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# Oral Presentations

**Oral Session 1: Topic Highlights****OP-01-01****Cryo-electron microscopy structure of full length TSH receptor in complex with TSH receptor blocking human monoclonal autoantibody K1-70™**

Ricardo Núñez Miguel, Paul Sanders, Lloyd Allen, Michele Evans, Matthew Holly, William Johnson, Andrew Sullivan, Jennifer Miller-Gallacher, Jane Sanders, Jadwiga Furmaniak & Bernard Rees Smith Rsr Limited

**Objectives**

The crystal structures of the TSH receptor (TSHR) leucine rich repeat domain (LRD) bound to TSHR stimulating monoclonal autoantibody M22™ or to TSHR blocking monoclonal autoantibody K1-70™ and antibody free have been solved previously. Cryo-electron microscopy (cryo-EM) was now used to solve the structure of full length TSHR in complex with K1-70™.

**Methods**

Recombinant human TSHR expressed in CHO cells was incubated with K1-70™ Fab, the complex solubilised in 10mM Tris pH7.5, 50 mM NaCl, 0.5g/l NaN<sub>3</sub>, 2% LMNG, 0.2% CHS and purified to homogeneity by affinity and size exclusion chromatography. Cryo-EM imaging was performed on a Titan Krios 300kV with a K3 Direct Electron Detector.

**Results**

The cryo-EM TSHR- K1-70™ structure was determined to a global resolution of 3.3Å. A model was built using the solved crystal structure of the TSHR LRD- K1-70 complex and the AlphaFold model of the TSHR. Model rebuilding and refinement were done in COOT v0.9 and Discovery Studio 2021 suite of programs. The cryo-EM structure shows full length TSHR in a monomeric state with all three domains; LRD, hinge region (HR) and transmembrane domain (TMD) visible. The binding arrangements of K1-70™ Fab with the LRD are similar to those observed in the crystal structure. The LRD and HR form the TSHR extracellular domain (ECD) in a similar arrangement to that seen in the crystal structure of the FSHR ECD and in the cryo-EM structure of the LH/CGR. The structure shows the TSHR ECD positioned on top of the extracellular surface of the TMD. The HR helix and the HR C-terminus form interactions with the TMD N terminus, extracellular loops 1 and 2 and the extracellular part of helix 7. The relative positioning of the ECD and TMD in the TSHR is similar to that seen in the cryo-EM structure of the LH/CGR inactive state. In particular the TSHR P10 region (amino acids 405-414), highly conserved in glycoprotein hormone receptors, is in a similar conformation to that seen in the LH/CGR inactive state structure. The structure and spacial arrangements of the TMD helices in the two cryo-EM structures are similar except that the TSHR extracellular end of helix 6 is displaced by approximately 6.5Å compared to the LH/CGR inactive state structure.

**Conclusions**

Our high resolution structure of full length TSHR in complex with K1-70™ provides an excellent basis for understanding the mechanism of TSHR activation.

DOI: 10.1530/endoabs.84.OP-01-01

**OP-01-02****A randomized, double-blind, placebo-controlled trial of Vitamin D supplementation in patients newly diagnosed with graves' disease**  
Diana Grove-Laugesen<sup>1</sup>, Eva Ebbelohj<sup>2</sup>, Klavs Hansen<sup>3</sup>, Torquil Watt<sup>4</sup> & Lars Rejnmark<sup>5</sup>

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**Objective**

Vitamin D has potential immunomodulatory effects. We studied whether vitamin D3 supplementation affects the course of Graves' disease (GD).

**Methods**

In a double-blind, placebo-controlled design, we randomized patients with a first time diagnosis of GD hyperthyroidism to daily supplementation with vitamin D3 70 mg (2800 IU) or placebo, as add-on to standard treatment with anti-thyroid drugs (ATD). The intervention was continued 12 months after cessation of ATD. Primary outcome was treatment failure (defined as either relapse of

hyperthyroidism within 12 months after ATD cessation, failure to taper of ATD within 24 months of treatment, referral for radioiodine treatment or thyroidectomy). Secondary outcomes included the risk of relapse of hyperthyroidism after achieving euthyroidism and the influence of age, sex, smoking status, and vitamin D status. Data was analyzed using an intention-to-treat approach. Results

A total of 278 patients were randomized. At baseline, participants were 44 ± 1 years old, 79% were females, 35% had vitamin D insufficiency (<50 nmol/l), and 22% were smokers. The risk of treatment failure was 41% (95%CI, 33% to 50%) in the vitamin D group and 32% (95% CI, 24% to 40%) in the placebo group. This corresponded to a relative risk (RR) of 1.30 (95%CI: 0.95 to 1.78, P=0.10) with vitamin D supplementation. The relapse rates were also similar in the two groups (RR of relapse with vitamin D: 1.50 (95% CI, 0.92 to 2.44), P=0.10). Effects of the intervention showed a significant interaction with smoking status (P=0.01). In non-smokers, vitamin D supplementation showed an unfavorable effect on risk of treatment failure (odds ratio (OR) 2.03; 95%CI, 1.15 to 3.59, P=0.02) and relapse (OR 2.09; 95% CI: 1.04 to 4.18, P=0.04); no effect was found among smokers. The effect of intervention was not affected by age, sex, or vitamin D status.

**Conclusion**

In GD, the course of the disease is not improved with vitamin D supplementation. On the contrary, we observed trends for increased risk of treatment failure and relapse of hyperthyroidism with vitamin D supplementation. Given the enormous interest in immune-modulating benefits of vitamin D mainly based on association studies, our findings are important and raise concern of uncritical use of high-dose vitamin D supplementation in Graves' disease.

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**OP-01-03****The relationship between thyroid function and lipid metabolomics and response to combination thyroid hormone replacement**

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**Objective**

Thyroid hormones are essential for maintaining metabolic balance and particularly influence lipid synthesis and degradation. Metabolomics and in-depth lipid profiling may enable us to assess for differential effects of thyroid hormones and provide insight into tissue thyroid status, that cannot be captured by levels of serum free thyroid hormones alone.

**Methods**

4,347 children from the Avon Longitudinal Study of Parents and Children who had thyroid function and plasma NMR metabolomics measured at age 7 were studied. Linear regression was performed to assess the association between thyrotropin (TSH), free tri-iodothyronine (FT3) or free thyroxine (FT4) and lipid metabolite levels. Analyses were adjusted for sex and BMI. We then studied 542 individuals from the WATTS trial where individuals were randomised to receive combination thyroid hormone replacement (liothyronine and levothyroxine (LT3 + LT4)) or standard levothyroxine (LT4) to compare selected metabolites and response to treatment as assessed by quality of life measures including general health questionnaire (GHQ).

**Results**

Multiple associations after correction for multiple testing were observed between TSH, FT3 and FT4 and lipid metabolites (P<0.001). The classic inverse association between TSH and free thyroid hormones was often not observed. Most robust and consistent associations were observed for FT3. The strongest lipid associations for FT3:FT4 ratio were taken forwards as markers of tissue T3 status and studied in the WATTS trial. After correction for multiple testing, 9 metabolomic markers of tissue T3 status were associated with improved GHQ in patients randomised to combination thyroid hormone replacement (LT3 + LT4) but not to standard treatment (LT4).

**Conclusion**

Our analysis has shown the broad and substantial impact of thyroid hormones, especially FT3, on lipid metabolomics and shown how each hormone has a different metabolomic signature, with TSH alone being unable to capture this. Metabolomic markers may reflect individuals who might benefit from combination thyroid hormone replacement. This work has key implications for monitoring treatment response in hypothyroidism and thyroid hormone replacement.

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## OP-01-04

**Selenoprotein deficiency disorder predisposes to aortic aneurysm formation**

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**Objectives**

Mutations in *SECISBP2* cause deficiency of selenoproteins, resulting in a multisystem disorder with abnormal circulating thyroid hormone and selenium levels and features due to lack of specific selenoproteins or loss of antioxidant selenoenzymes. Having observed early-onset, aneurysmal thoracic aortic dilatation in four patients with this disorder, we studied zebrafish and murine *Secisbp2* mutant models to determine whether the aortic phenotype and selenoprotein deficiency are causally related.

**Methods**

Analyses of histology, selenoprotein deficiencies, oxidative stress, DNA damage and apoptosis in aortae and aortic vascular smooth muscle cells (VSMCs) from two patients following surgery, and in aortae from zebrafish *Secisbp2* mutant and morpholino knockdown or VSMC-targeted, *Secisbp2*-deficient mouse models, were undertaken.

**Results**

Progressive, early-onset (age 10 to 41yrs) aneurysmal ascending aortic dilatation occurred in four patients with biallelic mutations in *SECISBP2*, but without defects in known, thoracic aortopathy, genes. Histology of aneurysmal aortae showed cystic medial necrosis, with deficiency of antioxidant selenoenzymes, oxidative membrane lipid and DNA damage and apoptosis in both medial wall of aorta and cultured, medial VSMCs. Ventral aortic dilatation increased tissue H<sub>2</sub>O<sub>2</sub> content, lipid peroxidation and DNA damage were observed in adult, *Secisbp2*<sup>Q333X/Q333X</sup> mutant and embryonic *Secisbp2* morpholino-knockdown zebrafish, with coexpression of *Secisbp2* mRNA in the morpholino model rescuing these abnormalities. Tamoxifen treatment of Myh11-Cre<sup>ER12</sup>/*Secisbp2*<sup>fllox/fllox</sup> mice, conditionally abolished *Secisbp2* and selenoprotein expression in the medial layer of murine aortae. Infusion of angiotensin II, an established model of thoracic aortic aneurysm (TAA) formation, caused markedly diminished survival (60%) of homozygous mice compared to wild-type or heterozygous littermates (90%), due to development of TAAs with aortic cystic medial necrosis. Loss of antioxidant selenoenzymes, oxidative stress, DNA damage and apoptosis, were features common to aortae from patients, zebrafish and mouse models of *SECISBP2* deficiency. Reversal of oxidative damage and apoptosis by exposure of human aortic VSMCs to antioxidants, implicates excess ROS in their pathogenesis.

**Conclusions**

We have documented thoracic aortic aneurysm formation in patients with *SECISBP2* mutations and similar aortopathy in zebrafish or mice with global or

VSMC-targeted inactivation of *Secisbp2*. We suggest that *SECISBP2* is a novel genetic aetiology of TAA, with oxidative stress and cell death secondary to deficiency of antioxidant selenoenzymes mediating aortic degeneration.

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## OP-01-05

**Sarcopenia as a prognostic factor in patients with advanced thyroid cancer treated with tyrosine kinase inhibitors**

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**Objective**

Recent studies have shown that sarcopenia at cancer diagnosis is associated with a poor survival in patients with solid tumors. Up to now, few data are available among patients with advanced thyroid cancer, especially those treated with tyrosine kinase inhibitors (TKI). We retrospectively evaluated sarcopenia by Skeletal Muscle Index (SMI) in a cohort of advanced thyroid cancer patients before and during TKI treatment and investigated its association with treatment outcome.

**Methods**

Fifty-eight patients (28 females and 30 males age at the time of TKI treatment 67.5 ± 13.8 years) with advanced thyroid cancer were divided into Sarcopenia (SG) and Non-Sarcopenia Groups (NSG) based on SMI values. SMI was measured by CT as the cross-sectional area of skeletal muscles at the third lumbar vertebra level, normalized by height squared (cm<sup>2</sup>/m<sup>2</sup>). Radiological evaluation was performed at baseline and on average every 3-6 months thereafter. Response to treatment was assessed according to the Response Evaluation Criteria in Solid Tumors v.1.1.

**Results**

The prevalence of pre-treatment sarcopenia was 20.7% and it was correlated with Body Mass Index (23.8 ± 3.7 kg/m<sup>2</sup> SG vs 28.2 ± 6.1 kg/m<sup>2</sup> NSG, *P* = 0.004), BMI categories (*P* = 0.01) and duration of first TKI treatment (19.1 months SG vs 28.78 months NSG, *P* = 0.012). A significant reduction in SMI values was observed already after 3 months of treatment (*P* = 0.002) and still after approximately 1 year of therapy (*P* < 0.0001). At the end of the period of observation sarcopenia prevalence was 38.5%. The development of 12 months-sarcopenia was predicted by a lower SMI (*P* = 0.029) BMI (*P* = 0.02) and weight (*P* = 0.04) and by presence of bone metastases (*P* = 0.02). The best basal SMI cut-offs able to predict sarcopenia occurrence were 37.6 cm<sup>2</sup>/m<sup>2</sup> (AUC = 0.94 95% CI: 0.8358-1; *P* < 0.0001) for females and 51.4 cm<sup>2</sup>/m<sup>2</sup> (AUC = 0.88 95% CI: 0.697-1; *P* < 0.0001) for males; the best BMI cut-off was 26.5 kg/m<sup>2</sup> (AUC = 0.920, 95% CI: 0.586-0.932, *P* = 0.003). The median progression-free survival (PFS) was 8.46 ± 6.87 months and 24.39 ± 18.96 months in SG and NSG (*P* = 0.008), respectively. At multivariate analysis, pre-treatment sarcopenia significantly affected treatment outcome, resulting the parameter that has the greatest impact on PFS (HR 4.29; 95% CI, 1.21–15.11, *P* = 0.02).

**Conclusions**

This is the first study that evaluated sarcopenia prevalence and its change over time in Caucasian patients with advanced thyroid cancer under TKI therapy. Sarcopenia seems to be a prognostic factor of TKI treatment outcome, suggesting the importance of the assessment of the nutritional status and body composition in advanced thyroid cancer patients.

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## OP-01-06

**Modeling braf-induced thyroid cancer development and cell re-differentiation using pluripotent stem cell-derived organoids**

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#### Introduction

Due to their remarkable self-organizing structures and functional properties, organoid has become a powerful tool to model diseases 'in a dish'. The use of organoids in cancer research emerged to better understand tumor behavior. Originally, adult stem cells (aSC)-derived organoids were derived from primary tumors while recent studies have reported the generation of cancer models arising from healthy cells by controlling oncogene expression. SC-derived cancer organoids can access the effects of oncogenes and early events driving tumorigenesis, role of cancer stem cells in tumor induction, genomic stability, effect of treatments and screening of new therapeutics.

#### Objectives

To generate a PTC organoid model by inducing the *Braf*<sup>V637E</sup> mutation in mouse ESC-derived functional thyroid follicles to better understand the oncogenic events driven by *Braf*-oncogene and develop a drug screening tool.

#### Methods

TRE-Nkx2-1-Pax8\_bTg-NES-*Braf*<sup>V637E</sup>-ERT<sup>2</sup> mESCs were differentiated into thyroid follicles with doxycycline and hrTSH/cAMP. After follicle enrichment *Braf*<sup>V637E</sup> activation was induced with 4-Hydroxytamoxifen (4OHT). Organoids were then treated with agents previously described to inhibit *Braf* oncogenesis (MEK, PI3K and histone deacetylase (VPA) inhibitors).

#### Results

TRE-Nkx2-1-Pax8\_bTg-NES-*Braf*<sup>V637E</sup>-ERT<sup>2</sup> mESCs were able to differentiate into functional thyroid follicles with iodinate-Tg (Tg-I) luminal accumulation. Starting six hours after 4OHT addition time-dependent dedifferentiation was observed; as evidenced by a decrease in mRNA expression of *TSHR*, *Tg*, *Nis* and *Tpo*. It was associated with an increase in ERK phosphorylation and proliferation. Activation of *Braf*<sup>V637E</sup> disrupted follicular organization and decreased Tg-I accumulation, <sup>125</sup>I uptake and organification. Transcriptomic analysis revealed hyperactivation of PI3K-AKT-mTOR, TNF, cytokine signaling and promotion of Epithelial Mesenchymal Transition. Isolated inhibition of MEK and PI3K resulted in partial increase of *Slc5a5/Nis* levels, whereas treatment with VPA resulted in complete recovery. The combination of MEK and PI3K inhibitors resulted in complete re-expression of *Nis*, *Tg*, *TSHR*, *Tpo* and reorganization into functional follicles confirmed by the detection of Tg-I.

#### Conclusions

We demonstrate the generation of an mESC-derived organoid model that recapitulates the transcriptomic and 3D-histological features of PTC. The combination of MEK and PI3K inhibitors promoted *Nis* reexpression and thyrocyte redifferentiation with recovery of follicular functionality. This mESC-derived PTC *in vitro* model opens new opportunities to study early mechanisms of carcinogenesis while providing a simple and efficient tool for screening new treatments for thyroid cancer.

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the available evidence, however the extent to which this is applied in clinical practice is unknown. The aim of the study was to document the demographic, work-related characteristics, and therapeutic choices of European physicians in the case of euthyroid infertile women positive for TPOAb.

#### Methods

The data presented here derive from THESIS (Treatment of Hypothyroidism in Europe by Specialists, an International Survey). THESIS developed and used a questionnaire to document the management of hypothyroidism by physicians in European countries. Twenty-eight out of the 29 invited Countries (all in Europe plus Israel and Turkey) accepted to participate. The questionnaire included eight questions about respondent characteristics and 23 questions about the use of thyroid hormones in different clinical settings. Here, we report the results on the use of thyroid hormones in infertile euthyroid women with positive TPOAb.

#### Results

Physicians ( $n=16,733$ ) were invited via national endocrine societies to participate; 5,406 valid responses were received. Almost half (2316/5406, 42.8%) of all respondents replied that thyroid hormones may be indicated in biochemically euthyroid patients with female infertility with high level of thyroid antibodies. The proportion of physicians responding positively to this question varied across countries between 23 and 84% (median=41%). In multivariate analysis male gender (OR: 0.8; CI: 0.7-0.9) and respondents older than 60 years (age >60 OR: 0.7; 0.6-0.8) were least inclined to prescribe LT4 for this indication. Conversely respondents with a high workload of thyroid patients ["weekly" (OR: 1.4; CI: 1.0-1.9), "daily" (OR: 1.9; CI: 1.4-2.5)] and practicing in Eastern Europe (OR: 1.6; CI: 1.3-1.9) were most likely to prescribe LT4 to euthyroid infertile women. Respondents' age, gender and geographical location had a striking impact: middle-aged female thyroid physicians practicing in Eastern Europe were seven times more likely to prescribe LT4 for euthyroid, infertile, TPOAb positive women than older male thyroid physicians practicing in Northern Europe (OR: 7.0; CI: 5.4-8.9).

#### Conclusions

Notwithstanding current evidence against systematic treatment, a remarkable number of thyroid specialists would use LT4 for euthyroid, infertile women with positive TPOAb. This practice varied widely across countries and correlated with sex, age, workload, and geography, significantly influencing the patient's management. Our study draws attention to the intriguing associations between clinical decisions and physician demographic characteristics.

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## OP-02-08

### Real world study on the impact of thyroid hormone treatment on pregnancy outcomes in women with subclinical hypothyroidism without tpoab

Georgiana Sitoris<sup>1</sup>, Pierre Kleynen<sup>2</sup>, Flora Veltri<sup>2</sup>, Malika Ichiche<sup>2</sup>, Serge Rozenberg<sup>3</sup> & Kris Poppe<sup>4</sup>

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#### Context/Objective

Evidence on the impact of thyroid hormone treatment (LT4) on pregnancy outcomes in women with subclinical hypothyroidism (SCH) without TPOAb remains scarce. We assessed this relationship in a single centre in a real-world setting.

#### Design, Setting, Participants

Cross-sectional study in 1460 women screened for TSH, free T4 and thyroid peroxidase antibodies (TPOAb) at median 13 (11-17) weeks of gestation, during the period 2013-2014. Exclusion criteria were twin and assisted pregnancies, women treated with LT4 before screening, overt hyperthyroidism, TPOAb positivity and hypothyroxinaemia. The impact of LT4 on pregnancy outcomes was investigated in group of 53 women with SCH (TSH  $\geq 3.74$ ); LT4 was initiated at median 13 (10-22) weeks and at a mean dosage of  $45.3 \pm 16.3$   $\mu$ g/day. Women with SCH and not treated with LT4 served as controls ( $n=18$ ). The prevalence of pregnancy morbidities in these two groups was compared with that in a reference (REF) group of 1389 women (TSH < 3.74 mIU/l) using a  $\chi^2$  test; results were adjusted for confounders and a p-value  $\leq 0.025$  was considered as significant.

#### Results

In the SCH control group, the prevalence of pre-eclampsia and gestational diabetes was (borderline and significantly) higher vs that in the REF group (16.7% vs 5.0%;  $P=0.026$  and 27.8% vs 18.9%;  $P=0.014$ ) but in the SCH treated group comparable vs the REF group (7.6% vs 5.0% and 22.6% vs 18.9%;  $P=0.610$  and 0.547, respectively). The prevalence's of the other outcomes (preterm birth, blood loss at birth, emergency C-section and altered birth weight) were comparable between the intervention and REF group.

## Oral Session 2: Pregnancy

### OP-02-07

#### Use of levothyroxine for euthyroid women with positive thyroid antibodies and infertility: Results of thesis (treatment of hypothyroidism in europe by specialists: an international survey)

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#### Objectives

The use of levothyroxine (LT4) treatment aiming to improve fertility in euthyroid women with positive thyroid peroxidase antibodies (TPOAb), is not supported by

## Conclusions

Women with SCH without TPOAb had a higher prevalence of pre-eclampsia and GDM compared with euthyroid women. However, in women with treated SCH, the prevalence of those outcomes was comparable with that in the euthyroid reference group, even when treatment was started late first / early second trimester. This is the first real-world study that shows a beneficial impact of LT4 on clinical pregnancy outcomes and therefore, adds some evidence to the current guidelines, proposing to treat women with SCH and no TPOAb.

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**OP-02-09****Hypothyroidism and the risk of preeclampsia: A national and regional study of 1,014,775 pregnancies in Denmark**

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## Objectives

Maternal hypothyroidism in pregnancy has been proposed to increase the risk of preeclampsia, but uncertainties persist regarding the underlying causal mechanisms. Thus, it remains unclear if an increased risk of preeclampsia in hypothyroid pregnant women is caused by the lack of thyroid hormones or by the thyroid autoimmunity *per se*.

## Methods

We performed a national and regional study in the Danish population. The national study was register-based, and the study population included all singleton pregnancies in Denmark from 1999-2015 ( $n=1,014,775$ ). The regional study included the biochemical measurement of TSH, thyroid peroxidase antibodies (TPO-Ab), and thyroglobulin antibodies (Tg-Ab) (ADVIA Centaur XPT, Siemens Healthineers) among pregnant women in The North Denmark Region Pregnancy Cohort which was established from 2011-2015 ( $n=14,573$ ). Information on outcome of preeclampsia was obtained from hospital diagnoses in the Danish National Hospital Register. The associations between maternal thyroid function and thyroid autoimmunity and outcome of preeclampsia were evaluated using logistic regression (adjusted odds ratio (aOR) with 95% confidence interval (CI) adjusting for potential confounders (e.g. maternal age, smoking, diabetes).

## Results

In the nationwide study cohort, altogether 2.2% of pregnant women with no history of thyroid disease (reference group) were diagnosed with preeclampsia in the pregnancy. The prevalence of preeclampsia was 3.0% among pregnant women with hypothyroidism (aOR of 1.3 (95% CI: 1.2-1.4)) and 4.3% among women with newly diagnosed hypothyroidism in the pregnancy (aOR 1.7 (95% CI: 1.3-2.1)). In the regional cohort, altogether 2.4% of women with early pregnancy TSH in the range from 0.1-2.49 mIU/l (reference group) were diagnosed with preeclampsia in the pregnancy. The prevalence of preeclampsia was 3.2% among women with early pregnancy TSH in the range from 2.5 to 4.99 mIU/l (aOR 1.1 (95% CI: 0.8-1.5)), and 6.4% among women with TSH at or above 5.0 mIU/l (aOR 2.3 (95% CI: 1.2-4.4)). Considering thyroid autoimmunity, preeclampsia was diagnosed in 2.2% of women who were positive for TPO-Ab (> 59 U/ml) and/or Tg-Ab (> 33 U/ml) in the early pregnancy with an aOR of 0.9 (95% CI: 0.6-1.2).

## Conclusions

In two large cohorts of Danish pregnant women, maternal hypothyroidism was consistently associated with a higher risk of preeclampsia. Biochemical assessment of maternal thyroid function revealed that the severity of hypothyroidism was important. Furthermore, results did not support an association between thyroid autoimmunity *per se* and preeclampsia.

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**OP-02-10****Phthalate exposure is associated with thyroid function during pregnancy through a novel pathway as a hcg disruptor**

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Phthalate exposure is associated with thyroid function during pregnancy through a novel pathway as a hCG disruptor.

## Objectives

hCG stimulates thyroid function in pregnancy and phthalates are known thyroid disruptors. We investigated if phthalate exposure could act as a thyroid disruptor through affecting hCG.

## Methods

This study was embedded in the prospective Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study. Pregnant women were enrolled at median gestational week of 10 (with 95% recruited before week 14). Urinary concentrations of phthalate metabolite, serum thyroid function measurements and hCG were measured. We used linear regression and causal mediation analysis to investigate the potential mediation by hCG in the association of phthalates with maternal FT4.

## Results

In total, 2004 women were included. Out of the 14 phthalate metabolites, higher MEP, MBP, MBzP and all metabolites of DEHP (MEHP, MEHHP, MEOHP, MECPP and MCMHP) were associated with lower hCG concentrations, with the largest effect estimate corresponding to a 0.15 IU/l decrease in hCG concentrations per 1 log-unit increase in urinary MBP concentrations ( $\mu\text{g/g}$  creatinine). We identified that of the 5 phthalate metabolites (MEP, MBP, MEHHP, MEOHP and MECPP) which were previously shown to be negatively associated with FT4 concentrations, hCG mediated 34% (MEOHP,  $P=0.004$ ) to 60% (MBP,  $P=0.03$ ) of the association of all five phthalate metabolites with FT4.

## Conclusions

This is the first study to suggest phthalates act as a hCG disruptor. We also show that higher phthalate exposure during early pregnancy is associated with lower hCG concentrations, resulting in lower FT4 concentrations which is likely mediated through reduced stimulation of the thyroid gland.

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**OP-02-11****Association of thyroid function and tpob positivity with the risk of postpartum depression: A population-based cohort study, systematic review, and meta-analysis**

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## Background

Postpartum depression (PPD) is a common mental health disorder with a major impact on maternal health and wellbeing and offspring development. Thyroperoxidase antibody (TPOAb) positivity is a major risk factor for postpartum thyroiditis and via this link, it is hypothesized that TPOAb positivity is a risk factor for PPD. However, the results of currently available single center studies are heterogeneous and affected by major study limitations.

## Objective

To examine the association of TPOAb and thyroid function with the risk of PPD.

## Methods

In the Generation R Study, a population-based prospective birth cohort in Rotterdam, The Netherlands, we measured TSH, FT4, and TPOAb in blood samples collected between 8-18 weeks of pregnancy. Postpartum depressive symptoms were assessed with the Edinburgh Postpartum Depression Scale (EPDS) at 2 months postpartum and with the Brief Symptom Inventory (BSI) at 2, 6, and 36 months postpartum. In addition, we performed a systematic review of

literature assessing the association of thyroid function and/or TPOAb positivity with risk of PPD and an aggregate data meta-analysis on included studies to generate a pooled risk estimate.

#### Results

There was no association of TSH or FT4 levels with the risk of postpartum depression (log<sub>e</sub> TSH OR:0.79, 95%CI 0.56-1.13, *P*=0.20; FT4 OR:1.02, 95%CI 0.96-1.08, *P*=0.57) in Generation R study. There was also no association of TPOAb positivity with PPD (OR:0.79, 95%CI 0.45-1.31, *P*=0.39). Additional analyses assessed an impaired thyroidal response to hCG stimulation and defined the combined effects of a high hCG with either a high TSH or low FT4 as an alternative marker of TPOAb positivity. We identified that an impaired thyroidal response to hCG stimulation was associated with a lower risk of PPD (*P* for interaction TSH=0.04 and FT4=0.06). In our systematic review, two out of 1219 identified articles were included and they were both eligible for a meta-analysis. The risk of PPD (pooled OR) for TPOAb positivity was 1.20 (95%CI 0.46-3.16).

#### Conclusions

Our original study is by far the largest study on this topic showing that neither TPOAb positivity nor TSH or FT4 were associated with PPD. Our systematic review revealed high heterogeneity and suboptimal methodological quality in the current literature, but overall does not support a link with PPD. Although TPOAb-positive women should be monitored for postpartum thyroiditis, there does not seem to be an indication to screen for postpartum depression. Further research should focus on other factors potentially involved in the etiology of PPD.

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## Oral Session 3: Advanced Thyroid Cancer

### OP-03-12

#### Clinicopathological and epidemiological features of thyroid cancer patients intended to initiate systemic therapy

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#### Objectives

The majority of patients with differentiated thyroid carcinoma (TC) have an excellent prognosis, with a 5-year survival rate of 98.3%, and only rarely reaches an advanced stage of disease. The present study aims to identify the clinicopathological and epidemiological features at the time of diagnosis of a group of patients who required to be treated with systemic therapy with tyrosine-kinase inhibitors (TKI).

#### Methods

We retrospectively evaluated 136 patients with thyroid carcinoma who were addressed to TKI between 2012 and 2022 and followed at the Endocrine Unit of the University Hospital of Pisa. Demographic, clinical and pathological data were collected at the moment of the initial diagnosis and at the moment the systemic therapy was started.

#### Results

Sex distribution was equal: 69 females (50.7%) and 67 males (49.3%). The mean age was advanced both at diagnosis (59.01 ± 10.44 years) and when systemic therapy was started (68.85 ± 8.52 years). The histotypes (available in 135 cases) were distributed as follows: 72 papillary thyroid carcinomas (PTC) (53.8%), 27 follicular TC (20.1%), 29 poorly differentiated TC (21.6%), 6 Hürthle cell TC (4.5%). The histological variants of PTC (available in 51 cases) were: 21/51 classical (41.2%), 14/51 tall cell (27.5%), 15/51 follicular (29.4%), 1/51 solid (1.9%). The histological variants of FTC (available in 16 cases) were: 6/16 widely invasive (37.5%), 4/16 minimally invasive (25%), 6/16 oxyphil (37.5%). Regarding TNM classification, primary tumors were frequently classified as T3 and T4 (40.4% and 30.3%, respectively). At initial diagnosis 73/136 patients (55.3%) presented with lymph node metastasis (LNM) and 51/136 patients (39.8%) with distant metastasis (DM). At the moment of starting systemic therapy, 114/136 patients (84.4%) had LNM and 126/136 patients (92.6%) had DM. AJCC stage at diagnosis (available in 120 cases) was distributed as follows:

31/120 at I stage (25.8%), 25/120 at II stage (20.8%), 15/120 at III stage (12.5%), 10/120 at IVA stage (8.3%), 39/120 at IVB (32.6%). At TKI inception 120/136 patients (88.2%) were at stage IVB, 7/136 patients at stage IVA (5.1%), 2/136 patients at stage III (1.6%) and 7/136 patients at stage II (5.1%).

#### Conclusions

Male sex, advanced age, lymph node and distant metastases at the time of diagnosis are the most relevant features associated to the need of starting systemic therapy with TKI.

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### OP-03-13

#### Thyroid cancer relapse as a challenge to a surgeon: The efficacy of radioguided occult lesion localization (roll) technique

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#### Introduction

Reoperation due to recurrent or persistent thyroid cancer (TC) poses a real challenge to a surgeon who must properly estimate the benefit risk-ratio. Radioguided occult lesion localization (ROLL) technique may facilitate the localization and sufficient and safe removal of nonpalpable TC relapse.

#### Aim

To analyze the efficacy of the ROLL technique in reoperation of TC relapse.

#### Material and methods

A retrospective study of 254 TC patients (186 papillary TC, 24 follicular TC, 34 medullary TC, 10 poorly differentiated TC) reoperated on from 2002 and 2018 was performed. There were 180 (70.9%) women and 74 (29.1%) men, mean age 47.2 years (median 50.7 years) with 7.82 years of the mean follow-up (median 5.81 years). All the relapses were preoperatively marked by ultrasound-guided administration of technetium Tc-99m albumin colloid (0.1 ml).

#### Results

The main localization of relapse was the thyroid bed – central neck compartment (178; 70.1%). Recurrence diameters ranged from 2 to 17 mm. In 71% of cases intralesional administration of the radiotracer was used and in 29% the radiotracer was applied in the lesion area. There was no difference in the efficacy of the relapse removal between these groups (*P*=0.926). In 55 patients (21.7%) multifocal recurrence was found and removed. No differences in the efficacy of relapse removal were observed depending on the histological type and multifocal nature of recurrence (*P*=0.08). In 19 patients (7.5%) relapse was not found in ROLL procedure (necessity of reoperation). No differences were found between the recurrent laryngeal nerve paresis after the primary and secondary operation (*P*=0.7) as in the case of postoperative hypoparathyroidism (*P*=0.4). More than 1 reoperation was performed in 42 patients. In this subgroup, 1 death (2%) was observed, disease progression was noted in 12 patients (29%) and complete remission was found in 28 (67%). In the whole group, complete remission was observed in 198 patients (78%) during the follow-up.

#### Conclusions

The ROLL technique in TC is a safe and effective procedure that facilitates intraoperative localization and removal of TC recurrence.

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## OP-03-14

**Entrectinib in patients with ntrk fusion-positive (ntrk-fp) thyroid cancer: Updated data from startrk-2**

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**Objectives**

*NTRK* gene fusions are oncogenic drivers in many solid tumours, including thyroid cancers. In the phase 2 study STARTRK-2 (NCT02568267), entrectinib (a CNS-active tropomyosin receptor kinase [TRK] inhibitor) demonstrated efficacy in patients with *NTRK*-fp thyroid cancer (objective response rate [ORR]: 40%; clinical cut-off: 31 Oct 2018; *n* = 5). We report updated data from a larger cohort with longer follow-up.

**Methods**

Adult patients with TRK-inhibitor naïve, locally advanced/metastatic *NTRK*-fp thyroid cancer, with or without baseline CNS metastases, were enrolled. Tumour responses were assessed by blinded independent central review (BICR) per RECIST v1.1 at Week 4 and every 8 weeks thereafter. Primary endpoints: ORR and duration of response (DoR). Secondary endpoints included progression-free survival (PFS), overall survival (OS), intracranial (IC) efficacy and safety. Enrolment cut-off: 31 July 2019; clinical cut-off: 31 August 2020.

**Results**

The efficacy-evaluable population included 13 patients with ≥ 1 year of follow-up: 10 (77%) had papillary and three (23%) had other types of thyroid cancers. Median age was 55.0 years (range: 26–78); 7 patients (54%) had received ≥ 2 prior lines of therapy; and 7 patients (54%) had investigator-assessed baseline CNS metastases. Median survival follow-up was 36.1 months. Responses for all patients and patients with papillary *NTRK*-fp thyroid cancer are shown in the Table. Median DoR, PFS and OS for all patients were 13.2 months (95% CI: 7.9–NE), 19.9 months (95% CI: 6.5–33.8) and 19.9 months (95% CI: 14.5–NE), respectively. In patients with BICR-assessed baseline CNS metastases (*n* = 6), IC-ORR was 50% (3/6; 95% CI: 11.8–88.2) and median IC-PFS was 15.0 months (95% CI: 6.3–NE). In the safety-evaluable population (*n* = 16; all treated patients), 10 patients (63%) had a Grade ≥ 3 treatment-related adverse event (TRAE). There were two deaths (13%) due to TRAEs. TRAEs leading to dose reduction, interruption and discontinuation occurred in 31%, 38% and 19% of patients, respectively.

**Conclusions**

In this updated analysis with more than twice the number of patients previously reported, ORR was numerically higher compared with the prior analysis and entrectinib demonstrated durable systemic and IC responses. No new safety signals were identified.

|                          | <b>NTRK-fp thyroid cancer<br/>All patients<br/>(N = 13)</b> | <b>Papillary thyroid<br/>cancer (n = 10)</b> |
|--------------------------|---|--|
| <b>ORR, n (%) 95% CI</b> | 7 (54) 25.1–80.8  | 5 (50) 18.7–81.3                             |
| Complete response (CR)   | 1 (8)   | 1 (10)                                       |
| Partial response         | 6 (46)  | 4 (40)                                       |
| Stable disease           | 2 (15)  | 2 (20)                                       |
| Progressive disease (PD) | 1 (8)   | 1 (10)                                       |
| Non-CR/non-PD            | 1 (8)   | 0  |
| Missing/unevaluable      | 2 (15)  | 2 (20)                                       |

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## OP-03-15

**Assessment of adrenal function in a large series of patients confirms that adrenal insufficiency is a common cause of fatigue during treatment with multikinase inhibitors (MKIS)**

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**Background**

Fatigue is one of the most frequently reported adverse events in patients treated with multikinase inhibitors (MKIs). It is present in about 59% and 24% of patients treated with lenvatinib and vandetanib, respectively, associates with a decreased quality of life and often leads to treatment discontinuation. In 2018, for the first time, a correlation between fatigue and primary adrenal insufficiency (PAI) was demonstrated in 12 patients treated with lenvatinib and vandetanib (Colombo *et al.*, JCEM 2018). These data were confirmed more recently by the evaluation of adrenal function in 13 patients treated with lenvatinib (Monti *et al.*, Thyroid 2022).

**Aim**

To assess adrenal function in a larger series of patients who developed fatigue during MKIs treatment.

**Methods**

Adrenal function has been monthly evaluated, for an average follow-up of 38 months (6-161 months), in 32 patients receiving MKIs and developing fatigue during treatment (23 on Lenvatinib, 7 on Vandetanib and 2 on Selpercatinib). In particular, cortisol and ACTH levels were evaluated together with 250-µg ACTH stimulation test (performed at the time of ACTH elevation above the normal limits).

**Results**

A high percentage of patients with fatigue, 23/31 (74%), had a significant elevation of basal ACTH with normal cortisol levels during MKIs treatment (18/23 on lenvatinib, 4/7 on vandetanib and 1/2 on selpercatinib). Moreover, 8/23 patients (6 on lenvatinib and 2 on vandetanib) showed an impaired response to the ACTH test, thus confirming PAI diagnosis. The onset of PAI occurred after an average period of 14 months (range 3-61) from the start of MKIs treatment. Cortisone acetate (CA) replacement therapy was recommended in 9 patients (all the patients with an impaired cortisol response to ACTH stimulation and in 1 patient with only ACTH elevation and fatigue degree 2 according to the CTCAE version 4.03), and improved fatigue in 6 of them (67%).

**Conclusions**

Data on a large series confirm that a high percentage of patients on MKIs show ACTH levels above the upper normal limit. Fatigue can be totally or partially related to the development of PAI in these patients. The first evidence of reduced adrenal function is the progressive rise of basal ACTH levels with cortisol values within normal limits. Moreover, a reduced cortisol response to the 250-µg ACTH stimulation test is observed in some of these patients. Replacement therapy with CA improves fatigue and, therefore, adherence to therapeutic regimens, avoiding dose reductions or discontinuation.

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## OP-03-16

**A clinical and molecular study of a real-world cohort of braf v600e anaplastic thyroid carcinoma treated with dabrafenib and trametinib**

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#### Introduction and objectives

Anaplastic thyroid cancer (ATC) has a very low overall survival (OS) and progression free survival (PFS) due to fast growth and resistance to non-target therapies. A recent phase II study showed a dramatic increase in OS and PFS of *BRAF* V600E mutated ATC patients treated with Dabrafenib and Trametinib (DT). However as commonly reported in melanoma durable responses in ATC may be compromised by resistance mechanisms. Until now only a few case reports and a small case series has been published. We present our experience with DT in *BRAF*-positive ATC patients and compare the outcomes with usual therapy. In addition, we studied the molecular alterations at baseline and during progression in DT group.

#### Materials and methods

ATC patients treated between May 2018 and October 2021 were included. *BRAF* positive patients underwent next generation sequencing (NGS) at baseline and at progression. Bioinformatic analyses filtered and selected relevant somatic genomic variants. Patients were classified in the following groups: *BRAF* WT under compassionate care (CC); *BRAF* wild type (WT) under multimodal therapy (MT) with surgery, radiotherapy ± chemotherapy or lenvatinib and *BRAF* V600E treated with DT. Response was assessed monthly in the first 6 months and then every 3 months by RECIST 1.1: OS, PFS, duration of response (DOR) was estimated with Kaplan-Meier method and compared with log-rank test.

#### Results

27 ATC patients were included (CC = 10, MT = 8 and DT = 9). Median follow-up was 24, 96 and 410 days for CC, MT and DT respectively due to differences in survival time. Median OS was 39, 156 and 475 days for CC, MT and DT respectively ( $P < 0.001$ ). At 12 months only patients in the DT group were alive (71%). Median PFS and DOR were 270 and 215 days, respectively (MT group < 32 days) ( $P < 0.001$ ). In the DT group only 1 patient needed trametinib dose reduction to 225 mg/d and no G3 adverse events were reported. Molecular profiling by NGS showed that the nine patients in the DT group had a *BRAF* V600E and a *TERT* mutation at baseline. In one of the four clinical disease progressions during DT treatment a pathogenic *NRAS* mutation was found.

#### Conclusions

Our results show a significant real-world efficacy of DT in both OS and PFS compared with contemporary standard treatment with a good safety profile. Molecular profiling allowed the patient with RAS mutations to change treatment strategy.

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## Oral Session 4: Basic 1

### OP-04-17

#### Age and diet regulate intrahepatic thyroid hormone concentration in a mouse model of non-alcoholic fatty liver disease (NAFLD)

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#### Background

Previously, we showed that thyroid hormone (TH) supplementation reduced hepatosteatosis in patients with non-alcoholic fatty liver disease (NAFLD) and was more effective in older patients. We hypothesized that intrahepatic TH concentrations may decrease with age. To investigate this issue, we examined the effects of age on intrahepatic TH concentrations in a mouse model of NAFLD.

#### Methods

Intrahepatic TH levels and deiodinase activity were measured in 18-24 week old (Young group) and 108-120 week old (Old group) mice. Mice were fed normal chow diet (NCD) or Western diet with fructose in the drinking water for 8 weeks (WDF). Liver TH concentrations ( $T_4$  and bioactive  $T_3$ ) were measured by LC-MS/MS. Deiodinase 1 (Dio1) and 3 (Dio3) activity was measured by conversion of 125I-labelled  $rT_3$  and  $T_3$  respectively. Results were analyzed by two-way ANOVA investigating the effect of age (young vs. old), diet (NCD vs WDF) and the interaction between age and diet.

#### Results

Intrahepatic triglycerides significantly increased in both in old and young mice fed WDF. Intrahepatic  $T_4$  concentrations were significantly lower in old mice fed

NCD ( $P < 0.0001$ ) and old mice fed WDF ( $P = 0.0002$ ) without an interaction effect. Intrahepatic  $T_3$  concentrations also were significantly lower in older mice ( $P = 0.0053$ ) and mice receiving WDF ( $P = 0.0014$ ) without an interaction effect. As previously described deiodinase 1 (Dio1) activity increases with a WDF diet ( $P = 0.03$ ) and decreases with age ( $P = 0.0018$ ). Old mice fed WDF had higher Dio1 activity than mice fed NCD; however, their Dio1 activity was lower than young mice fed WDF. Deiodinase 3 (Dio3) activity was increased in older mice ( $P = 0.0021$ ) with a significant interaction effect. Interestingly, young mice fed WDF had increased Dio3 activity whereas old mice had decreased Dio3 activity.

#### Conclusions

Age and WDF are both factors that decrease intrahepatic TH levels. The decrease in intrahepatic  $T_3$  concentration in old mice fed WDF could explain why TH supplementation was more effective in reducing hepatosteatosis in older patients.

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### OP-04-18

#### Differential impact of intrathyroidal IL-4 expression on thyroiditis development in C57BL/6J and NOD.H2H4 THYR-IL4 mice

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#### Introduction

Interleukine-4 (IL-4) a T-helper type 2 cytokine (Th2), has been implicated in the pathogenesis of autoimmune thyroid diseases (AITD). However, the role of IL-4 in Hashimoto's thyroiditis (HT) pathogenesis remains controversial. In this study, we investigated whether a constitutive IL-4 overexpression in the thyroid tissue (Thyr-IL4) could influence the development of thyroiditis in resistant (C57BL/6) or susceptible (NOD.H2<sup>h4</sup>) mouse strains.

#### Methods

Thyr-IL4 C57BL/6 parental strain and NOD.H2<sup>h4</sup> mice were exposed to 0.05% of NaI supplemented water during 8 and 16 weeks. Disease development was evaluated by measuring serum TgAbs, as well as quantifying the immune cell infiltration by immunostaining, flow cytometry and cytokine mRNA expression. Thyroid function was also evaluated through serum TSH levels as well as mRNA expression of thyroid differentiation markers.

#### Results

After 16 weeks of NaI treatment circulating TgAbs were significantly higher in transgenic susceptible NOD.H2<sup>h4</sup> animals. NOD.H2<sup>h4</sup> Thyr-IL4 mice developed also intense lymphocytic infiltration. Moreover the relative mRNA expression of IFN $\gamma$ , IL-10, TGF $\beta$ , TNF $\alpha$ , IL-17 and IL-13 was also significantly increased in treated transgenic animals compared to WT mice. Chronic administration of iodide induced an important increase in serum TSH levels in transgenic NOD.H2<sup>h4</sup> animals with the development of large colloid goiter. In addition, as expected in the escape from the Wolff-Chaikoff block, mRNA expression of the iodide symporter Nis was reduced in both WT and Thyr-IL4 animals. In contrast, for the thyroiditis resistant parental strain, no circulating TgAbs could be detected in the serum of WT and transgenic C57BL/6 mice. As previously reported, WT C57BL/6 animals did not show thyroidal leukocyte infiltrates 16 weeks after NaI treatment. Surprisingly, the transgenic parental strain shown intense leukocyte infiltration scattered throughout the thyroid tissue associated with enhanced expression of IFN $\gamma$ , TGF $\beta$ , TNF $\alpha$ , IL-5 and IL-13. Thyr-IL4 C57BL/6 animals developed also thyroid goiter with increased TSH levels. However, instead of a correct Wolff-Chaikoff escape present in WT animals, the Nis mRNA expression remained elevated in transgenic mice.

#### Conclusions

We have shown that prolonged expression of IL-4 in the thyroid associated to a chronic administration of iodide exacerbate thyroiditis disease in spontaneous NOD.H2<sup>h4</sup> mice and can induced non-autoimmune thyroid infiltrate in C57BL/6 resistant genetic background.

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### OP-04-19

#### A thyroid hormone-independent role for transthyretin in neural stem cells of the postnatal mouse subventricular zone?

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Transthyretin (TTR) distributes thyroxine in the cerebrospinal fluid of mammals. Choroid plexus epithelial cells produce and secrete TTR, and were long recognized as the only CNS source of TTR. However, research over the last years has reported neuronal-specific expression as well, but without a clear function. Recently, we found Ttr transcripts in cells of the adult mouse subventricular zone (SVZ), the largest neural stem cell (NSC) region, but the protein was undetectable. We therefore investigated in more detail what role TTR might play in the SVZ, and when. We mapped temporal-spatial Ttr expression by re-analysing publicly available single-cell RNA-Seq data obtained from dissected mouse SVZs at E14-E17-P2-P7-P20-P61. We observed a peak in Ttr expression in NSCs, neural progenitors and differentiating cells at postnatal day 7 (P7). That is one week prior to when thyroxine serum levels peak and T3 activates SVZ-NSCs that start generating neurons and glia at a constant rate. RNAscope on P7 brain sections confirmed that few Ttr transcripts are present in a many SVZ-progenitors, oligodendrocyte precursors and neuroblasts. Unexpectedly though, no protein was detectable using commercially available antibodies, signal amplification and appropriate controls. This might suggest TTR is rapidly secreted to affect nearby cells. To test this hypothesis, we prepared neurospheres from dissected SVZ-progenitors at P7. After 7 days of proliferation, cells were dissociated, and allowed to differentiate for 1 or 5 days. In parallel with controls, we treated them once at day 0 of differentiation with a low (2.5 µg/ml) or a high dose (25 µg/ml) of human recombinant TTR, or with 5 nM T3. Low TTR doses reduced cell mitosis at day 1, as did T3. After 5 days, we counted a 30% lower proportion of differentiated neuroblasts with the highest TTR dose. That proportion had dropped 3-fold in the presence of T3. Proportions of oligodendroglia after 5 days of differentiation were only significantly higher in T3 conditions. As a result, the neuron/glia balance shifted in favour of oligodendrogenesis under T3, and borderline-significantly following high TTR doses. Altogether, the murine SVZ represents a novel region containing cells that express Ttr, with a peak at P7, despite seeming absence of the protein itself, precluding deducing its exact role. Single-cell RNA-Seq on treated neurospheres could reveal how exogenous TTR affects intracellular pathways, and whether its action is TH-dependent or not. This can help unravelling the pathophysiology of familial amyloid polyneuropathy, in which misfolded TTR proteins cause neurodegeneration.

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## OP-04-20

### Rewiring of liver diurnal transcriptome rhythms by triiodothyronine (T3) supplementation

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Cellular 24-hour rhythms depend on transcriptional programs controlled by a set of circadian clock genes/proteins. Systemic factors like humoral and neuronal signals, oscillations in body temperature, and food intake align physiological circadian rhythms with external time. Thyroid hormones (THs) are major regulators of circadian clock target processes such as energy metabolism, but little is known about how fluctuations in TH levels affect the circadian coordination of tissue physiology. In this study, a high triiodothyronine (T<sub>3</sub>) state was induced in mice by supplementing T<sub>3</sub> in the drinking water, which affected body temperature, and oxygen consumption in a time-of-day dependent manner.

24-hour transcriptome profiling of liver tissue identified 37 robustly and time independently T<sub>3</sub> associated transcripts as potential TH state markers in the liver. Such genes participated in xenobiotic transport, lipid and xenobiotic metabolism. We also identified 10 – 15 % of the liver transcriptome as rhythmic in control and T<sub>3</sub> groups, but only 4 % of the liver transcriptome (1,033 genes) were rhythmic across both conditions – amongst these several core clock genes. In-depth rhythm analyses showed that most changes in transcript rhythms were related to mesor (50%), followed by amplitude (10%), and phase (10%). Gene set enrichment analysis revealed TH state dependent reorganization of metabolic processes such as lipid and glucose metabolism. At high T<sub>3</sub> levels, we observed weakening or loss of rhythmicity for transcripts associated with glucose and fatty acid metabolism, suggesting increased hepatic energy turnover. In sum, we provide evidence that tonic changes in T<sub>3</sub> levels restructure the diurnal liver metabolic transcriptome independent of local molecular circadian clocks.

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## OP-04-21

### CRYO-electron microscopy structures of human thyroid peroxidase (TPO) in complex with tpo antibodies

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#### Objectives

Thyroid peroxidase (TPO) is a key enzyme in the synthesis of thyroid hormones and is a target for autoimmune responses in autoimmune thyroid disease. TPO autoantibody (TPOAb) binding epitopes have been mapped on the peroxidase domain (POD) and the complement control protein like domain (CCP). This study aimed to solve the molecular structures of TPO bound to TPO antibodies.

#### Methods

An extracellular domain (ECD) of human TPO (amino acids; aa 1-839) was expressed in insect cells. Fabs were prepared from two TPO antibodies; a human monoclonal autoantibody 2G4 and a mouse monoclonal antibody 4F5. The structures of TPO-2G4 and TPO-4F5 complexes were determined by cryo-electron microscopy using a Titan Krios 300kV with a Falcon 3 Direct Detector.

#### Results

The structure of TPO-2G4 was solved at 3.92 Å and TPO-4F5 at 3.4 Å resolutions. The solved ECD structure comprises the POD, the CCP and an incomplete epidermal growth factor like domain (EGF). The POD Arg396 and Arg491 hold the haem group and form salt bridges with the two carboxylate groups of the haem. His494 acts as the proximal histidine interacting with the iron ion of the haem. The enzyme active site is located at the distal side of the haem and is lined by Gln235, Asp238, His239 and Glu399. A calcium ion is coordinated by Asp240, Thr321, Phe323, Asp325 and Ser327. 2G4 and 4F5 bind to TPO in different orientations. For both antibodies the binding epitopes are located exclusively on the POD. The binding interface for 2G4 is larger (2148 Å<sup>2</sup>) than for 4F5 (1959 Å<sup>2</sup>). 2G4 interacts with aa 194-277 and 604-628 whereas 4F5 with aa 461-659 with three common residues Glu604, Ala607 and Asp608 for both epitopes. In both complexes the antibody heavy chains make greater contributions to the interface than the light chains. 2G4 and 4F5 binding sites on the POD are distant from the CCP and EGF with no contacts between antibodies and the CCP or EGF. Any conformational movement of the CCP towards antibody epitopes on the POD would be prevented by a disulphide bond between POD Cys768 and CCP Cys 794.

#### Conclusions

Human TPO molecular structure has now been solved. This should be helpful in assessing the autoimmune responses to TPO in more detail and developing TPO enzyme inhibitors for therapeutic applications.

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## Oral Session 5: Autoimmunity

### OP-05-22

#### Update on the role of caveolin-1 in cell homeostasis and oxidative stress in hashimoto's thyroiditis and graves' disease

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Caveolin-1 (cav1) is a member of the tyrosinase multiprotein complex required for thyroid hormones synthesis which is decreased in Hashimoto's thyroiditis (HT) and excessive in Graves' disease (GD). Both pathologies are characterized by oxidative stress (OS), the production of reactive oxygen species (ROS) exceeding the antioxidant defenses. The aim of this study is to correlate cav1 expression and OS in HT and GD. Primary cultures of human thyrocytes were treated or not with Th1 (Interleukin-1 $\alpha$  and Interferon $\gamma$ ) or Th2 (Interleukin-4) cytokines. Thyroid samples from HT and GD patients were compared to parathyroid tissues of patients with multinodular goiter (controls). Orbital fats were obtained from controls or patients with Graves' orbitopathy (GO). Samples were processed for RT-PCR, Western blots or immunohistochemistry to analyze the expression of T4, cav1, 4-hydroxynonenal (HNE), caspase-6, catalase, peroxiredoxin 1 (PRDX1), sirtuin-1 (sirt1), NADPH oxidase (NOX)4 and NOX2. In HT, the glands comprised a mix of normal and altered follicles. The altered follicles, located within inflammatory zones, did not express cav1 and were unable to form and store T4 in the follicular lumen, T4 being detected inside the cytoplasm. They also highly expressed HNE, an OS marker, and presented numerous dead cells labelled with caspase-6. In primary cultures, Th1 cytokines decreased cav1 but also catalase and PRDX1 which detoxify H<sub>2</sub>O<sub>2</sub> and sirt1 known to positively regulate antioxidants expression. At the opposite, in GD thyroids, all the follicles were able to form and store T4. Cav1 protein was increased as compared to controls and properly located at the apical pole. The high HNE expression indicative of OS correlated with an increase of NOX4 continually generating H<sub>2</sub>O<sub>2</sub>. However, antioxidants like catalase were upregulated to cope with ROS production so that there were few dead thyrocytes. Interleukin-4 which could mimic GD did not influence cav1 expression. Of interest, in GO orbital adipocytes, NOX4 was also increased, as well as NOX2, inducing OS further aggravated in these cells by a reduction of catalase. In conclusion, cav1 expression is diametrically opposed in HT and GD. In HT, the downregulation of cav1 by Th1 cytokines induces tyrosinase disruption, hypothyroidism and intracellular H<sub>2</sub>O<sub>2</sub> production responsible for OS still aggravated by a Th1-induced decrease of sirt1 and antioxidant defenses. In GD, cav1 overexpression could be correlated with hyperthyroidism and NOX4 could be the prime target to prevent OS.

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### OP-05-23

#### Cut-offs for thyroid peroxidase and thyroglobulin antibodies in early pregnancy are not similar and may differ from non-pregnant individuals: evidence from 10,905 danish pregnant women

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#### Objectives

Thyroid disease in women of reproductive age is predominantly of autoimmune origin, and thyroid peroxidase antibodies (TPO-Ab) as well as thyroglobulin antibodies (Tg-Ab) are key markers of autoimmune hypothyroidism. Adding to this, much focus in pregnancy is on euthyroid women who are positive for thyroid

autoantibodies. The observational findings are diverse, and evidence to substantiate the applied cut-offs for definition of thyroid autoantibody-positivity in early pregnant women is warranted.

#### Methods

The North Denmark Region Pregnancy Cohort, 2011-2015, includes biochemical assessment of thyroid function, TPO-Ab, and Tg-Ab (ADVIA Centaur XPT, Siemens Healthineers) in stored blood samples from 14,030 early pregnant women. Antibody cut-offs recommended by the manufacturer (non-pregnant) were 60 U/ml for both TPO-Ab and Tg-Ab. Within the cohort, euthyroid singleton pregnant women with no history of thyroid disease were identified for establishment of antibody cut-offs (reference cohort). TPO-Ab and Tg-Ab showed skewed distributions with 50.7% and 66.4% of the values below the detection limits of 28 U/ml and 15 U/ml, respectively. Thus, antibody cut-offs (95% percentiles) were established by Regression on Order Statistics.

#### Results

Altogether 10,905 pregnant women were included in the reference cohort, and the established cut-offs for thyroid autoantibodies within the cohort were 59 U/ml (TPO-Ab) and 33 U/ml (Tg-Ab). The cut-offs were then applied in the full cohort of 14,030 pregnant women showing that 1,545 women (11.0%) were TPO-Ab positive, 1,870 (13.3%) were Tg-Ab positive, and 1,079 (7.7%) were TPO-Ab and Tg-Ab positive. Considering maternal thyroid function by antibody-status (Table), TSH was higher and free T4 lower among antibody-positive as compared to antibody-negative women with each of the applied cut-offs.

#### Conclusions

In a large cohort of Danish pregnant women, cut-offs for TPO-Ab and Tg-Ab in early pregnancy were established while considering the skewed distributions. The established cut-offs were not similar for TPO-Ab and Tg-Ab, and the cut-off for Tg-Ab was lower than recommended in non-pregnant individuals. The findings are important regarding classification of exposure in pregnancy outcome studies and specifically regarding the assessment of thyroid autoimmunity *per se*.

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### OP-05-24

#### Sirolimus for graves' orbitopathy: A novel drug for the management of patients with moderate-to-severe graves' orbitopathy?

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#### Background

Sirolimus is an immunosuppressive drug with anti-fibrotic and anti-proliferative activities. *In vitro*, sirolimus inhibits differentiation of orbital fibroblasts from patients with Graves' orbitopathy (GO), suggesting a possible use in clinical practice.

