smaller infarct size and improved recovery of LVDP, while hyperthyroidism was detrimental with enlarged infarct size (infarct size: control 48±9% vs. hypo 15±5% vs. hyper 71±10% of ventricular mass, $P<0.05$). $TR\alpha^0$ mice were also protected against IR injury, as infarct size was reduced and functional recovery of LVDP was improved compared to WT (infarct size: WT 51±14% vs. $TR\alpha^0$ 17±7% of ventricular mass, $P<0.05$). Moreover, hyperthyroidism did not increase infarct size in $TR\alpha^0$ hearts (infarct size: $TR\alpha^0$ 17±7% vs. $TR\alpha^0$hyper 22±6% of ventricular mass, n.s.). Strikingly, lack of canonical TRα signaling confered cardioprotection, as $TR\alpha^{GS}$ mice also showed smaller infarct sizes and improved recovery of LVDP compared to WT (infarct size: WT 51±14% vs. $TR\alpha^{GS}$ 18±6% of ventricular mass, $P<0.05$). Interestingly, these effects were independent from cardioprotective signaling pathways. In summary, hypothyroidism and the lack of canonical TRα signaling are cardioprotective in IR injury and protection is not due to favorable changes in hemodynamics or classical cardioprotective signaling.

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## TRANSCRIPTOME ANALYSIS OF THE DEVELOPING RAT THYROID GLAND FROM FETAL LIFE TO PREPUBERTY

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The structure and function of the thyroid gland is established early in life, laying the foundation for lifelong thyroid function. A range of early- and adult-life thyroid disorders may arise from perturbed thyroid development rather than simple perturbed thyroid function later in life. Yet, the development and maturation of the thyroid gland remains poorly understood, not least at the molecular level. Using bulk-RNA-barcoding and sequencing (BRB-seq) technology, we show that the developing rat thyroid gland undergoes marked transcriptional changes during establishment of thyroid gland function. We found that 1003 genes are differentially regulated throughout development and that they cluster in 8 patterns with a distinct transcriptional profile over the course of development (gestation day 21, postnatal days 3, 6, 16 and 22). Highly expressed genes in fetuses and neonates were primarily related to development, morphogenesis, cell proliferation and cell division. The immaturity of the gland, even after onset of fetal thyroid function, was supported by near absence of follicular lumen in fetal thyroid glands at gestational day 21. In contrast to the earlier time points, gland structure was more mature at 2 weeks of age, where there is a peak in serum thyroid hormone concentrations. Here, gene expression of genes important in thyroid hormone synthesis also peaked: thyroperoxidase, thyroglobulin and Duox1. We also identified 12 genes with sexually dimorphic expression patterns. Some of these are related to epithelial cell-cell contact and thyroid hormone synthesis and thus may indicate a sex-specific susceptibility of the developing thyroid gland to external stressors such as environmental chemicals. Our results show that even after the onset of fetal thyroid function, and during the first weeks of life in the rat, the thyroid gland is still a developing and maturing gland undergoing a distinct transcriptional reprogramming. Disruptions to this transcriptional programming could alter development and thus susceptibility to disease in adult life.


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# Oral Session 3

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## TUMOR VOLUME DOUBLING TIME IN ACTIVE SURVEILLANCE OF PAPILLARY THYROID MICROCARCINOMA: A MULTICENTER COHORT STUDY IN KOREA

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**Background:** Some papillary thyroid microcarcinomas (PTMCs) may progress with tumor enlargement or development of new lymph node (LN) metastasis during active surveillance (AS). This study evaluated the relevant predictors of disease progression, especially new cervical LN metastasis.

**Methods:** This was a long-term follow-up study conducted using a previous multicenter cohort of AS in Korea. After excluding 54 (14.2%) patients with a short follow-up duration, 326 PTMC patients were evaluated for tumor kinetics, including changes in tumor volume (TV) and TV doubling time (TVDT).

**Results:** During a median follow-up duration of 4.9 years, 17 (5.2%) patients showed a maximal diameter increase of ≥3 mm after a median 4.0 years of follow-up, while 9 (2.8%) showed newly developed LN metastasis after a median 2.2 years of follow-up. Both events occurred exclusively. The prevalence of new development of LN metastasis was higher in patients with TVDT <5 years (7.4%) than in those with TV ≥50% (3.2%). Furthermore, only TVDT <5 years was significantly associated with LN metastasis ($P$ = 0.002). In univariate and multivariate analyses, TVDT <5 years was an independent risk factor for disease progression with respect to new development of LN metastasis (hazard ratio [HR] = 6.51, confidence intervals [CI] 1.73–24.50; $P$ = 0.002) and tumor enlargement (HR = 20.89, CI 5.78–75.48; $P$ < 0.001). Finally, 86 (22.6%) patients underwent delayed surgery, and the most common reason was anxiety.

**Conclusions:** TVDT <5 years is the best predictor of disease progression during AS in terms of new LN metastasis development as well as tumor enlargement. Determination of TVDT in the early phase of AS could help in predicting disease progression for the precise tailoring of the follow-up intensity of AS and in determining if early surgical intervention is needed.

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## CABOZANTINIB VERSUS PLACEBO IN PATIENTS WITH RADIOIODINE (RAI)-REFRACTORY DIFFERENTIATED THYROID CANCER (DTC) WHO HAVE PROGRESSED AFTER PRIOR VEGFR-TARGETED THERAPY: RESULTS FROM THE PHASE 3 COSMIC-311 TRIAL

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**Objectives:** Cabozantinib, an inhibitor of VEGFR2, MET, AXL, and RET, showed activity in patients with RAI-refractory DTC. This phase 3 study (NCT03690388) evaluated cabozantinib versus placebo in patients with RAI-refractory DTC who had progressed during/after prior VEGFR-targeted therapy.

**Methods:** In this double-blind, phase 3 trial, patients were randomized 2:1 to cabozantinib (60mg QD) or matched placebo, stratified by prior lenvatinib treatment and age. Patients with RAI-refractory DTC must have received lenvatinib or sorafenib and progressed during/after treatment with ≤2 prior VEGFR inhibitors. Patients randomized to placebo could crossover to open-label cabozantinib upon disease progression per blinded independent radiology committee (BIRC). Primary endpoints were objective response rate (ORR) in the first 100 randomized patients and progression-free survival (PFS) in all randomized patients. PFS and ORR were assessed by BIRC per RECIST v1.1. The study was designed to detect an ORR for cabozantinib versus placebo (2-sided α=0.01) and a hazard ratio (HR) for PFS of 0.61 (90% power, 2-sided α=0.04). A prespecified interim PFS analysis was planned for the ITT population at primary ORR analysis.

**Results:** As of 19 Aug 2020,125 vs 62 patients were randomized to cabozantinib and placebo; median age was 66 years and 63% received prior lenvatinib. Median follow-up was 6.2 months in the ITT population. The trial met the primary endpoint for PFS at interim analysis with cabozantinib demonstrating significant improvement over placebo (median not reached vs 1.9 months; HR 0.22, 96% CI 0.13-0.36; P<0.0001); PFS benefit was observed in all prespecified subgroups including prior lenvatinib (yes, HR 0.26; no, HR 0.11) and age (≤65 yr, HR 0.16; >65 yr, HR 0.31). ORR was 15% for cabozantinib vs 0% for placebo (P=0.0281) but the difference was not statistically significant. Overall survival favored cabozantinib vs placebo (HR 0.54, 95% CI 0.27-1.11). Adverse events (AEs) of any grade (cabozantinib vs placebo) included diarrhea (51% vs 3%), palmar-plantar erythrodysesthesia (46% vs 0%), hypertension (28% vs 5%), fatigue (27% vs 8%), and nausea (24% vs 2%); grade 3/4 AEs were experienced by 57% versus 26%; dose reductions due to AEs occurred in 57% vs 5%; and treatment discontinuations due to AEs unrelated to DTC occurred in 5% vs 0%. There were no treatment-related deaths.

**Conclusions:** Cabozantinib showed a clinically and statistically significant improvement in PFS over placebo in patients with RAI-refractory DTC after prior VEGFR-targeted therapy with no unexpected toxicities. Cabozantinib may represent a new standard of care in patients with previously treated DTC.

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## FIRST RESULTS OF A SYSTEMATIC POPULATION-BASED APPROACH TO IDENTIFY PATIENTS WITH NTRK GENE FUSION AND RET GENE FUSION/MUTATION-POSITIVE THYROID CANCERS WHO COULD BENEFIT FROM TREATMENT WITH TRK AND RET INHIBITORS

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The TRK inhibitor, larotrectinib and RET inhibitor selpercatinib were recently approved by the United States Food & Drug Administration. We aimed to identify patients with *NTRK* gene fusion and *RET* gene fusion/mutation-positive thyroid cancers, who could benefit from larotrectinib or selpercatinib.

We identified patients for *NTRK* gene fusion and *RET* gene fusion/mutation screening in the Calgary prospective thyroid cancer database (N=482). Patients were pre-screened with either MassARRAY BRAF Test, Colon Panel, Melanoma Panel, or ThyroSPEC™ Test. Patients whose tumours were negative for other mutations were then screened for *NTRK* gene fusions and *RET* gene fusions/mutations with the Oncomine™ Comprehensive Assay v3 (OCAv3).

We identified 42 patients with radioactive iodine (RAI)-resistant distant metastases. After excluding 22 patients during pre-screening, 20 patients underwent OCAv3 screening, resulting in the detection of four patients with *NTRK* gene fusions and four patients with *RET* gene fusions (8/20, 40% of patients with tumours negative for other mutations). We also identified 42 patients with American Thyroid Association (ATA) high and ATA intermediate risk of recurrence and 2 patients with medullary thyroid carcinoma. During pre-screening we found one patient with an *NTRK* gene fusion and one patient with a *RET* gene fusion and excluded 33 patients. The remaining 9 patients received OCAv3 screening. We found two further patients with an *NTRK* and a *RET* gene fusion, respectively (4/12, 33% of patients with tumours negative for other mutations).

Our findings indicate a high rate of *NTRK* and *RET* gene fusions in patients with thyroid cancer with RAI-resistant distant metastasis, ATA high, or ATA intermediate risk of recurrence, whose tumours are negative for other mutations. This highlights the importance of early screening for *NTRK* gene fusions and *RET* gene fusions/mutations, to enable intervention with a TRK inhibitor, such as larotrectinib, or a RET inhibitor, such as selpercatinib.

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## EFFICACY OF [18F]FDG-PET/CT IN EVALUATION OF CYTOLOGICAL INDETERMINATE THYROID NODULES PRIOR TO SURGERY (EFFECTS): A RANDOMISED-CONTROLLED MULTICENTRE TRIAL

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**Objectives:** Approximately 75% of thyroid nodules with indeterminate cytology (atypia of undetermined significance or follicular lesions of undetermined significance (Bethesda III, AUS/FLUS) and (suspicious for a) follicular neoplasm (Bethesda IV, FN/SFN) or Hürthle cell neoplasm (Bethesda IV, HCN/SHCN) are benign. Avoiding unbeneficial diagnostic thyroid lobectomies for these nodules is crucial. [$^{18}$F]FDG-PET/CT has shown promise as an additional diagnostic to improve preoperative differentiation.

**Methods:** In this triple-blinded, multicentre, randomised-controlled trial in the Netherlands (NCT02208544), 132 patients with an indeterminate thyroid nodule underwent one [$^{18}$F]FDG-PET/CT of the neck and were randomised to the [$^{18}$F]FDG-PET/CT-driven or standard management group in a 2:1 ratio. In the [$^{18}$F]FDG-PET/CT-driven group, patient management was based on the undisclosed [$^{18}$F]FDG-PET/CT result: in case of a visually [$^{18}$F]FDG-positive nodule, diagnostic surgery was advised; in case of a [$^{18}$F]FDG-negative nodule, watchful waiting was recommended with a confirmatory ultrasound after one year. In the standard management group, diagnostic lobectomy was advised to all patients. The primary outcome was the accurate reduction in unbeneficial management, i.e., diagnostic surgery for benign nodules or watchful waiting for malignant and borderline nodules. Intention to treat analysis was performed.

**Results:** In the [$^{18}$F]FDG-PET/CT-driven group, the rate of unbeneficial patient management was 42% (38/91) as compared to 83% (34/41) in the standard management group ($p<0.001$). [$^{18}$F]FDG-PET/CT-driven management resulted in a 40% (25/63) reduction in unbeneficial diagnostic surgeries for benign thyroid nodules. In the standard management group, 2.9% (1/35) of benign nodules did not undergo surgery ($p<0.001$). There were no cases of unbeneficial watchful waiting for malignant/borderline nodules. Overall sensitivity, specificity, NPV and PPV (95% confidence interval) of [$^{18}$F]FDG-PET/CT were 94.1% (80.3%-99.3%), 39.8% (30.0%-50.2%), 95.1% (83.5%-99.4%) and 35.2% (25.4%-45.9%), respectively. The benign call rate was 31.1%. In the 101 non-oncocytic nodules (60 AUS/FLUS and 40 FN/SFN), the reduction in unbeneficial surgeries for benign nodules was 48% (23/48) in the [$^{18}$F]FDG-PET/CT-driven group, compared to 0% (0/28) in the standard management group ($p<0.001$). Sensitivity, specificity, NPV, PPV and benign call rate were 92.0% (74.0%-99.0%), 50.0% (38.3%-61.7%), 95.0%

(83.1%-99.4%), 37.7% (25.6%-51.0%) and 39.6%, The benign call rate in HCN/SHCN nodules was only 3% (1/31) and no reduction in unbeneficial management was seen.

**Conclusions:** Implementation of [$^{18}$F]FDG-PET/CT-driven management in the preoperative workup of indeterminate thyroid nodules accurately reduces the rate of unbeneficial diagnostic surgeries for benign nodules. As nearly all HCN/SHCN nodules are [$^{18}$F]FDG-positive, application of [$^{18}$F] FDG-PET/CT should be limited to non-oncocytic nodules to optimise diagnostic yield and use of resources.

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## ADVANCED DIFFERENTIATED THYROID CANCER, CLINICAL PRESENTATION, TREATMENT, AND PROGNOSTIC FACTORS (ERUDIT STUDY)

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**Introduction:** Advanced differentiated thyroid carcinoma (aDTC), herein defined as locally unresectable and/or metastatic, is one of the most common late-stage endocrine neoplasias. However, available data about its natural history are limited.

ERUDIT is a multicenter, observational, retrospective study of patients diagnosed with aDTC in Spain and Portugal. The study describes its natural history from the initial diagnosis until the advanced stages, focusing on specific characteristics of this subpopulation, its treatment, response patterns, and medical specialties involved in its management.

**Objectives:** To describe demographics and clinical characteristics of our aDTC cohort at first diagnosis, the efficacy of upfront, rescue and palliative therapies used, and the prognostic factors associated to disease evolution.

**Materials & Methods:** Clinical records from patients ≥18 y-o diagnosed with aDTC (including poorly differentiated DTC) with first evidence of advanced disease documented between January 2007 and August 2017 were retrospectively reviewed until death or lost to follow-up.

**Results:** 213 aDTC patients were identified in 23 sites with median age at initial DTC diagnosis 63 y-o and 59% being females. Of these, 54% presented with *de novo* aDTC (52% metastatic, being lung in 71% of cases) while the rest being recurrent from early DTC (eDTC). Most patients received upfront surgery (98.1%) with R0/R1 outcome in 82% and followed ablative radioiodine (RAI) in 89% of the cases. With most patients (62%) showing ATA structural incomplete response after first RAI, median (95%CI) relapse/progression-free survival [(RP)FS] into advanced disease was 2.3 (1.8-2.9) years. Overall survival (OS) of the entire cohort from the initial treatment was 10.4 (8.1-16.0) years with significant differences seen according to timing of aDTC diagnosis (recurrent better than *de novo;* log-rank p<0.0001), surgical outcome (R0/R1 better then R2*;* log-rank p<0.0001), and response to first RAI (excellent response better than biochemical/structural incomplete; log-rank p=0.0212). In total, 78% of patients were initially diagnosed or developed RAI-refractory DTC (RR-DTC) along study follow-up, mostly (69%) due to loss of RAI avidity, with median (Q1-Q3) time to RR-DTC diagnosis 27.6 (9.5-50.6) months. Median (95%CI) OS of RR-DTC patients was 4.7 (3.4-8.0) years, being statistically different (log-rank p=0.0142) from those not becoming refractory.

**Conclusions:** In our aDTC population, half of patients were *de novo* metastatic at diagnosis, with the majority receiving surgery and RAI as upfront treatments. Despite (RP)FS into aDTC was 2.3 years, OS for the entire and RR-DTC cohorts reached 10.4 and 4.7 years, respectively; suggesting a tangible prognosis improvement in the late years.

# Oral Session 4

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## ONCOGENIC MUTATIONS IN PI3K/AKT/MTOR PATHWAY EFFECTORS ASSOCIATE WITH WORSE PROGNOSIS IN BRAFV600E-DRIVEN PAPILLARY THYROID CANCER PATIENTS

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**Purpose:** The extent to which routine genomic sequencing can identify relevant secondary genomic alterations among BRAFV600E-mutant papillary thyroid carcinoma (PTC) is unknown. Such markers would prove highly valuable for prognostic purposes.

**Experimental Design:** We reviewed clinicopathological data of 225 patients with BRAFV600E-mutant PTC and integrated them with genomic data derived from targeted next generation sequencing on tumor specimens. We defined patient subgroups based on bona fide secondary oncogenic events (separate from BRAFV600E) and compared their clinical features and outcomes with those without additional oncogenic alterations.

**Results:** Additional oncogenic alterations were identified in 16% of tumors. Patients in the "BRAF+additional mutations" group were more likely to be at high ATA risk of recurrence (48.6 vs. 17.6%; P= 0.0009), had larger baseline tumor (2.7 vs. 1.9 cm; P= 0.0005) and more advanced stage at presentation (14.3% vs. 1.1% stage 4; P<0.0001). Importantly, over a 65-month follow up, disease-specific mortality was increased when additional mutations were identified (13.8% vs. 1.4% in the BRAF only group; P= 0.005). Separately, we identified a subcluster of patients harboring oncogenic mutations in key effectors of the PI3K/AKT/mTOR pathway, which were independently associated with disease-specific mortality [odds ratio (95% confidence interval) = 47.9 (3.5-1246.5); P= 0.0043].

**Conclusions:** Identification of additional PIK3/AKT/mTOR alterations in patients with BRAFV600E-mutant PTC provides important and actionable prognostic risk stratification. These data support genomic profiling of PTC tumors to inform prognosis and clinical strategy.

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## CANONICAL THYROID HORMONE AND TRA ACTION STIMULATES HEPATOCYTE PROLIFERATION

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**Background and Aims:** T3 is a potent stimulus for hepatocyte proliferation via the Wnt/β-catenin signaling pathway. However, the mechanism by which T3 increases β-catenin signaling is not fully understood and a contribution of noncanonical T3 signaling was suggested. We studied TRβ mutant mouse models to determine the underlying mode of TRβ action in T3-induced hepatocyte proliferation.

**Methods:** Wild-type (WT) mice, global TRβ knockout mice ($TR\beta^{KO}$), TRβ mutant mice with either specifically abrogated DNA-binding ($TR\beta^{GS}$) or abrogated direct PI3K activation ($TR\beta^{147F}$) were rendered hypothyroid and treated with T3 for 6 h or 7 days. Hepatocyte proliferation was assessed by Bromo-desoxy-uridine (BrdU) incorporation and Ki-67 staining. To test for noncanonical action of TRβ, rapid T3-mediated activation of β-catenin signaling was studied in primary murine hepatocytes. Microarray-based gene set enrichment analysis (GSEA) and qRT-PCR were used to analyze the effect of TR signaling on gene expression.

**Results:** T3-induced hepatocyte proliferation was markedly increased in WT and $TR\beta^{147F}$ mice, the mouse models with functional canonical signaling, compared to $TR\beta^{KO}$ and $TR\beta^{GS}$ mice (BrdU positive cells 25.9±2.0% and 24.7±2.9% in WT and $TR\beta^{147F}$ vs. 9.0±1.2% and 9.7±1.1% in $TR\beta^{KO}$ and $TR\beta^{GS}$ mice, respectively). 30 minutes of T3-treatment did not activate β-catenin signaling in WT primary murine hepatocytes, indicating that noncanonical TR signaling plays only a minor role. Interestingly, microarray analysis and GSEA revealed that genes of the Wnt/β-catenin pathway, e.g. *Fzd8* (Frizzled receptor 8) and *Ctnnb1* (β-catenin) itself, were positively enriched only in T3-treated WT and $TR\beta^{147F}$ mice. Additionally, T3 repressed genes encoding for anti-proliferative Btg2 and pro-apoptotic Bid only in these mice.

**Conclusion:** Direct comparison of TRβ mutant mouse models showed that canonical T3/TRβ action alone stimulated hepatocyte proliferation. T3 induced key genes of the Wnt/β-catenin pathway via canonical TRβ signaling, which increases induction of CyclinD1 and, ultimately, hepatocyte proliferation. Absence of rapid noncanonical T3 effects on the activation of the Wnt/β-catenin pathway in primary murine hepatocytes further supports the relevance of only canonical rather than noncanonical signaling of TRβ for hepatocyte proliferation. Thus, our results simplify the previously proposed model of T3-induced hepatocyte proliferation as only canonical signaling of TRβ is required.

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## HYPOTHYROIDISM AFFECTS EXPRESSION OF TUMOR SUPPRESSIVE AND ONCOGENIC MICRORNAS INVOLVED IN BREAST CANCER DEVELOPMENT AND PROGRESSION

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**Introduction:** Studies suggest that altered thyroid hormone (TH) levels correlate with incidence of breast cancer (BC) - one of the most frequent malignancies in women. However, different published data are inconsistent and TH status is still not considered either as a BC risk - or prognostic factor. microRNAs contribute to the initiation and progression of breast cancer. Here, we hypothesized that hypothyroidism may globally regulate mammary miRNome, thereby affecting preneoplastic changes.

**Material and Methods:** Female Wistar-Kyoto rats were divided into 2 groups (N=3 rats per group): hypothyroid (thyroidectomized) and control rats (sham operated). Analyses of miRNA expression changes in mammary glands was performed by Illumina Small RNA Sequencing. PanCancer analysis of expression of miRNAs in BC was performed using publically available TCGA data from 1085 cancer patients, and starBase. Gene ontology analysis was performed using DAVID. The study was approved by the local ethics committee (Second Warsaw Local Ethics Committee for Animal Experimentation) no. WAW2/126/2020.

**Results:** The induction of hypothyroidism in thyroidectomized rats was confirmed by serum evaluation of T4 (avg. <0.5 μg/dl) and TSH (avg. 42.06 ng/ml). Expression profile of miRNA in mammary glands of thyroidectomized rats revealed 81 differentially expressed miRNAs, including 42 downregulated and 39 upregulated (FDR<0.05; FC threshold: 60%). A comparison of expression profiles of thyroidectomized rat mammary glands and breast cancer patients showed 35 common miRNAs with significantly changed expression. Opposite direction of expression changes was indicated for 24 miRNAs. The latter included top-altered tumor suppressor miRNAs which were upregulated (FC>2.0) in hypothyroid mammary glands and downregulated (FC<0.5) in BC: miR-133a-3p, miR-144-3p, miR-411-5p, miR-494-3p, miR-379-5p, miR-497-5p and miR-99a-5p. These miRNAs are involved in induction of apoptosis, anti-metastasis and anti-invasion processes, suppression of cell proliferation and EMT process. In contrast, the oncomiRs most downregulated in hypothyroid mammary glands and upregulated in BC included: miR-183-3p, miR-200c-3p, miR-200b-5p, miR-200b-3p miR-331-3, miR-183-5p. These oncomiRs are responsible for enhanced cell proliferation and cell apoptosis inhibition.

**Discussion:** The study suggests that hypothyroidism induces expression changes in rat's mammary gland miRnome which may exert cancer protective effect. In particular, hypothyroidism stimulates the expression of tumor suppressive miRNAs, while concomitantly decreasing the expression of oncomiRs, possibly preventing the changes observed in breast cancer. Elucidation of the observed hypothyroidism-induced changes in mammary miRnome requires further research.

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## COMBINED MUTATIONAL AND CLONALITY ANALYSES SUPPORT THE EXISTENCE OF INTRA-TUMOR HETEROGENEITY IN PAPILLARY THYROID CANCER

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Intra-tumor genetic heterogeneity (ITH) refers to the coexistence of genetically different subclonal populations within the same tumor. Despite its potential impact, ITH has been scantly investigated in papillary thyroid cancer (PTC). In recent years, it has become evident that multiple alterations can be concomitantly present in PTC and associated to worst disease outcome. We have previously characterized a large series of PTCs by a custom Mass Array panel, finding at least one molecular alteration in 71% of cases. Interestingly, in 19% of cases two or more mutations were detected, and a minority of cases showed subclonal mutations, consistent with ITH. In the present study we aim to investigate ITH in PTC by HUMARA clonality assay and to correlate the clonality status with the clinico-pathologic characteristics of the tumors. We selected 53 female cases with a tumor purity >70% and at least one point mutation. Of 53 PTCs analyzed, 42 resulted informative for the assay. HUMARA assay revealed 21 polyclonal and 21 monoclonal PTCs, though 5 monoclonal cases were excluded because the ipsilateral normal thyroid tissue showed a skewed pattern of X-inactivation. Cases with single mutation (n=26) resulted either poly- or monoclonal. In particular, the majority of cases (12/15, 80%) with normalized mutated allelic frequency ~50% (range 45-55%) were clonal, consistent with the existence of a single mutated clone in the tumor. In contrast, 3/15 (20%) cases showed a polyclonal pattern, suggesting the presence of the same mutation in two or more clones. Differently, all cases harboring one subclonal mutation (n=11) resulted polyclonal. In these cases, we hypothesized the presence of different clones, one harboring the detected mutation and one or more with unknown genetic drivers. Among tumors with double mutations, all cases (n=4) with both mutations showing a normalized allelic frequency ~50% were monoclonal, suggesting the presence of a single clone. On the other hand, all cases harboring subclonal double mutations (n=7) resulted polyclonal, consistent with the presence of two clones with different mutations. Finally, no significant differences in the clinico-pathological characteristics were found between monoclonal and polyclonal tumors. In conclusion, the present study adds evidence to the concept of ITH in PTC. The heterogeneity found in some tumors warrants attention, since the occurrence of this phenomenon is likely to affect response to targeted drugs.

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## AN EARLY WAVE OF MACROPHAGE INFILTRATION FOLLOWED BY CD3+ T-CELLS CHARACTERIZES THE ONSET OF ORBITAL INFLAMMATION IN PRECLINICAL MOUSE MODEL OF GRAVES' ORBITOPATHY

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Graves' orbitopathy (GO) is an autoimmune driven manifestation principally of Graves' disease (GD) where pathogenic autoantibodies to the TSH-receptor activate orbital fibroblasts/preadipocytes in the orbital tissue to induce inflammation and extracellular matrix deposition. There are significant limitations to study immunological and proinflammatory mediator expression in early and during disease progression in GO patients, which would help in understanding disease pathogenesis and testing new therapeutic concepts. We have developed a robust mouse model of GD/GO induced by electroporation immunization of plasmid encoding human TSHR A-subunit, comprising of multiple injections over a course of 15 weeks to fully recapitulate the orbital pathology. In this study, we investigated kinetics of GO development in the model by serial analysis of immunological and cellular parameters during course of orbital inflammation.

Following immunization, groups of female BALB/c mice were sacrificed at different time points during disease progression. Pathogenic anti-TSHR antibodies with thyroid stimulating properties developed early after the second immunization step with concomitant induction of hyperthyroidism. Examination of orbital tissue showed early wave of infiltration of macrophages followed subsequently by $CD3^+$ T-cells into the orbital tissue. Examination of antigen specific T-cell activity using recombinant preparations of purified human A-subunit protein showed high $CD8^+$ T-cell proliferation during this early phase of disease onset, whereas effector $CD4^+$ and effector $CD25^+FOXP3^+$ regulatory T-cells were downregulated. The early phase of disease was also characterized by abundant presence of proinflammatory cytokines IFN-g and TNF-a in the orbital tissue. Moreover, as the disease progressed there was significant increase in browning of orbital fat tissue, which may be dependent on the proinflammatory mileau and/or the increased thyroid hormone levels during the established hyperthyroid status.

This work revealed that in our GD/GO mouse model anti-TSHR antibodies and the inflammatory state of the immune system accompanied by orbital inflammation and tissue remodeling are induced early and subsequently decline during the course of the disease. These findings provide new insights into the development of orbital inflammation in the model and have relevance to the timelines for testing new therapeutic interventions for the condition.

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## Case Reports

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### DRAMATIC CLINICAL IMPROVEMENT AFTER TOTAL THYROIDECTOMY IN A PATIENT AWAITING HEART TRANSPLANT WITH AMIODARONE-INDUCED THYROTOXICOSIS: A CASE REPORT

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**Introduction:** Amiodarone is a widely used antiarrhythmic drug that contains large amounts of iodine and it is a highly lipophilic drug extensively distributed into tissues, namely in adipose tissue and skeletal muscle where it accumulates in large amounts during long-term treatment. Considering that this agent has a long elimination half-life, the maintenance of a state of iodine excess in amiodarone-treated patients leads frequently to thyroid function changes. Amiodarone-induced thyrotoxicosis has been many times associated with serious cardiovascular derangements.

**Case Report:** A 53-year-old male was admitted to the Cardiology department due to acutely decompensated heart failure (HF). He was followed at the HF outpatient consultation and was included in the heart transplant waiting list due to a dilated cardiomyopathy with severe biventricular dysfunction, at ambulatory with NYHA III+ class. The patient also had permanent atrial fibrillation (AF). He was medicated with lisinopril 2.5mg qd, spironolactone 100mg qd, bisoprolol 2.5mg qd, furosemide 80mg bid, dapagliflozin 10mg qd, atorvastatin 40mg qd and warfarin. On admission, the patient was normotensive, with heart rate of 123 bpm, hypoxemic ($O_2$Sat 87%), with signs of pulmonary and systemic congestion. The blood work-up revealed: TSH - 0.02 µUI/mL(0.35-4.94), free T3 - 4.09 ng/dL(1.71-3.71) and free T4 - 2.46 ng/dL(0.70 -1.48), with negative TSH receptor antibodies. Besides the persistent rapid ventricular response AF, there were no other symptoms of hyperthyroidism. He had no history of familiar thyroid disease. Thyroid ultrasound revealed rare infracentimetric bilateral nodules, but the pattern of thyroidal vascularization was not described. After reviewing previous prescriptions, it was found that the patient took amiodarone between January/2019 and December/2019. He initiated methimazole 10mg bid and propranolol 40mg qid) with no clinical nor analytical response and, for that reason, prednisolone 40mg qd was added to the therapy. Methimazole dosage was progressively escalated until methimazole 20mg bid, but the thyroid function remained uncontrolled. The patient was then submitted to total thyroidectomy (after preoperative near-normalization of thyroid function with Lugol`s iodine) and medicated with levothyroxine. Two months after surgery, there was a marked clinical improvement to NYHA class II, with heart rate of 70-80bpm, and reversal of the associated heart failure cachexia. For that reason, it is being discussed the transitory withdrawal of the patient from the cardiac transplant list.

**Conclusion:** A careful history and a high index of suspicion are needed to correctly diagnose an amiodarone-induced thyrotoxicosis. In advanced heart failure patients with this thyrotoxicosis, not responding to medical therapy, the total thyroidectomy may be the key to achieve clinical stabilization and improve prognosis.

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### UNUSUAL COMPLICATION OF A SUBSTERNAL GOITER – GASTROINTESTINAL BLEED

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**Introduction:** Substernal goiters represent a common and challenging clinical scenario in medical practice. Symptoms often include dysphagia, dyspnea and hoarseness, being vascular compressive symptoms unusual. When present, they may relate to a superior vena cava syndrome that is often slowly progressive. Its gradual worsening allows the development of collateral circulation that rarely affects the upper esophagus. The authors report on a case of patient admitted for an upper gastrointestinal hemorrhage, whose source was downhill upper esophageal varices that developed in the context of the substernal goiter.

**Case Report:** A 81 year old woman, with atrial fibrillation, COPD, heart failure and a long term history of toxic multinodular substernal goiter was admitted to the hospital for a normocytic normochromic anaemia (Hb 8.3g/dL) of acute onset, developing in the setting of a gastrointestinal hemorrhage (melenas). Patient was hemodynamically unstable at admission and required volume support with iv fluids and blood products (1 Unit of erythrocyte concentrate). Upper endoscopy revealed the presence of tortuous esophageal mucosa with bulging of the proximal segment and downhill varices with red spots suggesting recent bleeding. (figure 1) Neck and Thoracic CT scan documented a substernal asymmetric goiter (right lobe - 9x6.7x5.6cm; left lobe 12.5x7.5x9cm), reaching the azygos vein crossing, compressing trachea, esophagus and both brachiocephalic veins. This caused an extensive collateral venous circulation, giving rise to the esophageal varices (figure 1). Despite surgical indication for a total thyroidectomy, performance status, comorbidities and patient's wishes did not allow for an adequate treatment approach.

**Conclusion:** We report a rare case of downhill variceal haemorrhage caused by a compressive substernal goiter. The patient presented with gastrointestinal bleeding – melenas - and hemodynamic instability. Timely surgical

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treatment of substernal goiters are recommended in order to prevent complications, especially in the elderly, in whom prognosis may be dismal.

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## REFRACTORY GRAVES DISEASE OR FACTITIOUS THYROTOXICOSIS

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**Introduction:** Graves disease is the most frequente cause of primary hyperthytoidism in women of childbearing age. Clinical severity varies significantly, being neuropsychiatric symptoms common. Patients with underlying psychiatric disease predisposition may be more susceptible to new onset and agrravation of anxiety and panic disorder, as well as to the development of desadaptive behavior. The authors report on a patient with Graves disease and factitious thyrotoxicosis.

**Case Report:** A 43 year of female with past medical history of generalized anxiety disorder, asthma and arterial hypertension, was referred to the Endocrinology department for thyrotoxicosis. She was diagnosed with Graves disease at the age of 21 (post-partum period) and underwent medical therapy (methimazol and lithium), followed by radioactive iodine ablation (10mCi), subtotal thyroidectomy and two additional radioactive iodine ablation treatments (cumulative activity of 25mCi) due to refractory thyrotoxicosis. Patient-reported medication included methimazole 30mg/day. Blood workup: TSH <0.005, freeT4 3.1ng/dL (NR 0.93-1.7), freeT3 5.2pg/mL (NR 2.3-4.2), TSH-receptor antibodies <0.8.Whole body $^{123}$i scan did not reveal tissue uptake and Thyroglobulin was <0.1ng/dL. This was compatible with factitious thyrotoxicosis, justifying referral to a psychiatric consultation, where follow up ensued.

**Conclusion:** This case reports highlights the emotional and psychiatric impact of Graves disease in a patient with underlying anxiety disorder. Early recognition and referral to psychiatry should always be considered when symptoms are severe at disease onset and when clinical and biochemical response is inadequate. This patient certainly underwent excessive therapy, whose associated risks would have been avoided if appropriate psychiatric evaluation and follow up would have been undertaken.

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## CARDIOVASCULAR EVENTS IN THE COURSE OF SUBACUTE THYROIDITIS – A REPORT OF THREE CLINICAL CASES

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**Introduction:** In the clinical course of subacute thyroiditis (ST), the combination of long-lasting inflammation and hyperthyroidism could increase the risk for cardiovascular (CV) events. We report three clinical cases of patients diagnosed with ST and treated with methylprednisolone (a starting dose of 24 mg/day with decreasing the dose for 4 mg every 5 days).

**Case Reports:** Patient 1 complained of chest pain during a clinical examination for ST. Electrocardiogram (ECG) did not show signs of acute ischemia, nevertheless he was admitted because of slighlty elevated troponin I 0.16 ml/L (normal level <0.1 mg/L). After 2 days, further elevation of troponin I to 0.898 mg/L and dynamic ECG changes were observed. Percutaneous coronary intervention in two coronary arteries was performed. In addition to methylprednisolone, aspirin, ticagrelor, perindopril, rosuvastatin and bisoprolol were prescribed. The patient recovered fully with a mild transient hypothyroidism.

**Table 1.** Clinical characteristics of three male patients with subacute thyroiditis (for Abstract 168)

| Parameter | Patient 1 | Patient 2 | Patient 3 |
|---|---|---|---|
| Age (years) | 53 | 81 | 60 |
| TSH (mIU/L; ref. range 0.59–4.23) | 0.01 | 0.02 | 0.01 |
| Free $T_4$ (pmol/L; ref. range 11.5–22.7) | 52.0 | 36.5 | 32.8 |
| Free $T_3$ (pmol/L; ref. range 3.5–6.5) | 16.44 | 10.2 | 6.9 |
| Sedimentation rate (mm/h) | 105 | 55 | 42 |
| Cardiovascular risk factors | Smoking | Arterial hypertension, hyperlipidemia | Arterial hypertension, hyperlipidemia, type 2 diabetes, ex-smoker |

Patient 2 presented 13 days after starting treatment for ST with acute dysarthtria and left-side hemiparesis. He was diagnosed with lacunar ischemic stroke and succesfully treated with thrombolysis without neurological consequences. Treatment of ST was also successful with disapperance of thyroid pain and complete normalization of thyroid function.

Patient 3 complained of malaise and growing tender mass in the right thyroid lobe for four months. In this period, he was evaluated twice at emergency department – first because of temporary loss of vision in the right half of the visual field that lasted several hours and 2 months later because of transient paresis in the left hand. Both times, he was diagnosed with transitory ischemic attack. Clopidogrel was added to aspirin for 3 weeks, along with treatment of pre-existing diseases. Diagnosis of ST was confirmed with fine-needle biopsy. Treatment with methylprednisolone led to a quick clinical and laboratory improvement of ST.

**Conclusion:** Although the course of ST is usually mild, we must not overlook the associated pathology that could lead to life-threatening complications. Further research should focus on the combined effect of inflammation and hyperthyroidism on cardiovascular events.

# COVID

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## SUBACUTE THYROIDITIS AT THE TIME OF SARS-COV-2 PANDEMIC

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**Background:** Subacute thyroiditis (SAT) has been related to acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We evaluated the incidence and the severity of SAT due to SARS-CoV-2.

**Methods:** A cross-sectional, retrospective study was conducted at the Endocrinology Unit of University-Hospital of Pisa, Italy. All patients

experiencing SAT arisen within a period of 15 days earlier and yet untreated, assessed from January 2016 to December 2020, were included in the study. SAT cases from 2016 to 2019 (N=152) were defined as "pre-SARS-CoV-2" , while 2020 SAT patients were classified as "pos-SARS-CoV-2" (N=13) or "neg-SARS-CoV-2" (N=24) according to positive or negative test for SARS-CoV-2 at SAT onset or within a period of 45 days earlier.

**Results:** While in the years 2016-2019 most SAT cases were observed in the 3rd quarter, in 2020 there were two peaks, in the 2nd and in the 4th quarters, superimposable to the two main outbreaks of SARS-CoV-2. Compared to the same quarters of the years 2016-2019, in the 2nd and the 4th quarters of 2020 we observed higher levels of free thyroxine (FT4), C-reactive protein (CRP) and thyroglobulin (Tg). Compared to pre-SARS-CoV-2, pos-SARS-CoV-2 had higher FT4 (28.1 vs 24.1 nmol/L), CRP (8.0 vs 3.6 mg/L) and Tg (155 vs 60 mcg/L) (P<0.05 for all) and resulted more frequently in hypothyroidism at 3 months (9/10 vs 30/152) (P<0.001). Neg-SARS-CoV-2 patients showed a clinical picture intermediate between pre-SARS-CoV-2 and pos-SARS-CoV-2 patients.

**Conclusions:** Most SAT cases in 2020 were associated with SARS-CoV-2 infection and were more severe than those previously observed.

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## SUBACUTE THYROIDITIS AND COVID 19

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Sars-COV-2 is the cause of acute respiratory illness. It is often complicated by respiratory distress syndrome, polyhedral insufficiency, disruption of the normal functioning of vital organs and systems. Prescribing anti-inflammatory therapy for COVID19 masks both clinical signs and laboratory manifestations of other acute and subacute diseases that may appear in patients with COVID19.

The aim of the study. To assess the features of laboratory and instrumental parameters of subacute thyroiditis (STh) in unvaccinated COVID19 in women.

**Materials and Methods:** The study involved 16 patients with STh, aged 24 to 46 years. Group 1 consisted of COVID19 convalescent patients (n=8); 2nd group - with STh without COVID19 in medical history (n=8).

The levels of erythrocytes (Er), the average volume of erythrocytes (MCV), hemoglobin, leukocytes, lymphocytes, band cells, platelets, erythrocyte sedimentation rate (ESR), thyroid-stimulating hormone (TSH), free thyroxine (T4fr), thyroid peroxidase antibodies (TPO antibodies), C-reactive protein (CRP) in the peripheral blood were determined; ultrasound examination of the thyroid gland (Toshiba SSA-790A (Japan)), thyroscintigraphy (Mediso "AnyScanS") with 99mTc-pertechnetate, with the calculation of the uptake index of the radiopharmaceutical (RF) were conducted as well.

**Results:** The results are shown in Table 1.

As a result of the study, it was found that patients with COVID19 convalescents with clinical signs of STh had significantly lower leukocyte counts ($4.7\times10^9$/l vs $9.7\times10^9$/l, p=0.032), ESR values (24mm/h vs 67mm/h (p=0.027), the highest CRP values (37mg /l vs 19mg/l, p=0.025), T4fr (74.34pmol/l vs 19.0 pmol/l, p=0.027), increased TPO antibodies (31.0U/ml vs 19.0U/ml (p=0.041)), Uptake index (0.05% vs 0.15%, (p=0.01)). Negative Spearman correlation dependences were obtained in group 1: decrease in Uptake index, is associated with an increase in CRP indices (r=-0.735). T4fr (r=-0.768). In Group 2, a decrease in the Uptake index is associated with an increase in ESR (r =-0.787), leukocyte count (r=-0.681), lymphocytes (r=-0.619).

**Conclusion:** Laboratory and instrumental indicators of STh in a patient with COVID19 are characterized by leukopenia, relatively low ESR values, increased CRP levels, the lowest Uptake index, which is significantly distinguishable from those of group 2. An increase in CRP, T4fr is associated with a decrease in the Uptake index thyroscintigraphy data.

**Table 1.** Comparative characteristics of indicators of groups 1 and 2 (for Abstract 85)

| Indicator | Group 1 Me [25;75] | Group 2 Me [25;75] |
|---|---|---|
| Leukocytes (x$10^9$/л) | 4.7[4.3;6.2]* | 9.7[8.4;11.6] |
| Band cell (%) | 3.0[2.0;3.20] | 4.0[4.0;5.0] |
| Lymphocytes (%) | 24.0[19.0;27.0]* | 40.0[32.0;41.0] |
| ESR (mm/h) | 24.0[23.0;27.0]* | 67.0[66.0;70.0] |
| CRP (mg/l) | 33.0[29.0;37.0]* | 19.0[15.0;20.0] |
| T4fr (pmol/l) | 74.34[60.3;67.0]* | 59.0[75.0;88.1] |
| TSH (mU/l) | 0.05[0.07;0.20] | 0.07[0.02;0.12] |
| TPO antibodies (U/ml) | 31.0 [29.00;31.00]* | 19.00[14.00;20.00] |
| Uptake index (%) | 0.07[0.05;0.10]* | 0.15[0.10;0.240] |

*p<0.05

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## THE PROBABILITY OF DEVELOPMENT OF SUBACUTE THYROIDITIS POST COVID-19 INFECTION

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**Background:** -Subacute thyroiditis is a form of thyroiditis that can be a cause of both thyrotoxicosis and hypothyroidism. It is uncommon, but individuals of both sexes can be affected. Subacute granulomatous, which is the most common form, is accompanied with muscle aches, fever and malaise.

-Coronavirus disease (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2.

**Objective:** To evaluate the occurrence of subacute thyroiditis as a result of Covid-19.

**Methods:** In total,1357 cases were enrolled in the study. Ages varied from 18-70 years old. 426 of the patients, who did not have thyroid issues prior to catching Covid-19, developed subacute thyroiditis. These patients were put into 2 groups. The first group included 150 patients, who were prescribed steroids alongside antibiotics and the second group consisted of 276 patients, all of whom got a symptomatic treatment.

**Result:** 1.≈31,4% of the patients developed subacute thyroiditis as a result of catching Covid-19.

2. Antibiotics and steroids do not have a connection to the development of subacute thyroiditis.

**Conclusion:** The thyroid gland is an organ with rich vascularity, so as a result, the formation of (micro)thrombosis, which is typical to Covid-19, causes aseptic inflammation zones, which in their turn form lymphoid infiltrative hotspots, appearing as subacute thyroiditis.

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## THYROID FUNCTION IN COVID-19 PATIENTS

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**Introduction:** It has been recognized that the main target organs attacked by SARS-CoV-2 are the lungs and immune system, but it is unclear whether SARS-CoV-2 has an effect on thyroid function. At present, one year after the pandemia onset, whether COVID-19 has an effect on thyroid function is yet unclear. Some patients with serious diseases other than thyroid disorders demonstrated abnormal levels of thyroid hormones, which are collectively called as nonthyroidal illness syndrome (NTI) or euthyroid sick syndrome. The most typical alterations are decreased plasma triiodothyronine (T3) level, low or normal plasma thyroxine (T4) level, and normal or slightly decreased TSH level.

**Material and Methods:** In this retrospective study, the thyroid hormone (FT3, FT4) and thyrotropin (TSH) of patients with COVID-19 were compared with those in control groups. All COVID 19 patients were treated at KGH, also control group patients and healthy ones were from GH policlinic facilities.

**Results:** Patients received no thyroid hormone replacement therapy, and the levels of the thyroid hormones returned to normal.Of the patients with COVID-19 and measured thyroid hormones, 80,95% (51/63) had lower-than-normal TSH and/or FT3 levels. The isolated lower serum TSH had 23,80% (15/63), isolated lower FT3 values 33,33% (21) and both TSH and FT3 levels 23,80% (15/63) of the patients with COVID-19.

The clinical classification of the 63 confirmed COVID-19 cases with thyroid assessment was severe in 33,33% (23/63), and critical in 66,66% (42/63) cases. The more severe the COVID-19 infection was, the lower the TSH and fT3 levels were with statistically significant differences

**Conclusions:** The FT3 and FT3/TSH levels of the patients with COVID-19 were significantly lower in the severe and critical group compared with non-COVID-19 pneumonia patients with a similar degree of severity. Lower TSH also indicates there might be a unique effect of COVID-19 on TSH-secreting cells. Shortcomings of this study was that hormones were not assessed at admission according to the retrospective nature of the study, also the thyroid hormones were tested while most patients were receiving glucocorticoid

**Table 1.** Comparison of TSH, FT3 and FT4 between COVID-19, Healthy control and Non Covid-19 pneumonia patients (for Abstract 105)

| Letter name | COVID-19 (N=63) | Healthy control (N=60) | Non COVID-19 (N=60) |
|---|---|---|---|
| TSH | 0,61 **,## | 1,99 | 1,62 |
| FT3 | 2,63 **,# | 4,15 | 3,63 |
| FT4 | 11,76 | 12,69 | 14,76 |

**p<0,1 compared with healthy control, # p<0,05, ##p<0,01 compared with Non COVID-19 pneumonia patients

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## THYROID FUNCTION IS AFFECTED BY COVID-19 DISEASE SEVERITY AND TREATMENT

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**Background:** Moderate-to-severe Covid-19 disease seems to affect thyroid function with different mechanisms: non-thyroidal illness syndrome, thyroiditis and cytokine storm. We aimed to analyse thyroid function in hospitalised Covid-19 patients according to disease severity and treatments received.

**Methods:** 174 patients hospitalised in sub-intensive care units for Covid-19 during the 1st (W1; Mar-Aug 2020, n=130) or 2nd (W2; Oct-Dec 2020, n=44) pandemic waves were assessed at hospital admission (baseline) for thyroid function, inflammatory markers and treatments received. Thyroid ultrasound was also performed after 2-3 months in 52 patients.

**Results:** W1 and W2 did not differ in mean age (68 years), male predominance (65%), median length of hospitalisation (20 days) and death rate (25%). However, W2 patients required more non-invasive ventilation (75% vs 47% W1) than intubation (0%, vs 13% W1, P<0.01). Drugs administered for Covid-19 were also significantly different: W2 patients, compared with W1, received more steroids (P<0.01) and heparin (P=0.04), but little if any paracetamol (P<0.01), hydroxychloroquine (P<0.01), IL-1R antagonist anakinra (P<0.01) and anti-virals (P<0.01).

After excluding patients with a history of thyroid dysfunction and amiodarone, thyroid analysis was performed on 151 total W1&W2 patients. Heparin treatment did not affect FT4 (P=0.36) since administered at least 8 hours before testing. However baseline TSH was significantly reduced in 36 patients commenced with early steroid treatment at hospitalisation compared with those untreated (0.41 vs 1.15 mIU/L, P<0.01), thus they were excluded from further analysis. Lower baseline serum TSH concentrations were associated with greater need of oxygen support during hospitalisation (P=0.02). TSH also positively correlated with lymphocyte count (P<0.01) and albumin (P=0.01), but not IL-6 and CRP. Serum FT3 concentrations correlated positively with albumin (P<0.01) and negatively with D-dimer (P<0.01), length of hospitalisation (P=0.04) and death rate (P=0.03). Thyroid ultrasound showed focal areas of thyroiditis (FT) in 17/52 (33%). 10/52 (19%) had low TSH; FT were present in 6/10 (60%) patients with low TSH, versus 11/42 (26%) normal TSH (P=0.04).

**Conclusions:** Steroids, mainly administered during W2, massively impact on serum TSH concentrations; heparin does not impact on serum FT4 concentrations if administered several hours before testing.

Baseline abnormal thyroid function correlates with increased death rate, length of hospitalisation, albumin and D-dimer. In particular low serum concentrations of TSH at baseline correlate with lymphopenia and are associated with increased need of oxygen support during hospitalisation. Of interest, low serum concentrations of TSH are also associated with the presence of focal areas of thyroiditis at ultrasound.

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## NEW ONSET GRAVES' DISEASE AFTER SARS-COV-2 INFECTION- CHANCE OR ASSOCIATION?

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**Introduction:** COVID-19 infection can have effects on multiple endocrine organs. It is now well known that severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection can lead to inflammation of thyroid gland leading to subacute thyroiditis. However in contrast to it, onset of Graves' disease after SARS- CoV-2 infection is very rarely reported. To the best of our knowledge only five such cases have been earlier reported in literature.

**Case Report:** A 40 year male presented with symptoms of weight loss, tremors and palpitation for last one month. There was no history of any thyroid disorder or autoimmune disorder among family members or self. A thyroid function test which was done 8 months ago for routine health checkup was found to be normal. He has been diagnosed with mild SARS- CoV-2 infection two months prior to current presentation and was treated symptomatically then. He had not received any steroids during that time. There was no history of sore throat or throat pain in past month. On clinical evaluation, he was afebrile, had tachycardia (pulse rate-108 beats/min) along with fine tremors on outstretched hands. Thyroid gland was normally palpable without evidence of tenderness. No obvious ophthalmopathy or dermopathy was present. Rest systemic examination was normal. Biochemical assessment showed presence of thyrotoxicosis with elevated free triiodothyronine (9.28pg/ml, normal: 2.02—4.4pg/ml) and free thyroxine levels (2.65ng/dl, normal: 0.93—1.7ng/dl) with suppressed thyroid stimulating hormone levels (<0.005mIU/ml, normal: 0.27–4.2mIU/ml). Complete blood count, renal function tests along with serum electrolytes and hepatic function tests were normal. Technetium 99m thyroid scintigraphy showed diffuse increased tracer uptake in bilateral lobes of thyroid. Anti –TSH receptor antibody was positive. Based on above findings, a diagnosis of Graves' disease was made. The patient was started on antithyroid drugs and beta blockers. As the patient did not had any prior thyroid related illness with a completely normal thyroid function test within a year, we postulate that perhaps SARS- CoV-2 infection might have triggered autoimmune thyroid disease in our case. Whether our finding is a mere coincidence or represents a genuine association remains to be seen.

**Conclusion:** New onset Graves' disease may represent a new facet of endocrine manifestations following SARS- CoV-2 infection. Though our assumption remains speculative, further studies are required to conclusively ascertain the temporal association of prior SARS- CoV-2 infection and onset of Graves' disease.

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## CAN COVID-19 TRIGGER SUBACUTE THYROIDITIS?

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**Introduction:** The actual coronavirus disease 2019 (COVID-19) pandemia, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has reached more than 123 million confirmed cases worldwide since December 2019.

**Objective:** The potential interaction between SARS-CoV-2 and the thyroid is poorly understood. Up to now, there have been reported a few cases that described the association between COVID-19 and subacute thyroiditis (SAT, de Quervain thyroiditis).

**Methods:** Here, we present a case series of seven patients with clinical manifestations and physical examination in favor of SAT after recovering from COVID-19 disease. Demographic, clinical, biochemical, and imaging data were presented.

**Results:** The mean ± SD patient age was 41.0±15.0 years at diagnosis; 5 patients were male. At the time of diagnosis, thyroid function tests revealed hyperthyroidism together with consistent ultrasonographic evidence suggesting SAT. Elevated C-reactive protein, erythrocyte sedimentation rate were found in laboratory evaluations. Anti-thyroid peroxidase, anti-thyroglobulin, and thyroid-stimulating hormone receptor antibodies were negative in all patients. Both IgM and IgG were positive for COVID-19 infection, but the PCR tests were negative in all patients. Treatment with corticosteroids resulted in rapid clinical resolution. Patients were followed up for 2 month.

**Conclusion:** Our goal is to alert clinicians about this new entity and recognize the symptoms of thyroid dysfunction, which could go unnoticed during the height of this pandemic.

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## THE POSITIVE RATE OF COVID-19 TEST IN PATIENTS WHICH PERFORMED FOR SCREENING BEFORE THYROID FINE NEEDLE ASPIRATION BIOPSY

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**Objectives:** Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is spreading rapidly around the world. COVID-19 infection presents with clinical symptoms ranging from asymptomatic infection to mild upper respiratory tract disease and severe interstitial pneumonia, and it may cause mortality in some patients. Asymptomatic infection is highly contagious, potentially leading to viral spread. We aimed to determine the rate of patients positive for COVID-19 PCR performed for screening before thyroid fine needle aspiration biopsy (FNAB). In this way, we aimed to see the frequency of asymptomatic cases among patients with thyroid nodules and to determine the frequency of health care professionals' to encounter and thus the contact with COVID-19 with positive patients during the invasive procedure.

**Materials and Methods:** The patients who applied to Ankara City Hospital, Endocrinology and Metabolism Diseases outpatient clinic between 15.03.2020 and 15.08.2020 and who underwent routine COVID-19 PCR test before FNAB for screening was evaluated retrospectively. FNAB was performed in patients with multinodular goiter (MNG) and papillary thyroid cancer (PTC). Age, gender, history of hypertension and type 2 diabetes, and use of levothyroxine (LT4) or antithyroid drug were obtained from the records.