#### Methods

We performed a retrospective investigation aimed at evaluating the effects of sirolimus as a second-line treatment for moderate-to-severe, active GO, compared with methylprednisolone. The investigation entailed data analysis of unselected, consecutive patients with moderate-to-severe, active GO, treated off-label with sirolimus (2 mg orally on first day, followed by 0.5 mg/day for 12 weeks) or methylprednisolone [500 mg iv/weekly (6 weeks), 250 mg/weekly (6 weeks)], as a second-line treatment, over a period of 18 consecutive months. The primary objective was the overall GO outcome at 24 weeks based on a composite evaluation. Secondary objectives at 24 weeks were: 1) improvement in quality of life, evaluated using a specific questionnaire (GO-QoL); 2) reduction of proptosis; 3) reduction of the clinical activity score (CAS); 4) improvement of eye ductions; and 5) reduction of eyelid aperture.

|                  | Antibody-positive<br>TPO-Ab > 59 U/ml |        |             | Antibody-negative<br>TPO-Ab ≤ 59 U/ml |        |             |
|------------------|---------------------------------------|--------|-------------|---------------------------------------|--------|-------------|
|                  | n                                     | Median | 95% CI      | n                                     | Median | 95% CI      |
| TSH (mIU/l)      | 1,545                                 | 1.73   | 1.67-1.79   | 12,485                                | 1.07   | 1.05-1.09   |
| Free T4 (pmol/l) | 1,545                                 | 15.61  | 15.46-15.71 | 12,485                                | 16.03  | 15.99-16.07 |
|                  | Tg-Ab > 33 U/ml                       |        |             | Tg-Ab ≤ 33 U/ml                       |        |             |
|                  | n                                     | Median | 95% CI      | n                                     | Median | 95% CI      |
| TSH (mIU/l)      | 1,870                                 | 1.64   | 1.58-1.69   | 12,160                                | 1.06   | 1.05-1.08   |
| Free T4 (pmol/l) | 1,870                                 | 15.57  | 15.46-15.67 | 12,160                                | 16.04  | 16.00-16.09 |

**Results**

Data from 30 patients (15 per group) were analyzed. Baseline demographic and clinical features did not differ between the two groups. The proportion of overall GO responders (primary outcome) was significantly greater in the sirolimus group compared with the methylprednisolone group (80% vs 26.6%; OR: 11; 95% CI from 1.9 to 60.5;  $P=0.0059$ ). The total GO-QoL score at 24 weeks was greater in the sirolimus group (mean difference 5.5 points; 95% CI from 1.4 to 9.6,  $P=0.010$ ). The proportion of proptosis responders was greater in the sirolimus group (80% vs 13.3% in the methylprednisolone group; OR: 26; 95% CI from 3.6 to 183.4;  $P=0.0011$ ), as was the proportion of CAS responders (86.6 vs 33.3%, OR: 13; 95% CI from 2 to 81.4;  $P=0.0062$ ). In contrast, eyelid width and eye ductions responders did not differ between the two groups. No serious adverse events were observed, with no differences between the two groups.

**Conclusions**

Given the limitations of a retrospective investigation, sirolimus seems to be an effective second-line treatment for GO. Further randomized clinical trials are needed to confirm our observation and to investigate whether sirolimus can be employed as a first line treatment. To our knowledge, apart from two previous case reports, this is the first report on sirolimus for GO in a patient series.

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**OP-05-25****Intestinal barrier permeability in patients with hashimoto's thyroiditis associated to non-endocrine autoimmune disorders**Camilla Virili<sup>1</sup>, Ilaria Stramazzo<sup>1</sup>, Silvia Capriello<sup>1</sup>, Nunzia Brusca<sup>1</sup>, Maria Flavia Bagagli<sup>1</sup>, Cristina Nocella<sup>2</sup>, Roberto Carnevale<sup>3</sup> & Marco Centanni<sup>1</sup>

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**Objective**

Intestinal barrier (IB) is one of the wider human body surface, whose integrity allows the uptake of nutrients also preserving from the entrance of harmful compounds. An increased permeability of IB, known as "leaky gut", has been demonstrated in patients with intestinal inflammatory and/or autoimmune disorders as well as in patients with autoimmune diseases involving organs far from the intestine, as in patients with Hashimoto's thyroiditis (HT). The permeability of IB in patients with Hashimoto's thyroiditis who associate further non-endocrine autoimmune disorders has not been described yet and this is the aim of our study.

**Methods**

The study group encompassed 93 patients bearing HT (median age=48 years; M=12; F=81); 33 of them associated another non-endocrine autoimmune disorder (HT+POLY) [13 gastric atrophy (HT+GA), 13 non-segmental vitiligo (HT+V) and 7 celiac disease (HT+CD)]. The chronic use of interfering drugs, metabolic, gastrointestinal or chronic disorders, pregnancy, smoking habit, and unbalanced diets have been positively excluded in the enrolled patients. The evaluation of gut permeability was performed by dosing serum zonulin, a regulator of intestinal tight junctions, and LPS, a structural component of the cell membrane of Gram negative bacteria, index of endotoxemia. All subjects underwent venous blood sampling between 8:00 and 9:00 and fasting for at least 12 hours. The serum was stored at -20°C until all the samples were simultaneously measured by ELISA kits.

**Results**

The concentration of zonulin was higher in patients HT+POLY than in patients with isolated HT ( $P<0.0001$ ); a similar result was observed for LPS concentrations ( $P=0.0004$ ). The highest concentrations of zonulin as compared to HT may be observed in HT+CD ( $P<0.0001$ ), followed by HT+GA ( $P<0.01$ ) and by HT+V ( $P<0.05$ ). On the contrary, the highest concentration of LPS as compared to HT may be observed in HT+V ( $P<0.01$ ), followed by HT+GA ( $P<0.05$ ). In the whole sample and in patients with isolated HT, zonulin and LPS concentrations significantly correlated ( $P<0.0001$ ;  $r=0.4431$  and  $r=0.4409$ , respectively), a correlation lost in patients with HT+POLY. This was due to a greater increase of LPS over zonulin levels (37% vs 31%) in HT+POLY group.

**Conclusions**

The association of HT with further autoimmune disorders is characterized by a leaky gut, even when the disease does not involve directly the gastrointestinal tract. The higher concentration of LPS in polyautoimmune patients indicates a more severe systemic inflammatory state.

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**OP-05-26****Prospective single blind, usa-based, multicenter comparison of tsh-receptor antibody immunoassays**Mark Lupo<sup>1</sup>, Amy Little<sup>1</sup>, Burak Hatun<sup>2</sup> & George Kahaly<sup>3</sup>

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**Objective**

The performance of two functional bioassays and two immunoassays for the measurement of TSHR-Ab was assessed in this multicenter study.

**Methods**

Two-hundred two subjects (median age 54 years, 162 female, 80%), with well-documented thyroid disorders and controls were prospectively enrolled in a consecutive, unselected manner. Antibody measurements were performed in a blinded manner using the Bridge (Siemens, performed at the Dartmouth-Hitchcock Medical Center Laboratory in Lebanon, NH, USA) and Cobas (Roche) automated assays as well as stimulating (TSAb) and blocking (TBAb) TSHR-Ab cell-based bioassays (Quidel) according to manufacturer's instructions. The Cobas and the two bioassays were performed at the Johannes Gutenberg University, Mainz, Germany.

**Results**

The four assays were negative in controls ( $n=10$ ), patients with euthyroid nodular disease ( $n=11$ ) and non-autoimmune thyrotoxicosis ( $n=21$ ). In patients with Graves' disease (GD), independent of disease duration, TSHR-Ab positivity was present in 72/114 (63%), 69 (61%), and 91 (80%) for the Bridge, Cobas immunoassays and TSAb bioassay, respectively ( $P<0.001$ ). Concordant positive results in the two immunoassays and TSAb bioassay were noted only in 69/114 (61%) patients with GD and TSAb positivity. Of the 45 discordant findings, 26 were TBAb positive as well as Bridge and Cobas positive. One was Bridge positive only, clinically in remission. Of the remaining discordant samples, 18 were TSAb positive, four of which were new diagnoses and untreated; all Bridge negative, one Cobas positive. Eight TSAb positive were on low dose methimazole, but only in two was Cobas positive and in one Bridge positive. Five TSAb positive were clinically in remission, one only Bridge positive. The two immunoassays highly correlated in GD patients ( $r=0.8$ ,  $P<0.001$ ), with a lower correlation between the TSAb bioassay and the Bridge- ( $r=0.49$ ,  $P<0.001$ ) or Cobas ( $r=0.52$ ,  $P<0.001$ ) assays. In 27 patients with Hashimoto's thyroiditis (HT), four were TSAb positive (15%), of which three and one were Bridge and Cobas positive, respectively. In 160 patients with autoimmune thyroid disease (AITD), TBAb were present in 32 (20%) HT 11/27 (41%) and GD 21/133 (16%). Thirty (94%) and 28/32 (88%) of the TBAb positive samples were also Cobas and Bridge assay positive but TSAb bioassay negative.

**Conclusions**

Both in newly diagnosed GD and patients on low dose methimazole, substantial differences in sensitivities were observed among the TSHR-Ab assays. Clinically, this may affect initial diagnosis and decisions to discontinue anti-thyroid drugs. In patients with AITD and TSAb negative, both Bridge and Cobas immunoassays were positive in the presence of blocking TSHR-Ab measured in a TBAb bioassay.

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**Oral Session 6: Hypothyroidism****OP-06-27****Evaluation of dried blood spot thyroglobulin as a biomarker for iodine status in pregnant women and newborns**Camilo Fuentes Peña<sup>1</sup>, Claudia Riedel Soria<sup>2</sup> & Rodrigo Moreno-Reyes<sup>3</sup>

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**Background**

Thyroglobulin (Tg) is a promising biomarker for monitoring iodine nutrition in children and pregnant women. The determination of Tg in dried blood spots (DBS) offers many logistical advantages, simplifying the collection, transport, and storage of samples. The present study aimed to validate an ELISA assay for Tg determination in newborns.

**Methods**

Tg-DBS samples were measured in 196 newborns born from healthy pregnant women. The different criteria for validation, such as sensitivity, linearity, and

intra- inter-assay coefficients of variability, were determined with the appropriate standards and quality control materials. We also assessed the effect of hematocrit on DBS-Tg measurements. DBS-Tg stability was assessed at different storage conditions over 12 months. Finally, we studied the effect of anticoagulants heparin and EDTA on the analytics performances of the assay. Clinical performances were confirmed by comparing Tg values using the Bland-Altman plot and Passing-Bablok regression.

#### Results

The limit of detection (LoD) of the DBS-Tg ELISA assay was 2.41 µg/l, and the limit of quantification (LoQ) was 5.62 µg/l. The intra- and inter-assay variability was 6.9%-10.2% and 9.9%-19.6%, respectively. The DBS-Tg values were linear, with a  $r > 0.99$  ( $P < 0.001$ ). Cord Blood samples with different hematocrit values ranging between 39% to 65% were obtained. DBS-Tg concentrations decrease with increasing hematocrit values. DBS-Tg from healthy adults was stable at a concentration of 25 µg/l over 12 months of storage at -20°C and 4°C. The samples collected with heparin showed a better correlation compared to EDTA. The correlation between Plasma-Tg Immulite 2000 (siemens) and DBS-Tg ELISA assay was good ( $n=91$ ;  $r = 0.71$ ,  $P < 0.0001$ ). The agreement analysis showed proportional but not constant differences between the two methods.

#### Conclusion

The results obtained show the validity of the Elisa Tg DBS test in neonates over a wide range of Tg concentrations, making its use in population studies possible.

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## OP-06-28

### The optimal ranges of thyroid function based on the risk of cardiovascular disease and mortality: an individual participant data meta-analysis

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#### Background and Objective

Thyroid function reference ranges are statistically defined by the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles, which do not account for potential risk of clinical outcomes. We aimed to define the optimal ranges of thyroid-stimulating hormone (TSH) and free thyroxine (FT4), based on the risk of cardiovascular disease (CVD) and mortality.

#### Methods

We performed an individual-participant data analysis and identified prospective cohorts with baseline TSH and FT4 concentrations as well as CVD outcomes and mortality through the Thyroid Studies Collaboration, supplemented with a systematic literature search. The primary outcome was a composite of incident CVD (coronary heart disease, stroke, heart failure), CVD mortality and all-cause mortality, while secondary analysis assessed these outcomes separately. In a one-step approach, we analyzed TSH and FT4 in percentiles, using cohort-stratified Cox proportional hazard models or cause-specific hazard models with nonlinear associations assessed by restricted cubic splines. For the two-step analysis, we used thyroid function quintiles and pooled estimates from each cohort using a random-effects model. All analyses were adjusted for age and sex, and further adjusted for cardiovascular risk factors (e.g. diabetes), and stratified by age (< 70, ≥ 70 years) and sex.

#### Results

We included individual participant data on 123,892 participants from 26 cohorts (51.5% women median age of 60 years, median follow-up of 10.3 years). TSH was not associated with CVD events ( $P=0.18$ ) and only marginally associated with the composite outcome ( $P=0.02$ ) with individuals in the lowest quintile had a hazard ratio (HR) of 1.04 (0.99-1.08) compared to those in the third quintile. In contrast, FT4 was nonlinearly associated with the composite outcome as well as secondary outcomes in a J-shape pattern (all  $P < 0.001$ ). Participants with FT4 in the second quintile (i.e. 20<sup>th</sup>-40<sup>th</sup> percentiles) had the lowest risk of composite outcome while individuals with FT4 in the highest quintile had a HR of 1.18 (95%CI 1.08-1.29). Overall, compared to men and younger individuals, the FT4 concentrations with the lowest risk of the aforementioned outcomes were lower in women and older individuals. In individuals aged ≥ 70 years, ten-year absolute risk estimates of composite outcome increased more than 5% for women with

FT4 concentrations >85<sup>th</sup> percentile while 5% and 10% for men with FT4 concentrations >75<sup>th</sup> and >90<sup>th</sup> percentiles respectively.

#### Conclusion

Overall TSH was not associated with CVD events. FT4 between the 20<sup>th</sup> and 40<sup>th</sup> percentiles represented the optimal health ranges based on the risk of cardiovascular disease and mortality, which has potential implications for treatment targets when managing clinical and subclinical thyroid disease.

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### OP-06-29

#### Central hypothyroidism ensuing endonasal transsphenoidal pituitary surgery: the role of a post operative trh stimulation test

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#### Objectives

The goal of this study was to evaluate the value of a TRH stimulation test to assess the risk of permanent central hypothyroidism after transsphenoidal pituitary surgery.

#### Methods

A retrospective cohort study was performed, evaluating the results of a routinely performed TRH stimulation test executed in the first 2 weeks after transsphenoidal pituitary surgery in patients admitted to the Ghent University Hospital between 1/1/2010 and 31/12/2019. The presence of central hypothyroidism was evaluated 6-18 months post-surgery by extracting the diagnosis from the patient file, combined with double checking based on the presence of T4 substitution therapy and corresponding thyroid function tests within this timeframe.

#### Results

After exclusion of patients with central hyperthyroidism and patients who received additional radiotherapy within the first 18 months after the surgery, 116 patients were included, 18 of whom developed permanent central hypothyroidism. Permanent postoperative central hypothyroidism was significantly associated with a low TSH at the start of the TRH stimulation test, as well as with a low maximum TSH and a low maximum TSH increase from baseline after TRH administration. A peak TSH of <2.0 mU/l during the test had a sensitivity of 100% and a specificity of 87.2% to identify patients at risk for permanent central hypothyroidism.

#### Conclusions

Our data support the use of a postoperative TRH stimulation test with a cut-off value of 2.0mU/l as maximum reached TSH value during the test as a screening tool to identify patients at risk of permanent central hypothyroidism after transsphenoidal pituitary surgery.

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### OP-06-30

#### Levothyroxine replacement therapy overuse and factors guiding successful treatment discontinuation: short and long-term observation data of a large cohort

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#### Background

Levothyroxine (LT4Rx) is one of the most prescribed drugs worldwide the vast majority of patients receive long-term treatment. However, in a recent study of 291 subjects, it was found that 60% were euthyroid 2 months after LT4-Rx discontinuation<sup>1</sup>.

#### Aim of the study

A prospective clinical cohort follow-up study was carried out. In 688 subjects (82% females) aged 48.01 ± 15.96 (range 17-84years) with 8.59 ± 6.98 years on LT4Rx without a solid diagnosis of hypothyroidism, treatment was abruptly interrupted. The indications for treatment were nodule(s) (33%), undefined (27%),

post-partum (7%) and Hashimoto's thyroiditis (33%). A short period of follow-up was initiated in 54% of subjects (≤ 4 months, Group A) and long-term follow-up in the rest (up to 60 months, Group B). The subjects were evaluated when LT4Rx was discontinued, 2-4 months later, and at the end of follow-up. At each time point, estimation of TSH, FT4 levels, and thyroid ultrasound was performed. A TSH value of ≥4.5IU/ml was considered as underlying hypothyroidism.

#### Results

Among the entire cohort, 158 subjects became hypothyroid, while the remaining 530 remained euthyroid off LT4-Rx (23 vs. 77%,  $P < 0.001$ ). On subgroup analysis, 40% of subjects comprising Group A became hypothyroid, whereas the corresponding value for Group B was 3%. In Group A, the rationale for LT4Rx, LT4 dose, LT4 dose/BMI, TSH levels, and the existence of thyroid autoantibodies (ATA) were significantly different in those who became hypothyroid. No difference among any parameters evaluated was observed in Group B. Subjects with a diagnosis of Hashimoto's thyroiditis, positive ATA, higher TSH values, and higher LT4 dose were significantly more likely to become hypothyroid. Furthermore, in Group A, 15.4% became hypothyroid with baseline TSH > 3IU/ml vs. 5.4% with baseline TSH < 3IU/ml ( $P < 0.001$ ); the corresponding values for Group B were 44.4% vs. 10.0%, ( $P < 0.001$ ), respectively.

#### Conclusions

These findings suggest considerable overuse of thyroxine administration. In cases of uncertainty, the existence of nodules, low-normal TSH level a relatively small T4 dose, and absence of ATA are strong indicators of euthyroid patients' overuse of the LT4Rx and, accordingly, treatment discontinuation is strongly advised. Furthermore, in the case that a subject does not become hypothyroid 2-4 months post treatment discontinuation, the likelihood of developing hypothyroidism long-term is insignificant.

#### Reference

1. Livadas S, *et al.* Thyroid 2018.

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### OP-06-31

#### Does subclinical hypothyroidism add on any symptoms? evidence from a danish population-based study

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#### Background

Few studies have scrutinized the spectrum of symptoms in subclinical hypothyroidism.

#### Methods

From three DanThyr cross-sectional surveys performed in the period 1997 to 2005, a total of 8,903 volunteers participated in a comprehensive investigation including blood samples and questionnaires on previous diseases, smoking habits, alcohol intake, and education. From the three surveys we included patients with unknown subclinical hypothyroidism ( $n = 376$ ) and euthyroid controls ( $n = 7,619$ ). For further comparison we also added patients newly diagnosed with autoimmune overt hypothyroidism ( $n = 140$ ) recruited from a previous study. We explored to which extent patients with subclinical hypothyroidism reported 13 previously identified hypothyroidism-associated symptoms (tiredness, dry skin, mood lability, constipation, palpitations, restlessness, shortness of breath, wheezing, globulus sensation, difficulty swallowing, hair loss, dizziness/vertigo, and anterior neck pain). In various uni- and multivariate regression models we searched for circumstances predicting why some patients have more complaints than others.

#### Results

Subclinically hypothyroid patients did not report higher hypothyroidism score ((median, IQR), 2 (0-4) vs. 2 (0-4),  $P = 0.25$ ) compared to euthyroid controls. Within the group of subclinical hypothyroid patients: co-morbidity had the highest impact on symptoms (tiredness, shortness of breath, wheezing; all  $P < 0.001$ ); TSH level had no impact on symptom score; low age was accompanied

with higher mental burden (tiredness,  $P < 0.001$ ; mood lability,  $P < 0.001$ ; restlessness,  $P = 0.012$ ), whereas shortness of breath was associated with high BMI ( $P < 0.001$ ) and smoking ( $P = 0.007$ ).

#### Conclusion

Patients with a thyroid function test suggesting subclinical hypothyroidism do not express thyroid disease related symptoms more often than euthyroid subjects. In subclinical hypothyroidism, clinicians should focus on undiagnosed co-morbidity and should optimize treatment of known concomitant diseases rather than expecting symptomatic relief following levo-thyroxine substitution.

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## Oral Session 7: Thyroid Cancer Basic

### OP-07-32

#### The micro-rna content of extracellular vesicles in papillary thyroid cancer: from identification in mouse thyroid tumour to detection in patient plasma

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#### Background

Papillary thyroid carcinoma (PTC) is the most frequent subtype of thyroid cancers. Despite good prognosis in most cases, postsurgery recurrences and metastases can occur. Moreover, differential diagnosis between benign and malignant nodules is still challenging. Gaining knowledge about extracellular vesicles (EVs) in PTC could have a double benefit: a better understanding of PTC clinical behaviour, and the discovery of accessible (and accurate) diagnostic tools.

#### Aims

The goals of this project are to (i) identify miRNAs actors and markers, released via EVs by the tumor, (ii) decipher the mechanisms by which they impact thyroid cancer and its microenvironment, and (iii) evaluate the diagnostic value of circulating miRNAs.

#### Methods and results

Using a mouse model mimicking human PTC, we isolated EVs from dissociated control- and early and late PTC-tissue by differential ultracentrifugations. Vesicles in the high-speed pellet were characterized in-depth and sequencing was performed to identify tumor-derived EV-miRNAs. We focused on 4 miRNAs differentially more abundant in EVs from PTC tissues. *In silico* analysis revealed their enrichment in immune-related pathways, consistently with the massive recruitment of macrophages observed in our model. We investigated the distribution of EV-miRNAs according to their cellular source. Using the Nanoview technique, we showed that the number of EVs bearing epithelial and immune markers was increased in PTC tissues. The 4 miRNAs were mostly expressed, and deregulated, in epithelial cells. We thus propose that their increased abundance in epithelial-EVs could affect the immune microenvironment. In parallel, the miRNAs candidates were quantified in tissues, in plasma and in plasma-EVs from patients treated for thyroid diseases. Two miRNAs, miR-146b-5p and miR-21a-5p were more abundant in tissue and plasma-EVs isolated from patients with PTC, as compared to benign diseases.

#### Conclusions

We provided a gradual tissue- and EV-miRNAs profiling during PTC development which allowed the identification of EV-miRNAs that could (i) support the establishment of a permissive microenvironment for tumor development and (ii) contribute to thyroid cancer diagnosis.

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### OP-07-33

#### Hürthle cell tumors vs oncocytic variants of the follicular cell derived thyroid tumours: a comprehensive analysis based in transcriptome, proteome and cnv profiling

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#### Objectives

Hürthle cell (oncocytic) lesions can be metaplastic or neoplastic events. The neoplastic entities -formerly, oncocytic variant of follicular tumours; Hürthle cell neoplasm (HCN) - include HC adenomas and HC carcinomas and have been recognized as a separate class of tumours by the WHO. The remaining of the thyroid tumours demonstrating "mitochondrion-rich cells" and oncocytic morphology are referred under the umbrella term of "oncocytic variant". However, this rigid separation between the two groups of tumors, characterized by the presence of a mitochondrial-rich cytoplasm, is not clarified, and is not universally accepted. This brings the question if the "oncocytic cells" are different from "mitochondrion-rich cells", or if they represent a sort of continuum? Thus, we conducted a comparative transcriptomics, proteomic and CNV analysis in the group of HCNs vs mitochondrial-rich non-Hürthle thyroid tumours (other thyroid tumours demonstrating oncocytic morphology).

#### Methods

Eighteen thyroid tissue samples obtained from the biobank of the pathology department. The cohort of 12 HCN (7 HCC, 5 HCA) and 6 non-HCNs (2 OV-PTC, 3 WT-UMP, 1 NIFTP with oncocytic morphology). We have compared the groups HCNs vs non-HCNs. RNA was sequenced using Ion AmpliSeq Transcriptome Humane Gene Expression Kit and analysed using Transcriptome Analysis Console Software. For proteomic analysis, liquid Chromatography Analysis/Mass Spectrometry were done by using the software Proteome Discoverer 2.4.0.305. Shallow whole-genome sequencing of DNA samples was performed using Illumina platform. BWA was used to map sequencing reads to human reference. Then QDNaseq was used to access the CNV among the genome regions. Enrichment analyses was performed for RNA, proteomic and CNV, with package *enrichGO* and with annotation of the *org.Hs.eg.db* package. For all plots, R and the package "ggplot2" were used. Statistical analyses were performed also using R, in particular the nonparametric Wilcoxon rank-sum test.

#### Results

The mitochondrial translation system related pathways for RNA sequencing, extracellular matrix related, metabolic process and Immune response related pathways, for proteomics and plasticity related pathways for CNV analysis were found to be significantly increased in the HCN. HCN display, a higher number of gains (chromosomes 2, 4 and 12,  $P < 0.0001 - 0.004$ ) and lower number of losses (chromosome 3,  $P < 0.001 - 0.004$ ) when compared with the non-Hürthle group.

#### Conclusions

Our results revealed some significant differences between the two groups in the omics platform and in the CNV analyses. Our results suggest that HCNs vs mitochondrial-rich non-Hürthle thyroid tumours may be just "different phases" of a spectrum that ends with the Hürthle cell phenotype.

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### OP-07-34

#### CDK4 phosphorylation status and rational use of CDK4/6 inhibitors in advanced thyroid cancers

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## Introduction and objectives

The cyclin-dependent kinases CDK4 and CDK6 are key regulators of the cell cycle entry, by phosphorylating the onco-suppressor retinoblastoma protein (pRb). CDK4/6 inhibitors (CDK4i) are already established as standard first-line treatment against advanced Estrogen Receptor-positive breast tumors, and have emerged as novel drugs to treat various pRb-proficient chemotherapy-resistant cancers. Presence of activating T172-phosphorylation of CDK4 in breast tumors correlates with their sensitivity to the CDK4i palbociclib (Raspé E, *et al.* EMBO Mol Med. 2017; 9,1052-1066). The molecular characterization of metastatic differentiated (DTC), poorly differentiated (PDTC) and anaplastic thyroid carcinomas (ATC) suggests that CDK4i could be considered for treating advanced thyroid cancers. We aimed to investigate the CDK4 activation state in thyroid cancer and its relationship with the sensitivity to CDK4i.

## Methods and results

Sensitivity to three CDK4i was assessed (by BrDU incorporation and viability assays) in 11 ATC-, 2 PDTC- and 7 WDTC-derived cell lines. All except 3 cell lines were sensitive to CDK4i with either full or partial inhibition of DNA synthesis. CDK4 post-translational modifications were investigated using 2D-gel electrophoresis. As seen previously in breast cancer, detection of CDK4 T172-phosphorylation also predicted sensitivity to CDK4i in thyroid cancer cell lines. The three resistant cell lines were characterized by barely detectable pRb phosphorylation and high expression of CDK4 inhibitor p16, whereas in all sensitive cell lines, phosphorylated pRb was detected. A cohort of fresh-frozen primary tissues was also analyzed by 2D-gel electrophoresis. Consistent with their quiescent state, phosphorylated CDK4 could not be detected in 14 of 17 non-malignant thyroid tissues. CDK4 phosphorylation was detected in 29 of 32 DTC, in 8 lymph node metastases, in 17 of 19 PDTC and in 12 of 20 ATC. Analysis by RNA-sequencing revealed that in comparison to tumors with CDK4 phosphorylation, tumors without phosphorylated CDK4 presented lower pRb levels and the highest p16 levels. However, no pRb mutations were found in these samples. Palbociclib combination with MEK/BRAF inhibitors as evaluated by clonogenic assay was highly effective, being able to completely arrest proliferation. The combined drugs were shown to prevent known resistance mechanisms, most notably Cyclin E-CDK2 activation, as observed by immunoprecipitation assays.

## Conclusion

The presence of the phosphorylated CDK4 (the actual CDK4i target) and the inhibition of all ATC cancer cell lines support CDK4i as a very promising option to treat or control at least some ATC, which presently are incurable and lead to patients death within few months.

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## OP-07-35

**RET fusion genes in a large cohort of papillary thyroid carcinomas**

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## Objectives

RET fusion genes are known driver mutations in papillary thyroid carcinomas (PTCs) and have been described mainly in pediatric PTCs, in which they represent the most common genetic alteration. The aims of this study were to identify RET fusion genes in PTCs (from pediatric as well as adult patients), to correlate them with clinical and histopathological features and to determine the prognostic significance of RET fusion genes based on long-term follow-up of patients with PTC harboring this mutation.

## Methods

The cohort consisted of 920 PTC samples (fresh frozen tissues) from pediatric and adult patients. Based on the detected mutation, samples were triaged. Samples positive for the BRAF, HRAS, KRAS, NRAS or NTRK fusion gene mutations were excluded from the further RET fusion gene analyses. Samples were analyzed for the presence of RET fusion genes using Real-Time PCR (LC480, Roche) or using the FusionPlex Comprehensive Thyroid and Lung panel (ArcherDx) by next generation sequencing (MiSeq, Illumina).

## Results

RET fusion genes were detected in 108 (11.7%) PTCs, from which 34/121 (28.1%) were from pediatric and adolescent patients (7-20 years old) and 74/799 (9.3%) were from adult patients. The mean age of diagnosis was 33.0 ± 17.1 years. A total of 20 types of RET fusions were found, including the following partner genes: CCDC6, NCOA4, PRKARIA, SOSTM1, IKBKG, RASAL2, TPR, ACBD5, RUFY2, BBIP1, AFAP1L2, AKAP13, TRIM27, SPECCIL, FBXO41, GOLGA5, SSBP2, ZMYM2, ERC1, KIAA1217. The RET fusion-positive carcinomas were associated with infiltrative tumor growth, numerous intrathyroidal micrometastases, psammoma bodies, lymph node and distant metastases. Lymph node metastases were found in 31/34 (91.2%) pediatric cases and in 52/71 (73.2%) adult patients. Distant metastases were identified in 9/34 (26.5%) pediatric patients and 11/69 (15.9%) adult patients. Patients responded well to radioiodine treatment, radioiodine-refractory PTCs harboring RET fusions were rare. Three patients (2.8%) died of the disease, in two cases was carcinoma positive for NCOA4/RET and in one case for CCDC6/RET with a TERT C250T co-alteration.

## Conclusion

In summary, RET fusion genes are an important genetic marker in PTCs associated with aggressive tumor behavior and frequent metastases. RET fusions occurred approximately three times more frequently in pediatric and adolescent patients, in which carcinomas were more advanced, than in adult patients. In conclusion, the genetic molecular testing of RET fusions is important for patient's diagnosis and prognosis and also for possible targeted therapy.

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## OP-07-36

**Loss of tumor cell MHC class ii expression as driver of relapse to dabrafenib and trametinib in mouse BRAF-mutant anaplastic thyroid cancer**

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## Objectives

BRAF<sup>V600E</sup>-anaplastic thyroid cancers (ATCs) show remarkable responses to dabrafenib and trametinib (dab/tram) an effect that may be in part immune-mediated. Murine BRAF<sup>V600E</sup>-ATCs regress upon BRAF inhibition. We find that recurrences are frequent and associated with loss of Mhc class II (MhcII) expression. Our goal was to investigate the mechanisms of loss of antigen presentation by tumor cells and whether this contributes to disease recurrence.

## Methods

We developed primary ATC cell lines from mouse dox-inducible BraF-ATCs (TPO-Cre/LSL-rtTA\_GFP/tetO-mycBRAF<sup>V600E</sup>/p53<sup>fl/fl</sup> (BRAF/p53) as well as ATC lines derived from recurrent tumors arising in mice after dox-withdrawal. A syngeneic orthotopic model with BraF<sup>V600E</sup>/Tp53<sup>-/-</sup> (TBP3743) ATC cells was developed. RNAseq of BRAF/p53 ATC cells from mice treated with or without dox and from dab/tram or vehicle treated TBP3743 ATCs was performed.

Primary and recurrent cell lines were treated with vehicle, interferon  $\gamma$  (IFN $\gamma$ ), tram or IFN $\gamma$ +tram and analyzed by FACS, RT-PCR and western blotting.

#### Results

Dox withdrawal or dab/tram treatment resulted in ATC infiltration by CD4<sup>+</sup> T-helper cells and increased MhcII expression in tumor cells. RNAseq of ATC cells showed activation of IFN $\gamma$  transcriptional output and of the antigen presentation pathway (MhcII > MhcI). IFN $\gamma$  induced MhcII expression in primary cell lines only upon MEK inhibition with trametinib but this was ineffective in recurrent cell lines. Loss of induction of MhcII in recurrences was not due to incomplete ERK pathway inhibition or to interference with upstream IFN $\gamma$  signaling. Recurrent cell lines had markedly attenuated basal and IFN $\gamma$  induced expression of *Ciita*, the master transcriptional regulator of genes in the MhcII signaling pathway. We developed homozygous CRISPR KO of *Ciita* in TBP3743 cells to study the impact of *Ciita* loss *in vivo*. *Ciita* KO cells lost expression of MhcII *in vitro* and *in vivo*, which did not impact tumor growth but rendered the *Ciita*<sup>-/-</sup> TBP3743 cells completely refractory to dab/tram therapy.

#### Conclusions

- IFN $\gamma$  induction of MhcII expression requires MEK inhibition in primary BRAF<sup>V600E</sup>-ATC cells but the combination fails to induce MhcII in recurrences.
- Absence of MhcII expression is associated with attenuated *Ciita* expression.
- In *Ciita* Crispr KO TBP3743 ATC cells MhcII expression is absent and *Ciita*<sup>-/-</sup> ATCs are resistant to dab/tram *in vivo*.

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## Oral Session 8: Basic 2

### OP-08-37

#### Resistance to thyroid hormone alpha: molecular, biochemical and physiological approach to diagnosis and therapy

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#### Objectives

*THRA* mutations cause Resistance to Thyroid Hormone  $\alpha$  (RTH $\alpha$ ), an underdiagnosed disorder with hypothyroid features but near-normal thyroid function tests (TFTs). We developed a pathway, combining molecular analyses, new biomarkers and physiological measurements, to better diagnose and treat this disorder.

#### Methods

Structural and functional analyses of *THRA* variants, discovered by next generation sequencing in specific projects (eg 100K Genome, Deciphering Developmental Delay, Genetics of Obesity study) or unbiased investigation of patients, identified an RTH $\alpha$  cohort ( $n=32$ ). In this cohort, we measured plasma metabolites or proteins and analysed facial images using artificial intelligence (AI) to differentiate RTH $\alpha$  from controls. We measured resting energy expenditure (REE) during thyroxine therapy of the disorder.

#### Results

17 different, heterozygous *THRA* variants, in individuals investigated for diverse causes (growth retardation, developmental delay, autism, dysmorphic facies) localised to the hormone binding domain of TR $\alpha$ 1, with 14 being homologous to *THRB* mutations causing RTH $\beta$ . Varying transcriptional impairment or morphological and skeletal abnormalities when variants were expressed in mammalian cells or developing zebrafish and reduced *KLF9* expression in variant-containing, patients' blood cells, led to their classification as pathogenic. 12 novel TR $\alpha$  mutations (R228C, R266L, D268N,  $\Delta$ 268-272, T275M, G278R, V282L, L287P, I299T, H381Q, P399S, L400Tfs\*) were identified. Mutations occurred *de novo* in 20/32 patients, including at a mutation hotspot (G291S) in five, unrelated, cases. With TFTs being near-normal (concentrations in reference range: TSH 100%; FT4 85%; RT3 70%; FT3 50%) in patients, 'omics technologies identified plasma metabolites or proteins, whose relative levels distinguish RTH $\alpha$  cases from controls with 95% accuracy. Validating this, plasma concentrations of the most important metabolites and protein differed significantly (RTH $\alpha$  vs Controls: Asymmetric dimethylamine, ( $P=3.7E-05$ ), pregnenolone sulphate ( $P=1.98E-07$ ), or Factor XIII ( $P=1.1E-11$ )). AI-guided scores of facial features in RTH $\alpha$  cases and controls differed significantly, generating a classifier with a receiver operating characteristic of 0.966. Thyroxine therapy, in TSH-suppressive dosage, raised REE from low ( $Z$  scores  $-3.58$  to  $-0.02$ ) to higher levels.

#### Conclusions

*In silico* analyses of *THRA* variants of unknown significance, identifies TR $\alpha$  mutations whose loss-of-function is confirmed using transcriptional, zebrafish model and patient cell-based assays. New biomarker and AI-guided dysmorphic feature analyses in individuals with mutant genotypes diagnoses RTH $\alpha$ , enabling thyroxine therapy to correct subnormal energy expenditure.

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## OP-08-38

**Brain effects of combined levothyroxine (T4) and 3-iodothyronamine (TIAM) replacement therapy in a murine model of hypothyroidism**

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Adult-onset hypothyroidism is associated with cognitive dysfunction, and a significant fraction of hypothyroid patients still shows persistent disturbances despite standard T4 replacement therapy. Experimental results showed that tissue levels of T4 metabolites, particularly TIAM, are decreased in hypothyroidism and remain low after T4 replacement. Thyroid hormones are important for the promotion of neurogenesis and neuron migration to different areas of the cerebral cortex, including hippocampus, and it is known that hippocampus-dependent memory is impaired in the hypothyroid mice. We aimed to evaluate the effects of hypothyroidism and different replacement treatments on neurogenesis in the subgranular zone of the dentate gyrus (SGZ). Six-week-old C57BL/6J male mice were given methimazole and potassium perchlorate (0.20 mg/g/die and 0.30 mg/g/die) in drinking water for 49 days while the control littermates received water. At day 21 mice were implanted with subcutaneous ALZET® osmotic pumps delivering replacement treatments for 28 days. Animals were divided in 4 groups: euthyroid; hypothyroid; hypothyroid treated with T4 (0.04 µg/g BW/die); hypothyroid treated with T4 + TIAM (0.04 µg T4 & 0.004 µg TIAM/g BW/die). Specific markers were used to quantitate cell proliferation (Ki67) and the presence of neuroblasts/immature neurons (doublecortin-DCX) through immunofluorescence analysis performed in the SGZ. Then, following hippocampal RNA isolation, we analysed gene targets involved in neurogenesis pathway using a PrimePCR pre designed 96-well collection panel (Bio-Rad). A 49-day period of adult-onset hypothyroidism induced a reduction of around 20% in the number of DCX positive newly generated cells. Compared to hypothyroidism, T4 treatment increased the number of cells by 45.58% (mean diff = -106.9;  $P < 0.05$ ) while the T4 + TIAM treatment produced a 58.20% increase (mean diff = -141.6;  $P < 0.01$ ). One-way ANOVA revealed a global significant effect among the 4 groups ( $P = 0.003$ ). Real time PCR highlighted significant changes in the expression of genes related to neurogenesis as ANOVA revealed an upregulation in the expression of *Ngf*, *Kdr*, *Ntf3*, *Mapk1/3* and *Neurog2* genes in T4 + TIAM treated mice compared to T4 treated mice ( $P < 0.05$ ). Our results indicate that cognitive dysfunctions related to adult-onset hypothyroidism could be due in part to impaired hippocampal neurogenesis. Cellular markers and gene expression analysis suggest the presence of underlying molecular mechanisms activated only by the combination of T4 and TIAM. Further investigations are required to elucidate the potential pathophysiological and clinical relevance of these findings.  
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## OP-08-39

**Stochastic epigenetic mutations as possible explanation for phenotypical discordance among twins with congenital hypothyroidism**

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Congenital Hypothyroidism (CH) is the most common congenital endocrine disease and avoidable cause of severe mental retardation. The CH pathogenesis may include the contribution of genetic and environmental factors. However, causal mutations have been found in a minority of cases. Moreover, the elevated frequency of discordance for CH phenotype between monozygotic (MZ) twins

suggests the involvement of non-Mendelian mechanisms. Aim of this study was to investigate the role of epigenetics in CH pathogenesis. We performed a genome-wide DNA methylation analysis in the peripheral whole blood of 23 twin pairs (10 monozygotic and 13 dizygotic), of whom 4 concordant and 19 discordant for CH at birth, using the Illumina HumanMethylation450K BeadChip array. Differential methylation analysis failed to identify differences in methylation levels between cases and controls. In order to detect rare epigenetic differences not shared among subjects, we then analyzed the distribution of Stochastic Epigenetic Mutations (SEMs). Interestingly, the median number of hypomethylated SEMs resulted significantly increased in cases compared to controls, independently from the zygosity, the thyroid morphology, the CH outcome or the genetic background. The prioritization analysis for CH performed on the genes that were epimutated exclusively in cases identified SLC26A4, FOXI1, NKX2-5 and TSHB as the genes with the highest score. Furthermore, the analysis of significantly SEM-enriched region led to the identification of two genes (FAM50B and MEG8) that were epigenetically dysregulated in cases. Collectively our data indicate that epigenetic modifications are rare events in CH pathology. However, a significant increase of hypomethylated SEMs was identified in hypothyroid twin pairs, suggesting that thyroid defects could be caused by an increased expression of predisposing factors. The role of genes that resulted epigenetically dysregulated in CH cases should be further investigated.  
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## OP-08-40

**Disrupted trans-placental thyroid hormone transport in a human model for MCT8 deficiency**

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## Objectives

During prenatal neurodevelopment, maternal-to-fetal thyroxine (T4) transfer is critical, particularly during the first half of pregnancy when the fetal thyroid gland is immature. Transcellular transport of thyroid hormones (TH) is facilitated by TH transporters. Monocarboxylate transporter 8 (MCT8) is a specific TH transporter that is crucial for transport of TH with a prominent expression at the blood-brain barrier. MCT8 deficiency is a rare disorder consisting of severe intellectual and motor disability and abnormal thyroid function tests. The transporter facilitating trans-placental TH transport is unknown. With the blood-brain barrier maturing around 18 weeks, the placental barrier may be as relevant as the blood-brain barrier for regulation of TH bioavailability for the fetal brain. We hypothesized that, should MCT8 be relevant in the placenta (a fetal-derived barrier), defective trans-placental transport of TH in MCT8 deficiency could be another additive mechanism of the disease, which might be overcome by the 3,3',5-triiodothyronine (T3) analogue 3,3',5-triiodothyroacetic acid (TRIAC).

## Methods

We tested T4 transport in human term placentas using an *ex vivo* placental perfusion model. We added 10 µM silychristin (a MCT8-specific inhibitor) to

mimic MCT8 deficiency or vehicle as control, together with 100 nM T4 in the maternal circulation of the perfusion system. Samples were taken from both maternal and fetal circulations at different time points during a 3-hour perfusion. Next, we tested whether 100 nM TRIAC was able to cross silychristin-treated ("MCT8-deficient") placentas. T4 and reverse T3 (rT3) concentrations in perfusates were measured by radioimmunoassays. TRIAC concentrations were measured by liquid chromatography-mass spectrometry (LC-MS)/MS.

#### Results

Maternal-to-fetal T4 transfer was substantially reduced in the presence of silychristin (with  $4.2 \pm 1.2$  nM vs  $10.6 \pm 0.6$  nM (control) ( $P < 0.01$ ) fetal T4 after 3h-perfusion). TRIAC maternal-to-fetal transfer was achieved with TRIAC appearing in the fetal circulation ( $0$  nM to  $17.1 \pm 2.5$  nM after 3h-perfusion).

#### Conclusions

MCT8 has a major role in maternal-to-fetal T4 transport. Possibly, impaired transport of T4 across the placenta in MCT8 deficiency is a key element in disturbing early fetal brain development. TRIAC is efficiently transported across the placenta and may provide preclinical support for future clinical studies of TRIAC treatment in mothers carrying fetuses with MCT8 deficiency.

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## OP-08-41

### BOREALIN/CDC48 is necessary for an adequate thyroid morphogenesis and aging

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#### Background

Previously, we identified a novel gene, *BOREALIN/CDC48* in congenital hypothyroidism. Patients with *BOREALIN* mutations had thyroid dysgenesis, from asymmetric lobes to athyreosis (Carré *et al. Hum Mol Genet* 2017). Borealin is a major component of the Chromosomal Passenger Complex, an essential regulator of mitosis. We demonstrated a new feature of *BOREALIN*: involvement in the adhesion and the migration of the thyrocytes.

#### Objective

Further understand the role of Borealin in thyroid development and function.

#### Methods

*Borealin* +/- mice were studied during development, at 4 and 18 months. *Borealin*-/- mice were not available because they die at E5.5. We documented thyroid morphology, performed immunohistology with thyroid markers (Nkx2-1, Thyroglobulin, T4) and we analyzed the thyroid function. We used a well-established model with antithyroid drug induced hypothyroidism which was applied to the *Borealin* +/- and wild-type mice.

#### Results

First of all, *Borealin* +/- mice did not develop hypothyroidism at the adult stage (4-month-old) but they were significantly more sensitive to antithyroid drugs with a more profound hypothyroidism (T4: 41% less for *Borealin* +/- vs wild-type,  $P < 0.01$ ). Four months-old *Borealin* +/- thyroids were significantly more hyperplastic with larger follicles surfaces in comparison with wild-type thyroids ( $P < 0.05$ ). Thus, the *Borealin* +/- mice remain euthyroid at the expense of developing goiters. For elder mice, thyroid morphology of *Borealin* +/- was altered with heterogeneity in size of follicles with predominantly very large follicles and thyroids significantly more hyperplastic compared with wild-type ( $0.34$  mg/g thyroid weight/animal weight vs  $0.23$  mg/g,  $P < 0.05$ ). We found that thyroids of *Borealin* +/- were significantly hyperplastic at E9.5 in comparison with wild-type, and hypoplastic from E11.5 to E17.5 ( $P < 0.05$ ). Thyroid development thus was abnormal in *Borealin* +/- compared to wild-type. In addition, transcriptome analysis of thyroids were performed at different stages. Specific pathways were disturbed in *Borealin* +/- thyroids at E13.5, at 4 and 18 months, mainly adhesion and motility pathways. At 18 months, *Borealin* +/-

thyroid are enriched in cytoskeleton, cell cycle and thyroid cancer gene sets compared to wild-type.

#### Conclusion

Borealin is involved in crucial steps of the thyroid lifetime cycle. These data demonstrate the involvement of Borealin in the structural organization of the thyroid gland and consolidate the role of Borealin in thyroid development and function, which supports its involvement in thyroid dysgenesis of patients with congenital hypothyroidism. Impaired Borealin function plays also a role in morphologic deregulation in thyroids along time.

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## Oral Session 9: Thyroid Cancer Clinical

### OP-09-42

#### Childhood papillary thyroid carcinoma: long-term postoperative outcome and prediction of recurrent disease in 189 patients consecutively treated at the mayo clinic during 1936 through 2020

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#### Objectives

Childhood papillary thyroid cancer (CPTC) often presents with advanced disease but rarely results in cause-specific mortality (CSM). Controversy remains regarding optimal management and association with non-thyroid second primary malignancies (NSPM). We analyzed outcome in 189 CPTC patients and assessed the influence of radioiodine remnant ablation (RRA) and the utility of the American Thyroid Association risk-groups (ATA-R) in predicting tumor recurrence (TR).

#### Methods

All patients were operated by specialist surgeons. 88% had bilateral thyroidectomy (BT); at first surgery 86% had neck nodes removed, 17% had pT4a tumors and 78% were pN1, 58% with 5 or more regional metastases (RM). During 1951-2020 RRA was given to 43% of TNM stage I patients having BT with curative intent. Mean follow-up was 29 yr (range 1-71). TR and CSM details were derived from a computerized database and analyzed as previously described (WJS 43: 329, 2018).

#### Results

During 1936-50, 9/19 died from cancer; one from PTC and 8 from NSPM (7 having received radiation therapy); 15 died from all causes, as compared to expected 6 ( $P < .001$ ). During 1951-2020 none of 170 patients died of PTC but 3 died from NSPM; 13 died from all causes, as compared to 13 expected ( $P = .97$ ). In 169 patients operated with curative intent 25-yr TR rate was 37%; 57 patients (34%) had recurrence: 81% RM and 19% distant metastases (DM). Patient ages < 11 and pT4a tumors were significantly ( $P < .05$ ) associated with postop RM; tumor size > 4 cm and pT4a tumors with postop DM ( $P < .002$ ). BT+RRA did not significantly improve the 20-yr TR rates of 28% and 3% seen with BT alone for RM ( $P = .75$ ) and DM ( $P = .99$ ). We applied the ATA-R to 167 patients who had no DM at presentation and had complete tumor excision. 61 were classed as ATA-low risk, 49 as intermediate and 57 as ATA high-risk. 25-yr TR rates for low, intermediate, and high-risk groups were 24%, 34% and 53% ( $P = 0.0015$ ). 25-yr rates for RNM and DM for the 3 risk groups were 19, 34 and 40% ( $P = 0.05$ ) and 0, 4 and 19% ( $P < .001$ ).

#### Conclusions

During the I<sup>131</sup> era (1951-2020), we have noted no excess all-causes mortality in CPTC but we have not found that RA reduces postop TR after BT. Given that 78% present with pN1 disease, persistent/recurrent disease within RNM may be expected. The ATA-R high-risk group seems capable of predicting DM; perhaps in future years many CPTC patients classed as low-risk may require less than BT.

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### OP-09-43

#### Novel somatic mutations in sporadic MTC (spMTC); clinical utility of NGS in precision medicine

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#### Objectives

Distant metastases in MTC adversely affect disease prognosis. Somatic mutations in spMTC as well as Variant Allele Frequency (VAF) have been related to tumor burden, disease course and the response to TKIs. The aim of this study is to report three cases of nucleotide (nt) insertions/deletions (indels) in the *RET* oncogene in relation to disease course/response to TKIs.

#### Methods

Of 195 spMTC patients followed-up in Dept. Clinical Therapeutics (Athens), 39 presented with persistent/metastatic disease. In 11/39 FFPEs were obtained. DNA was extracted and NGS libraries were prepared. In silico tools were used to estimate the pathogenic effect of novel mutations.

#### Results

8/11 patients-72.7%, harbored *RET* somatic mutations while 3/11-27.3% *HRAS*. In 3 female patients *RET*-indels were detected. **A:** 32 y.o, diagnosed in 2018, stage IVA. Disease progression two-years later (mediastinum); thoracic surgery was performed. PET-CT following surgery revealed persistent disease (pre-carinal LNs). Due to threatening of vital structures, Vandetanib was initiated. Despite disease stabilization SAEs were recorded. NGS: deletion of 54 nts (*RET*-exon-10, p.Ile590\_Gly607del, VAF: 36.34%), not previously reported. Therefore, treatment with Selpercatinib was initiated and a remarkable disease remission was documented. **B:** 80 y.o, diagnosed in 1990. Disease progression 20-years later, when the patient was referred to our Unit (metastases: cervical/mediastinal LNs, right breast-lump, liver, bones). LN-dissection, excision of the right breast-lump and local treatments, were performed. After a 12-months disease stabilization, enlargement in liver metastases was documented. Vandetanib was initiated achieving stabilization. NGS: deletion/insertion of 2 nts (*RET*-exon-11, p. [Leu633 =; Cys634Arg], VAF: 48.85%). **C:** 72 y.o, MTC diagnosis-2008, stage I. Disease progression two years later (cervical-LNs); LN-dissection was performed. During a 12yrs f-up, biochemical persistence (Calcitonin ~ 500 pg/ml) in the absence of metastases has been documented. NGS: insertion of 21 nts (*RET*-exon-11, p.Cys634\_Ala640dup, VAF: 8.79%), not previously reported.

#### Conclusions

Deletions in the cysteine-rich region of *RET* are related to disease aggressiveness. Molecular mechanisms possibly involved may be related to the shortening of crucial for the oncogenic activity *RET* protein cysteine residues and the formation of stronger disulfide bonds. NGS, beyond of providing pivotal information regarding disease progression and response to TKIs, contributes to the identification of novel mutations even of low VAF, thus being an indispensable tool in precision medicine implementation.

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#### OP-09-44

##### Dynamic risk stratification in long-term clinical outcome of papillary thyroid cancer patients

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#### Introduction and Objective

The initial assessment of differentiated thyroid cancer (DTC) patients is based on the American Thyroid Association (ATA) risk stratification criteria. Dynamic risk stratification (DRS) of DTC patients takes into consideration the response to initial treatment being reassessed at 1–2 years and revealed significant shifts in the

risk categories of DTC patients. We aimed to evaluate the long-term outcome of papillary thyroid cancer (PTC) patients according to the ATA risk class and DRS at the first follow-up.

#### Methods

We retrospectively evaluated 704 patients with low ( $n=372$ ) and intermediate ( $n=332$ ) risk PTC. At the first follow-up patients were divided in four subgroups according to the response to the initial therapy: excellent response (ER) 320/372 (86%), biochemical incomplete response (BIR) 31/372 (8.3%), indeterminate response (IR) 11/372 (3%), and structural disease (SD) 10/372 (2.7%) in low-risk group and ER 202/332 (60.8%), BIR 51/332 (15.4%), IR 23/332 (6.9%), SD 56/332 (16.9%) in intermediate-risk group.

#### Results

After a median follow-up of 9 years in the excellent subgroup of PTC patients, persistent remission was observed in 98.8% of low-risk patients and in 93.1% of intermediate-risk patients, while recurrent disease was observed in 1.2% of low-risk patients and in 6.9% of intermediate-risk patients ( $P=0.005$ ). The BIR patients showed an excellent response in 58% of cases in low-risk group and in 33.3% of cases in intermediate-risk group ( $P=0.038$ ). The rate of persistent disease (BIR+SD) was significantly higher ( $P=0.038$ ) in intermediate-risk patients than in low-risk patients (66.7% vs 42%, respectively) due to the higher rate of SD in intermediate-risk than in low-risk patients (15.7% vs 6.5%, respectively). All patients with IR had an excellent response at the last follow-up, regardless the initial ATA risk class. Finally, in patients with SD, after additional treatments, the rate of excellent response was only slightly not statistically significant between low and intermediate risk group (80% vs 46.4%, respectively) ( $P=0.08$ ).

#### Conclusions

The long-term outcome according to the dynamic risk stratification of PTC patients is still poorly characterized. In our study we found a better clinical outcome of low-risk PTC patients for each subgroup of response to initial therapy, suggesting that the initial ATA risk class should be taken into account in the long-term management of PTC patients.

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#### OP-09-45

##### The phenotype correlated with *RET* V804 germline mutation is characterized by the presence of medullary thyroid cancer alone

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#### Background

Genotype-phenotype correlations between various *RET* mutations and clinical manifestations of MEN 2 syndrome are well established. A discussion is still open if the FMTC phenotype really exists or if it is just a MEN2A variant. Aim of this study was to verify if the phenotype corresponding to the V804M germline mutation is restricted to FMTC.

#### Methods

During the last 25 years, we have identified 200 families with a hereditary form of MTC and 993 subjects have been studied for the presence of a *RET* germline mutation. Among these families, 43 had a Cys634 mutation, 32 had a Cys mutation in exon 10, 15 had the M918T mutation and 110 cases had a *RET* mutation at non cysteine codons. Among these latter 54 families had a V804M/I germline mutation. All patients were annually submitted to clinical and biochemical examinations (e.g., abdomen ultrasound, plasma and urinary

epinephrine and norepinephrine, serum parathyroid hormone, vitamin D and calcium measurements) to ascertain any parathyroid and adrenal gland involvement.

#### Results

A total of 226 subjects have been screened: 54 subjects were index cases, 97 were *RET* gene carriers and 75 were negative for the presence of any *RET* germline mutation. Only 3 families showed the presence of additional endocrine neoplasia. In one family there was 1 subject with hyperparathyroidism that was cured with the surgical removal of one single parathyroid adenoma. In another family, a second germline mutation of TMEM127 was found correlating with the presence of pheochromocytoma even in family without *RET* germline mutations. The third family showed several cases with both MTC and pheochromocytoma but the genetically analysis did not find any other gene alteration in genes commonly involved in familial pheochromocytoma. However, the genealogic tree clearly showed a strict segregation of MTC and pheochromocytoma in one branch of the family while the other had only MTC.

#### Conclusions

These data strongly support the possibility that in these 3 families MTC was incidentally associated with other endocrine neoplasia. If this the case, the V804 mutation correlates with FMTC and there is no reason to screen gene carriers for pheochromocytoma and hyperparathyroidism.

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### OP-09-46

#### Interim outcome analysis of prospective ATA recurrence risk (RR) stratification for postoperative treatment and follow up of differentiated thyroid cancer (DTC)

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#### Objective

The 2016 ATA RR assessment recommendations for patients with DTC were based on retrospective studies. We present the first outcomes of a prospective assessment of thyroid cancer management according to the ATA RR stratification.

#### Methods

Using the Calgary prospective thyroid cancer database, we identified 612 patients with differentiated thyroid cancer (DTC) treated at our centre between April 2017 and December 2021. Each case was reviewed by the thyroid cancer (endocrinology) triage group and patients were prospectively assigned a modified ATA recurrence risk and AJCC 8<sup>th</sup> edition stage. Initial risk stratification guided the indication of radioiodine dose and other adjuvant therapies. Patients were assessed for their response to treatment (RTT) at 6, 12, and 24 months postoperatively.

#### Results

The 612 patients of our study cohort comprise 435 (71%) females and 177 (29%) males with a median age at diagnosis of 48 years. Of these patients, the ATA recurrence risk was as follows: low-risk  $n = 323$  (53%), intermediate-risk  $n = 178$  (29%), high-risk  $n = 111$  (18%). 280 patients (46%) received total thyroidectomy (TTX) and radioiodine (RAI), 230 (38%) received TTX alone, and 102 (17%) received lobectomy alone. 542 patients (89%) had at least 1-year follow-up. The RTT at 1 year was excellent response to treatment (ERT) in 86% of patients with lobectomy, 75% for patients with TTX only, and 53% for TTX & RAI (Table 1). Among the patients who were initially deemed to be low risk of recurrence, 79% had ERT, 17% had indeterminate response (IRT), 3% had biochemical incomplete response (BIR), and 1% had structural incomplete response (SIR). These are significantly better interim outcomes compared to the intermediate-risk group, which showed 60% ERT, 26% IRT, 7% BIR, and 7% SIR. Lastly, the high-risk group had the worst outcomes, with 42% ERT, 18% IRT, 11% BIR, and 29% SIR.

#### Conclusions

The 2015 ATA risk stratification system is a useful tool for predicting disease status at 1-year post-treatment in patients with DTC. The 2015 ATA guidelines and modified ATA recurrence risk stratification treatment recommendations reduce thyroid cancer overtreatment by including lobectomy as a definitive treatment option for low-risk thyroid cancers and selective use of RAI for intermediate and high-risk patients.