**Results:** Asymptomatic COVID-19 infection was detected in 29 (2.43%) of 1195 patients who underwent FNAB. The mean age was 51±13.1 years. Thyroid autoantibodies were positive in 13(44.8%) patients. 4 (27.9%) patients had type 2 diabetes and 6 (20.7%) patients had hypertension. Clinical diagnosis was MNG in 24 (82.8%) and PTC in 5 patients (17.2%).

**Conclusion:** The healthcare professionals work devotedly against COVID-19 infection, demonstrating a great example of struggle worldwide. This brings increased infection risk. FNAB is an invasive procedure requiring close contact and sometimes the contact time is prolonged depending on the number of nodules. It should be known by the clinician that COVID-19 infection is associated with a high risk of transmission in asymptomatic patients, and this risk is also high in the early stages of the disease. The rate of 2.43% in the population can not be underestimated and indicates for the importance of the use of personal protective equipment and taking infection prevention measures for healthcare workers.

**Keywords:** SARS-CoV-2, COVID-19, asymptomatic infection, FNAB, screening

# Diagnostic Tools in Thyroid Cancer

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## THE DIAGNOSTIC VALUE OF BASAL AND CALCIUM-STIMULATED PROCALCITONIN FOR THE DIAGNOSIS OF MEDULLARY THYROID CANCER

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**Introduction:** calcitonin (CT) is the reference marker for the diagnosis of a medullary thyroid carcinoma (MTC), but its measurement presents some pitfalls. Recently, procalcitonin (proCT) has been proposed alternatively or in addition to CT in MTC diagnosis.

**Methods:** basal CT and proCT levels (bCT and bproCT) were measured on the serum, presurgically withdrawn, of a series of consecutive MTC (n=43) and non-MTC (n=75) patients retrospectively collected from tissue and serum Bank. Another set of consecutive 33 patients, were subjected to calcium-stimulation test and then underwent surgery, with final diagnosis of MTC (n=20) and non-MTC nodular disease (n=13). This second set of patients came from 3 tertiary-level institutions. BproCT, bCT and stimulated proCT and CT (sproCT and sCT) were measured. Only sporadic MTC were considered, being patients carrying a germline *RET* mutation excluded.

**Results:** median bproCT values were significantly higher in MTC than non-MTC patients (0.64 ng/ml, IQR: 0.24-5.91 ng/ml and 0.04 ng/ml, IQR: 0-04-0.04 ng/ml, respectively, P<0.01). Median bproCT values paralleled T stages, being 0.35 ng/ml (IQR: 0.18-0.89 ng/ml) in T1, 5.41 ng/ml (IQR:1.43-10.29 ng/ml) in T2 and 15.9 ng/ml (IQR: 6.02-33.56 ng/ml) in T3 (P<0.01) and were higher in lymph-node positive patients at diagnosis (N1a+N1b) than in N0 patients (11.70 ng/ml, IQR: 2.92-26.48 ng/ml and 0.35 ng/ml, IQR: 0.12-0.92 ng/ml respectively, P<0.01). A positive correlation was found between bproCT and bCT (P<0.01, $R^2$=0.75) and tumor size (P<0.01, $R^2$=0.39). At the receiver operating characteristic (ROC) curve analysis, the cut-off of bproCT able to differentiate between non-MTC and MTC patients was >0.07 ng/ml (sensitivity: 85.7%, specificity: 98.9%, positive predictive value (PPV): 98.2%, negative predictive value (NPV): 90.6%, AUC: 0.942 and P<0.01). While bproCT was > 0.07 ng/ml in 38/39 (97.4%) of MTC larger than 10 mm, bproCT was above the said cut-off only in 15/23 (65.2%) patients with a tumor <10 mm. A positive correlation was found between sproCT and sCT (P<0.01, $R^2$=0.46). A sproCT >0.19 ng/ml was able to identify an MTC with the following accuracy: sensitivity: 90.0%, specificity: 100.0%, PPV: 100.0%, NPV: 86.7%, AUC: 0.938 (P<0.01). In patients with bCT between 10 and 100 ng/L, bproCT performance was as follows: sensitivity: 70.4%, specificity: 100.0%, PPV: 100.0% and NPV: 40.0%.

**Conclusions**: proCT cannot replace CT as the standard of care in the MTC diagnosis, because it often fails in the identification of small size MTCs. Besides that, it has a very high specificity and it can be used in combination with to CT in MTC diagnosis, particularly in the context of mildly elevated basal CT levels.

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## ULTRASOUND PREDICTORS OF MALIGNANCY IN PAPILLARY THYROID CANCERS DEPENDING ON THE TUMOR SIZE

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**Background:** Ultrasonography is the main diagnostic tool for assessing the malignancy risk of thyroid nodules. The prognostic value of the high-risk ultrasound characteristics has been extensively analyzed. Although the size of the nodule is not among the ultrasound predictors of malignancy, it has been taken into account in determining the indications for FNAB (EUTIRADS).

**Objective:** To examine the ultrasound characteristics of malignant nodules in patients with papillary thyroid carcinoma and to analyze the incidence of suspicious ultrasound features in cancers with a diameter less and more than 10 mm.

**Patients and Methods:** A total of 302 patients (F:M=4.92:1; mean age 49.16±1.4 2 years) diagnosed with papillary thyroid carcinoma were included in the study. 319 malignant thyroid nodules were analyzed. An ultrasound neck examination, FNAB with cytological analysis and histological examination of the surgically removed thyroid nodules were performed.

**Results:** Compared to subcentimeter tumors, cancers with a diameter greater than 1 cm were more common in men than in women (OR=2.801; p=0.03) and were solitary (OR=3.353; p=0.003). They were characterized by higher frequency of irregular shape (OR = 2.145; p<0.05), presence of microcalcifications (OR = 3.422; p=0.001), intranodal blood flow (OR=14.123; p<0.0001) and regional lymph node metastases at diagnosis (OR=12.853; p<0.0001). Marked hypoechogenicity and posterior acoustic shadowing were more common in cancers with a diameter less than 10 mm (OR=4.496; p<0.0001 and OR=2.790; p=0.01 respectively). No differences between the two groups were seen in terms of the presence of autoimmune thyroiditis, localization within the gland, presence of posterior acoustic enhancement, peripheral and macrocalcifications and taller than wide shape in transverse scan.

**Conclusion:** Ultrasound characteristics suggestive of malignancy occur with a different frequency in thyroid cancers with a diameter less and more

than 10 mm. Possible cause of this difference is the change in tissue characteristics during tumor growth. The data from this and other studies focused on the problem can be useful in further stratification of the malignancy risk in patients with EUTIRADS 5 nodules.

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## SERUM TSH LEVEL AS A PREDICTOR FOR MALIGNANCY IN INDETERMINATE NODULES

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**Background:** Studies suggest an association between serum thyroid-stimulating hormone (TSH) levels and an increased risk of differentiated thyroid cancer.

**Aim:** To investigate the utility of TSH levels in predicting malignancy in thyroid nodules characterized as AUS/FLUS by fine needle aspiration cytology.

**Methods:** We collected clinical data of all patients who underwent thyroidectomy for AUS/FLUS thyroid nodules at our hospital between 2016 and 2020. We evaluated TSH values by chemiluminescent immunoassay before the surgery. Thyroid cancer was diagnosed by histopathology. We excluded patients receiving thyroid medication and those with Non Invasive Neoplasm with Papillary-like Nuclear Features (NIFTP) diagnosis.

**Results:** A total of 112 patients (116 nodules) were included. Mean age was 52.8±11.7 years and 88,4% (n=99) were female. Malignancy was found in 26.7% (n=31) of the nodules. Mean TSH value was similar between patients with thyroid cancer (2,7μU/mL) and patients with benign nodules (2,1μU/mL) (p>0,05).

**Conclusion:** In our analysis TSH value was higher in patients with thyroid cancer but we couldn´t establish a significant association.

# Follow-Up in Differentiated Thyroid Carcinoma

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## BONE MINERAL DENSITY IN ADULT SURVIVORS OF PEDIATRIC DIFFERENTIATED THYROID CARCINOMA: A LONGITUDINAL FOLLOW-UP STUDY

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**Objectives:** Survivors of pediatric differentiated thyroid carcinoma (DTC) receive thyrotropin (TSH) suppressive therapy to minimize disease recurrence. However, knowledge about long-term effects of subclinical hyperthyroidism on bone mineral density (BMD) in pediatric DTC survivors is scarce, as is the information regarding long-term consequences of permanent hypoparathyroidism on BMD. We evaluated BMD in pediatric DTC survivors and investigated if BMD was affected by subclinical hyperthyroidism and/or permanent hypoparathyroidism during long-term follow-up.

**Methods:** In this nationwide longitudinal study, we determined BMD in the lumbar spine and femur by dual energy X-ray absorptiometry (DXA) in 65 pediatric DTC survivors. Measurements were repeated after 5 years of follow-up in 46 pediatric DTC survivors. At both visits, we determined biochemical parameters and markers of bone resorption (C-terminal telopeptide of type I collagen (β-CTX)) and formation (N-propeptide of type I collagen (PINP) and osteocalcin (OC)).

**Results:** First measurement of BMD was done in 65 DTC survivors after a median follow-up of 17 years (IQR 8.0-25.5) after diagnosis. Median age at diagnosis was 15 years (IQR 13.0-17.0). Twenty-five percent of the survivors had subclinical hyperthyroidism. In most survivors, BMD T- and Z-scores were within the reference range. The 46 survivors, in whom a 2nd BMD measurement was done, no aberrant median BMD parameters were present. However, in the 2nd evaluation, after a median follow-up time of 23.5 years, osteopenia and osteoporosis were found in 17.4% and 2.2%, respectively. In the 13 survivors with hypoparathyroidism, BMD values did not differ after 5 years of follow-up compared to baseline values or in comparison with the 33 survivors without hypoparathyroidism. During follow-up, turnover markers β-CTX and PINP remained stable.

**Conclusions:** This longitudinal study of pediatric DTC survivors demonstrated normal and stable median lumbar spine and femur BMD values after a median time of 17 and 23.5 years after diagnosis. Osteopenia was still found in 17.4% after prolonged follow-up despite intensive follow-up. Based on these reassuring data, monitoring of bone mass during follow-up may be done at low frequency in pediatric DTC survivors.

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## THE IMPACT OF PREGNANCY ON DISEASE OUTCOME IN WOMEN WITH PERSISTENT DIFFERENTIATED THYROID CARCINOMA

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**Background:** Pregnancy does not cause differentiated thyroid cancer (DTC) recurrence in patients without structural or biochemical evidence of disease at the time of conception. On the contrary, pregnancy might be associated with an increased progression risk in patients not cured before pregnancy, but scanty data are available on this topic.

**Aim:** The aim of the study was to determine whether pregnancy could significantly influence the outcome in DTC patients in persistence before pregnancy, but with a biochemical and structural stable disease.

**Methods:** This was a retrospective evaluation of all women followed for DTC at a tertiary Italian thyroid cancer center who had a pregnancy after initial treatments between 2003 and 2020. Subjects included were required to have biochemical and/or structural persistence within 12 months before pregnancy.

**Results:** We enrolled 8 patients with papillary thyroid carcinoma (PTC) (7 conventional variant PTCs and 1 sclerosing variant PTC) with a mean age at diagnosis of 27.6 yo (range 21-35) and a mean time between PTC diagnosis and pregnancy of 60 months (range 12-120).

Among the 7 patients with structural disease, 2 patients had lung metastases and 5 had neck lymph node metastases; only 1 patient showed biochemical persistence of disease without radiological evidence.

The treatment for PTC included total thyroidectomy, lymphadenectomy and radioactive iodine ablation (RAI) in all women. In accordance with the 8th edition of the American Joint Commission on Cancer (AJCC) and 2015 American Thyroid Association (ATA) Guidelines, 75% of women included had a AJCC Stage I and an intermediate risk of recurrence, whereas the 25% had a AJCC Stage II and an ATA high risk of recurrence.

Evaluation of Dynamic Risk Stratification (DRS) during the 24 months of follow-up showed 88% patients with structural incomplete response and 12% with a biochemical incomplete response.

During a mean follow-up of 153 months, none of the patients showed biochemical (serum thyroglobulin -Tg- or Tg antibody measurements) and radiological disease progression either during pregnancy or within 6 months after delivery, and no additional treatments were needed.

**Conclusions:** In conclusion, the present findings demonstrate that pregnancy is not associated with clinically significant disease progression in patients affected with PTC with stable local and/or distant persistence before conception. It remains to be established if the growth factors produced during pregnancy may worsen the disease outcome in DTC patients with rapid disease progression.

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## DO ULTRASENSITIVE THYROGLOBULIN IMMUNOASSAYS OVERCOME ULTRASOUNDS IN THYROID CANCER FOLLOW-UP?

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**Introduction:** Differentiated Thyroid Cancer (DTC) is the most common endocrine neoplasm, with an increasing incidence and a long-life expectancy. The possibility to carry out routine follow-ups with the only use of high-sensitive immunoassays for thyroglobulin (Tg) measurement has been recently proposed for low and intermediate risk DTC with a good 1-year response to treatment. The aim of this study was to evaluate the serum Tg levels in a series of patients with histologically proven local recurrences.

**Methods:** 50 consecutive patients with a histologically ascertained DTC recurrence and routinely followed-up in our centre were enrolled, and their clinical, histological and biochemical data were retrospectively collected. Median follow-up duration was 3.5 years (IQR: 1.55–7.6 years). Immunohistochemistry (IHC) analysis was performed on the recurrences in all patients with negative biochemistry.

**Results:** limited to AbTG negative patients (35/50), serum Tg tested negative in 5/35 (14.3%), indeterminate in 7/35 (20.0%) and positive in 23/35 (65.7%) patients. No difference between patients with negative serum Tg and detectable (≥0.2 ng/ml) serum Tg was observed in recurrence size documented at neck US (P=0.62), age at recurrence (P=0.13) and histological aggressiveness of the primary tumor (P=1.00). False negative rate of serum highly sensitive Tg according to their 1-year response to the treatment were: 14.3% in patients with an excellent response; 16.7% in patients with a biochemical incomplete response; 15.4% in patients with a structural incomplete response; 16.7% in patients with an indeterminate response. A positive, although weak, correlation was found between the recurrence size at neck US and serum Tg levels ($R^2$= +0.16, P=0.046) in patients with a detectable serum Tg (≥0.2 ng/ml), but no statistical difference in the recurrence size at neck US was found between patients with negative serum Tg and detectable serum Tg (P=0.91). False-negative rate in the 11 patients that presented the recurrence after more than 7.6 years (corresponding to the third interquartile in the median follow-up duration of this series) with negative TgAb rose up to 27.3%. The IHC confirmed the cytoplasmatic expression of Tg protein by tumor cells in recurrences in all patients with negative biochemistry

**Conclusions:** Serum Tg is not detectable in a not-negligible percentage of patients with a proven DTC recurrence, even when measured with a high-sensitive immunoassay and neck US remains essential to exclude the presence of a recurrence. Causes of this phenomenon are still unknown.

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## CLINICAL MANAGEMENT OF METASTATIC CERVICAL LYMPH NODES AFTER INITIAL TREATMENT IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

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The long-term outcome of loco-regional disease in patients with differentiated thyroid carcinoma is still to be clearly defined and the clinical management of suspicious cervical lymph nodes detected on ultrasound is still debated. The American Thyroid Association guidelines state that, in this context, the main challenge is to differentiate between low-volume metastatic disease that progresses with an aggressive behavior and that which remains stable.

Our study aimed to evaluate the rate of suspicious lymph nodes, detected after initial treatment (i.e. thyroidectomy with or without cervical lymphadenectomy and radioiodine treatment), that remained stable during follow-up. Secondary endpoints were the analysis of predictive factors of a more aggressive behavior of these metastases and the final outcome of the disease that progressed.

We retrospectively evaluated 95 patients who had a confirmed finding of suspicious cervical lymph nodes on ultrasound with a minimum follow-up of twelve months, and we divided patients in two groups according to the behavior of the metastatic lymph nodal disease. Aggressive disease was defined as a growth of at least 5 mm in the longest diameter at ultrasound of at least one suspicious lymph node detected from baseline to the end of follow-up, or a positive PET scan result.

After a mean follow-up of more than 9 years from initial treatment, 75/95 (79%) had a stable disease, while 20/95 (21%) had a progressive loco-regional disease. Patients with an aggressive disease were more frequently male (50% vs 25.3%, p=0.03), older (mean age 54.3 vs 38 years old, p=0.0003), and had a larger tumor size at surgery (32 vs 20 mm, p=0.005). No differences between the two groups were found regarding TNM, histology, PTC variant, extrathyroidal extension, multifocality, rate of central and/or lateral neck dissection at primary surgery, RAI ablation, ablative RAI dose, stimulated thyroglobulin at first RAI, positive thyroglobulin antibodies after initial treatment, finding of distant metastases at the end of follow-up.

Sixteen out of 20 patients with an aggressive disease had a structural disease at the end of follow-up, despite further treatments (i.e. lymphadenectomy, external beam radiotherapy, radioiodine treatment, tyrosine kinase inhibitors).

Our findings indicate that a vast majority of patients with metastatic cervical lymph node disease can be safely followed with serial biomarker evaluation and ultrasound, without needing further treatments. Around 20% of patients, in particular male, older patients, and with a larger tumor size at surgery may have lymph node metastases with an aggressive behavior requiring additional treatments.

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## INDEPENDENT PREDICTORS OF POOR PROGNOSIS IN WELL-DIFFERENTIATED THYROID CANCER

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**Introduction:** With the widespread use of US-FNAB, more indolent form of TC and early tumor stages are found, which can lead to overdiagnosis and overtreatment. The aim of this study is to identify the clinical and paraclinical indicators that would allow us to identify differentiated thyroid cancers at low risk from those at intermediate and high risk in order to offer them an appropriate initial treatment.

**Methods:** A retrospective chart review was conducted. Demographic, clinical, imaging and histological features were gathered from patients who underwent thyroid surgery from 2010 to 2017. The cohort was divided into two study groups based on the presence or absence of malignancy. A subgroup analysis was done among patients with WDTC. The histologic subtypes were recorded as a binary result: positive for high-risk tumors (FTC and intermediate and high-risk PTC) and negative for low-risk tumors (low-risk PTC). Logistic regression models were used to assess the correlation between the demographic, clinical and imaging variables and the histopathology result.

**Results:** 756 patients were included. 42.3% had a WDTC and 12.5% had a high-risk tumor. Malignancy was associated with younger age, absence of tracheal deviation, pre-operative serum TSH levels, hypoechogenicity, microcalcifications and irregular margins ($p < 0.05$). Male sex, radiation history, pre-operative serum calcitonin levels, substernal extension, tracheal compression, solid nodule, absence of halo and central vascularization were not statistically significant. Younger age (OR 2.504, p= 0.046), pre-operative serum TSH levels (OR 1.412, p= 0.025) and microcalcifications (OR 3.465, p= 0.002) were independent predictors of malignancy. Only microcalcifications (OR 2.287, p= 0.025) was associated with an increased risk of diagnosing a high-risk tumor.

**Conclusion:** Younger age, higher pre-operative serum TSH levels and microcalcifications were independent predictors of high-risk thyroid cancer, enabling to identify high-risk tumors that could benefit from total thyroidectomy. Clinical factors of poor prognosis, such as male sex and history of radiation, were not predictors of high-risk thyroid cancer.

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## ROLE OF [18F] FDG-PET/CT IN THE EVALUATION OF RESPONSE AND IN PREDICTING OUTCOME OF PROGRESSIVE METASTATIC RADIOIODINE-REFRACTORY DIFFERENTIATED THYROID CANCER PATIENTS TREATED WITH LENVATINIB

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**Introduction:** Metastatic lesions in radioiodine-refractory differentiated thyroid cancer (RAI-R DTC) that are positive at the [$^{18}$F] FDG PET/CT have a poor prognosis. In these cases, lenvatinib represents the best therapeutic option. In this study we investigated the role of [$^{18}$F] FDG PET/CT in the evaluation of metabolic response and in the prediction of the outcome in progressive locally advanced and metastatic RAI-R DTC patients treated with lenvatinib.

**Patients and Methods:** Thirty-three progressive locally advanced or metastatic RAI-R DTC patients, referred to our Center and treated with lenvatinib from December 2014 up to October 2016, were evaluated at baseline and during follow-up (Tg/TgAb, whole-body CT scan, [$^{18}$F] FDG PET/CT).

**Results:** Nineteen/33 (57.6%) patients had the greatest metabolic response to lenvatinib at the first [$^{18}$F] FDG-PET/CT scan, performed after 4 weeks of treatment, while 5/33 (15.1%) patients showed this response later during follow up. Moreover, 66.7% of patients had a metabolic response at [$^{18}$F] FDG PET/CT scan performed after 4 weeks of treatment and a morphological response at CT scan performed after 8 weeks of treatment and a more significant association was found during follow-up. We also observed a correlation between the metabolic response at first [$^{18}$F] FDG-PET/CT scan and the biochemical response (Tg/TgAb) at the same time in 60.6 % of patients and also between the morphological response at first CT scan and Tg/TgAb values after 4 weeks of treatment in 90% of patients. The median overall survival (OS) was significantly longer in patients with metabolic response at first [$^{18}$F] FDG PET/CT performed after 4 weeks of treatment (36.53 vs 11.28 months), at last [$^{18}$F] FDG PET/CT performed during follow up (40.00 vs 8.98 months), and in patients with morphological response at last CT scan performed during follow up (37.22 vs 9.53 months) than in those without metabolic and morphological response, respectively. Moreover, the most significant predictor of a better OS was the metabolic response.

**Conclusions:** Based on our data, [$^{18}$F] FDG-PET/CT scan can predict the early and long-term response to lenvatinib treatment and its impact on the OS of patients with RAI-R DTC.

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## FDG PET-CT AS FIRST LINE NUCLEAR MEDICINE IMAGING MODALITY FOR PATIENTS WITH ELEVATED THYROGLOBULIN LEVELS SUSPICIOUS FOR RECURRENT OR PERSISTENT DIFFERENTIATED THYROID CANCER

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**Purpose:** To investigate the potential role of [$^{18}$F]fluorodeoxyglucose (FDG) PET/CT as a first line nuclear medicine imaging modality in adult patients with differentiated thyroid cancer (DTC) presenting with detectable thyroglobulin (Tg) after thyroidectomy and radioiodine-131 [$^{131}$I] thyroid remnant ablation.

**Materials and Methods:** Retrospective analysis of FDG PET/CT results performed in patients with first presentation of detectable Tg levels (≥0.20 ng/mL) after initial treatment with total thyroidectomy and [$^{131}$I] thyroid remnant ablation for pT1-3aN0-1bM0 DTC. Results of FDG PET/CT and management of patients after FDG PET/CT were extracted from patient record files. The positive (PPV) and negative (NPV) predictive value of neck ultrasound (US), FDG PET/CT and US in combination with FDG PET/CT for recurrent or persistent DTC were calculated.

**Results:** Thirty-one patients (25 female) underwent a FDG PET/CT as the first nuclear medicine imaging modality because of detectable Tg suspicious for recurrent or persistent DTC. Median Tg level at time of FDG PET/CT was 2.9 ng/ml (range 0.30-23.0), after a median follow-up time of 1.8 years (range 0.1-19.0) after $^{131}$I treatment. FDG PET/CT was positive in 18 patients (58%), of which 16 patients showed uptake suspicious for lymph node metastases and 3 (additional) for lung metastases. DTC was confirmed (true positive) in 17 of 18 FDG PET positive patients. In 6 of 13 FDG PET/CT negative patients DTC

was found during further follow-up, of which 2 histologically proven, (false negative). The PPV and NPV of neck US, FDG PET/CT, or US in combination with FDG PET/CT for DTC were: 75.0% and 28.6% (US, n=26), 94.4% and 53.8% (FDG PET/CT, n=31), 100% and 50% (combination, n=26). 61% of patients with FDG positive lesions were treated surgically within 3 months (n=11/18) after which Tg declined, without need further iodine treatment.

**Conclusions:** FDG PET/CT detects metastatic disease in 58% of DTC patients with detectable but low Tg levels allowing surgical treatment for these patients. We recommend to use FDG PET/CT, in combination with neck ultrasound, in such patients because it may prevent unnecessary treatment with $^{131}$I and subsequent adverse effects.

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## TSH SUPPRESSION THERAPY IN PATIENTS WITH THYROID CANCER INDUCES A HYPERCOAGULABLE STATE

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**Introduction:** Treatment of differentiated thyroid carcinoma (DTC) for high-risk patients consist of thyroidectomy, $^{131}$I-therapy and TSH-suppression therapy (THST). During this treatment patients go from eu- to hypo- and subclinical hyperthyroidism which could affect hemostasis. It appears that severe hypothyroidism can increase tendency to bleed, while hyperthyroidism is thrombogenic. As DTC-patients have a higher risk for cardiovascular disease (CVD), we hypothesized that changes in hemostasis could play a role in the pathophysiology of CVD.

**Methods:** Paired samples of DTC-patients were obtained consecutively during euthyroidism (V1, n=6), hypothyroidism (V2, n=21) and subclinical hyperthyroidism (V3, n=21). We measured selected hemostatic proteins, performed functional tests of hemostasis (a thrombin-generation test and a plasma-based clot-lysis test), and assessed markers of in vivo activation of hemostasis (thrombin-antithrombin complexes [TAT], plasmin-antiplasmin complexes [PAP], and D-dimer levels). Data is presented as median with (interquartile range [IQR]). We used the Wilcoxon's paired test.

**Results:** Between V1 and V2, only factor (F) VIII and FXI decreased minimally and PAP increased slightly (Table 1). Changes in the other parameters were not detected. Between V2 and V3, all selected hemostatic proteins increased (Table 1). Furthermore, ex vivo thrombin-generation increased (626.0(IQR: 477.0-836.0) vs. 832.5(IQR: 632.25-1025.5) nM*min, P=.023) and clot-lysis time decreased (60.6(IQR: 55.6-60.6) vs. 76.7(IQR: 70.0-93.9) min, P<.001). TAT and D-dimer levels remained unaltered.

**Conclusions:** During hypothyroidism, no clear changes in hemostatic parameters occurred. During THST, more profound changes in individual hemostatic proteins and in ex vivo functional hemostatic tests occurred, resulting in a shift towards a more hypercoagulable and hypofibrinolytic state. However, unchanged levels of TAT and D-dimer indicated no evidence of acute ongoing activation of coagulation. Since hypercoagulable and hypofibrinolytic changes are associated with an increased risk for thrombosis and CVD in other clinical settings, this may play a role in CVD during THST.

**Table 1.** Changes in hemostatic proteins with different thyroid-hormone levels (for Abstract 103)

| | Euthyroidism (V1) | Hypothyroidism (V2) | P* | Hyperthyroidism (V3) | P† |
|---|---|---|---|---|---|
| VWF | 85.0*(78.0-114.5)* | 69.0*(53.3-96.3)* | ns | 128.0*(95.0-169.0)* | <.001 |
| Fibrinogen | 2.7*(2.4-3.1)* | 2.6*(2.4-3.0)* | ns | 3.3*(2.7-3.6)* | .001 |
| FV | 103.7(*94.1-116.1)* | 107.2*(87.5-117.8)* | ns | 120.9*(107.2-129.7)* | .003 |
| FVIII | 170.5*(148.5-224.5)* | 116.5*(85.3-134.5)* | .046 | 182.0*(164.0-212.0)* | <.001 |
| FIX | 86.6*(51.2-93.3)* | 87.9*(74.6-100.4)* | ns | 112.7*(98.2-133.8)* | .003 |
| FXI | 118.9*(100.2-142.5)* | 98.3*(82.3-108.5)* | .043 | 128.0*(110.5-136.7)* | .001 |
| tPA | 4.9*(4.5-10.1)* | 7.05*(4.3-9.9)* | ns | 8.7*(7.3-10.8)* | .004 |
| PAI-1 | 8.4*(5.2-12.7)* | 9.8*(7.1-16.6)* | ns | 17.4*(10.7-33)* | .001 |
| PAP | 190.0*(144.0-207.0)* | 266.5*(211.8-312.0)* | ns | 197.5*(163.0-266.3)* | .002 |

Data are presented as median+IQR. *Abbreviations:* VWF=von Willebrand factor, F=factor tPA=tissue-plasminogen-activator, PAI-1=plasminogen-activator inhibitor-1, PAP=plasmin-antiplasmin complexes, ns=not significant.