**Table 1.** RRT at 1-Year Evaluation for 542 Patients who had TTX/lobectomy and one-year follow-up

| Patients Number | All       | TTX + RAI | TTX only  | Lobectomy |
|-----------------|-----------|-----------|-----------|-----------|
| ERT             | 363 (67%) | 126 (53%) | 157 (75%) | 80 (86%)  |
| IRT             | 107 (20%) | 59 (25%)  | 38 (18%)  | 10 (11%)  |
| BIR             | 30 (6%)   | 21 (9%)   | 7 (3%)    | 2 (2%)    |
| SIR             | 42 (8%)   | 34 (14%)  | 7 (3%)    | 1 (1%)    |

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### Oral Session 10: Young Investigators Session / Clinical + Translational

#### OP-10-47

#### Putative protective role of anti-nuclear antibodies in graves' orbitopathy

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#### Objectives

A sporadic association between thyroid and non-organ-specific autoimmunity has been reported, which could be relevant for Graves' orbitopathy (GO), an autoimmune disease affecting orbital connective tissue. We investigated whether there is an association between GO and anti-nuclear antibodies (ANAs).

#### Methods

We performed a cross-sectional investigation in 265 consecutive patients with Graves' disease (GD) who came to our observation over 36 consecutive months to undergo radioiodine treatment. One-hundred and fifty-eight of them had GO, whereas 107 had no GO. The primary outcome was the prevalence of ANAs in patients with GO vs those without GO. The secondary outcomes were: 1) relationship between ANAs and GO features; 2) prevalence of ANAs in GD compared with nonautoimmune hyperthyroidism, namely 78 consecutive patients with toxic nodular goiter (TNG) who came to our observation over the same period to undergo radioiodine treatment.

#### Results

ANAs were detected in 212 (80%) GD patients, in all cases at low titres, namely 1:80 (98 patients, 46.2%) and 1:160 (114 patients, 53.7%). The prevalence of detectable ANAs did not differ between patients with GO (79.7%) and those without GO (80.3%; OR 0.96; 95% CI from 0.51 to 1.77;  $P = 0.9$ ). However, the prevalence of patients with higher ANA titres (1:160) was greater in GO patients (51.5 vs 38.3%), although the difference was only nearly statistically significance (OR 0.58; 95% CI from 0.33 to 1.02;  $P = 0.059$ ). Within GO patients, proptosis was significantly lower in ANA-positive patients (mean difference -1.42; 95% CI from -2.51 to -0.32;  $P = 0.011$ ). Although CAS and eyelid aperture were lower in ANA-positive patients, differences were only nearly statistically significant (CAS: Mann-Whitney U -1.557;  $P = 0.077$ ; eyelid aperture: mean difference -0.98; 95% CI from -2.01 to 0.05;  $P = 0.062$ ). The distribution of Gorman's score for diplopia did not differ between ANA-positive and ANA-negative patients. The proportion of patients with detectable ANAs in TNG patients was significantly greater than that in GD patients (91 vs 80%; OR 0.39; 95% CI from 0.17 to 0.9;  $P = 0.028$ ).

#### Conclusions

Within GD, ANAs are not more frequent in GO. However, they seem to exert a protective role on GO severity and on thyroid autoimmunity in general. A possible explanation is that ANA-related autoimmunity has an immunological phenotype different from the one of thyroid autoimmunity, in which ANA-positive patients may

have a switch of the T-cell population that could result in a milder clinical GO picture. Further studies are needed to investigate the mechanisms underlying our observations.  
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**OP-10-48****ABSTRACT WITHDRAWN**

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**OP-10-49****TSH-receptor antibodies among 1,024 early pregnant women in the north denmark region: cut-off, prevalence, and follow-up**

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**Objectives**

Measurement of TSH-receptor antibodies (TRAb) in hyperthyroid, pregnant women is an important tool when distinguishing between the autoimmune Graves' disease and the physiological gestational hyperthyroidism. Evidence to support a cut-off for TRAb in early pregnancy is needed along with information on the frequency and follow-up of TRAb-positive as opposed to TRAb-negative hyperthyroidism.

**Methods**

Within the North Denmark Region Pregnancy Cohort (2011-2015), TRAb was measured (BRAHMS TRAK Human, Kryptor Compact, Thermofisher Diagnostics Aps) in stored blood samples from the early pregnancy among all women with low TSH (<0.1 mIU/l) (*n*=443) and among randomly selected women (*n*=606). Method- and pregnancy-specific cut-off (95-percentile) for TRAb was established using Regression on Order Statistics. Each woman was followed in the years after the pregnancy for later diagnosis and treatment of thyroid disease (median follow-up: 8.1 years, range: 4-10 years) using Danish nationwide registers. Comparison of groups was performed using Mann-Whitney U Test or Fisher's exact test as appropriate. Thermofisher Diagnostics Aps supported the biochemical measurements of TRAb.

**Results**  
The established cut-off for TRAb was 1.0 IU/l. Altogether 28 (4.6%) of randomly selected women and 29 (6.5%) of women with low TSH were TRAb-positive. Considering women with low TSH (Table), TRAb-positive women had lower TSH, higher free T4, and lower β-hCG, and they less often terminated the pregnancy with live birth. Among women with low TSH and no known thyroid disease (*n*=414), maternal follow-up after the pregnancy showed that diagnosis of thyroid disease was more frequent among TRAb-positive (52.5%) as compared to TRAb-negative women (8.4%).

**Conclusions**

In a large cohort of Danish pregnant women, most women with low TSH in early pregnancy were TRAb-negative and rarely later diagnosed with thyroid disease. The results warrant further studies on TRAb in early pregnancy to substantiate a pregnancy-specific cut-off and to extend the findings on prevalence and follow-up.

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**OP-10-50****Outcomes of lenvatinib therapy in poorly differentiated thyroid carcinoma**

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**Introduction and objectives**

Poorly differentiated thyroid carcinoma (PDTC) is a rare but aggressive thyroid tumor. PDTC frequently presents in advanced stages and conventional treatments are usually less effective than in differentiated thyroid carcinoma (DTC). Lenvatinib is a multi-kinase inhibitor approved for the treatment of radioiodine-refractory DTC, with significant improvement in progression free survival. Despite the inclusion of a minority PDTC patients in SELECT trial, specific studies have been scarce. We intend to share the results of real-world PDTC patients under lenvatinib therapy.

**Materials and methods**

Retrospective study of all PDTC patients treated with lenvatinib between 2019 and 2021 in our institution. Histological diagnosis were reviewed according to the Turin criteria. Stable disease (SD), partial and complete response (PR and CR) were assessed on computed tomography studies, performed every 3 months. The following clinical end-points were analyzed: median overall survival (OS), disease specific survival (DSS), progression-free survival (PFS), best overall response (BOR), disease control rate (DCR) and duration of response (DoR). Thyroglobulin (Tg) levels were measured every 1-3 months.

**Results**

7 patients, 5 females, with median age at diagnosis of 58yr (IQR 12) and median age at lenvatinib start of 61yr (IQR 10). Two patients had inoperable disease and 6 had distant metastasis (lung, liver and bone). Previous treatments were performed in 5 patients: neck surgery and radioiodine therapy (*n*=5), neck radiotherapy (*n*=3) and sorafenib (*n*=2). The 2 patients with inoperable disease were treated with lenvatinib in neoadjuvant setting. Six patients had measurable Tg before treatment. Median follow-up was 22 months (IQR 19) and mean duration of lenvatinib therapy was 10.4 ± 6.9 months. Mean starting dose and mean overall dose were 15.1 ± 5.1 mg/day and 15.9 ± 4.6 mg/day, respectively (*P*=0.438). The median OS was 12 months (IQR 1) and DSS was 85.7% between 3 and 9 months and 66.7% at 12 months. Two patients died due to progression of disease. The median PFS was 9 months (IQR 6) and mean Tg-based PFS was 8.8 months. The BOR was SD with a DCR and relative PFS of 100%, 60% and 50% at 3, 6 and 12 months of follow-up, respectively. The median DoR was 6 months (IQR 6) and mean Tg-based DoR was 7.8 months.

**Conclusion**

Lenvatinib has shown consistent results in radioiodine-refractory thyroid cancer. However, real-world analysis are still scarce. Our work specifically directed to PDTC revealed encouraging results in this otherwise orphan population. We encourage the design of prospective studies to evaluate multi-kinase inhibitors in the treatment of PDTC, which can increase therapy approaches and allow significant increase in outcomes.

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**OP-10-51****Hypocalcemia is a frequent and life-threatening effect during lenvatinib treatment**

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|                  | Early pregnancy hyperthyroidism (TSH < 0.1 mIU/l) |             |     | TRAb < 1.0 IU/l |             |         |
|------------------|---|-------------|-----|-----------------|-------------|---------|
|                  | Median  | 95% CI      | n   | Median          | 95% CI      | p-value |
| TSH (mIU/l)      | 0.004   | 0.004-0.012 | 29  | 0.037           | 0.032-0.041 | <0.001  |
| Free T4 (pmol/l) | 22.7  | 20.1-25.2   | 29  | 20.2            | 19.9-20.5   | 0.019   |
| β-hCG (IU/l)     | 57.0  | 49.1-78.0   | 29  | 100.5           | 93.5-106.5  | <0.001  |
| Live births      | n   | %           | n   | %               | p-value     |         |
|                  | 24  | 82.8        | 395 | 95.4            | 0.015       |         |

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#### Objectives

Lenvatinib treatment is responsible for several adverse events (AEs). Hypocalcemia has been described in the registration study in 7% of patients, being of grade  $\geq 3$  in 2.7% of cases. No real-life studies are available, and the actual impact of this AE during Lenvatinib treatment is still unknown. Aim of our study is to evaluate the frequency of hypocalcemia in our series of patients treated with Lenvatinib and the possible predictors of this side effect.

#### Methods

We included all patients who were treated with Lenvatinib for progressive radioiodine refractory thyroid cancer at our Institution. We excluded patients who had a follow-up of less than 6 months and for whom information about calcium levels was not available.

#### Results

We included 25 patients who received Lenvatinib treatment for a mean of 29 months (range 6-68 months). Hypocalcemia was recorded in 6/25 patients (24%) and it was of grade  $\geq 3$  in 2/25 patients (8%). Hypocalcemia occurred after a mean of 5 months (range 0.5-13 months) from the start of Lenvatinib. It was managed with calcium oral supplementation or intravenous treatment, when necessary, and Lenvatinib was transiently withdrawn in 2/6 patients. No significant differences were found among who developed and who did not develop hypocalcemia in terms of gender (females were 50% and 57.9% respectively), age at start of Lenvatinib (69.8 vs 65.9 years old), starting dose of Lenvatinib (mean dose was 17.7 mg vs 16.6 mg), length of treatment (mean duration was 23 vs 31 months), post-thyroidectomy hypoparathyroidism (16.7% vs 10.5%). All patients who had hypocalcemia and for whom a bone densitometry evaluation was available had osteoporosis (3/3), while among patients who did not experience hypocalcemia 4/6 (66.7%) had osteoporosis ( $P=0.28$ ). Finally, 2 patients with normal post-surgical PTH levels, developed a grade  $\geq 3$  hypocalcemia with low (12.6 ng/l) or inappropriately normal (46.9 ng/l, n.v.13-64 ng/l) PTH levels during the hypocalcemic crisis.

#### Conclusions

Hypocalcemia is a frequent AE during Lenvatinib. Since it can be a life-threatening AE, monitoring of calcium levels following the start of treatment is mandatory and we recommend a particular caution during the first year of treatment. Oral calcium supplementation can correct hypocalcemia if promptly diagnosed; however, up to 10% of patients may have a severe hypocalcemia requiring intravenous treatment and Lenvatinib transient interruption. Further studies are needed to get more insights into the pathogenesis of hypocalcemia during Lenvatinib, though an inappropriate response of PTH to hypocalcemia has been observed.

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### OP-10-52

#### Outcome of sporadic medullary thyroid cancer (MTC) patients with a biochemical persistent disease after initial treatment

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#### Introduction

MTC is a rare neuroendocrine tumor arising from thyroid parafollicular cells. After initial treatment, patients should be divided according to their clinical status in cured, biochemical persistence (BIO) and structural persistence (STR) of the disease. Concerning BIO patients less is known about the structural disease appearance rate and the time elapsed between the first evaluation after surgery and the appearance of structural disease.

#### Method

We retrospectively reviewed data of 592 consecutive patients with sporadic MTC, followed at the Endocrine Unit of the University Hospital of Pisa, from 2000 to 2018. After surgery, all patients were reclassified according to clinical, biochemical [basal (bCT) or stimulated (sCT) calcitonin], neck ultrasound and,

whenever indicated, other imaging procedures. When bCT or sCT was above upper normal level according to gender thresholds, without evidence of structural disease, patients were considered BIO.

#### Results

After a median time of 5 months after surgery (IQR 3-8), 132/592 (22.3%) patients showed BIO. Among these, 89/132 (67.4%) and 43/132 (32.6%) patients were considered BIO for elevated bCT or sCT, respectively. Median time follow-up of the BIO group was 103 months (IQR 50.5-152.5). The appearance of structural disease occurred in 60/132 (45.5%) patients, after a median time of 37.5 months (IQR 16-61.5). In patients who were considered BIO for the presence of elevated bCT, the appearance of structural disease was more frequent than those with a positive sCT (56.2% vs 23.3%,  $P<0.001$ ). This finding was still more evident when comparing patients with bCT  $> 150$  pg/ml to those  $< 150$  pg/ml (82.6% vs 37.6%,  $P<0.001$ ). Neck was the most common site of structural disease appearance (41/60, 68.3%), followed by bone (9, 15%), liver (8, 13.3%), lungs (6, 10%) and mediastinal lymph nodes (5, 8.3%). Half of patients in whom structural disease appeared (30/60 – 50%), were submitted to other treatments and of these, 11/30 (36.6%) started systemic therapies.

#### Conclusions

In our large cohort of sporadic MTC patients with BIO, about half of them showed the appearance of a structural disease, prevalently located in the neck, after a median time of 3 years of follow-up. Patients with elevated bCT at first evaluation, particularly those with bCT  $> 150$  pg/ml, had the higher risk of structural disease appearance.

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### Oral Session 11: Young Investigators Session / Basic OP-11-53

#### CD3+CD8+CD20+ T cells as a marker of the inflammatory phase in thyroid autoimmune and related polyautoimmune disorders: a pilot study

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#### Objectives

Human CD3<sup>+</sup>CD20<sup>+</sup> T cells represents 3-5% of circulating T cells and may be detected in all lymphatic organs and in the cerebrospinal fluid. In healthy individuals CD3<sup>+</sup>CD20<sup>+</sup> T cells have been shown to produce higher levels of IL-17A and/or IFN- $\gamma$  than those of CD3<sup>+</sup>CD20<sup>-</sup> T cells. Some reports described the role of CD3<sup>+</sup>CD20<sup>+</sup> T cells in autoimmune disorders such as multiple sclerosis and rheumatoid arthritis possibly due their ability to produce these inflammatory cytokines. This study is aimed at describing the behavior of CD3<sup>+</sup>CD20<sup>+</sup> T lymphocytes in patients in the most frequent autoimmune disorder i.e., Hashimoto's thyroiditis, isolated or associated to further autoaggressive disorders in a frame of poly-autoimmunity.

#### Methods

The study group encompasses 65 patients bearing HT aged from 23 to 69 years (M=14; F=51), 42 of them associate another non-endocrine autoimmune disorder [16 with gastric atrophy (HT+GA), 15 with nonsegmental vitiligo (HT+V) and 11 with celiac disease (HT+CD)]. Twenty sex- and age-matched healthy subjects act as control group (HD). The chronic use of interfering drugs, severe or chronic disorders, pregnancy and lactation were used as exclusion criteria. Whole blood samples (100 microliters) were stained with the fluorescent-labelled antibodies. Red blood cells were then lysed by adding 1 ml of hypotonic buffer and samples were acquired on a FACs ARIA II Flow Cytometer (BD).

#### Results/Conclusions

The percentages of CD3<sup>+</sup>CD20<sup>+</sup> and that of CD3<sup>+</sup>CD4<sup>+</sup>CD20<sup>+</sup> lymphocytes were similar in HD HT and poly-autoimmune patients. The subpopulation CD3<sup>+</sup>CD8<sup>+</sup>CD20<sup>+</sup> was higher in the whole group of autoimmune patients as compared to HD ( $P=0.0089$ ). Patients with isolated HT showed higher percentages of CD3<sup>+</sup>CD8<sup>+</sup>CD20<sup>+</sup> than in HD patients although not reaching statistical significance. However dividing HT group based on thyroid function, hypothyroid patients showed a doubled CD8<sup>+</sup>CD20<sup>+</sup> percentages than HD patients ( $P=0.0115$ ). The presence of associated autoimmune disorders did not change the CD8<sup>+</sup>CD20<sup>+</sup> cells subset but the co-presence of GA increased this cells percentage as compared to HD ( $P=0.0257$ ) unlike the patients with H+CD all in gluten-free diet, in whom the CD8<sup>+</sup>CD20<sup>+</sup> subset was similar to the one in HD. These preliminary findings indicate that CD8<sup>+</sup>CD20<sup>+</sup> cells may behave differently in HT patients with or without poly-autoimmunity and may be a provisional marker of inflammatory phase of auto aggressive disorders.

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**OP-11-54****Study of target tissue-resident immune cells in graves' disease and orbitopathy (star-GO): preliminary findings with a novel extensive immunophenotyping panel**

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**Background and Aims**

Graves' disease (GD) and orbitopathy (GO) are characterised by the presence of pathological anti-thyrotropin receptor antibodies. In thyroid autoimmunity, especially GD and GO, a dysregulation of several T cell subpopulations has been proposed, especially T regulatory (Treg) and T helper 17 (Th17) cells, determining (auto)immunity inhibition and enhancement, respectively. T follicular cells (Tf) within germinal centres (GC: aggregates of lymphocytes within tissues) are also crucial, since supporting the maturation of GC-B cells that will later generate antibodies. The STAR-GO project aims to characterise immune signatures of GD/GO in relation with disease activity by analysing tissue-resident lymphocytes, more specific than those blood-derived.

**Materials and Methods**

Lymphocytes were derived from blood sampling and ultrasound-guided-fine-needle aspiration (US-FNA) of thyroid and neck lymph nodes (LNs) performed in the following patients: 6 GD early-onset (GD-E; newly diagnosed or recently relapsed) 2 active GO (GO-A; ongoing orbital inflammation), 3 inactive GO (GO-I; absent orbital inflammation) and 2 Hashimoto's thyroiditis (HT). Lymphocytes were immunophenotyped by flow cytometry (BD FACS Symphony0) with a 21 surface/intracellular staining panel.

**Results**

In the LNs analysis, B cells were more abundant in GD and GO, while T cells in HT. Among T cells, both GD and GO had increased Th17 cells compared with HT, while both GO-A and GO-I showed a peculiar increase of Tf-helper cells (Tfh). GC-B cells were highly abundant in GD-E, GO-A and especially in HT, while very low in GO-I; as a consequence GO-I patients had the lowest GC-B/GC-Tfh ratio (0.1), compared with GD-E (0.62), GO-A (0.70) and HT (0.94). The immunophenotyping of PBMC and thyroid did not show particular differences among the patient groups.

**Conclusions**

Our preliminary results show that:

- Neck LN sampling with US-FNA is an effective tool for the immunophenotyping of patients with thyroid autoimmunity, more specific and informative than blood;
- GD and GO patients showed increased numbers of B cells, while HT patients of T cells, likely reflecting the different disease pathogenesis (predominantly humoral vs cell-mediated);
- Increased Th17 cells identify both GD and GO patients, while Tfh cells seem particularly important for GO;
- As expected, GO during its inactive phase (GO-I) showed a low GC activity.

We are currently increasing the number of subjects to verify our findings and further distinguish the different phases of GD/GO disease activity.

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**OP-11-55****Role of NADPH oxidase 4 (NOX4) in resistance to metabolic iodine-131 radiotherapy in metastatic thyroid tumors carrying the BRAFV600E mutation**

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Radioiodine therapy (RAI): which is the cornerstone of the treatment of distant metastasis from differentiated thyroid cancers (DTC), is based on the expression of the iodine transporter NIS. The majority of DTC are papillary with BRAF<sup>V600E</sup> mutation in 45% to 60% of cases. This mutation is associated with RAI refractory DTC with a low NIS expression and a low differentiation score. The absence of RAI uptake is a major challenge for the treatment of patients. A promising approach for the treatment of RAI-refractory patients is to re-enhance RAI uptake by promoting tumor redifferentiation. We showed that BRAF<sup>V600E</sup> controls NADPH oxidase NOX4 and that NOX4-derived ROS contribute to NIS repression. Deletion of NOX4 promotes reactivation of NIS. This reversibility suggests a contribution of an epigenetic mechanism. Our hypothesis is that NOX4 generates specific oxidative DNA damage, promoting longer retention of epigenetic modifiers, such as DNMTs, at sites of DNA damage via the interaction with DNA repair proteins, contributing to stably preventing the transcription of genes from interfering with the repair process. Our objectives are 1- to determine the molecular and mechanistic events induced by NOX4-derived ROS that contribute to the reversible regulation of genes involved in differentiation/dedifferentiation process and 2- to evaluate the role of NOX4 in the resistance mechanism to BRAF/MEK inhibitors. Our data suggest a key role of NOX4 in oxidative DNA damage, which promotes the recruitment to chromatin of an epigenetic complex in which DNA methyl transferase 1 (DNMT1) and the DNA mismatch repair system (MMR) cooperate.

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**OP-11-56****Focusing on the role of the enigmatic TRα2 isoform in modulation of thyroid hormone action**

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Thyroid hormones (TH) are important regulators of human metabolism and development, which modulate expression of target genes via nuclear thyroid hormone receptors (TRs). Different isoforms of these classical TRs including TRβ isoforms TRβ1 and TRβ2 as well as TRα1 were shown to be functional TH-responsive transcription factors and have been extensively studied. In contrast, the cellular function of TRα2, an alternative splice variant of TRα1, is poorly understood and it is even speculated whether the TRα2 protein is of biological relevance *in vivo* at all or the mRNA might only act as transcriptional sink to quickly regulate TRα1 production. Characterization of specific isoform functions has in particular been hampered by the lack of reliable antibodies. We aim to address the function and physiological relevance of the poorly characterised TRα2 isoform by using a tagged TRα2 variant. ChIP-sequencing analyses will be applied to generate genome-wide DNA binding profiles specific for both TRα isoforms, and different proteomic approaches will be used to identify isoform-specific protein interactions mediating TRα1 and TRα2 functions in gene regulation. These analyses will not only unravel the role of TRα2 for TH signalling but also elucidate whether this isoform may be a possible target to modulate TH action. To gain first insights into TRα2-specific functions, co-immunoprecipitation followed by mass spectrometric analysis of peptides bound by GFP-tagged TRα isoforms transiently overexpressed in HEK293 cells has been performed. In this analysis, well-known interacting proteins such as TR-corepressor NCOR1 were found, confirming the validity of the experimental approach. Moreover, novel putative shared as well as isoform-specific binding proteins have been identified that are suggesting a potential so far unknown specific function of TRα2. Interestingly, during first cellular analyses, we also observed different subcellular localisation of the two isoforms. While TRα1 appears to be evenly distributed in the cell nucleus, TRα2 accumulated in distinct nuclear speckles, which we will further investigate. To uncover cell-type specific functions of TRα2, protein interactions and DNA binding profiles will next be analysed in other cell types. Moreover, we aim to verify the identified DNA binding profile and protein interactions found for ectopic expression of TRα2 by performing the same analyses in different induced pluripotent stem cell-derived cellular models expressing endogenously tagged TRα isoforms.

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## OP-11-57

**Monocarboxylate 8 transporter and deiodinase 2 deficiency impairs neurogenesis in the adult mouse subventricular zone leading to cellular and functional alterations**Victor Valcárcel-Hernández<sup>1</sup>, Pieter Vancamp<sup>2</sup>, Sylvie Remaud<sup>3</sup> & Ana Guadaño-Ferraz<sup>1</sup><sup>1</sup>Instituto de Investigaciones Biomédicas Alberto Sols Csic-Uam, Department of Endocrine and Nervous System Pathophysiology, Madrid, Spain; <sup>2</sup>Muséum National D'histoire Naturelle, Cnrs, Umr 7221, Paris, France; <sup>3</sup>Muséum National D'histoire Naturelle; Cnrs; Umr 7221, Paris, France

Thyroid hormones (THs) play a crucial role orchestrating neurodevelopment, but also regulate adult brain function. Recently, the potent effects that THs exert in adult neurogenic niches have started to be uncovered in rodents. These include an important role in the modulation of progenitor generation, especially controlling whether a neural stem cell (NSC) determines to become a neuronal or an oligodendroglial progenitor in the adult subventricular zone (SVZ), the largest NSC niche in the mammalian brain. A complex network of regulators tightly modulates TH availability and action, including transmembrane transporters, deiodinases and receptors. Among the TH-transporters, there is only one that is TH-specific, the monocarboxylate transporter 8 (MCT8). Deficiency of MCT8 leads to an ultra-rare but devastating disease, the Allan-Herndon-Dudley Syndrome (AHDS). Patients exhibit a plethora of endocrine and severe neurological disturbances and so far, no effective treatment for their neurological symptoms exists. Its complexity, along with its low prevalence and severe symptomatology, makes animal models and biomarkers of the disease a crucial step in the research for potential strategies to alleviate the patients' severe conditions. Using a well-validated animal model of AHDS, the *Mct8/Dio2* KO mice, we aimed to characterize how a reduced T3 availability structurally and functionally affected the neurogenic and gliogenic capacity of the adult SVZ-NSCs. To this end, we analysed the expression of cell markers by immunohistochemistry to study the balance between neurons and glia in the SVZ, both *in-vivo* and using *ex-vivo* neurosphere cultures. These studies revealed severe alterations in the neuroglial balance, with an increase of the neuron/glia ratio in the SVZ in adult *Mct8/Dio2* KO mice. We also observed that MCT8/DIO2 deficiency reduced NSC proliferation two-fold and hampered migrating of proliferating neuronal progenitors. Moreover, we tested the effects of administering exogenous THs and TH-analogues on neurospheres prepared from dissected SVZs. Neither the neuron/glia balance, nor proliferative activity responded to TH treatment in MCT8/DIO2 deficient neurospheres. Also, behaviour consequences of the observed NSCs alterations were studied using the olfactory memory and odour discrimination tests, as potential non-invasive biomarkers of the disease. These tests revealed that *Mct8/Dio2* KO mice did not recognize new odours and failed to memorize them. Altogether, these results indicate that MCT8/DIO2 deficiency severely hampers TH-dependent regulation of adult SVZ-neurogenesis and suggest potential biomarkers for future preclinical studies.

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## OP-11-58

**Integrated genomic, phenomic, functional and structural mapping of variants in thyroid hormone transporter MCT8**Stefan Groeneweg<sup>1</sup>, Ferdy Van Geest<sup>2</sup>, mariano martín<sup>3</sup>, Mafalda Dias<sup>4</sup>, Jonathan Frazer<sup>4</sup>, Rosalie Sterenborg<sup>5</sup>, linda de rooij<sup>6</sup>, anna dolcetta-capuzzo<sup>6</sup>, alexander teumer<sup>7</sup>, Marcel Meima<sup>8</sup>, Marco Medici<sup>9</sup>, juan pablo nicola<sup>3</sup>, debora marks<sup>4</sup> & W. Edward Visser<sup>10</sup><sup>1</sup>Academic Center for Thyroid Diseases, Academic Center for Thyroid Disease, Department of Internal Medicine, Erasmus Medical Center Rotterdam, The Netherlands, Department of Internal Medicine, Rotterdam, Netherlands; <sup>2</sup>Erasmus MC, Academic Center for Thyroid Disease, Department of Internal Medicine, Rotterdam, Netherlands; <sup>3</sup>Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina; <sup>4</sup>Department of Systems Biology, Harvard Medical School, Boston, MA, USA; <sup>5</sup>Academic Center for Thyroid Disease, Department of Internal Medicine, Erasmus Medical Center Rotterdam, The Netherlands, Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Center, Nijmegen, The Netherlands, Netherlands; <sup>6</sup>Academic Center for Thyroid Disease, Department of Internal Medicine, Erasmus Medical Center Rotterdam, The Netherlands; <sup>7</sup>Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany, Dzhk (German Center for Cardiovascular Research), Partner Site Greifswald, Greifswald, Germany; <sup>8</sup>Dept of Internal Medicine, Academic Center for Thyroid Diseases, Erasmus MC, Rotterdam, Rotterdam, Netherlands; <sup>9</sup>Radboud University Medical Center,Nijmegen, Erasmus Medical Center, Rotterdam, Department of Internal Medicine, Radboud University Medical Center, Nijmegen, Netherlands; <sup>10</sup>Erasmus Medical Center, Academic Center for Thyroid Diseases, Department of Internal Medicine, Academic Center for Thyroid Diseases, Rotterdam, Netherlands

## Background

MCT8 deficiency is caused by loss-of-function (LoF) mutations in thyroid hormone (TH) transporter MCT8. Patients have developmental delay and abnormal thyroid function tests (TFTs). The large phenotypic variability is not understood. Moreover, phenotypes arising from LoF mutations could be employed to enhance understanding of physiology in the general population. Also, computational disease variant classifiers have poor predictive power to ascertain impact of MCT8 variants. We conducted a generalizable approach that addresses all abovementioned challenges.

## Methods

We systematically integrated genetic, clinical and biochemical data from 371 patients with MCT8 deficiency, accrued through combination of data from our well-phenotyped global cohort and meta-analysis of all reported cases. We assessed the impact of common genetic variation in MCT8 on TFTs in ~70k individuals. We evaluated impact of 108 patient mutations and 304 MCT8 variants in a full alanine-scanning by TH transport assays. We linked three distinct LoF classes (mild, moderate, severe) to phenotypic outcomes and mapped all variants onto our homology model. Utilizing these data and conservation analyses, an MCT8 deficiency-specific variant classifier was constructed using artificial intelligence methods.

## Findings

Linking the different LoF classes to phenotypic outcomes, we observed a clear genotype-phenotype relationship across a range of disease features. Functional impact of variants strongly associated with survival of patients (median survival mild LoF: 71yrs; moderate LoF: 60yrs; severe LoF: 21.4yrs). Similar observations were noted for developmental (e.g. motor function), clinical (e.g. seizures) and biochemical (e.g. fT4, but not T3) features. Beneficial effects of the TH analogue Triac on several disease outcomes were independent of LoF category. By cross-referencing functional alanine-scanning data with patient mutants, we could infer the underlying mechanisms for the majority of variants. Our MCT8-specific classifier largely outperformed (AUC 0.95) commonly used prediction tools. Common genetic variation in MCT8 was associated with lower serum fT4, but not with TSH or T3 concentrations, resembling the genotype-phenotype relationships in patients.

## Interpretation

The combination of deep phenotyping data from patients with MCT8 deficiency with a battery of functional and computational tests and with outcomes in population cohorts, enabled us to: (i) understand the divergent clinical phenotypes of MCT8 deficiency, (ii) assess therapy effectiveness, (iii) advance structural insights of MCT8, (iv) create a high-quality disease variant classifier, together also leveraging information on the role of MCT8 in non-affected individuals in the population.

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## Oral Session 12: Nodules and Diagnostic

## OP-12-59

**Autonomously functioning thyroid nodules present intermediate malignancy risk according to european thyroid imaging and reporting data system; a comprehensive clinical, cytological and molecular characterization**Aglaiia Kyrilli<sup>1</sup>, nunzia tacelli<sup>2</sup>, Laetitia Lebrun<sup>3</sup>, Isabelle Salmon<sup>4</sup>, Gilles Russ<sup>5</sup>, Rodrigo Moreno-Reyes<sup>6</sup> & Bernard Corvilain<sup>7</sup><sup>1</sup>Cliniques Universitaires de Bruxelles- Hôpital Erasme, Endocrinology, Brussels, Belgium; <sup>2</sup>Cliniques Universitaires de Bruxelles - Hôpital Erasme, Radiology, Brussels, Belgium; <sup>3</sup>Cliniques Universitaires de Bruxelles - Hôpital Erasme, Anatomopathology, Brussels, Belgium; <sup>4</sup>Hôpital Erasme, Anatomie Pathologique, Brussels, Belgium; <sup>5</sup>Thyroid and Endocrine Tumors, Institute of Endocrinology, Pitié Salpêtrière Hospital, Sorbonne University, Paris, France; <sup>6</sup>Cliniques Universitaires de Bruxelles - Hôpital Erasme, Nuclear Medicine, Brussels, Belgium; <sup>7</sup>Cliniques Universitaires de Bruxelles - Hôpital Erasme, Hub-Erasme University Hospital, Université Libre de Bruxelles, Endocrinology, Brussels, Belgium

## Objectives

To systematically characterize autonomously functioning thyroid nodules (AFTN) by clinical, biological and imaging methods, cytology and histology when indicated.

**Design**

Prospective, single-center study conducted from March 2018 until September 2021, in 901 consecutive patients with 67 AFTN evaluated.

**Methods**

Enrolled patients underwent  $^{99m}\text{TcO}_4$  scintigraphy evaluation of thyroid function, ultrasound (US) using European Thyroid Imaging and Reporting Data System (EU-TIRADS),  $^{125}\text{I}$  scintigraphy in case of normal serum TSH, and fine needle aspiration (FNA) biopsy with molecular analysis and surgery when indicated.

**Results**

The median serum TSH of patients with AFTN evaluated was 0.41 (0.03-0.97) mU/l and more than half of the patients were euthyroid. The median AFTN size measured by US was 27.0 (21.1-35.0) mm. 28.3% of AFTN were classified as EUTIRADS score 3 and 72.7% as EUTIRADS score 4, indicating that the majority of AFTN had intermediate risk for malignancy according to US. Out of the 46 AFTN (31 with EU-TIRADS score 4 and 15 with score 3) subjected to cytological evaluation, 18 (39.2%) yielded indeterminate FNA results. DNA sequencing revealed *THSR* and *GNAS* mutations in 50% of the samples. No malignancy was detected at final histology ( $n=12$ ), but one non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was observed.

**Conclusions**

AFTN frequently present with normal serum TSH, intermediate malignancy risk according to US, and yield indeterminate FNA results. No malignant AFTN was detected in this study, but further prospective studies addressing the risk of malignancy of AFTN as a primary outcome are needed.

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**OP-12-60****Population study on thyroid nodule ultrasound (TUS) reports quality with adherence to guidelines**

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**Objectives**

To date, there has been no population-wide data looking at the adherence to published TUS reporting guidelines for thyroid nodule malignancy risk assessment. In our health care region in 2018, two radiology groups worked closely with endocrinologists to improve the quality of their TUS reports by adhering to the 2015 ATA or the 2017 TIRADS guidelines. We aim to present the improvement in TUS reports quality with these dedicated changes.

**Methods**

We analyzed the TUS reports of 981 patients from two prospective databases covering a population of 1.5 million people. We measured the reports' utility score (UtS; range 0–6), which is calculated based on the number of nodule characteristics provided in the report, and rate of ATA or TIRADS classification given by TUS, to assess the TUS report quality. The TUS report quality of the two radiology groups and other radiology groups were compared to each other. Since the two radiology groups implemented their adherence to guidelines in 2018, we then divided the databases into pre-2018 TUS reports and 2018-onwards reports and compared each group's later reports to its earlier ones.

**Results**

The two groups that implemented strict adherence to ATA or TIRADS guidelines had significantly higher UtS compared to other groups among the 2018-onwards reports. There was also a significant improvement in the UtS of the guideline-adhering radiology groups when comparing their TUS reports from pre-2018 vs those from 2018-onwards (Table 1). Additionally, the two groups also had higher rates of including an ATA or TIRADS classification in their report, and overall, they had a substantially higher likelihood of providing TUS reports with both a clinically useful UtS of 4 or greater and an ATA or TIRADS classification. This allows for clinicians to better estimate the risk of malignancy of thyroid nodules.

**Conclusions**

Our data indicates that with dedicated adherence to the ATA or TIRADS guidelines, radiology groups can significantly improve the quality and utility of their TUS reports to better help clinicians manage thyroid nodules in a risk stratified manner, and to also help avoid unnecessary anxiety and additional testing and surgery.

**Table 1.** Mean UtS and percentage of reports with an ATA or TIRADS classification for pre-2018 TUS reports vs 2018-onwards TUS reports.

| pre-2018               | Mean UtS | Classification reporting rate | Number of reports |
|------------------------|----------|-------------------------------|-------------------|
| Radiology group 1      | 3.62     | 39.4%                         | 71                |
| Radiology group 2      | 2.8      | 11.5%                         | 87                |
| Other radiology groups | 2.49     | 32.2%                         | 171               |
| 2018-onwards           | Mean UtS | Classification reporting rate | Number of reports |
| Radiology group 1      | 5.77     | 97.0%                         | 133               |
| Radiology group 2      | 5.58     | 93.3%                         | 178               |
| Other radiology groups | 3.28     | 61.8%                         | 259               |

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**OP-12-61****Comparison of accuracy of  $^{18}\text{F}$ -fluorocholine PET-CT and PET/MR to  $^{99m}\text{Tc}$ -sestamibi for the localization of hyperfunctioning parathyroid tissue in hyperparathyroidism**

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**Objectives**

The aim of this prospective study was to compare the diagnostic performance of  $^{99m}\text{Tc}$ -sestamibi SPECT scintigraphy (sestaMIBI),  $^{18}\text{F}$ -fluorocholine PET/CT or PET/MR (FCH) for preoperative localization of hyperfunctioning parathyroid gland.

**Materials and Methods**

60 patients with biochemical evidence of primary ( $n=57$ ) or tertiary ( $n=3$  kidney transplanted patients) hyperparathyroidism were imaged prospectively with dual phase/dual tracer  $^{99m}\text{Tc}$ -sestaMIBI SPECT/CT, early FCH (FCH<sub>E</sub>) acquired by PET/CT ( $n=60$ ) and late FCH (FCH<sub>L</sub>), acquired by PET/CT ( $n=18$ ) or by PET/MR ( $n=36$ ). All imaging were interpreted independently by two nuclear medicine physicians and two radiologists. The results were classified into 3 categories (positive, inconclusive or negative) based on the nodular aspect of tracer uptake and the visualization of corresponding nodules on the CT or MRI. The imaging results were confronted to the surgical and histopathological findings and the follow-up.

**Results**

FCH<sub>E</sub> was positive in 51/60 (85%) patients, inconclusive in 8 and negative in 1 compared to 48/54 (88.9%), 5 and 1 for FCH<sub>L</sub> and 41/60 (68%), 13 and 6 for sestaMIBI. FCH (FCH<sub>E</sub> and FCH<sub>L</sub>) detected 15 additional positive foci and 18 additional inconclusive foci and it confirmed 19 inconclusive foci. 45 patients underwent surgery and 54 lesions were removed (34 adenomas, 20 hyperplasia and 4 normal glands). FCH<sub>E</sub> and FCH<sub>L</sub> PET/CT and PET/MR correctly localised 48 lesions compared to 39 correctly localised by sestaMIBI. Per-lesion sensitivity was 88.88% for FCH<sub>E</sub> and 87.75% for FCH<sub>L</sub> – with respectively sensitivity of 100% for acquisition by PET/CT and 82.85% for acquisition by PET/MR – vs 72.22% for sestaMIBI. At follow-up, 34 patients were considered cured after surgery, while 5 patients had a biological recurrence of hypercalcaemia. Biological control is requested for 4 patients and 2 patients are lost to follow-up.

**Conclusion**

$^{18}\text{F}$ -fluorocholine with PET/CT or PET/MR appears to be superior to sestaMIBI for lesion detection and localization of hyperfunctioning parathyroid tissue, particularly in patients with multiple lesions.

**Key words:** Hyperparathyroidism; parathyroid adenoma; MIBI SPECT;  $^{18}\text{F}$ -fluorocholine; PET/CT; PET/MR.

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**OP-12-62****Progressive diastolic dysfunction in survivors of pediatric differentiated thyroid carcinoma**

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#### Background

Pediatric differentiated thyroid cancer (DTC) has an excellent prognosis, but has unknown late effects of treatment. Initial cardiac evaluation showed subclinical diastolic dysfunction in 20% of adult survivors. In this follow-up study, we determined the clinical course of this finding.

#### Methods

This multicenter study, conducted between 2018 and 2020, re-evaluated survivors 5 years after the first evaluation. The primary endpoint was echocardiographic diastolic cardiac function (depicted by the mean of early diastolic septal and early diastolic lateral tissue velocity [ $e'$  mean]). Secondary endpoints were other echocardiographic parameters and plasma biomarkers.

#### Results

Follow-up evaluation was completed in 47 (71.2%) survivors. Of these 47 survivors (87.2%) were women, median age 39.8 years (range 18.8-60.3). The median follow-up after initial diagnosis was 23.4 years (range 10.2-48.8). Between the first and second evaluation, the  $e'$  mean significantly decreased by 2.1 cm/s (SD 2.3 cm/s,  $P < 0.001$ ). The median left ventricular ejection fraction did not significantly change (58.0% vs. 59.0%, *NS*). In the best explanatory model of  $e'$  mean, multivariate linear regression analysis showed that body mass index and age were significantly associated with  $e'$  mean ( $\beta$  coefficient -0.169, 95% confidence interval (CI) [-0.292;-0.047],  $P = 0.008$  and  $\beta$  coefficient -0.177, 95% CI [-0.240;-0.113],  $P < 0.001$ , respectively). The TSH value was not significantly associated with the  $e'$  mean.

#### Conclusions and relevance

In these relatively young survivors of pediatric DTC, diastolic function decreased significantly during 5 year follow up, possibly more pronounced than in normal ageing. This finding requires further follow-up to assess clinical consequences.

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#### OP-12-63

##### Real-world performance of a novel dual-component molecular assay in cytologically indeterminate thyroid nodules: a single institutional experience

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#### Objective

We developed a novel dual-component molecular assay as an ancillary method to improve clinical decision-making in patients with cytologically indeterminate thyroid nodules. The assay includes next-generation sequencing (NGS) based detection of mutations in 23 thyroid cancer related genes and digital polymerase chain reaction (dPCR) evaluation of the expression levels of a microRNA strongly associated with thyroid cancer. The method was designed as a "rule-out" test and preliminary results in a surgical cohort showed a negative predictive value comparable to a negative diagnostic cytology (100%). The aim of this study was to describe the performance of this approach in a real-world cohort of patients undergoing thyroid nodule fine-needle aspiration cytology, whose treatment is defined by the current clinical practice and based on clinical and ultrasonographic data.

#### Methods

Patients with a cytologically indeterminate nodule (TIR3A and TIR3B according to of the Italian Consensus for Thyroid Cytology) were prospectively enrolled from January 2017 to January 2022 in the thyroid cancer unit at Policlinico Umberto I of Rome. For each consenting patient, residual cytological material from thyroid nodule aspirates was collected washing the needle with a nucleic acid preservative solution, after the preparation of standard cytology smears, with no dedicated passes. The samples were stored at -20°C until molecular testing. Patients underwent either surgery or clinical monitoring based on cytology diagnosis, molecular test results, clinical features, and patient's preference. Patients who did not proceed to surgery were monitored clinically for sonographic evidence of growth, development of suspicious US characteristics, or suspicious lymph node appearance. Median follow-up of these patients was 34 months.

#### Results

In total, 326 cytologically indeterminate thyroid nodules were consecutively collected since January 2017. Molecular analysis was performed on 218 thyroid aspirates from 211 consenting patients (TIR3A,  $n = 163$ ; TIR3B,  $n = 55$ ), displaying a 58% of positive call rate. The benign call rate of the assay was significantly higher in TIR3A (60%) than in TIR3B (44%) nodules ( $P = 0.0411$ ). Among the 63 patients undergoing surgery (cancer prevalence 36%), the molecular assay showed high sensitivity (96%) and negative predictive value (96%). There was only 1 false-negative test result, representing a low-risk neoplasia.

#### Conclusion

These data confirm that the used dual-component molecular test can increase the diagnostic accuracy of thyroid cytology alone by reducing the number of nodules that will be classified as indeterminate and increasing those that can be reliably classified as benign, thus avoiding a substantial number of diagnostic surgeries.

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#### Oral Session 13: Basic 3

##### OP-13-64

##### Metabolic and neuroinflammatory consequences of hypothyroidism in two mouse strains with different metabolic adaptability capacities, the C57BL/6J and the WSB/EIJ strains

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Thyroid hormones (TH), among their pleiotropic actions, play a central role in the regulation of metabolism and in cognitive functions. Indeed, hypothyroidism is associated with a decrease in energy expenditure and lipid metabolism, and an impairment of memory. Metabolic deregulations induced by a high fat diet (HFD) generate peripheral inflammation, which promotes the development of neuroinflammation in various brain regions. This inflammatory state can disrupt neuronal homeostasis, leading to the alteration of synaptic plasticity and to memory disorders. Furthermore, a link between hypothyroidism and the development of neuroinflammation has previously been shown, particularly in the hippocampus, a brain structure rich in TH receptors. Our objective was to evaluate whether

metabolic deregulations induced by hypothyroidism favor the development of neuroinflammation and thereby promote memory deficits. We compared the response to induced hypothyroidism in two mouse strains, the wild-derived WSB/EiJ mouse strain characterized by an obesity resistance due to its high metabolic flexibility phenotype and the C57BL/6J mice, prone to HFD-induced obesity. Adult mice were fed with a low-iodine diet supplemented with 6-n-propyl-2-thiouracil (PTU) for 7 weeks to induce hypothyroidism. Our results show that hypothyroidism, characterized by a decrease in serum T4 levels, led to metabolic deregulations, as an alteration of lipid metabolism in the liver of both strains. However, the decrease in hepatic lipid synthesis was compensated in WSB/EiJ mice by a mobilization of lipid reserves from white adipose tissue, but not in the C57BL/6J mice. No peripheral inflammatory response to hypothyroidism was observed in both strains. In the hippocampus of C57BL/6J mice treated with PTU, the decrease in intracellular T3 availability was accompanied by an activation of glial cells, a hallmark of neuroinflammation, associated with an impairment of spatial memory. In contrast, no inflammatory response was observed in the hippocampus of WSB/EiJ mice, which appeared to maintain their thyroid status by locally increasing T3 availability via compensatory mechanisms. Our results shed the light on the fact that serum thyroid status does not always reflect central thyroid status. Moreover, they demonstrated that the described link between hypothyroidism and neuroinflammation does not seem to be the consequence of metabolic deregulations induced by hypothyroidism but rather of an imbalance in the central thyroid signaling. Thus, our results emphasize the importance of maintaining central thyroid homeostasis to protect against the development of neuroinflammation, and in extension, of neurodegenerative diseases, given that neuroinflammation favors cognitive and memory impairments.

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### OP-13-65

#### 3,3',5-triiodo-L-thyronine and 3,5-diiodo-L-thyronine differentially modulate hepatic mitochondrial quality control in hypothyroid rats

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#### Objective

The maintenance of healthy and functional mitochondrial network via mitochondrial quality control (QC) mechanisms, is critical throughout life to respond to physiological adaptations and stress. Due to their role in energy production, mitochondria are exposed to high amounts of reactive oxygen species making their DNA (mtDNA) particularly vulnerable to oxidative damage. Mitochondrial dysfunction causes altered QC mechanisms (i.e. altered biogenesis, dynamics, autophagy/mitophagy) and mtDNA damage and depletion, and in some cases, mtDNA release. When this occurs, mtDNA released from mitochondria into the extracellular and cytosol environment plays a central role in the damage-associated molecular patterns (DAMPs) through the activation of cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, acting as an inflammatory trigger. Both 3,5-diiodo-L-thyronine (3,5-T2) and 3,3',5-triiodo-L-thyronine (T3) have been shown to influence the mitochondrial QC system. However, the underlying mechanisms are poorly understood and likely differentiated when comparing the two iodothyronines. Here, by using a rat model of chemically induced hypothyroidism, we investigated the effect of administration of either 3,5-T2 or T3 on some key factors related to inflammation, mtDNA damage and mitochondrial QC system in the liver.

#### Methods

Hypothyroidism was induced by propylthiouracil and iopanoic acid; 3,5-T2 and T3 were intraperitoneally administered to hypothyroid rats for 1 week at 25 and 15 µg/100 g BW, respectively. Factors linked to hepatic inflammation (i.e. cGAS-STING pathways) were investigated. The status of mtDNA damage/repair and mitochondrial QC mechanisms (biogenesis, dynamics, and mitophagy) were studied.

#### Results

We showed an increase in mtDNA damage in the liver of hypothyroid rats accompanied by a significant reduction of mtDNA copy number, suggesting a reduction in mitochondrial biogenesis. Moreover, in hypothyroid rats, we found increased protein expression of both cGAS and cSTING, indicating activation of DAMPs pathways. The administration of either 3,5-T2 or T3 affected QC

mechanisms ameliorating mitochondria fitness. Both iodothyronines enhanced mitochondrial copy number, reduced the mtDNA lesion frequency and oxidative damage, induced mtDNA repair mechanism and mitochondriogenesis, being T3 more effective than 3,5-T2. Also mitochondrial dynamics and autophagy were influenced. Of note, 3,5-T2, but not T3, reverted the activation of inflammatory triggers.

#### Conclusion

The reported data highlight new molecular mechanisms underlying the effect elicited by the administration of naturally occurring iodothyronines to hypothyroid rats on liver pathways related to QC to preserve mitochondrial health.

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### OP-13-66

#### Generation of human thyroid organoids from embryonic stem cells to rescue hypothyroidism

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Organoids are tiny, self-organized three-dimensional versions of an organ that are produced *in vitro*. Human organoids offer unique opportunities to model the development, physiology, and diseases of human tissues while complementing animal models and reducing the need for animal testing. In the thyroid field, there is a lack of a functional human *in vitro* thyroid model which allows to further explore various aspects related to thyroid development and disease. Thus, the ability to generate TH-producing human follicles from embryonic stem cells would open new perspectives for the human thyroid research field. Here, by transient overexpression of thyroid transcription factors, *NKX2-1* and *PAX8*, in human embryonic stem cells (hESCs), followed by time-dependent treatment with cAMP, hrTSH, Dexamethasone and TGF-beta inhibitor, we aimed to recapitulate thyroid developmental stages and generate the first *in vitro* functional thyroid derived from human ESC. Stepwise transcriptomics and histological analysis evidenced that the generated protocol recapitulates the gland developmental steps, cell expansion and follicular organization, and finally results in T4 production. In addition, these *in vitro*-grown follicles were able to maintain histological organization, promote vascular formation, and synthesize and release THs after three weeks of transplantation into the renal capsule of hypothyroid mice. This model opens a new window to better understand thyroid development processes as well as mechanisms/variants causing congenital hypothyroidism. Also, it can be considered as screening tool to test the toxic effects of compounds, in particular endocrine disruptors. Finally, although this model still needs improvement to be therapeutically applicable, it provides a proof-of-concept that generating autologous human thyroid tissue to maintain TH levels is within reach.

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### OP-13-67

#### A single cell atlas of the T3-responsive transcriptome during early cortical neurogenesis in human cerebral organoids

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Perturbation of thyroid hormone (TH) function during prenatal brain development can cause a spectrum of neurological disorders as evident in pediatric patients with untreated congenital hypothyroidism or patients with mutations in *THRA* and *SLC16A2* genes. However, scarcity and inaccessibility of human brain tissue from early developmental stages has been a major roadblock for experimental approaches to decipher local TH action in early development. Advanced *in vitro* models including human cerebral organoids (hCOs) hold great promises of accelerating our understanding of early human brain development and the hormonal signals that govern the complex processes underlying cortical neurogenesis. In this study, we differentiated hCOs from human induced pluripotent stem cells (hiPSC) in media containing either 1.5 nM T3 (BASAL group) or 20 nM T3 (CHRONIC group) for 9 weeks. Starting at day 13 of differentiation, hCOs were harvested at various developmental stages and analyzed by single cell RNA-seq for T3 treatment effects on the transcriptome. In addition, a subset of hCOs from the BASAL group was acutely treated with 50 nM T3 for 48 h (PULSE) at various stages and similarly analyzed by single cell RNA-seq. To avoid hiPSC line-inherent bias, all treatments were replicated for three different hiPSC lines resulting in 27 multiplexed single cell libraries. Global analysis of all cells collected during this study identified 31 neural cell clusters. Following cluster annotation, we could assign more than 95% of all cells to a major lineage trajectory from neural stem cell state towards excitatory neurons. Our single cell atlas comprised major cortical cell types including various radial glia cell (RGC) populations, three intermediate progenitor (IP) subtypes, as well

as nine excitatory neuron subtypes. We defined developmental and T3-responsive expression profiles for genes related to TH action with cell type-specific resolution. For a subset of genes, smFISH was used to correlate spatial mRNA expression patterns with the laminar cell type distribution. We identified partially overlapping gene signatures in response to acute and chronic T3 treatment. Cell type- and developmental stage-specific gene set enrichments highlighted T3 effects on metabolic pathways in RGC, cell cycle regulation in IP and synapse function in neurons. T3-induced gene signatures in hCOs showed limited overlap with published mouse data sets. Chronic T3 treatment did not cause differentiation of aberrant cell types but altered the relative proportion of neuronal cell types in late stage hCOs. This comprehensive atlas of the T3-responsive transcriptome will serve as a unique resource to propel the use of hCOs in delineating the regulatory logic of local TH action in human brain development.

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### OP-13-68

#### ABSTRACT WITHDRAWN

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# Poster Presentations

Saturday, 10th September 2022

## Poster Session 1: COVID &amp; Thyroid Disease

## PS1-01-01

**Autoimmune and inflammatory thyroid diseases following SARS-CoV-2 vaccines: an update from a systematic review of the literature**Solange Grunenwald<sup>1</sup> & Philippe Caron<sup>2</sup><sup>1</sup>Chu Larrey, Service D' Endocrinologie, Department of Endocrinology and Metabolic Diseases, Chu Larrey, Toulouse Cedex 9, France; <sup>2</sup>Chu Larrey, Endocrinology, Toulouse Cedex 9, France

Since the emergence of the Covid-19 pandemic in 2019, a massive vaccination campaign has been undertaken around the world, and SARS-CoV-2 vaccine-induced thyroid diseases became more frequently described in the literature. Subacute thyroiditis is reported in 52 patients, mean age  $45.5 \pm 1.8$  years, mainly in women ( $n = 39$ ), after the first (62%) or the second (38%) dose of mRNA (60%), inactivated whole-virus (25%) and adenoviral-vectored (13.5%) vaccines. The mean time to onset is  $9.0 \pm 0.8$  days, and the most frequent symptom is neck pain (97%). Thyrotoxicosis is confirmed by increased free T<sub>4</sub> ( $30.0 \pm 2.8$  pmol/l), free T<sub>3</sub> ( $34.3 \pm 10.8$  pmol/l) concentrations, with high ESR ( $53 \pm 3$  mm/hour) and CRP ( $105 \pm 14$  mg/l), heterogeneous thyroid gland with hypochoic areas and decreased blood flow, decreased uptake on thyroid scan, and in rare patients on post-surgical pathology ( $n = 1$ ) or on cytology after FNA ( $n = 5$ ). Patients were initially given NSAIDs (52%) and/or oral glucocorticoids (48%), 10% patients are followed without treatment. In most patients, thyroid function returns to normal and subacute thyroiditis does not relapse. Graves' disease is more frequent in women ( $n = 22$ ) than in men ( $n = 10$ ), mean age  $46.2 \pm 2.6$  years. Hyperthyroidism is reported as new onset, recurrent or exacerbation of well controlled disease, after the first (62%) or the second dose (34%) of mRNA (72%) or adenoviral-vectored (28%) vaccines. Mean time to thyrotoxicosis onset is  $15.1 \pm 2.6$  days and patients present palpitations (53%), weight-loss (34%), tremor (22%). Thyrotoxicosis is confirmed by increased free T<sub>4</sub> ( $43.3 \pm 4.0$  pmol/l), free T<sub>3</sub> ( $39.0 \pm 20.1$  pmol/l) concentrations, with positive anti-TSH-receptor or thyroid stimulating immunoglobulins, markedly increased uptake of the radiotracer activity and increased vascularity of normal sized or enlarged thyroid gland. Patients are treated with beta-adrenergic blockers (32%), antithyroid drugs (89%), and 11% have no treatment. The discussed underlying pathogenic mechanisms of SARS-CoV-2 vaccine-induced thyroid diseases are molecular mimicry (SARS-CoV-2 proteins sharing a genetic homology with a large heptapeptide human protein) or autoimmune/inflammatory syndrome induced by adjuvants (ASIA), usually occurring in genetically susceptible individuals. The benefits of SARS-CoV-2 vaccination far weight the potential vaccine-induced side effects, but clinicians should be aware of possible thyroid adverse-effects, and can advise patients to seek medical assistance when experiencing anterior neck pain, fever or palpitations after SARS-CoV-2 vaccination. Further studies are warranted to clarify the clinical features, predisposing factors, management, and to investigate the etiopathogenesis of SARS-CoV-2 vaccine-induced thyroid diseases.

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## PS1-01-02

**Post covid-19 complication in patient with chronic autoimmune thyroiditis**Iryna Kostitska<sup>1</sup>, Iryna Cherniavska<sup>1</sup> & Antonina Piddubna<sup>2</sup><sup>1</sup>Ivano-Frankivsk National Medical University, Endocrinology, Ivano-Frankivsk, Ukraine; <sup>2</sup>Bukovinian State Medical University, Endocrinology, Alergology and Immunology, Chernivtsi, Ukraine

## Introduction

The information on prognosis morbidity chronic autoimmune thyroiditis and relationship with coronavirus disease 2019 (COVID-19) is lacking but endocrine involvement has been increasingly reported. The symptoms of thyroid's dysfunction can be due to relationship after SARS-CoV-2 infection. In addition, the patients with COVID-19 leading to an increased risk of post COVID-19 complications of chronic autoimmune thyroiditis. As such, post COVID-19 complications outcomes among people with autoimmune disorders remain poorly understood.

## Case report

We present clinical case of patient with chronic autoimmune thyroiditis with severe hypothyroidism which developed few weeks after resolution of COVID-19 infection. We discuss clinical presentation, diagnostic evaluation and principle of treatment of post COVID-19 complication in patient with Hashimoto thyroiditis. A 58-year-old female with a past medical history of chronic autoimmune

thyroiditis diagnosed two years ago after COVID-19 pneumonia who later manifested hypothyroidism. She was diagnosed with COVID-19 infection with nasopharyngeal reverse transcriptase polymerase reaction (RT-PCR) at an outpatient clinic 68 days ago. An IgG against SARS-CoV-2 was positive. After tree weeks for the treatment of COVID-19 pneumonia the patient complaining of worsening depression, dry skin, hair loss extreme and fatigue. Laboratory examinations showed significant increased for thyroid-stimulating hormone (TSH) 108 mIU/l (range 0.27–4.2), free thyroxine (T<sub>4</sub>) level 0.01 ng/dL (range 0.93–1.7), anti-thyroid peroxidase antibody > 800 IU/ml (normal less than 34) and anti-thyroglobulin antibodies > 1000 IU/ml (normal less than 4.0). After additional examination the patient with Hashimoto thyroiditis was diagnosed severe hypothyroidism. The patient was prescribed treatment with levothyroxine a starting dose of 25 µg/day followed by titration at a daily dose of 175 µg. After 3 months, the therapy resulted in patient's improvement of the general conditions and compensation of hypothyroidism (TSH- 4.1 mIU/l, free T<sub>4</sub> – 1.0 ng/dL).