*P-values represent comparison of V1-V2.

†P-values represent comparison of V2-V3.

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## CLINICAL OUTCOMES OF PAPILLARY THYROID CARCINOMA CO-EXISTING WITH GRAVES' DISEASE: A PROPENSITY SCORE-MATCHED ANALYSIS

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**Background:** It is well known that patients with Graves' disease (GD) have higher risk of developing thyroid cancer than general populations. Previous studies have been suggested conflicting opinions on the prognosis and features of thyroid cancer in patients with GD. The aim of this study is to evaluate whether co-existing GD affects clinical outcomes of papillary thyroid cancer (PTC).

**Methods:** From January 2000 to December 2013, 8,274 patients underwent thyroidectomy for PTC at Asan Medical Center, Seoul, Korea and 188 patients among them had co-existing GD. We compared response to therapy classification and recurrence free survival (RFS) between patients with GD and others by using 1:4 propensity score matching analysis.

**Results:** After propensity score matching, 188 PTC patients with GD and 701 PTC patients without GD were included. Of the total 889 patients, the median age was 48.6 years (Inter Quatile Range [IQR] 38.0–54.5) years, and 87.4% were female. Median tumor size was 0.8cm (IQR 0.5–1.0) and 345 patients (39.8%) had cervical lymph node metastasis. None of the patients had synchronous distant metastasis. There was significant difference in response to therapy between two groups ($P < 0.05$). PTC patients with GD showed less excellent response to therapy than control group (69.1% vs. 82.6%) and showed more indeterminate response (28.7% vs. 15.3%). There was no significant difference in biochemical incomplete disease (2.1% vs. 1.7%) and structural incomplete disease (0.0% vs 0.4%) between two groups ($P$ =0.94 and $P$ =0.85, respectively). During the median 9 years of follow-up, a total of 24 (2.7%) had structural recurrence; 3 patients (1.6%) in patients with GD and 21 (3.0%) patients in control group. The 5-year-RFS in the patients with GD group was 98.4% and 97.4% in control group, respectively. There was no significant difference in RFS in Kaplan-Meier survival curve between two groups ($P = 0.135$).

**Conclusion:** Our findings indicate that clinical outcomes of PTC patients co-existing with GD are not different those without GD.

# Genetics

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## DUOX2 HETEROZYGOUS GENE MUTATION IN A FEMALE PATIENT WITH INAPPROPRIATE TSH SYNDROME

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**Background:** DUOX2 is a member of the nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) enzyme family which is expressed in the apical membrane of follicular thyroid cells. Sufficient hydrogen peroxide ($H_2O_2$) production is required for the synthesis of thyroid hormones. DUOX2 produces $H_2O_2$ which is, the principal electron acceptor for iodination and coupling reactions catalyzed by thyroperoxidase (TPO). DUOX2 gene mutations are transmitted in an autosomal recessive pattern. Although it causes variable phenotypes, most patients have goiter and hypothyroidism. In this report, we present a case with inappropriate TSH syndrome and DUOX2 heterozygous gene mutation.

**Case:** A 23-year-old female patient was admitted to our outpatient clinic with complaints of palpitations and nervousness. She had no history of chronic illness. Her mother had undergone thyroidectomy and her brother had hyperthyroidism. On physical examination her blood pressure was 120/80mm/hg, body mass index was 30 kg/m² and, systemic findings were normal. Laboratory tests were as follows; TSH 1.96 µU/mL(0.27-4.2), fT4 2.02 ng/dL (0.87-1.7), fT3 3.74pg/mL (2-4.4), antithyroglobulin 20.4 IU/mL (<60), antithyroid peroxidase 30U/Ml(<60), cortisol 17.6µg/dL(6.2-19.4), ACTH 17.6pg/mL(7.2-63.3), FSH 6.3U/L (2.5-10.2), LH 14.4U/L (1.9-12.5), Estradiol 211 ng/L (19.5-144.2), alfa subunit 0.4 ng/mL (<1.2). The presence of heterophile antibodies against fT3 and fT4 was ruled out. On thyroid ultrasonography, thyroid size was normal, the parenchyma was minimally heterogeneous, the color flow doppler pattern was 2 and there was no nodule. Pituitary magnetic resonance imaging showed the pituitary gland height was 10 mm, there was no adenoma. Thyroid hormone resistance was considered in the patient whose TSH suppression was achieved in the T3 suppression test and TSH level increased by 8-fold in the TRH stimulation test. Genetic testing revealed that the patient was heterozygote for c.1921G>A(p.Glu641Lys) variant in exon 16 of DUOX2 gene while PAX8, POU1F1, PROP1, TG, THRA, THRB, TPO, TRHR, TSHB, TSHR, SLC5A, GNAS, HESX1 genes were normal.

**Conclusion:** DUOX2 gene mutation was found in our case, who was considered to have thyroid hormone resistance clinically and laboratory. It has been observed that maternal DUOX2 mutation carriers are euthyroid and hypothyroidism is not permanent in adulthood. In this case, we think that the importance of the DUOX2 mutation might be more clear after the genetic analysis of her family.

**Keywords:** Inappropriate TSH syndrome, DUOX2 Gene Mutation

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## PREVALENCE OF PATHOGENIC GERMLINE DICER1 VARIANTS IN YOUNG INDIVIDUALS WITH GOITRE

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**Context:** DICER1 syndrome encompasses a variety of benign and malignant manifestations including multinodular goitre (MNG), which is the most common manifestation among individuals carrying pathogenic *DICER1* variants.

**Objective:** To estimate the prevalence of pathogenic *DICER1* variants in young individuals with MNG.

**Design and Setting:** Danish individuals diagnosed with nodular goitre based on thyroidectomy samples in 2001-2016 with the age limit at time of operation being ≤ 25 years were offered germline *DICER1* gene testing.

**Results:** Six of 46 individuals, 13% (CI [3.3;22.7], p <0.05), diagnosed with nodular goitre under the age of 25 years had pathogenic germline variants in *DICER1*. They were found in different pathoanatomical nodular goitre cohorts i.e. nodular goitre (n=2), colloid nodular goitre (n=3) and hyperplastic nodular goitre (n=1).

**Conclusions:** We recommend referral of patients with MNG in need of surgery aged <20 years and patients with MNG in need of surgery aged 20-25 years with a family history of goitre or an additional DICER1 manifestation to genetic counselling. As the individuals were found in different goitre cohorts, referral should not be limited by goitre pathology.

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## A NOVEL THYROTROPIN-RELEASING HORMONE RECEPTOR (TRHR) MISSENSE VARIANT (L206P) IN A FAMILY WITH CENTRAL CONGENITAL HYPOTHYROIDISM

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**Introduction:** Isolated central congenital hypothyroidism (CeH) is rare condition which escapes diagnosis on TSH-based screening programs.

Recognized causes include *IGSF1*, *TBL1X* and *IRS4* gene variants with X-linked inheritance and biallelic variants in the *TSHB* and *TRHR* genes with autosomal recessive inheritance. To date only few *TRHR* homozygous variants have been reported.

**Case Report:** Here we report a novel variant in the TRHR causing CeH.

The proband had been misdiagnosed at the 17th week of pregnancy as primary hypothyroidism due to the co-existence of a Hashimoto's thyroiditis and treated with L-T4. After delivery, L-T4 dose was adjusted by a reflex TSH strategy. Poor compliance with treatments further delayed diagnosis. She came to our attention 16 years later aged 44, because of persistent reduced FT4 levels with a borderline low TSH, while taking L-T4 75 mcg/day.

The investigations performed at referral, were consistent with CeH: TSH and PRL levels did not changed after TRH iv injection. No other associated pituitary hormone defects were found. A pituitary MRI showed a 3.5 mm lesion compatible with a microadenoma. NGS analysis showed a homozygous variant (p.L206P) in the *TRHR*, substituting a highly conserved leucine with a proline residue in transmembrane helix 5. This variant was neither found in ExAC nor 1000G.

Familial studies revealed biochemical features of CeH segregating with the homozygous p.L206P variant in her sister. The heterozygous mother had borderline low FT4 levels (Table 1). The father refused any investigation, thus we could not rule out a paternal deletion, since parents were not consanguineous. None of the affected patients had neurological deficits despite late diagnosis and treatment. Both the sisters had short stature (154 and 156 cm) but in line with their expected target.

**Conclusions:** the p.L206P variant expands the spectrum of gene variants causing CeH. The co-existence of a primary thyroid disease and the inappropriate use of the reflex-TSH strategy were the main reasons for a delayed diagnosis in this family.

**Table 1.** (for Abstract 101)

| | | proband | sister | mother |
|---|---|---|---|---|
| age (years) | | 44 | 32 (15th week of pregnancy) | 63 |
| baseline | TSH mU/ml | 4.6 | 1.42 | 2.1 |
| | FT4 pmol/l (11.5-24.5) | 5.3 | 4.6 | 12.1 |
| | FT3 pmol/l (2.9-7.1) | 2.1 | 3.2 | 4.1 |
| | Total cholesterol mg/dl | 213 | | on statins |
| | LDL mg/dl | 118 | | |
| | HDL mg/dl | 76 | | |
| | CPK U/l <190 | 754 | | |
| | TPO-Ab/Tg-Ab | positive | negative | negative |
| on treatment | TSH | 0.02 | 0.04 | |
| | FT4 pmol/l | 15.9 | 8.1 | |
| | FT3 pmol/l | 3.1 | 2.9 | |
| | Total cholesterol mg/dl | 198 | | |
| | LDL mg/dl | 104 | | |
| | HDL mg/dl | 75 | | |
| | CPK U/l <190 | 150 | | |
| | LT4 µg | 125 | 100 | |



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## GENETIC VARIANTS IN THE DNMT1 (RS2228611) AND DNMT3B (RS2424913) GENES ARE NOT ASSOCIATED WITH GRAVES' DISEASE IN THE POLISH CAUCASIAN POPULATION

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**Objectives:** Graves' disease (GD) is a complex disorder with endogenous, environmental and genetic factors involved in its pathogenesis. Recently, it has been suggested that also epigenetic modifications, including DNA and histone methylation that are regulated by the enzymes known as DNA methylotransferases (DNMTs), may take part in this process. In line with this hypothesis, two candidate gene studies conducted in Asian populations reported significant associations between the DNMT1 (rs2228611) and DNMT3B (rs2424913) single nucleotide polymorphisms (SNPs) and GD. The aim of this study was to evaluate the association between these variants and GD in the Polish Caucasian population.

**Methods:** A total of 1352 unrelated individuals including 642 GD patients (513 females and 129 males, mean age 44.6 ± 15.6 years) and 711 healthy controls (591 females and 120 males, mean age 29.8 ± 9.6 years) were included in the study. The DNA material was isolated from the patients' whole blood samples and the studied variants were genotyped using a polymerase chain reaction with pre-designed TaqMan SNP genotyping assays on the QuantStudio 12K Flex Instrument, according to the manufacturer's protocol. The overall genotyping call rates were 95.7% (rs2228611) and 95.2% (rs2424913), respectively. The genotype distribution was tested for Hardy-Weinberg equilibrium (HWE). Chi-square test was used to compare allele frequencies between GD patients and healthy controls as well as between GD patients stratified by age, sex, presence of Graves' orbitopathy and smoking status.

**Results:** We did not observe any associations between the rs2228611 (OR=1.19, 95%CI=0.84-1.69, P=0.30) or rs2424913 (OR=1.04, 95%CI=0.89-1.22, P=0.55) variants and GD. No differences were neither found in allele frequencies for these variants between GD patients stratified by a specific disease phenotype (all P>0.05).

**Conclusions:** The genetic variants in DNMT1 (rs2228611) and DNMT3B (rs2424913) are not associated with GD or disease phenotype in the Polish Caucasian population. A direct analysis of DNA methylation pattern in GD patients should further verify the role of DNA methylation in GD pathogenesis.

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## A GENETIC VARIANT IN THE PSORS1C1 GENE LOCATED WITHIN THE MHC REGION CONTRIBUTES TO GRAVES' DISEASE SUSCEPTIBILITY IN CAUCASIANS INDEPENDENTLY FROM HLA-DRB1*03

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The *Psoriasis susceptibility 1 candidate 1* (*PSORS1C1*) gene, located within the major histocompatibility complex (MHC) region at the human chromosome 6p21, is an autoimmunity-related locus that has been recently associated with normal-range thyrotropin levels and Graves' disease (GD) in the Japanese population. As the effects of MHC variants on autoimmune disorders often significantly vary across different ethnic populations, we aimed to investigate the association between the *PSORS1C1* rs1063646 variant and GD in the Caucasian population, as well as to evaluate the genotype-phenotype correlation, and to assess linkage disequilibrium (LD) between rs1063646 and HLA-DRB1*03, a major risk variant for GD in Caucasians.

rs1063646 was genotyped in 647 GD patients (517 females and 130 males) and 516 healthy controls (399 females and 117 males) from the Polish Caucasian population using a polymerase chain reaction with a pre-designed Taqman SNP genotyping assay on the QuantStudio 12K Flex Instrument according to the manufacturer's protocol. The genotype distribution was tested for Hardy-Weinberg equilibrium (HWE). The overall genotyping call rate was 97.5 %. Allele frequencies were compared between GD patients and controls as well as between specific subgroups of GD patients stratified by gender, age of GD onset, presence of Graves' orbitopathy (GO) and smoking status, using the chi-square test. HLA-DRB1*03 was genotyped using low-resolution single specific primer-polymerase chain reaction (SSP-PCR) method in 403 GD patients and LD between rs1063646 and HLA-DRB1*03 was calculated using PLINK v1.07 software.

The C allele was significantly more frequent in GD patients than in healthy controls (OR=1.53, 95%CI=1.20-1.94, $P=6.0x10^{-4}$). Phenotype-specific analyses showed no difference in allele frequencies between: (i) male and female GD patients, (ii) children and adolescents with age of GD onset ≤18years and adults with age of GD onset >18 years, (iii) GD patients with and without GO, and (iv) smoking and non-smoking GD patients (all P>0.05). LD analysis indicated no LD between rs1063646 and HLA-DRB1*03 ($r^2=0.001$, D'=0.20).

The rs1063646 *PSORS1C1* variant is associated with GD in the Caucasian population, and it is not related to any specific phenotype of the disease. This association is independent from the effect of HLA-DRB1*03, which indicates that multiple genetic variants within the MHC region are involved in the genetic susceptibility to GD.

## Iodine and Pregnancy

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### DIETARY IODINE INTAKE, THYROID STATUS AND EDUCATIONAL PERFORMANCE: A STUDY OF HIMALAYAN HOUSEHOLDS

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Iodine deficiency is believed to lead to mental impairment that 'reduces intellectual capacity at home, in school and at work' (WHO 2021). In 2006, with only 51% of households consuming iodine sufficient salt, UNICEF identified India at the top of 16 "make-or-break" countries that needed accelerated Universal Salt Iodation program. Here we describe a study conducted during 2003-2006 in Uttarakhand Himalaya, an endemic goitre zone. Surprisingly data on thyroid function status from this region is almost non-existent.

**Objective:** Assessment of iodine intake and thyroid status vis-à-vis literacy and school drop-out rate (a measure of intellectual capacity)

**Design:** Epidemiological observational survey.

Study site: Rural. 16 villages of Chamoli district, Uttarakhand Himalaya (1500-3400m), India

**Study population:** 1963 males & females, 5-60 years (total population 10,638) Year 2003-2006

**Method:** Informed consent and IEC approval were obtained. Goitre grading was carried out by palpation (PAHO, 1986). Questionnaire was used for iodised salt usage and demographic information. Salt samples were collected for assessment of dietary iodine (WHO protocol). Urine and blood samples were collected from subsets of population within fixed hours of the day for UIC and thyroid hormone estimation respectively.

Plasma TSH was determined by commercial IRMA kit, free $T_4$ by ERBA ELISA kit and total $T_4$,$T_3$ using Radioimmunoassay (published in-house methods).

Data on literacy rate and school drop-outs were obtained from State Economics & Statistics Department and Census 2011.

**Results:** 81.77$\pm$ 3.96% of surveyed population used un-iodised salt. Salt iodine content was below recommended level 3.28$\pm$0.69ppm. Palpable non-visible goitre was 2.62% and visible goitre 0.28% of surveyed population.

Thyroid hormone levels were in normal range (TSH 2.75$\pm$0.17mIU/L, T4 126$\pm$4.3nmol/L, T3 2.27$\pm$0.3 nmol/L, FT4 10.61$\pm$0.9 pmol/L). Median UIE was 80.08 ug/L (mild iodine deficient)

According to Census 2011 average literacy rate for Chamoli district was 91.99% with male 95.63% & female 87.20%. Rural area: male 92.91%, female 70.08%; Drop-out rate secondary level was 8.34% (2008-2009) which is considerably less compared to other districts/states of India. Average school drop-out rate of Uttarakhand state (2011) i.e. 9.0 % was much lower as compared to other developed states of India viz. Punjab, Gujarat, Maharashtra, Andhra Pradesh, Karnataka.

**Conclusion:** The normal thyroid status, in conformity with the educational performance, despite low iodine consumption argues for a 'Precise Salt Iodation' rather than 'Universal Salt Iodation'.

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### ASSESSMENT OF THE THYROID STATUS OF PREGNANT WOMEN IN THE REGION OF MILD IODINE DEFICIENCY

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**Relevance:** Our earlier studies of the state of iodine deficiency in the population of the Tyumen region showed the presence of mild iodine deficiency in the body of residents of Tyumen. It is known that iodine deficiency during pregnancy in women can cause thyroid diseases, which can adversely affect both the woman's body and the development of the fetus and child. In this connection, it seems relevant to assess the state of thyroid status in pregnant women in Tyumen.

**Purpose:** To study the state of the thyroid status of pregnant women in the 1st trimester of pregnancy living in the territory of mild iodine deficiency (Tyumen).

**Material and Methods:** In 2019, a survey was carried out of 264 women in the 1st trimester of pregnancy registered at the antenatal clinic No. 2 in Tyumen. Laboratory assessment of thyroid status was carried out by testing thyroid-stimulating hormone (TSH), free thyroxine fraction (fT4) and antibodies to thyroperoxidase (AT-TPO) in the blood. The average age of pregnant women was 29 ± 5.2 years (from 18 to 43 years). The average gestational age on examination is 9.9 weeks. Due to the fact that in Russia currently does not have developed a trimester of specific reference values for thyroid status indicators for pregnant women; the standards recommended by the European Thyroid Association (2014) were used.

**Results:** The data obtained showed that 207 pregnant women (78.4%) had TSH values within the reference values. An increase in TSH levels above the upper limit of the reference interval (2.5 mIU / L) was detected in 57% of women (21.5%), while subclinical thyrotoxicosis was diagnosed in 3 pregnant women. In 16 pregnant women, the TSH content exceeded 4.0 mIU / L, one woman was diagnosed with overt hypothyroidism with a TSH level above 10 mIU / L. An increase in AT-TPO titers was recorded in 28 people, of whom the TSH level above 2.5 mIU / L was determined in 21.4%. Also, when analyzing a group of pregnant women with euthyroidism, an increase in AT-TPO titers was determined in 10%.

**Findings:** The revealed changes indicate the need to include examination of the functional state of the thyroid gland for all pregnant women, as well as the need to develop a trimester of specific reference intervals, taking into account living in a region with mild iodine deficiency.

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### IODINE STATUS OF PREGNANT WOMEN IN THE MILD IODINE DEFICIENCY REGION

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**Relevance:** The Tyumen region is a region of mild iodine deficiency. It is known that iodine deficiency during pregnancy in women can cause impaired growth and development of the fetus, reduce the intelligence and mental abilities of the child in the future. In Russia, in order to prevent iodine deficiency in the population of pregnant women, in addition to using iodized salt in the diet, it is recommended to take potassium iodide preparations in physiological doses (200 μg). Thus, the study of iodine status in the population of pregnant women is relevant.

**Purpose:** To study the iodine status in the population of pregnant women living in the territory of mild iodine deficiency using the example of the Tyumen region (Russia).

**Material and Methods:** A prospective study was carried out, the sample was continuous, 264 pregnant women in the 1st trimester who were registered



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in the antenatal clinic No. 2 in Tyumen in 2019 were included.The study of the iodine status was carried out according to the WHO criteria (2007). The determination of iodine excretion in the urine was carried out by the cerium-arsenite method, neonatal thyroid-stimulating hormone was investigated as part of the neonatal screening for congenital hypothyroidism, in addition, thyroglobulin and antibodies to thyroglobulin were studied in the 1st trimester of pregnancy.

**Results:** The average age of pregnant women was 29 ± 5.2 years. The average gestational age on examination is 9.9 weeks.

The median concentration of ioduria in pregnant women in the 1st trimester was 158 μg / l. Ioduria less than 20 μg / L was not detected, 4.5% of women had less than 50 μg / L.

The median thyroglobulin was 17.7 ng / ml. Belgian colleagues proposed a median TG of less than 20 ng / ml as a criterion for iodine deficiency, while other authors suggested indicators of 13 and 10 ng / ml.

nTTG above 5 μIU / L in children of pregnant women in the study group was 3.125%, which does not reach the target of 3% recommended by WHO.

**Findings:** In terms of the median ioduria, against the background of group prophylaxis in the population of pregnant women, the optimal iodine supply was achieved. However, the level of neonatal hyperthyrotropinemia corresponds to mild iodine deficiency. Further large observational studies in the population of pregnant women are needed to evaluate thyreglobulin as a criterion for iodine deficiency.

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## DEFINITIONS AND PREVALENCE OF THYROID DYSFUNCTION IN PREGNANCY: AN INDIVIDUAL PARTICIPANT DATA META-ANALYSIS

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**Objective:** Thyroid dysfunction during pregnancy is associated with adverse pregnancy and child outcomes. Diagnosing thyroid dysfunction during pregnancy is complicated by changes in maternal thyroid physiology and considerable differences in thyroid function test outcomes between populations and immunoassays. Current international guidelines recommend calculating local, trimester-specific reference ranges in pregnancy while fixed upper limits of TSH are provided as an alternative. While exclusion of TPOAb positive women, twin pregnancies and thyroid medication users is ubiquitously accepted, the relevance and impact of stratification per trimester remains unknown. The aims of this study were to assess the prevalence of thyroid disease during pregnancy and to quantify the effects of different methodologies.

**Methods:** This was an individual patient data meta-analysis performed using data collected in the Consortium on Thyroid and Pregnancy. Participants with a twin pregnancy, known thyroid disease, thyroid medication use or TPOAb positivity were excluded before calculating cohort-specific reference ranges based on the 2.5-97.5 percentiles. We assessed trimester-specific reference ranges and fixed TSH upper limits. Primary outcomes were prevalence of thyroid disease in pregnancy and differences in reference range cut-offs.

**Results:** After exclusions, the individual participant data of N=60,887 pregnant women were included, The overall prevalence was hypothyroidism 0.4% (0.0–1.1%), subclinical hypothyroidism 2.7% (1.0-5.6%), hypothyroxinemia 1.9% (0.7–2.5%), subclinical hyperthyroidism 1.3% (0.4-6.5%) and hyperthyroidism 0.8% (0–1.5%). Upper limits of TSH differed markedly between cohorts, ranging from 2.26-5.37. The prevalence of hypothyroxinemia, but not other entities, differed per trimester (first, second and third trimester: 1.9%, 1.6% and 2.8%, respectively) changing the diagnosis in up to 53% of women initially considered to have hypothyroxinemia. The use of a fixed TSH upper limit of 4.0 mU/L resulted in a substantial shift by which 30% (0-85%) were no longer considered to have subclinical hypothyroidism while 1.1% (0-6.6%) of the total population would be newly diagnosed with subclinical hypothyroidism.

**Conclusion:** By quantifying differences in disease prevalences according to various methodologies, we identified that trimester-specific reference ranges predominantly affect the diagnosis of hypothyroxinemia while a fixed TSH upper limit considerably affects the diagnosis of subclinical hypothyroidism. For other disease entities the differences were negligible. Future studies are required to identify if these differences also translate into different risks of adverse pregnancy outcomes.

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## WHAT IS THE ROLE OF THYROID AUTOIMMUNITY IN WOMEN WITH INFERTILITY AND EUTHYROIDISM UNDERGOING ASSISTED REPRODUCTION?

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**Introduction:** the prevalence of thyroid autoimmunity (AI) in women with infertility is high and reflects a general immune imbalance, which may lead to implantation failure, higher miscarriage rate and infertility in euthyroid patients.

**Objectives:** to evaluate the impact of thyroid AI on the results of assisted reproduction in euthyroid women.

**Methods:** retrospective study of women with infertility, in euthyroidism, followed in a Human Reproduction Department, between May/2016-January/2020. TSH, anti-thyroid antibodies, ovarian stimulation protocol, reproductive technique, number of retrieved oocytes, number of metaphase II (MII) oocytes, number of transferable and cryopreserved embryos and beta-hCG levels were obtained. Functional ovarian reserve was assessed by anti-Müllerian hormone (AMH) levels with antral follicle count (AFC). Exclusion criteria: prior thyroidectomy, radioactive iodine treatment, cervical surgery/radiotherapy, oophorectomy, malignant/autoimmune pathology, chronic kidney disease, liver disease, polycystic ovary syndrome and current medication with levothyroxine, methimazole or propylthiouracil. We separated women into two groups: antagonist protocol (ANP) and long agonist protocol (AGP), and compared the results of assisted reproduction, between those with positive and negative AI. $p<0.05$ was considered statistically significant.

**Results:** 279 women, 221 submitted to ANP and 58 submitted to AGP, were evaluated. In the ANP and AGP groups, positive AI was present in 15.8% and 15.5% of cases, respectively.

In the ANP group, patients with positive and negative AI did not differ significantly in age [34.6±3.3years; 33.9±3.5years (p=0.204)], body mass index (BMI) [(23.1±3.8kg/m$^2$; 23.8±3.8kg/m$^2$ (p=0.665], AFC [9 (IQR:9); 11 (IQR:7) (p=0.222)] and AMH [2.6ng/mL (IQR:2.1); 2.5ng/mL (IQR:2.3) (p=0.541), respectively. TSH levels were significantly higher in the positive AI group [2.0±1.0uUI/mL; 1.5±0,70uUI/mL (p=0.002)].

In the AGP group, there were no significant differences in patients with positive and negative AI in age [36years (IQR:7.5); 36years (IQR:5.5)

(p=0.594)], BMI [(23.4kg/m$^2$ (IQR:5.9); 22.4kg/m$^2$ (IQR:7.0) (p=0.096], AFC [8.9±3.8; 7.8±4.1 (p=0.501)] and AMH [1.4ng/mL (IQR:1.3); 0.9ng/mL (IQR:0.9) (p=0.344), respectively. TSH levels were significantly higher in the positive AI group [1.9±0.6uUI/mL; 1.4±0.6uUI/mL (p=0.002)].

In both groups, patients with positive and negative AI did not differ significantly in the reproductive technique used (p=0.605;p=0.673), number of retrieved oocytes (p=0.955;p=0.134), number of MII oocytes (p=0.378;p=0.121), and number of transferable (p=0.634;p=0.515) and cryopreserved embryos (p=0.931;p=0.261), respectively.

The result of the immunological pregnancy test did not differ significantly between patients with positive and negative AI, in both groups.

**Conclusions:** In this study we found that thyroid autoimmunity and TSH levels in the normal range, in women with infertility, did not have impact on the results of assisted reproduction.

# Molecular and Morphological Features in Thyroid Cancer

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## HOW WAS NIFTP DIAGNOSED: FOLLICULAR ADENOMA OR PAPILLARY THYROID CARCINOMA?

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One of the criteria established for the diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is the presence of nuclear alterations of papillary thyroid carcinoma (PTC). Objective score was defined for the nuclear alterations necessary to diagnose NIFTP and to differentiate it from follicular adenoma (FA). Using less rigorous criteria for the nuclear alterations of PTC, noninvasive follicular neoplasms without these alterations that were diagnosed as FA would probably continue with the same diagnosis after application of the current nuclear score. It is unlikely that the diagnosis would be changed to NIFTP. In this situation, NIFTP should indeed be diagnosed as PTC. Using a stricter criterion, it is possible that noninvasive follicular neoplasms without nuclear alterations of PTC that were diagnosed as FA would be diagnosed as NIFTP after application of the current nuclear score. The study of Hirokawa et al. exemplifies the latter situation. Among the tumors previously diagnosed as FA by these authors, 30% now had a diagnosis of NIFTP because they exhibited score 2 (rarely score 3). In contrast to Hirokawa et al., we were less strict about the nuclear alterations that characterize PTC. The diagnosis of FA was only made when nuclear alterations of PTC were absent or when they were very subtle. We thus hypothesize that the frequency of NIFTP among cases of FA diagnosed by us before 2016 would be very low. We revised 50 tumors diagnosed as FA before 2016 that could be potentially diagnosed as NIFTP: (i) full encapsulation or partial encapsulation or clear demarcation from adjacent thyroid parenchyma; (ii) follicular growth pattern without well-formed papillae or psammoma bodies and < 30% solid/trabecular/insular growth pattern; (iii) no vascular or capsular invasion; (v) no tumor necrosis or high mitotic activity. After application of the current nuclear score, none of these cases previously diagnosed as FA had their diagnosis changed to NIFTP because all exhibited score 0-1. These results show that the strictness of the nuclear criterion used before 2016 changes how NIFTP was being diagnosed up to that time. A high frequency of NIFTP is observed among FA when a strict nuclear criterion was used for PTC, while this frequency is very low when a less rigorous definition was used. Conversely, in the first situation, the frequency of NIFTP might be low among cases previously diagnosed as PTC. In the second situation, the frequency of NIFTP would be higher among cases previously diagnosed as PTC.

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## PITFALLS IN THE MANAGEMENT OF PAPILLARY MICROCARCINOMA – A TALL CELL VARIANT CASE REPORT

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Papillary thyroid microcarcinoma is associated with a good prognosis. Distant metastases occur in about 1-2% of cases. In contrast, the tall target variant is regarded as more aggressive. Below we present the case of a 51-year-old patient with low-stage thyroid cancer in whom massive bone spread was detected.

A 51-year-old patient underwent a total thyroidectomy due to nodular goiter in 2020. The histopathological examination revealed in the right lobe of the thyroid a papillary microcarcinoma (conventional and tall cell variant) with a diameter of 5.3 mm (a surgical margin of 1.3 mm), without vascular invasion, and one lymph node without tumor metastases (pT1aN0 according to AJCC 2017). Laboratory tests performed at the request of the multidisciplinary consultation team revealed an increased concentration of thyroglobulin - 456.2 ng/mL (TSH 18,000 mIU/L, anti-thyroglobulin antibodies 15.6 IU/mL). The neck ultrasound did not show any remnant thyroid tissue or suspected lymph nodes. The patient was referred for diagnostic whole-body scintigraphy. Native Thyroglobulin concentration was 221.7 ng/mL; the rhTSH stimulated thyroglobulin concentration reached 245.1 ng/mL (TSH 109.0 mIU/L). The chest X-ray showed no infiltrative changes. The whole-body scintigraphy followed by SPECT/CT (after the administration of 148 MBq $^{131}$I) showed uptake of $^{131}$I in the thyroidectomy bed, in small lymph nodes of the IV left cervical compartment and multiple foci in bones. The patient was referred for radioactive iodine (RAI) palliative therapy after rhTSH preparation. She received activity of 5500 MBq of $^{131}$I in February 2021. The patient is scheduled for the first post-therapeutic assessment in April 2021, and the next RAI treatment is planned in 6 months.

**Conclusions:** the detection of a more aggressive subtype of papillary thyroid microcarcinoma, such as tall cell variant, even if low-advanced in pathology, should prompt an increased vigilance and consideration of more aggressive therapeutic approach.

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## UNUSUAL SUBCUTANEOUS GLUTEUS METASTASES IN AN OLIGOMETASTATIC TCV-PTC PATIENT

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**Introduction:** Among all papillary thyroid cancer (PTC) variants, tall cell variant (TCV-PTC) is the one of the most aggressive with a high prevalence of somatic BRAFV600E mutation. When present, distant metastases usually occur in latero-cervical (LC) and mediastinal lymph nodes, lungs and more rarely in liver and bone. Subcutaneous and intramuscular metastases are extremely rare and considered a sign of a very diffuse metastatic involvement and related to poor prognosis. Here, we present a patient with TCV-PTC and oligometastatic disease but with an unexpected subcutaneous metastatic lesion in the left gluteus.

**Case presentation:** In 2005, a 62-year-old female was submitted to total thyroidectomy for a 3.5 cm Thy5 nodule. Histology revealed a TCV-PTC, without extrathyroidal extension and lymph nodes metastases. Low activites of radioiodine (i.e., 1.1 GBq – 30 mCi) were administered for remnant ablation and post therapeutic WBS showed only the presence of a small remnant. Thyroglobulin (Tg) levels increased from 2.46 mcg/L in 2006 to 16 mcg/L in 2009 and, 2 years after surgery, a LC lymph node metastasis appeared and enlarged over time. For this reason, high activities of radioiodine were administered but without evidence of radioavid structural disease. Therefore, in 2010 lymph node dissection and external beam radiotherapy were performed: thereafter the patient continued to show detectable but stable values of Tg without structural disease. In 2018 another centimetric cervical lesion adjacent to the trachea was discovered at neck ultrasound and kept in active surveillance. However, Tg values progressively increased up to 43 mcg/L. In December 2019, the patient referred the presence of a small palpable mass in the left gluteus: a total body CT scan was performed and confirmed the presence of a 2.5 cm nodule in the left gluteus without any other distant metastases. After FNAC positive for malignancy, surgery was performed, and histology confirmed the subcutaneous metastasis of PTC. Moreover, molecular analysis showed the mutation BRAFV600E. After surgery Tg values decreased to 9.2 mcg/L and up to now the patient is in active follow up, without further metastatic lesions except for the small lesion in the neck.

**Conclusions:** Subcutaneous metastases in PTC patients are very rare and often are the consequence of a very advanced metastatic disease. The peculiarity of this case is in the late development, 14 years after the first diagnosis, of an unusual subcutaneous metastasis in a patient with an oligometastatic TCV-PTC.

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## TSHR MRNA QUANTIFICATION IN CIRCULATING THYROID CANCER CELLS SHOWS POTENTIAL IN DETECTING AGGRESSIVE FORMS OF DTC

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**Background:** Diagnosing thyroid cancer is routinely performed by fine needle aspiration biopsy (FNAB), which although a reliable non-surgical method, cannot classify around 15-30% of cases as clearly benign or malignant and presents discomfort for the patient. A serological test may present a non-invasive and less costly alternative.

**Objective:** We examined the performance of measuring thyroid-stimulating hormone receptor (TSHR) mRNA, an analyte suggested by Cleveland Clinic, Ohio, in circulating tumour cells (CTC) isolated from peripheral blood samples and assessed its diagnostic and prognostic performance in a Serbian cohort of patients.

**Methods:** We collected samples from 36 patients (17 benign, 19 malignant out of which 10 PMC, 6 PTC and 3 HTC) undergoing surgery for thyroid nodules. Total RNA was isolated using Trizol from mononuclear cell fraction and reverse transcribed with random hexamer primers. TSHR mRNA was quantified on droplet digital PCR (ddPCR) using thyrocyte-specific primers [2]. ddPCR was the method of choice because of the very low amount of the desired molecule compared to the background.

**Results:** Mean ± SD of TSHR mRNA normalized expression levels were 0,138 ± 0,114 in the benign and 0,225 ± 0,256 in the malignant group. Using ROC analysis we determined the cut-off value for the levels of TSHR mRNA for discriminating benign from malignant disease. The test had a Sensitivity = 52.6%, Specificity = 82.4%, PPV = 76.9%, NPV 60.9% and Diagnostic Accuracy = 66.7%. The diagnostic accuracy and sensitivity slightly increase at the expense of specificity if suspicious ultrasound features (hypoechogenicity, microcalcifications, intranodular hypervascularity, irregular margins) are added as a marker of malignancy, i.e. Sensitivity = 63.2%, Specificity = 76.5%, PPV = 75%, NPV 65% and Diagnostic Accuracy = 69.5%. The TSHR mRNA test results positively correlate with the aggressiveness (Pearson correlation coeficient 0,570; p=0,011) defined using the following markers of tumour severity i.e. thyroid capsule invasion, extrathyroidal extension (Ei), intraglandular dissemination (ID) and metastasis to the lymph nodes (LNM). Interestingly, the TSHR mRNA test correctly classified all samples with thyroid capsule invasion, Ei or LNM as malignant.

**Conclusions:** Combining the testing of TSHR mRNA in CTC on ddPCR with suspicious US features creates a non-invasive marker of cancer with diagnostic accuracy of around 70%. Moreover, TSHR-mRNA test detects 100% of the most aggressive differentiated thyroid cancer cases. Wider clinical studies are warranted to assess the usefulness of this promising blood-based biomarker in detecting aggressive DTC.

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## THE ROLE OF MIRNAS EXPRESSION IN THYROID CANCER

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**Objectives.** Thyroid cancer is the most prevalent endocrine malignancy and in the past several decades, the incidence of thyroid cancer worldwide has been steadily increasing. Deregulation of miRNA expression has been described in a variety of tumors, including thyroid cancer. The aim of our study is to evaluate the role of different miRNAs in thyroid cancer.

**Methods:** A retrospective case-control study included 112 patients with surgically treated suspected thyroid cancer (Bethesda III-VI). Control group included 58 patients with benign nodules. Study group included 54 patients with thyroid cancer (47- papillary thyroid carcinoma (PTC), 3 – follicular (FTC), 2 – medullary (MTC), 2 -anaplastic). The expression of 12 miRNA (miR144, miR145, miR146, miR155, miR183, miR199, miR221, miR31, miR551, miR375, miR451, miR7) were determined using quantitative real-time PCR.

**Results:** Up-regulation of miR146 ($p<0.001$), miR221 ($p=0.03$), miR155 ($p<0.001$), miR375 ($p<0.001$), miR31 ($p<0.001$), miR551 ($p<0.001$) and down-regulation of miR7 ($p<0.001$) and miR145 ($p<0.001$) were significantly associated with thyroid cancer. Over-expression of miR31 ($p<0.001$), miR155 ($p<0.001$), miR551 ($p<0.001$), miR146 ($p<0.001$) were significantly correlated with PTC, over-expression of miR183 ($p=0,02$) with FTC, miR7 ($p<0.001$) and miR375 ($p<0.001$) – with MTC. The recurrent ($p=0,003$) and metastatic ($p<0.001$) tumors were statistically significantly associated with down-regulation of miR7.

**Conclusion:** Expression of miRNA could be used for risk stratification of thyroid nodules.

**Keywords:** thyroid cancer, molecular genetic testing, miRNA

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## BILATERAL TESTICULAR METASTASES OF MEDULLARY THYROID CARCINOMA IN AN ADULT MALE WITH MULTIPLE ENDOCRINE NEOPLASIA 2A SYNDROME: A CASE REPORT

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**Introduction:** Medullary thyroid cancer (MTC) is a rare endocrine tumor, which can be sporadic or familial, as a component of multiple endocrine neoplasia type 2 syndrome (MEN2). Overall, 10% of MTC cases presents metastasis at diagnosis or during follow-up. Testicular metastases are exceptional and only one case of unilateral testis' involvement by metastatic MTC has been reported so far. We described the first known case of asymptomatic bilateral testicular MTC metastases, discovered incidentally at testicular ultrasound performed for unrelated reason.

**Case Report:** The case was a Latin-American 32-year-old man affected by MEN2A syndrome (RET heterozygous germline mutation c.T1852>C, p.Cys618Arg of exon 10) characterized by MTC with cervical and mediastinal lymph node metastases, treated with total thyroidectomy, central and lateral lymph node dissections and external-beam radiotherapy. The patient had also a bilateral pheochromocytoma, treated with bilateral adrenalectomy in two times. At our first evaluation, the patient was asymptomatic, except for premature ejaculation. For this, he underwent andrological and urological examination. No palpable nodule in the testes were found, but ultrasound (US) imaging showed two symmetrical hypoecoic lesions of 5 mm in the lower part of left testis and in the middle part of the right testis, without clear vascular pattern. The patient underwent enucleoresection of both testicular lesions. Histopathology revealed the presence of atypical cells with focal granular cytoplasm and "salt and pepper" chromatin, morphologically resembling MTC. Immunohistochemistry showed reactivity for chromogranin A, calcitonin (CTN), carcinoembryonic antigen (CEA), neural cell adhesion molecule (CD56), transcriptional thyroid factor 1 (TTF-1), synaptophysin, cytoplasmatic pattern (OCT4), CK-Pan ("dot-like" pattern), thus confirming the diagnosis of metastases from MTC. No reactivity was showed for anti-Placental alkaline phosphatase (PLAP), CD30, CD117, Inhibin, α-FP. The MIB-1 proliferation index was about 10%. As expected, in the postoperative period serum CTN and CEA levels decreased significantly.

**Conclusion:** Although exceptional, testis should be considered as an unusual, but a possible site of metastases in patients with diffuse metastatic MTC. In this setting, testicular ultrasound is helpful to reveal an asymptomatic and often unnoticed secondary lesions to the testis, and it could be considered an useful tool for the evaluation and follow-up of metastatic male MTC patients.

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## LYMPHOCYTIC THYROIDITIS AND AGGRESSION MARKERS OF PAPILLARY THYROID CANCER

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**Objectives:** To determine the relative frequency and prognostic significance of the presence of chronic lymphocytic thyroiditis (CLT) in patients with papillary thyroid carcinoma (PTC).

**Methods:** Retrospective study of patients with papillary thyroid carcinoma followed by multidisciplinary team of Endocrine Diseases, who underwent thyroidectomy from 2016 to 2020. Patients were divided into 2 groups (with and without CLT. Tumor features (size, angioinvasion, capsular infiltration, mono / multifocality and ganglion metastases) were analyzed. CLT was confirmed by histopathological diagnosis. Lymphadenectomy was performed only in patients with proven ganglion metastases (by ultrasound or cytology) or when suspected during surgery.

A value of $p<0.05$ was considered statistically significant.

**Results:** 128 patients underwent total thyroidectomy for papillary thyroid carcinoma, 111 were women (86.7%). Of these, 37 (28.9%) had lymphocytic thyroiditis. The distribution by sex and age was similar between groups. (p = 0.149; p = 0.783).

The group with lymphocytic thyroiditis seemed to have less invasion of peri-thyroid tissues (27% vs 30.8%), less vascular invasion (13.5% vs 23.1%) and, a smaller tumor size (15 mm vs 17 mm). However, there was no statistically significant difference between groups (p = 0.684; p = 0.223; p = 0.241, respectively).

Multifocality (29.7% vs 23.1%), the presence of lymphatic invasion and ganglion metastases (18.9% vs 17.6%) was higher in this group. However, it was not possible to demonstrate a correlation between the presence of lymphocytic thyroiditis and perineural invasion (p = 0.106), lymphatic invasion (p = 0.858), the presence of multifocality (p = 0.431) and the presence of ganglion metastases (p = 0.648).

The presence of CLT did not correlate with a lower degree of PTC (p> 0.05).

Many studies have shown that the presence of CLT slows the growth and spread of thyroid carcinoma and can be considered a protective factor. However, this is a controversial topic, given the contradictory results obtained in other investigations.

Our study suggests that the tumor size, the invasion of peri-thyroid tissues and the vascular invasion, were lower in the group with CLT, thus suggesting a better prognosis.

The coexistence of autoimmune disease and papillary thyroid cancer did not limit the number of foci, with a higher prevalence of multifocality in the group with CLT.

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## CALCITONIN RECEPTOR EXPRESSION IN FOLLICULAR CELL-DERIVED THYROID TUMORS AND MEDULLARY THYROID TUMORS: CLINICAL IMPLICATIONS

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**Background:** Human Calcitonin receptor (hCTR) is expressed in several human primary tumours and tumoral cell lines. The putative function of CTR in this context is still largely unknown and different results have been reported according to the different affected tissue. Only few papers are available regarding its expression in medullary thyroid cancer (MTC). No data are available regarding follicular cells-derived thyroid cancer.

**Aims:** aims of this work were to: identify and semiquantify by immunohistochemistry the expression of hCTR protein in a series of follicular cells-derived and MTC tumor samples and their matched lymphnode metastases; confirm and quantify by real-time PCR the hCTR mRNA expression in follicular cells-derived tumors frozen tissues samples, previously analyzed by immunohistochemistry; identify which hCTR isoform is expressed; correlate the hCTR expression score with clinicopathological and molecular features of samples.

**Methods:** we analyzed by immunohistochemistry 92 follicular cell-derived primary thyroid tumor samples and 138 MTCs, using a monoclonal antibody 31/01-1H10, previously used in other studies. For real-time PCR a commercial assay was used to identify the hCTR total transcript while an home-designed assay was specifically designed for hCTRC1b and hCTR C1a isoforms.

**Results:** hCTR expression was positively detected in 74/92 (80.4%) follicular cells-derived tumors and in 125/138 (90.6%) MTC tumors. Higher hCTR expression was significantly associated with less aggressive tumoral features both series. When we analyzed the hCTR immunoexpression in primary tumors and corresponding loco-regional lymphnode metastases, we observed 11/18 positive cases (61.1%) in the PTCs and all positive cases in MTCs. No statistically significant differences were found between concordant and not concordant cases. Moreover when we analyzed the mRNA pattern of expression of all hCTR transcripts we found that the hCTRC1b isoform was the most expressed in follicular cells-derived tumors. In a subgroup of 7 PTC samples we could also compare the expression levels of all transcripts both in primary tumors and adjacent thyroid tissue: only hCTRC1b isoform showed higher expression levels in tumor samples compared to normal thyroid.

**Conclusions:** Our study confirms that hCTR is expressed not only in MTCs but also in follicular cells derived thyroid tumors and that higher expression levels are associated with a more differentiated status and less aggressive clinical behavior. For the first time we demonstrated that hCTR is expressed both in primary tumor tissues and loco-regional lymphnode metastases in PTCs and MTCs. Moreover both isoforms are expressed in normal and tumoral tissue, with higher expression levels in malignant samples, but only the isoform hCTRC1b is overexpressed in a tumoral context.

# Nodules and Thyroid Cancer

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## PREDICTIVE FACTORS FOR THYROID COMPLICATIONS IN CANCER PATIENTS AFTER RADIATION THERAPY

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**Objectives:** Survival after childhood cancer has greatly improved over the last decades. Radiation therapy plays an important role in the treatment of several types of malignancies. As a result, childhood cancer survivors are at an increased risk of adverse outcomes including thyroid neoplasms, due to the radio-sensitivity of the thyroid gland.

The aim of this study was to assess the incidence and timeframe of thyroid complications in cancer patients and to identify risk factors for the development of hypothyroidism and thyroid cancer.

Methods: We performed a retrospective study which included 282 subjects who received irradiation to the neck, craniospinal or total body irradiation. Patients were subgrouped into 4 diagnostic clusters: Leukemia, Hodgkin's disease, Central Nervous System (CNS) and Head or Neck tumors (HNT).

**Results:** Hypothyroidism was observed in 56,7% patients, on average 6,8 ± 6 years after the treatment. The prevalence of hypothyroidism was lowest in leukemia patients and the events occurred after a shorter interval in HNT than in other diagnostic groups. Neck and craniospinal irradiation presented a 3.5-fold increased risk for development of hypothyroidism compared to total body irradiation. Moreover, the risk of hypothyroidism tended to be higher among patients who received higher radiation doses (>35Gy).

Papillary Thyroid Cancer was diagnosed in 8,5% of patients, on average 18,5 ± 5 years after radiotherapy. Female gender, younger age and low irradiation doses were independently associated with the finding of thyroid cancer. HNT patients had the lowest incidence of thyroid cancer (1 case). Two reasons can contribute to this outcome: these patients were exposed, on average, to higher doses of radiation (>50Gy) and these tumors were often diagnosed in adulthood when the thyroid gland is less radiosensitive.

**Conclusions:** In light of the results, we developed a risk-based protocol for surveillance of thyroid complications in cancer survivors followed at our Department. Our results further support the evidence for lifelong surveillance of cancer survivors with a history of radiation exposure. They highlight the need for thyroid function and ultrasound screening in accordance with a well-defined risk-based protocol.

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## EFFICACY AND SAFETY OF PERCUTANEOUS ETHANOL INJECTION FOR THE TREATMENT OF BENIGN CYSTIC THYROID NODULES: A 3-YEAR EXPERIENCE AT A TERTIARY CARE CENTER

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**Objectives:** To assess the efficacy and safety of ultrasound-guided percutaneous ethanol injection (PEI) for treating benign cystic thyroid nodules.

**Methods:** Retrospective analysis of euthyroid patients treated with PEI for purely (>90% of cystic component) or predominantly cystic (50%-90% of cystic component) thyroid nodules from 3/1/2018 to 3/31/2021. Reductions of more than 50% (≥50%) in nodular volume defined efficacy. Safety was considered as no or minor PEI-related complications.

**Results and conclusions:** The study involved 40 patients, 29 (72.5%) of them were women. Mean age (standard deviation) at the time of 1st PEI was 50 (±16) years. At initial observation, 4 (10.0%) patients were symptomatic (2 with dysphagia, 1 with dysphonia and 1 with cervical discomfort). PEI was used in 25 (62.5%) patients with predominantly cystic nodules. The median largest diameter of the nodules was 39.5 (range, 32.3-49.0) mm and the median initial volume was 13.7 (range, 9.0-24.1) mL. During PEI sessions, the median amount of fluid drained was 8.5 (range, 4.8-17.5) mL, and the median amount of ethanol instilled was 4.0 (range, 2.0-7.8) mL. During a median follow-up of 14 (range, 7-24) months, 38 (95.0%) patients revealed decrements of ≥50% in nodular volume, with a median time of 4 (range, 1-10) weeks after the 1st PEI. The last follow-up ultrasound revealed a median volume of 1.9 (range, 0.4-3.2) mL, which corresponds to a volume decrease of 11.8 mL (86.1%). All of symptomatic patients went into remission and did not recurred. After the 1st PEI, the 2 patients who manifested dysphagia went into remission after 3 and 4 weeks, respectively. In the other 2 patients, dysphonia and cervical discomfort resolved after 5 weeks and 1 week, respectively. Fifteen (37.5%) patients performed more than one session, even though in 5 of them (33.3%), one session was enough to achieve reductions of ≥50% in nodular volume. In 3 of the 4 symptomatic patients, one session was sufficient to go into remission. Surgery was not necessary in all cases. No severe adverse reactions were reported. Only 4 (10.0%) patients revealed mild and transitory adverse effects: 3 (7.5%) with burning sensation at the injection site, and 1 (2.6%) patient developed local hematoma.

In our cohort, PEI was a safe and effective option to treat benign cystic thyroid nodules with aesthetic issues or with compressive symptoms who declined or were not good candidates to surgery (elderly patients and/or those with multiple comorbidities).

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## VALUE OF SHEAR WAVE ELASTOGRAPHY AS A TOOL FOR FURTHER DIAGNOSTIC PROCEDURE WITH THYROID NODULES

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**Aim:** Shear wave elastography yields a quantitative assessment of the elasticity of a thyroid nodule by evaluating the speed of propagation of the shear-wave in the tissues, and provides a numerical value expressed in meters per second (m/s). Therefore the dignitiy of thyroid nodules can be elucidated further. The aim of the study was to test a new design using ESAOTE my lab 9 programme Q elaxto 2 D in the evaluation of thyroid nodules ea. for using thermal ablation for volumen reduction or evalating the dignity before thyroid surgery.

**Methods:** 11 patients with indeterminated thyroid nodules were assesed before the decision to thyroid surgery. For the diagnostic procedure convential ultrasound (ESAOTE, Milano Italy) was equipped with ML 6-15 linear probe working in the range of 6-15 MHz. In all elastosonographic procedures the features of the tissue were quantified using the programme Qelaxto 2 d software which was installed on the machine. The investigators were experienced in the field of thyroidology for years and performed the convential und shear wave elastography in the same session. The evaluated parameters were measured as mean value by 10 consequent measurements. All patients also assessed laboratory testing, Tc scintigraphy, MIBI scintigraphy and often fine needle aspiration cytology. The results could be confirmed to histologic characterisation.

**Results:** 11 patients were used for the evaluation of the programme. Subsequent thyroid surery was performed on all patients because of cold nodules. 8 patients had a benign diagnosis (follicular or oncocytic adenoma) and 3 patients had the histiologic diagnosis of a thyroid carcinoma (2 paillary and 1 follicular thyroid carcinoma. In the patients with benign thyroid carcinomas the mean value of the shear wave elastography was 1,7 ± 0,8 m/sec and with the malignant disease 5,4 ± 1,6 m/sec (t-test p< 0,002).

**Discussion:** The used share wave elastography is a further diagnostic tool for the evaluation of thyroid nodules. So far this parameter could help to distingiush between benign and malignant lesions as shown in comparison to the final histologic findings but must be evaluated in larger patient cohortes.

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## DO THE ROUND SOLID ROUND ISOECHOIC THYROID NODULES HAVE A HIGHER RISK OF MALIGNANCY?

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**Objectives:** Isoechogenity and ovoid-round shape are generally considered to be associated with a low risk of malignancy. According to commonly used guidelines, such nodules, without sonographic features of higher risk, is usually considered for FNAB when they are larger than >20-25

**Table 1.** Comparison of Solid Round Isoechoic Nodules (SRIN) and Solid Ovoid Isoechoic Nodules (SOIN) without any high risk sonographic features (for Abstract 162)

| | | SRIN (n=165) | SOIN (n=116) | p |
|---|---|---|---|---|
| Age (years) | | 53±10.2 | 48.2±10.9 | 0.22 |
| Diameter (largest) mm | | 13 (6-25) | 18.4±4.6 | 0.07 |
| Nodule volume ($mm^3$) | | 1098 (108-7812) | 2017±1769 | <0.01 |
| Serum TSH level (mIU/L) | | 1.5 (0.1-16) | 1.2 (0.2-4) | 0.08 |
| Cyto-pathological results | Bethesda classification Thy 1 | 40 (24%) | 23 (19.8%) | 0.38 |
| | Bethesda classification Thy 2 | 89 (53.9%) | 76 (65.5%) | 0.05 |
| | Bethesda classification Thy 3a | 18 (10.9%) | 9 (7.8%) | 0.38 |
| | Bethesda classification Thy 3b | 4 (2.4%) | 6 (5.2%) | 0.33 |
| | Bethesda classification Thy 4 | 1 (0.6%) | 2 (1.7%) | 0.57 |
| | Bethesda classification Thy 5 | 13 (7.9%) | 0 | 0.0009 |
| Not operated | | 135 (81.8%) | 110 (94.9%) | 0.0013 |
| Histo-pathological results | - Malignant | 25 (15.2%) | 2 (1.7%) | 0.0001 |
| | - Benign | 5 (3%) | 4 (3.4%) | 1.000 |

TSH: Thyroid Stimulating Hormone

mm. However, small solid round isoechoic nodules (SRIN) were reported to be associated with increased risk of malignancy in few studies. Thus, we aimed to evaluate the malignancy risk of <25 mm SRIN detected at thyroid ultrasonography(TUS) and compare it with solid ovoid isoechoic nodules (SOIN).