## Conclusions

Our clinical case suggests that the temporal relationship between post COVID-19 complications and the severe hypothyroidism manifestations in the patients with Hashimoto thyroiditis. Further studies are needed to clarify the link between combined effects of SARS-CoV-2 infection on the thyroid gland and the immune system.

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## PS1-01-03

**Incidence of subacute thyroiditis and autoimmune thyroid disease during COVID-19 pandemic**Tim Medved<sup>1</sup>, Nastja Medle<sup>2</sup> & Simona Gaberscek<sup>3</sup><sup>1</sup>Faculty of Medicine, University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; <sup>2</sup>Faculty of Medicine, University of Ljubljana, Slovenia; <sup>3</sup>Faculty of Medicine, University of Ljubljana, University Medical Centre Ljubljana, Department of Nuclear Medicine, Slovenia

## Objectives

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is causing coronavirus disease (COVID-19), enters host cells via the angiotensin-converting enzyme 2 receptor. Its expression is higher in thyroid gland than in lungs. In the literature, an association between SARS-CoV-2 infection and subacute thyroiditis/autoimmune thyroid disease has been implicated. Therefore, we aimed to explore the influence of COVID-19 pandemic on the incidence of subacute thyroiditis, Hashimoto's thyroiditis and Graves' disease.

## Methods

In our retrospective study we reviewed medical records of all patients who were referred for the first time to our thyroid department from 1 April 2019 to 31 May 2019 (before COVID-19) and from 1 April 2020 to 31 May 2020 (during COVID-19). Our institution has a stable catchment area of 1,000,000 inhabitants. Therefore, number of new cases may be considered the incidence of the disease. In each patient, thyroid specialists performed clinical examination and thyroid ultrasound. Levels of thyrotropin (TSH), free thyroxine, free triiodothyronine, thyroid peroxidase antibodies, thyroglobulin antibodies and, if applicable, TSH receptor antibodies as well as sedimentation rate were measured.

## Results

In the two months period before COVID-19, we examined 946 patients (224 men/722 women) with the mean age 52.0 19.0 years, and in the two months period during COVID-19, we examined 576 patients (154 men/422 women) with the mean age 53.7 18.3 years. Between the two periods, patients did not differ with respect to sex and age ( $P = 0.201$  and  $P = 0.438$ , respectively). Before COVID-19, we found 8 patients with subacute thyroiditis (0.8% from all in that period), while during COVID-19, we found 10 patients with COVID-19 (1.7% from all in that period). The incidence of subacute thyroiditis did not differ significantly between the two periods ( $P = 0.189$ ). Before COVID-19, we diagnosed 435 (46% from all) patients with Hashimoto's thyroiditis and 51 (5.4% from all) patients with Graves' disease, while during COVID-19, we diagnosed 273 (47.4%) patients with Hashimoto's thyroiditis and 22 (3.8% from all) patients with Graves' disease. Incidence of Hashimoto's thyroiditis and Graves' diseases did not differ significantly between the period before and during COVID-19 ( $P = 0.629$  and  $P = 0.205$ , respectively).

## Conclusions

Although we diagnosed absolutely and relatively more patients with subacute thyroiditis during COVID-19 than before, the results were not statistically significant and cannot be easily attributed to SARS-CoV-2 infection. A longer observation period would probably yield different results. In addition, we did not find any difference in the incidence of Hashimoto's thyroiditis and Graves' disease during COVID-19 pandemic.

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**PS1-01-04****Subacute thyroiditis: The severity depends on the causative agent and the treatment requires a lower glucocorticoid dose than that recommended by the guidelines**

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Subacute thyroiditis (SAT) is an inflammation of the thyroid gland characterized by varying degrees of severity regarding thyrotoxicosis, inflammation and the incidence of hypothyroidism. The present study aims to identify whether the severity of SAT depends on the causative agent involved and to identify what is the optimal therapy. To this purpose we retrospectively evaluated 402 patients referred to of the University Hospital of Pisa because of a SAT from January 2011 to December 2020. 32 patients did not receive pharmacological treatments, 16 were treated with non-steroidal anti-inflammatory drugs (NSAIDs) and 354 with glucocorticoids; among these the most common initial dose ( $n = 286$ ) was 25 mg of prednisone and most patients were treated for 90 days ( $n = 277$ ). 88 patients experienced definitive hypothyroidism. Patients with higher levels of free thyroxine (FT4), C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and thyroglobulin (Tg) experienced more frequently hypothyroidism at the end of inflammatory process. Patients treated with glucocorticoids experienced a shorter duration of neck pain (14 days,  $P < 0.001$ ) than patients treated with NSAIDs (28 days) and those not treated (28 days). The more adequate prednisone's starting dose to obtain neck pain remission was 25 mg/day. The third quarter of the year was characterized by the highest number of cases compared to the other quarters in all years except in 2012 and 2015, when there was a high incidence of cases in the first quarter of the year, and in 2020 where most of the SAT cases occurred in the second and fourth quarters. In these same quarters SAT was more severe with regard thyrotoxicosis, inflammatory indices and incidence of hypothyroidism. In a previous study we observed that SAT clusters in 2020 occurred within a month of the Covid-19 waves. Therefore, similarly we have evaluated the Italian epidemiological situation of the winter seasons 2011-12 and 2014-15, in which an anomalous circulation of the influenza virus A.H3N2 emerged; therefore it is might be responsible for SAT clusters in the first quarter of 2012 and 2015. We conclude that the differences about the severity of SAT depend on the different agent responsible and glucocorticoid therapy for SAT is effective even at lower doses than recommended by the latest guidelines.

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**PS1-01-05****Subacute thyroiditis and COVID-19 vaccination**

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**Aim**

The aim of this study is to find out whether Subacute Thyroiditis may occur after Covid-19 Vaccination.

**Materials and methods**

This investigation was designed and held in "Kanakaner-Zeytun" Medical Center and "Muratsan" University Hospital. 25 women (age:  $35 \pm 7$ ) with proved diagnoses of Subacute Thyroiditis were included in the study. The detailed history of disease was collected in order to understand how many of them survived Covid-19, whether they have been vaccinated and which vaccine had been done.

**Results**

It has been found that 18 from 25 patients had been vaccinated via vaccines of Chinese production. 7 participants had not been vaccinated at all. 7 from 18 patients furtherly had symptoms of Covid-19 and also developed Subacute Thyroiditis. Although 11 patients (61%) had not any complain after vaccination, during 4-6 weeks clinically Subacute Thyroiditis had appeared.

**Conclusion**

61% of vaccinated patients with Subacute Thyroiditis developed Covid-19 without any significant clinical symptom. 28% of vaccinated patients had Covid-19 with huge manifestation of different symptoms. Vaccinated patients who developed painful swallowing, hoarseness, tenderness when gentle pressure is applied to the thyroid gland, elevated temperature, fatigue, feeling weak must be observed in order to exclude Subacute Thyroiditis after Covid-19.

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**PS1-01-06****The peculiarities of the subacute thyroiditis treatment course during the COVID-19**

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**The Purpose of The Research**

The aim of the study is to reveal whether there are any peculiarities of treatment of the subacute thyroiditis, which was caused by Covid-19.

**Materials and Methods**

The research has been conducted in "Muratsan" Hospital Complex and Armenian-American Wellness Center during 2020-2021. Among forty five patients who participated in the study were women about  $35 + 10$  years old and all of them were diagnosed with subacute thyroiditis with various degrees of severity after Covid-19. All of them underwent a general blood test: TSH, FT4, ultrasound of the thyroid gland. A Glucocorticoid treatment was prescribed - Prednisolone 30-40 mg in  $\frac{2}{3}$  dose in the morning,  $\frac{1}{3}$  dose at 12:00-13:00pm. Afterwards, within 2-3 months the dose was gradually reduced and removed.

**Results**

After the withdrawal of the prescription the 22 (45%) out of the 45 patients had a recurrence of the disease, moreover 8 (36,4 %) of them had the recurrence of the disease 3-4 times. New inflammatory of the disease foci have appeared in the thyroid gland. As the Glucocorticoid treatment was not effective and 50% of them had recurrence, it was decided to add Arbidol to the treatment according to the following regimes: Arbidol 200 mg 1 piece x 2 for 5 days, 1 piece x 1 for 5 days. The results were unexpected. All the patients had stable remission.

**Conclusion**

In case of subacute thyroiditis caused by Covid-19, it is more preferable to add antiviral drugs to the Glucocorticoid treatment in order to avoid the recurrence. After the Covid-19 viremia persists in the blood and it may cause a subacute thyroiditis (6-8 weeks later).

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**PS1-01-07****The course and outcome of subacute thyroiditis: A retrospective analysis and predictive model**

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**Background**

Subacute thyroiditis (SAT) is a destructive thyroiditis which is presumably caused by a viral infections or post-viral inflammatory response. Thyroid dysfunction evolves through a set of stages (hyperthyroidism - hypothyroidism - euthyroidism) and is usually temporary, although some patients may develop permanent hypothyroidism. The risk factors for permanent hypothyroidism remain unclear.

**Methods**

A retrospective analysis of patients with SAT of the University Hospital of Brussels from 2001 to 2020. Firstly, a description of the patient characteristics, inflammatory and thyroid parameters is provided. Secondly, a predictive model for the need to initiate thyroid hormone therapy (THT) is developed by means of a logistic regression analysis. The optimal model configuration was selected by a forward sequential analysis maximizing the classification accuracy while the prediction performance was validated by a leave-one-out cross validation.

**Results**

35 patients were included, with a female predominance (ratio 4.8/1). Thyrotoxicosis was detected in the majority of patients (91%), while hypothyroidism developed in 71% (of which 27% subclinical). Thyroid replacement therapy was initiated in approximately half of patients (18/35). The discontinuation of THT was attempted in 10/18 patients, and was successful in most of these (8/10). The logistic regression model selected the predictors Age, Season and Regimen, and demonstrated a maximum accuracy of 86%, classifying 30 of 35 patients correctly in the outcome measure for what the model fit is concerned, while during a leave-one-out cross validation the accuracy is 74% predicting 28 of 35 patients correctly.

**Conclusion**

THT was initiated for hypothyroidism in half of the patients, which is higher than previously reported. A scoring model, applicable in clinical practice to identify patients who may benefit from THT, was developed.

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**PS1-01-08****Graves' orbitopathy and subacute thyroiditis related to SARS-CoV-2 infection or vaccination: the experience of a single centre in Milan, Italy**  
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**Background**

The Covid-19 pandemic caused by the severe-acute-respiratory-syndrome-coronavirus-2 (SARS-CoV-2) determined millions of deaths worldwide, thus at the end of 2020 a massive vaccination campaign has been launched. SARS-CoV-2 infection and vaccines have been associated with several thyroid disorders, especially subacute thyroiditis (SAT), Graves' disease (GD) and orbitopathy (GO). We studied the occurrence of SAT and GO following SARS-CoV-2 infection or vaccination in our Centre.

**Methods**

We recorded all consecutive cases of SAT (new diagnosis) from February 2020 and GO (new diagnosis or sudden worsening) from June 2021 onwards, noting if occurred within 1 month after SARS-CoV-2 infection or vaccines.

**Results**

Up to March 2022 we have recorded 25 SAT and 28 GO. The onset of SAT occurred within 1 month from SARS-CoV-2 vaccination in 8/25 (32%) and from SARS-CoV2 infection in 4/25 (16%). Among the 28 GO, 11 (39%) occurred within 1 month from SARS-CoV2-vaccination (9 new diagnosis and 2 worsening) and 1 from SARS-CoV2-infection (new diagnosis). Interestingly, 5 (18%) had a GO onset apparently unrelated to SARS-CoV2-vaccination, however had developed GD hyperthyroidism within one month from it. The 19 patients developing SAT or GO after SARS-CoV-2 vaccination had received Pfizer ( $n = 12$ ), Moderna ( $n = 3$ ) or AstraZeneca ( $n = 4$ ); symptoms developed following the first, second or third dose in 8 (42%, mean +13 days), 5 (26%, mean +17 days) and 6 (31% mean +10 days) cases, respectively. A previous documented SARS-CoV-2 infection several months before the vaccination had occurred in 1/19 patients (5%). The mean age of patients was  $54 \pm 18.17$  years (range 21-83 years) and females were 14/19 (73%). A previous history of thyroid disease was present in 3/19 (16%): one subclinical hypothyroidism, one euthyroid nodular goitre, one euthyroid Hashimoto's thyroiditis. A family history of thyroid disorders was present in 10/19 (52%) patients.

**Conclusions**

SARS-CoV-2 vaccines seem to be associated with the onset of SAT and the onset or worsening of GO. Possible mechanisms involve the interaction of the spike protein with the ACE-II receptor expressed in thyroid tissue, a cross-reactivity of the spike protein with thyroid self-proteins or an immune reaction induced by adjuvants (ASIA syndrome). Many patients had a positive family history for thyroid disorders, thus a genetic predisposition is likely involved. Until more safety data about SARS-CoV-2 vaccines will be available, caution and strict monitoring of injected individuals is suggested, especially those predisposed to thyroid disorders or autoimmunity.

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**PS1-01-09****Subacute thyroiditis after SARS-CoV-2 vaccine**

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**Introduction**

Subacute thyroiditis (SAT) is an inflammatory disorder of thyroid associated with viral infections. Rarely, cases of SAT have also been described post-vaccination, but there are not many reports related to exposure to the COVID-19 vaccine. Here, we present a case of subacute thyroiditis after receiving the first dose of a SARS-CoV-2 vaccination inactivated COVID-19 vaccine (Sinovac-CoronaVac, developed by Sinovac/China National Pharmaceutical Group).

**Case report**

A 36-year-old male presented to our outpatient endocrinology clinic with complaints of anterior neck pain with irradiation to right ear, fever and fatigue. In his past medical history, he had mild Covid-19 infection in October 2020 and did not report any past medical history for thyroid disease or preceding upper respiratory system infection. He has received his first dose of COVID-19 vaccine 5 days previously. On his physical examination his heart rate was 90/min; body temperature was 37.5 C. On palpation, the thyroid gland was painful and enlarged. The nasopharyngeal swab polymerase chain reaction (PCR) test for SARS-CoV-2 done twice was negative. At laboratory investigations, his thyroid function test was compatible with subclinical hyperthyroidism thyroid stimulating hormone - 0.1 IU/ml (normal range: 0.39-4.2 IU/ml); free thyroxine -1.65 ng/ml (normal range 0.8-2.0 ng/dl). Thyroid autoantibody levels were normal: anti-thyroglobulin antibody - 1.2 IU/ml (normal <4 IU/ml), thyroid peroxidase antibody - 3 IU/ml (normal <20 IU/ml) and anti-TSH receptor antibody - 0.1 IU/l (normal range 0-1.5 IU/l). Levels of erythrocyte sedimentation rate (ESR) and C-reactive protein were elevated (ESR -38 mm/h; CRP - 30 mg/l). Thyroid ultrasound revealed unilateral focal hypoechoic areas with decreased blood flow. Based on clinical symptoms and laboratory examinations, the patient's diagnosis was considered to be subacute thyroiditis. He was advised to rest, and nonsteroidal anti-inflammatory (NSAIDs) drugs were prescribed; however, the symptoms persisted. Methylprednisolone 24 mg was initiated, and his symptoms rapidly improved after medication.

**Conclusion**

Post-vaccination SAT cases have so far been rarely reported despite mass vaccination programs in all countries. Roughly 58% of the world population has been already fully vaccinated against covid-19, and there are only a handful number of cases of SAT reported. The development of thyroiditis may occur within a few days, as opposed to more days in case of post-viral illness. Clinicians need to be aware that there is a possibility of SAT post-vaccination and must consider this as a differential diagnosis in a patient presenting with anterior neck pain or fever with a recent vaccination history for adequate patient management and cure.

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**Hypothyroidism****PS1-02-10****Modern study of life quality of women with subclinical hypothyroidism**

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**Objective**

To study the life quality of patients with subclinical hypothyroidism by using questionnaire SF-36 before and after replacement therapy.

**Methods**

All patients were collected at the Department of Endocrinology 3rd clinics of Tashkent Medical Academy. The study included 55 women with subclinical hypothyroidism and 20 women for the control group. The age surveyed had an average of  $37.6 \pm 8.83$  years. Patients included in the study do not take replacement therapy with thyroid hormones. All patients were divided into 2 groups: group 1 included 55 patients with subclinical hypothyroidism and the 2nd group of 30 women without thyroid disease. All patients had a test that assessed the quality of life with questionnaire SF-36 and ultrasound investigation of the thyroid gland (USI), conducted research analysis: thyroid hormone-binding free T4, TSH, and anti-thyroid peroxidase antibody.

**Results**

The first group of patients with subclinical hypothyroidism were obtained test results: TSH -6.9 free T4- 14.2, TPOAb - 87.6, thyroid gland volume V-24.8  $\text{cm}^3 \pm 7.8$  and a second group members TSH -1.7 mU/l free T4-15.6 pmol/l and TPOAb -18.4. After replacement therapy in the first group, TSH and TPOAb levels were 1.9 mEd/l and 24.6 respectively. Average thyroid gland volume in the first and second groups was V-19.8  $\text{cm}^3 \pm 4.3$  and V-14.6  $\text{cm}^3 \pm 3.6$  respectively. The most common cause of subclinical hypothyroidism was Hashimoto's thyroiditis with 83, 6%. In 26, 6% members of the second group were diffuse enlargement of the thyroid gland I degree. Practically on all scales of the questionnaire SF-36, quality of life parameters in patients with compensated hypothyroidism was significantly lower, than in the group of healthy women. This study shows the relationship between increased rates of TSH and the deterioration of the quality of life in women with subclinical hypothyroidism.

**Conclusion**

In patients with subclinical hypothyroidism in almost all parameters the quality of life is worse than in women without thyroid disease, especially scales are worse, than in healthy women. While comparing the quality of life in patients with hypothyroidism the rates of role physical functioning, vitality, social functioning,

and psychological health of patients with hypothyroidism were significantly less compared with women with euthyroid goiter.

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### PS1-02-11

#### Ventricular extrasystoles as a consequence of hypothyroidism

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#### Background

Hypothyroidism is a clinical condition caused by a deficiency of thyroid hormones or a decrease in their biological activity. Premature ventricular contractions (PVCs) are early depolarizations of the myocardium originating in the ventricles. Rhythms with frequent and apparently idiopathic PVCs are considered to be a benign condition that can be managed with conservative measures. Beta blockers are considered to be quite effective.

#### Objective

To demonstrate the positive effect that the compensation of hypothyroidism had on the "treatment" of PVC.

#### Methods

The study, which lasted for six months, involved 73 women (aged 19y-53y), who were diagnosed with PVCs after 48 hours of Holter monitoring. In addition, TSH and FT4 levels in the blood were checked. TSH levels were ranging from 3.7-6.2 IU/ml, however FT4 levels were normal, therefore the patients were also diagnosed with hypothyroidism. They were treated with Levothyroxine exclusively (no Beta blockers were prescribed). Medication doses were calculated individually based on the patient's body weight.

#### Result

Approximately 15 days after the start of Levothyroxine administration, the patients started experiencing significantly less tachycardia. Moreover, according to the second Holter monitoring, which was performed after 2 months, VPCs were either absent or considerably decreased.

#### Conclusion

We can conclude from this research that besides compensating hypothyroidism, Levothyroxine is highly likely to also "treat" benign ventricular extrasystoles.

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### PS1-02-12

#### Characteristics of specialists delivering clinical care for hypothyroidism in europe: the "THESIS" study (treatment of hypothyroidism in europe by specialists: an international survey)

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#### Objectives

Some hypothyroid patients are critical of their care, although this appears to vary between countries. The quality of thyroid care delivered, and the perceived patient experience may be influenced by characteristics of specialists such as age, sex, experience, and workplace setting. Here, we aimed to explore geographical differences in thyroid specialist characteristics.

#### Methods

THESIS is a large European questionnaire survey of thyroid specialists treating patients with hypothyroidism. Participants were reached via the endocrine societies of 26 European countries plus Israel and Turkey. The questionnaire included questions about respondent demographic characteristics. Geographic

regions were defined according to the UN Statistics Division definition (Eastern Europe, Western Europe, Northern Europe, Southern Europe, Western Asia). Data on gross national income per capita (GNIPC) in US dollars from the World Bank were used.

#### Results

A total of 16,733 invitations yielded 6,058 responses (93% endocrinologists). The median response rate was 43.5% and differed significantly between countries (6.8%-95.2%,  $P < 0.01$ ). The mean age, the proportion of female respondents, and GNIPC were not associated with response rates. The proportion of females ranged between 36.6-93.9% (median 63.7%). The lowest proportion of females was in Northern Europe (45.6%) and the highest in Eastern Europe (77.2%). The proportion of females differed between regions (moderate association,  $P < 0.001$ , Cramer's  $v$  0.21). GNIPC was inversely associated with the proportion of female respondents ( $P < 0.001$ ,  $r^2=0.42$ ). An inverse correlation was noted between respondent age and proportion of females (Cochran-Armitage test for trend,  $P < 0.001$ , two-sided). The sample included 794 (16.2%) respondents working exclusively in private practice. The distribution of respondents in private practice differed between countries with a strong association ( $P < 0.001$ , Cramer's  $v$  0.40). Decreasing GNIPC correlated with an increasing proportion of respondents working privately ( $P < 0.011$ ,  $r^2=0.23$ ). The proportion of respondents who reported treating over 100 hypothyroid patients/year was 62.1%. As GNIPC increased the percentage of respondents who treated more than 100 patients/year decreased ( $P < 0.01$ ,  $r^2=0.36$ ).

#### Conclusion

Differences in characteristics of specialists who treat hypothyroid patients exist both at the country and the regional levels, with the greatest differences noted between Northern and Eastern Europe. The most significant determinant for these differences is GNIPC, although other factors, not addressed in this survey (organization of healthcare, culture) may also play a role. These differences may influence the management of hypothyroidism and patient satisfaction.

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### PS1-02-13

#### Sex hormones levels and chronic autoimmune thyroiditis in a cohort of obese male patients

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#### Introduction and Aim

Obesity is associated with an increased risk of hypothyroidism and chronic autoimmune thyroiditis (AIT). However, little is known about the underlying pathogenic mechanisms. Recently it has been evaluated the role played by sex hormones in the onset of AIT and, analysing the data from a large cohort of normal/overweight males, a positive correlation between an increased ratio of estradiol/testosterone levels (E2/T) and AIT was found. Since obese males are more likely to have decreased testosterone and increased circulating estradiol levels, we investigated whether there was a causal relationship between an imbalance of the E2/T ratio and AIT in obese male patients.

#### Patients and Methods

We retrospectively evaluated anthropometric data (weight, height, BMI), thyroid assessment (thyroid ultrasonography and TSH, FT3, FT4, TgAb, TPOAb levels) and gonadal hormones [total testosterone (T), estradiol (E2), gonadotropins] of 337 obese male patients (age:  $50 \pm 11.5$  years, BMI:  $46 \pm 7.6$  Kg/m<sup>2</sup>). AIT was defined in different ways: the serum TPOAb and/or TgAb positivity ( $>100$  IU/mL) and/or characteristic US features (diffuse parenchymal hypoechoogenicity and/or heterogeneous echogenic pattern of the thyroid gland) and/or hypothyroidism.

#### Results

AIT prevalence in our cohort was 6.5%. The prevalence of AIT did not correlate with age ( $P = 0.9$ ), BMI ( $P = 0.9$ ), hypogonadism ( $P = 0.7$ ), E2 ( $P = 0.1$ ) and T ( $P = 0.4$ ) levels, while an increased E2/T ratio was observed in subjects with AIT, although this difference was not statistically significant ( $P = 0.07$ ). By ROC curve analysis (AUC 0.614; CI 95%:0.51-0.73,  $P = 0.045$ ) we identified a E2/T ratio cut-off value significantly associated with AIT: 63.6% of subjects with E2/T  $\geq 18.8$  had AIT while this was observed only in 36.4% of subjects with a E2/T ratio  $< 18.8$  ( $P = 0.035$ ). This cut-off also predicted the absence of AIT with an excellent diagnostic accuracy (96% NPV). Then we carried out a multivariate statistical analysis and the E2/T ratio  $\geq 18.8$  was confirmed as a parameter independently associated with AIT (OR 2.80; CI 95%:1.17-7.21,  $P = 0.024$ ).

#### Conclusions

Our results suggest that higher E2/T ratios were significantly associated with AIT among obese male patients. Since estrogens and androgens play an important role

in autoimmunity due to their capacity of modulating the immune response via estrogen and androgen receptors, such mechanisms could provide a possible explanation for the correlation between the imbalance of the physiological E2/T ratio and the higher prevalence of AIT observed in obese male patients.

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## PS1-02-14

### A case of late-onset dys-hormonogenic goiter with hypothyroidism due to a homozygous mutation of SLC26A7 gene

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#### Introduction

Congenital hypothyroidism (CH) is the most common neonatal endocrine disorder, affecting up to one in 1500 to 2000 newborns, if mild forms of hypothyroidism with eutopic and normal-sized thyroid gland are included. It is caused by either dysgenesis or dys-hormonogenesis. Recently a novel iodide transporter, SLC26A7 (a member of the SLC26 transporter family), whose dysfunction affects thyroid hormonogenesis in humans, has been identified. The main purpose of this study is to present a case of dys-hormonogenic CH due to a homozygous mutation of SLC26A7 gene (c.1883delC, p.P628Qfs\*11), which has never been described before.

#### Case Report

Here we report a case of a 19-year-old young Tunisian male, who presented with severe hypothyroidism and a voluminous diffuse goiter appeared 2 months after his arrive in Italy at the age of 18. No other signs and symptoms of hypothyroidism, mental retardation or delayed growth were observed. Neck US confirmed the presence of diffuse goiter and no nodules or lymphadenopathies were documented. Autoimmune and infiltrative thyroid diseases were excluded and no iatrogenic/toxic causes were detected. The perchlorate discharge test showed a partial organification defect and a hormone replacement therapy was started, leading to an overt reduction of the goiter size (thyroid volume from 93.98 ml to 41.43 ml). With LT4 therapy, a rapid increase of serum FT3 compared to serum FT4 levels was observed. After stopping LT4 therapy for 2 weeks, 1 mg of iodine was administered but worsening of hypothyroidism was observed. NGS analysis showed a not yet identified homozygous mutation of SLC26A7 gene (c.1883delC, p.P628Qfs\*11), which results into a stop codon in position 639 and the synthesis of a truncated protein.

#### Conclusions

In literature, most of CH patients with homozygous mutations of SLC26A7 were detected by neonatal screening or within the first years of life. We describe the first case of CH due to a homozygous mutation of SLC26A7 gene diagnosed during late adolescence, when the patient was 19 years old. Although the neonatal screening test was not available, the absence of intellectual retardation and his harmonic growth suggest a late onset of hypothyroidism. The administration of 1 mg of iodine for 2 weeks did not correct hypothyroidism. We suppose that other environmental factors or genetic polymorphisms of other genes involved in thyroid hormone synthesis might influence the transport of iodine into the lumen of the thyroid follicles and might have a role on the timing of onset and on severity of hypothyroidism.

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## PS1-02-15

### Use of iodine in the treatment of congenital hypothyroidism with an *in situ* thyroid gland and of non-autoimmune subclinical hypothyroidism

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#### Introduction

Congenital hypothyroidism (CH) with *in situ* thyroid gland (GIS) and non-autoimmune subclinical hypothyroidism (NASI) are functional defects of thyroid gland occurring at birth and after birth, respectively. In recent years, a higher incidence of these disorders has been documented. The etiology remains unclear,

with only an almost 50% of cases attributable to mutations in known dys-hormonogenesis-associated or TSH-receptor genes. Although replacement therapy with levothyroxine (L-T4) is the treatment of choice for hypothyroidism, previous studies have reported an improvement/normalization of thyroid function in some cases of dys-hormonogenic CH after iodine administration.

#### Objective

The objective of this study was to evaluate the effect of treatment with physiological doses of iodine in a group of children with CH and GIS and NASI.

#### Patients and Methods

34 children, 17 with CH and GIS (mean age 10.22 ± 3.15 years) and 17 with NASI (mean age 10.70 ± 4.36 years) were given iodine for 9 months, after stopping for 4 weeks L-T4, when taken. 3 children with CH and 1 with NASI had mutations of DUOX2, whereas the etiology was unknown in the remaining children. Iodine treatment was initiated with a daily dose of 50 µg, increasing to a maximal 150 µg/d. At the beginning of the study, all patients presented serum TSH between 4 and 12 µU/ml, free thyroid hormones within the normal range, undetectable anti-TG and anti-TPO antibodies and a thyroid of normal size and normoechoic pattern at ultrasound. The same parameters were evaluated every 3 months for 12 months.

#### Results

Treatment with iodine failed to normalize TSH. Under the lowest dose of iodine, TSH did not change as compared to the baseline in both groups. After increasing the daily dose, there was a progressive increase in serum TSH in both groups, that became statistically significant ( $P = 0.038$ ) when basal TSH values of whole group of patients were compared with those after 150 mg/d of iodine. Other parameters of thyroid function did not change after treatment in both groups. None of children developed serum and ultrasound markers of thyroid autoimmunity.

#### Conclusions

This study shows a failure of treatment with physiological iodine doses to correct CH with GIS and NASI. These results, which are apparently in contrast with the data reported in the literature, are probably due to the lower doses used in our study. Moreover, the increase of serum TSH levels observed during treatment may reflect the spontaneous course of the disease rather than a detrimental effect of iodine.

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## PS1-02-16

### Evolution of congenital hypothyroidism with *in situ* thyroid gland in children and adolescents: Clinical and biochemical features at diagnosis and after retesting

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#### Background

In recent years, increasing cases of congenital hypothyroidism (CH) with *in situ* thyroid gland (GIS) are identified. Outcome of children affected from CH with normally sited thyroid of normal size is still unknown. The objective of our study is to describe the natural history of this specific form of CH.

#### Methods

We retrospectively evaluated clinical, biochemical and instrumental data of 89 patients with diagnosis of CH and GIS, referred to our center after positive neonatal screening. After 3 years of age, 48 patients performed a clinical reassessment after withdrawal of levothyroxine therapy (L-T4), through biochemical evaluation with thyroid function profile (TSH, FT3, FT4), imaging evaluation with neck ultrasound and, in most cases, a scintiscan with 123-I and perchlorate discharge test. We evaluated the need for L-T4 therapy at retesting and during follow-up.

#### Results

In the first year of follow-up, 15 patients showed a transient CH. Among the other 74 patients, 48 performed clinical reassessment: 10 had overt hypothyroidism (20,8%), 20 showed hyperthyrotropinemia (41,7%) and 18 were euthyroid (37,5%) after L-T4 withdrawal for 4 weeks. 32 patients performed a scintiscan with 123-I and perchlorate discharge test: 6 patients presented a partial iodine organification defect, while 4 patients had a total iodine organification defect. 28 children (58,3%) resumed therapy immediately after clinical reassessment, while 20 (41,7%) suspended it. Follow-up data after retesting (median duration of 10,36 years) were available in 44 patients. Among children who had suspended therapy at retesting, 4 resumed therapy during follow-up, while in the group of children who had resumed therapy at retesting, 9 suspended it. At the end of follow-up, 22 patients (50%) were untreated and 22 (50%) were still taking L-T4 therapy. We

observed no statistical differences between children who suspended or continued L-T4, in first serum TSH levels, sex ratio, or birth weight. Serum TSH at clinical reassessment showed a significant difference between two groups ( $P < 0.01$ ).

#### Conclusions

Over a third of patients with CH and GIS had a normal thyroid function off L-T4 therapy when retested after 3 years of age. During subsequent follow-up, half of patients underwent to suspension of L-T4 therapy because of normal thyroid function. Therefore, a clinical reassessment after 3 years of age should be performed to avoid unnecessary prolonged treatment. However, it is not possible to predict whether these subjects will need therapy again, so long-term follow-up studies are needed to better understand natural history of disease.

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### PS1-02-17

#### Isolated hypothyroxinemia - not only in pregnancy

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Isolated hypothyroxinemia is rarely and briefly mentioned in medical textbooks and seldom discussed at endocrine conferences. It is most often, but not exclusively encountered in pregnancy. The author presents cases of isolated hypothyroxinemia encountered at a private endocrine practice in Budapest, Hungary and a specialized medical practice in Berlin, Germany. In the last seven years the author encountered about thirty cases: app. 60% in women who were 17-85 years old and app. 40% in men who were 50-86 years old. Women with isolated hypothyroxinemia in fertile age had a long history of infertility, miscarriage, primary and/or secondary amenorrhea and psychiatric symptoms. Most of these patients responded well to thyroxin supplementation and their symptoms improved or disappeared. The majority of patients over 60 years of age did not show any symptoms. They were predominantly closely followed and received thyroxin supplementation only in a few cases. In summary, isolated hypothyroxinemia seems to be a rare entity which is easily dismissed. In women of fertile age isolated hypothyroxinemia should receive more attention. An fT4-value below the reference range should not be regarded automatically as laboratory error even if the TSH is adequate.

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### PS1-02-18

#### Human cerebral organoids as *in vitro* platform to assess thyroid hormone system disrupting chemicals

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Thyroid hormones (TH) play a fundamental role in brain development. Changes on TH availability during pregnancy and early childhood can lead to neurological disorders. Accordingly, concerns are mounting that exposure to environmental compounds capable of interfering with TH action can disrupt neuronal

development. Current approaches to categorize chemicals as thyroid hormone system disrupting chemicals (THSDC) are mainly based on determination of changes in circulating TH concentrations *in vivo*. Since such tests are not adequate to capture alterations at cellular and molecular levels during human brain development, there is an urgent need to establish and validate human *in vitro* models for THSDC assessment. Human cerebral organoids (hCOs) derived from induced pluripotent stem cell (hiPSC) present a promising model system as hCOs recapitulate tissue complexity and critical developmental processes while providing an infinite source of material. We used single cell hCO transcriptome data to extract lists of TH-responsive genes to be used as molecular markers of TH action in RT-qPCR assays. Since hCOs show dynamic changes in cell composition during their development, these lists included broad response genes as well as cell type-specific markers. We analyzed expression patterns of marker genes following acute 48 h treatment of hCOs at different stages with T3 (3-300 nM). We further characterized expression profiles following co-exposure of hCOs to T3 and the MCT8 inhibitor silychristin (SC) or the pan-deiodinase inhibitor iopanoic acid (IA). In addition, we established a sensitive LC-MS/MS method to determine basal TH concentrations in media and the TH metabolite pattern (rT3, 3,3'-T2; 3,5-T2; 3-T1; T0) following TH treatment and co-exposure experiments. RT-qPCR assays of whole hCOs showed significant gene expression changes in a TH concentration-dependent manner for most of the selected marker genes. SC co-treatment attenuated the T3-induced expression response for a subset of genes (i.e., CADM2, DBP and DIO3) in acute T3 treatment schemes. IA co-treatment enhanced T3-induced expression levels in longer-term T3 treatment schemes. Cell type composition (progenitors, neurons) had a clear influence on T3-induced expression responses as evident from comparison of T3 effects in early and late stage hCOs. hCOs effectively metabolize T3 to 3,3'-T2. Notably, metabolic degradation of T3 was reduced upon co-treatment with either SC or IA. Thus, these reference compounds affected both molecular markers and local T3 metabolism. Our study demonstrates the utility of hCOs as a promising platform for THSDC assessment, identifies critical aspects (validation, endpoints, reference compounds) and highlights pitfalls for the assay validation process.

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## Thyroid Cancer CLINICAL 1

### PS1-03-19

#### Is isthmus tumor location in papillary thyroid carcinoma a high-risk factor in comparison with tumor located in thyroid lobes?

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#### Introduction

Thyroid isthmus has lack of normal parenchyma, and it connects both of thyroid lobes with lymphatic drainage. It is known that isthmus papillary thyroid cancer (PTC) presents frequent extrathyroidal extensions (ETE), multifocality and lymph node involvement. Therefore, many surgeons prefer total thyroidectomy and central compartment neck dissection (CCND) for the radical treatment of PTC located in thyroid isthmus. The purpose of this study is to figure out the clinical implication of isthmus tumor location in PTC compared with PTC located in unilateral thyroid lobe.

#### Materials and Methods

A total 1500 patients with PTC who underwent total thyroidectomy and lymph node dissection were reviewed. 160 were isthmus PTC patients (isthmus cancer group). 1340 were patients with PTC located in unilateral thyroid lobe and 1:5 propensity score matching in age, sex and tumor size were performed. Finally, 800 patients (matched-unilateral cancer group) were selected to compare with isthmus cancer group.

#### Results

After matching, the median follow up period of total patients was 122 months. 10-year cumulative RFS rate were 99% for isthmus cancer group and 96% for matched-unilateral cancer group ( $P = 0.260$ ), respectively. There were no differences in age, sex, tumor size, ETE, tumor multifocality and the number of metastatic lymph node between two groups. However, the ratio of patients with lateral neck node metastasis was lower in isthmus cancer group ( $P = 0.032$ ) and it mainly occurred bilaterally ( $P < 0.001$ ) in comparison with matched-unilateral cancer group. The tumor location of isthmus did not increase the risk of ETE, lymph node metastasis and tumor multifocality in multivariate analysis. It did not worse RFS in Cox regression, neither.

## Conclusion

The isthmus location is not an independent risk factor in papillary thyroid carcinoma, therefore, it is necessary to reconsider the preference of total thyroidectomy in thyroid isthmus cancer.

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## PS1-03-20

### Changes in clinical status after second <sup>131</sup>I treatment in patients with differentiated thyroid cancer (dTC) not cured with the initial treatment

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## Objectives

Currently <sup>131</sup>I treatment after surgery is suggested in selected intermediate-risk and in high-risk patients with DTC. In patients with radio-avid structural disease and in those with biochemical disease a second <sup>131</sup>I treatment could be considered. However data about the impact of the second <sup>131</sup>I treatment on clinical status remains controversial.

## Methods

Clinical data of 231 DTC patients followed at the Unit of Endocrinology of the University Hospital of Pisa, who experienced the second <sup>131</sup>I treatment for indeterminate/biochemical (BiR) or structural incomplete response (StR), were collected.

## Results

Most of patients were females (64.9%). Median age was 41 and median tumor size was 2.1 cm. Most of cases were pT1 according to TNM 8<sup>th</sup> edition (45.5%) and pN1 disease accounted for 51.5% of the cases. CV-PTC was the most frequent histology (59.3%). Multifocality and bilaterality were frequently observed (77.7%), like mETE (70%); while in 32.4% of the cases vascular invasion was diagnosed. All patients were submitted to the first <sup>131</sup>I treatment either with low (61%) or high (39%) <sup>131</sup>I activities. Eight patients (3.5%) performed neck surgery for lymph node metastases before second <sup>131</sup>I treatment and, for this reason, were excluded from the study. Therefore 223 patients performed a second <sup>131</sup>I treatment: 65.5% (n = 146) because of BiR and 34.5% (n = 77) because of StR, either for radio-avid disease or for positive neck ultrasound. Regarding BiR patients (n = 146) the second <sup>131</sup>I treatment led to excellent response (ExR) in 13.7%, while BiR persisted in 64.4%. In the remaining 32 patients (21.9%) a StR was detected, either because radio-avid (40.4%) or for the identification of lymph node metastases by neck ultrasound (59.4%). Conversely in patients who performed the second <sup>131</sup>I treatment for an initial StR (n = 77), 6.5% showed an ExR, 15.6% a BiR and 77.9% a persistent StR. During the follow-up, overall, 58.3% (n = 130) experienced further treatments, while 41.7% (n = 93) were followed-up without any other treatment. After a median follow-up of 9.6 years, ExR accounted for 22.9%, BiR for 48.8% and StR for 28.7% of all study group.

## Conclusions

Only 11.2% of patients can be cured by a second <sup>131</sup>I treatment. Other treatments, any type, performed in about 60% of patients, increased the percentage of cured patients up to 23%, during a follow-up of about 10 years.

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## PS1-03-21

### Risk of structural recurrence in differentiated thyroid carcinoma (DTC) patients without evidence of disease after initial treatment: insights into risk factors and comparison with american thyroid association guidelines

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## Background

The last American Thyroid Association guidelines (ATA-2015) defined the risk of structural recurrence in patients with DTC cured after initial treatment and according to the initial histology. However, these data resulted from several studies including different patients with different kinds of treatment. We retrospectively evaluated a large population of patients with DTC, treated and followed in a tertiary referral center, with the aim to characterize the rate of structural recurrence, the clinical-pathological factors involved in the recurrence and to compare our results with those proposed by the ATA-2015.

## Patients and Methods

We evaluated epidemiologic and clinical-pathologic data of 1331 consecutive patients between January 2010 and September 2012, followed at the Operative Unit of Endocrinology of the University Hospital of Pisa. Data were collected at the time of first <sup>131</sup>I treatment after surgery for all patients, and thereafter during the follow-up according to the standard of care.

## Results

At the first control after <sup>131</sup>I treatment (median time 8 months), 36 patients (2.7%) were lost at follow-up and 82 patients (6.2%) showed a structural incomplete response and for this reason were excluded from the study. In the remaining (n = 1213) who showed an excellent, indeterminate, or biochemical incomplete response, only 34 patients (2.8%) (Group A) showed a structural recurrence during the follow-up (median 7 years). Compared to the patients who did not show any structural recurrence (Group B), patients of group A showed more frequently larger tumor size (> 4 cm), aggressive histology, minimal extrathyroidal extension, vascular invasion and a more advanced TNM stage. When compared with ATA-2015, the overall recurrence rate in our group is significantly lower, both if considering the histologic ATA-2015 categories and when classifying patients in three groups according to initial histology and rate of recurrence reported (≤ 5%, 6-20%, > 20%).

## Conclusions

In a large population of DTC patients without evidence of structural disease after the initial treatment, treated and followed with a uniform modality over time, the structural recurrence rate is a rare event (2.8%). However, several clinical and histologic factors were significantly associated with the risk of structural recurrence. The recurrence rate, in this series was lower than that proposed by ATA-2015. One possible reason is that these patients were followed-up at the same tertiary referral center with the same clinical strategy.

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## PS1-03-22

### Low risk differentiated thyroid carcinoma (DTC) can be safely treated with thyroidectomy alone: real-life experience in a medium-long term follow-up

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## Background

Since 2006 according to the European consensus for the management of patients with DTC, in tumors ≤ 1 cm, treatment with thyroidectomy alone without <sup>131</sup>I treatment was suggested. Therefore, in our institution, we applied this suggestion in clinical practice, although prospective studies were not still available. Aim of this study is to evaluate the rate of the events occurring in a medium-long term follow-up.

## Patients and Methods

We evaluated clinical-pathologic data of 378 consecutive DTC patients at low risk of recurrence treated with total thyroidectomy, without lymph node dissection and <sup>131</sup>I treatment after surgery, between 2006 and 2012. Events during the follow-up were defined as structural if abnormal findings at neck US appeared and were cytologically confirmed. Biologic events were defined in TgAb negative patients if LT4-Tg increased >5 ng/ml or >2 ng/ml in two consecutive evaluations, or TgAb appeared; conversely in TgAb positive patients if TgAb increased > 50% in two consecutive evaluations and if the increasing trend over time was constant.

## Results

Females accounted for 75.4% of our study group median age was 50 (IQR 40.75-59). Mean tumor size was 0.45 ± 0.27 cm. Most of patients had a unifocal T1a tumor (98.9%), and 73.3% had CV-PTC, 24.1% FV-PTC, 2.4% aggressive variants of PTC and in 1 case an FTC was diagnosed. After a median follow-up of 7.7 years, no structural events occurred. Regarding biologic events, 16/378

(4.2%) were highlighted, 12 (3.2%) for increase in LT4-Tg values and 4 (1.1%) for the increase in TgAb values, over time. Mean detection time of biologic events was 41 months (median 17 months). No patients performed additional treatments, surgery or <sup>131</sup>I treatment and currently were followed-up with active surveillance. Conclusions

In a real-life experience in the management of low-risk DTC we obtained similar results than the recently reported prospective ESTIMABL2 trial, although with a mean tumor size slightly smaller, but in longer follow-up time. These data confirmed that also in a medium-long term follow-up, low-risk DTC, particularly PTC, treated with total thyroidectomy without <sup>131</sup>I treatment showed an excellent outcome, without any additional treatment performed over time.

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### PS1-03-23

#### Comparison between minimal and gross ETE for risk of recurrence in papillary thyroid carcinoma: a propensity score matching study

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#### Background

The presence of extrathyroidal extension (ETE) is associated with locoregional recurrence and distant metastases in papillary thyroid carcinoma (PTC). ETE is classified into gross ETE (gETE) and minimal ETE (mETE). The mETE was recently excluded from the T3 category in the TNM staging system. The purpose of this study is to compare the recurrence risk of mETE and gETE in PTC patients through propensity score matching analysis. And a comparison in the same way for papillary thyroid microcarcinoma (PTMC) in subanalysis was done.

#### Methods

We assessed 4452 patients with DTC who underwent thyroid surgery from January 2009 to December 2015 at Seoul St. Mary's Hospital (Seoul, Korea). Clinicopathological characteristics and long-term oncologic outcomes between mETE and gETE in PTC were compared using propensity score matching to reduce selection bias. The mean follow-up duration was 122.7 ± 22.5 months.

#### Results

There was no statistically significant difference in recurrence when comparing mETE group and gETE group before propensity score matching ( $P = 0.072$ ). In multivariate Cox regression analysis, mETE and gETE were not associated with an increased risk of recurrence. After propensity score matching, there was no statistically significant difference in recurrence when comparing mETE group and gETE group ( $P = 0.668$ ). Moreover, gETE did not show statistical significance compared with mETE in univariate and multivariate Cox regression analysis. Lymphatic invasion features and positive lymph nodes were shown as independent risk factors for tumor recurrence. Similar results were shown in PTMC subanalysis.

#### Conclusions

There was no statistically significant difference in tumor recurrence and DFS between the mETE and gETE groups after propensity score matching. The results of this study suggest that, like gETE, PTC patients with mETE should be carefully followed up because of equal risk of recurrence. Furthermore, we might reconsider the T staging system classification.

Keywords: Minimal ETE, Gross ETE, Papillary thyroid carcinoma, Recurrence risk, Propensity score matching

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### PS1-03-24

#### Central lymph node ratio predicts prognosis of N1b papillary thyroid cancer

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#### Background

To date, no staging system has been related to the number of metastatic lymph node or lymph node ratio. Lymph node ratio (LNR) was defined as the number of

metastatic LNs divided by the total number of LNs dissected. The purpose of this study was to determine how central LNR had a prognostic value to predict recurrence in N1b papillary cancer.

#### Methods

This study included 331 patients who underwent total thyroidectomy with lateral neck dissection for N1b papillary thyroid cancer at Seoul St. Mary's Hospital between January 2012 and December 2017. The mean follow-up duration was 82.1 ± 20.4 months. Hazard ratios of the cut-off LNR values for cancer recurrence were calculated for relevant covariates using multivariate Cox regression analyses. Kaplan-Meier analyses were also utilized to assess the effects of estimated LNR cut-off values on disease-free survival (DFS).

#### Results

The patients enrolled was divided into two groups according to central lymph node ratio, and the cut-off value of 0.56 was determined by ROC curve. Age, sex, tumor size, lymphatic invasion, vascular invasion, the number of positive LNs, T stage, TNM stage, and recurrence were factors that statistically relevant to central LNR. Multivariate Cox regression analyses revealed that central lymph node ratio higher than 0.56 was an independent prognostic factor for recurrence in N1b papillary thyroid cancer (hazard ratio [HR]: 6.177, 95% confidence interval [CI]: 1.763-21.639,  $P = 0.004$ ). In addition, tumor size was independently prognostic for recurrence (hazard ratio [HR]: 1.951, 95% confidence interval [CI]: 1.140-3.340,  $P = 0.015$ ). Kaplan-Meier analyses demonstrated that higher LNR of central compartment with cut-off value of 0.56 reduced disease-free survival (log-rank  $P < 0.001$ ).

#### Conclusion

Central LNR was an independent prognostic factor in N1b PTC patients.

Keywords: Papillary thyroid cancer, central lymph node, lymph node ratio, Disease-free survival

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### PS1-03-25

#### Low-intermediate risk thyroid cancer: no benefit of postsurgical iodine?

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#### Objectives

Evaluate the benefit of postsurgical radioactive iodine (RAI) in patients with differentiated thyroid cancer (DTC) at low to intermediate risk of recurrent disease in whom not routine use of RAI is recommended

#### Methods

We retrospectively evaluated 1316 patients with DTC diagnosed between 2009-2015. ATA low (LR) and intermediate (IR) risk were included in the study (PTC: tumor size > 1 cm, microscopic extrathyroidal invasion, <5 microscopic N1 (< 2 mm), >5 N1 of <3 cm, aggressive histology, vascular invasion; FTC minimally invasive with tumor size > 1 cm). These patients were categorized into either treated or untreated with I-131. The response to therapy was evaluated by ongoing ATA risk stratification system. Treatment effect was assessed by inverse-probability weighted regression adjustment (IPWRA), by balancing the distribution of factors influencing treatment assignment between I131-treated or untreated patients.

#### Results

A total of 469 patients (119 males and 350 females) were selected. The mean age at diagnosis was 46.1 ± 14.3 years old. 328 patients (69.9%) were treated with I131 while 141 (30.1%) untreated. In the overall group, biochemical or structural disease was observed in 44 (9.4%) patients after a median time of 17.5 months from the diagnosis. Persistent/recurrent disease was more frequent in I131-treated than in untreated patients (12.5% vs 2.1%, respectively,  $P < 0.001$ ). This result was in line with the bias that I131-treated patients displayed a more aggressive pathology at diagnosis. By the inverse-probability weighted regression adjustment (IPWRA) analysis, the estimated percentage of recurrent disease was 10% (95% CI = 6.3-12.9%) in I131-treated and 16% (95% CI = 11.1-20.71%) in untreated patients ( $P = 0.02$ ). Hence, if all patients would be treated with I131, the estimated risk of relapse would be reduced by 40% (RR = 0.6; 95% CI = 0.40-0.92,  $P = 0.018$ ). Multivariate logistic regression analysis identified

as factors independently associated with persistent/recurrent disease: pN1 (OR = 3.56; 95% CI 1.55-8.17), male sex (OR = 2.45; 95% CI 1.12-5.36) and microscopic extrathyroidal extension (OR = 3.28; 95% CI 1.5-7.15).

#### Conclusions

Radioiodine administration in LR and IR is frequently defined on a case-by-case basis as suggested by retrospective studies. To make the outcome conditionally independent of the treatment assignment, in our matching method we observed that treatment with I131 in low/intermediate DTC reduces the absolute risk of persistent/recurrent disease by 6%. Only prospective clinical trial may definitively answer the question whether consider or not consider I131 therapy for low-to-intermediate DTC. Until then, a careful evaluation of the whole range of "aggressive" features of the tumor should guide RAI decision making.

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### PS1-03-26

#### Age as a prognostic factor in ata low and intermediate risk thyroid cancer patients

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#### Background and Aim

American Thyroid Association (ATA) guidelines do not consider age at diagnosis of differentiated thyroid cancer (DTC) as a prognostic factor in the estimation of risk of recurrence and persistent disease. While age at diagnosis has already been assessed in high-risk patients, it remains to be established whether there is a correlation between age at diagnosis and long term outcome in DTC patients.

#### Methods

We retrospectively evaluated 863 DTC patients with a median follow-up of 10 years, 52% of them classified as low risk (449/863) and 48% as intermediate risk (414/863). For each ATA-risk class patients were divided into subgroups based on age at diagnosis (<55 or ≥55 years).

#### Results

Age had no impact on clinical outcome of ATA low risk patients. Intermediate risk patients ≥55 years had a higher risk of recurrence ( $P = 0.0125$ ), death ( $P = 0.013$ ) and worse long term outcome ( $P = 0.006$ ) at univariate analysis. Multivariate analysis confirmed the impact of age on mortality together with T stage in intermediate risk patients (OR = 5.35, 95% IC 1.44-25.55,  $P = 0.02$  and OR = 13.34, 95% IC 3.74-54.2,  $P < 0.01$  for age and T stage, respectively). Age ≥55 years was the only independent risk factor associated with recurrences in intermediate risk patients (OR: 5.04, 95%IC 1.31-24.18,  $P = 0.02$ ). Age at diagnosis was not confirmed as a long term outcome predictor at multivariate analysis in intermediate risk patients, where only T stage was significantly associated with final outcome (OR: 5.45, 95%IC 2.63-11.21,  $P < 0.001$ ).

#### Conclusion

Age at diagnosis is a predictor of recurrence and death only in ATA intermediate risk patients. This finding suggests that age at diagnosis should be considered as an additional feature to improve the initial risk stratification.

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### PS1-03-27

#### Clinical significance and prognosis of solid / trabecular component areas of papillary thyroid carcinoma

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#### Introduction

the presence of areas with a solid / trabecular growth pattern in differentiated papillary thyroid carcinoma (PTC) represents a source of controversy regarding clinical and prognostic significance and usually requires a more aggressive

therapeutic approach. The aim of this study was to compare clinicopathological characteristics, treatments and prognosis of PTC with and without solid component (SC). We also aimed to evaluate whether SC affects patient outcome in the absence of aggressive features.

#### Material and methods

We studied 300 patients with PTC with follow-up for more than 5 years. All PTC with any percentage of SC were included. Poorly differentiated thyroid carcinoma was excluded from this analysis. Study data were analyzed using the Chi-square and Student's T-test. Global survival (GS) and disease-specific survival (DSS) were analyzed using Kaplan-Meier survival curves.

#### Results

There were 300 cases of PTC, 99 (33.0%) with SC and 201 (67.0%) without SC (mean follow-up time 9.5 years). Mean tumor size was larger in the presence of SC ( $35.2 \pm 17.5$  vs  $25.3 \pm 16.2$  mm, respectively for PTC with SC and PTC without SC,  $P < 0.001$ ). A SC was not associated with a higher prevalence of extrathyroidal extension (27.3% vs 28.9%,  $P = 0.775$ ) or locoregional invasion (4.1% vs 4.5%,  $P = 0.100$ ). Vascular invasion was more frequent in PTC with SC (43.4% vs 18.9%,  $P < 0.001$ ). The prevalence of lymph node metastases was similar between the two groups (24.2% vs 24.9%,  $P = 0.905$ ), but distant metastases were more frequent in the group of PTC with SC (19.2% vs 8.5%,  $P = 0.007$ ). Total activity of radioactive iodine (RAI) was significantly higher in PTC with SC ( $P = 0.001$ ). Disease recurrence rates were similar between both groups (12.1% vs 10.9%,  $P = 0.763$ ). At last follow-up, GS and DSS were similar in patients with and without SC (93.9% vs 98.0%,  $P = 0.084$ ; 99.0% vs 99.5%,  $P = 0.549$ , respectively). A subanalysis excluding the cases with vascular invasion showed no differences in the percentage of distant metastases, OS and DSS between the groups. However, RAI was significantly higher in the group with SC ( $P = 0.026$ ).

#### Conclusion

Clinicopathologic features were similar between PTC with and without SC. There was a higher prevalence of vascular invasion in PTC with SC which might explain the higher incidence of distant metastasis in this group. However, the presence of SC was not related to less favorable prognosis or lower survival rates. In the absence of features of aggressiveness, the clinical impact of the SC alone is negligible.

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### PS1-03-28

#### Cardiometabolic risk factors in a cohort of algerian thyroid cancer survivals

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#### Objectives

Cardiovascular disease encountered the leading cause of death in thyroid cancer survivals exceeding death by cancer itself. Etiological factors are the therapy used and the duration of exposure to this it, the diagnosis of cancer itself, or the factors that led to this cancer. We aim to evaluate cardiometabolic risk factors in an Algerian cohort of thyroid cancer survivals.

#### Methods

The study includes a cohort of patients followed in the thyroid cancer register in the endocrinology department of Constantine University Hospital during the period between January 2020 and June 2021. Patients have received a complete examination in addition to an ECG and a fasting blood sample including fasting blood sugar, complete lipid profile, TSH, and FT4.

#### Results

33 patients have been included, mean age was 47 years (28-70), mean age at diagnosis of cancer was 42(25-64), 91% were women, type of cancer was 63.6% for papillary thyroid cancer and 27.3% of follicular variant of papillary thyroid cancer, 3% were follicular cancer and 6.1% were NIFTP. All patients underwent total thyroidectomy. Concerning TNM classification; 75.7% were T1 including 33.3% of T1a, 15% were T2 and 3% were T3, 15% were N1. According to the 8 AJCC staging system, 97% were stage 1 and 3% were stage 2. In accordance with the modified 2009 American Thyroid Association (ATA) risk stratification system 72.7% were at low risk, 21.2% at intermediate risk, and 6.1% at high risk. 60% of patients have received Radioiodine therapy. Concerning cardiometabolic risk factors, 36.4% had hypertension which was diagnosed after thyroid cancer surgery in 18.2%. 21.2% were diabetic or prediabetic with the diagnosis made after surgery in 6.2% of patients, dyslipidemia was diagnosed in 48.5% of patients and 78.8% of patients were overweight or obese. Just one patient was a current smoker.

#### Conclusion

In this cohort of Algerian thyroid cancer survivals, the risk of death by cancer is low in most cases, however, cardiometabolic risk factors are prevalent and more attention for cardiovascular prevention is needed for these patients.

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**Thyroid Hormone transporters and development****PS1-04-29****Cell-specific function of the thyroid hormone transporters MCT8 and oatp1c1 in murine brain barrier cells**

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Mice with combined deficiency in the thyroid hormone transporters Mct8 and Oatp1c1 (Mct8/Oatp1c1 dko mice) display a strongly diminished TH brain content and, consequently, a disturbed neuronal maturation and myelination as well as locomotor abnormalities while serum T3 levels are highly elevated. This phenotype can be explained by an impaired transport of T4 and T3 into the CNS in the absence of both transporters. Yet, the exact cell-specific function of Mct8 and Oatp1c1 in brain barrier cells remains to be investigated. To address this question, we generated mice lacking Mct8 and/or Oatp1c1 specifically in endothelial cells (= Endo del mice) by crossing conditional Mct8 flox and Oatp1c1 flox animals with mice expressing cre-recombinase under the constitutively active Tie2 promoter. In order to eliminate Mct8/Oatp1c1 specifically in astrocytes in the adult CNS (= Astro del mice), we took advantage of a Tamoxifen-inducible Aldh1l1 CreERT2 line and crossed them with conditional Mct8/Oatp1c1 flox mice. Both Mct8/Oatp1c1 Astro del as well as Endo del mice were phenotypically indistinguishable from their wildtype littermates. Monitoring Cre recombinase activity with a YFP reporter construct confirmed the cell-specific cre-mediated recombination in astrocytes and endothelial cells, respectively. Likewise, Mct8 protein expression was not affected in choroid plexus epithelial cells as well as in tanycytes excluding off-target effects. Mct8 and Oatp1c1 mRNA expression further was studied by fluorescence *in situ* hybridisation (FISH). Indeed, in both mouse models, a decreased mRNA expression for both TH transporters could be observed. To evaluate the functional outcome of the respective genetic modification, Mct8/Oatp1c1 Astro del mice were subjected to locomotor tests and revealed only mild coordination deficiencies in beam walk test whereas Mct8 Endo del mice showed a normal locomotor performance. To assess the cell-specific TH status in different brain cells, a first set of FISH experiments was conducted and already revealed normal TRH transcript levels in hypothalamic PVN neurons of Mct8/Oatp1c1 Astro del mice whereas TRH expression was found to be highly elevated in Endo del mice. Currently, further FISH studies are ongoing that include a variety of different TH-regulated target genes in different neural cell types and are expected to provide further information regarding the cell- and brain area-specific TH status. By this approach, we ultimately aim to clarify the function of Mct8 and/or Oatp1c1 in astrocytic vs endothelial TH transport.

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**PS1-04-30****Impact of thyroid hormone transport on the hippocampal gabaergic and glutamatergic system**

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Patients with inactivating mutations in MCT8, known as Allan-Herndon-Dudley syndrome (AHD), present a severe form of psychomotor retardation and, frequently, epileptic seizures of unknown etiology. These neurological symptoms are thought to arise due to an impaired transport of thyroid hormones (TH) across the blood brain barrier and/or into the neural cells. As a consequence of species-specific differences in the expression of the T4-specific organic anion transporter polypeptide 1c1 (Oatp1c1), the AHD pathology is only replicated in Mct8/Oatp1c1 double knockout (M/O-dKO) mice. Here, we address the question whether alterations in the hippocampus, a brain area central in epilepsy disorder, underpin the seizure susceptibility of MCT8 patients. To this end, we first characterize the inhibitory GABAergic system and the excitatory glutamatergic system in the hippocampus at different postnatal stages using the M/O-dKO mouse model. At P12, immunofluorescence studies showed a severe decrease in Gad67 levels, a general GABAergic marker, in M/O-dKO mice. Likewise, numbers of GABAergic neurons positive for parvalbumin were strongly reduced, while there was a significant increase in somatostatin positive interneurons in M/O-dKO mice. These cellular changes are transient as at P120, no differences were visible in M/O-dKO mice any longer. In contrast, qPCR analysis of hippocampal homogenates at P12 and P120 revealed an increased expression of GABA transporters and GABA transaminase in adult M/O-dKO animals, indicating increased GABA reuptake and metabolism, respectively. On the other hand, the expression levels of kainite receptor subunits such as GluR6 or

KA1 were increased in adult M/O-dKO mice, suggesting an abnormal glutamate signaling in the hippocampus. Together, these results point to an altered development of both the inhibitory and the excitatory system that can potentially impact seizure susceptibility in the absence of Mct8 and Oatp1c1.

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**PS1-04-31****The role of type 3 deiodinase in a human model for early brain development**

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**Background**

Disrupted thyroid hormone (TH) homeostasis has devastating effects on human neurodevelopment. THs are critical signaling molecules in neurodevelopment, acting on differentiation of neural cells, migration, synaptogenesis and myelination, with deiodinases governing intracellular TH concentrations in a spatiotemporal manner. It is remarkable that fetal neural cells, while being key target cells of TH, exhibit strong activity of the TH inactivating enzyme DIO3. Currently, the molecular mechanisms underlying TH action in brain are mainly derived from animal models. We utilized human induced pluripotent stem cell (hiPSC) technology to investigate the role of DIO3 in a human model for early brain development.

**Methods**

We generated neural progenitor cells (NPCs) and neural networks from hiPSCs as a model for early human brain development. hiPSCs-derived neural cells contained all the key players in TH cellular signaling. We inactivated DIO3 in different neural cells using iopanoic acid (IOP), a small molecule that blocks DIO3 activity. Cells were cultured with different T3 concentrations (0-3-10 nM). We used immunocytochemistry, gene expression and DIO3 and metabolism assays as readouts.

**Results**

We observed a high DIO3 activity in NPCs and neural networks, being the highest in NPCs. Inactivation of DIO3 caused increased gene expression of T3-dependent genes such as *KLF9* in neural progenitor cells and neural networks. We also examined the differentiation potential of neural progenitor cells to neural networks in absence of DIO3. Our preliminary immunocytochemistry data showed an increase in cells containing neuronal nuclear protein (NeuN), a biomarker for neurons, when the activity of DIO3 is diminished.

**Conclusion**

Our preliminary results suggest that impaired DIO3 activity may lead to excessive TH action in neural cells and therefore compromising normal brain development. We are currently investigating further to validate this findings. We also aim to inactivate DIO3 through genetic modification using the CRISPR interference technology. Our model represents a versatile tool to investigate cellular TH regulation and action for early human brain development.

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**PS1-04-32****Three-dimensional spheroids: a new approach for the identification and characterization of novel markers for tumor-initiating cells subpopulations in thyroid cancer**

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Thyroid cancer (TC) is the most common endocrine malignancy, with an increasing number of diagnosis in the last decades. Of all TC histotypes, the

poorly differentiated and anaplastic TCs (PDTC and ATC) have a very poor prognosis because of their invasiveness and metastatic behavior, as well as their insensitivity to radioactive-iodine treatment. In the last years, the stem-cell model has been proposed to further explain TC carcinogenesis. According to this model, only a subset of cancer cells, identified as Cancer Stem-like Cells (CSCs) or Tumor Initiating Cells (TICs), give rise to progenitor cells that may drive tumor growth. These cells exert stem-like properties, tumorigenic potential and the ability to grow as non-adherent spheres. An intriguing candidate for the identification of putative TICs in TC, especially for the most dedifferentiated forms, is EpCAM (epithelial cell adhesion molecule), a transmembrane glycoprotein that is highly expressed in TICs of other tumors of epithelial origin, playing a role in balancing cell proliferation and differentiation. We are aiming to investigate the biology of putative thyroid TICs, by *in vitro* characterization of thyrosphere-forming cells. We've developed a standardized thyrosphere model, based on PDTC and ATC cell lines displaying different genetic background. The main methodologies applied to obtain the 3D cultures are the hanging-drop and coating with poly(2-hydroxyethyl methacrylate) non-adhesive substrate. In appropriate growth condition, all the cell lines tested were able to generate thyrospheres when seeded at clonal density. We applied the ELDA web-tool to estimate the frequency of TICs with potential stem-like properties able to generate the 3D spheres, and we observed the highest stem cell frequency on spheres derived from FRO (ATC cell line). The screening of eight cell lines by Western Blot revealed that only FRO express EpCAM. Immunofluorescence on FRO-derived 3D spheres have shown an increase in EpCAM cleavage according to a radial gradient, and a variable expression of E-cadherin, a typical epithelial marker, in EpCAM-expressing cells. Moreover, immunofluorescence on cryosections of healthy and pathological patient-derived tissue samples have shown homogeneous expression and cleavage of EpCAM in the healthy epithelium, while a highly heterogeneous distribution is seen in tumor sections. To conclude, EpCAM displays a distinct expression among TC cell lines and human tissues, and appears to correlate with the ability to generate 3D spheres *in vitro*. This thyrosphere model is a promising approach to better investigate the biology of aggressive TCs, including the sensitivity of thyroid TICs to different anticancer drugs.

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#### PS1-04-33

##### Transcriptomic signature of human embryonic thyroid reveals transition from differentiation to functional maturation

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The human thyroid gland acquires a differentiation program as early as weeks 3–4 of embryonic development. The onset of functional differentiation, which manifests by the appearance of colloid in thyroid follicles, takes place during gestation weeks 10–11. By 12–13 weeks functional differentiation is accomplished and the thyroid is capable of producing thyroid hormones although at a low level. During maturation, thyroid hormones yield increases and physiological mechanisms of thyroid hormone synthesis regulation are established. In the present work we traced the process of thyroid functional differentiation and maturation in the course of human development by performing transcriptomic analysis of human thyroids covering the period of gestation weeks 7–11 and comparing it to adult human thyroid. We obtained specific transcriptomic signatures of embryonic and adult human thyroids by comparing them to non-thyroid tissues from human embryos and adults. Remarkable upregulations of signaling growth factors such as IGF1 and FGF, already found to be involved in animal models of thyroid development, as well proteins involved in the cAMP pathway regulation and activity were highlighted in this transcriptomic analysis of human fetal/embryonic thyroids. This gives clues to explain how this pathway, crucial for the differentiation of thyroid cells, is activated in the absence of TSH during embryonic/fetal development. We defined a non-TSH (thyroid stimulating hormone) dependent transition from differentiation to maturation of thyroid. The study also sought to shed light on possible factors that could replace TSH, which is absent in this window of gestational age, to trigger transition to the emergence of thyroid function. We propose a list of possible genes that may also be involved in abnormalities in thyroid differentiation and/or maturation, hence leading to congenital hypothyroidism.

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#### PS1-04-34

##### The dawning of deiodinases: an outer ring deiodinase activity in the social amoeba *Dictyostelium discoideum* with high affinity for reverse t3

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##### Background

Conservation of genes involved in TH transport, metabolism and action can reveal clues about the origins of TH signaling. A deiodinase homologue (*DdDio*) was previously identified in the social amoeba *Dictyostelium discoideum* (Singh, 2014). *Dictyostelium* cells live as single cell amoebae in soil where they feed on bacteria. However, upon starvation a developmental program is initiated that results in the formation of a multicellular fruiting body consisting of a stalk of dead cells that supports a sporehead in which 80% of the cell population survives as spores. *DdDio* knock-out cells are disturbed in aggregation and developmental gene expression, indicating a potential role for *DdDio* in development. In this study we determined whether *Dictyostelium* has deiodinating activity towards iodothyronines.

##### Methods

Lysates from *Dictyostelium* cells in the early culmination phase were prepared and measured for deiodinase activity using several radiolabeled iodothyronines as substrate.

##### Results

We found limited iodine release with T3 and T4, but efficient outer ring deiodination of rT3, 3,3'-T2 and 3',5'-T2. We did not detect any inner ring deiodination. For rT3 as substrate, we found a Vmax of 1.2 (± 0.1) pmol/min.mg total lysate protein, a Km of 21 (± 2) nM, and an IC50 of 19 (± 0.5) nM in cis-inhibition studies. The *Dictyostelium* deiodinase activity is dependent on DTT as a co-factor and fully inhibited by 0.1 mM iopanoic acid, but not by 0.1 mM PTU.

##### Conclusion

Our results show that the *Dictyostelium* deiodinase has high affinity and activity towards certain iodothyronines. To our knowledge, this is the most distant species from humans in which iodothyronine deiodination has been found to date, pushing the root of iodothyronine metabolism back to at least 1 billion years. Future studies will have to reveal whether iodothyronines are produced and have signaling functions in *Dictyostelium*. Singh *et al. Dev Biol* 2014, 396: 256-68

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#### PS1-04-35

##### Spatiotemporal expression of the thyroid hormone transporter MCT8 during cortical neurogenesis in human cerebral organoids

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Thyroid hormones (TH) play a critical role during nervous system development and patients carrying coding variants of *THRA* or *SLC16A2/MCT8* genes present a spectrum of neurological phenotypes presumably resulting from perturbed local TH action during early brain development. Monocarboxylate transporter MCT8 is a well-characterized TH membrane transporter and MCT8 mutations have been associated with the Allan-Herndon-Dudley Syndrome. Recent advances in stem cell biology allow for generation of human cerebral organoids (hCOs) from induced pluripotent stem cells (hiPSC). By recapitulating many aspects of human neocortex development, hCOs provide a tractable model to probe local TH action during cortical neurogenesis. The primary aim of this study was to map the spatiotemporal expression of MCT8 during hCO differentiation. hCOs were generated from healthy hiPSC lines and cultured for up to 10 weeks to a stage

corresponding to human cortex development at midgestation. The developmental expression profile of MCT8 protein was analyzed by immunofluorescence staining of cryosections prepared from hCOs of different stages. Expression of *SLC16A2* mRNA was analyzed by single molecule fluorescent *in situ* hybridization (smFISH) and quantitative RT-PCR. Already along with the initial formation of rosette-like structures containing neuronal progenitors, MCT8 protein was detectable in SOX2+/Nestin+ radial glia cells (RGCs). During subsequent development of the ventricular zone, MCT8 immunostaining in RGCs became enhanced in apical endfeet at the ventricular surface. MCT8 expression was also detected in EOMES+ intermediate progenitors and HOPX+ outer RGCs located in the inner and outer subventricular zone, respectively. In addition to the diverse progenitor cell types, we detected robust MCT8 protein expression in TBRI1+/CTIP2+ deep layer neurons and at later developmental stages in SATB2+ upper layer neurons. The spatial expression of *SLC16A2* mRNA across cortical cell layers (detected by smFISH) was highly concordant with the MCT8 protein expression profile. When comparing the spatial expression profile of *SLC16A2* and *THRA* mRNAs, we observed enhanced *THRA* probe staining in the cortical plate zone and smFISH confirmed that excitatory neurons express much higher levels of *THRA* mRNA than neuronal progenitors. Our study shows that MCT8 is expressed at comparable levels in both neuronal progenitors and excitatory neurons during early cortical neurogenesis in hCOs whereas *THRA* mRNA is clearly expressed at higher levels in neurons compared to progenitors. The similarity of expression profiles in hCOs and human embryonic cortex tissue suggests that hCOs provide a promising system for studies on neuronal effects resulting from targeted disruption of MCT8 function.

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### PS1-04-36

#### Thyroid hormone signaling in a human cellular model for early brain development

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#### Background and Objective

Disrupted thyroid hormone (TH) signaling has devastating effects on human neurodevelopment. The molecular mechanisms underlying TH regulation and action are largely based on animal models. However, animal models are limited in revealing some of the most fundamental aspects of neurodevelopment that are unique to humans. We employed human induced pluripotent stem cell (iPSC) technology to study the effects of T3 on neurodevelopmental processes in a human model for fetal brain development.

#### Methods and Results

We differentiated iPSC-derived neural precursor cells into 2D neural networks, consistent 60:40 ratio of neurons to glia and mimicking fetal brain developmental. Neural networks were cultured in different T3 concentrations (0, 3, 10, 30 nM). We quantified T3-responsive gene expression (KLF9) by qPCR, and differentiation potential and synaptogenesis by immunohistochemistry. Neuronal electrophysiological function was assessed by calcium imaging.

#### Results

This well-established model in the field of neuroscience expressed key players of TH signaling (e.g. MCT8 DIO3). We observed a dose-dependent upregulation of KLF9. Synapse formation was increased by T3 (0.54 vs 4.26 in 0 nM vs 3nM T3; to 1.97 vs 2.1 synapse/100um<sup>2</sup> in 10 nM and 30 nM T3, respectively). Calcium imaging data showed neurons cultured under 3 nM T3 had highest number of action potentials, lower variance in the amplitude and mean firing time, all indicating maturity of iPSC-derived neurons, compared to 0, 10 or 30 nM T3.

#### Conclusion

In a human model for early brain development, we identified T3 as a critical signaling molecule for synaptogenesis and neuronal function. In our model, concentrations lower or higher than 3 nM T3 had adverse effects on synapse formation and neuronal electrophysiological characteristics. This study provides the molecular underpinnings of actions of TH in human brain. Our model represents a useful tool to advance understanding of TH signalling in human brain in health and disease.

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## Miscellaneous

### PS1-05-37

#### The solid study (simplification of low level internal dosimetry):

##### preliminary findings

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#### Background

Diagnostic thyroid scans using technetium-99m pertechnetate are widely used to differentiate the causes of hyperthyroidism. Only 1-5% of the administered isotope is taken up by the thyroid after 20 minutes resulting in high background radioactivity.

#### Objectives

To determine the effective radiation dose and calculated organ doses after a standard administration of Technetium-99m pertechnetate in patients referred for diagnostic scans.

#### Methods

All patients participating in the study gave their written informed consent. We used SPECT imaging and 2D whole body imaging to determine the radio-pharmaceutical retention within regions of specific uptake i.e. thyroid, stomach, bladder, salivary glands. IDAC 2.1 was used to determine effective doses.

#### Results

The effective dose calculated for the standard 78.4 MBq administration was 4.89 mSv. The highest organ doses expressed as tissue dose per administered activity (mGy/MBq) were observed in the thyroid (0.448) followed by salivary glands (0.018), oesophagus (0.011), bladder wall (0.009), reproductive system (0.007) and lymphatic nodes (0.006). Lowest organ doses were in the lens (0.002) and skin (0.002).

#### Conclusions

These are preliminary results from the Simplification of Low Level Internal Dosimetry study (SOLLID). As more data are accumulated we hope to develop a simple method of determining tissue doses which will help guide clinicians ordering nuclear medicine scans.

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### PS1-05-38

#### TSH is superior to T4 for the assessment of thyroid function

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#### Background

Morbidity and mortality are associated with thyroid hormone levels in populations. This has invited a debate on what is the better parameter for assessment of thyroid function: the controlling hormone thyrotropin (TSH) or peripheral thyroid hormone measurements (thyroxine (T4) and triiodothyronine (T3)).

#### Aim

To assess the ability of TSH and total T4 (TT4) to discriminate between subtle differences in thyroid function.

#### Methods

Monthly collection of blood samples over one year in 35 subjects leading to 420 samples in 15 euthyroid and 20 mildly hypothyroid subjects. The latter group was classified as subclinically hypothyroid (SH) by two independent tests of thyroid function prior to inclusion. None of the participants received current treatment for thyroid disease. We measured TSH and total T4 in serum. Reference ranges with our assays were 0.4-4.5 mU/l for TSH and 60-140 nmol/l for TT4.

#### Results

The true thyroid state was confirmed by the mean of 12 repeated measurements, which was 1.19/7.23 mU/l for TSH in the euthyroid/SH subjects and 106/85 nmol/l for TT4. No single TSH measurement was above the upper limit of the reference range for TSH in the euthyroid group, and thus 100% of test results conformed to the euthyroid state. In the SH group, 86% of test results were in keeping with the true thyroid state while 14% were within the reference range. For

TT4, 99% of test results were within the reference range among euthyroid subjects while this was 96% for the SH group. An overlap between the two groups was markedly more pronounced for TT4 compared to TSH. Bootstrap estimates based on 1000 replications showed an estimated area under the curve of 0.999 (95%-CI: 0.995; 1.000) for TSH and 0.853 (0.736; 0.935) for TT4. There was no confidence interval overlap between participant groups for TSH, and hence markedly better performance of TSH compared to TT4.

#### Conclusion

The two groups differed more clearly when evaluated by TSH than by TT4. The TSH measurements were outside the reference range for 86% of individuals with SH compared 4% of TT4 measurements. Thus, our findings point to a higher diagnostic power for TSH compared to TT4 for separating individuals with mild hypothyroidism, and TSH is the most sensitive and accurate index of thyroid status at an individual level.

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### PS1-05-39

#### The effect of obesity-related allostatic changes on cardio-metabolic risk in euthyroid children

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#### Purpose

The hormonal thyroid changes related to obesity, even when in the euthyroid state, may contribute to the unfavorable cardio-metabolic profile of obese patients. In this retrospective study we aim to investigate the biochemical thyroid changes and the association between serum TSH, FT4, FT3 and cardio-metabolic risk factors in euthyroid obese youths.

#### Methods

Four hundred ninety-one Caucasian euthyroid obese children and adolescents aged 9.93±2.90 years were recruited. Each patient underwent clinical and auxological examination and laboratory workup including an OGTT and the measurement of thyroid function and lipid profile. Homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride to high density lipoprotein cholesterol ratio, total cholesterol to HDL ratio, atherogenic index of plasma, insulinogenic index, area under the glucose and insulin curve were calculated.

#### Results

We found that TSH was positively correlated with BMI-SD values, increasing FT3 levels was associated with increasing degree of obesity whereas FT4 levels resulted negatively associated with BMI-SD values. TSH values were associated with increasing LDL levels. A significant association between serum TSH, hypercholesterolemia and hyperinsulinemia was found. In addition, FT4 level resulted negatively correlated with fasting plasma glucose and FT3 levels positively correlated with the area under the curve of insulin and negatively correlated with HDL levels.

#### Conclusions

Taken together, our data showed that thyroid hormones influence obesity, lipid and glycemic parameters in euthyroid youths. These findings could carry implications regarding optimal TSH levels in obese children and confirm the importance of evaluating the thyroid function as possible adjunctive cardio-metabolic risk factor related to obesity.

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### PS1-05-40

#### Thyroid hormone variability and cardiovascular morbidity in hyperthyroid patients treated with long-term antithyroid drug therapy

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#### Objectives

The variability of thyroid hormone levels during long-term antithyroid drug (ATD) therapy and its possible association with adverse health outcomes has not been previously studied. The aim of this study was to evaluate the association of thyroid function test (TFT) variability and cardiovascular disease (CVD) morbidity during long-term ATD therapy. Furthermore, clinical factors related to high TFT variability during the ATD therapy were identified.

#### Design

Retrospective cohort study.

#### Methods

Patients ( $n = 394$ ) treated for hyperthyroidism with a first-line long-term ATD therapy at Tampere University Hospital between March 2016 and December 2018 were included and followed up until March 2019. The medians and coefficients of variation (CVs) of the follow-up thyroid-stimulating hormone (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) measurements were determined and evaluated in relation to baseline clinical factors. The associations of TFT variability and baseline clinical factors with CVD-associated hospital visits were assessed with logistic regression analyses.

#### Results

In the multivariable analyses, age (odds ratio [OR] 1.06, 95 % confidence interval [CI] 1.02-1.09), median fT4 (OR 1.09, 95 % CI 1.0-1.16) and median fT3 values (OR 1.34, 95 % CI 1.09-1.65), and fT4-CV (OR 1.02, 95 % CI 1.01-1.04) were independent risk factors for CVD morbidity, whereas baseline positive thyrotropin receptor antibodies (TRAb) protected from CVD morbidity (OR 0.29, 95 % CI 0.13-0.66). When the patients with baseline TRAb positivity were studied separately, fT4-CV was associated with CVD morbidity (OR 1.03, 95 % CI 1.00-1.05), but median fT4 or fT3 levels were not. The patients with positive baseline TRAbs or thyroid peroxidase antibodies (TPOAbs) had higher fT4-CV and median fT4 levels, compared to the patients with negative TRAb or TPOAb measurements ( $P = 0.002$  and  $P = 0.024$ , respectively).

#### Conclusions

During long-term ATD therapy for hyperthyroidism, fT4 variability is associated with an increased CVD morbidity. Patients with autoantibody-related hyperthyroidism have a higher variability of fT4 values compared to patients without thyroid autoantibodies. Although positive TRAbs are associated with a lower CVD morbidity compared to hyperthyroidism with negative autoantibodies, the variability of fT4 is associated with CVD morbidity also in patients with positive TRAbs.

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### PS1-05-41

#### I-131 treatment of patients with autonomously functioning thyroid nodules and normal TSH blood level

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#### Objectives

The purpose of this study was to evaluate the results of radionuclide therapy with iodine-131 (I-131) in patients (pts) with autonomously functioning thyroid nodules (AFTNs) and a normal thyroid stimulating hormone (TSH) blood level.

#### Methods

In this study 110 cytological benign AFTNs in 98 pts (83 female and 15 male) with normal TSH level have been treated with a fixed I-131 doses (370 MBq). Clinical exam, ultrasonography with color Doppler (US), fine needle aspiration biopsy (FNAB), TSH, FT4, FT3, anti-TPO, anti-Tg, anti-TSH receptor and thyroid scan (scintigraphy) have been performed in all pts before and 6 months after I-131 therapy.

#### Results

The median age of the pts was 60 (range 35 - 90) years. AFTNs were located more frequently in the right thyroid lobe (62 nodules) than in the left lobe (48 nodules). In 16 pts a solitary AFTN has been found on ultrasonography and the other 82 patients had AFTNs in multinodular goiter. 12 pts had two AFTNs. On post I-131 therapy thyroid scan in 72 AFTNs complete therapy effect has been observed, but in 38 AFTNs a scintigraphically partial effect has been noted. Statistical analysis showed a significant reduction in the thyroid ( $P = 6,9774E-13$ ) and AFTNs ( $P = 0,0002$ ) volume after I-131 therapy. TSH value significantly increased ( $P = 5,7746E-05$ ) and FT4 value significantly decreased ( $P = 2,5938E-05$ ) after I-131 therapy. FT3 ( $P = 0,3622$ ), anti-TPO ( $P = 0,7793$ ) and anti-Tg ( $P = 0,2272$ ) and anti-TSH receptor ( $P = 0,1171$ ) values did not change significantly.

## Conclusion

This study shows that radionuclide therapy with I-131 in pts with AFTN and normal TSH blood level is a simple, cheap and very effective modality. The effect of the I-131 therapy on AFTNs can be evaluated with a thyroid scan 6 months after I-131 therapy.

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**PS1-05-42****Not all automated FT4 immunoassays measure accurately in samples of pregnant women and hemodialysis patients**

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## Objectives

Free thyroxine (fT4) measurements are performed to detect thyroid disorders and monitor treatment. FT4 is measured using automated immunoassays (IAs) which face established analytical challenges due to low serum concentrations in the picomolar range and the precarious equilibrium between free and bound T4 (to thyroxine-binding globulin (TBG) and (pre-)albumin). Furthermore, IAs are known to be affected by the composition of the serum sample (matrix-effects), which can lead to falsely high or low results thereby affecting clinical decisions. Isotope-dilution liquid chromatography tandem-mass spectrometry (ID-LC-MS/MS) is not influenced by these matrix effects and considered the gold standard technique. The goal of this study is to unravel the accuracy of a variety of automated FT4 IA methods using samples of pregnant women and hemodialysis patients, conditions characterized by an altered serum matrix.

## Methods

FT4 was measured in healthy controls, pregnant women and hemodialysis patients using a method performed following reference method criteria (ID-LC-MS/MS, Radboud Nijmegen) and 5 commercially available automated immunoassays (Alinity (Abbott), Atellica (Siemens), Cobas (Roche), Lumipulse (Fujirebio) and UniCel DXI (Beckman Coulter)). Method comparisons (Bland Altman plots and Passing and Bablok analyses) between ID-LC-MS/MS and IAs were performed for samples of healthy controls, pregnant women and hemodialysis patients.

## Results

We collected 30 serum samples from each of the three groups. Mean gestational age in the group with pregnant women was 24.8 weeks. The fT4 IAs deviated +7 to +29% more from the LC-MS/MS method in pregnant women vs healthy controls (false high) and -16 to -27% more from the LC-MS/MS method in hemodialysis patients vs healthy controls (false low).

## Conclusion

All tested and frequently used automated IAs are less accurate in measuring fT4 in the altered serum matrix of pregnant women and hemodialysis patients compared to healthy controls. This may lead to an overestimation of fT4 concentrations in pregnant women and to an underestimation of fT4 concentrations in hemodialysis patients when using IAs. Physicians and laboratory specialists should be aware of this phenomenon to avoid drawing wrong conclusions and manufacturers are encouraged to improve their fT4 assays.

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**PS1-05-43****Treatment of hyperthyroidism reduces the systemic oxidative stress load, as measured by biomarkers of rna and dna damage**

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## Background

Increased oxidative stress has been linked to both hypo- and hyperthyroidism. Whole-body oxidative stress can be estimated by the oxidized guanine nucleosides, 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), derived from RNA and DNA, respectively. These biomarkers have been associated with increased morbidity and mortality in several diseases but are not well explored in humans with thyroid disorders.

## Methods

We measured urinary excretion of 8-oxoGuo and 8-oxodG in 51 hyperthyroid patients (toxic nodular goiter (TNG),  $n = 30$ ; Graves' disease (GD),  $n = 21$ ) before, or shortly after, initiation of therapy and when stable euthyroidism had been achieved for at least 12 months. Patients with TNG were older (mean:  $59 \pm 12$ SD years) than those with GD ( $50 \pm 8$  years). Mean follow-up time was  $17.2 \pm 4.6$  and  $22.9 \pm 8.9$  months for TNG and GD, respectively. All patients with TNG were treated with radioiodine, except for one who underwent thyroidectomy. GD patients were treated with methimazole and two of whom also received radioiodine.

## Results

Both oxidative stress markers correlated positively with age (8-oxoGuo:  $P < 0.001$ ; 8-oxodG:  $P = 0.003$ ). After adjustment, the baseline urinary excretions correlated with the severity of the disease, reflected by the plasma levels of thyroxine (8-oxoGuo:  $P = 0.002$ ; 8-oxodG:  $P = 0.021$ ), and were significantly higher in GD than in TNG ( $P = 0.001$  for both biomarkers). Treatment significantly affected the excretions of the oxidative stress markers. In TNG, 8-oxoGuo decreased from geometric mean (GM) 2.11 nmol/mmol (95% CI: 1.85-2.39) to 1.91 nmol/mmol (95% CI: 1.67-2.19),  $P = 0.001$ , while 8-oxodG decreased from 1.65 nmol/mmol (95% CI: 1.41-1.93) to 1.48 nmol/mmol (95% CI: 1.27-1.74),  $P = 0.026$ . In GD, 8-oxoGuo decreased from 2.25 nmol/mmol (95% CI: 1.95-2.59) to 1.79 nmol/mmol (95% CI: 1.63-1.97),  $P = 0.0003$ , while 8-oxodG decreased from 2.02 nmol/mmol (95% CI: 1.73-2.38) to 1.54 nmol/mmol (95% CI: 1.31-1.81),  $P = 0.001$ . When euthyroid, no between-group differences were found.

## Conclusion

Treatment of hyperthyroidism significantly decreased the systemic oxidative stress load by 10-25%, as measured by the urinary excretion of nucleic acid metabolites. The higher values in patients with GD could be due to the more severe hyperthyroidism seen in this condition. Our findings may signify a key factor, explaining the higher morbidity and mortality linked to patients with hyperthyroid diseases, as shown in observational studies. Link to publication: <https://pubmed.ncbi.nlm.nih.gov/33901280/>

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**PS1-05-44****The effect of testosterone supplementation on the hpt axis in euthyroid hypogonadal adult men: a prospective observational study**

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## Background and Objective

It is known that androgens decrease and estrogens increase TBG. In female to male transsexuals under testosterone replacement, decrease of TBG has been shown, but also an increased T3/T4 ratio despite stable free T4 and TSH, suggesting increased conversion to T3 by testosterone. We wanted to study the HPT axis in hypogonadal cis-males before and after testosterone replacement.

## Method

Prospective observational study in adult male patients with hypogonadism in the setting of an outpatient endocrine clinic. Serum samples were taken prior to (visit

1) and at the first consultation (visit 2) under testosterone replacement. Males with history of thyroid disorder, having abnormal thyroid function at visit 1, treated with betaHCG, or taking drugs at baseline or during follow-up knowing to affect thyroid function were excluded. Paired t-test or Wilcoxon matched-pairs signed rank test was applied where appropriate.

#### Results

A cohort of  $n = 24$  hypogonadal male patients was studied. Mean age was  $52.9 \pm 15.4$  years,  $n = 17$  patients were treated for structural hypogonadism ( $n = 8$  hypogonadotropic,  $n = 9$  hypergonadotropic),  $n = 7$  patients were treated for functional hypogonadism. Median time between visit 1 and 2 was 2.53 months [range 1.15-11.5]. As expected, total and free testosterone increased significantly (see Table). TSH was not different, but T4 (total and free) decreased. Total T3 was not different, and T3/T4 ratio increased between visit 1 and 2.

#### Conclusion

In euthyroid hypogonadal cis-males, testosterone replacement did not alter TSH, decreased total T4 and free T4, but not total T3. The increased T3/T4 ratio suggests increased conversion and/or differential binding to TBG.

|                               | Visit 1            | Visit 2             | n  | p value           |
|-------------------------------|--------------------|---------------------|----|-------------------|
| Testosterone (ng/dL)          | 249.1 [15.1-649.1] | 368.3 [122.9-1297]  | 24 | <b>0.0003</b>     |
| Free testosterone (ng/dL)     | 3.95 [0.20-7.30]   | 6.45 [2.20-16.90]   | 24 | <b>&lt;0.0001</b> |
| TSH (mIU/l)                   | 1.54 +/-0.85       | 1.46 +/-0.74        | 24 | 0.6490            |
| Total T4 ( $\mu$ g/dL)        | 7.45 [4.70-12.40]  | 6.90 [4.50-11.30]   | 24 | <b>0.0294</b>     |
| Free T4 (ng/dL)               | 1.32 +/-0.16       | 1.24 +/-0.19        | 18 | <b>0.0337</b>     |
| Total T3 (ng/dL)              | 117.5 +/-30.4      | 123.2 +/-24.5       | 24 | 0.1010            |
| T3/T4 ratio ( $\times 10^3$ ) | 15.35 [8.94-22.34] | 16.15 [11.56-27.71] | 24 | <b>0.0101</b>     |

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#### PS1-05-45

##### ABSTRACT WITHDRAWN

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#### PS1-05-46

##### Body composition determinants of thyroid function and volume in euthyroid patient with obesity

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The aim of our study was to investigate the relationship between anthropometric and body composition parameters and thyroid function (measured with TSH and free T4), as well as thyroid volume, in patients with obesity.

#### Methods

This is a cross-sectional study including patients with obesity consecutively referred to our tertiary endocrine center for diverse pathologies. TSH and free T4 was measured in all patients and thyroid ultrasound was performed by an experienced investigator, to estimate thyroid volume. Whole body DXA scans were available to evaluate total and regional body composition (lean and fat mass). We excluded from our analysis patients with abnormal thyroid function (TSH > 10 or < 0.5 mIU/l).

#### Results

In our group of 221 patients (147 women) mean BMI was  $37.03 \pm 7.37$  kg/m<sup>2</sup> and mean age was  $51.9 \pm 18.95$  years. As expected, TSH positively correlated with BMI ( $r=0.142$ ,  $P < 0.05$ ), while free T4 was negatively associated with total lean mass ( $r=-0.143$ ,  $P < 0.05$ ), legs lean mass ( $r=-0.136$ ,  $P < 0.05$ ) and total fat-free mass ( $r=-0.169$ ,  $P < 0.05$ ). Thyroid volume was significantly higher in men ( $P < 0.01$ ) and correlated with total lean mass ( $r=0.333$ ,  $P < 0.001$ ), %fat mass ( $r=0.213$ ,  $P = 0.002$ ), weight ( $r=0.179$ ,  $P < 0.05$ ) and height ( $r=0.225$ ,  $P < 0.01$ ), but not with BMI. Lean mass remained positively associated with thyroid volume in a multivariate linear regression analysis which also included age, gender and %fat, together explaining about 41% of its variation.

#### Conclusions

In patients with obesity, lean mass is a factor independently associated with thyroid volume

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## Sunday, 11th September 2022

### Poster Session 2: Hypothyroidism treatment

#### PS2-06-47

##### Prescription of levothyroxine and concurrent use of anti-arrhythmic and bone-anti-resorptive drugs in Belgium: retrospective study in a large population sample

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#### Background and objective

Increased prescription of levothyroxine (LT4) has been observed in the USA and countries in Europe and has been related to increased thyroid function screening and a lower treatment threshold, increasing risk for overdiagnosis and overtreatment. We wanted to study the evolution of LT4 prescriptions in Belgium, and compare the use of anti-arrhythmic drugs (AAD) and bone-anti-resorptive drugs (BAD) between LT4-users and non-LT4-users.

#### Method

Retrospective study, using reimbursement data from the largest health insurance company (Christelijke mutualiteit/Mutualité Chrétienne), representing 42% of all Belgian residents during years 2001 to 2018. Individuals with history of thyroid surgery or concurrent use of thiazole drugs were excluded (2.1% of cohort). Data extracted: age, gender, socio-economic status (SES, defined by right for increased reimbursement), number of persons with at least one package of reimbursed LT4 that was delivered in the public pharmacy, dose, BAD, AAD.

#### Results

Whereas 2.1% of individuals were prescribed LT4 in year 2001, this rose to 5.1% in year 2018 (2.5-fold increase). The increase was most pronounced for individuals aged  $\geq 65$  and especially  $\geq 80$ yo (11.2% in year 2018 vs 4.3% in year 2001). Furthermore in year 2018 and for the total cohort, the proportion of LT4-users was higher in females (8.2% vs 1.9% in males, year 2018) and higher in individuals with increased reimbursement (7.4% vs 4.7%, year 2018). In 10.9% only the lowest commercially available dose of LT4 (25  $\mu$ g) was prescribed, with highest proportion (14.4%) in the  $\geq 80$ yo subgroup. Finally, in individuals aged  $\geq 65$ yo in year 2018 use of AAD was higher among LT4-users than non-LT4-users (58.6% vs 50.4%; OR = 1.39;  $P < 0.00001$ ). Also use of BAP was higher among LT4-users, especially in males (2.6% vs 1.9%; OR = 1.3;  $P < 0.0001$ ; females: 10.4% vs 9.8%; OR 1.03;  $P = 0.026$ ).

#### Conclusions

In a large representative Belgian sample, we confirm increasing prescription of LT4, most pronounced in the elderly, raising concerns regarding potential overdiagnosis. The high proportion of lowest LT4 dose and higher concurrent use of anti-arrhythmic and bone-anti-resorptive drugs among older LT4-users could point to overtreatment, but warrants further study.

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#### PS2-06-48

##### Pseudomalabsorption of levothyroxine: munchausen's syndrome, compliance defects or fraud?

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**Objectives**

Factitious disorder imposed on self or Munchausen's syndrome (MS), is a psychiatric disorder in which physical symptoms are intentionally produced without any practical benefit. Patients with MS represent a diagnostic dilemma as laboratory and clinical results can be inconsistent with the history and physical exam. The aim of this study was to evaluate the role of the levothyroxine overload test to differentiate MS from malabsorption and to treat life-threatening hypothyroidism.

**Methods**

1 mg of levothyroxine was administered to patients referred to our Department for severe hypothyroidism and suspect malabsorption. TSH, FT4 and FT3 were measured at baseline, after 360 minutes and 2 to 7 days.

**Results**

we studied 9 hypothyroid patients (1 male and 8 females, mean age  $46.2 \pm 14.8$  years). Three female had Hashimoto's thyroiditis while the remaining patients had undergone thyroidectomy for cancer ( $n = 5$ ) or goiter ( $n = 1$ ). Although at referral all patients had TSH  $> 100$  mU/l with low or even undetectable FT4/FT3, at the time of testing three of them had TSH 4.5, 23.6 and 39.1 mU/l, respectively. All patients adequately absorbed levothyroxine as shown in the table (data expressed as mean  $\pm$  SD). The huge variability of results depended on the severity of their hypothyroidism, as we observed that the rate of change of FT4/FT3 was positively and that of TSH negatively correlated with baseline TSH levels ( $P < 0.01$ ;  $r^2 = 0.69$ ;  $0.67$  and  $0.62$ ), while did not correlate with patients' weight or BMI. In patients with baseline TSH  $> 200$  mU/l, the TSH did not change significantly at 360 minutes, but declined few days after the overload (range 32-62%). In 5/9 patients additional levothyroxine loads were administered to achieve euthyroidism. None of the patients had adverse events or reported side effects. Three patients fulfilled the criteria for MS and were referred to a Psychiatrist. In 3 patients we suspected malingering, as they were asking for social assistance for disabled patients and were lost to follow-up after showing them the results of their testing. The remaining patients had an inadequate compliance or were taking levothyroxine with interfering drugs/foods; their thyroid function improved on follow up after proper instructions.

**Conclusion**

in patients with a severe hypothyroidism apparently unresponsive to treatment we suggest supervised levothyroxine administration before starting the workout for malabsorption. Subsequent overloads of levothyroxine might be given to rapidly achieve euthyroid.

|            | baseline         | 360 minutes       | % of change from baseline |
|------------|------------------|-------------------|---------------------------|
| TSH mU/l   | $129.0 \pm 91.2$ | $102.3 \pm 91.57$ | $-31.4 \pm 19.7$          |
| FT4 pmol/l | $7.3 \pm 6.7$    | $25.6 \pm 8.7$    | $+947 \pm 1276.0$         |
| FT3 pmol/l | $2.4 \pm 1.7$    | $3.2 \pm 1.6$     | $+82.6 \pm 105.3$         |

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**PS2-06-49****Faecal microbiota composition in patients with increased need of levothyroxine**

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**Objectives**

Variations of gut microbiota composition have been described in patients with different thyroid disorders. The site of oral levothyroxine (T4) absorption is represented by the small intestine and it has been demonstrated that some bacterial strains possess enzymatic activity that may affect iodothyronine deconjugation, process probably involved in thyroid hormone enterohepatic recycling. The relationship between gut microflora composition and oral T4 requirement has, as yet, not been analyzed, and represents the aim of the present study.

**Patients and Methods**

Twenty two hypothyroid adult patients (19F/3M) treated with oral levothyroxine at a stable dose for at least two years, have been subdivided into two groups on the basis of their T4 requirement: A) group, showing an increased need for T4 (10 patients; 9F/1M; median dose 1.76 mg/kg/day) or a normal one. B) group (12 patients; 10F/2M; median dose 1.36 mg/kg/day). All patients had TSH values between 0.8 and 2 mU/l. All patients took T4 in fasting state, avoiding all food and drugs interfering with T4 absorption and action. Moreover, none of included

patients followed unbalanced diets, had disorders, or used drugs, interfering with faecal microbial composition. From each patient faecal samples were provided. Microbiota composition was determined via 16s rDNA sequencing of the hypervariable region V3-4 on Illumina MiSeq. Alpha and beta-diversity indices and all statistical analysis were computed in Qiime2.

**Results**

Alpha-diversity indices showed an increased relative abundance of Firmicutes in BEN group while an increased relative abundance of Bacteroidetes and Proteobacteria characterized MAL group. Beta-diversity analysis, revealed a significant difference between the microbial populations of patients belonging to the two groups through weighted UniFrac analysis ( $P = 0.008$ ). The ANalysis of COmposition of Microbiomes (ANCOM) between the different taxonomic units revealed that Bacteroidetes phylum is significantly associated to the increased need for T4, while Firmicutes phylum is associated to a normal T4 absorption. The Linear discriminant analysis with Effect Size measurement (LEfSe) allowed the identification of potential biomarkers associated to the increased need for T4, mostly belonging to the class of beta-Proteobacteria.

**Conclusions**

Despite the low number of patients, typical of a pilot study, this is the first characterization of faecal microbiota composition of hypothyroid patients with the specific focus on T4 requirement.

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**PS2-06-50****TSH levels assessment in hypothyroid patients treated with liquid or tablet L-thyroxine**

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**Objective**

Tablet levothyroxine (L-T4) is the common used therapy for hypothyroidism. In order to assess a good compliance to the therapy, treated patients are monitored with the execution of annual thyroid hormonal test. The stability of TSH levels in hypothyroid patients (with no malabsorption issues), treated with liquid L-T4, compared to that of the patients treated with tablet L-T4, remained a poorly understood topic. This study has the purpose to deepen this issue.

**Methods**

We selected patients who reported the following criteria: a) normal serum TSH levels at the basal evaluation; b) no malabsorption or drug interference issues; c) in treatment with liquid or tablet L-T4. Hypothyroid patients (matched by gender and age) were compared according to the used drug formulation; 653 subjects were in treatment with liquid L-T4, and 329 subjects with tablet L-T4. We performed a two year follow-up, during which the serum TSH, FT3, FT4 levels were annually measured.

**Results**

Gender, age, body mass index, history of chronic autoimmune thyroiditis, initial TSH level, and L-T4 dose were the parameters evaluated at the first abnormal TSH value. These parameters, at the time of initial normal TSH, were not associated significantly with time to abnormal TSH values. After 1 year, TSH values were normal in 86% of the patients who were in treatment with L-T4 liquid formulation, whereas only in 80% of patients who followed a tablet L-T4 therapy. After 2 years, normal TSH values were registered in 84% of patients who were treated with L-T4 liquid formulation, while only in 74% of patients who received tablet L-T4 ( $P < 0.05$ ).

**Conclusion**

These data showed that in the long term follow-up, liquid L-T4 can permit to maintain more efficiently normal TSH levels in hypothyroid patients, than tablet L-T4.

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**PS2-06-51****ABSTRACT WITHDRAWN**

DOI: 10.1530/endoabs.84.PS2-06-51

**PS2-06-52****The effect on serum tsh levels in patients in treatment with proton pump inhibitors after the switch from oral L-T4 tablet to a liquid L-T4 formulation**Poupak Fallahi<sup>1</sup>, Silvia Martina Ferrari<sup>2</sup>, Giusy Elia<sup>3</sup>, Francesca Ragusa<sup>3</sup>, Sabrina Rosaria Paparo<sup>3</sup>, Valeria Mazzi<sup>3</sup> & Alessandro Antonelli<sup>3</sup><sup>1</sup>University of Pisa, Department of Translational Research of New Technologies in Medicine and Surgery, Pisa, Italy; <sup>2</sup>University of Pisa, Department of Clinical and Experimental Medicine, Pisa, Italy; <sup>3</sup>University of Pisa, Department of Surgical, Medical and Molecular Pathology and Critical Area, Pisa, Italy**Objective**

The treatment with proton pump inhibitors (PPI; omeprazole, pantoprazole, lansoprazole) used for gastritis, gastric ulcer, etc. could lead to L-thyroxine (L-T4) malabsorption issues, that is induced by the increased gastric pH. Many factors like age, way of assumption (during breakfast or with food), other drugs interferences, drug-kinetics, adherence to therapy, could impair the L-T4 absorption.

**Methods**

The study involved 27 hypothyroid patients in treatment with L-T4 tablet formulation. All patients were switched to an oral liquid L-T4 formulation, maintaining the same dosage of L-T4.

**Results**

We showed that circulating thyroid-stimulating hormone (TSH) levels could be normalized or decreased, after the switch from L-T4 tablet to a liquid formulation, at the same dose. Furthermore, a worsening of TSH levels, with a relapse in the hypothyroid range, was recorded in 15 of these patients who, for different reasons, were switched back to take L-T4 in tablets, with the same dosage.

**Conclusion**

Finally, our study suggests that the L-T4 malabsorption issue caused by PPI, could be solved with the assumption of the L-T4 in a liquid formulation. However, this field needs to be further investigated involving also other conditions of altered L-T4 absorption.

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**PS2-06-53****Whole-body oxidative stress measured by biomarkers of RNA and DNA damage is higher in hypothyroid women, even after treatment, than in healthy individuals, measured by biomarkers of RNA and DNA damage**Kamilla R. Riis<sup>1</sup>, Camilla B. Larsen<sup>1</sup>, Bjarke R. Medici<sup>2,3</sup>, Christian Z. Jensen<sup>2</sup>, Kristian H. Winter<sup>1</sup>, Emil L. Larsen<sup>3</sup>, Andreas Brønden<sup>3</sup>, Christina Ellervik<sup>4,5,6</sup>, Jeppe L. la Cour<sup>2</sup>, Laszlo Hegedüs<sup>1</sup>, Thomas H. Brix<sup>1</sup>, Henrik E. Poulsen<sup>3,7</sup>, Jens Faber<sup>2</sup>, Filip K. Knop<sup>2,3</sup>, Birte Nygaard<sup>2</sup> & Steen J. Bonnema<sup>1</sup>

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**Introduction**

Hypothyroidism has been associated with oxidative stress. Urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), respectively, represent whole-body RNA and DNA oxidation. While these biomarkers have been associated with increased morbidity and mortality in various diseases, they have been only sparsely explored in patients with thyroid disorders.

**Method**

In 45 women with newly diagnosed hypothyroidism (overt:  $n = 23$ ; subclinical:  $n = 22$ ), urinary excretions of 8-oxoGuo and 8-oxodG, corrected for creatinine, were measured before or shortly after initiation of levothyroxine (LT4), and again after 6-12 months of euthyroidism. Eighteen healthy women were included as controls.

**Results**

The hypothyroid women and the controls had a mean age of  $47.4 \pm 11.4$  (SD) and  $45.2 \pm 13.1$  (SD) years, respectively. There was no difference in age, BMI, and smoking status between patients and controls. In the patients, none of the biomarkers changed significantly by achievement of euthyroidism. Thus, the geometric mean of 8-oxoGuo was 1.63 (95%CI: 1.49-1.78) nmol/mmol creatinine at baseline and 1.67 nmol/mmol (95%CI: 1.53-1.83) at euthyroidism ( $P = 0.39$ ), while 8-oxodG was 1.28 nmol/mmol (95%CI: 1.14-1.44) and 1.32 nmol/mmol (95%CI: 1.18-1.48), respectively ( $P = 0.47$ ). In the control group, the geometric mean of 8-oxoGuo was 1.23 nmol/mmol creatinine (95%CI: 1.07;1.42), while 8-oxodG was 1.04 nmol/mmol creatinine (95%CI: 0.88;1.23). Thus, the patients at euthyroidism, compared with control subjects, showed a significantly higher level of both 8-oxoGuo ( $P < 0.001$ ) and 8-oxodG ( $P = 0.03$ ). Among patients, a multiple regression analysis demonstrated a negative correlation between TSH and 8-oxoGuo, and a positive correlation between free T4 and 8-oxoGuo, at baseline. A positive correlation between baseline free T4 and 8-oxodG was also demonstrated.

**Conclusion**

In hypothyroid women, no significant effect of LT4 treatment was demonstrated on the oxidative stress biomarkers 8-oxoGuo and 8-oxodG. The excretion of these biomarkers was significantly higher in patients than in healthy individuals. It is speculated whether the increased oxidative stress burden reflects the impact of thyroid autoimmunity per se.

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**PS2-06-54****Treatment of hypothyroidism in europe by specialists: an international survey (THESIS): analysis of contested indications for prescribing among uk participants**Younes R Younes<sup>1</sup>, Petros Perros<sup>2</sup>, Laszlo Hegedüs<sup>3</sup>, Enrico Papini<sup>4</sup>, Endre V Nagy<sup>5</sup>, Roberto Attanasio (ORCID: 0000-0002-1417-287X)<sup>6</sup>, Roberto Negro<sup>7</sup> & Benjamin C.T. Field (ORCID: 0000-0002-1883-1588)<sup>8</sup>

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**Objectives**

We present findings from the UK arm of the THESIS collaboration, a pan-European survey of endocrinologists' opinions and current practice in the management of hypothyroid and euthyroid disorders.

**Methods**

UK clinical members of the Society of Endocrinology and the British Thyroid Association were invited to participate in an online survey.

**Results**

272 of 1295 (21%) eligible members completed the survey. More than 50% of respondents stated that combined treatment with levothyroxine + liothyronine could be considered for levothyroxine-treated patients whose symptoms persist despite normalisation of serum thyroid stimulating hormone (TSH) concentration. However, only 40% are currently prescribing such treatment, and just 23% would consider taking it themselves. Endocrinologists who reported managing larger numbers of patients with hypothyroidism were more likely than those managing smaller numbers to consider prescribing combined levothyroxine + liothyronine (> 100 patients annually, 65%; 51-100 patients annually, 54%; ≤ 50 patients annually, 48%;  $P = 0.0302$ ). Just 5.4% prescribe desiccated thyroid extract, and those most likely to do so are aged over 60 years ( $P = 0.043$ ). 27% would recommend levothyroxine for euthyroid female infertility with high titre thyroid peroxidase antibodies, despite recent trials finding no benefit. Most respondents stated that they have no influence over brand or formulation of levothyroxine dispensed to their patients and expect no major differences in efficacy between formulations.



## Conclusions

For UK endocrinologists, the role of combined levothyroxine + liothyronine in treatment of hypothyroidism remains a live issue for further research, brought into focus recently by the cost-led withdrawal of access to liothyronine in many regions. The importance of facilitating efficient dissemination of research findings in the UK is highlighted by the proportion of respondents who would currently consider prescribing thyroid hormone treatment in euthyroid female infertility with high titre antibodies, an indication for which evidence of efficacy is lacking.

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## PS2-06-55

### Free T3 levels and clinical status of patients with levothyroxine-compensated primary hypothyroidism: can we do better?

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## Objectives

To evaluate the relationship between free T3 (FT3) levels with symptoms suggestive of thyroid dysfunction, and quality of life (QoL) outcomes, in patients with compensated primary hypothyroidism (PH).

## Methods

Cross-sectional study encompassing Portuguese levothyroxine(LT4)-treated patients for PH who presented Thyroid-stimulating hormone (TSH) and free T4 (FT4) within the normal range. Pregnant women, patients with central hypothyroidism and/or with significant comorbidities (Charlson Comorbidity Index) were excluded. Blood samples for thyroid function tests were collected at the time of clinical evaluation. The Portuguese versions of the "Quality of Life Questionnaire for Patients with Thyroid Disease" (ThyPRO-39br) and the "MOS Short Form Health Survey 36 item v2" (SF-36v2) were applied.

## Results

Two hundred and one patients were included, 176 (87.6%) were female, with a median age of 5 (minimum;maximum: 19;87) years. Chronic autoimmune thyroiditis was the most common cause of PH ( $n = 89[44.3\%]$ ). The median daily dose of LT4 was 100 µg (1.51µg/kg/day; minimum; maximum: 25; 250 µg). Globally, the most reported symptoms were asthenia ( $n = 134[66.7\%]$ ), cutaneous xerosis ( $n = 127[63.2\%]$ ) and muscle fatigue ( $n = 107[53.2\%]$ ). In the ThyPRO-39br questionnaire, 138 (68.6%) patients did not attribute a detrimental effect on their QoL to PH. Asthenia ( $n = 114[56.7\%]$ ), depressed mood ( $n = 112[55.7\%]$ ) and cutaneous xerosis ( $n = 94[46.8\%]$ ) were the most frequent complaints. With regard to the SF-36v2 questionnaire, most patients did not describe significant physical disability in activities of daily living. Around a third reported decreased vitality and energy, and approximately half of the patients described stability, compared to their health status in the previous year. Patients with complaints of constipation ( $P = 0.010$ ) and depressed mood ( $P = 0.039$ ) had significantly lower FT3 levels than patients without these symptoms. The same trend was observed in patients with asthenia, muscle fatigue, cutaneous xerosis, periorbital edema, dysphonia, weight gain, cold intolerance and memory loss, although without statistical significance.

## Conclusions

Most patients with normal TSH and FT4 do not have a negative impact on their QoL, at least significantly. Despite the restoration of "euthyroidism", there are a panoply of symptoms that remain, which may be related to a lower conversion of T4 to T3 (symptomatic patients have lower FT3 levels). The persistence of these symptoms makes us question the potential benefit of T3 therapy in this subset of patients.

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## PS2-06-56

### Polychlorinated biphenyl congeners PCB 118 and PCB 126 induce upregulation PD-L1 expression in human thyrocytes through aryl hydrocarbon receptor (AHR) pathways

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## Objective

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that that have been reported to cause a variety of toxic effects, including inflammation and cancer, through binding to the aryl hydrocarbon receptor (AhR). In turn, AhR promotes xenobiotic detoxification and antioxidant defense, by up-regulating specific responsive genes, the so-called "AhR gene battery". AhR is also involved in modulation of immune response, by regulating programmed cell death 1(PD-1) ligand (PD-L1) levels. PD-L1 has an important role in regulating immune responses by binding to PD-1 on immune cells, contributes to maintaining immune tolerance by down-regulating of T-cell immune responses and cytokine production, and might favor the escape from immune surveillance of cancer cells. The aim of this study is to investigate the effects of PCBs on PD-L1 expression on thyroid cells

## Methods

Primary thyrocytes were obtained from patients undergone surgery for benign thyroid disease (solitary thyroid nodule). Cultured cells were exposed for 24 h to increasing concentrations (2.5 and 5 µM) of 2 dioxin-like PCBs: the 2,3',4,4',5-pentachlorobiphenyl (PCB 118) and the 3,3',4',4',5 Pentachlorobiphenyl (PCB 126). Gene silencing of AhR was performed by using a specific siRNA. mRNA and protein levels of PD-L1, AhR, IL-1beta and IL-6 were evaluated by real-time qPCR, ELISA and Western Blot.

## Results

In cultured thyrocytes, exposure to PCB 126 and PCB 118 at 2.5 and 5 µM concentrations significantly induced the increase of both mRNA and protein levels of AhR and PD-L1 ( $P < 0.01$  and  $P < 0.001$ , at 2.5 and 5 µM respectively for mRNA expression and  $P < 0.05$  at 5 µM for protein levels). On the contrary, the knockdown of AhR before PCBs treatments reduced PD-L1 mRNA and related protein levels, indicating the involvement of this receptor in the regulation of PD-L1 enhanced by PCBs. In the same *in vitro* model, PCB exposure induced the increase of both mRNA and protein levels of inflammatory cytokines IL-1beta and IL-6 ( $P < 0.01$  and  $P < 0.001$ , at 5 and 10 µM respectively for mRNA expression;  $P < 0.05$  and  $P < 0.01$  at 5 and 10 µM for protein levels).

## Conclusion

Our data demonstrated that PCB 118 and PCB 126 may promote PD-L1 expression in thyrocytes. Such effects can be partially attributed to the activation of the AhR. These results suggest that the PD-1/PD-1L pathway is activated in thyrocytes in the context of inflammatory/toxic stimuli and point to a new mechanism that need to be further deepen to understand the effect of PCBs on thyrocytes.

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## Graves' Disease 1

## PS2-07-57

### Post-alemtuzumab graves' disease remitting after switch to ocrelizumab

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## Objectives

Graves' hyperthyroidism is a frequent complication of alemtuzumab treatment in MS. We present the case of an MS patient who developed clinically and biochemically overt alemtuzumab-induced Graves' disease, remitting 6 months after treatment with ocrelizumab and methylprednisolone pulse-therapy.

## Methods

A 49-year-old man diagnosed with active multiple sclerosis who had undergone alemtuzumab treatment, and developed Graves' hyperthyroidism which remitted after ocrelizumab and pulse-therapy with methylprednisolone. Clinical symptoms

of Graves' disease and thyrotropin antibody titres were frequently evaluated as well as multiple sclerosis clinical and paraclinical parameters.