**Methods:** Between 2017-2021, SRIN with the diameters ≥ 5 mm and smaller than 25 mm at TUS, were retrospectively selected and enrolled in the study. Age, size, nodule volume, serum TSH levels, cytopathological and histo-pathological results were recorded**.** Malignacy were defined as Bethesda Thy 4,5 nodules, mostly confirmed with histopathology or repeated FNABs, and/or at least two years of follow up for Bethesda Thy 3a-3b nodules.

**Results:** Study is in progress, for the moment, 236 patients were enrolled. 137 of the patients have SRIN, 99 of them had SOIN. The mean age, the median largest diameter, nodule volume, TSH level, cytopathological and histopathological results were given below. There was a significant difference in terms of Bethesda classifications and histopathological results between SRIN and SOIN.

**Conclusions:** Limited data, showed a relatively high malignancy rate in small round isoechoic nodules. We found a higher malignancy rate for SRIN (≤ 25 mm) compared to SOIN. Thus round isoechoic nodules should be evaluated with more caution and biopsied, regardless of commonly used sonographic guidelines.

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## NONDIAGNOSTIC FINE-NEEDLE ASPIRATION BIOPSY ON THYROID NODULES: SHOULD BE THE RISK OF MALIGNANCY IGNORED?

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**Background:** Although The Bethesda System for Reporting Thyroid Cytopathology recommends the repetition of fine-needle aspiration biopsy (FNAB) in nondiagnostic results (category I), the malignancy risk reported in these cases is low(1-4%), which may justify the high number of patients that will only maintain clinical surveillance. However, recent studies have shown a not negligible malignancy rates in nodules with non-diagnostic FNAB (5-14%).

**Aim:** To determine the rate of malignancy of thyroid nodules with nondiagnostic FNAB and compare it with the risk in diagnostic FNAB.

**Methods:** Retrospective study including all FNABs performed between january 2016 and december 2019. According to the results, we divided FNABs into two groups: nondiagnostic FNAB (category I of the Bethesda System for Thyroid Cytopathology) and diagnostic FNAB (other categories). Demographic data, the ultrasound (US) characteristics, the respective EU-TIRADS classification, the follow-up for each nodule (repeat FNAB, surgery, clinical/US surveillance or loss of follow-up), the cytological results according to Bethesda's classification and their histology were recorded. The nodule was only considered malignant with an histologically confirmed diagnosis.

**Results:** From 1497 FNABs performed, 495 (33.1%) had a nondiagnostic result. Significant differences in age and gender were found between the 2 groups: patients with diagnostic FNAB were younger (median age 56 [P25-P75:47-66] vs 61 [P25-P75:51-70] years,p<0.001) and were more frequently female (85.8%vs76.6%,p<0.001). Of the nondiagnostic FNABs, 230(46.5%) repeated cytology, 53(10.7%) underwent surgery, 187(37.8%) maintained clinical/US observation and 25(5%) lost the follow-up. After considering the FNAB repeat results and respective follow-up, 205 nodules(72.4%) had a cytologic or histologic diagnosis and 16 were malignant (14 papillary thyroid carcinomas[PTC], 1 medullary thyroid carcinoma, 1 undifferentiated thyroid carcinoma). There was no significant difference in rate of malignancy between nondiagnostic vs diagnostic FNAB (16[7.8%] vs 65[6.5%],p=0.290) although this is higher in nondiagnostic group. PTC were slightly more frequent in nondiagnostic FNABs but no statistical result was found (6.8%vs5.8%,p=0.331).

**Conclusions:** In this sample, the rate of nondiagnostic FNAB results was 33.1% and malignancy was confirmed in 7.8% of cases, which is about two times higher than the one described by the Bethesda's classification, but in accordance with recently published studies. These results support the importance of the recommendation in repeat the FNAB in insufficient cytologies.

However, when compared with diagnostic biopsies, we did not find differences in the rate of malignancy in nondiagnostic biopsies.

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## THE ROLE OF ELASTOGRAPHY IN THE ASSESSMENT OF COLD SOLID THYROID NODULE

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**Objectives:** The prevalence of thyroid nodules detected by ultrasound (US) is reported to be up to 50% in general population and approximately 5-10% of them is malignant. Diagnostic assessment includes laboratory tests, thyroid US and scintigraphy, where suspicious nodules are characteristically cold when using $^{99m}$Tc-pertechnetate as a tracer. A useful tool for US-based risk stratification of thyroid nodules is Thyroid Imaging Reporting and Data System (TIRADS) and recently, a complementary role of elastography was shown. Our aim was to evaluate a diagnostic value of elastography using carotid artery pulsation in assessment of cold solid thyroid nodule.

**Methods:** In 32 patients, 25 females in 7 males (mean age, 49.6±17.4 years), we evaluated solitary or dominant solid thyroid nodule that was cold on scintigraphy with $^{99m}$Tc-pertechnetate. In every patient, thyrotropin (TSH) was measured, thyroid and nodule volume were calculated using standard formula and TIRADS score was estimated on the basis of US characteristics. Elastography using carotid artery pulsation was performed and elasticity contrast index (ECI) of thyroid nodule and paranodular tissue was assessed. In every nodule, fine needle biopsy was performed and cytology was reported using Bethesda classification.

**Results:** Mean TSH level was 1.8 mIU/L (range, 0.44-6.74 mIU/L). Mean thyroid volume was 31.1±20.3 ml and mean nodule volume was 14.4±16.1 ml. In 13 (40.6%) nodules TIRADS score was 4 or 5, and in 19 (59.4%) nodules TIRADS score was 3 or less. Mean ECI of thyroid nodules was significantly higher compared with mean ECI of paranodular tissue (1.89±0.85 and 1.09±0.37, respectively, $p<0.001$). Suspicious Bethesda category (4 or 6) was confirmed in 6 patients; their TIRADS score was 4 or 5. Compared to patients with unsuspicious Bethesda category, patients with suspicious Bethesda category did not have significantly higher mean ECI of thyroid nodule (2,21±1.58 and 1.81±0.61, respectively, p=0,31), but they were significantly younger (29.3±8.3 and 54.3±15.5, respectively, $p<0.001$). Patients with suspicious or unsuspicious cytology did not differ with respect to nodule volume or TSH concentration. Comparison of nodules with TIRADS score 4 or 5 and those with TIRADS score 3 or less did not confirm difference in ECI, patient's age, nodule volume or TSH concentration.

**Conclusions:** Our results show a significantly higher ECI in cold solid thyroid nodules than in surrounding thyroid tissue. However, elastography with ECI evaluation does not contribute significantly in assessment of those nodules. Data based on larger number of nodules is needed to further evaluate the value of elastography.

# Peripheral Actions of Thyroid Hormones

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## LYMPHOCITIC SUBPOPULATIONS INFILTRATING ORBITAL TISSUES IN GRAVES' ORBITOPATHY (GO) AND THEIR RELATION WITH GO ACTIVITY

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**Objective:** Graves' orbitopathy (GO) is a disfiguring condition most commonly associated with Graves' hyperthyroidism. The pathogenesis of GO is believed to reflect an autoimmune aggression against antigens expressed both in thyrocytes and orbital fibroblasts. The immune mechanisms responsible for GO occurrence and persistence are known only in part. We recently reported a significant correlation between the activity of GO and the number of B and T cells infiltrating orbital tissues. The aim of the present investigation was to determine which lymphocytic subpopulation are involved in the immune aggression to orbital tissues.

**Methods and Results:** We designed an observational, cohort study, aimed at evaluating the immunohistochemical phenotype of orbital lymphocytes and relate it with the activity GO, assessed with the clinical activity score (CAS). The study population included 94 patients (20 men and 74 women), all Caucasians (age: 50.2±11.8 yr), who underwent orbital decompression surgery. Orbital tissues samples were collected and subjected to histology and immunohistochemistry for CD20 (B cells); CD19 (B precursor); CD25 (B memory); CD3 (T cells), CD4 (T helper), CD8 (T cytotoxic) and CD56 (natural killer). An ophthalmologic evaluation was performed in all patients prior to surgery. A lymphocytic infiltrate was detected in 61.7% of tissue samples, staining positive for CD20 (79.3%), CD19 (43.1%), CD25 (43.1%), CD3 (62.0%), CD4 (86.2%) and CD8 (79.3%), suggesting the presence of a mixed lymphocytic population. No staining for CD56 was observed. In a simple linear regression model, the total number of lymphocytes (P=0.05), as well as the number of CD25-positive (P=0.05), CD3- positive (P=0.02), CD4- positive (P=0.01), and CD8- positive (P=0.05) cells correlated significantly with CAS, whereas the number of CD20-positive and CD19-positive cells did not. Multiple regression model including the other variables correlated with CAS (age, duration of hyperthyroidism, duration of GO, previous thyroid treatment, time since last glucocorticoid administration) confirmed a significant correlation for CD4-positive cells (P=0.005).

**Conclusion of the study:** Our findings show a T cell predominance in the relationship between the lymphocytic infiltrate in orbital tissue and GO activity and highlight a major role of T helper cells, possibly enhancing our understanding of the relationship between GO immunological features and clinical expression.

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## ROLE OF MACROPHAGE - FIBROBLAST INTERACTION FOR IMMUNOPATHOLOGY OF GRAVES` ORBITOPATHY

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Graves' disease (GD) is a thyroid disease caused by autoantibodies directed mainly against the Thyrotropin Receptor (TSHR) leading to autoimmune hyperthyroidism. Graves' orbitopathy (GO), also known as thyroid eye disease, is an inflammatory eye disease that develops in 30-50% of GD patients. Graves' orbitopathy is characterized by inflammation, remodeling, and expansion of the retroocular connective tissue. Orbital fibroblasts (OF) are considered the central cell type in inflammation and tissue remodeling in GO. Low oxygen tension could be an important component of the OF microenvironment during GO, especially when enlargement of connective/ fat and muscle tissues within the limited space of the orbita constricts vessels or even outpaces vascularization. Hypoxia is known to attract inflammatory cells and therefore can maintain inflammation and recruitment of immune cell like macrophages (MQ). Few is known about the specific contribution of macrophages to progression of orbitopathy. Therefore, we investigate the role and interaction of MQ and OF in context of inflammation and hypoxia. We analyzed the expression levels of hypoxic marker HIF-1α, macrophages marker CD68, proinflammatory cytokine TNFα and recruitment proteins CCL2, CCL5 and CCL20 in fat biopsies of Ctrl/GO tissue, we analyzed mRNA expression by real-time PCR. We found that HIF-1α, CD68, TNFα, CCL2, CCL5 and CCL20 mRNA expression was increased in the fat tissue of GO patients. Next, we analyzed the cytokine profile of the supernatants from fat biopsies with a multiplex ELISA. We could show that TNFα, CCL2 and CCL20 were enhanced secreted solely under hypoxia while CCL5 was induced on protein level under normoxia as well as hypoxia in GO tissue. To investigate inflammatory processes in OF we stimulated the OF with TNF-α or co-cultured the OF with M1 macrophages from a THP-1 cell line under normoxic and hypoxic conditions. We found that OF expressed hypoxic marker HIF-1α, hypoxia target gene VEGF and immune marker ICAM-1 as well as cytokines CCL2, CCL5 and CCL20 most pronounced upon TNFα stimulation and hypoxia. Induction of HIF-1α and CCL2 in OF was additionally elevated by co-culture with M1 macrophages under hypoxia compared to hypoxia alone. Infliximab a TNFα neutralizing Antibody prevented the effect. In summary, our results indicate that the inflammatory milieu in the orbital tissue is characterized by TNFα positive macrophages. This suggest that the orbital fibroblasts promote the inflammatory process by expression of chemokines necessary for recruitment of macrophages. Our data show a cumulative effect of hypoxia and macrophage migration in the presence of orbital fibroblasts. Infliximab could be a possibility to minimize the inflammatory process and thus prevent the progression of the GO pathogenesis.

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## HYPOTHYROIDISM INDUCES TOLERANCE TO EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is a devastating disease that causes more than 400.000 deaths per year, mainly children under the age of 5. In some impoverished countries where malaria prevails there are also endemic areas of iodine deficiency. Malaria causes low T3 syndrome, but the role of the thyroidal status in experimental models of malaria has not yet been examined. Using the CM model of *Plasmodium berghei* ANKA infection in mice we found that hypothyroidism conferred protection from CM. Unlike euthyroid animals that suffer altered consciousness, seizures, paralysis and coma, dying after 6-7 days, hypothyroid animals (fed a low iodine diet complemented with antithyroidal drugs), do not develop CM and survive until succumbing to anemia. We did not find differences in parasitemia, indicating that hypothyroidism induces survival by a disease tolerance mechanism. Although hypothyroid mice display spleen atrophy, after infection they develop a strong immune response with a larger increase in the number of the different splenic immune cell populations. The infection caused blood–brain barrier disruption both in eu- and hypothyroid mice. Brain immune cell infiltration and activation, as well as a pathogen burden, were also similar in both groups. However, the number of microhemorrhages, a hallmark of CM, was markedly reduced in hypothyroid mice. Other features of CM, including increased brain volume, crushing of the cerebellum and brainstem herniation as a consequence of edema were absent in hypothyroid-infected mice. Fatal outcome in CM is also associated with compression of the cerebral arteries and impairment of the cerebral blood flow (CBF) consequence of brain swelling. Infected euthyroid mice showed a strong reduction of CBF, while CBF was quite similar in uninfected and infected hypothyroid mice. The host´s metabolic state controls tissue damage to achieve disease tolerance compatible with survival. Tolerance to CM is associated with a distinct metabolic profile in the brain and with a resilient metabolism in the infected hypothyroid mice, which in contrast to euthyroid mice, display little reduction in $O_2$ consumption, $CO_2$ output, respiratory exchange rate or energy expenditure upon infection. Sirtuin-1, a master regulator of metabolism, might be an important component of the tolerance mechanism, as hypothyroid mice treated with the Sirtuin-1 inhibitor EX-527 displayed increased lethality, brain compression and collapse of cerebral arteries, while treatment of euthyroid mice with the SRT1720 activator resulted in reduced compression, partial recovery of CBF and increased survival. This suggests that the utilization of Sirt1 activators might be useful for the treatment of human CM, a major clinical problem in developing countries.

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## DIFFERENTIAL DERANGED LIVER PATHWAYS IN GENETICALLY DETERMINED THYROID DYSFUNCTIONS: INTRA- AND EXTRAHEPATIC FACTORS

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**Objectives:** Alterations in thyroid function play a major role in the pathogenesis of liver diseases. Thyroid hormones (THs) have been associated with the development of insulin resistance (IR), a signature of the metabolic syndrome, predisposing to other diseases such as diabetes as well as non-alcoholic fatty liver disease and hepatocellular carcinoma. The pathogenic mechanisms appear complex and not reducible exclusively to the impaired TH signalling. Indeed, both intra- and extrahepatic factors may be involved.

**Methods:** By a transcriptomic, proteomic, morphological and functional approach, we obtained new details on the metabolic consequences of mild and severe thyroid dysfunction associated to single or double null heterozygous mutations of the Pax8 and Nkx2-1 genes, two of the master regulators of thyroid development and function in both humans and mice. We characterized thyroid function in 3 months old females of the different genotypes evidencing different dysthyroidisms, impacting the whole animal body, other endocrine axis at differential levels and liver metabolism.

**Results:** Nkx2-1+/- mice display elevated circulating T3 levels and develop liver steatosis with a concomitant induction of hepatic IR, TH signaling (as suggested by the increased expression of Dio1 and Tr**β1), and lipogenic genes.** On the other hand, while double heterozygote for both Nkx2-1- and Pax8-null mutations (DHTP) mice display hypothyroidism, Pax8+/- mice are euthyroid, but the two genotypes share hepatic and skeletal muscle IR and liver local hypothyroidism. In addition, both DHTP and Pax8+/- mice show, in liver, activation of the Akt pathway and increased expression of glutathione peroxidase 4, but this is associated with oxidative stress and reduced mitochondrial COX activity in DHTP mice only. Importantly, Pax8+/- mice, but unexpectedly not DHTP ones, display activation of the hepatic (and renal) gluconeogenic pathway, hypercortisolemia, and net fasting hyperglycaemia and hyperinsulinemia associated, in liver, to AMPK activation and mitofusin 2, OPA1 and peroxiredoxin 3 increased representation levels. DHTP mice, on the other hand, show increased levels of AMPK with a concomitant activation of Ser2448mTOR, central core of the mTORC1 complex, involved in autophagy inhibition.

**Conclusions:** Our data indicate that mutations in Pax8 and Nkx2-1 genes, in single or double heterozygosity, may produce, in young female mice, multiple dysmetabolisms depending on the extent to which TH levels are affected, even under systemic euthyroidism. The data indicate differential affected pathways in liver with implications for energy and nutrient sensing within the complex network controlling glucose and lipid metabolism, mitochondrial oxidative capacity and autophagy. The obtained information opens new perspectives in targeting liver dys/metabolism in the management of thyroid dysfunction in terms of prevention/counteraction of IR, diabetes and related comorbidities.

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## THYROID HORMONE DEFICIENCY MODIFIES HEPATIC LIPID DROPLET MORPHOLOGY AND MOLECULAR PROPERTIES IN LITHOGENIC-DIET SUPPLEMENTED MICE

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**Objective:** Thyroid hormones have been associated with a hepatic lipid lowering effect and thyroid function has been shown to play a substantial role in development of non-alcoholic fatty liver disease. Hepatic lipid droplets differ in the number, size and molecular properties depending on metabolic state or pathological condition. However, in how far thyroid hormone deficiency affects hepatic lipid droplet morphology and molecular properties is still poorly understood. Therefore, we performed a study in mice using a lithogenic diet model of steatohepatitis and modulated the thyroid hormone status.

**Methods:** Male and female three months old C57BL/6 mice were divided into a euthyroid (control), a lithogenic (litho) and a lithogenic+thyroid hormone deficient (litho+hypo) group and treated for six weeks. Hepatic transmission electron microscopy and gene expression analysis of lipid-droplet associated proteins were performed.

**Results:** Increased mean diameters of hepatic lipid droplets and a shift towards raised electron-density in lipid droplets was observed under thyroid hormone deficiency. Furthermore, thyroid hormone deficiency altered hepatic expression of genes involved in lipophagy and triacylglycerol mobilization. Interestingly, while the impact of thyroid hormone deficiency on lipid droplet morphology seems to be sex-independent, hepatic lipid droplet-associated gene expression differed significantly between both sexes.

**Conclusion:** This study demonstrates that thyroid hormone deficiency alters hepatic lipid droplet morphology and hepatic gene expression of lipid droplet-associated proteins in a lithogenic diet mouse model of steatohepatitis.

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## MILD EXERCISE INDUCES THYROID HORMONE ACTION ASSOCIATED WITH BDNF SIGNALING IN BOTH CORTEX AND MUSCLE UNDER DISTINCT NUTRITIONAL CONDITIONS

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**Objectives:** Thyroid hormone has an important role in energy metabolism. We have recently shown that short-term fasting with exercise results in beneficial metabolic changes in rats and humans. In rats we showed that this is accompanied by a normalization of serum fT4 levels and increased beta hydroxy butyrate (BHB) levels. We have shown that the BHB-responsive brain derived neurotrophic factor (BDNF) is upregulated significantly in muscle in this condition. Here we studied BDNF signaling in gastrocnemius muscle and the prefrontal cortex and we set out to explore the eventual consequences for local T3 production in muscle vs brain. We also used rat L6 muscle cells to further investigate the relationship between glucose deprivation, T3, BHB, and BDNF signaling.

**Methods:** Rats housed at 28°C (thermoneutrality) were subjected to 66h of fasting, with or without mild 30 min exercise sessions on a treadmill (twice daily, 15 m/min, 0° inclination). BDNF protein and phosphorylation of downstream factors was measured by Western immunodetection in gastrocnemius muscle and prefrontal cortex. In the same tissues, the expression of various

genes involved in thyroid hormone transport, metabolism, and action, was measured by Q-PCR. Undifferentiated or differentiating Rat L6 muscle cells (3 days) were glucose deprived and treated with 100 nM T3 or 8 mM BHB and assayed for BDNF expression, and related signaling factors.

**Results:** Increased BDNF signaling by exercise consistently decreased D3 expression in both prefrontal cortex and gastrocnemius muscle. However, exercise-induced BDNF signaling increased in the prefrontal cortex of chow-fed rats and in gastrocnemius muscle of fasted rats. The strong increase in D2/D3 ratio explains the strong modulation in various T3-responsive genes in gastrocnemius muscle in this condition. In analogy, exercise modulated the expression of T3-responsive genes in the chow fed-rat prefrontal cortex. Surprisingly, the increased exercise-induced response to T3 and low D3 levels are not correlated with BDNF signaling in the fasted rat cortex, despite the high BHB levels. In L6 myoblasts and myotubes, glucose deprivation strongly induced BDNF expression, which T3 downregulated in the undifferentiated but not in the differentiated cells, which needs to be explored further in future studies.

**Conclusion:** This ongoing study has brought to light the association between BDNF signaling and local T3 action during short-term combined exercise with fasting with proven beneficial effects, opening interesting avenues for studying the central and peripheral relationship between ketones, T3 and BDNF signaling.

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## THYROID PHENOTYPE OF SBP2-DEFICIENT MODELS RECAPITULATES THE THYROID SIGNATURE OF PATIENTS WITH SBP2 BIALLELIC MUTATIONS

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Selenium is incorporated as selenocysteine (Sec) into a family of selenoproteins (SPs) with diverse, biological roles. Sec incorporation is mediated by a multiprotein-complex including SECIS-binding protein-2 (SBP2). Individuals with biallelic SBP2 mutations exhibit a multisystem disorder with a characteristic thyroid signature caused by abnormal thyroid hormone (TH) metabolism due to deficiency of Sec-containing deiodinases: high T4, normal or low T3, high rT3 and normal TSH. Since murine Sbp2 knockout (KO) is embryonic lethal, we used sbp2 morpholino-mediated zebrafish knockdown (KD) and sbp2-KO embryos to gain insights into human SBP2-thyroid pathology. Embryonic thyroid development was normal in both sbp2-KD and -KO models as evidenced by normal expression of early (*nkx2.4*, *pax2a*) and late (*tg*, *slc5a5*) thyroid markers. However, thyroid function tests performed by immunofluorescence and ELISA assays documented abnormal TH levels in both models. When compared with controls, sbp2-KD showed increased number of T4-producing follicles and low pronephric T3 signal, accompanied with normal/slightly increased expression of *tshba*. Significant changes in TH levels were also been observed in the whole sbp2-KD larvae (high T4, low T3, low T3/T4 ratio). Interestingly, the co-injection of morpholino and *sbp2* mRNA in rescue experiments was able to quite complete normalize the thyroid function of sbp2-KD, confirming the specificity of the observed thyroid phenotype. Consistently, as both heterozygous and siblings, the homozygous sbp2-KO larvae displayed normal *tshba* expression, high T4 and low T3 levels resulting in a diminished T3/T4 ratio. The enzymatic activity of dio2 was tested measuring T3 concentration in basal condition and after treatment with db-cAMP, a potent activator of *dio2* expression. When compared with control vehicle (1% DMSO), *dio2* mRNA levels were dramatically increased in all groups following exposure with 100µM of db-cAMP. As a consequence, T3 conversion increase +310% and +300% in control and rescued larvae (for KD model), and +370% and +350% in siblings and heterozygous (for KO model). In contrast to the high *dio2* levels, only a negligible increment of T3 concentration was seen in both sbp2-KD and KO homozygous larvae after db-cAMP administration. Similar results were obtained following levothyroxine supplementation (20nM of L-T4). In comparison with the significant increment of T3 levels seen in ctrl (+92%) and rescued (+80%), as well as in sib (+173%) and het (+ 156%), T4/T3 conversion was strongly reduced in both sbp2-KD and KO homozygous larvae. In conclusion, zebrafish sbp2-KD and -KO present impaired T4 to T3 conversion due to reduced activity of dio2, representing a useful *in vivo* model that recapitulates the thyroid phenotype of human SBP2 deficiency, enabling their pathogenesis to be further elucidated.

# Primary Thyroid Disease

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## THYROID PEROXIDASE ANTIBODIES DO NOT PREDICT FIRST USE OF ANTIDEPRESSANTS IN THE GENERAL POPULATION

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**Context:** Associations between autoimmune hypothyroidism and symptoms of depression and anxiety have been reported. Conflicting results exist on the influence of thyroid autoimmunity per se.

**Objective and Design:** We investigated whether autoantibodies against thyroid peroxidase (TPOAbs) were associated with the first use of antidepressants using data from The Danish General Suburban Population Study (GESUS) and Danish national registers. We followed 8361 individuals from the time of TPOAb measurement in GESUS (Jan. 2010 – Jul. 2011) until the first redeemed prescription for an antidepressant, death, emigration, or end of follow-up (Dec. 2018). Positive TPOAbs were defined according to the specific assay as Ab-titer > 60 IU/ml. We used cox proportional hazard regression to estimate cause-specific hazard functions.

**Results:** At baseline, we excluded the same proportion in both groups due to previous antidepressant use (TPOAb-positive: 21% vs TPOAb-negative: 20%, p = 0.26). TPOAb-positive were older (57 vs 56 yrs., p<0.001) and more often female (74% vs 48%, p<0.001). During 63587 person-years of follow-up, 670 persons redeemed a prescription for an antidepressant. Mean follow-up time did not differ between groups (7.6 vs 7.6 yrs., p=0.60). Comparing TPOab-positive with TPOab negative yielded an unadjusted hazard ratio (HR) for antidepressant use of 1.15 (95% CI, 0.92 to 1.43, p=0.21). After adjusting for sex, age, and levothyroxine use at baseline, TPOab-positive had an HR for antidepressant use of 0.99 compared to TPOab-negative (95% CI, 0.79 to 1.25, p=0.96). Restricting the analysis to the upper third with the highest TPOab titers yielded an adjusted HR of 0.71 compared with TPOAb-negative (95% CI, 0.46 to 1.08, p=0.11).

**Conclusion:** In a large Danish cohort of 8361 individuals with 63587 person-years of follow-up and 670 first-time prescriptions for antidepressant use, TPOAb-positivity was not associated with the first use of antidepressants.

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## SCREENING FOR THYROID DYSFUNCTION IN LOW-RISK ADULTS

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**Objective:** The evaluation of thyroid function by TSH measurement is accepted for individuals with a clinical suspicion of thyroid dysfunction or who are at high risk for this condition. It can be noticed that the recommendation

of evaluating thyroid function based only on age in asymptomatic individuals who are not at high risk is controversial. The aim of this study was to report the result of screening for thyroid dysfunction by TSH measurement in adults at low risk for the condition but older than 35 years. Methods: Initially, 2,532 apparently healthy adults (age > 18 years), not including pregnant women, were interviewed. Of these, 2,327 subjects agreed to participate in the study. Nine hundred and one subjects were excluded because of high-risk factors for thyroid disease or conditions potentially interfering with thyroid function detected upon clinical evaluation. The final sample consisted of 1,426 apparently healthy adults considered low risk for thyroid dysfunction without known interfering factors. TSH was obtained in all subjects. Thyroid hormones were measured in those with TSH < 0.4 mIU/L or > 4.5 mIU/L. Investigation for underlying thyroid disease was performed when this could result in the potential indication for treatment of subclinical dysfunction.