#### Results

Seven months after the second alemtuzumab course the patient developed a symptomatic Graves' hyperthyroidism with very high thyrotropin antibody titres, which was treated with anti-thyroid drugs. Two years later ocrelizumab was started along with methylprednisolone in pulse-therapy due to a multiple sclerosis relapse, with subsequent remission of the hyperthyroidism. Six months later the antibodies decreased and the anti-thyroid drugs were stopped. 24 months later he remains euthyroid.

#### Discussion

This case highlights the possibility of remission of post-alemtuzumab Graves' disease after methylprednisolone pulse-therapy and ocrelizumab with avoidance of further medical or surgical treatment.

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## PS2-07-58

### High IgG4 serum levels in graves' disease compared with nonautoimmune hyperthyroidism

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#### Objectives

A role of IgG4 in Graves' disease (GD) has been proposed, although the available data are conflicting. In addition, an association between Graves' orbitopathy (GO) and IgG4-related disease has been postulated, but no firm demonstration has been obtained. Thus, the aim of the present study was to investigate the role of IgG4 in GD.

#### Methods

We performed a cross-sectional investigation in 351 consecutive patients (F=284, M=67; age 48.2±15 yr.), of whom 306 had Graves' disease and 45 had toxic nodular goiter (TNG). All patients came to our observation over 36 consecutive months to undergo radioiodine treatment. IgG4 were measured in all patients. The primary outcome was the level of IgG4 in GD patients compared with TNG. Secondary outcomes were: 1) the prevalence of IgG4 levels above the upper limit of normal range (66 mg/dL); 2) the prevalence of IgG4 levels above the cut-off value considered diagnostic for IgG4-RD (135 mg/dL); and 3) within GD patients, the levels of IgG4 and the prevalence of high IgG4 (≥66 mg/dL or ≥135 mg/dL) in patients with and without GO.

#### Results

IgG4 concentrations in GD patients (53 mg/dL, IQR: 26-94.7) were significantly greater than those in patients with TNG (56 mg/dL; IQR: 25-59; Mann Whitney U: 5.41;  $P = 0.02$ ). In addition, the prevalence of patients with IgG4 levels above the upper limit of normal range was greater in GD (112/306, 36.6% vs 8/45, 17.7% in TNG; OR: 2.67, 95%CI from 1.2 to 5.9;  $P = 0.016$ ). The prevalence of patients with IgG4 levels above the cut-off value diagnostic for IgG4-RD was nearly statistically significantly greater in GD patients (46/306, 15% vs 2/45, 4.4% in TNG; OR: 3.8, 95%CI from 0.89 to 16.2;  $P = 0.071$ ). Limited to GD patients, IgG4 levels and prevalence of high values (≥66 mg/dL or ≥135 mg/dL) did not differ between patients with GO and those without GO.

#### Conclusions

IgG4 serum levels are higher in patients with GD compared with nonautoimmune hyperthyroidism, with an apparent greater prevalence of values that can be considered relevant. The possible clinical implications of our findings remain to be investigated.

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## PS2-07-59

### TPO antibody status prior to first radioactive iodine therapy as a

predictive parameter for early hypothyroidism in graves' disease  
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#### Introduction

Several studies identified pre-treatment parameters that could help in the prediction of the thyroid functional outcome after radioactive iodine therapy (RAI) in Graves' disease. However, the role of the thyroid peroxidase antibody (TPO Ab) status is not well studied.

#### Objectives

We investigated if a positive TPO Ab status before radioactive iodine (RAI) therapy in patients with Graves' hyperthyroidism is a predictive factor for developing hypothyroidism post RAI.

#### Methods

We performed a retrospective study of patients with Graves' hyperthyroidism with known TPO Ab status, receiving a first administration of RAI. Patients from four thyroid outpatient centres in Belgium receiving a first RAI therapy between the years 2011 and 2019 were studied. Clinical, laboratory, imaging, and treatment data were recorded from medical charts. Hypothyroidism and cure (defined as combined hypo- and euthyroidism) were evaluated in period 1 (≥2 and ≤9 months, closest to 6 months post RAI) and period 2 (>9 months and ≤24 months post RAI, closest to 12 months post RAI).

#### Results

One hundred fifty-two patients were included of which 105 (69%) were TPO Ab positive. Compared to TPO Ab negative patients, TPO Ab positive patients were younger, had a larger thyroid gland, and had more previous episodes of hyperthyroidism. There was no difference in THS receptor antibody titer at diagnosis, pre-treatment with anti-thyroid drugs or administered RAI activity. In period 1, 89% of the TPO Ab positive group developed hypothyroidism vs 72% in the TPO Ab negative group ( $P = 0.007$ ). In period 2, the observation was similar: 88% vs. 72% ( $P = 0.019$ ). Cure rate was similar in both groups in period 1 (91% vs. 85%,  $P = 0.238$ ) and in period 2 (94% vs 86%,  $P = 0.146$ ).

#### Conclusion

A positive TPO Ab status in patients with Graves' hyperthyroidism receiving a first administration of RAI is associated with a higher risk of early hypothyroidism. The underlying mechanism warrants further investigation. Future studies investigating pre-treatment parameters affecting outcome after RAI in patients with Graves' disease should incorporate TPO Ab status as a variable.

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## PS2-07-60

### Polymorphisms in proinflammatory cytokines' genes and lipid profile in patients with graves' disease

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#### Objective

Graves' Disease (GD) is one of the most common autoimmune disorders. Some SNP in pro-inflammatory cytokines have already been linked to an increased susceptibility of developing GD. Thyroid hormones are crucial modulators of lipid metabolism. A pro-inflammatory state, may contribute several metabolic changes, including disturbances in lipid metabolism. Our aim was to evaluate if SNP in pro-inflammatory cytokines also contribute to disturbances of lipid profile in GD patients.

#### Methods

Lipid profiles (total cholesterol [TC], high density lipoprotein [HDL], low-density lipoprotein [LDL] and triglycerides [TG], apolipoprotein A-I [Apo A-I], apolipoprotein B [ApoB] and lipoprotein (a)) were assessed in a case-control study comprising 98 Graves' disease patients treated with methimazole. Genetic variants in IL6-174 G/C (rs1800795), TNFA-308 G/A (rs1800629), IL1B-511 C/T (rs16944), and IFNGR1-56 T/C (rs2234711) were discriminated by real-time PCR

using TaqMan SNP genotyping assays. The associations of genetic variants with lipid profile were evaluated with analysis unadjusted and adjusted for age and sex. Results

Within our sample of 97 patients with GD, 91.8% were females, with a mean age of  $44.4 \pm 15.1$  years. The mean TSH level of our population was 0.4 (0.0-1.3)  $\mu$ UI/L, and the mean levels of FT3 and FT4 were 3.0 (2.6-3.5) ng/ml and 1.1 (0.9-1.4)  $\mu$ g/mL, respectively. Seven subjects (9.2%) had diabetes and thirty-three patients (43.4%) had prediabetes. The mean level of total cholesterol was 193.7 (149.1-238.3) mg/dL, the mean level of HDL cholesterol was 59.9 (43.5-76.3) mg/dL and the of LDL cholesterol's level was 117.1 (84-150.2) mg/dL. The A allele in TNFA-308 G/A was associated with significant lower levels of HDL cholesterol ( $P = 0.037$ ). TNF- $\alpha$  A allele also had higher levels of fasting insulin (0.042), IL1B-511 C/T (rs16944), IL6-174 G/C (rs1800795) and IFNGR1-56 T/C (rs2234711) polymorphisms showed no association with lipid profile.

#### Conclusions

In patients with GD, we found lower levels of HDL among those with A allele in TNFA-308 G/A. This polymorphism may contribute to a higher atherogenic risk in patients with GD.

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### PS2-07-61

#### Management of graves' disease among endocrinologists in russia

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#### Objective

We aim of the study was to investigate the clinical approach to Graves' disease (GD) treatment among endocrinologists in Russia.

#### Materials and methods

An online questionnaire survey was conducted among doctors from Sept till Dec 2021. The questions covered the principles of diagnosis, treatment and dynamic observation of adults with GD.

#### Results

104 endocrinologists participated in the study. 99% of respondents chose the thyrotropin receptor antibody (TRAb) test to clarify the etiology of thyrotoxicosis, while 60.6% chose thyroid scintigraphy. Antithyroid drug (ATD) treatment was chosen as the first-line treatment by 88.5% of responders, radioactive iodine (RAI) by 13.5%. Titration ATD regimen is preferable compared to the "block-replace" 72.1% vs 28.8%. Most doctors (95.2%) initiate therapy with moderate doses of thiamazole (20-30 mg). Most of them perform dynamic monitoring of the level of transaminases (57.7%) and complete blood count (78.8%). ATD treatment is prescribed for a period of 12-18 months by 88.5% of doctors, up to 24 months by 10.6%. 89.4% of respondents monitor thyroid hormones, 82.7% of respondents TRAb and 49% perform thyroid ultrasound before ATD withdrawal. Repeated courses of ATD treatment are prescribed by up to 61.5% of respondents. About 63.5% of the surveyed doctors stated difficulties with referral to radical treatment due to the limited number of specialized institutions. The main limiting factors in RAI are the patient's unwillingness and planning pregnancy (81.6% and 24.3%, respectively); thyroidectomy is associated with patient unwillingness (69.2%), as well as doctors' fear of developing complications (49%).

#### Conclusion

The results of the study demonstrated that in their practice, doctors, as a rule, follow international recommendations for the treatment of GD. This study is unique in its kind, since no similar work has been carried out in Russia before.

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### PS2-07-62

#### Multifocality in sporadic and familial medullary thyroid cancer: analysis of prevalence and possible predictive roles

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#### Aims

Surgery is the only curative treatment for medullary thyroid cancer (MTC) patients and the gold standard practice is still represented by total thyroidectomy and prophylactic central neck compartment lymphnode dissection. As it happened for differentiated thyroid cancer, in the last years some authors proposed less aggressive and extent surgical modality as hemithyroidectomy and ipsilateral neck compartment dissection in unilateral and solitary sporadic MTC cases. Few studies are available regarding the prevalence of multifocality in MTC and its pre and postsurgical role. Aims of this study were to assess the prevalence of multifocality in familial and sporadic MTC patients and to correlate the presence of multifocality with clinical and pathological parameters.

#### Patients and Methods

We retrospectively analyzed data from 389 consecutive MTC patients followed-up at our department from 2005 to 2018. Multifocality was defined as the presence of at least more than one tumoral focus both in the same thyroid lobe or in the contralateral one. Independent sample t-test and chi-squared were used for correlations. A p-value  $\leq 0.05$  was considered statistically significant. Statistical analysis was performed using 22.0 SPSS statistical package

#### Results

Multifocality was found in 89/389 cases (22.9%), particularly in 45/311 (14.5%) of sporadic cases and 44/78 (56.4%) of familial ones. All multifocal tumors were also bilateral in familial cases, while tumor bilaterality was present in half of sporadic cases (8.7%). When we correlated the presence of multifocality with clinical and pathological features we found a strong correlation with mutated RET ( $P < 0.001$ ), advanced stage III/IV ( $P < 0.001$ ), tumor extrathyroidal extension ( $P < 0.001$ ), presence of neoplastic emboli ( $P = 0.001$ ), tumor bilaterality ( $P < 0.001$ ), presence of central neck compartment lymphnode metastases ( $P < 0.001$ ), younger age at MTC diagnosis ( $P < 0.001$ ), higher pre-surgical calcitonin levels ( $P = 0.01$ ). When we analyzed separately the group of familial and sporadic cases, we found almost the same associations, with the exception of age at MTC diagnosis in both group ( $P = 0.1$  in sporadic and  $P = 0.3$  in familial cases) and of pre-surgical CT levels in familiar one ( $P = 0.06$ ). In this last group a correlation was also found between multifocality and with bigger tumor dimension ( $P = 0.01$ ).

#### Conclusions

According to our data, as expected, the majority of hereditary cases were multifocal and bilateral, while sporadic cases were multifocal in a rather low percentage of cases (14.5%) and only in a subgroup they were bilateral (8.7%). In the era of personalized medicine, we could start to propose lobectomy in sporadic MTC patients with single intrathyroidal lesions.

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### PS2-07-63

#### Corticosteroids and mycophenolic acid have a synergistic effect on chemokines secretion in orbital cells from patients with graves' ophthalmopathy

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#### Objective

An enhancement of the response rate has been recently showed in patients with Graves' ophthalmopathy (GO) treated with corticosteroids and mycophenolic acid. In GO, retro-orbital cells showed a cytokine-induced secretion of Th1 and Th2 chemokines. Since no data are present in literature about the effect of corticosteroids and mycophenolic acid on the chemokines secretion in GO orbital cells, we aim to deepen out this topic.

## Methods

We established primary cultures of myoblasts, preadipocytes and fibroblasts obtained from GO patients, and we tested increasing concentrations of mycophenolic acid or corticosteroids in order to study the effect on the secretion of either the Th1 (CXCL10) and Th2 (CCL2) chemokines.

## Results

As regard the Th1 chemokines, we observed that CXCL10 was undetectable in the supernatants of the retro-orbital primary cultures cells, while IFN $\gamma$  induced its release in a dose-dependent manner, and TNF $\alpha$  alone had no effect. By contrast, TNF $\alpha$  induced in a dose-dependent manner the release of the Th2 chemokine CCL2, whose amount is low in basal conditions, while IFN $\gamma$  alone had no effect on the CCL2 secretion. The combination of TNF $\alpha$  and IFN $\gamma$  had a significant synergistic effect on the CXCL10 and CCL2 secretion. Increasing concentrations of mycophenolic acid or corticosteroids (in a pharmacological range), were able to reduce the chemokines secretion in a dose-dependent manner, even in the presence of the IFN $\gamma$  and TNF $\alpha$  stimulation. In addition, in presence of IFN $\gamma$  and TNF $\alpha$ , the combination of corticosteroids and mycophenolic acid had a higher effect on the inhibition of the release of chemokines.

## Conclusion

Our investigation showed the important role of mycophenolic acid and/or corticosteroids in the inhibition of the secretion of both Th1 (CXCL10) and Th2 (CCL2) chemokines. These findings suggest a possible therapeutic role of these drugs.

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## PS2-07-64

## The effect of glucocorticoids on hyaluronan production of orbital fibroblasts

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## Objectives

Glucocorticoids (GCs) are the first line therapy in moderate to severe, active thyroid eye disease (TED). While their beneficial immunosuppressive effects are well known, direct connective tissue effects are less characterized. The accumulation of extracellular matrix component hyaluronan (HA) and increased proliferation rate of fibroblasts are hallmarks connective tissue changes in TED. We examined the effect of methylprednisolone (MP), prednisolone (P), hydrocortisone (HC) and dexamethasone (DEX) on HA synthesis and proliferation of orbital (OF) and dermal fibroblasts (DF) in culture.

## Methods

In the presence or absence of MP, P, HC or DEX, mRNA expression of hyaluronan synthases (HAS1, HAS2, and HAS3) and HA production were measured by RT-PCR and ELISA respectively. TED orbital ( $n = 4$ ), non-TED orbital ( $n = 4$ ) and dermal fibroblastlines ( $n = 4$ ) were used. The effect of GCs on proliferation was measured using a BrdU incorporation assay.

## Results

After 24-hour GC treatment (1  $\mu$ M), HA production of TED and non-TED OFs, as well as DFs decreased by 61.1 %, 62.9 % and 77.8 % for DEX, 57.5 %, 63.8 %, 72.6 % for MP, 53.8 %, 58.7 %, 72.2 % for P, and 50.7 %, 52.7% and 72.3 % for HC, respectively ( $P < 0.0001$  for all GCs tested). GCs reduced *HAS3* expression ( $P < 0.0001$ ) in all cell cultures irrespective of the site of origin, while only DEX could decrease *HAS2* expression in all fibroblast cultures tested (TED OFs  $P = 0.0239$ , non-TED  $P = 0.0044$  DFs  $P < 0.0001$ ). The proliferation rate was not influenced by GCs, except a 27 % and 18 % reduction in non-GO OFs and DFs after DEX treatment ( $P = 0.0165$  and  $P = 0.0227$ , respectively).

## Conclusions

We confirmed that glucocorticoids act directly on HAS mRNA expression and HA production of human OFs. We assume that the reduction in HAS gene expression and inhibition of HA production contribute to the beneficial effect of glucocorticoids in TED.

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## PS2-07-65

## Hyperthyroidism in graves' disease and subacute thyroiditis and association with cardiovascular events

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## Objectives

Overt hyperthyroidism is reported to be associated with an increased mortality risk and an increased risk of cardiovascular (CV) complications such as an acute coronary syndrome requiring percutaneous coronary intervention, ischemic and non-ischemic stroke, atrial fibrillation and venous thromboembolism. Increased rate of acute CV events in the course of hyperthyroidism is well documented in Graves' disease (GD) but not in subacute thyroiditis (ST). Last year we reported three patients with an acute CV event in the course of ST. The aim of this study was to systematically compare the characteristics of hyperthyroidism and the incidence of CV events in patients with GD and ST.

## Methods

Our retrospective study included 505 patients that were evaluated for overt hyperthyroidism due to GD or ST in a tertiary referral center from January 2019 to December 2020. The diagnosis of GD and ST was based on clinical evaluation, laboratory tests including measurement of TSH receptor antibodies and/or sedimentation rate, and on thyroid ultrasound. In selected cases, thyroid scan and fine needle aspiration biopsy were also performed. Continuous data are presented as mean ( $\pm$ SD).

## Results

Table 1 presents the characteristics of patients with GD or ST.

**Table 1.** Clinical characteristics of patients with hyperthyroidism due to Graves' disease or subacute thyroiditis.

| Parameter                               | Graves' disease<br>( $n = 390$ ) | Subacute thyroiditis<br>( $n = 115$ ) | p-value          |
|---|----------------------------------|---------------------------------------|------------------|
| Sex (female/male;<br>% female)          | 301/89 (77.2%)                   | 81/33 (70.4%)                         | 0.22             |
| Age (years)                             | 46.6 ( $\pm$ 15.8)               | 46.0 ( $\pm$ 10.6)                    | 0.91             |
| Body mass index<br>(kg/m <sup>2</sup> ) | 24.6 ( $\pm$ 6.3)                | 24.8 ( $\pm$ 4.4)                     | 0.43             |
| Arterial<br>hypertension (%)            | 51 (13.1%)                       | 5 (4.3%)                              | <b>0.01</b>      |
| Smoking (%)                             | 108 (27.7%)                      | 9 (7.8%)                              | <b>&lt;0.001</b> |
| Free T <sub>4</sub> (pmol/l)            | 43.0 ( $\pm$ 27.9)               | 36.5 ( $\pm$ 15.1)                    | <b>0.01</b>      |
| Free T <sub>3</sub> (pmol/l)            | 18.5 ( $\pm$ 8.6)                | 12.3 ( $\pm$ 6.3)                     | <b>&lt;0.001</b> |

In the GD group, 13 (3.3%) patients suffered a CV event within 3 months before or after the diagnosis of GD (acute coronary syndrome in 6, atrial fibrillation in 4, pulmonary thromboembolism in 2 and cerebrovascular accident in 1 patient). No CV events were registered in the ST group.

## Conclusions

The patients in the GD group were significantly more hyperthyroid; in this group, there were significantly more patients with hypertension and smokers. In our cohort of patients with overt hyperthyroidism, CV events were registered only in the GD group. Acute CV events in the course of ST seem to be an extremely rare complication.

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## PS2-07-66

## Influence of thyroperoxydase and thyroglobulin antibodies on the presentation and the evolution of medically treated graves' disease

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#### Introduction

Thyrotropin receptor stimulating antibodies (TRAb) are responsible for Graves' disease (GD) manifestations. Other thyroid antibodies, namely thyroperoxidase (TPOAb) and thyroglobulin (TGAb) antibodies are highly prevalent in GD, but their roles in GD presentation and evolution are controversial.

#### Methods

We retrospectively analysed TPOAb and TGAb levels and evolution in 88 consecutive patients with newly diagnosed GD between 2000 and 2018. The patients were treated with anti-thyroid drugs (ATD) in a block-and-replace regimen for at least 12 months and followed-up after ATD discontinuation for at least one year or until disease relapse.

#### Results

The mean age at diagnosis was  $41.4 \pm 12$  years, 67/88 (76.1%) of patients were women and 16/88 (18.2%) of patients were smokers. Thyroid eye disease (TED) was observed in 21/88 (24%) patients. Mean duration of medical treatment and follow-up were  $24.6 \pm 13.2$  months and  $80.3 \pm 58.2$  months, respectively. During follow-up, 51/88 (58%) of patients relapsed. At diagnosis, 64/88 (73%) of patients were TPOAb positive and 45/88 (51%) of patients were TGAb positive. The presence of TPOAb or TgAb antibodies at diagnosis did not influence the relapse ( $P = 0.356$  and  $P = 0.641$ , respectively). There was less TED in patients TgAb positive at diagnosis than in patients without TGAb (15.6% vs. 32.6%,  $P = 0.061$ ). Logistic regression showed that the absence of TGAb at diagnosis increased the risk of TED by a factor 2.6, without reaching statistical significance ( $P = 0.066$ ). TPOAb and TGAb were both positive in 39/88 (44%) of patients, 25/88 (28.5%) of patients had only TPOAb, 6/88 (7%) of patients had only TGAb, and neither antibody was positive in 18/88 (20.5%) of patients. There was no difference in terms of GD relapse (56.4%, 65.4%, 40% and 55.6% respectively,  $P = 0.722$ ) or TED occurrence ( $P = 0.119$ ) between the four groups. The positivity of TPOAb and/or TgAb at treatment discontinuation or at the last visit before relapse was not predictive of relapse. We observed lower titers of TGAb during treatment in patients who relapsed, however this difference was not significant ( $P = 0.076$ ). In relapsing patients, TRAb, TPOAb and TGAb significantly decreased during the treatment and increased after ATD discontinuation until relapse.

#### Conclusion

TPOAb and/or TGAb positivity and evolution were not significantly associated with GD presentation or relapse after a first course of ATD. There is a non significant trend of more recurrence of GD in patients with low levels of TgAb but a slight non significant protection against TED in cases of TGAb positive at diagnosis.

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#### Objectives

Finding novel tumor markers capable to recognize aggressive papillary thyroid carcinoma (PTC) forms are of great importance. We evaluated hsa-microRNA-204-3p (miR-204-3p) for differential diagnosis of thyroid tumors and assessed its prognostic usefulness.

#### Methods

MiR-204-3p levels were determined in 77 PTC cases of diverse histological variants, 12 cases of follicular thyroid adenoma (FTA), and 89 matched nonmalignant thyroid epithelial tissues (NMT) using RT-qPCR. The results were evaluated in comparison with the clinicopathological features of the patients.

#### Results

The relative expression of miR-204-3p is lower in PTC compared to the levels in paired NMT, with the median down-regulation of 65% ( $P < 0.05$ ). The level of miR-204-3p down-regulation depends on PTC subtype – PTC cases with classical variant architecture had significantly more down-regulated values of miR-204-3p (median down-regulation was 85%) than all other PTC subtypes (median down-regulation in follicular variant was 41%, in cases with mixed architecture 60%, in rare variants, that included Warthin-like, tall cell, solid, clear cell, and oxyphilic cases, was 45%) ( $P < 0.05$ ). Lower levels of relative miR-204-3p expression in PTC associate with the presence of metastasis to regional lymph nodes, intraglandular tumor dissemination and a degree of tumor infiltration through the gland ( $P < 0.05$ ). On the other hand, miR-204-3p is up-regulated in FTA (median up-regulation 178%). Up-regulated expression of miR-204-3p is a sensitive (83.3%) and specific (74.7%) marker for distinction of FTA from PTC (cut off = 1.08,  $P < 0.001$ , AUC = 0.821).

#### Conclusions

MiR-204-3p is a useful marker for PTC detection, particularly its classical variant, and can help in risk stratification of PTC patients.

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#### PS2-08-68

##### NOX-derived oxidative stress is high in neoplastic thyroid lesions and correlates with ata risk in papillary thyroid cancer

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Oxidative stress (OS) can have an impact both in the pathogenesis and in the progression of TC, as it has been shown to induce oncogenes and inhibit tumor suppressors. The main source of cellular ROS is represented by NADPH oxidases (NOXs). Aims of the study were to investigate the NOX-derived OS in TC samples, benign nodules and corresponding normal tissues, and to correlate the level of OS with histological classification, genetic profile and clinical and prognostic features of patients. Twenty-four papillary (PTCs), 6 follicular (FTCs) and 3 anaplastic thyroid tumors (ATCs), 4 noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFPTs), 8 follicular adenomas (FAs), 4 Hurtle adenomas (HAs) and contra-lateral normal tissues were included in the study. H2O2 generation by NOXs in the cytoplasmic fraction of tumoral and normal tissues was measured using the fluorescent Amplex Red Reagent. The molecular profile of tumor samples was characterized by a custom AmpliSeq DNA/RNA NGS panel (Illumina) in which the most common mutations/fusions found in TC are included. The median of H2O2 generation considering all tumors and benign lesions was significantly higher than that obtained in all normal tissues analyzed. When compared with the corresponding normal tissues, differences in H2O2 production were found only in PTCs and FTCs ( $P = 1.2 \times 10^{-6}$ ,  $5.8 \times 10^{-5}$ , respectively). Interestingly, when we stratified PTCs for genetic variants, only PTCs with mutations in TERT and BRAF or BRAF alone showed an increased H2O2 generation compared with the corresponding normal tissues ( $P = 0.03$  and  $6.1 \times 10^{-5}$ , respectively). Moreover, H2O2 production in FTCs resulted higher compared to FA and to PTCs ( $P = 0.003$ ). Finally, the

## Thyroid Cancer BASIC

### PS2-08-67

#### Down-regulated HSA-MIR-204-3p may identify high-risk papillary thyroid carcinoma patients

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H2O2 production in PTCs with high/intermediate ATA risk resulted significantly higher compared with that obtained in low ATA risk PTCs ( $P = 0.02$ ). In conclusion, our data indicate that thyroid tumors are exposed to a higher OS compared to normal tissues. Moreover, the level of NOX4-generated ROS correlates with BRAF and TERT mutations and with worst tumor presentation in PTCs. The high OS associated with thyroid tumors may have diagnostic, prognostic and therapeutic relevance.

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## PS2-08-69

**Use of long noncoding rnas for molecular diagnosis of thyroid cancer**  
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### Objective

Improved molecular testing for common somatic mutations and the identification of mRNA and microRNA expression classifiers are promising approaches for the diagnosis of thyroid nodules. However, there is a need to improve the diagnostic accuracy of such tests in identifying thyroid cancer. Recent findings have revealed a crucial role of lncRNAs in gene modulation. Thus, we aimed to evaluate the diagnostic value of selected lncRNAs from The Atlas of Noncoding RNAs in Cancer (TANRIC) thyroid cancer dataset.

### Methods

lncRNAs in TANRIC thyroid cancer dataset that have significantly increased or decreased expression in thyroid cancer tissues were selected as candidates for thyroid cancer diagnosis. Surgical specimens from patients who underwent thyroidectomy were used to determine the separation capability of candidate lncRNAs between malignant and benign nodules. Fine needle aspiration samples were obtained and screened for candidate lncRNAs to verify their diagnostic value.

### Results

LRRC52-AS1, LINC02471, LINC02082, UNC5B-AS1, LINC02408, MPPE2-AS1, LINCNEF, LOC642484, ATP6V0E2-AS1, and LOC100129129 were selected as the candidate lncRNAs. LRRC52-AS1, LINC02082, UNC5B-AS1, MPPE2-AS1, LINCNEF, and LOC100129129 expression levels were significantly increased or decreased in malignant nodules compared to those in benign nodules or paired normal thyroid tissues. The combination of LRRC52-AS1, LINC02082, and UNC5B-AS1 showed favorable results for the diagnosis of thyroid cancer from fine needle aspirates, with 89 % sensitivity and 100 % specificity.

### Conclusions

lncRNA expression analysis could be a promising approach for advancing the molecular diagnosis of thyroid cancer. Further studies are needed to identify lncRNAs of additional diagnostic value.

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## PS2-08-70

**Biochemical and clinical characteristics of 16 belgian families with germline insttctdelg mutation affecting codon 666 of the retgene: a retrospective cohort study**

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### Background

Medullary thyroid carcinoma (MTC) can result from an inherited predisposition related to variants in the *RET* gene. The insTTCTdelG variant in codon 666 has been previously associated with MTC in one family, but larger studies are not available. We aim to describe biochemical and clinical characteristics associated with this genetic variant in sixteen Belgian families.

### Methods

Retrospective study of sixteen families in Belgium, evaluating biochemical and clinical characteristics of index patients (IPs) and their carrier-relatives (CPs).

### Results

Familial genetic cascade screening of sixteen IPs identified 108 additional family members carrying the variant (total cohort  $n = 124$ , 55% females, 45% males), with age ranging from 6y to 83y (mean  $42 \pm 18$ y) at first clinical evaluation. Twenty-six (22%) CPs had elevated baseline calcitonin at screening. In the total cohort, 39 (31%) patients underwent thyroidectomy, resulting in 34 patients with histological diagnosis of MTC, 2 with C-cell hyperplasia (CCH), 3 with negative histology. The age at diagnosis ranged from 8y to 83y (mean  $48 \pm 17$ y), with 30 (76%) females affected compared to 10 (24%) males. Five IPs and 14 CPs were diagnosed in stage I, 3 IPs and 1 CPs in stage II, 2 IPs and 1 CP in stage III, 3 IPs and 2 CPs in stage IVa, 2 IPs and 1 CP were diagnosed with MTC without available staging data. Thyroidectomy was combined with prophylactic lymph node resection in 23 (59%) patients, with therapeutic lymph node resection in 8 (21%) patients, and without lymph node resection in 8 (21%) patients. Postoperatively, 28 (71%) patients were in remission, 7 (18%) were diagnosed with biochemical recurrent disease, 3 (7%) with structural locoregional recurrent disease. One patient with MTC was also diagnosed with pheochromocytoma, 1 patient with MTC with primary hyperparathyroidism, and 1 patient with pheochromocytoma and primary hyperparathyroidism. One IP and 2 CPs had clinical disease without thyroidectomy (1 due to fear, 2 because of stable very low risk clinical disease based on calcitonin +/-imaging and shared clinical decision). In the CP subgroup, MTC or CCH was diagnosed in 22 (20%) patients (mean age  $49 \pm 17$ y) for a total follow up time of 446 patient y (diagnostic risk of 0.04% per y follow-up per patient).

### Conclusion

This retrospective study on the insTTCTdelG-RET666 variant, which is the largest to date, suggests that this variant is pathogenic and associated with low risk MTC/CCH and rarer other MEN2-A manifestations.

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## PS2-08-71

**Stabilin-1 Macrophages in thyroid cancer microenvironment**

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Papillary thyroid carcinoma (PTC) is the most frequent histological subtype of thyroid cancers and BRAF<sup>V600E</sup> genetic alteration is found in 60% of this endocrine cancer. BRAF<sup>V600E</sup> tumors are associated with poor prognosis resistance to radioiodine therapy and tumor progression. Histological follow-up by anatomic-pathologists reveals that 2/3 of surgically-removed thyroid do not present malignant lesions. Continued fundamental research into the molecular mechanisms of thyroid cancer downstream of BRAF<sup>V600E</sup> remains thus central to better understand the clinical behaviour of tumours, to improve differential diagnosis between thyroid cancer subtypes, to propose new therapies, and to avoid unnecessary surgery. To study PTC we used a mouse model in which expression of BRAF<sup>V600E</sup> is specifically switched on in thyrocytes by doxycycline administration. Upon daily IP doxycycline injection thyroid tissue rapidly acquired histological features mimicking human PTC. Transcriptomic analysis revealed that two pathways: cytokine/cytokine receptor interaction and immune system were highly enriched upon BRAF<sup>V600E</sup> induction. Multiplex immunofluorescence indeed confirmed the recruitment of abundant macrophages among which a population of CD206<sup>+</sup>/Iyve-1<sup>+</sup>/Stab-1<sup>+</sup> was dramatically increased. We decided to focus on this subpopulation of alternatively-activated macrophages by genetically inactivating the gene coding for the scavenger receptor Stabilin-1. We will present the results of our inactivation of Stabilin-1 in the context of *in situ* BRAF<sup>V600E</sup>-dependent thyroid cancer and of the expression of Stabilin-1 in human thyroid cancers and other thyroid pathologies.

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**PS2-08-72****The extracellular matrix actively influence thyroid cancer cells sensitivity to TKIS**

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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. As in other cancer types, despite the promising results obtained in pre-clinical studies, the efficacy of Targeted Therapy is highly variable and therapy resistance is not uncommon. This may be due to a lack in the understanding of the complex signaling pathways and feedbacks that cancer cells with a specific genetic background exchange with all the other components of the tumor microenvironments, such as immune cells, fibroblasts, endothelium, and extracellular matrix (ECM). The aim of the present study is to unravel the mechanisms by which cancer associated fibroblasts (CAFs) and ECM can influence the tumor response to different anticancer drugs and to identify new potential druggable pathways. For these purposes, we developed an *in vitro* model in which a panel of 12 thyroid cancer cell lines with different genetic background is used to activate fibroblast and generate ECMs. The ECMs are then used as culture substrate for the different thyroid cancer cell lines. We used this model to compare how the ECMs produced under different conditions can influence TC cells response to different TKI currently used in clinical practice, such as vemurafenib, lenvatinib and dabrafenib. First, our experiments confirmed that the genetic background of TC cells can significantly influence the degree of activation of CAFs and the type and amount of ECM that they produce. Moreover, the various ECMs differentially influence TC cells growth and their sensitivity to TKIs, irrespectively of their genetic background. These data show that ECM can either actively influence the therapeutic response, and play an active role in the clonal evolution of thyroid cancer, by differentially supporting subpopulations with diverse genetic background. The *in vitro* modulation of crosstalk between TC cells and fibroblasts may open new perspectives on the complex mechanisms of therapy resistance and eventually allow the development of efficient therapeutic strategies.

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**PS2-08-73****MiR-145 and miR7 as a potential diagnostic markers of thyroid cancer**

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**Objective**

Thyroid nodules are the most common thyroid disease and preoperative differentiation of benign and malignant lesions is an important diagnostic purpose. In about 1/3 cases FRA and cytological examination shows indeterminate diagnosis. Deregulation of miRNA expression has been described in a variety of tumors, including thyroid cancer. The aim of our study is to evaluate the association between miRNAs expression and risk of thyroid cancer in patients with thyroid nodules.

**Methods**

In retrospective case-control study we have studied 112 patients with suspected thyroid cancer (FNA Bethesda III-VI). All patients underwent thyroidectomy with histological verification of the diagnosis. The expression of miR144, miR145, miR146, miR155, miR183, miR199, miR221, miR31, miR551, miR375, miR451 and miR7 were determined using quantitative real-time PCR. Logistic regression and receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic efficacy and build mathematical diagnostic models.

**Results**

Study group included 54 patients with thyroid cancer and control group of 58 patients with benign nodules. In our study up-regulation of miR146, miR221, miR155, miR375, miR31, miR551 and down-regulation of miR7 and miR145

were significantly associated with thyroid cancer. The most diagnostic value had expression of miR145 with an area under the ROC curve (AUC) value of 0,982 (95%CI: 0,953-1,0), miR146 – 0,945 (95%CI: 0,896-0,994), miR375 – 0,913 (95%CI: 0,855-0,971) and miR7 – 0,900 (95%CI: 0,842-0,958). After logistic regression we build mathematical diagnostic models based on the expression of miR145 and miR7. The AUC value for model was 0,99 (0,96-1,00), the sensitivity (Se) was 98,1% (89,9-100), the specificity (Sp) was 96,6% (88,1-99,6).

**Conclusion**

The expression of miR145 and miR7 has a high diagnostic value in detecting of thyroid cancer

**Key words:** thyroid cancer, molecular genetic testing, miRNA

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**PS2-08-74****EIF1AX gene variants in the context of thyroid tumorigenesis**

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**Objectives**

Variants in the *EIF1AX* gene have been reported in malignant as well as benign thyroid nodules and their clinical significance is still unclear due to their low prevalence. The aim of this study was to identify *EIF1AX* variants in a large cohort of different types of thyroid nodules and to correlate them with clinical and pathological data.

**Methods**

The study consisted of 904 thyroid nodule samples. The cohort included 577 papillary thyroid carcinomas (PTCs), 16 anaplastic thyroid carcinomas (ATCs), 8 poorly differentiated thyroid carcinomas (PDTCs), 18 follicular thyroid carcinomas (FTCs), 10 oncocytic carcinomas, 35 borderline lesions (e.g., follicular tumor of uncertain malignant potential - FT-UMP), 55 follicular thyroid adenomas (FTA) and 185 benign nodules. DNA isolated from fresh frozen

thyroid tissues was used for preparation of libraries using the Nextera XT DNA Library Prep Kit (Illumina, USA) and analyzed using next-generation sequencing (MiSeq, Illumina, USA). Variants in the *EIFIAX* gene (exons 1, 2, 5, 6) were visualized in Integrative Genomics Viewer (Broad Institute, USA) and evaluated by VarSome platform (Saphetor SA, Switzerland).

#### Results

*EIFIAX* gene variants were detected in 18 of 904 thyroid samples (2%) - 2 ATCs, 3 PTCs, 2 FTCs, 2 FTAs, 2 FT-UMPs, 1 oncocytic adenoma and 6 other benign nodules. The most frequent *EIFIAX* A113\_splice variant was found in 6 of 18 (33.3%) positive samples (1 ATC, 2 PTC, 1 FTA, 1 FT-UMP and 1 goiter). Variants in codon 9 (G9R, G9V, G9D) of *EIFIAX* gene were found in 3 benign nodules and 1 ATC, in which coexisted with variants of *TERT*, *TP53* and *NRAS* genes. The coexistence of *EIFIAX* variants with mutations in other genes (5× *TERT*, 9× *RAS* - 4 × simultaneously) was found in a total of 10 of 18 cases, and often correlated with more aggressive disease. On the other side, 8 *EIFIAX*-positive samples which did not possess other driver mutations were benign nodules, FTAs or FT-UMPs.

#### Conclusion

In summary, *EIFIAX* gene variants were detected in 18 cases. In most cases, the *EIFIAX* variants co-occurred with known variants of other genes and were associated with more aggressive tumor behavior. In accordance with literature, distinct *EIFIAX* variants may be related to different types of thyroid tumors and extension of the positive cohort could provide more accurate insight into the understanding of this gene. Supported by AZV NU21-01-00448 and MH CR RVO 00023761.

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### PS2-08-75

#### PD-L1 and MCL-1 markers and the relationship with prognostic characteristics of differentiated thyroid carcinoma

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#### PD-L1 AND MCL-1 MARKERS AND THE RELATIONSHIP WITH PROGNOSTIC CHARACTERISTICS OF DIFFERENTIATED THYROID CARCINOMA

##### Objectives

MCL-1 and PD-L1 proteins are related to carcinogenesis mechanisms in differentiated thyroid carcinoma (DTC). Tumor antigens stimulate the expression of PD-1 in immune cells, which binds to PD-L1 of tumor cells, inducing immune escape from the tumor. MCL-1, an anti-apoptotic member of the BCL-2 family and has a high oncogenic potential. The relationship of these markers with prognosis in DTC remains unknown. We aim to evaluate the clinical utility of immunohistochemical expression (IHC) of markers MCL-1 and PD-L1 in DTC and to investigate its relevance in the long-term prognosis.

##### Methods

120 patients with DTC after total thyroidectomy and radioiodine therapy followed for a minimum of 2 years after complete treatment were included. Demographic features, tumors histopathological characteristics, initial risk classification of persistence/recurrence, factors associated with outcome, initial response to therapy, persistence or disease-free at the end of follow-up were evaluated. These data were related to IHC expression of MCL-1 and PD-L1 and presence of *BRAFV600E* mutation.

##### Results

Among the patients, 100(83.33%) were women and 20 men, age at diagnosis 46.64±16.73 years; 37 (30.8%) patients were at high risk, 45(37.5%) of

intermediate risk and 38(31.7%) of low risk of disease recurrence/persistence. At the end of follow-up, 65(57.5%) were disease-free and 48(42.5%) had persistent disease. The largest tumor diameter was 2.99±1.79 cm; 103(85.8%) patients had papillary thyroid carcinoma (PTC) and 17(14.2%) had follicular thyroid carcinoma (CFT), with a follow-up time of 124.86±65.36 months. *BRAF* V600E mutation was detected in 49 (59.8%) patients and absent in 33(40.2%). Strong/moderate expression of PD-L1 was associated to tall cell variant PTC ( $P = 0.0274$ ). Strong MCL-1 expression was associated with the presence of the *BRAF* V600E mutation ( $P = 0.0468$ ); and weak expression of MCL-1 was associated with multifocality ( $P = 0.0290$ ). No patients with CFT presented weak MCL-1 expression, and all tumors with weak expression were PTC ( $P = 0.0409$ ).

##### Conclusions

PD-L1, marker of higher proliferation and progress of tumor cells was associated with more aggressive PTC variant. The anti-apoptotic marker MCL-1 was associated with CDT carrying the *BRAFV600E* mutation. Additionally, lower anti-apoptotic marker expression was related to multifocal and papillary thyroid cancer. Additional studies are needed to confirm the possible role of these markers in the tumorigenesis and evolution of DTC.

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### PS2-08-76

#### Double mutation of the TERT promoter in extremely aggressive papillary thyroid carcinoma

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##### Objectives

*BRAFV600E* mutation is present in 50% of aggressive papillary thyroid carcinoma(PTC). *TERT* promoter (*pTERT*) mutations (C228T, C250T) are related to cancer growth and reduced overall- and disease-free survivals in differentiated thyroid carcinomas(DTC). We report a patient with an extremely aggressive PTC presenting in the primary lesion two *pTERT* mutations (C228T and C250T), and absence of *BRAFV600E*.

##### Case Report

Female, 39-year-old, euthyroid patient referred a 10-year multinodular goiter, developed compressive symptoms during last year. Cytology of largest nodule was not suggestive of malignancy. Total thyroidectomy was performed due to compression. Histopathology revealed multifocal PTC follicular variant with areas of classic form within the hyperplastic nodules (5.3 cm in right lobe; 3.5 cm in left lobe; two 0.9 cm foci in isthmus) (pT3N0M0). Patient received adjuvant radioiodine ablation therapy(RIT); 150mCi 131I; Tg=68ng/dL, anti- TgAb undetectable, TSH=73mUI/dL. Two years later, a whole-body 131I scintigraphy(WBS) demonstrated diffuse iodine-avid pulmonary metastases; Tg=1,100ng/dL, negative TgAb. After five years, 18F-FDG PET/CT images revealed hypermetabolism in cervical lymph node metastases bilaterally and in pulmonary nodules; Tg=2,991ng/dL; TgAb undetectable. After seven years, patient presented pathological fractures (humerus, right tibia), soft tissues infiltration. 18F-FDG-PET/CT revealed widespread lungs metastases, cervical lymph nodes, left breast, gluteus maximus, mediastinal, axillary, and inguinal lymph nodes. Hypermetabolic metastatic lytic bone lesions were noted in skull, mandible, clavicle, ribs, humerus, femur, pelvis, T2 vertebra. Patient received three adjuvant RIT (1223mCi), without response. Palliative radiotherapy was performed in skull, humerus, left leg with partial reduction. There was lung metastases reduction with



sorafenib, and progression of the other. Patient progressed with bronchial obstruction from lung metastases and died from respiratory failure eight years after the initial diagnosis. Molecular analysis of primary tumor tissue revealed absence of *BRAF* V600E mutation, evaluated by qPCR in COBAS Z 480 System (Roche) and presence of both *pTERT* C228T and C250T mutation, detected by pyrosequencing and validated by droplet digital PCR.

#### Conclusions

The *pTERT* mutations C228T and C250T have been described as mutually exclusive, indicating that one mutation is enough for telomerase activation and exert its action on thyroid tumorigenesis. However, in this case report we observed the presence of both *pTERT* mutations.

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## Thyroid Hormone ACTION

### PS2-09-77

#### Correlation between plasmatic long pentraxin PTX3 and nodular thyroid disease: a preliminary report

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#### Introduction

The long pentraxin-3 (PTX3) is a key component of humoral innate immunity that is expressed in various cell types during stress and tissue injury. PTX3 also acts like an oncosuppressor regulating tumor-promoting inflammation and it is implicated in tissue repair and autoimmunity. Autoimmune disease, tissue remodelling and oncogenesis often coexist in the thyroid. PTX3 role in thyroid disease is still unknown. Aim of the study is to evaluate if plasmatic levels of PTX3 in patients submitted to thyroidectomy for benign or malignant nodular disease are higher than normal.

#### Materials and methods

After informed consent, patients over 18 years old with nodular disease of the thyroid who were eligible for thyroid surgery were enrolled in this study. All patients underwent total or hemi-thyroidectomy at Humanitas Mater Domini Clinical Institute in Castellanza (VA). A blood sample was taken on the day of surgery and another one was taken 45 days after surgery to evaluate plasmatic PTX3 level. Blood samples were centrifuged and PTX3 levels were evaluated with ELISA test. In this preliminary report, we evaluated the data of the first 53 consecutive patients enrolled in the study.

#### Results

We found that preoperative plasmatic PTX3 levels were significantly higher than normal in patients with thyroid disease ( $P < 0.05$ ). Plasmatic PTX3 mean value was 4.54 ng/ml (range 1.06 – 8.63 ng/ml), when normal value is considered 2 ng/ml with 1 ng/ml of standard deviation. At 45 days follow-up PTX3 mean value was reduced to 3.40 ng/ml (range 0.89 – 9.21 ng/ml); this reduction was statistically significant ( $P < 0.05$ ).

#### Conclusions

For the first time, at the best of our knowledge, we observed a correlation between elevated PTX3 plasmatic levels and nodular disease of the thyroid. We hope to identify if plasmatic PTX3 could be used as a marker for nodular thyroid disease.

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### PS2-09-78

#### Synthetic analogs of 3-iodothyronamine (TIAM) prevent $\beta$ -amyloid neurotoxicity in an *in vitro* model of Alzheimer's disease (AD)

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We have already reported that synthetic analogs of 3-iodothyronamine (TIAM), a naturally occurring thyroid hormone derivative, share a pleiotropic activity with the endogenous parent compound, including autophagic flux promotion (ATG),

neuroprotection, and metabolic reprogramming. Our study aimed to determine whether the TIAM lead analog SG2 and its derivatives SG22 and SG23, developed as prodrugs of SG2, can protect human glioblastoma U87 MG cells from the toxic effects induced by  $A\beta_{25-35}$ . Indeed, in our experimental setting, exposure to 10  $\mu$ M  $A\beta_{25-35}$  for 48h produced a significant reduction of U87 MG cells viability ( $35.24\% \pm 2.29$ ). To examine whether pretreatment with test compounds, namely SG2, SG22 and SG23, prevents  $A\beta_{25-35}$  neurotoxicity, U87 MG cells were exposed to 10  $\mu$ M test compound for 24h, followed by treatment with 10  $\mu$ M  $A\beta_{25-35}$  for 48h. In another set of experiments, the effect of post-treatment with test compounds in rescuing U87 MG cells' viability after exposure to  $A\beta_{25-35}$  was also evaluated. Next, to ascertain whether the exposure of U87 MG cells to  $A\beta_{25-35}$  may further reduce their ATG activity, the expression of ATG-related genes was analyzed. Our results indicate that pretreatment with SG2, SG22 or SG23 efficiently prevented  $A\beta_{25-35}$  cytotoxicity in U87 MG cells, whereas post-treatment with the compounds restored cell viability to a lesser extent. Using qPCR we found that treatment with 10  $\mu$ M  $A\beta_{25-35}$  caused a significant ( $P < 0.05$ ) overexpression of potent ATG inhibitors mTOR and sirtuin-5 (Sirt5), as well as a reduced expression of pro-ATG genes, including sigma-1 receptors (Sig-1R) and nuclear sirtuins (Sirt1 and Sirt6). Notably, pretreatment with 10  $\mu$ M SG2, SG22 or SG23 prevented the effects of  $A\beta_{25-35}$  on the expression of ATG-related genes. Moreover, in U87 MG cells exposed to test compounds, the expression of pro-ATG genes was particularly pronounced as compared to U87 MG control cells. Consistently, in thyronamine-like analogs treated cells an increased expression of the specific ATG marker LC3 was also observed. Taken together our results provide strong evidence that SG2 and its prodrug analogs SG22 and SG23 effectively prevent  $A\beta_{25-35}$  toxicity in U87 MG cells by activating ATG. Future studies will address the capability of these novel compounds to extend the lifespan and promote ATG in an *in vivo* model of Alzheimer disease of *C. elegans*.

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### PS2-09-79

#### Ectopic expression of human TRA and TRB mutants disentangle the isoform-specific regulation of gene expression during zebrafish development

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Thyroid hormone receptors (TRs) modulate various physiological functions in many organs. The conservation of TR $\alpha$  and TR $\beta$  isoforms during evolution of vertebrates suggests different roles for these TRs in thyroid hormone-dependent regulation of gene expression. TR $\alpha$  and TR $\beta$  are widely distributed and overlapping in several tissues, and their functional divergence is mainly attributed to their variable tissue expression patterns and intrinsic properties of specific TR isoforms have not been extensively investigated. Here, we want to verify whether the two TRs can be interchangeable in their tissue actions by comparing the phenotypical alterations in zebrafish embryos ubiquitously expressing human TR (hTR) mRNAs, injected in zygotes at 1-2 cells stage at a concentration of 50 pg/embryo. We previously showed that zebrafish TRs (zTR) can efficiently interact with their human homologues and the microinjection of wild-type hTRs does not affect the normal embryonic development. Conversely, the dominant-negative hTR $\alpha$ 1-E403X or hTR $\beta$ 2-E464X mRNAs results in distinct phenotypes despite their ubiquitous expression in zebrafish tissues. Embryos injected with hTR $\alpha$ 1-E403X present aberrant angiogenesis, reduced heart looping and bradycardia, whereas only the embryos expressing hTR $\beta$ 2-E464X show the typical RTH $\beta$  biochemical signature (high T4/T3 with unsuppressed *tshba* levels) confirming a dominant-negative effect on the pituitary negative feedback. Then, we generated mutant chimeras by switching the  $\alpha$ 1 and  $\beta$ 2 functional domains: N-terminus A/B domain (A/B), DNA binding domain (DBD), hinge region (HR) and ligand binding domain (LBD). Multi-step PCR was used to generate wild-type and mutant chimeric fragments where the hTR $\alpha$ 1 domain has been replaced with that of hTR $\beta$ 2, and *vice versa*. Using *tshba* expression as marker of TR $\beta$ 2-dependent activity, we attempted to discriminate which domain dictates the specific function. Several experimental replicates indicate the chimeric E403X mutant containing the A/B of hTR $\beta$ 2 linked to DBD-HR-LBD of hTR $\alpha$ 1 as functionally similar to the hTR $\beta$ 2-E464X on *tshba* expression, suggesting that the A/B domain of TR $\beta$ 2 is both required and sufficient to mediate this specific transcriptional effect, and that the variable structural properties of TRs represent another relevant factor dictating the isoform-specific actions *in vivo*.

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**PS2-09-80****Deiodinase type I (DIO1) regulation in non-alcoholic fatty liver disease (NAFLD)**

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**Background and Aim**

Altered hepatic thyroid hormone (TH) signalling is associated with the onset and progression of liver diseases. Local hepatic hypothyroidism is related to a higher incidence of developing non-alcoholic fatty liver disease (NAFLD) in humans and animal models. Thyroid hormone treatment proved to be a promising therapy, slowing the progression of NAFLD to non-alcoholic steatohepatitis (NASH), a more advanced stage of the disease characterized by inflammation and occasional fibrosis. Hepatic thyroid hormone activity is regulated by TH transporters, deiodinases, and receptors. Among these, deiodinase type 1 (*Dio1*) is a major player, it converts the prohormone thyroxine (T4) to the bioactive form T3 within the hepatocytes. Unfortunately, the precise regulation of *Dio1* in liver diseases remains incompletely understood.

**Methods**

We studied *Dio1* expression in different hepatic disease models, including male C57BL/6 mice fed with high-fat diet (HFD) for 18 weeks and treated with metformin for the last four weeks of treatment, male C57BL/6 mice fed with choline-deficient HFD for 4 and 8 weeks at thermoneutrality, and finally, male C57BL/6 mice treated with carbon tetrachloride to induce liver failure.

**Results**

*Dio1* expression is rapidly induced by HFD and remained elevated throughout the treatment without alterations by metformin treatment. Notably, none of the other conditions resulted in an induction of *Dio1*, despite a similar degree of liver lipid deposition.

**Conclusion**

Our results show that *Dio1* is rapidly induced by HFD, an effect that seems to be independent of insulin sensitivity, as it was not reversed by metformin treatment. This effect was not visible in other animal models with a similar degree of hepatic lipid deposition, suggesting that other factors such as liver inflammation and fibrosis may prevent the HFD induced *Dio1* induction. We suggest *Dio1* increases as part of a protective response in NAFL and early NASH.

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**PS2-09-81****An experimental framework for probing molecular mechanisms of local thyroid hormone action during cortical neurogenesis in human cerebral organoids**

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Recently, human cerebral organoids (hCOs) emerged as powerful three-dimensional *in vitro* models recapitulating key aspects of early human cerebral cortex development. Improved protocols for hCO generation from induced pluripotent stem cells (hiPSC) paved the way for new disease modeling approaches and enable multimodal analyses of perturbed neurogenesis resulting from manipulation of signaling pathways or gene function. These prospects prompted us to adopt this technology as a model system to delineate the regulatory logic of local TH action during early human brain development and to model the consequences of impaired TH function for cortical neurogenesis. Here, we report results from our efforts to develop experimental strategies for targeted perturbation of TH signaling in hCOs and highlight critical quality measures to prevent experimental artifacts. Titration of graded amounts of exogenous TH to culture media is commonly regarded as the simplest means for manipulating TH signaling. Yet, we found that the variable amounts of TH present in culture media and commercial supplements (i.e. B27) can confound experimental outcomes and that rigorous analytical verification of TH levels is required to ensure reproducible endpoint measurements. Patient-derived hiPSC lines carrying genetic variants conferring reduced functionality of specific proteins gained great popularity for disease modeling. However, differences in the inherent propensity of individual hiPSC lines to generate cerebral cortex tissue *in vitro* can result in enormous variation and hamper robust phenotypic comparisons. We therefore developed CRISPR/Cas9-based strategies for generation of isogenic wildtype and mutant hiPSC lines to minimize this source of variation. In developing hCOs, progenitor and neuronal cell types position themselves in three-dimensional *in vivo*-like laminar structures along with lineage specification. We validated a panel of canonical cell type and layer markers to distinguish germinal zones and a cortical plate-like zone and define the timing of the sequential appearance of deep and upper layer neurons consistent with the *in vivo* inside-out manner of cortical layer emergence. Due to the complexity of the tissue, we apply single cell transcriptome techniques to comprehensively capture the temporal and cell type-specific expression of TH transporters, receptors and deiodinases in developing hCOs and use single molecule fluorescent *in situ* hybridization to register cell type-specific expression patterns in the spatial context of the laminar cortical organization. Collectively, we present a strategy combining various endpoint measurements and quality measures to faithfully exploit the enormous potential of hCO for studies on TH action in a human model system.

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**PS2-09-82****Novel central actions of thyroid hormone in the control of body temperature**

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The ability of thyroid hormones to regulate body temperature is well established. While the active hormone T3 can act peripherally to induce thermogenesis in fat and muscle, it also acts centrally in the brain to increase body temperature through the sympathetic nervous system. Most remarkably, recent studies show that T3 treatment in mice causes an elevated body temperature even at 10°C, far below

thermoneutrality, suggesting that the T3 effect constitutes pyrexia rather than hyperthermia. Therefore, mice with induced hyperthyroidism seem to have an altered temperature set point in the brain; however, the precise neuroanatomical substrate has remained unknown. The goal of this research project is to identify the brain region where T3 acts to regulate the body temperature setpoint. Using PET/CT scans of mice treated with T3, several candidate regions have been identified. Among these, the Zona Incerta (ZI), has been associated with the control of body temperature previously. To test whether this region constitutes the missing link between the central T3 effect and pyrexia, we studied the ZI using well-established mouse models. Preliminary data show no difference in cell number of dopaminergic neurons in the ZI when comparing offspring of wild-type mice to those of mice with a mutation in thyroid hormone receptor  $\alpha 1$ , indicating no developmental effect of thyroid hormones on ZI dopaminergic neurons. However, further studies will be needed to illuminate the acute actions of T3 in this enigmatic brain region.

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## PS2-09-83

### FAM83B is involved in thyroid cancer cell differentiation and migration

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*FAM83B* has been recently identified as an oncogene involved in the development and progression of several malignancies, but its role in thyroid cancers (TC) is still unclear. In the present study, we examined the expression of *FAM83B* and its possible involvement in cell migration and differentiation, in neoplastic and normal thyroid tissues and in TC human cell lines. We found that *FAM83B* expression in TC varies according to tumor histotype, being significantly downregulated in more aggressive TCs and in metastatic tissues. *In vitro* experiments showed that *FAM83B* levels in cell lines recapitulate patients' samples variations, and that its total and cytoplasmic levels decrease upon the induction of migration, together with an increase in its nuclear localization. Similar variations were detected in the primary tumor and in the metastatic tissues obtained from a patient with a follicular TC. *FAM83B* knock down experiments confirmed a role for *FAM83B* in thyroid differentiation and migration, as demonstrated by the reduction of thyroid differentiation markers *PAX8* and *NIS* and the increase of the mesenchymal marker Vimentin. Moreover, the silencing of *FAM83B* significantly increased cells migration abilities, while not affecting the oncogenic RAS/MAPK/PI3K pathways. Our data indicate for the first time a role for *FAM83B* in TC cell differentiation and migration. Its expression is reduced in more dedifferentiated tumors and the decrease in its expression and its nuclear relocalization could favour distant migration, suggesting that *FAM83B* should be considered a possible diagnostic and prognostic biomarker.

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## PS2-09-84

### Molecular mechanisms underlying action of triac in resistance to thyroid hormone BETA (RTH $\beta$ )

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### Background

Resistance to thyroid hormone (TH) beta (RTH $\beta$ ), caused by mutations in *THRB*, is characterized by elevated serum (F)T4 accompanied by non-suppressed TSH concentrations. Disease features arise from variable resistance to TH action in tissues expressing Thyroid Hormone Receptor (TR)  $\beta$  (hypothalamus, pituitary, liver) and from thyrotoxic effects in tissues expressing TR $\alpha$  (heart, bone, brain). In symptomatic patients, treatment mainly involves beta blockade to ameliorate tachycardia. In a subgroup of patients, the T3-analogue TRIAC has been employed to alleviate thyrotoxic symptoms. TRIAC preferentially activates TR $\beta$  rather than TR $\alpha$ , and has been proven to suppress TSH, thereby lower circulating TH concentrations. Despite its clinical use for decades, the exact mechanism by which TRIAC works in RTH $\beta$ , is as yet unclear. Here, by linking clinical observations with molecular studies, we investigated whether TRIAC exerts its effects through activation of mutant TR $\beta$  or by stimulating residual wild-type TR $\beta$ .

### Methods

We collected clinical and biochemical data from 17 RTH $\beta$  patients treated with TRIAC in 3 centres. A proof-of-concept study was done with two TR $\beta$  mutants (G432del and R438fsx445). G432del and R438fsx445 were studied in the TR $\beta$ 2 pituitary isoform background. Transcriptional activity was measured using two TRE-luciferase reporters (DR+4-TRE, TSH $\alpha$ -TRE) and interaction with cofactors (NCoR1 and SRC1) tested in mammalian two-hybrid assays with increasing doses of T3 or TRIAC (0 to 10000 nM).

### Results

All patients showed clear clinical and biochemical responses to TRIAC treatment. In contrast, our *in vitro* studies showed that G432del and R438fsx445 TR $\beta$  mutants mediated no transcriptional responses on either TRE at any concentration of TRIAC and T3 tested. In addition, TRIAC did not induce co-repressor (NCoR1) release or promote co-activator (SRC1) recruitment for the G432del mutant. Data from thirteen other receptor mutants in TRIAC-treated patients, will be included in the presentation.

### Conclusion

Despite beneficial effects *in vivo*, our preliminary results, utilizing a battery of functional assays, indicate that TRIAC does not activate mutant TR $\beta$ 2 *in vitro*. We are currently testing *in vitro* and *in vivo* in a zebrafish model the expanded panel of mutant receptors, found in patients that responded favourably to TRIAC. Our studies will provide insights into mechanisms of action of TRIAC in RTH $\beta$  and may shape future studies developing therapies for RTH syndromes.

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## PS2-09-85

### 3-Iodothyroacetic acid release in a mouse model of ischemia-induced synaptic dysfunction

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The endogenous thyroid hormone derivative 3-iodothyronamine (T<sub>1</sub>AM), and its metabolite 3-iodothyroacetic acid (TA<sub>1</sub>) are known to stimulate learning and induce hyperalgesia in mice. Recently, it has been demonstrated that exogenous T<sub>1</sub>AM is able to rescue synaptic impairment after transient ischemia in the entorhinal cortex (EC), a brain area crucially involved in learning and memory, and early affected during Alzheimer's disease. However, it is still controversial whether T<sub>1</sub>AM and its metabolites are produced and released locally at brain

level. Here, we sought to assess the release of T<sub>1</sub>AM and TA<sub>1</sub> in a mouse model of ischemia-induced EC synaptic dysfunction. EC brain slices were obtained from 3-4 months old C57BL/6 male mice (*n* = 5), using a vibratome (Leica VT1200S). All steps were performed in ice-cold oxygenated artificial cerebrospinal fluid (aCSF). Slices were perfused at 2-3 ml/min rate with oxygenated aCSF at 33 ± 1 °C. Field excitatory postsynaptic potentials were evoked by a concentric bipolar stimulating electrode in the layer II of the EC. After 10 min of stable baseline recordings, slices were perfused with deoxygenated glucose-free aCSF to obtain a transient oxygen-glucose deprivation (OGD). After 10 min of OGD slices were reperfused with aCSF for 50 min. Effluent aCSF was collected over 10 min intervals during the whole duration of experiments, and assayed by tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS). In additional experiments electrical stimulation was omitted and/or amine oxidase inhibitors were included in aCSF. T<sub>1</sub>AM release was not observed in any experiment, however a significant release of its metabolite TA<sub>1</sub> was detected during OGD (0.41 ± 0.04 ng/ml) and during the first 10 min of reperfusion with aCSF (0.24 ± 0.04 ng/ml), but not at the baseline. Notably, no TA<sub>1</sub> release was observed if EC were subjected to OGD in the absence of electrical stimulation. Moreover, preliminary experiments showed that semicarbazide, an amine oxidase inhibitor, was able to block TA<sub>1</sub> release. We conclude that in functional, but not in electrically silent, EC, simulated ischemia elicits TA<sub>1</sub> release. TA<sub>1</sub> might be produced by oxidative deamination of an endogenous precursor, possibly T<sub>1</sub>AM. To our knowledge, this is the first report of TA<sub>1</sub> production and release in a pathophysiological relevant condition.

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## PS2-09-86

### Quantification of CD5L as circulating marker of peripheral thyroid hormone action

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The quantification of TSH from a serum or plasma sample constitutes the cornerstone of assessing the thyroid axis in the clinical routine. In addition, T4 and/or T3 levels may be determined to diagnose pathological conditions. However, these data do not necessarily reflect the local action of thyroid hormone in the target tissues and are sensitive to distortion, as best known from pregnancy when chorionic gonadotropin disturbs the regular feedback control. Recently, we identified CD5L as a liver-derived thyroid hormone-responsive biomarker in mice and men, and observed a positive correlation to circulating thyroid hormone in human subjects. [1] In order to enable large-scale clinical analyses, we decided to develop a reliable and robust CD5L-specific sandwich ELISA. To this end, CD5L was recombinantly expressed in HEK293 cells, purified to homogeneity and used to develop monoclonal antibodies. Specificity of the antibodies was tested by commercial CD5L preparations, and a pair of monoclonals was selected for sandwich assay detection that allowed sensitive and robust CD5L quantification. Performance parameters were determined by measuring standard curves four times in double determination; one log level of concentration differences was achieved by a manual assay design, and two log levels were spanned by an automat-based assay design, each with a coefficient of variation below 10% and a relative error below 20%. Stability of CD5L in serum samples was verified by repeated freeze-thaw cycles, and linear dilution experiments indicated reproducible detection over a range of 100- to 32,000-fold with a coefficient of variation below 6%. Given this positive achievement, we are confident to next turn to clinical samples and test whether CD5L assessment may indeed provide the clinically needed additional information on peripheral thyroid hormone action in different and challenging clinical constellations. Supported by the DFG-funded CRC/TR296, LocoTact.

[1] Nock, S., K. Johann, L. Harder, E.K. Wirth, K. Renko, C.S. Hoefig, V. Kracke, J. Hackler, B. Engelmann, M. Rauner, J. Köhrle, L. Schomburg, G. Homuth, U. Völker, G. Brabant, and J. Mittag. 2020. CD5L Constitutes a Novel Biomarker for Integrated Hepatic Thyroid Hormone Action. *Thyroid*. 30:908-923.

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## Nodules & Cancer

### PS2-10-87

#### Toetva- scarless thyroid surgery in hungary

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In thyroid surgery recently is a very important issue to maintain good cosmesis of the scar which can always be visible. Even smaller incisions have been utilized in order to improve satisfaction of the patient. In the last decades new endoscopic techniques have been applied using breast or axillary approach with unfavourable cosmetic outcome. In the last couple of years there has been a development of a new endoscopic technique known as transoral endoscopic thyroidectomy vestibular approach (TOETVA) which is suitable for patients with small thyroid carcinomas without extrathyroidal extension, for benign nodules up to 4-5 cm, and also for parathyroid adenomas. Metastatic thyroid diseases and large substernal goiters should be operated with conventional open surgery.

#### Methods

From June 2018 to October 2021, a total of 12 patients with thyroid cancer or nodule (size of 1-5 cms) were operated with TOETVA in the National Institute of Oncology. Lobectomy was performed in 11 cases, and 1 patient had isthmusectomy.

#### Results

10 patients were female, 2 were men, mean age was 48 years (41-72). 10 right sided, 1 left sided and 1 isthmusectomy were performed. The histological report referred papillary thyroid cancer (size 0,8 cm-1,7 cm) in 4 cases, follicular neoplasm in 5 cases, and colloid nodules in 3 patients. TOETVA patients had no drain placement, and were discharged on the 1st postop day. The average operating time was 107 minutes (76-124 min). Injury of the recurrent laryngeal nerve was not detected. Any other complications were not detected.

#### Conclusion

A TOETVA is the only scar-free, and effective procedure of the thyroid gland, which provides good cosmetic outcome. The long operative procedure time will be shortened with experience after a learning curve of 15-20 operations. The surgeon must be a high volume surgeon on the field of thyroid surgery.

**Key words:** (TOETVA) transoral endoscopic thyroidectomy vestibular approach, thyroid surgery

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## PS2-10-88

### Neoadjuvant effects of apatinib in progressive, metastatic differentiated thyroid cancer (DTC)

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#### Purpose

Recently, apatinib, an orally anti-angiogenic tyrosine kinase inhibitor (TKI) is reported to be useful for treatment of progressive RAIR-DIC. The aim of this study was to evaluate the effect of apatinib and the combination therapy with radioactive iodine (RAI) in patients with progressive metastatic DTC.

#### Methods

Five patients (all female mean age 62 ± 8 years, ranged from 51 to 69 years) with progressive distant metastatic DTC (dmDTC) after total thyroidectomy (TTE) and neck lymph node dissection were treated with apatinib at a dose 500 mg per day after <sup>18</sup>F-Fluorodeoxyglucose (<sup>18</sup>F-FDG) PET/CT. The effects of apatinib on DTC were evaluated at 4 ± 1 months after treatment with apatinib. RAI therapy was then initiated. The response to apatinib and the combination therapy with RAI treatment was evaluated by Response Evaluation Criteria in Solid Tumours (RECIST version 1.1) and metabolic activity using serum thyroglobulin (Tg) and <sup>18</sup>F-FDG PET/CT.

#### Results

Positive <sup>18</sup>F-FDG PET/CT results were found in all patients before apatinib therapy. The immunohistochemical analysis of primary tumour tissues showed high expression of vascular endothelial growth factor receptor-2 (VEGFR-2). Four patients with follicular thyroid carcinoma (FTC) showed partial response (PR) with significant decrease in tumour size and maximum standardized uptake value

(SUVmax) after  $4 \pm 1$  month's treatment with apatinib. Further significant reduction of tumour size and SUVmax were observed in three patients after combination therapy with apatinib and RAI. Only one patient with both FTC and papillary thyroid cancer (PTC) demonstrated progressive disease (PD) after treatment with apatinib alone, however, a decrease in tumour size and SUVmax as well as serum Tg levels was achieved after the combination with RAI therapy and apatinib.

#### Conclusions

Apatinib had significant neoadjuvant antitumour effects on progressive distant metastatic DTC. Moreover, beneficial complementary effects were shown when apatinib combined with RAI therapy.

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### PS2-10-89

#### Follicular thyroid neoplasm with papillary-like nuclear features (NIFT-P) showed peculiar ultrasonographic features compared to follicular variant of papillary thyroid carcinoma (FV-PTC), follicular carcinoma (FTC) and follicular adenoma (FA)

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#### Background

Up to 2016, NIFT-P was considered a non-invasive encapsulated follicular variant of papillary thyroid carcinoma (FV-PTC) and was reclassified and downgraded to a pre-malignant lesion. However, if these nodules have peculiar ultrasonographic features able to pre-operatively suggest their histology is still a matter of discussion. We evaluated a large series of NIFT-P, FV-PTC, FTC, and FA to characterize their ultrasonographic features.

#### Methods

We reviewed pre-operative digital ultrasound images and reports of NIFT-P ( $n = 116$ ), FV-PTC ( $n = 170$ ), FTC ( $n = 76$ ), and FA ( $n = 90$ ) evaluated at the Endocrine Unit of the University Hospital of Pisa, and surgically treated at the Endocrine Surgery Unit of the same hospital. For each nodule we evaluated the following ultrasonographic features: size, composition, echogenicity, shape, margins, and calcifications.

#### Results

In table 1, the differences in ultrasonographic appearance among the four considered histologies, were highlighted.

|                          |                    | NIFT-P <sup>a</sup><br>( $n = 116$ ) | FV-PTC <sup>b</sup><br>( $n = 170$ ) | $p^{a \text{ vs } b}$ | FTC <sup>c</sup><br>( $n = 76$ ) | $p^{a \text{ vs } c}$ | FA <sup>d</sup><br>( $n = 90$ ) | $p^{a \text{ vs } d}$ |
|--------------------------|--------------------|--------------------------------------|--------------------------------------|-----------------------|----------------------------------|-----------------------|---------------------------------|-----------------------|
| Nodule max diameter (cm) | Median (IQR)       | 2.1 (1.3-3.7)                        | 2.3 (1.5-3.7)                        | 0.27                  | 3.1 (1.8-4.4)                    | <0.01                 | 2.7 (1.8-4.4)                   | <0.01                 |
| Composition              | Solid              | 94.8                                 | 97.1                                 | 0.16                  | 96.1                             | 0.15                  | 94.4                            | 0.10                  |
|                          | Cystic             | -                                    | 0.6                                  |                       | 2.6                              |                       | 3.3                             |                       |
|                          | Mixed              | 5.2                                  | 2.4                                  |                       | 1.3                              |                       | 2.2                             |                       |
| Echogenicity             | Anechoic           | -                                    | 0.6                                  | 0.85                  | 2.6                              | <0.01                 | 3.3                             | <0.01                 |
|                          | Hypoechoic         | 31                                   | 31.8                                 |                       | 53.9                             |                       | 57.8                            |                       |
|                          | Isoechoic          | 63.8                                 | 61.8                                 |                       | 40.8                             |                       | 36.7                            |                       |
| Margins                  | Hyperechoic        | 5.2                                  | 5.9                                  | <0.01                 | 2.6                              | 0.02                  | 2.2                             | 0.60                  |
|                          | Well defined       | 96.6                                 | 84.7                                 |                       | 88.2                             |                       | 97.8                            |                       |
|                          | Lobed or irregular | 3.4                                  | 15.3                                 |                       | 11.8                             |                       | 2.2                             |                       |
| Calcifications           | Absent             | 94                                   | 70                                   | <0.01                 | 76.3                             | 0.01                  | 87.8                            | 0.61                  |
|                          | Macro              | 1.7                                  | 9.4                                  |                       | 5.3                              |                       | 4.4                             |                       |
|                          | Peripheral         | 0.9                                  | 6.5                                  |                       | 3.9                              |                       | 1.1                             |                       |
|                          | Micro              | 2.6                                  | 4.7                                  |                       | 9.2                              |                       | 4.4                             |                       |
|                          | Hyperechoic spots  | 0.9                                  | 9.4                                  |                       | 5.3                              |                       | 2.2                             |                       |
| Taller than wide         | Yes                | 1.7                                  | 10                                   | <0.01                 | 13.2                             | <0.01                 | -                               | 0.21                  |
|                          | No                 | 98.3                                 | 90                                   |                       | 86.8                             |                       | 100                             |                       |
| Halo                     | Yes                | 91.4                                 | 81.2                                 | 0.02                  | 69.7                             | <0.01                 | 68.9                            | <0.01                 |
|                          | No                 | 8.6                                  | 18.8                                 |                       | 30.3                             |                       | 31.1                            |                       |

#### Conclusion

While FV-PTC and FTC showed significantly more frequent ultrasonographic features suspicious for malignancy; most NIFT-P showed ultrasonographic features suggestive for benignity, similar to FA.

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### PS2-10-90

#### ABSTRACT WITHDRAWN

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### PS2-10-91

#### Comparison of the clinicopathological features and oncologic outcomes of the classic papillary thyroid carcinoma with tall cell features and tall cell variant

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#### Background

The tall cell variant (TCV) of papillary thyroid carcinoma (PTC) (TCVPTC) is the most common aggressive variant of PTC. Classic PTC with tall cell features (TCF) is defined as PTC with noticeable tall cells but the percentage of these cells is lower than that required for the diagnosis of TCVPTC. We aimed to investigate the potential differences between TCVPTC and classic PTC with TCF with respect to clinicopathological characteristics and long-term oncologic outcomes.

#### Methods

We retrospectively assessed 509 patients with TCVPTC or classic PTC with TCF who underwent thyroid surgery between January 2013 and December 2018 at the Seoul St. Mary's Hospital (Seoul, Korea). Clinicopathological characteristics and long-term oncologic outcomes between TCVPTC and classic PTC with TCF were compared in terms of disease-free survival. The mean follow-up duration was  $70.7 \pm 21.7$  months.

## Results

The mean tumor size was significantly larger in the TCVPTC group. There was no significant difference between the TCVPTC and classic PTC with TCF groups with respect to disease-free survival. Tumor size >2 cm [odds ratio (OR), 1.922;  $P = 0.019$ ], bilaterality (OR, 1.668;  $P = 0.030$ ), extrathyroidal extension (ETE) (OR, 2.352;  $P = 0.002$ ), and lateral LN metastasis (OR, 1.700;  $P = 0.045$ ) were significantly associated with TCVPTC compared with classic PTC with TCF.

## Conclusions

TCVPTC and classic PTC with TCF have similar clinicopathological characteristics and long-term oncologic outcomes. Therefore, we suggest a potential re-classification of classic PTC with TCF from low-risk to intermediate-risk category in the American Thyroid Association (ATA) risk stratification system.

**Keywords:** Tall cell features, Tall cell variant, Papillary thyroid carcinoma, Disease-free survival

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## PS2-10-92

### Incidence of non-diagnostic and undetermined cytologies in ultrasound-guided fine needle aspiration biopsy specimens of thyroid nodules – a single-center cohort

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## Objectives

Ultrasound-guided fine needle aspiration biopsy (FNAB) is a commonly performed diagnostic procedure for evaluation of thyroid nodules. The Bethesda System for Reporting Thyroid Cytopathology comprises six categories. Bethesda categories I and III present a challenge for deciding on further diagnostic and therapeutic procedures. Category I includes non-diagnostic or unsatisfactory results that may be due to both aspiration of cystic material from simple cysts or aspiration of too few thyroid cells to render a diagnosis. Category III includes atypia of undetermined significance or follicular lesions of undetermined significance. In the literature, the rate of Bethesda I ranges from 6.1% to 26.8%, while the rate of Bethesda III ranges from 3.7% to 27.4%. In these studies, 164 to 1399 specimens were included. The aim of our study was to evaluate the incidence of categories I and III in ultrasound-guided FNABs of thyroid nodules in the tertiary medical center with a very large number of thyroid patients.

## Methods

Our retrospective study included 2167 specimens provided by ultrasound-guided FNAB. The selection of nodules for the FNAB was based on clinical findings, laboratory tests, thyroid scintigraphy with  $^{99m}\text{Tc}$ -technetium-pertechnetate and ultrasound malignancy risk stratification according to the European Thyroid Association Guidelines. Simple cysts were excluded from the study. FNABs were performed by seven thyroid specialists from January 2019 to April 2021. For the procedure, 21-gauge and, rarely, 23-gauge needles were used. All ultrasound-guided FNABs were performed with an on-site cytologist, who used the aspirated material to immediately perform an air-dried smear. All cytology reports were performed by the same very experienced cytologist using the Bethesda System for Reporting Thyroid Cytopathology.

## Results

Among the 2167 samples, there were 168 (7.7%) non-diagnostic or unsatisfactory (Bethesda I), 1750 (80.8%) benign (Bethesda II), 37 (1.7%) atypias of undetermined significance or follicular lesions of undetermined significance (Bethesda III), 119 (5.5%) follicular neoplasms or suspicious for a follicular neoplasm (Bethesda IV), 25 (1.2%) suspicious for malignancy (Bethesda V) and 68 (3.1%) malignant (Bethesda VI).

## Conclusions

To the best of our knowledge, this study includes the largest number of ultrasound-guided FNAB specimens of thyroid nodules. When compared with the literature data, the incidence of Bethesda I and Bethesda III categories in our center is in the lower part of the reported range or lower, respectively. Most likely, this is due to the high frequency of FNABs in our center and the experienced cytologist who performs smears on site immediately after FNAB.

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## PS2-10-93

### Elastography in the assessment of cold solid thyroid nodules

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## Introduction

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

## Methods

In 39 patients 31 females and 8 males (mean age  $51.9 \pm 16.8$  years), we evaluated solitary or dominant solid thyroid nodule that was cold on scintigraphy with  $^{99m}\text{Tc}$ -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 parannodular tissue was assessed. In every nodule, fine needle biopsy was performed and cytology was reported using Bethesda classification system. Patient and nodule characteristics were compared according to cytology result.

## Results

Mean TSH level was  $1.73 \pm 1.13$  mIU/l. Mean thyroid volume was  $29.6 \pm 18.9$  ml and mean nodule volume was  $13.4 \pm 14.9$  ml. Males had significantly larger nodule volume than females ( $28.4 \pm 22.7$  vs  $9.6 \pm 9.3$  ml,  $P = 0.05$ ). Mean ECI of thyroid nodules was significantly higher compared with mean ECI of parannodular tissue ( $1.81 \pm 0.84$  vs  $1.09 \pm 0.34$ ,  $P < 0.001$ ). Suspicious Bethesda category (4 or 6) was confirmed in 20.5% (8/39) of patients. Compared with unsuspected nodules, nodules with suspicious Bethesda category were confirmed in significantly younger patients ( $38.6 \pm 18.8$  vs  $55.4 \pm 14.6$  years,  $P = 0.01$ ), their proportion was significantly higher in males than in females ( $P = 0.02$ ) and their TIRADS score was significantly higher ( $P < 0.001$ ). Patients with suspicious or unsuspected cytology did not differ with respect to mean ECI of thyroid nodule ( $2.2 \pm 1.3$  and  $1.7 \pm 0.6$ ,  $P = 0.33$ ), nodule volume ( $P = 0.71$ ) or TSH concentration ( $P = 0.87$ ).

## Conclusion

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

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## PS2-10-94

### Follow-up and incidence of malignancy in eu-tirads 3 nodules with indication of fine needle aspiration cytology. a single-center descriptive study

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**Background**

Current guidelines recommend repeat evaluation of a benign thyroid nodule after 1 to 2 years, which is only based on expert opinion. Objective data are needed to evaluate this recommendation.

**Methods**

This is a retrospective, descriptive study to describe the follow-up and incidence of malignancy in EU-TIRADS 3 nodules for which a fine needle aspiration cytology (FNAC) was indicated between 2017 and 2022. PET-positive thyroid nodules and thyroid nodules of which FNAC was indeterminate (Bethesda 1) were excluded.

**Results**

In total, 112 EU-TIRADS 3 nodules were included with a mean diameter of  $3.17 \pm 1.16$  cm. Mean age of the study population was  $54 \pm 15$  years with a female/male ratio of 2.39. FNAC showed the following results; Bethesda 2 (97 nodules, 86.6%), Bethesda 3 (10 nodules, 8.9%), Bethesda 4 (4 nodules, 3.6%), Bethesda 5 (1 nodule, 0.9%), Bethesda 6 (no nodule). Of the 97 cases with Bethesda 2 classification, 13 cases underwent a total or hemithyroidectomy, 34 cases were followed for a median time of 24 (12-38) months and 50 cases were lost to follow-up. FNAC was repeated in 16 of the 34 cases which were in follow-up with the following results: Bethesda 1 (3 nodules, 18.8%), Bethesda 2 (11 nodules, 68.8%), Bethesda 3 (no nodule), Bethesda 4 (1 nodule, 6.3%), Bethesda 5 (1 nodule, 6.3%). The 2 nodules in which the repeat FNAC showed Bethesda 4 and 5 were resected and were benign. Anatomopathological examination of the 13 resected thyroid nodules with Bethesda 2 showed a malignancy in 2 cases of which one with distant metastasis. Of the 10 nodules with Bethesda 3, 5 underwent a thyroidectomy and all were benign. Of the 4 nodules with Bethesda 4, 3 underwent a thyroidectomy of which one nodule was malignant. The one case with Bethesda 5 underwent a thyroidectomy and showed a benign nodule.

**Conclusion**

In our single-center sample of EU-TIRADS 3 nodules only 3 (2.7%) cases had a pathology proven malignancy which is consistent to the given range described in the EU-TIRADS guidelines (2-4%). However, in 2 out of the 3 malignant thyroid nodules FNAC was benign (Bethesda 2). In the follow-up of 34 EU-TIRADS 3 nodules with Bethesda 2, 16 underwent a repeat FNAC of which 2 revealed a higher Bethesda classification which were eventually found to be benign on anatomic pathological examination.

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**PS2-10-95****Contribution of cyto-histological genetic profile to a precocious diagnosis in thyroid neoplasm**

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**Introduction**

The diagnostic gold standard for thyroid nodular disease is ultrasound-guided fine-needle aspiration cytology (US-FNAC), being the most accurate, cost-effective and minimal invasive preoperative test to distinguish benign from malignant nodules, aiming to resolve patient management. However, up to 30% of FNACs are classified as indeterminate nodules, turning difficult the decision on patient management to avoid unnecessary surgeries. The development of thyroid cancer has been associated with the activation of oncogenes (*TERTp*, *BRAF* and *RAS* (*NRAS*, *HRAS* and *KRAS*)), and several studies recommend the use of molecular tests for a more accurate diagnosis of malignancy.

**Aim**

The aim of this study was to compare the cyto-histologic genetic profile (*TERTp*, *BRAF* and *RAS* (*NRAS*, *HRAS* and *KRAS*)), by using a paired series of cytology and histology samples, to establish whether the molecular profile defined by US-FNAC is reliable to further characterize indeterminate nodules.

**Material and Methods**

The series in this study was composed by a cytology and corresponding formalin-fixed paraffin-embedded (FFPE) tissue from 231 consecutive patients with

thyroid nodules that underwent surgery between 2012 and 2020. The genetic alterations were examined by polymerase chain reaction (PCR), followed by DNA sequencing. The association of the genetic alterations with clinicopathologic features was evaluated.

**Results/Discussion**

Our series of 231 patients included 80.1% females (mean age 53.7;SD= 15.9) and 19.9% males (mean age 57.8;SD=6.9); cytology was non-diagnostic in 4.3%, benign in 18.2%, indeterminate in 36.8% and malignant in 40.7%; histology result presented 17.3% benign and 82.7% malignant nodules. In histology, the eighty-five indeterminate nodules correspond to 17.6% benign and 82.4% malignant lesions. Mutation frequencies in cytology and histology specimens were, respectively, *TERTp*: 4.3% v 8.2%; *BRAF*: 20.8% v 22.5%; *NRAS*: 4.3% v 5.2%; *HRAS*: 5.2% v 7.8%; *KRAS*: 1.7% v 1.7%. In indeterminate nodules, mutation frequencies in benign and malignant histology were: *TERTp*: 2.4% v 8.2%; *BRAF*: 2.4% v 9.4%; *NRAS*: 3.5% v 4.7%; *HRAS*: 10.6% v 16.5%; *KRAS*: 2.4% v 3.5%. A good cyto-histologic agreement was obtained for molecular alterations (96.2%,  $k = 0.657$ ), suggesting that US-FNAC can contribute to anticipate the molecular profile of the tumor. Indeterminate nodules showed more *TERTp* and *BRAF* mutations in malignant histology. Several statistically significant associations between the clinicopathological and molecular features of the tumors were found; *TERTp*, *BRAF* and *TERTp*+ *BRAF* mutations were associated with aggressiveness, extra thyroidal invasion, and lymph node metastases. On the contrary, *RAS* mutations were associated with a better patient outcome.

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**PS2-10-96****Prospective, observational study on radioiodine treatment in DTC patients with intermediate RISK or micro lymph node metastases**

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There are much controversy adjuvant radioiodine treatment (131-I-th) in intermediate or low risk patients with lymph node micrometastases. The first results of ESTIMABL2 trial showed no radioiodine benefit in low risk DTC, however intermediate patients were not included in this study.

**Aim**

Observational study was to evaluate effects of 131-I-th in intermediate/low risk DTC patients.

**Patients**

There were 342 women (85%), the median age at diagnosis was 52. Most patients, 298 (98%), were diagnosed with papillary cancer, 117 (29%) had extrathyroid extension, 133 (33%) vascular invasion and 249 (61,6%) lymph node metastases. Median 131-I activity was 100 mCi and all patients were treated after rhTSH stimulation. Median time from first operation to 131-I-th was 5 months. Media time of follow-up after treatment 14 months.

**Results**

In posttherapy scintigraphy only in 9 patients there was suspicion uptake in lymph nodes. In none of these patients persistent disease was confirmed. During first follow-up 318 (79%) had excellence response and 1 structural recurrence. Thereafter 5 (1,2%) of patients recurred.

**Conclusion**

Our results show that in a selected group of patients with low/intermediate risk, there are excellent treatment results. The question whether 131-I-th may be omitted in this group of patients should be confirmed in a prospective randomized trial.

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**Monday, September 12th, 2022****Poster Session 3 Case Reports****PS3-11-97**

**Van wyk-grumbach syndrome: an unusual presentation of long-standing primary hypothyroidism mimicking ovarian tumor**  
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#### Introduction

Children with long-standing primary hypothyroidism usually have growth retardation, neurodevelopmental impairment, and delayed puberty. However, paradoxical precocious puberty, namely Van Wyk-Grumbach syndrome (VWGS), has been described. The postulated mechanism is high levels of TSH stimulate gonadal FSH receptors, resulting in ovarian over-stimulation and precocious puberty. Since the enlarged ovarian cyst is commonly found in VWGS, malignancy is often suspected. Non-recognition can lead to misdiagnosis and unnecessary surgery. Here we report a girl with long-standing hypothyroidism and gonadotropin-independent precocious puberty (GIPP) who was first misdiagnosed with ovarian tumors.

#### Case report

A 9-year and 10-month-old girl presented with slow-progressive abdominal distension, heavy vaginal bleeding, and pelvic pain for a month. Her thelarche and menarche occurred at the age of nine years with regular periods and cycle length of 4 weeks, suggesting precocious puberty. Physical examination showed breast Tanner stage IV with no pubic or axillary hair. A midline cystic mass was palpated at the lower abdomen. A pelvic ultrasound found a pubertal-size uterus with endometrial thickness and large bilateral ovarian cysts (8.5\*4.2\*7 cm and 8.1\*4.7\*8 cm in right and left ovary, respectively) with multiple internal septations. The bilateral ovarian tumors were initially suspected. However, no tumor markers were elevated, except slightly increased LDH (266 IU/l (135-214)). In addition, the patient was disproportionately short (height at -4.5SDS with upper/lower ratio of 1.39:1 and weight at -2.0 SDS) and had signs of hypothyroidism, including mild pallor, puffy eyelids, flat nasal bridge, dry skin, gross motor and speech delays, and muscular pseudohypertrophy. Her skeletal age was markedly delayed (5 years). Severe primary hypothyroidism was diagnosed by high TSH and low FT4 (TSH 621 uIU/ml (0.60-4.84), FT4 0.13 ng/dL (0.97-1.67), TPOAb 6.9 IU/ml (<40), TGAb 37.6 IU/ml (<125), thyroglobulin level 1.1 ng/ml (5-40)). Baseline LH was below 0.1, FSH 6.62 IU/l, and estradiol 6.22 pg/ml, indicating GIPP. Thyroid scan revealed an ectopic lingual thyroid. VWGS was diagnosed based on the concurrence of severe primary hypothyroidism, GIPP, and delayed bone age. After levothyroxine replacement, she had no further episode of vaginal bleeding, and bilateral ovarian cysts were gradually resolved.

#### Conclusion

VWGS is an unusual presentation of long-standing primary hypothyroidism. The combination of precocious puberty, short stature, and delayed skeletal maturation is a clue for diagnosis. Ovarian cysts progressively regress with LT4 replacement. Physicians need to be aware of this condition to avoid unnecessary surgery due to suspected ovarian tumors.

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### PS3-11-98

#### Complicated treatment with lenvatinib for hurthle cell carcinoma

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#### Introduction

The tyrosine kinase inhibitor (TKI) lenvatinib, used in radioiodine-refractory differentiated thyroid cancer, is usually well-tolerated. However, severe side effects can occur. We describe a life-threatening complication under lenvatinib, followed by a challenging treatment with levothyroxine (LT4).

#### Case Report

A 62-year-old man was diagnosed with a bone-metastasized Hurthle cell thyroid carcinoma (pT3N0M1). He underwent a total thyroidectomy, external neck radiation and radioiodine after LT4 withdrawal (555 MBq; stimulated TSH 104.7 mU/l, stimulated thyroglobulin 2.7 µg/l, thyroglobulin antibodies <10 U/l). A I131 scintigraphy showed radioiodine-refractory disease. His medical history included a sleeve gastrectomy with conversion to gastric bypass and repeat surgical interventions for small bowel obstruction, eventrations and complicated abdominoplasty. He required LT4 250µg/day (weight 85 kg) to obtain a low normal TSH (table). Ten months after starting lenvatinib resulting in stable disease, he presented with an ischemic stomach pouch with perforation and fistulisation. An urgent laparotomy was performed and lenvatinib was stopped. Afterwards, he developed diarrhoea, for which cholestyramine 8g/day was started. Two months later, overt hypothyroidism was diagnosed (table, time 0)

and the LT4 dose was doubled. An oral challenge with 1000µg LT4 showed an adequate increase of FT4, excluding deterioration of mucosal gastrointestinal malabsorption. After stopping cholestyramine, his thyroid hormone levels returned to baseline under LT4 250µg/day.

#### Conclusions

Treatment with lenvatinib can result in gastro-intestinal perforation and fistulisation, which is attributed to the anti-angiogenic effect. Caution is needed, especially in patients with a history of complex abdominal surgery. The need for high doses of LT4 should be reminiscent of either reduced gastrointestinal absorption or availability, such as interaction with cholestyramine. Cholestyramine, an anionic exchange resin used in the treatment of bile acid diarrhoea, binds LT4 in the intestine thereby reducing its absorption. The passage of LT4 through the enterohepatic circulation further contributes to this interaction. This interference can be minimized by providing at least four hours between ingestion of the two agents.

m=months, d=days, hr=hours, reference range TSH (0.27-4.20 mU/L), FT4 (11.6-21.9 pmol/L)

**Table.** Evolution of thyroid function tests and treatments.

| Timeline | TSH (mU/l)  | FT4 (pmol/l) | LT4 dose (µg) | Interventions   |
|----------|-------------|--------------|---------------|---|
| -5m      | 0.49        | 23.7         | 250           | 12m post laparotomy for ischemic stomach pouch with stop lenvatinib |
| -2m      |             |              | 250           | start cholestyramine 8g/day   |
| 0        | <b>83.3</b> | <b>8.9</b>   | <b>250</b>    | <b>on cholestyramine 8g/day</b>                                     |
| + 1d     |             |              | 500           |   |
| + 2d     |             |              |               | PO LT4 1000 µg  |
| 0min     |             | 11.2         |               |   |
| + 1hr    |             | 28.3         |               |   |
| + 2hr    |             | 27.1         |               |   |
| + 4hr    |             | 32.2         |               |   |
| + 41d    | 1.39        | 20.5         | 250           |   |

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### PS3-11-99

#### The clinical outcome of covid-19 infection in a patient with the history of multinodular goiter: case report

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#### Introduction

The thyroid gland and COVID-19 infection with its associated inflammatory-immune responses are known to be engaged in complex interplay. Until now, the evidence on the outcome of COVID-19 in patients with a history of thyroid cancer remains scarce, and most of the recommendations given are based on common sense.

#### Case report

A 71-year-old man was admitted to Mikaelyan University hospital with general weakness shortness of breath, hiccup and tumor-like mass on the anterior part of neck. The patient had a history of tumor-like mass for 20 years. The patient presented with weakness and fatigue that became severe two days before hospitalization. Examination revealed Ps=77bpm, BP=125/70 mmHg, T=36.5°C, SpO<sub>2</sub> 84 % (O<sub>2</sub>-), BMI=19.0kg/m<sup>2</sup>. On the anterior surface of the neck, from the middle line to the left there was a tumor-like mass with mobile, smooth, elastic surface of about 7\*8 cm. According to the results of the ultrasound scan, there were evidence of many metastases in the liver and the right kidney. Thyroid ultrasound showed significant diffuse changes and foci of cystic degeneration. There was a pronounced blood circulation in color Doppler mode. Parajugular lymph nodes up to 1.8 cm in size were visualized. The Chest X-ray revealed bilateral pneumonia and lung metastasis. Therefore, nasal swabs were collected for the COVID-19 PCR test, and the result was positive. Lab results: CRP-107.71 mg/l /N 0.0-5.0/, D-dimer-0.492ug FEU/ml /N0-0.5/, Ferritin-225.8ng/ml/N 13-350/, TSH-1.11uIU/ml /N 0.3-4.5/, FT4-12.5 pg/ml /N 8.9-17.2/. Diagnosis: COVID-19, bilateral pneumonia, severe course, respiratory insufficiency stage. Multinodular goiter, euthyroidism. Lungs, liver and right kidney



metastases. This patient underwent treatment for COVID-19, that included infusion therapy, dexamethasone 12 mg with dose decrease to 4 mg, ceftriaxone, heparin 10000U daily, aspirin, famotidine, oxygen. Finally, he was discharged in good general health condition after eight days. The patient was recommended to perform fine needle aspiration biopsies of thyroid nodules and total thyroidectomy afterwards.

#### Conclusion

As there are few cases of combination of thyroid tumor and COVID-19 reported worldwide, our clinical case can contribute the management of such patients. In our clinical case, a step-by-step management of the patient led the favorable outcome of COVID-19.

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## PS3-11-100

### Differentiation of parathyroid gland from a lymph node in clinical practice

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#### Introduction

How to differentiate lymphadenitis from parathyroid gland?

#### Case report

The patient, a 45-year-old woman, applied to "Muratsan" Polyclinic complaining of asthenia, dry skin, easy fatigability, muscle and bone pain, palpitations. Anamnesis was gained and an examination was carried out. According to the

| Test            | Result  | The result after 3 months | Normal range     |
|-----------------|---|---------------------------|------------------|
| TSH             | 5.42 uIU/mL   | 0.43 uIU/mL               | 0.27-4.2 uIU/mL  |
| FT4             | 1.28 ng/dL  | 1.68 ng/dL                | 0.93-1.7 ng/dL   |
| Vitamin D       | 23.43 ng/mL   | 34.3 ng/mL                | 30-70 ng/mL      |
| PTH             | 96.06 pg/ml   | 103 pg/ml                 | 15-65 pg/ml      |
| Ca              | 1.26 mmol/l   | 1.21 mmol/l               | 1.2-1.32 mmol/l  |
| P               | 1.3 mmol/l  | 1.21 mmol/l               | 0.84-1.45 mmol/l |
| Neck ultrasound | A well-defined, homogeneous isoechoic lesion with 35x20 mm in measures (a parathyroid adenoma or lymph nodes?) was detected in the projection of the parathyroid gland from the right side. |                           |                  |
| Densitometry    | Osteoporosis: T score – 2.7   |                           |                  |

\*The examinations have been carried out on the background of 100 mg of levothyroxine. Levothyroxine 150 mg 1x1. vitamin D 5000IU for 3 months, CaD3 1000 mg 1x1 for 3 months were prescribed followed by the same results of the laboratory tests. Due to the high PTH level, the secondary hyperparathyroidism was denied and the patient was directed to scintigraphy. Adenoma of the parathyroid gland was confirmed and surgical treatment was advised.

patient's indication in the anamnesis, total thyroidectomy was done a year before, followed by radioiodine therapy with regard to papillary carcinoma of the thyroid gland. A normal menstrual period.

The following examinations have been carried out\*

#### Conclusion

As the appearance of the parathyroid gland and the lymph node is similar in the sonography, it is advised to check PTH, vitamin D, Ca<sup>2+</sup>, deny the secondary hyperparathyroidism, then to direct either to scintigraphy or to FNA.

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## PS3-11-101

### Carpal Tunnel Syndrome in Subclinical Hypothyroidism

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#### Introduction

Is treatment with Levothyroxine indicated for subclinical hypothyroidism concomitant with Carpal Tunnel Syndrome?

#### Case Report

The patient, a 35-year-old woman, applied to "Muratsan" Polyclinic in Armenia with complaints of weakness, fatigue, dry skin, pain in hands, swelling, numbness, all these intensified at night. The above mentioned complaints appeared approx a year ago.

The following findings have been determined:

#### Conclusions

In case of Subclinical Hypothyroidism and Carpal Tunnel Syndrome it is recommended to prescribe Levothyroxine for 3 months, thereafter the patient's condition should be assessed. If the clinical symptoms subside or disappear on the background of the treatment, continue treatment with Levothyroxine, if not then stop the treatment.

| Test                     | Result   | Normal range    |
|--------------------------|--|-----------------|
| TSH                      | 5.6 Uiu/ml   | 0.27-4.2 uIU/ml |
| FT4                      | 1.2 ng/dl  | 0.93-1.7 ng/dl  |
| Anti-TPO                 | 16 IU/ml   | < 34 IU/ml      |
| CRP                      | Normal range   |                 |
| RF                       | Normal range   |                 |
| ASLO                     | Normal range   |                 |
| CBC                      | Normal range   |                 |
| Thyroid gland ultrasound | Diffuse Goiter   |                 |
| Electroneuromyography    | No conductance disturbance; however there is pressure on the median nerve. |                 |

DS: Diffuse Goiter, Subclinical Hypothyroidism. Carpal Tunnel Syndrome. No medication was prescribed by neurologists as there was no conductance disturbance. Levothyroxine 50 mg was prescribed by endocrinologist once daily. After 3 months a follow-up was conducted and the patient's condition was assessed. As a result of the prescribed treatment, all the clinical symptoms related to the Carpal Tunnel disappeared.

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**PS3-11-102****Do not rush to overdiagnose when a newly formed node is detected**Anna Chivchyan<sup>1</sup>, Lilit Kambulyan<sup>2</sup>, Armine Khroyan<sup>3</sup>, Elena Aghajanova<sup>4</sup> & Artashes Tadevosyan<sup>5</sup><sup>1</sup>Yerevan State Medical University, Pediatric Endocrinology, Yerevan, Armenia; <sup>2</sup>Yerevan State Medical University, Yerevan State Medical University, Endocrinology, Yerevan, Armenia; <sup>3</sup>Yerevan State Medical University, Koryun 2, Endocrinology, Yerevan, Armenia; <sup>4</sup>Yerevan State Medical University; <sup>5</sup>Yerevan State Medical University, Yerevan State Medical University, Armenia

28 years old woman has presented to the Armenian-American Wellness Center complaining of muscular weakness, fatigue, palpitation, dysphagia, dry skin and hair fall. The patient was visited by the endocrinologist and referred to the following tests: ultrasound of the thyroid gland, blood test for TSH, FT4 and anti-TPO.

**Results**

Diffusely enlarged thyroid gland with diffuse heterogeneous echotexture and the presence of hypoechoic micronodules. In the left lobe a large nodule 7x8 mm is detected, hypoechoic with uneven outline and prominent hypervascularisation. (TIRAD-S- 4a)

| Test     | Results      | Result after 3 months | Normal range    |
|----------|--------------|-----------------------|-----------------|
| TSH      | 5,87 uIU/ml  | 1,2 uIU/ml            | 0,27-4,2 uIU/ml |
| FT4      | 0,83 ng/dl   | 1,3 ng/dl             | 0,93-1,7 ng/dl  |
| Anti-TPO | 208,21 IU/ml | < 34 IU/ml            | Diagnosis       |

Autoimmune thyroiditis, hypothyroidism The presence of Tr-4 a is a direct indication for FNA but conservative treatment has been taken as a start with Levothyroxine 50 mg/kg/day The next follow up was in 3 months Ultrasound of the thyroid gland - in the left lobe 4x5 mm. TIRAD-S-2 Conclusion:

In case the node of the thyroid gland is based on hypothyroidism and has rather no big dimensions, it is preferable not to rush to perform FNA and consider a treatment with Levothyroxine for 3-6 months and repeat the tests. In case of no changes in the size of the node perform FNA.

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**PS3-11-103****Suspicious thyroid nodule in de quervain's thyroiditis**Carmen Sorina Martin<sup>1</sup>, Bianca Dumea<sup>2</sup>, Ovidiu Parfeni<sup>3</sup>, Theodor Mustata<sup>3</sup>, Anca Sirbu<sup>4</sup> & Simona Fica<sup>4</sup><sup>1</sup>Umf Carol Davila, Elias Hospital, Elias Hospital, Bucharest, Romania; <sup>2</sup>Elias Hospital, Endocrinology, Bucharest, Romania; <sup>3</sup>Elias Hospital, Endocrinologie, Bucharest, Romania; <sup>4</sup>Carol Davila University of Medicine and Pharmacy, Elias Hospital, Endocrinology, Bucharest, Romania**Introduction**

De Quervain's thyroiditis, also known as subacute granulomatous thyroiditis, is a self-limiting inflammatory disorder of the thyroid gland. It is presumed to be caused by a viral infection and many patients have a history of an upper respiratory infection 2-8 weeks prior to the onset of thyroiditis. It is the most common cause for neck pain or discomfort, and it usually has a predictable course of thyroid function evolution. On ultrasonography the thyroid gland might be slightly enlarged or normal with a diffuse or focal hypoechoic appearance.

**Aim(s)**

We report the case of a 50-year-old female patient referred for the work-up of a TIRADS 5 thyroid nodule with satellite enlarged lymph nodes.

**Materials and methods**

Anamnesis revealed a progressive onset low anterior neck pain after an upper respiratory infection. On physical examination the thyroid gland was enlarged and tender. Laboratory assessment showed subclinical hyperthyroidism with suppressed TSH (0.05 µIU/ml) and normal levels of FT4 and TT3. Anti-thyroid peroxidase antibodies, anti-thyroglobulin and anti-TSH receptor antibodies were negative. Our patient associated high levels of inflammatory markers: erythrocyte sedimentation rate level was 57 mm/h and C-reactive protein was 24.9 mg/dl. Neck ultrasound identified multiple thyroid nodules with a left dominant nodule of 2.75/1.5/1.5 cm with a high index of sonographic suspicion for thyroid cancer and multiple left lymph nodes.

**Results**

Thus, the diagnosis of subacute granulomatous thyroiditis was established and treatment with oral corticosteroids was initiated. Thyroid ultrasound-guided fine needle aspiration cytology was performed and the result showed follicular cells without nuclear atypia and several multinucleated giant cells. One month after treatment onset, follow-up ultrasound examination showed complete resolution and disappearance of the thyroid nodules. The patient became hypothyroid and replacement treatment with Levothyroxine was initiated.

**Conclusion**

De Quervain's thyroiditis can sometimes show on ultrasound the presence of ill-defined hypoechoic thyroid lesions that may be interpreted as thyroid nodules. Thus, it is important to properly identify these transient lesions to improve the treatment and follow-up of these patients and de Quervain's thyroiditis should be included in the differential diagnosis of thyroid nodules.

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**PS3-11-104****Parathyroid adenoma apoplexy mimicking a thyroid bleeding cyst, a seemingly innocent condition may harbor a threatening one**Jasmine Van de Kerckhof<sup>1</sup>, Jacqueline Bijens<sup>2</sup>, Frank De Geeter<sup>3</sup>, Catherine Dick<sup>2</sup>, Pascale De Paepe<sup>4</sup> & Annick Van Den Bruel<sup>5</sup><sup>1</sup>General Hospital Sint-Jan Bruges, Universal Hospitals Leuven, Ent, H&n Surgery Department, Bruges, Belgium; <sup>2</sup>General Hospital Sint-Jan Bruges, Ent, H&n Surgery Department, Bruges, Belgium; <sup>3</sup>General Hospital Sint-Jan Bruges, Department of Nuclear Medicine, Bruges, Belgium; <sup>4</sup>General Hospital Sint-Jan, Bruges, Department of Anatomopathology, Bruges, Belgium; <sup>5</sup>Sint Jan Brugge Oostende, General Hospital Sint Jan, Bruges, Endocrinology, Brugge, Belgium**Introduction**

Primary hyperparathyroidism most commonly presents with hypercalcemia. Rarely, parathyroid apoplexy or haemorrhage, mimicking a thyroid bleeding cyst is the first presentation of a parathyroid adenoma<sup>(1,3)</sup>.

**Case Report**

A 55-year-old woman presented to the ENT department with a sudden onset painful goiter. Ultrasound revealed a sharply defined hypoechoic nodule in the right thyroid lobe measuring 24.1 x 17.8 x 18.2 mm. The patient was referred to the endocrinology department for fine-needle aspiration (FNA). Repeat ultrasound showed a partially cystic nodule located posterior to rather than in the right thyroid lobe, suggesting parathyroid adenoma bleeding rather than thyroid nodule bleeding. FNA was deferred. Lab testing confirmed hypercalcemia and hyperparathyroidism. <sup>99m</sup>Tc-Perchnetate/SestaMIBI one month after initial presentation showed no uptake in the nodule, which was interpreted as a cold thyroid nodule.<sup>18</sup> F-fluorocholine-PET/CT two months after presentation showed uptake in the nodule, suggestive of a parathyroid adenoma. The patient was referred for parathyroidectomy along with right thyroid lobectomy in case of thyroid adhesion. At surgery, the right inferior parathyroid was strongly fused with the thyroid. A right hemithyroidectomy and resection of the parathyroid adenoma was performed. Pathology showed a parathyroid adenoma, approximately 17 mm in diameter, with an eccentrically located cystic structure (5 mm in diameter), filled with red blood cells and surrounded by a thickened fibrous capsule. The cyst wall contained numerous macrophages with iron pigment deposition.

**Conclusion**

Diagnostic workup includes dedicated ultrasonography to raise the suspicion of a parathyroid adenoma haemorrhage and to discern it from thyroid nodule haemorrhage. A negative <sup>99m</sup>Tc-Perchnetate/SestaMIBI scan has been reported in most cases of parathyroid apoplexy<sup>(2,3)</sup>. Scarce blood supply as well as replacement of metabolically active cells by hemorrhage may contribute to the absence of SestaMIBI uptake. To our knowledge, this is the first parathyroid adenoma apoplexy case in which <sup>18</sup>F-Choline-PET/CT has been performed. In conclusion, cervical pain/haemorrhage along with hypercalcemia point to the diagnosis of parathyroid apoplexy, mimicking a thyroid bleeding cyst. Expedite work-up with ultrasound and if available <sup>18</sup>F-Choline-PET/CT allows for timely surgery, minimizing the risk of recurrent and more severe bleeding. Hence, a two-step process with a localized parathyroid adenoma haemorrhage preceding massive life threatening haemorrhage has been reported<sup>(4)</sup>.

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**PS3-11-105****Pseudomalabsorption of levothyroxine**

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Pseudomalabsorption of Levothyroxine Hypothyroidism due to non-compliance with levothyroxine therapy (pseudomalabsorption) is rare. The diagnosis is considered in patients with persistent refractory hypothyroidism despite treatment high doses of levothyroxine. Intestinal malabsorption, drug and dietary interference with levothyroxine absorption and nephrotic syndrome should be

excluded. The diagnosis of pseudomalabsorption can be demonstrated by using levothyroxine absorption protocol, showing a rapid decrease in thyroid-stimulating hormone and increase in thyroxine. There are however few data on the sensitivity and specificity of the test in large cohorts of hypothyroid patients. Treatment of pseudomalabsorption is controversial, with reports using parenteral, intramuscular or single weekly oral dosing of levothyroxine.

#### Case

We report 46 years old women who presented with persistent clinical and biochemical signs of hypothyroidism after thyroidectomy despite treatment with levothyroxine doses in excess of weight based calculations. Digestive, liver and kidney diseases were excluded. Levothyroxine absorption test was performed and percent absorption was calculated, which was nonconclusive 55% (normal  $\geq 60\%$ ). For further diagnosis and management patient had been hospitalized in our clinic for 5 days. Daily PO Levothyroxine administration was supervised by nurse resulting in improvement of TSH and serum thyroxine compared to pre-admission and admission day results confirming pseudomalabsorption. Patient and the care givers were appropriately instructed. Patient was referred for psychiatric evaluation.

#### Conclusions

Non-compliance with medical therapy should be considered in patients with treatment refractory hypothyroidism. Supervised PO levothyroxine administration with pre and post admission TSH and thyroxine measurement could be considered as the tool to exclude or confirm pseudomalabsorption. Apart from the routine diagnostic approach, there is also a need for psychiatric evaluation and care.

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## Graves' disease 2 and Orbitopathy

### PS3-12-106

#### Alterations in the gut microbiota are associated with the humoral immune response in graves' disease

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#### Background

Graves' disease (GD) is characterized by lymphocytic infiltration and autoimmune activation. The gut microbiota plays a pivotal role in immune regulation. The underlying mechanism of the gut microbiota in GD autoimmunity remains elusive. The present study aimed to investigate the role of the gut microbiota in the humoral immunity of GD.

#### Methods

A total of 45 healthy controls (HCs) and 68 GD patients [52 without treatment (U\_GD) and 16 with treatment (T\_GD)] were enrolled in the study. B-cell subset distribution and CD32b expression on B cells were analyzed by flow cytometry. Cytokines were measured by enzyme-linked immunosorbent assays. The gut microbial composition was analyzed by 16S rRNA gene sequencing.

#### Results

In the discovery cohort, we observed aberrant B-cell subset distribution, decreased CD32b expression and elevated proinflammatory cytokines in U\_GD patients compared with HCs. The diversity and structure of the microbial community in U\_GD patients were different from those of HCs, and some alterations in gut microbiota were significantly correlated with changes in humoral immunity. Moreover, we identified a fecal microbiome index that could be used to distinguish U\_GD patients from HCs in the validation cohort, whereas the structure of the microbial community and B-cell activation-related cytokines in T\_GD patients were similar to those of HCs.

#### Conclusions

The interaction between the microbiota and humoral immunity is involved in the development of GD, and antithyroid drug therapy could relieve the disease by rebuilding homeostasis of gut microbiota.

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### PS3-12-107

#### Macrophage-orbital fibroblasts interaction in context of hypoxic signaling for inflammatory processes during graves' orbitopathy

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#### Introduction

The inflammatory eye disease Graves' orbitopathy (GO) is the main complication of Graves' disease in patients. In previous studies we have shown that hypoxia and HIF-1 dependent pathways could play an important role in the pathogenic process of GO. Hypoxia is known to attract inflammatory cells and therefore maintains inflammation and recruitment of immune cells like macrophages (MQ). However, few is known about the specific contribution of MQ to the progression of orbitopathy. Therefore, we investigated the role and interaction of MQ and orbital fibroblasts (OF) in context of inflammation and hypoxia.

#### Methods & Results

We analyzed the expression levels of hypoxic marker HIF-1 $\alpha$ , MQ marker CD68, proinflammatory cytokine TNF $\alpha$  and recruitment proteins CCL2, CCL5 and CCL20 in fat biopsies of control and GO patients by real-time PCR. We found that HIF-1 $\alpha$ , CD68, TNF $\alpha$ , CCL2, CCL5 and CCL20 mRNA expression was increased in the fat tissue of GO patients. Next, we analyzed the cytokine profile of supernatants from fat biopsies with a multiplex ELISA. We could show an enhanced secretion of TNF $\alpha$ , CCL2 and CCL20 only under hypoxia while CCL5 was induced on protein level under normoxia as well as under hypoxia in GO tissue. An immunofluorescence stain of CD68 and TNF $\alpha$  was used to demonstrate the source of TNF $\alpha$  in the orbital tissue. The immunofluorescence indicated that TNF $\alpha$  secretion occurs in conjunction with CD68 positive MQ. To further, investigate the inflammatory interaction of MQ and OF, we stimulated OF with TNF $\alpha$  or co-cultured them with M1-MQ from a THP-1 cell line under normoxic and hypoxic conditions. We found that OF expressed hypoxic marker HIF-1 $\alpha$ , hypoxia target gene VEGF and immune marker ICAM-1 as well as chemokines CCL2, CCL5 and CCL20 most pronounced upon TNF $\alpha$  stimulation and hypoxia. M1-MQ enhanced the induction of HIF-1 $\alpha$  and CCL2 in OF in addition to hypoxia alone, whereas proinflammatory inhibitors Etanercept and dexamethasone reduced this effect. Furthermore, we found that OF-macrophage co-culture enhanced adipogenic differentiation and adiponectin secretion under hypoxia. PX-478, a HIF-1 $\alpha$  inhibitor, reduced the adipogenic differentiation of OF significantly.

#### Conclusion

In summary, our results show that hypoxia and macrophage-OF interactions have a cumulative effect. The findings indicate that the inflammatory milieu and hypoxic signaling in the orbit are characterized by TNF $\alpha$  positive macrophages, which interact with OF which results in constant inflammation and tissue remodeling. A combination of anti-inflammatory treatment and HIF-1 $\alpha$  reduction could be an effective treatment option for GO.

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### PS3-12-108

#### The incidence and risk factors of radioiodine-induced graves' disease following treatment of thyroid autonomous tissue

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#### Introduction

Radioiodine (I-131) therapy is an effective treatment for thyroid autonomy but may induce Graves' disease (GD) but in up to 5% of patients. GD is characterised by antibodies against TSH receptor (TSHRab). We set out to evaluate the incidence and risk factors of I-131-induced GD in patients with thyroid autonomy treated with I-131.

## Methods

We retrospectively reviewed patients with solitary toxic adenoma or toxic nodular goiter who were negative for TSHrAb and received I-131 between January 2013 and December 2018. Prior to treatment, antibodies against thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) were measured, and the uptake of iodine-123 (I-123) at 20-hours or technetium-99m-pertechnetate (Tc-99m) was determined. Patients were treated with median activity of 747 MBq I-131 (range 478–1140 MBq) and followed-up for 12 months. We monitored for *de novo* occurrence of GD by measuring thyroid function and TSHrAb concentration. Patients' characteristics influencing the occurrence of I-131-induced GD were analysed; p-value of <0.05 was considered statistically significant.

## Results

A total of 1551 patients (277 males, 1274 females) with an average age of 68.6 ± 14.0 years (range 14–95) were included. Prior to I-131 therapy, TPOAb and/or TgAb concentration were raised in 14.5% (225/1551) of patients. The median I-123 uptake, measured in 64.7% (1004/1551) patients, was 29.0%, and the median Tc-99m uptake, measured in 35.3% (547/1551) patients, was 0.82%. An increase in TSHrAb concentration was observed in 4.4% (68/1551) patients at 4.1 ± 3.3 months following I-131 application; of those, 63.2% (43/68) presented with overt hyperthyroidism. Patients with *de novo* occurrence of GD were significantly younger (63.1 ± 14.3 vs 68.7 ± 13.8 years,  $P < 0.01$ ) and more likely to be positive for TPOAb and/or TgAb before I-131 application than those with negative TSHrAb (47.1% vs 13.0%,  $P < 0.001$ ). Furthermore, they had significantly higher median concentrations of TPOAb (41.6 KU/l vs 30.3 KU/l,  $P < 0.001$ ) and TgAb (15.0 KU/l vs 15.0 KU/l,  $P < 0.001$ ). Additionally, their median uptake of I-123 before treatment was significantly higher (32.5% vs 29.0%,  $P < 0.05$ ), but their Tc-99m uptake did not differ (0.89% vs 0.82%,  $P = 0.72$ ). There was no significant difference in applied activity of I-131 (median, 741 vs 747 MBq,  $P = 0.09$ ), or gender ( $P = 0.47$ ).