**Results:** None of the 1,100 adults older than 35 years had TSH concentrations ≤ 0.1 mIU/L or ≥ 10 mIU/L. TSH between 0.1 and 0.4 mIU/L was observed in 24 subjects, all with normal free T4 and T3 concentrations. Only two subjects would be potential candidates for treatment of subclinical hyperthyroidism. TSH between 4.5 and 7 mIU/L was observed in 26 subjects, all with normal free T4. Among adults younger than 65 years, one was TPOAb positive and only he would be a potential candidate for treatment of subclinical hypothyroidism. Thus, significant TSH abnormality was not detected in any of the subjects and only three (0.27%) would be potential candidates for treatment of subclinical thyroid dysfunction. Among the 416 adults older than 60 years, only two subjects (0.5%) with TSH between 0.1 and 0.4 mIU/L would be potential candidates for treatment. Considering the postmenopausal women, treatment would be potentially indicated in only one (0.3%). Even among the women older than 60 years, only one (0.5%) would eventually be a candidate for treatment. The results of this study do not support screening for thyroid dysfunction based only on age in low-risk asymptomatic adults.

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## ADVERSE CHILDHOOD EXPERIENCES AND NEGATIVE EMOTIONAL RESPONSES: RISK FACTORS FOR THYROID AUTOIMMUNE DISEASES?

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**Background:** Stressful condition and negative emotions can contribute to the overproduction of proinflammatory cytokines and promote immune dysregulation, which, in turn, increases the risk of various diseases, including autoimmune disorders. The present study was aimed at evaluating the relationship, if any, between psychological and individual characteristics (stress, coping and emotional intelligence) and thyroid autoimmunity.

**Method:** We enrolled 174 HT patients (gender: 157 female; 12 male; age: M=47,01; SD=13,36) and 133 euthyroid subjects (gender: 111 female, 21 male; aged M=45,56; SD=12,69) as controls. All subject had no personal and/or familial history of psychiatric disorders. All participants fell a set of psychological self-report questionnaires in order to measure psychological stress (MSP), coping strategy (Coping Inventory for Stressful Situations, CISS), trait emotional intelligence (TEIQue-SF) and Adverse Childhood Experiences (ACEs questioner

**Results:** We found significant differences between patients and controls. MSP test showed greater levels of overall stress (P=0.001) and stress subcategories, including psycho-physiologic sensations (p= 0.000), effort and confusion (p=0.02), depressive anxiety (p= 0.01), pain and physical problems (p=0,000), in HT patients than controls. Also, HT patients showed minor well-being (p= 0.020) and self-control (p=0.047) compared to controls at TEIQue Test. Concerning coping strategies, HT patients seem to have more difficulties in adequately managing the emotional area (p=.004), which involves greater emotional responses, self-preoccupation, and a fantasizing tendency. Noteworthy, it emerged that HT patients have had a greater number of traumatic experiences in childhood (p= 0,01) than controls, with particular reference to physical abuse (p=.001), parental divorce (p=0,02) and presence in the family of subjects suffering from mental illness (p=0.05) or substance abuse (p=0.38). The patha analysis on HT patients confirmed that the total stress is influenced by adverse childhood experiences (β = .12, p = .04) and by trait emotional intelligence (β= -.16, p =0.02). It is conceivable that traumatic events occurring early in life might have had an influence on the subsequent development of autoimmune disease.

**Conclusions:** Our data suggest a correlation between psycho-social and immune factors. HT patients seem to have a very delicate psycho-affective equilibrium, difficulties in emotion regulation and impulse control, as well as in managing stress. Traumatic experiences in childhood and negative emotional responses may favor the subsequent development of autoimmunity, acting as an exogenous trigger in susceptible subjects. Autoimmunity, even in conditions of euthyroidism, can in turn negatively impact the psychological well-being of patients, who in fact appear less confident and optimistic.

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## THYROID FUNCTION AND SIZE IN INDIVIDUALS WITH DETECTABLE BUT NOT INCREASED LEVELS OF THYROID ANTIBODIES

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**Objectives:** Hashimoto's thyroiditis (HT) is an autoimmune thyroid disease that is most frequently associated with hypothyroidism. The disease is usually characterised by increased level of thyroid peroxidase antibodies (antiTPO) and/or thyroglobulin antibodies (antiTg) as well as by hypoechoic ultrasound pattern. In clinical practice, however, various forms of HT are observed. Patients with HT may only have hypoechoic ultrasound pattern, while antiTPO and/or antiTg are not increased, and vice versa, patients with HT may only have increased levels of antiTPO and/or antiTg, while thyroid ultrasound pattern is isooechoic. Another open issue regarding diagnosis of HT is assessment of antiTPO and/or antiTg levels. We aimed to explore the question if individuals with detectable and not elevated levels of antiTPO and/or antiTg differ from individuals with undetectable levels of antiTPO and/or antiTg and if undetectable levels of thyroid antibodies only exclude diagnosis of HT.

**Methods:** In this cross-sectional clinical study we included 307 non-pregnant female volunteers of reproductive age. Women with the known thyroid disease were not included in the study. In every volunteer, levels of antiTPO, antiTg and thyrotropin (TSH) were measured. We also performed thyroid ultrasound and calculated thyroid volume using standard formula.

**Results:** Mean age of all women was 29.8±7.1 years. Mean thyroid volume was 7.5±2.6 mL. Median TSH level was 1.6 mIU/L (range, 0.3-116.9 mIU/L). We detected the increased level of antiTPO and/or antiTg in 52 (16.9%) women, the detectable but not elevated level in 27 (8.8%), and undetectable level in 228 (74.3%) women. Women with increased levels of thyroid antibodies had significantly higher TSH level than women with detectable or undetectable levels of antibodies (6.0±18.9 mIU/L and 1.7±0.9 mIU/L, respectively, p<0.001). Women with increased levels of thyroid antibodies had significantly larger thyroid volume than women with detectable or undetectable levels of antibodies (8.7±2.7 mL and 7.3±2.5 mL, respectively, p<0.001). Women with detectable but not increased thyroid antibodies had higher - but not significantly different - TSH level than women with undetectable antibodies (2.0±1.1 mIU/L and 1.7±0.9 mIU/L, p=0.084). Women with detectable but not increased thyroid antibodies had significantly larger thyroid volume than women with undetectable antibodies (8.0±2.5 mL and 7.2±2.5 mL, respectively, p=0.044).

**Conclusions:** Presumably healthy individuals with detectable but not increased levels of antiTPO and/or antiTg do not have similar thyroid function and size as individuals with undetectable levels of antibodies. This raises the question whether HT can only be excluded by undetectable levels of thyroid antibodies.

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## FOLLICULAR THYROGLOBULIN REGULATES EXPRESSION AND LOCALIZATION OF SLC26A7, A NOVEL IODIDE TRANSPORTER AT THE APICAL MEMBRANE

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**Background:** Recently, *solute carrier family 26 member 7 (SLC26A7)* was identified as a novel responsible gene for congenital hypothyroidism. The encoded protein was found to be expressed on the apical membrane of follicular epithelium in the thyroid, which transfers iodide into the follicular lumen. We have recently reported that TSH downregulates Slc26a7 expression in the thyrocyte and translocates the protein to the plasma membrane. Thyroglobulin (Tg) stored in the follicular lumen is another potent regulator of follicular function that regulates the expression of thyroid-specific genes by an autocrine mechanism. In the present study, we investigated the effect of follicular Tg on Slc26a7 expression and localization in thyrocytes.

**Method:** Rat thyroid FRTL-5 cells were stimulated by follicular concentrations of Tg. Cellular mRNA and protein were purified and Slc26a7 expression levels were evaluated using real-time PCR and Western blotting, respectively. 5'-franking regions of the *SLC26A7* gene were cloned into pGL3-Basic and the promoter activity was evaluated by luciferase reporter gene assay following Tg stimulation. Changes in subcellular localization of Slc26a7 in FRTL-5 cells were analyzed using confocal laser scanning microscopy following Tg stimulation.

**Results:** Expression of Slc26a7 was significantly suppressed by the follicular concentration of Tg in a dose-dependent manner in both mRNA and protein. The promoter activity of *SLC26A7* was also suppressed by Tg. Tg inhibited TSH action to induce plasma membrane localization of Slc26a7.

**Conclusion:** As we have reported on other genes essential for the thyroid function, Slc26a7 expression was also significantly suppressed by follicular Tg. Tg suppressed the action of TSH to translocate Slc26a7 to the plasma membrane. Since follicular Tg suppressed the expression of Slc26a4 (pendrin), another iodide transporter expressed at the apical membrane, the results further support an idea that iodide secretion into the follicular lumen will be suppressed in the follicle where a high concentration of Tg is already accumulated in the colloid.

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## A NOVEL HYPOMORPHIC GENE VARIANT AND POLY-ALANINE STRETCH OF FOXE1 CO-SEGREGATE WITH ATHYREOSIS IN A LARGE ASIAN FAMILY WITH CONGENITAL HYPOTHYROIDISM

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**Objectives:** Congenital hypothyroidism (CH) due to thyroid dysgenesis (TD) is a heterogeneous disorder with a frequently missing heredity. However, a limited but significant portion of TD familial cases have been identified (2%). We describe a large Asian family with 7 of 10 siblings affected with severe CH due to athyreosis, which is likely to be the largest family with TD studied so far. To identify the potential cause of CH in this family, a next-generation sequencing (NGS) was performed.

**Methods:** A targeted NGS panel including 20 potential candidate genes for CH was applied to all members of the Asian family and further, we validated the results in our database of 370 CH patients and 1456 controls without thyroid diseases. *In silico* and *in vitro* analysis were used to predict possible changes in the FOXE1 variant protein structure and function.

**Results:** We identified a novel missense heterozygous variant in *FOXE1* gene affecting a highly conserved residue in the DNA binding domain of the transcription factor, that co-segregated with homozygosity of *FOXE1* allele containing 14 Alanine repeats (poly-A14) in all the children affected with athyreosis. No other variation in *FOXE1* or other candidate genes co-segregated with CH phenotype in this family. Functional studies demonstrated that this new variant is normally expressed but significantly impairs the FOXE1 transcriptional activity on the TG and TPO promoters. Similarly, the transcriptional activity of the poly-A14 was found to be significantly lower than that of the most frequent poly-A16 allele. Consistently with data indicating the highly polymorphic poly-A tract of *FOXE1* gene as a susceptibility factor for CH, the large cohort of CH patients was found to be significantly enriched with the homozygosity of poly-A14 allele (54% vs 32% in controls).

**Conclusions:** We propose that the poly-A14 is a hypomorphic *FOXE1* allele highly diffuse in the general population that once present in homozygosity and in combination with a rare loss-of-function *FOXE1* mutation can cause athyreosis and severe CH.

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## ASSOCIATION OF TWO MATRIX METALLOPROTEINASE-9 PROMOTOR POLYMORPHISMS AND ACETYLATED FORM OF C-JUN TRANSCRIPTION FACTOR WITH PAPILLARY THYROID CARCINOMA ADVANCEMENT

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**Objectives:** Papillary thyroid carcinoma, a common form of endocrine malignancy, presents a clinical challenge from a prognostic standpoint. Molecular alterations responsible for PTC advancement include MMP-9 genetic promotor polymorphism sequences that bind transcription factors with varying affinity, hence constituting predisposition towards MMP-9 expression levels. This work was aimed to examine how two promotor polymorphisms (1562 C/T single nucleotide transition and -131 (CA)n tandem repeats) as well as levels of c-jun transcription factor and its posttranslational modification acetylated at lys-271 influence MMP-9 expression and PTC progression.

**Methods:** Genotyping of -1562 C/T single nucleotide transition was performed by PCR-RFLP (polymerase chain reaction restriction fragment length polymorphism). The estimation of -131 (CA)n tandem repeat number was evaluated by resolving on urea-PAGE followed by silver staining. MMP-9 mRNA levels were quantified utilizing RT-qPCR, while protein levels of jun, c-jun and MMP-9 were scored by immunohistochemistry.

**Results:** Genotyping and immunohistochemical analysis revealed that a significant portion of PTC samples was heterozygous for the (CA)n tandem repeat number (80.8%), had a transcription-promoting T allele at -1562 (75.5%), and expressed high levels of c-jun (35.4%), acetylated c-jun (38.6%) as well as MMP-9 protein levels (36.7%). RT-qPCR revealed higher levels of MMP-9 mRNA in PTC compared to healthy subjects ($p=0.01$) as well as a positive correlation between MMP-9 mRNA and protein levels ($p=0.048$). The T allele at -1562 position was accompanied by elevated MMP-9 protein expression ($p=0.019$), while high acetylated c-jun levels accompanied high MMP-9 both on mRNA and protein levels ($p<0.05$). The -1562 C>T transition, MMP-9 and acetylated c-jun were associated with presence of extrathyroid invasion and degree of tumor infiltration, while the T allele and acetylated c-jun also correlated with T stage, all comparisons significant at the $p<0.05$ level.

**Conclusions:** We conclude that -1562 MMP-9 polymorphism and levels of acetylated c-jun affect PTC progression via modulation of MMP-9 levels. Genotyping MMP-9 at -1562 and estimating protein levels of MMP-9 and acetylated c-jun in PTC may prove beneficial in identifying high-risk patients.

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## PRO-INFLAMMATORY EFFECTS OF HYALURONAN (HA) FRAGMENTS ARE PREVENTED BY CURCUMIN IN HUMAN FIBROBLAST AND THYROCYTES

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**Background:** Lymphocytic infiltration and inflammation in autoimmune thyroid diseases (AITDs) results in accumulation of HA, contributing to the pathogenesis of both thyroidal and extra-thyroidal (ophthalmopathy, pretibial dermopathy and mixedema) manifestations of AITDs. HA fragments, originating from native HA during tissue inflammation and injury, in turn promote the expression of different mediators of oxidative stress and inflammation, by interacting with the Toll-like receptor 2 (TLR-2) and 4 (TLR-4) and CD44, via nuclear factor kappa-B (NF-kB). Curcumin (diferuloylmethane) is a phytochemical with anti-inflammatory and anti-oxidant properties. It has been reported to have suppressive effect on NF-kB signaling pathway in various cell types. This study was aimed at investigating the effects of curcumin treatment in cultured primary human thyrocytes and fibroblasts after exposure to 6-mer HA oligosaccharides (O-6-mer HA).

**Methods:** Cultured cells were treated with increasing concentrations of curcumin (2.5, 5, 10 μg/ml), with and without O-6-mer HA (50 μg/ml). mRNA and proteins expression for TLR-2, TLR-4, inducible nitric oxide synthases (iNOS), interleukin-1beta (IL-1beta), IL-6, matrix metalloproteinase 9 (MMP-9 ), and thyroid-specific genes [thyroglobulin (Tg) and sodium iodide symporter (NIS) in thyrocytes] were evaluated by real-time PCR and Western Blot, respectively. Protein quantification was assessed by densitometry analysis. NF-kB p50/65 activation was determined in nuclear extracts by DNA binding activity assay. The pro-inflammatory cytokines IL-1 beta and IL-6 levels were measured by ELISA. Levels of malondialdehyde (MDA) were measured in culture medium by a spectrophotometric method.

**Results:** In both cell lines 6-mer HA treatment induced the increase in mRNA and protein of TLR-2, TLR-4, CD44, as well as the activation of NF-kB, that in turn increased iNOS, IL-1beta, IL-6 and MMP-9 expression and MDA levels. Treatment with curcumin at increasing concentrations (2.5, 5, 10 μg/ml) decreased NF-κB activation and significantly reduced iNOS, IL-1beta, IL-6, MMP-9 and MDA levels in a dose-dependent manner ($p<0.05$; $p<0.001$; $p<0.0001$ respectively). In thyrocytes, treatment with curcumin significantly increased the mRNA expression of the Tg and NIS, decreased after exposure to O-6-mer HA. No effect was observed on CD44 and TLRs activation, thus suggesting that the suppressive effect of curcumin on NF-kB is mediated by receptors others than TLRs and CD44.

**Conclusions:** Curcumin is able to reduce pro-inflammatory and pro-oxidative effects of HA oligosaccharides in both thyrocytes and fibroblasts. Since it has been suggested that HA fragments contribute to develop inflammation and oxidative damage in both thyroidal and extra-thyroidal (i.e. dermal and orbital) tissues in the course of AITDs, curcumin could be beneficial in these disorders as a suitable adjunct to conventional pharmaceutical therapy.



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## MULTICELLULAR SPHEROIDS: A NEW MODEL TO SCREEN NOVEL DRUGS FOR INCURABLE FORMS OF THYROID CANCER

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Thyroid cancer (TC) is the most common endocrine malignancy and its incidence is increasing worldwide even if the mortality rate remains low. There is accumulating evidence that solid tumors contain a distinct subpopulation of Cancer Stem-like Cells (CSCs), or tumor-initiating cells (TICs), that play important roles in cancer initiation, progression, recurrence and metastasis. Whether thyroid cancer cells are derived from mutated adult stem cells or whether they display a newly acquired stem-like phenotype due to cancer mutations remains controversial, but they are believed to be one main actor in therapy resistance and disease progression. The aim of the project is to understand the biology of thyroid TICs, by *in vitro* characterization of thyrosphere-forming cells, and use this 3D model to investigate thyroid TICs sensibility to different anticancer treatments. To overcome the intrinsic limitations of primary cultures and fresh sample availability, we are currently developing a standardized thyrosphere model based on immortalized cell lines with different genetic background. Hanging drop cell cultures and coating with poly(2-hydroxyethyl methacrylate) non-adhesive substrate are the main methods to obtain thyrosphere-forming cells. In appropriate growth condition, all the cell lines tested are able to generate thyrospheres when seeded at clonal density, with an efficiency significantly higher that what has been previously reported. In particular, we've obtained a sphere-forming efficiency of 61.22% for B-CPAP, 52.22% for HTC/C3, 61.20% for SW579, 70.24% for FRO, 51.09% for SW1736 and 50.09% for HTH-74. Furthermore, we selected different anticancer drugs and analysed their effects on both 3D and 2D cultures. In this regard, we've tested two inhibitors so far: PLX-4720, a potent and selective inhibitor of ERK phosphorylation in BRAFV600E cells, and Cariporide, a selective inhibitor of NHE-1, a Na+/H+ exchanger. We evaluated the effects of these compounds by MTT colorimetric assay and observed that cell lines with different genetic background respond differently when seeded as adherent cells or as 3D thyrospheres. In conclusion, this 3D model can partially mimic the tumor complexity *in vitro*, and the most promising results shall be validated on patients-derived samples.

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## IN VITRO MODELING OF THYROID CANCER CELLS AND FIBROBLASTS INTERPLAY IN DRUG SCREENING

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Thyroid cancer (TC) is the most common endocrine tumor and its incidence has increased faster than in any other malignancy. Although TCs are usually well differentiated, disease recurrence or persistence is high, because of local and distant metastasis and therapeutic resistance. Among the different genetic alterations, BRAFV600E is the most frequent one. Several studies tried to establish a correlation between BRAFV600E and clinical outcome, with controversial results, probably due to the co-existance of TC cells subpopulations with different genetic background. Nevertheless, the activation of different BRAF downstream pathways influences immune response, extracellular matrix (ECM) remodeling and intra- and extra-cellular pH. All these alterations substantially modify tumor microenvironment and may enhance the survival of different TC populations and promote therapy resistance.

The aim of the study is to investigate the role of BRAF-induced cancer-associated fibroblasts (CAFs) activation and matrix remodeling in TC therapy resistance.

In particular, the use of conditioned media and co-cultures of TC cells with different genetic background and fibroblasts is used to generate different ECMs. The ECM themselves and their effects on cell growth, survival and sensitivity to various compound is then analyzed by different techniques, such as western blot, immunofluorescence, colony assay, sphere formation assays and proliferation assays.

Our results show that TC cells with BRAFV600E mutation can significantly increase the proliferation and activation of fibroblasts in respect with BRAF WT TC cells and normal thyrocytes. Similar to what observed in TC tissue samples, fibroblasts that have been conditioned with BRAFV600E TC cells can produce an ECM that is thicker and with different fiber pattern than the one produced from fibroblasts conditioned with BRAF WT TC cells and normal thyrocytes. The different ECMs differentially influence the survival of TC cells and their sensitivity to different drugs. In particular, the BRAF-induced matrix remodeling increase the survival of TC cells treated with compounds directed against the BRAF/RAS and PI3K/Akt pathways, while the matrix obtained with fibroblasts pre-treated with inhibitors of BRAF downstream targets sensitize TC cells to the same therapy.

The disruption of TC cells-fibroblasts crosstalk can thus be a promising field of investigation, and the screening of compounds that specifically act in this direction can allow the development of better therapeutic associations. In conclusion, our *in vitro* model can partially recapitulate the complex environment of human tumors and can be a useful tool for the screening of different anticancer drugs and the identification of synergistic combinations.

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## IN VITRO EVALUATION OF 3-IODOTHYRONAMINE (T1AM) AND 3-IODOTHYROACETIC (TA1) BLOOD BRAIN BARRIER (BBB) PERMEABILITY

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Recent reports highlighted the potential of 3-iodothyronamine (T1AM) as novel drug for the treatment of neurodegenerative diseases (NDDs). However, the pharmacological response to T1AM might be influenced by tissue metabolism, which is known to convert T1AM into its catabolite 3-iodothyroacetic acid (TA1). A critical aspect in the development of new drugs for NDDs is to know their distribution in the brain, which is fundamentally related to their ability to cross the blood-brain barrier (BBB). To this end, in the present study we used the immortalized mouse brain endothelial cell line bEnd.3 to develop an in vitro model of BBB and evaluate T1AM and TA1 permeability by LC-MS/MS analysis. Since the membrane-associated drug transporter P-glycoprotein (P-gp) plays an essential role in drug efflux from the brain, we also evaluated P-gp expression on bEnd.3 cells treated with test compounds.

**Methods:** In vitro BBB model: bEnd.3 cells were seeded on 24-well culture plates provided of a polyester membrane able to sustain cells attachment and growth, and to generate an upper and a lower compartment inside the well. Immunological detection of ZO-1 protein allowed to confirm the BBB formation. Paracellular selective permeability of our BBB model was assess by using 4 KDa and 70 kDa FITC-dextrans. The ability of T1AM and TA1 to cross the in vitro model of BBB was analyzed by using a well-characterized LC-MS/MS method. After incubating bEnd.3 cells with 1μM T1AM or TA1 Krebs-Ringer solution for 1, 2 and 4h at 37 °C, the media from upper and lower chambers were collected, and cell lysates were prepared according to a previously reported procedure. In all experiments, cell free pre-coated membranes with gelatin 1% were used as control. To assess the contribution of efflux pumps on the pharmacokinetics of test compounds, P-gp expression and inhibition by Verapamil inhibitor were also analyzed.

**Results:** Our results indicate that T1AM and TA1 show profound differences in the BBB permeability. T1AM efficiently crossed the BBB, whereas TA1 showed an almost negligible entry through BBB. Notably, in bEnd.3 cells a significant ($p<0.01$) and time dependent uptake of T1AM was observed, followed by oxidative deamination to produce TA1, which was subsequently released through P-gp activation.

**Conclusions:** Our results confirm the potential of T1AM as a novel drug for the treatment of NDDs, whereas for TA1 the observed reduced BBB penetrance suggests the need to exploit a novel strategy to improve the drug delivery to the brain.

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## THYROID HORMONE METABOLITE CONCENTRATIONS ARE NOT DETERMINED BY SERUM BINDING PROTEINS IN HEALTHY INDIVIDUALS

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**Objectives:** In blood, thyroid hormones are bound to the thyroid hormone binding proteins thyroxine-binding globulin (TBG), albumin and transthyretin to facilitate transport and to provide a reservoir to prevent urinary iodine loss. Binding properties have been extensively studied for T3 and T4 but less so for the other thyroid hormone metabolites (THMs) produced via deiodination or alternative metabolic pathways such as oxidative deamination. Recently we determined reference ranges in healthy individuals of nine THMs by liquid chromatography-tandem mass spectrometry. To assess whether the width of the THM reference interval is influenced by fluctuations of thyroid hormone binding proteins, we determined the binding protein characteristics in relation to the different THMs.

**Methods:** The distribution of $^{125}$I-labeled 3,3'-T2, T3, rT3, TA3 and TA4 to TBG, transthyretin and albumin was determined by electrophoresis. A radioligand binding assay was used to determine the rank order of affinity based on $IC_{50}$-values of TBG and transthyretin to T0, 3-T1, 3,5-T2, 3,3'-T2, T3, rT3, T4, TA3 and TA4. Serum TBG, transthyretin and albumin were measured in an earlier investigated cohort of 251 healthy subjects. Correlations of THM with TBG, transthyretin and albumin (linear model adjusted for gender and age) were determined.

**Results:** T3 and T4 are predominantly bound to TBG in contrast to 3,3'-T2 and rT3 which are predominantly bound to albumin, TA3 which is equally distributed over transthyretin and albumin and TA4 which is predominantly bound to transthyretin. With the radioligand binding assay, we found that the rank order of affinity was T4>TA4=rT3>T3>3,3'-T2=TA3>3-T1=3,5-T2>T0 for TBG and TA4>T4=TA3>rT3>T3>3,3'-T2>3-T1>3,5-T2>T0 for TTR. Apart from an expected correlation of TBG with serum T3 and T4, serum THMs did not correlate with TBG, transthyretin and albumin concentrations.

**Conclusions:** Concentrations of T0, 3-T1, 3,3'-T2, rT3 and TA4 are not influenced by fluctuations in serum TBG, transthyretin and albumin concentrations within the reference range. However, the effects of pathophysiological conditions that result in significant reductions in binding capacity of serum binding proteins on THM concentrations needs further investigation.

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## THE 3-IODOTHYROACETIC ACID DOES NOT CAUSE CHANGE IN PHOSPHORYLATION OF PROTEINS AFFECTED BY 3-IODOTHYRONAMINE

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The 3-iodothyroacetic acid does not cause change in phosphorylation of proteins affected by 3-iodothyronamine.

**Introduction:** The 3-iodothyroacetic acid (TA1) derives from the metabolism of 3-iodothyronamine (T1AM) and it is highly produced in cell culture medium supplemented with fetal bovine serum. Previous results indicated that, in models of brain cell lines, exogenous T1AM can increase the phosphorylation of the extracellular signal-regulated kinases (ERKs) and of the transcriptional factor cAMP response element-binding protein (CREB). Therefore, we evaluated whether TA1 can reproduce T1AM's effects in the same experimental conditions.

**Methods:** A hybrid line of cancer cells of mouse neuroblastoma and rat glioma (NG108-15) and a human glioblastoma cell line (U-87 MG) were used. Cell lines were treated with TA1 for 24h, ranging from 0.1 to 10 μM. Uptake, cell viability, and protein expression were assessed.

**Results:** TA1 was taken up by cells, even though only a slight reduction in medium concentration was recorded upon 24h of incubation. Cell viability was significantly increased by TA1 10 μM in U87-MG cell line ($p<0,05$), while NG108-15 cells were unaffected. Western blot analysis indicated that, upon infusion of pharmacological doses of TA1, neither the expression of Sirtuin 1, (p=NS) nor the post-translational modifications of ERK or CREB (pERK/total ERK, pCREB/total CREB, p=NS) were changed.

In conclusion, our preliminary results suggest that, in our experimental models, TA1 does not seem to mimic T1AM's effects.

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## 3,5-DIIODO-L-THYRONINE (T2) ADMINISTRATION AFFECTS VISCERAL ADIPOSE TISSUE INFLAMMATORY STATE IN RAT RECEIVING LONG-LASTING HIGH-FAT DIET

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**Objectives:** 3,5 diiodo-L-thyronine (T2), a naturally existing iodothyronine, has biological effects, but the underlying mechanisms are poorly understood. Its actions exerted in counteracting fat accumulation in rats on a high fat diet (HFD) are particularly relevant. Visceral white adipose tissue accumulation is associated with the inflammatory response that plays an important role in the development of many diseases related to obesity. In the present study, we focused our attention on T2 actions aimed at improving the adverse effects of long-lasting HFD such as the adipocyte inflammatory response.

**Methods:** For this purpose, three groups of rats were used: i) receiving a standard diet for 14 weeks; ii) receiving a HFD for 14 weeks, and iii) receiving a HFD for 14 weeks with a simultaneous daily injection of T2 for the last 4 weeks. In the adipose tissue, the expression of pro and anti-inflammatory cytokines, hypoxia and angiogenesis markers, and oxidative damage of DNA were measured.

**Results:** The results showed that T2 administration ameliorated the expression profiles of pro- and anti-inflammatory cytokines, reduced macrophage infiltration in white adipose tissue and influenced their polarization. Moreover, T2 improved the expression of hypoxia markers, all altered in HFD rats, and reduced angiogenesis by decreasing the pro-angiogenic miR126 expression. Additionally, T2 reduced the oxidative damage of DNA, known to be associated to the inflammatory status.

**Conclusions:** This study demonstrates that T2 is able to counteract some adverse effects caused by a long-lasting HFD and to produce beneficial effects on inflammation.

# Treatment of Thyroid Disease

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## BODY COMPOSITION COMPARISON IN PATIENTS WITH THYROTOXICOSIS- PRIOR TO AND DURING TREATMENT

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**Introduction:** Thyrotoxicosis is a catabolic state associated with increased energy expenditure, lipolysis and protein turnover. Hyperthyroidism-related weight loss reflects not only on the depletion of body fat but also on the loss of muscle mass. Its treatment leads to weight gain and subsequently to changes in body composition. There are few studies in literature assessing this topic.

**Objective:** The aim of this cross-sectional study was to investigate body composition redistribution in patients with newly diagnosed thyrotoxicosis at baseline and after normalization of the hormone levels.

**Material and methods**: 25 women over 40 years of age with thyrotoxicosis took part in this study. Participants were classified in two groups: group A (n=12) - patients with newly diagnosed hyperthyroidism, prior to treatment and group B (n=13) - patients in an euthyroid state, treated with antithyroid drugs for 6 to 18 months. Body composition parameters such as fat mass and appendicular skeletal muscle mass adjusted for body size (ASM/height2) were assessed by bioelectrical impedance analysis (BIA). Serum levels of thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured.

**Results:** Although insignificant, the weight gain in group B was due to both of the increase in fat (group A- 38,93%, group B- 40,20%) and appendicular muscle mass (ASM/h2: group A- 6,41 kg/m2 , group B- 7,14 kg/m2). The higher levels of muscle mass resulted in increased grip strength (group A- 25,33kg, group B- 30,46kg) and gait speed (group A- 0,79m/s, group B- 0,91m/s) in the euthyroid patients. The patients in group B had a lower level of visceral fat, compared to the patients in group A. There was a strong negative correlation between free thyroid hormones (FT3 and FT4) and the visceral fat level among the patients in group A ($p<0,028$; $p<0,044$). None of the body composition parameters in group B correlated with the levels of thyroid hormones.

**Conclusions:** Treatment of thyrotoxicosis leads to important changes in body composition - the weight gain in the patients who have achieved an euthyroid state during treatment is due to the increase in both adipose and muscle tissues. The study demonstrates that changes in body composition are accompanied by improvement in functional parameters such as grip strength and gait speed. Therefore, treatment of women with thyrotoxicosis also leads to better physical well-being and performance and could prevent the development of secondary sarcopenia.

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## USE OF THYROID HORMONES IN HYPOTHYROID AND EUTHYROID PATIENTS: A THESIS* QUESTIONNAIRE SURVEY OF BULGARIAN PHYSICIANS

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**Aim:** to assess the present use of thyroid hormones, with focus on levothyroxine (LT4) formulations, in hypothyroid and euthyroid patients by Bulgarian physicians.

**Materials and Methods:** This is a cross-sectional, questionnaire-based survey. 120 physicians participated (88 females /32 males). 74% were specialists in endocrinology and most of the remaining were general practitioners and internal medicine specialists. 62.4% of them was aged over 50 years. The Bulgarian translation of the original English questionnaire was employed for the THESIS survey. Its first part contained 8 questions exploring the physicians' characteristics (sex, age, specialty, years in medical practice, and others). The second part contained 24 questions revealing the preferences in the treatment of hypothyroidism. The statistical analysis was performed on the IBM SPSS 19.0 for Windows statistical package.

**Results:** One third (33.3%) of the participating physicians would not recommend LT4 to euthyroid patients. 96% of the respondents accepted and 100% prescribed LT4 as first line replacement therapy in hypothyroidism. A minority of respondents considered the use of triiodothyronine (LT3), alone (10%) or as a combination of LT3 + LT4 (6%). However, 34.2% of respondents would recommend LT4 + LT3 for a short period after prolonged hypothyroidism, and 24.2% also in case of persisting hypothyroid symptoms despite normal TSH. LT4 tablets were the preferred formulation by more than half of the respondents; one-fifth would prescribe soft-gel capsules, and one-sixth the liquid solution. 52.5% of the physicians would not administer iodine or selenium. Persistent symptoms despite optimal hormonal control were attributed to comorbidities, psychosocial factors, chronic fatigue syndrome, or unrealistic patients' expectations. 16 (13.3%) of the physicians who responded declared to be hypothyroid and 43.8% of them reported symptoms like tiredness or fatigue. 25% of them was in favor of a trial with LT4 + LT3 combination or desiccated thyroid extract (25%).

**Conclusion:** LT4 is the primary thyroid hormone used in our country for replacement therapy. The treatment with LT4+LT3 combination or desiccated thyroid is considered in their practice by part of the physicians. This study highlighted the persistence of differences between the hypothyroidism treatment guidelines and the real clinical life.

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## MANAGEMENT OF THYROTOXICOSIS INDUCED BY PD1 OR PD-L1 BLOCKADE

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**Background:** Thyrotoxicosis is a common immune-related adverse event in patients treated with PD1 or PD-L1 blockade. A detailed endocrinological assessment, including thyroid ultrasound and scintigraphy is missing, as are data on response to treatment and follow-up. Aim of the study was to better characterize the thyrotoxicosis secondary to immune checkpoint inhibitors, gaining insights into pathogenesis and informing management.

**Methods:** We conducted a retrospective study of 20 consecutive patients who had normal thyroid function before starting immunotherapy and then experienced thyrotoxicosis upon PD1 or PD-L1 blockade. Clinical assessment was combined with thyroid ultrasound, scintigraphy, and longitudinal thyroid function tests.

**Results:** Five patients had normal scintigraphic uptake (Sci+), no serum antibodies against the TSH receptor, and remained hyperthyroid throughout follow-up. The other 15 patients had no scintigraphic uptake (Sci-) and experienced destructive thyrotoxicosis followed by hypothyroidism (N= 9) or euthyroidism (N= 6). Hypothyroidism was more readily seen in those with normal thyroid volume than in those with goiter (P= 0.04). Among Sci- subjects, a larger thyroid volume was associated to a longer time to remission ($P<0.05$). Methimazole (MMI) was effective only in Sci+ subjects ($P<0.05$).

**Conclusion:** Administration of PD1 or PD-L1 blocking antibodies may induce two different forms of thyrotoxicosis that appear similar in clinical severity at onset: a type 1 characterized by persistent hyperthyroidism that requires treatment with MMI, and a type 2 characterized by destructive and transient thyrotoxicosis that evolves to hypo- or euthyroidism. Thyroid scintigraphy and ultrasound help differentiating and managing these two forms of iatrogenic thyrotoxicosis.

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## RISK OF OVER- AND UNDERTREATMENT WITH LEVOTHYROXINE AMONG GENERAL PRACTITIONERS IN COPENHAGEN DENMARK

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**Background:** There has been reported a decrease over time in thyroid stimulating hormone (TSH) levels when initiating levothyroxine (L-T4) therapy for hypothyroidism in both the United Kingdom and Denmark, where treatment most often is initiated with TSH levels below 10 mIU/L. The primary purpose of this study was to investigate whether this reduction of TSH threshold leads to an increased number of patients being overtreated.

**Method:** Retrospective cohort study comprising all inhabitants in Copenhagen who had TSH measurements requested by general practitioners (GPs), leading to a new prescription of L-T4 between 2001 and 2012. Not picking up a prescription for one year was regarded as ending of treatment and a redemption after that regarded as a new initiation. The database was linked to the Danish registers until 2015. Overtreatment and undertreatment were defined as TSH < 0.1 mIU/L or above 10 mIU/mL, respectively, in three consecutive measurements and analysed by Aalen-Johansen estimators and Cox proportional hazards models.

**Results:** In total 13,311 patients with 14,553 treatment initiations were included in the study. The risk of ever being over- or undertreated, before ending treatment or death, was 4.7% and 7.4% after 10 years. The hazard of overtreatment was higher among women as compared to men, higher among younger adults as compared to older and higher with lower initial TSH levels compared to higher levels (1.3 times higher with an initial TSH of 5 compared to a TSH of 10 mIU/L). The hazard of overtreatment decreased over the time period from 2001 to 2012. Among overtreated individuals, the chance of returning to normal TSH levels was about 24% after 2.5 years and about 54% after 10 years. In 18% of the cases, L-T4 therapy was initiated on TSH levels less than 5 mIU/L.

**Conclusion:** Although a still decreasing threshold for initiating L-T4 therapy is evident, the risk of overtreatment (and undertreatment) was low and lessened in the period 2001 – 2012 among Danish primary care patients. Nevertheless, as many as 18% were started on L-T4 with a normal TSH level. Focus on avoiding overtreatment and especially unnecessary medical treatment with thyroid hormone in patients is still needed.

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## A DECREASE OF PLASMA T3 FOLLOWING LEVOTHYROXINE SUBSTITUTION IN PATIENTS WITH AUTOIMMUNE THYROIDITIS IS ASSOCIATED WITH AN IMPAIRED QUALITY OF LIFE

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**Background:** Health related quality of life (HRQL) is reduced in a substantial number of patients on levothyroxine (LT4) substitution. There has been much interest in the importance of plasma triiodothyronine (T3), and whether HRQL correlates to this variable. In a prospective study, we investigated the dynamics in the plasma levels of thyroid hormones in patients newly diagnosed with hypothyroidism due to autoimmune thyroiditis (AIT). Especially, we focused on the correlation between HRQL and changes in plasma T3 following LT4 treatment.

**Methods:** Patients ≥18 years with AIT diagnosed within the last 3 months were enrolled. At diagnosis, LT4 treatment was initiated, with dose adjustment aiming at a normal plasma TSH. Thus, two sets of thyroid function tests were compared, i.e. at diagnosis and at euthyroidism. Patients were stratified, according to the change in plasma freeT3index (FT3i) following treatment. Group A were patients showing a decrease in plasma FT3i, while group B included patients with an increase (or no change) in plasma FT3i. The two groups were compared in terms of HRQL, measured at enrollment by the thyroid questionnaire ThyPRO-39.

**Results:** Sixty-seven patients (31 with overt and 36 with mild hypothyroidism) were included; 24 in group A and 43 in group B. Patients in group A were younger than in group B (45±15 vs. 52±14 years, p=0.047), and were less hypothyroid at diagnosis. In group A, freeT4index (FT4i) was 68.2±12.2 nmol/L vs. 59.5±14.4 in group B (p=0.011), FT3i was 1.66±0.22 nmol/L vs. 1.40±0.25 (p<0.001), and FT4i/logTSH was 78.7±27.7 vs. 63.0±27.7 (p=0.043). At euthyroidism, the FT4i/FT3i ratio was higher in group A than in group B (66.5±11.9 vs. 60.4±10.3, p=0.043). For the majority of the ThyPRO-39 scales, higher scores (i.e. worse HRQL) were found in group A than in group B. Significant differences were found for Anxiety (p=0.032) and Emotional Susceptibility (p=0.035). In a multiple regression analysis, Anxiety correlated positively with a decrease in plasma FT3i following treatment (p=0.012), while Emotional Susceptibility correlated negatively with age (p=0.006).

**Conclusion:** More than a third of patients with mild or overt hypothyroidism showed a decrease in plasma FT3i following LT4 substitution. These individuals tended to have an impaired HRQL, particularly in terms of anxiety, compared to patients showing a stable or a rise in plasma FT3i after treatment. Whether these findings reflect a causal relationship or are due to the selection of patients with pre-existing reduced HRQL remains to be clarified in future studies.

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## THE "VICIOUS CIRCLE" OF THYROID METABOLISM IN CHRONIC KIDNEY DISEASE

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Primary hypothyroidism is common in patients with chronic kidney disease and is associated with a high risk of adverse outcomes.

**Objective:** to study disorders of thyroid metabolism in patients with chronic kidney disease.

**Materials and Methods:** The study included 457 patients with glomerulopathies without cardiovascular diseases. The average age of patients was 47.17 ± 0.95 years. According to the results of the screening determination of TSH, groups of patients with a low level of TSH (<0.4 mIU / l) were identified - 2.9% (n = 13); with normal TSH (0.4-3.5 mIU / L) - 80.9% (n = 360); with subclinical hypothyroidism (> 3.5 mIU / L) - 16.2% (n = 72). TPO Antibodies were determined to detect autoimmune damage to the thyroid tissue.

**Results:** Subclinical hypothyroidism was detected in 16.2% of patients with different stages of CKD. The detection rate of patients with subclinical hypothyroidism with CKD stages III-IV is significantly higher and amounted respectively to 25.64% and 30.6%. The increase of TSH levels was not associated with the female sex and did not depend on age. In 90.4% of cases, the increase of TSH levels was not associated with TPO antibodies.

**Conclusions:** Subclinical hypothyroidism with different stages of CKD was found in 17.56%, increased TSH levels were associated with advanced stages of CKD. Patients with subclinical hypothyroidism had more disorders of the nitrogen-releasing function of the kidneys, protein and electrolyte metabolism.

**Table 1.** Indicators of complex laboratory functional examination of the kidneys of patients with subclinical hypothyroidism and normal value of thyroid-stimulating hormone (for Abstract 76)

| Laboratory parameters | The group with normal TSH (0,4-3,5 mIU / L) | The group with subclinical hypothyroidism (>3,5 mIU / L) | p |
|---|---|---|---|
| Total protein, g / l | 66,5 (60-72) | 62,5 (45-70) | p=0,02 |
| Albumin, g / l | 37,85 (33,45-41,10) | 35,55 (24,5-39,6) | p=0,007 |
| Total calcium, mmol/l | 2,26 (2,17-2,36) | 2,2 (2,03-2,31) | p=0,01 |
| Inorganic phosphorus, mmol/l | 1,16 (1,02-1,32) | 1,22 (1,15-1,35) | p=0,04 |
| Chlorides, mmol / l | 105 (103-107) | 105,6 (104-109) | p=0,045 |
| Urea, mmol/l | 6,6 (4,6-9,8) | 9,15 (5,7-12,1) | p=0,008 |
| Daily proteinuria, g / 24h | 1,28 (0,37-4,56) | 4,62 (0,33-9,2) | p=0,01 |
| albumin / creatinine ratio, mg / mol | 111,2 (40-360,4) | 346 (33,55-1016,15) | p=0,02 |
| GFR, ml / min/1, 73m2 | 66,59 (43,9-92,1) | 46,03 (34,46-85,4) | p=0,006 |

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## LEVOTHYROXINE MALABSORPTION OR PSEUDOMALABSORPTION? A QUESTION IN THE MANAGEMENT OF REFRACTORY HYPOTHYROIDISM

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**Introduction:** Refractory hypothyroidism is a clinical condition where hypothyroid patients fail to achieve euthyroid state even with much higher levothyroxine (LT4) dosage than would be expected. The levothyroxine absorption test (LT4AT) is an important tool for distinguishing LT4 malabsorption from other "pseudomalabsorption" conditions.

**Aim**: To review our institution's experience with the LT4AT and assess its effect on the management of patients with refractory hypothyroidism.

**Methodology:** We performed a retrospective study of all patients evaluated for refractory hypothyroidism who had performed a LT4AT in our tertiary center between Jan. 2014 to Dec. 2020. We evaluated its results and the impact on thyroid function management during follow-up.

**Results:** Ten female patients were included with a mean age of 39.8±12.6 years. Mean weight was 72.2±14.7kg and baseline LT4 requirement ranged from 2.5 to 5.3µg/kg per day. Most common cause of hypothyroidism was postsurgical in 50% (n=5) and autoimmune in 20% (n=2). In all but one individual, normal LT4 absorption was found (85-668% increase, IQR 286). The only patient with LT4 absorption impairment (maximal increase of 48% by hour 5) had previous history of *Helicobacter pylori* gastritis and prior "intestinal surgery" during childhood. No adverse events were reported during any of the LT4ATs. At follow-up [median 11.5 months (IQR 23)], 5 patients developed normal or suppressed TSH values and 5 had improved but persistent TSH elevations under the same dose of LT4 as before the LT4AT. The only patient with documented malabsorption obtained euthyroid state after increasing her oral LT4 dose.

**Conclusion:** The LT4AT is an effective and safe way to assess refractory hypothyroidism providing valuable information to distinguish LT4 malabsorption from "pseudomalabsorption". Our data suggest that most patients with suspicious LT4 malabsorption do present normal LT4AT

**Results:** This test provides relevant information for better discuss treatment adherence strategies with refractory hypothyroidism patients.

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## METABOLIC CHARACTERISTICS OF WOMEN AFTER THYROIDECTOMY ON LEVOTHYROXINE REPLACEMENT THERAPY

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**Introduction:** Thyroid stimulating hormone (TSH) measurement is recommended in the follow-up of patients with primary hypothyroidism being the most reliable marker for treatment monitoring. It has been speculated however that levothyroxine (L-T4) replacement monotherapy following thyroidectomy does not ensure complete restoration of euthyroid state despite maintaining TSH within reference range.

**Objective:** To compare the metabolic characteristics body mass index (BMI), blood glucose and lipids between women after thyroidectomy on replacement LT-4 therapy and healthy control group with similar TSH euthyroid values.

**Patients and Methods:** 45 women after thyroidectomy (mean age 55.3±8.8 years) and 147 healthy age- and TSH-matched control women were



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included in the study. 15 women had thyroid surgery due to Graves' disease, 11 because of thyroid cancer and 19 women had undergone thyroidectomy for benign nodular goiter. All women were clinically euthyroid with TSH between 0.4-4.2 mIU/l. Fasting morning levels of TSH, free thyroxine (FT4), blood glucose, total cholesterol, HDL, triglycerides were measured, LDL was calculated using the Friedewald equation. Women with significant comorbidities and taking medications affecting the studied parameters were excluded.

**Results:** Women after thyroidectomy had significantly higher levels of total cholesterol (6.10±0.2 vs 5.58±0.1 mmol/l, p=0.013) and LDL (3.56±0.1 vs 3.96±0.2 mmol/l, p=0.021) compared to euthyroid controls. No differences in BMI, blood glucose, HDL and triglycerides were found between groups. Women after thyroidectomy had considerably higher FT4 values than the control group (13.1±0.3 vs 11.1±0.1 pmol/l, p<0.001) having similar TSH levels (1.98±0.2 vs 1.88±0.1, p=0.599). No association between thyroid function tests and the investigated laboratory parameters was observed among thyroidectomized patients. Mean values of TSH, FT4, metabolic parameters and L-T4 daily dose were similar between treated women regardless of the indication for thyroidectomy.

**Conclusion:** Our study shows that total cholesterol and LDL levels are higher in women after thyroidectomy on L-T4 replacement therapy than in euthyroid healthy women. This finding is in accordance with the results of a recent meta-analysis suggesting that L-T4 alone is not sufficient to normalize total cholesterol and LDL levels in patients with primary hypothyroidism justifying further studies on cardiovascular risk assessment in those patients.

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## EXPLORING ENDOCRINOLOGISTS' VIEWS ABOUT NEW WAYS OF WORKING WITH HYPOTHYROIDISM – A QUALITATIVE STUDY IN THE UK

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**Objectives:** A sizeable subgroup of patients with hypothyroidism report persistent symptoms despite treatment. Recommendations exist to develop models of care to support them to address the demands of living with their condition and to develop the skills, knowledge, and experience to manage their health. This study explored clinicians' perceptions of barriers and facilitators to psychosocial and self-management models in secondary care.

**Methods:** Eighteen Endocrinologists in the UK were recruited via a snowballing sampling technique. In depth interviews were conducted and analysed using reflexive thematic analysis.

**Findings**: Patients with hypothyroidism are heterogenous subgroups with diverse support needs from diagnosis, but Endocrinologists report variations in will, skill and confidence to meet these needs. A lack of referral pathways and evidence-based guidelines for addressing psychosocial concerns were described as barriers to incorporating new models of care. Difficulty shifting the focus of the consultation towards psychosocial concerns and perceived challenges to professional identity were also described. Scientific unknowns and stalled conversations about treatments for hypothyroidism (T3) were described as key barriers to clinicians endorsing self-management, fuelling polarisation between patients and healthcare providers and patients' rigid treatment beliefs.

Multi-professional collaboration is required to develop interventions and clinician training to address gaps in provision, particularly between primary a secondary care. Self-management support should not be provided in place of medical treatment, and the needs of those for whom standard treatment may be ineffective should be addressed.

Addressing the cost of treatments (unregulated rises in generic medications), funding research (targeted trials of long-acting LT3), building bridges between patients and the healthcare system (through co-production) and increasing innovation and autonomy in healthcare may reduce barriers to the implementation of psychosocial and self-management models of care. The value of asset-based models of working to meet the changing demands upon healthcare should be recognised, alongside the threat that they may pose to the socio-political status-quo. NHS staff should be supported in a culture which promotes creativity and innovation and reduces burnout and fear blame, especially for those caught in the middle of opposing healthcare paradigms.

**Conclusion**: This study presents a rationale for change at all levels of the healthcare system to improve outcomes for patients with hypothyroidism and for the clinicians treating them. A whole-systems approach is needed to support Endocrinologists to place patients with hypothyroidism at the centre of their care.

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## USE OF THYROID HORMONES IN HYPOTHYROID AND EUTHYROID PATIENTS: A 2020 THESIS* QUESTIONNAIRE SURVEY OF MEMBERS OF THE DANISH ENDOCRINE SOCIETY (*TREATMENT OF HYPOTHYROIDISM IN EUROPE BY SPECIALISTS: AN INTERNATIONAL SURVEY)

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**Objectives:** The standard treatment of hypothyroidism is levothyroxine (LT4), which is available as tablets or soft-gel capsules in Denmark. This study aimed to investigate Danish endocrinologists' use of thyroid hormones in hypothyroid and euthyroid patients.

**Methods:** An e-mail with an invitation to participate in an online survey investigating practices about substitution with thyroid hormones was sent to all members of the Danish Endocrine Society (DES) on 27th February 2020. The initial e-mail was followed by three reminders between February and May 2020, and where after it was closed. Survey responses were collected and electronically stored by the Lime-Survey service.

**Results:** Out of 488 eligible DES members, a total of 152 (31.2%) respondents were included in the analysis. The majority (94.1%) of responding DES members use LT4 as the treatment of choice. Other treatment options for hypothyroidism are also used, as 58.6% prescribe combination therapy with liothyronine (LT3)+LT4 in their clinical practice. LT4+LT3 combination is preferred in patients with persistent symptoms of hypothyroidism despite biochemical euthyroidism on LT4 treatment. Over half of the respondents answered that thyroid hormone therapy is never indicated for euthyroid patients, but 42.1% will consider it for euthyroid infertile women with high antibody levels. In various conditions that could interfere with the absorption of LT4, most responding Danish endocrinologists prefer tablets to soft-gel capsules or liquid LT4 and do not expect a significant difference when switching from one type of tablet formulation to another. The Danish endocrinologists are nearly equally divided into two categories regarding supplementation; 57 (37.5%) answered that supplementation with selenium or iodine can be used if requested by the patient, while 62 (40.8%) stated that such supplementation should never be used.

**Conclusion:** The treatment of choice for hypothyroidism is LT4. Combination therapy with LT4+LT3 is considered for patients with

persistent symptoms. Even in the presence of conditions affecting bioavailability, responding Danish endocrinologists prefer LT4 tablets rather than newer LT4 formulations, such as soft-gel capsules.

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## THE INFLUENCE OF LEVOTHYROXINE FOR HYPOTHYROIDISM ON PHYSIOLOGICAL MECHANISMS DETERMINING BODY WEIGHT

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**Background and Aims:** When initiating levothyroxine (L-T4) substitution therapy for hypothyroidism, overweight patients typically expect body weight loss from L-T4-induced acceleration of adipose tissue combustion. However, despite the increase in resting energy expenditure (REE) observed after L-T4 treatment, it rarely causes body weight loss. This prompted us to investigate changes in appetite sensations and food intake during the first 6 months of L-T4 therapy in hypothyroid patients.

**Methods:** Eighteen newly diagnosed hypothyroid women with thyroid-stimulating hormone (TSH) >10 mU/l were investigated on three separate experimental days: at diagnosis, after normalisation of TSH (<4.0 mU/l) and after six months of treatment. The primary endpoint was *ad libitum* food intake, and key secondary endpoints included appetite and satiety sensations (as assessed by visual analogue scales (0-10 cm)), REE, body weight and body composition, 24-hour physical activity and pulse rate. Eighteen healthy controls, matched for sex, age and BMI, were subjected to a single experimental visit.

**Results:** In the hypothyroid women (mean age 44.4 (SD 6.4) years; mean BMI 29.0 (SD 13.4) kg/m$^2$), TSH decreased from a median of 46.9 (IQR 12.4;83.8) mU/l at diagnosis to 1.2 (IQR 0.2;3.2) mU/l after 1 month ($p$<0.001) and remained normal after 6 months (1.6 (0.5;2.1) mU/l). REE increased ($p$=0.006) from 1,380 (SD 171) kcal/day to 1,519 (SD 258) kcal/day and remained higher than baseline after 6 months (1,524 (SD 225) kcal/day). Daily physical activity increased significantly after 6 months. Body weight was unchanged (80.6 (IQR 72.2;91.4), 80.2 (IQR 72.2;89.8) and 81.0 (IQR 71.3;88.3) kg at baseline and after 1 and 6 months, respectively). Fat-free mass decreased by a mean of 0.8 kg ($p$<0.05) while fat mass was unchanged (0.09 kg $p$=0.87). Sensation of hunger in the fasted state increased ($p$=0.048) after 6 months whereas the observed increase in *ad libitum* food intake of 28 g did not reach statistical significance ($p$=0.18).

**Conclusions:** In these hypothyroid women, L-T4 treatment increased mean REE by 144 kcal/day corresponding to a combustion of 2.9 kg fat over 6 months; but body weight and fat mass remained unchanged after 6 months of treatment. As the daily level of physical activity increased in the same period, we propose that the increased sensation of hunger in the fasting state (combined with accompanying increased food intake) may constitute a culprit in the lack of body weight loss from substitution therapy with L-T4 in patients with hypothyroidism.

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