## Conclusion

We show that I-131-induced GD occurs in 4.4% of patients treated for thyroid autonomy. Younger patients and those with increased TPOAb and/or TgAb levels prior to I-131 therapy are at a higher risk of developing GD post-treatment. We therefore recommend monitoring these patients closely following I-131 application.

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## PS3-12-109

### Blocking the TSH receptor with human monoclonal autoantibody K1-70TM in patients with graves' disease – results from a phase I clinical trial

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## Objectives

TSH receptor (TSHR) autoantibodies (TRAb) which mimic the actions of TSH are responsible for hyperthyroidism in Graves' disease (GD) which is often associated with Graves' orbitopathy (GO). K1-70 is a TSHR specific human monoclonal autoantibody which binds to the TSHR with high affinity and prevents stimulation of the TSHR by TSH and TRAb. Safety, tolerability, pharmacokinetic, pharmacodynamic and immunogenic effects of K1-70 in patients with GD were assessed in a phase I clinical trial.

## Methods

K1-70<sup>TM</sup> was administered to 18 GD patients stable on anti-thyroid drugs in ascending doses of 0.2 mg, 1 mg, 5 mg and 25 mg by intramuscular (im) or 50 mg and 150 mg intravenous (iv) routes in 6 cohorts of 3 subjects each. The subjects were followed up for 100 days post dosing.

## Results

K1-70<sup>TM</sup> was well tolerated in all subjects at all doses with no reported deaths or Serious Adverse Events. The reported Adverse Events were mild or moderate and none were directly related to K1-70<sup>TM</sup>. No significant immunogenic responses were observed in any of the subjects. The iv administration resulted in improved systemic exposure compared to im administration indicating this was the correct dosage route for future stages of drug development. The half-life of K1-70<sup>TM</sup> given iv was about 500 hours. Subjects receiving higher doses of K1-70 (25 mg and above) demonstrated expected pharmacodynamic effects with fT3, fT4 and TSH progressing into hypothyroid ranges. At 28 days post dose 11/18 (61%) of patients were in a hypothyroid state while for higher dose cohorts 9/9 (100%) progressed to the hypothyroid state on or before day 28. This corresponded to clinically observed and patient reported improvements in symptoms of both GD and GO. Patients reported improvements in tremor, sleep, mental focus, toilet urgency, aches, pains and general wellbeing. Clinically significant reductions in exophthalmos measurements (>2 mm) were observed in subjects receiving higher doses of K1-70<sup>TM</sup>. In addition, patients reported improvements in

photosensitivity, gritty eyes sensation, conjunctival redness and gaze-evoked pain.

## Conclusions

Our phase 1 trial demonstrated that K1-70<sup>TM</sup> was safe and well tolerated in all subjects. Systemic exposure following iv administration was as expected and the risk-benefit profile for K1-70 was favourable for later phases of drug development. The pharmacokinetic/pharmacodynamic relationship exceeded the expectations of the phase 1 trial design and the beneficial effects of K1-70 on patients' eye signs support suggestions of a key role for TRAb stimulation of orbital TSHRs in GO.

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## PS3-12-110

### Linear mixed model analysis of quality of life scores in patients with thyroid eye disease treated with teprotumumab from three 24-week clinical trials

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## Objectives

Teprotumumab, an IGF1-receptor antagonist, has been shown in three clinical trials to markedly improve the clinical course of Thyroid Eye Disease (TED) or Graves' Orbitopathy (GO) with significant improvements noted in inflammation, proptosis, and diplopia. Furthermore, compared to placebo moderate-to-large improvements were noted in the total, appearance (AP), and visual function (VF) as measured by EUGOGO GO quality of life (GO-QOL) scores after 24 weeks of therapy. Based on these findings, we undertook an analysis to determine the major outcomes associated with these substantial changes in QOL.

## Methods

A total of 120 patients with moderate-to-severe TED who were treated with teprotumumab from the phase 2, phase 3 (OPTIC), and OPTIC-X controlled studies were examined with observation points on study day 1, week 6, 12, and 24. Linear mixed-effects models were employed to measure the impact of Demographics, Time on treatment, Proptosis (mm), Diplopia (Gorman Grade), presence/absence of Gaze Evoked Orbital Pain, and Spontaneous Orbital Pain on Total, AP, and VF GO-QOL scores, via hierarchical addition. Random effects accounted for within-patient response variability.

## Results

Mean age was 52 (SD 12) years and females represented 73% of the population examined. Total, AP and VF GO-QOL scores improved 27%, 33%, and 23% respectively from baseline to Week-24. Patients with proptosis improved Total, AP and VF GO-QOL by 44%, 37%, and 49% respectively. The final model indicated that within-patient variability accounted for 80% of Total GO-QOL Score variance. Improvements in Diplopia and Gazed Evoked Orbital Pain were significantly related to higher (improved) Total, AP, and VF scores ( $P < 0.001$  for all). Improvements in Spontaneous Orbital Pain were associated with higher Total and VF scores ( $P < 0.001$ ). Increasing age ( $P < 0.001$ ) and male sex ( $P = 0.02$ ) had a significant positive correlation with the AP Score. All scores were positively associated with time on study ( $P < 0.001$ ). Gazed Evoked Orbital Pain and Proptosis were found to interact significantly, such that an improvement in proptosis correlated to a greater improvement in the AP score in patients without Gaze Evoked Orbital Pain as compared with those that had Gaze Evoked Orbital Pain ( $P < 0.001$ ).

## Conclusions

These novel data indicate that improvement in Diplopia and orbital pain, particularly Gaze Evoked Orbital Pain are strong contributors to GO-QOL improvement in TED patients receiving teprotumumab. Gaze Evoked Orbital Pain may impact patients' perception of AP improvements through its interaction with proptosis reduction.

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## PS3-12-111

### Circulating marginal zone b cells and IGA serum levels as potential biomarkers of clinical activity of graves' orbitopathy

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#### Objectives

The Clinical Activity Score (CAS) is used to measure and classify Graves orbitopathy activity (GO). However, CAS is partly subjective, and the evaluation of its components is binary. As CAS is known to be correlated with TSH receptor antibodies (TRAb), we wanted to evaluate other immunological parameters in the peripheral blood as markers of GO activity.

#### Methods

The study included 32 patients (19 females, 13 males). CAS was evaluated by a single experienced physician. Patients were not treated for GO, except by local measures. All patients were euthyroid on therapy. We measured TRAb, immunoglobulins, and B lymphocyte subpopulations. Multicolour flow cytometric analysis of B cell subsets was performed using the backbone of six monoclonal antibodies (anti-CD38, - CD27, -CD21, -CD19, -IgM, -IgG). This approach provided phenotypic characterization of naïve B cells, circulating marginal zone B cells (MZB), CD21<sup>low</sup> B cells, class-switched B cells, transitional B cells and plasmablasts in peripheral blood. Ordinal regression was used for data analysis with CAS as a dependent variable.

#### Results

In the multivariate analysis, there was a positive association between CAS (1-4) and TRAb but a negative association between CAS and MZB and IgA.

#### Conclusions

Although this is just preliminary data from a small study, it seems that the activity of GO may be modulated by or associated with changes of some less often considered components of the immune system. While TRAb drives the inflammatory response and positively correlated with CAS, we found that IgA and the presence of MZB are associated with the less active form of GO. The role of MZB in autoimmunity is still unclear. However, it has been demonstrated that these cells may have a dual role in autoimmune pathophysiological processes. MZB may promote autoimmunity by rapid production of low-affinity antibodies with self-reactivity to clear pathogens and apoptotic cell debris. On the contrary, this subset of B cells may acquire a regulatory phenotype and produce IL-10 after activation of the TLR9 signal through direct contact with apoptotic cells. Our results indicate that the latter function of MZB may have implications in the pathogenesis of GO. The perplexing role of MZB in GO needs to be further investigated. Our findings may pave the new way to assess GO activity and opens new therapeutic options.

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### PS3-12-112

#### Alemtuzumab-induced graves' orbitopathy successfully treated with a single dose of rituximab

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#### Introduction

The humanized antibody anti-CD52+ alemtuzumab has been approved since 2014 for the treatment of relapsing-remitting multiple sclerosis (RRMS). Immune reconstitution after alemtuzumab induces thyroid autoimmunity in 34-41% of patients with RRMS, with Graves' disease (GD) accounting for 63-65% of cases. Graves' Orbitopathy (GO) may also occur in 13% of patients after alemtuzumab and is scarcely reported. Here we present the first case of alemtuzumab-induced GO resistant to high doses of i.v. methylprednisolone and successfully treated with a single low-dose of rituximab.

#### Case Report

A 37-year-old woman was diagnosed with RRMS in 2009 and initially treated with natalizumab and fingolimod. Alemtuzumab was administered in two courses 12 months apart (2016, 2017). In 2019 she developed GD and was started on methimazole. In 2020 she developed moderate GO and was treated with i.v. methylprednisolone, unsuccessfully. In Dec 2020 she presented clinical-activity-score (CAS) 8/10, proptosis 27.5/28 mm and intermittent diplopia and was treated with 500 mg i.v. rituximab; her CAS improved to 3/10 and 1/10 after one and two months, respectively. In Oct 2021 she underwent total thyroidectomy and in Dec 2021 her GO remained inactive and was scheduled for surgical decompression. Two years after alemtuzumab, her total lymphocytes count had not fully recovered yet (1.06x10<sup>9</sup>/l, n.v. 1.2-3.4). Just before rituximab infusion (3 years post-alemtuzumab), her peripheral B lymphocyte count was still reduced (76 cells/ $\mu$ L, n.v. 100-500) and total B cell depletion occurred immediately after therapy. At the time of thyroidectomy (1 year post-rituximab and 4 years post-alemtuzumab), her B cell numbers were still markedly depleted in both blood and thyroid tissue, compared with control subjects and patients with spontaneous GD. Our patient also presented reduced numbers of T regulatory (Treg) cells in both blood and thyroid tissue compared with controls.

#### Conclusions

Immune reconstitution after alemtuzumab is known to trigger GD and GO, despite determining a very good control of RRMS. Our patient developed both GD and active GO, despite a post-alemtuzumab B lymphocyte count persistently reduced. Rituximab therapy was effective in inactivating her GO and determined a further reduction of B lymphocytes count. We hypothesize that B cell depletion after rituximab ameliorates GO by interfering with the B cell antigen presentation to T cells. Interestingly, her GO and RRMS remained under control even in presence of reduced counts of Treg. More complete and long-term studies are needed to unravel the immunological mechanism involved in this complex scenario.

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### PS3-12-113

#### Temporal trends in the clinical presentation of graves' ophthalmopathy: a single – centre retrospective study

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#### Background

Graves' ophthalmopathy (GO) is an autoimmune disease that affects particularly the retrobulbar soft tissues and represents the most common extrathyroidal manifestation of Graves' disease (GD). Some studies suggest that GO in newly diagnosed patients in recent years has a trend towards a less severe clinical presentation, moreover we have no studies that focus the trend of clinical presentation of GO in the last decade on the population of our area.

#### Aim

Of our study was to evaluate the temporal trend of the clinical presentation of GO in the east part of Sicily over the last decade.

#### Methods

We selected 221 consecutive patients observed from January 2005 to December 2006 and from January 2015 to December 2016. 21 patients were excluded because underwent to surgical orbital decompression, 40 patients because were previously treated by oral or parenteral corticosteroids and/or radiotherapy and 49 patients because longer GO duration. Finally we studied 111 patients with Graves' Disease, diagnosed with GO according to EUGOGO criteria within 12 months from the Graves' Disease diagnosis. We compared 55 consecutive patients, 11 males (F) and 44 females (M) come to our observation from January 2005 to December 2006 [group 1 (G1)], with 56 patients, 15 males, and 41 females, referred to us from 2015 to 2016 [group 2 (G2)].

#### Results

We assumed that iodine and selenium intake were similar between the groups; sex, age, smoke, thyroid function, LDL cholesterol, diabetes, impaired fasting glycaemia (IFG), impaired glucose tolerance (IGT), time from GD diagnosed to start of therapy (TGDD) and GO different grade of severity were tested by univariate analysis evidencing only TGDD reduced in G2 vs G1,  $P = 0.057$ . We build a bivariate logistic regression model considering the effect of age, TGDD, Hertel measurements, CAS, eyes motility improvement and GO severity (considering moderate to severe and severe GO as a same group) at presentation respect the two different temporal range considered in the study. Go severity was

significantly reduced in G2 vs G1: Odds Ratio = 0.3; 95% CI 0.07-0.81,  $P = 0.02$ .

#### Conclusions

GO severity at presentation was significantly reduced in G2 as compared to G1. We were not able to find some factors related to GO severity decrement although TGDD was reduced by univariate analysis.

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## Pregnancy & Iodine

### PS3-13-114

#### The troubled discourse of hypothyroidism in pregnancy: sentiment analysis of the literature from 2011 to 2021

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#### Introduction/Aim

The diagnosis and management of hypothyroidism in pregnancy is a domain of shifting paradigms, with changes in guidelines, which may have led clinicians to confusion or angst (Endocrine Today; October 2019). Since sentiment analysis (SA) of medical texts can be implemented (Artificial Intelligence in Medicine 2015; 64: 17-27) we sought to use SA regarding this clinical issue.

#### Materials/Methods

We collected the English language abstracts of review articles in PubMed from 2011 to November 2021, using the Medical Subject Headings (MeSH) terms "hypothyroidism", "pregnancy" and "human" and performed SA with an online artificial intelligence tool (courtesy of Prof. Daniel Soper, California State University, Fullerton, CA, USA; <https://www.danielsoper.com/sentimentanalysis/default.aspx>). The results were evaluated by year with the Kruskal-Wallis and Chi square tests.

#### Results

From 2011 to 2015 a slight trend from negative to positive sentiment ( $P = 0.08$ ) was noted in the literature studied, while from 2018 wide and significant ( $P = 0.04$ ) sentiment fluctuations were noted by year.

#### Discussion

Regarding hypothyroidism and pregnancy uncertainties remain vis-à-vis screening and management bearing in mind the need to optimize perinatal outcomes. Researchers continue to debate the very definition of subclinical hypothyroidism, which can differ between nonpregnant or pregnant states. This situation is reflected in the sentiment of the published literature, especially in review articles, usually by experts in the field worldwide, which try to provide a critical evaluation of the data that is available from existing studies.

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### PS3-13-115

#### Higher thyroid FT3-to-FT4 ratio is associated with gestational diabetes mellitus and adverse pregnancy outcomes

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#### Aim

To determine the association between thyroid function and the risk for gestational diabetes mellitus (GDM) and adverse pregnancy outcomes.

#### Methods

This case-control study was a sub-analysis of the BEDIP-N study, in which 199 GDM women were matched for age and body mass index with 398 controls. Thyroid stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), and thyroid peroxidase (TPO) antibodies were measured at 6-14 weeks and 26-28 weeks pregnancy. TSH and fT4 were also measured in early postpartum in GDM women.

#### Results

TSH and TPO were not associated with the risk to develop GDM. The fT3-to-fT4 ratio at 26-28 weeks was positively associated with GDM risk with an adjusted odds ratio (aOR for smoking, education, parity, ethnicity, gestational weight gain and (family) history of diabetes or GDM) of 2.12 (95% CI 1.07; 4.23) comparing the highest with the lowest tertile. Higher fT3 levels and fT3-to-fT4 ratio were associated with a less favorable metabolic profile with higher BMI and more insulin resistance in pregnancy and postpartum. Women in the upper fT3 tertile and upper fT3-to-fT4 ratio had a higher rate of preeclampsia [respectively 4.6% (10) vs. 1.0% (2),  $P = 0.040$ , and 4.4% (9) vs. 0.5% (1),  $P = 0.020$ ], gestational hypertension [8.3% (18) vs. 3.1% (6),  $P = 0.034$  and 8.9% (18) vs. 2.0% (4),  $P = 0.003$ ], and caesarean sections [29.4% (63) vs. 16.1% (31),  $P = 0.002$  and 32.2% (65) vs. 12.7% (25),  $P < 0.001$ ].

#### Conclusion

Higher fT3-to-fT4 ratio late in pregnancy was associated with GDM, adverse pregnancy outcomes, and an adverse metabolic profile in early postpartum.

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### PS3-13-116

#### Iodine status in danish pregnant women after an increase in iodine fortification: a regional study

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#### Objectives

The Danish population was previously iodine deficient with regional differences, and a mandatory iodine fortification of salt was introduced more than 20 years ago. Despite iodine fortification and frequent use of iodine-containing supplements, the iodine status in Danish pregnant women was insufficient when evaluated in 2012 (median urinary iodine concentration (UIC): 101 µg/l). From July 1, 2019 the authorities implemented a mandatory increase from 13 to 20 ppm in the level of iodine added to salt in Denmark. The aim of the present study was to evaluate iodine status in Danish pregnant women after such increase in iodine fortification.

|                                     | unit | Overall | Iodine-containing supplements |                    | Nonusers |         | p    |
|-------------------------------------|------|---------|-------------------------------|--------------------|----------|---------|------|
|                                     |      | n = 147 | Users<br>n = 130              | Nonusers<br>n = 17 | 95% CI   | 95% CI  |      |
| Urinary iodine concentration        | µg/l | 77      | 80                            | 59                 | 61-96    | 61-97   | 0.08 |
| Urinary iodine/creatinine ratio     | µg/g | 116     | 119                           | 87                 | 103-133  | 106-146 | 0.03 |
| Estimated 24-hours iodine excretion | µg   | 127     | 130                           | 94                 | 112-145  | 115-159 | 0.03 |

**Method**

We performed a cross-sectional study in the North Denmark Region which is within the geographical part of Denmark with previously most severe iodine deficiency. From September 8 until October 12, 2021, pregnant women referred for routine obstetric ultrasound in the Department of Obstetrics, Aalborg University Hospital, were included. All women delivered a spot urine sample and filled out a questionnaire. UIC was determined after alkaline ashing by the cerium-arsenite method, and measurement of urinary creatinine concentration (Cobas 8000, Roche Diagnostics) was performed for calculation of urinary iodine/creatinine ratio and estimated 24-hours urinary iodine excretion. Results were reported as medians with 95% confidence intervals.

**Results**

Altogether 147 pregnant women were included in the study (median gestational week 20), and 130 women (88%) reported current use of iodine-containing supplements (Table). The overall median UIC was 77 µg/l (Table). When stratified by intake of iodine-containing supplements, median UIC as well as the creatinine-adjusted measures of urinary iodine status were higher in iodine-containing supplement users compared with nonusers (Table).

**Conclusion**

Despite a recent and considerable increase in mandatory iodine fortification in Denmark, iodine status in pregnant women within the North Denmark Region was insufficient and median UIC was even lower than previously found. Results call for detailed assessment of underlying factors and continued attention to ensure adequate iodine status during pregnancy in Denmark.

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**PS3-13-117**

**Iodine status during pregnancy in the veneto region: impact on maternal and newborn thyroid function and analysis of the nutritional and socio-economic determinants**

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**Objectives**

The aim was to evaluate the iodine nutritional status in pregnant women residing in Veneto region and its associations with diet, iodine supplements and social status (nationality and education).

**Methods**

292 consecutive pregnant women at the third trimester of pregnancy were enrolled (≥18 years old, and resident in Veneto). Exclusion criteria were a personal history of thyroid disease and the refusal of the informed consent. Every woman provided an early-morning spot urine sample (to assess iodine to creatinine concentration ratio, UI/Creat) and a blood sample (to measure TSH, FT4, FT3 and Thyroglobulin (Tg)) and were administered a questionnaire regarding diet habits, and the use of iodine supplements. The new-borns' TSH levels were obtained from the congenital hypothyroidism screening program, together with their data at birth.

**Results**

Use of iodized salt was spread to 72.5% of women. Median UI/Creat was 112.37 µg/g (IQR: 60.95-185.93 µg/g). Only 36.9% of women had a UI/Creat ≥ 150 µg/g and the frequency was higher among Italian than foreign women ( $P = 0.01$ ). UI/Creat was higher among higher educated women ( $P = 0.01$ ). The frequency of women with a UI/Creat (≥ 150 µg/g) was higher among regular cow's milk consumers ( $P = 0.046$ ) and among iodine supplement users ( $P = 0.0001$ ) than among their counterparts, but no association was found with iodized salt use. Only the combined use or the iodine supplement plus cow's milk guaranteed an adequate iodine intake (UI/Creat ≥ 150 µg/g) ( $P < 0.01$ ). At the multivariate analysis, only regular cow's milk and iodine supplement were independent

predictors of an adequate iodine status. There was no association between maternal thyroid function and UI/Creat levels. Median Tg values were lower among the iodine-sufficient group than among iodine-deficient women 8.20 µg/l and 11.61 µg/l, respectively ( $P = 0.019$ ). The weight at birth was lower in the offspring of women with UI/Creat <50 µg/g and ≥250 µg/g than in the iodine-adequate or mildly deficient group ( $P = 0.02$ ). The TSH at screening was higher among the offspring of women receiving an iodine supplement than among non-users ( $P = 0.04$ ), but both in the normal range.

**Conclusions**

The iodine prophylaxis program should be implemented to reach a better iodized salt coverage and education among childbearing-aged women, especially the foreign and lower educated. In the meanwhile, both the iodine supplement and cow's milk seem to be pivotal. Neonatal TSH levels resulted higher among the offspring of the women that took the iodine supplement than among non-users, suggesting a particular sensitivity to iodine in the fetal thyroid.

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**PS3-13-118**

**Which is the best pre-conceptual TSH cut-off in women submitted to assisted reproductive technology?**

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TSH is involved in the immunomodulation of early pregnancy stages. Thyroid guidelines have indicated 2.5 mU/l as the best TSH level below which women undergoing assisted reproductive technology (ART) need to be maintained, though this threshold is still controversial. Aims of the present study were to evaluate if pre-conceptual TSH is associated with an increased risk of miscarriage, to identify a TSH cut-off significantly associated with risk of miscarriage and to assess the impact of TSH levels on primary and surrogate outcomes. A series of 1484 infertile women (mean ± age 36.7 ± 4.1 years, mean ± SD BMI 22.7 ± 4) submitted to IVF in a single center from 2004 and 2014 was retrospectively studied. The majority of patients (60.8%) submitted to ART cycles had a primary infertility, while the remaining cases had a secondary infertility. Primary outcomes were biochemical pregnancy, clinical pregnancy, miscarriage and delivery. Surrogate outcomes were the number of oocytes, the number of embryos and the transfer of embryos. Embryo transfer was performed in 86% of cycles. A biochemical pregnancy was recorded in 369/1274 (29%) patients and 146 of them (39.5%) experienced a pregnancy loss. Moreover, among these 146 women, 52 (36%) were clinically pregnant and had a miscarriage in the first trimester, while in 94 patients (64%) a biochemical pregnancy without clinical evolution was documented. No significant differences in mean TSH levels were observed between women with different times of miscarriage. ROC curve analysis showed that a TSH of 3 mU/l is significantly associated with miscarriage ( $P = 0.001$ ), while a TSH of 2.3 mU/l is associated with a higher chance to have a biochemical pregnancy. These two TSH thresholds (2.3 or 3 mU/l) were also studied in relation to surrogate outcomes: we observed a weak association between  $TSH \leq 2.3$  mU/l with the number of retrieved oocytes ( $P = 0.04$ ) while no significant correlation was found with the number of either embryos obtained or transferred ( $P = NS$ ). In conclusion, lower pre-conceptual TSH levels appears to favor the embryo implantation and to reduce the risk of early pregnancy loss in women undergoing ART. TSH screening prior to IVF procedures and L-T4 treatment are strongly recommended in order to optimize TSH levels before ovarian stimulation.

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**PS3-13-119**

**The impact of iodine supplementation in pregnancy on iodine intake, thyroid function and thyroid volume in pregnant women from iodine sufficient region**

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#### Objective

The aim of the study was to assess the impact of iodine supplementation (IS) on iodine intake, thyroid function and thyroid volume (Tvol) in pregnant women (PW) from iodine sufficient region of Zagreb, the capital of Croatia. The secondary aim was to assess the impact of nodular or diffuse thyroid ultrasound (US) pattern on serum thyroglobulin (Tg) measurement.

#### Subjects and Methods

The study enrolled 91 PW taking IS (onIS) and 100 PW without IS (offIS) during pregnancy. Spot urine samples for urinary iodine concentration (UIC), blood samples for serum TSH, FT4 and Tg measurement and US was performed in PW in each trimester of pregnancy. Thyroid volume (Tvol) was measured with assessment of thyroid parenchyma and nodules by US. UIC was measured by Seal AA3 HR (Seal Analytical, Wisconsin, USA), TSH and FT4 by CLIA (Immolute 2000 XPi Siemens) and Tg by ECLIA (Roche COBAS e411). All PW were euthyroid with negative Tg and TPO antibodies. SPSS (26.0, SPSS Inc., Chicago, IL, SAD) was used for statistical analysis.

#### Results

Overall median UIC, TSH, FT4, Tg and Tvol in PW onIS vs offIS were: 174 vs 158 ng/mL, 1.9 vs 1.8 mU/L, 13.2 vs 12.1 pmol/L ( $P < 0.05$ ), 11.0 vs 13.6 ng/mL ( $P < 0.01$ ) and 12.3 vs 13.7 mL ( $P < 0.05$ ). Trimester (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) specific median UIC, TSH, FT4, Tg and Tvol in PW onIS vs offIS were: 173\*, 177, 178 vs 155\*, 163, 166 ng/mL, 1.8, 2.1\*, 2.1 vs 1.7, 1.7\*, 1.9 mU/L, 12.6\*, 13.3\*, 12.9 vs 11.2\*, 12.0\*, 12.6 pmol/L, 10.2, 11.0\*, 11.1\* vs 13.5, 14.5\*, 14.5\* ng/mL, 11.0\*, 12.0\*, 13.0\* vs 12.5\*, 14.0\*, 14.0\* mL ( $*P < 0.05$ ). Nodules < 1 cm were recorded in 15 (17%) PW onIS and 24 (24%) PW offIS, and diffuse US pattern in 12 (13%) PW onIS and 17 (17%) PW offIS. Median Tg in PW onIS with diffuse and nodular US pattern vs normal US was 13.4\* and 12.5 vs 10.6\* mg/L ( $P < 0.05$ ). Median Tg in PW offIS with diffuse and nodular US pattern vs normal US was 15.7\* and 15.0 vs 13.2\* mg/L ( $P < 0.05$ ).

#### Conclusion

Pregnant women in the city of Zagreb, Croatia, have sufficient iodine intake regardless of IS. Iodine supplementation in pregnancy increases UIC and FT4 and decreases Tg and Tvol. Thyroglobulin is a valuable biomarker for assessment of iodine nutritional status. Nodular and diffuse thyroid US pattern increases Tg values with significant difference for diffuse pattern.

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### PS3-13-120

#### The impact of thyroid function on neonatal outcome in women with polycystic ovary syndrome treated with metformin or placebo during pregnancy

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#### Objectives

Polycystic ovary syndrome (PCOS) and thyroid disorders have separately been linked to adverse pregnancy and neonatal outcomes. Even small variations in thyroid function within the normal range may influence fetal growth. Previous data have shown that metformin-treatment of pregnant women with PCOS reduced late miscarriages and preterm births, and resulted in less decrease of FT4 compared to placebo. Moreover, metformin exposure in utero altered the offspring anthropometrics leading to larger head size. Our aim was to investigate whether newborn anthropometrics are associated with maternal thyroid function in PCOS-pregnancies, and explore the potential modifying effect of metformin.

#### Methods

Post-hoc analyses of two randomized, double-blinded, placebo-controlled trials, in which pregnant women with PCOS were randomized to metformin or placebo, from first trimester to delivery. Maternal serum levels of thyroid stimulating hormone (TSH) and free thyroxine (fT4) were longitudinally measured at gestational weeks (gw) 5-12, 19, 32 and 36 in 309 singleton pregnancies. The

mean z-scores of birthweight, birth length, and head circumference were estimated in offspring. The associations between maternal thyroid parameters and offspring anthropometrics were studied using regression analyses.

#### Results

There were no associations between maternal TSH and fT4 during pregnancy and head circumference z-score of the newborns. However, in early pregnancy (gw 5-12), one pmol/l increase in maternal natural logarithm (ln)-transformed fT4 levels was associated with a reduction in birthweight z-score of 1.34 units ( $b = -1.34$ ,  $P = 0.010$ ) and a reduction in birth length z-score of 1.32 units ( $b = -1.32$ ,  $P = 0.035$ ). In the third trimester (gw 32), one pmol/l increase in maternal ln-transformed fT4 levels was associated with a reduction in birthweight z-score of 1.39 units ( $b = -1.39$ ,  $P = 0.018$ ). The aforementioned associations were observed independently of treatment group. For the placebo group only, significant negative linear associations were observed between TSH at gw (5-12) and gw36 and birth length z-score, where one mU/L increase in square root transformed TSH was associated with a 0.53- and 0.79-units decrease in birth length z-score respectively ( $b = -0.53$ ,  $P = 0.048$  and  $b = -0.79$ ,  $P = 0.030$  respectively).

Conclusions. In early pregnancy, maternal fT4 was inversely associated with birthweight and length. In late pregnancy, maternal fT4 was inversely associated with birthweight. Inverse associations were observed between maternal TSH in early and late pregnancy and birthweight in the placebo group. Subclinical changes in maternal thyroid function parameters may influence anthropometrics in babies born to women with PCOS.

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### PS3-13-121

#### Thyroid homeostasis in iodine deficient healthy pregnant women from 10 villages of bihar, india

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#### Background

Pregnancy is a physiological state characterised by enhanced thyroid hormone production resulting in increased demand for iodine. Iodine deficiency during pregnancy, is therefore, considered to be a risk factor for adequate growth and development in offspring. However our understanding of thyroid hormone homeostasis in iodine deficient environment is limited.

#### Objectives

1) To assess the current iodine status in pregnant women from an endemic zone 2) to assess the impact of iodine deficiency on homeostatic adjustments of thyroid hormones, pregnancy outcome and health of pregnant women.

#### Method

Epidemiological observational survey included pregnant women (18-48 year) from 10 villages of the Bihar State in India (2014-2018) in association with Government Hospital and Government Primary Health Centers. Total 900 healthy pregnant women fulfilling inclusion criteria were enrolled. A questionnaire survey was conducted for demographic socioeconomic and other parameters. Salt samples from relevant households were obtained for the measurement of iodine using State Govt UNICEF kits. Body weight, height and BMI were recorded. Blood and urine samples were procured from subsets of population for the assessment of UIC and thyroid hormones (TSH, FT<sub>4</sub>) respectively. UIC was measured spectrophotometrically. TSH & FT<sub>4</sub> were measured in DBS using ELISA and Chemiluminiscence methods. IEC approval & informed consent were obtained.

#### Results

Dietary iodine intake was less than 15ppm in 50% of pregnant women. UIC median values indicated iodine deficiency viz., 73, 82, 84 in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> trimester with 88%, 96% and 94% subjects iodine deficient, respectively. Evidently dietary iodine intake need not necessarily reflect UIC status of a population. TSH mu/l ranged as 1.3-5.1, 1.47-5.79, 2.32 -6.5 in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> tri indicating an increasing trend reflected in the quartile distributions. FT<sub>4</sub> pmol/l ranged from 7.02-18.44, 7.19-19, 4.59-16.9 indicating a decline in 3<sup>rd</sup> tri. While FT<sub>4</sub> values were comparable, TSH consistently was higher than the prescribed international guideline cut-offs. However, no individual showed overt symptoms of hypothyroidism. Pregnancy outcome (still birth, miscarriages) and BMI values were comparable with Indian/world normal figures.

#### Conclusion

TSH values were above the international standard cut offs and may represent an attempt to restore equilibrium ensuring adequate thyroxine supply in these iodine deficient women. According to population outliers the TSH values were apparently normal (within 2.5-97.5 percentile). Financial assistance from Apeejay Education Society, and, facilities from Swami Rama Himalayan University,



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### PS3-13-122

#### Preconception tsh levels and early gestation maternal thyroid function in a large cohort of women with hashimoto's thyroiditis (ht) diagnosed preconceptionally

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Several lines of evidence indicate an increased risk of adverse gestational outcomes in women with Hashimoto's thyroiditis (HT) already as of TSH levels at early pregnancy >2.5mU/l, and definitely greater for TSH concentration >4mU/l. The latest guidelines recommend preconception TSH levels (pre-C-TSH) in HT women be maintained below 2.5mU/l, this threshold being expected to prevent hypothyroidism occurrence at early gestation.

#### Objectives

To prospectively evaluate: i) whether maintaining pre-C-TSH values <2.5mU/l in HT women was effective in preventing the occurrence of early gestation thyroid insufficiency, defined by TSH >2.5mU/l (diagnostic criteria 1) or >4.0mU/l (diagnostic criteria 2) at early gestation; ii) the cut-off values of TSH that would best preconceptionally identify euthyroidism at early gestation in HT women, by means of Receiver-Operating Characteristic (ROC) curves.

#### Methods

Two-hundred and sixty women preconceptionally diagnosed with HT and pre-C-TSH <2.5 mU/l, whose thyroid function was prospectively monitored from preconception up to pregnancy term.

#### Results

Of the 260 women, 122/260 (46.9%) were on LT4 therapy (Hypo-HT group) and 138/260 (53.1%) were confirmedly euthyroid without LT4 (Eu-HT group) prior to conception. At 1<sup>st</sup> trimester, 37/122 (30.3%) Hypo-HT women had TSH > 2.5mU/l, with almost 2/3 of these women (24/37, 64.9%) displaying TSH values >4.0mU/l. Analogously, at 1<sup>st</sup> trimester evaluation TSH was >2.5mU/l in 42/138 (30.4%) EU-HT women, but >4.0 mU/l in 14/138 (10.1%) only. The optimal pre-conception TSH cut-offs found were 1.24mU/l and 1.73 mU/l for the diagnostic criteria 1 in Hypo-HT and Eu-HT women, respectively, and 1.74 mU/l and 2.07 mU/l for the diagnostic criteria 2 in Hypo-HT and Eu-HT women, respectively. Applying the above cut-offs, the relative risk reduction (RRR) of early gestational thyroid insufficiency was 82.7% and 94.0% for the diagnostic criteria 1 in Hypo-HT and Eu-HT women, respectively, and 82.5% and 97.9% for the diagnostic criteria 2 in Hypo-HT and Eu-HT women, respectively.

#### Conclusions

In our series, about one third of HT women whose pre-C TSH were in the range recommended by current guidelines (<2.5mU/l) required to increase or to start LT4 therapy once pregnant because of TSH levels exceeding the advisable TSH threshold at early pregnancy. In contrast, a more than halved rate of HT women whose pre-C serum TSH was below the calculated cut-offs was found to have unsatisfactory thyroid function at their first antenatal visit. The identification of population-specific pre-conception TSH cut-offs is in our view indicated, to optimize maternal thyroid function of HT women prior to conception.

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## Thyroid Cancer CLINICAL 2

### PS3-14-123

#### A case of warthin-like papillary thyroid cancer. surgical decision based on eu-tirads criteria

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#### Introduction

The Warthin-like variant of papillary thyroid cancer (WLPTC) is a rare subtype of papillary thyroid carcinoma (PTC) resembling Warthin tumours of the salivary

glands, and more commonly associated with Hashimoto's thyroiditis than other types of PTCs. The WLPTC is characterized by papillae lined by large oncocytic cells with cores having dense lymphoplasmacytic infiltrate. The prognosis of WLPTC is the same or less aggressive than that of classical PTC, but in some cases it is associated with poor outcome. The preoperative fine needle aspiration cytology (FNAC) is the most reliable technical approach for surgical decision, but it shows sometimes false negative result. The EU-TIRADS criteria (European Thyroid Association for ultrasound (US) assessment of thyroid nodules and stratification) can significantly increase diagnostic accuracy with a direct impact on treatment decisions.

#### Case

A 45-year-old male patient presented at outpatient clinic with Hashimoto thyroiditis for hormone replacement therapy. The first thyroid US showed an 8.1x7.6x10.4 mm irregular, hypoechoic nodule in the right lobe. A FNAC was taken from this target lesion, which resulted in a diagnosis of lymphocytic thyroiditis without suspicious malignant cells. Two years later the target nodule showed mild expansion in all dimensions: 14.4x13.2x15.7 mm. We re-assessed the following US features: non-oval shape, irregular margin, microcalcifications, marked hypo echogenicity and resulted in a EU-TIRADS 5 score. The repeated FNAC showed again a benign lymphocytic thyroiditis. Despite of the benign cytology result, thyroid lobectomy with adjunctive central lymph node dissection was recommended because of the growing tendency and high EU-TIRADS score. The histological diagnosis was WLPTC and lymphocytic thyroiditis of the nodule (pT1bN0Mx). The additional contralateral lobectomy resulted only lymphocytic thyroiditis without malignancy. Further genetic analysis is ongoing to decide on the need for the adjuvant radioiodine treatment.

#### Conclusion

In clinical practice, evaluation of thyroid nodules based on EU-TIRADS criteria can rarely overrule the FNAC results with an impact on surgical decision-making. In some cases, the growing tendency and the very suspicious US signs of malignancy (e.g. microcalcification) of a nodule results in a higher risk for malignancy, than that based on the FNAC results. Furthermore, in the case of WLPTC, when both the benign lymphocytic thyroiditis and malignant tumour can be seen in one nodule, the FNAC can be misleading when the aspiration is taken from the benign part of the lesion.

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### PS3-14-124

#### Evaluating prognostic markers for stage migration in medullary thyroid cancer

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#### Background

Medullary thyroid cancer (MTC) is a rare malignancy with a variable and sometimes unpredictable disease course. To predict the course of disease more accurately at diagnosis, we introduce dynamic risk stratification by analyzing factors related to "stage migration" within the first 5 years after diagnosis.

#### Methods

All patients diagnosed at the University Medical Center of Groningen between 1999 and 2015 were retrospectively studied. Patients were staged according to the 8<sup>th</sup> edition of the TNM classification and variables were collected. Stage migration was defined as i) an upgrade of the N and/or M stage, i.e., the development of i) a lymph node metastasis in a previously unaffected cervical compartment or ii) distant metastasi/es in an initially M0 staged patient or 2) death due to MTC progression, within 5 years after diagnosis. Clinical and pathological variables were then evaluated in univariate Cox regressions to find prognostic factors.

#### Results

Of the 75 included patients, 41 were male and 47 had sporadic MTC. The median age at diagnosis was 49 (IQR 32 – 58) years. A total of 29 (39%), 7 (9%), 9 (12%) and 30 (40%) of the patients were classified with stage I, II, III and IV, respectively. Sporadic MTC patients had a higher stage at diagnosis than hereditary patients ( $P < 0.001$ ). Five years after diagnosis, 61 (81%) patients were still alive. Eleven patients died due to MTC progression and 3 died from other causes. Twenty-one out of 75 included patients developed stage migration within 5 years after diagnosis, after a median time of 17.0 (IQR 11.5 – 30.5) months. Stage migration was a result of an upgrade of the N and/or M stage in 10 patients, disease-specific death in 8 patients and a result of both (upgrade of N and/or M stage followed by death) in 3 patients. Stage migration occurred in 1 (14%), 6 (67%) and 14 (47%) of stage II, III and IV patient(s), respectively. In univariate Cox regressions, sporadic MTC, palpable lymph node(s) at diagnosis, a

higher TNM stage, angioinvasion, a positive resection margin, and extrathyroidal growth significantly increased the risk of stage migration.

#### Conclusion

MTC is a dynamic disease where disease progression is always lurking. This stage migration model could improve the clinician's prediction of disease progression and thereby help to design a better patient-tailored follow-up strategy after initial treatment. To verify this method, further collaborative studies with larger datasets need to be performed.

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### PS3-14-125

#### Lymph nodes metastases in differentiated thyroid cancer patients: understanding their impact on the clinical outcome

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#### Background

Lymph node metastases at histology (pN1) are usual findings in differentiated thyroid cancer (DTC) patients. Their detection has an impact on the extent of surgery, the further treatments after surgery, and on the clinical outcome.

#### Material and method

We evaluated data of 1332 consecutive DTC patients who performed the first <sup>131</sup>I treatment between January 2010 and September 2012. According to their pN status, they were divided into 2 groups: Nx/N0 and N1. The latter was subdivided in central compartment (N1a) and latero-cervical compartment (N1b) metastases. Clinical outcome, according to 2015-ATA was defined as: post-operative and post-<sup>131</sup>I (median time from surgery: 6 months), first evaluation after <sup>131</sup>I (median time from <sup>131</sup>I: 8 months) and last evaluation (median time from <sup>131</sup>I: 83 months). Results

1064 (79.9%) patients were in the Nx/N0 and 268 (20.1%) in the N1 group. N1 patients were more frequently males (35.8 vs 27.3%,  $P < 0.01$ ) and younger (median age 40 vs 47,  $P < 0.01$ ). Several histologic features were significantly more frequent ( $P < 0.01$ ) in the N1 group: multifocality (63.4 vs 46.8%), mETE (67.9 vs 24.6%), vascular invasion (28 vs 8.9%) and intermediate ATA-risk (94.8 vs 36.5%). Structural incomplete response (StR) rate was higher in N1 group throughout the follow-up ( $P < 0.01$ ), although these patients experienced higher <sup>131</sup>I activities over time and more neck re-intervention. Also, N1b ( $n = 142$ , 53%) patients, compared to N1a ( $n = 126$ , 47%), had more frequently mETE (74.6 vs 59.5%,  $P < 0.01$ ) and vascular invasion (33.1 vs 22.2%,  $P < 0.05$ ) and lower prevalence of histologic thyroiditis (21.1% vs 35.7,  $P < 0.01$ ). Nevertheless, N1b patients experienced higher <sup>131</sup>I activities and more neck re-intervention during the follow-up. StR rate was significantly higher in N1b at post-operative (16.2 vs 6.3%), post-<sup>131</sup>I (26.1 vs 8.7%) and at first evaluation after <sup>131</sup>I (24.3 vs 9.6%). Conversely, at the last evaluation, significance was not reached (17.9 vs 10.4%,  $P = 0.09$ ).

#### Conclusions

pN1 status is related to more aggressive histologic features in DTC patients. Although more treatments were performed during the follow-up, the N1 patients had a higher StR rate at each time of the follow-up when compared with Nx/N0. Moreover, N1b patients, compared to N1a, experienced more frequent and aggressive treatments during the follow-up but still with a higher persistence of StR.

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### PS3-14-126

#### The prognostic role of chromosomal gains and loss in sporadic medullary thyroid carcinoma

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#### Background

Human cancer is characterized by the accumulation of somatic alterations including base substitutions, indels, structural rearrangements and somatic copy number alteration (CNA), either gain or loss, of chromosomes that can be responsible either for initiation and/or cancer progression. About 80% of Sporadic Medullary Thyroid Carcinoma (sMTC) harbor *RET* or *RAS* somatic alterations with a negative prognostic role for the presence of the *RET* mutation; a few studies about the role of chromosomal CNA in MTC have been published so far showing a variable frequency ranging from 50%-77%.

#### Objective

Aim of this study was to evaluate the global genomic profile of a series of 41 sMTC obtained by Comparative Genomic Hybridization array (array-CGH). The presence of CNAs was compared to the presence of a *RET* mutation and clinical and pathological features of MTC patients in order to investigate their role in tumor behaviour.

#### Methods and Results

Twenty-five cases (25/41, 61%) showed at least one CNA with a range of CNA per sample: [1 to 27] while 16 cases (16/41, 39%) did not show any CNA. In general, losses in chromosomal regions were more frequent than gains (91 vs 50 events). Chromosomes most frequently involved in CNA were chromosome 22 (31.7% of cases), chromosome 1 (29.3% of cases), chromosomes 3 (19.5% of cases), chromosomes 10 (17.1% of cases), chromosome 21 (14.6% of cases), while the remaining chromosomes were affected only by few CNAs. When we compared the presence of CNA with the presence of somatic *RET* mutations, we found that 18/23 *RET*+ cases (78.3%), showed the presence of at least one CNA, present in only 7/18 (38.9%) *RET*- cases ( $P = 0.02$ ). The CNAs in *RET*+ MTC patients were significantly associated with chromosomes 3 ( $P = 0.0035$ ) and 10 ( $P = 0.007$ ). We finally correlated the cases harboring at least one CNA with the outcome of sMTC patients (disease free, persistent disease at biochemical level and metastatic disease) and we found that patients present in a metastatic disease showed a higher rate of CNAs ( $P = 0.005$ ) and the chromosomes mainly affected by these CNAs were chromosomes 3 ( $P = 0.002$ ), 9 ( $P = 0.02$ ), 10 ( $P = 0.2$ ) and 16 ( $P = 0.02$ ).

#### Conclusions

In conclusion, MTC cases showing CNAs were mainly the ones harboring a *RET* mutation, suggesting that a higher level of chromosomal instability could be responsible for a higher rate of chromosomal alterations. Interestingly, the regions involved with loss and gain show the presence of important tumor suppressor genes and oncogenes, respectively, that could justify a role of these CNAs in the progression of the disease.

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### PS3-14-127

#### Dominant benign thyroid nodules (BTN) treated with percutaneous microwave thermoablation (PMWT): an effective tool to decrease thyroid nodule volume

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#### Objectives

Image-guided thermal-ablation (LTA; RFA) are well established therapy options in selected BTN. PMWT is a mini-invasive technique recently applied in thyroid disease; aim of this work is investigate its effectiveness as treatment in BTN.

#### Methods

From May 2021, 45 patients (30 F, 15 M, Aged 37-90, Mean 55.8) with BTN symptomatic/in growing, refusing/non eligible to surgery were enrolled. Inclusion criteria: nodule diameter  $\geq 2$  cm, mainly solid  $\geq 20\%$ , 2 FNA cytology pathologically confirmed as benign (TIR2 sec ITCCS 2014). Baseline were performed ECG, anesthesiology and ENT consults, laboratory assessment (serum levels of FT3, FT4, TSH, TPOAb, TgAb, calcitonin, blood count, clotting indexes).

Additionally 25/45 pts done Thyroid Scintiscan with  $^{99m}\text{Tc}$ -Pertechnetate (19 cold 3 hot no; 3 non focal findings). Ultrasound-guided PMWTA was carry out under local anesthesia through TATO antenna (18G x8 cm/17G x10 cm Terumo) delivering 10-15 W in 10-15 minutes, depends on the Volume of BTN. Physician effects an anamnestic evaluation, thyroid physical examination rating symptomatology with a Compressive Score (CS on a 10 cm visual-analog scale), assigned an Aesthetic Score (AS from 1-no palpable-visible nodule to 4 palpable-visible in all positions). US thyroid scan was performed to record Volume of BTN target (VnT) baseline and during follow-up scheduled at 1,3,6 months after procedure. Additionally volume reduction rate % (VRR) was calculated, and success rate fixed in a volume reduction  $\geq 50\%$ .

#### Results

No peri-procedural major complications were observed. 1/45 has developed in 10 days after PMWT transient thyrotoxicosis and atrial fibrillation pharmacologically reverted. 10 patients still missing the follow-up; the data will be available and presented during the meeting. Clinically was registered CS mean score from 4,2 (baseline) to 3 and 1,7 (1 and 3 months respectively), AS from 3,2 (baseline) to 2 (1 and 3 months). Interestingly both scores were stable at 6 months follow-up. Baseline mean VnT was 17,29 mL (range 7,7-1,6 mL) and mean VnT post-procedure was 8,79 mL (range 32,9-0,31mL). The estimated mean VRR at 1,3 6 months was 52% (range 12- 82%),58%(range 45-91%), 66% (range 45-94%), respectively. If consider as a therapeutic goal a volume reduction of  $\geq 50\%$  the success rate was approximately 82,9%. Neither a re-growth occurred in this short-term evaluation.

#### Conclusion

In BTN disease use of PMWT has shown effective nodule shrinking, well tolerated, safe and with low complication rate. Furthermore we registered a satisfactory clinical response. Our data need a validation in large series and long term follow-up.

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### PS3-14-128

#### Progrp as an additional screening marker in the diagnostic work up for medullary thyroid carcinoma

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#### Background

Medullary thyroid carcinoma (MTC) is a rare disease accounting for 1-3% of all thyroid carcinomas. Unfortunately, most patients present with metastasized disease: 70% with cervical lymph node metastasis and 5-10% with distant metastasis. Survival strongly correlates with stage of disease at diagnosis, illustrating the need for early diagnosis. Calcitonin is a well-established tumour marker for MTC, but its use in the screening phase is limited by a high rate of false positives. Among thyroid cancer types, progastrin-releasing peptide (proGRP), is specifically expressed in MTC. Therefore, proGRP is a potential tumour marker for MTC. We studied the value of proGRP as a screening marker for MTC, especially in the range where calcitonin is inconclusive.

#### Methods

To investigate the potential of proGRP as an additional tumour marker in the diagnostic work up for MTC we took a stepwise approach. First, we prospectively collected serum samples of patients with benign thyroid nodules (benign cohort) and measured serum proGRP concentration. Second, we aimed to establish a reference range and cut-off for proGRP in a clinical setting. Hereto, we measured serum proGRP in a cohort of people where blood was drawn for a vitamin B12 measurement (reference cohort). Third, we investigated the correlation between calcitonin and proGRP in a cohort of patients in whom calcitonin was measured. Serum proGRP and calcitonin concentrations were measured using Lumipulse G1200 (Fujirebio) and Immulite 2000XPI (Siemens) respectively.

#### Results

The mean serum proGRP concentration in the benign cohort ( $n = 48$ ) was 36.9 pg/ml (95% CI 33.2-40.5, range 17.8 – 62.3 pg/ml). In the reference cohort ( $n = 670$ ) mean serum proGRP was 43.9 pg/ml (95% CI 41.2-46.6). The 2.5%-97.5%

reference interval was 13.5 – 124.9 pg/ml, with a 90% CI of the upper limit of 103.5 – 154.5. There was a strong correlation between calcitonin and proGRP ( $r = 0.814$ ,  $P < 0.001$ ,  $n = 212$ ), especially in the calcitonin concentration range above 100 pg/ml ( $r = 0.810$ ,  $P < 0.001$ ,  $n = 54$ ). In the calcitonin concentration range of 10-100 pg/ml no correlation ( $r = -0.161$ ,  $P = 0.475$ ,  $n = 22$ ) was found. Conclusion

Mean proGRP concentration in the benign cohort was within reference ranges of the reference cohort, suggesting a potential role for proGRP as an additional screening marker for MTC. This was confirmed by the good correlation between proGRP and calcitonin in patients with a calcitonin above 100 pg/ml. Further research should determine whether proGRP can be employed as a screening tool to diagnose MTC.

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### PS3-14-129

#### Hypocalcaemia secondary to lenvatinib induced-hypoparathyroidism: a case-report

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In the last decades, multi-tyrosine-kinase inhibitors (mTKI) have emerged as an effective treatment for radioiodine refractory differentiated thyroid cancer. Due to their pleiotropic mechanism of action, these drugs may cause different side effects. Hypocalcaemia has been reported in up to 35% of patients treated with mTKI, but up to date little is known about its pathophysiology and relevance. We report the case of a 78 years old woman operated on for a papillary thyroid cancer infiltrating striated muscles, oesophagus, blood and lymphatic vessels. The extent of surgery was limited to hemithyroidectomy, because of tumour extension and infiltration to contiguous structures. Radioactive-iodine treatment was not performed due to the remnant tissue dimension. The patient was therefore started on lenvatinib at the initial dose of 10 mg per day, in order to avoid fistulisation. During the first months of therapy, the largest diameter of the main neoplastic lesions in the neck significantly reduced. Serum Tg levels decreased from 370  $\mu\text{g/l}$  to 49.6  $\mu\text{g/l}$ . The patient experienced grade I-II proteinuria, anorexia, fatigue, diarrhoea, nausea, mucositis and hypertension, according to CTCAE. After four months, the patient accessed the E.R. for sudden dyspnoea, muscular cramps and limb spasms. Blood exams revealed a grade III hypocalcaemia (corrected serum calcium: 6.6 mg/dL), due to primary hypoparathyroidism (serum PTH: 12.6 pg/ml; serum phosphorus: 4.7 mg/dL). The patient was treated with calcium infusions and oral vitamin D and magnesium supplementation. After discharge, the oral dose of carbonate calcium was of 6 g per day. Lenvatinib was discontinued for the duration of hospitalization and restarted three days after discharge, when serum calcium levels were proven to be stable (corrected serum calcium: 8.8 mg/dL). Calcium intake was titrated according to blood exams performed every 3-5 days. Two weeks after discharge, while taking calcium 3 g per day, the patient complained worsening of anorexia and stupor. Grade II hypercalcaemia (serum calcium: 11.7 mg/dL) was demonstrated. She was treated with an intravenous infusion of physiological solution and calcium supplementation was interrupted. During the subsequent follow up, the patient remained and still is eucalcemic without calcium supplementation. Though hypocalcaemia has been described as potential side effect, this is the first report of a lenvatinib-induced primary hypoparathyroidism. This case is of particular interest since the patient was submitted to hemithyroidectomy and the hypoparathyroidism was thus definitely not-related to surgery. Further studies are needed to clarify pathogenesis and relevance of this life-threatening adverse event.

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**PS3-14-130****The role of calcitonin wash-out in the diagnosis of small sporadic medullary cancer - case reports**Boyan Nonchev<sup>1</sup>, Antoaneta Argatska<sup>2</sup>, rosen dimov<sup>3</sup>, Veselin Chonov<sup>4</sup> & Elena Chobankova<sup>5</sup><sup>1</sup>Department of Endocrinology, Medical University of Plovdiv, Clinic of Endocrinology, Kaspela University Hospital, Bulgaria; <sup>2</sup>Medical University of Plovdiv, Department of Endocrinology, Plovdiv, Bulgaria; <sup>3</sup>Department of Special Surgery, Medical University of Plovdiv, Clinic of Surgery, Kaspela University Hospital, Bulgaria; <sup>4</sup>Department of General and Clinical Pathology, Medical University of Plovdiv, Bulgaria; <sup>5</sup>Clinic of Endocrinology, Kaspela University Hospital, Bulgaria**Introduction**

Medullary thyroid cancer (MTC) is a rare thyroid malignancy whose prognosis is highly dependent on the early diagnosis as well as on the available treatment options. Serum calcitonin represents the most sensitive test for the detection of MTC thus its measurement is strongly recommended in patients with known genetic or hereditary conditions associated with high risk of medullary hyperplasia or cancer. However, its diagnostic utility and routine clinical application in all patients with thyroid nodules are highly controversial especially in nodules less than 1 cm.

**Case Description**

Case 1: A 49-year-old woman was referred for further evaluation after finding increased levels of serum calcitonin on routine laboratory tests. Medical history and physical examination were unremarkable, the elevated levels of serum calcitonin were confirmed – 136.3 pg/ml. Neck ultrasound scan showed a solid hypochoic lesion in the dorsal part of the right lobe measuring 7.6/4 mm in size with type 3 vascularization, no suspicious lymph nodes were detected. FNAB and calcitonin wash-out measurement were performed. Cytological report fell in BETHESDA III category, but calcitonin levels from the wash-out were > 5000 pg/ml, suggesting the lesion was medullary cancer. The patient was referred for surgery, histology and immunohistochemical analysis confirmed the diagnosis of a micromedullary cancer.

Case 2: The second patient is a 49-year-old woman in whom slightly elevated levels of calcitonin were found on occasion. She did not have any significant medical conditions, family history or medication intake. On neck ultrasound examination a 4.8/4 mm solid hypochoic nodule in the ventral part of the right lobe was seen without any other abnormal neck findings. The cytological report from the FNAB was inconclusive but calcitonin wash-out measurement was > 5000 pg/ml. Very small focus of medullary cancer was confirmed on histological and immunohistochemistry tests following surgery. Postoperative calcitonin measurement was negative, no abnormal neck lymph nodes were detected during follow-up.

**Conclusions**

MicroMTC is generally defined as a medullary cancer ≤ 1 cm in the greatest dimension and represents a rare entity with increasing incidence over the past decade. Calcitonin wash-out measurement can be a useful tool in the comprehensive evaluation of micronodular thyroid lesions. Early detection of medullary thyroid cancer significantly reduces the risk for local and distant metastasis and increases the rate of disease-free survival of the patients with this potentially lethal neuroendocrine malignancy.

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of *RET*-negative tumor tissues. Other genetic alterations, chromosomal rearrangements or point mutations in minor genes, are very rare.

**Case Report**

We report a case of a 38-year-old woman with a nodule in the left lobe, cytologically suspected of MTC. Serum calcitonin was elevated at 217 pmol/l. The patient underwent total thyroidectomy and histopathological examination revealed a 1.3 cm MTC with positive immunohistochemical staining for calcitonin and chromogranin. Molecular genetic analysis detected neither germline *RET* mutation, nor *RET/RAS* somatic mutations in examined fresh frozen tumor tissue. A comprehensive NGS panel targeted especially fusion genes, but also other genetic changes, was used for subsequent analysis. Surprisingly, a common *V600E BRAF* mutation, typical for papillary thyroid carcinoma (PTC), was found. The mutation was confirmed by allele-specific real-time PCR performed from material isolated from both fresh frozen and FFPE tumor tissue. The histological examination demonstrated morphological features of MTC, no signs of mixed tumor and no evidence of PTC. Interestingly, beside positivity for calcitonin and chromogranin, it showed strong and diffuse CK19 expression, an immunohistochemical marker, typical for diagnosis of PTC.

**Conclusions**

Only two other cases of *BRAF V600E* in MTC patients have been reported. In all these studies, the results of molecular genetic analysis were verified by an alternate method, and the tumors were histologically confirmed as pure medullary thyroid carcinomas, without concurrent PTC. The study was supported by projects of the MHCR AZV NU21-01-00448 and MHCR-RVO (00023761).

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**PS3-14-132****Efficacy and safety of lenvatinib in a cohort of well-differentiated advanced thyroid carcinomas**Inês Damásio<sup>1</sup>, Joana Maciel<sup>2</sup>, Ana Figueiredo<sup>3</sup>, Helder Simões<sup>4</sup>, Tiago Nunes da Silva<sup>5</sup>, Joana Simões-Pereira<sup>6</sup>, Mariana Horta<sup>7</sup>, Rita Santos<sup>8</sup>, Sara Donato<sup>9</sup> & Valeriano Leite<sup>10</sup>

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**Introduction**

Treatment of differentiated thyroid carcinoma (DTC) remains a challenge in the setting of locally advanced or metastatic disease refractory to radiodine (RAI) therapy. SELECT trial demonstrated that Lenvatinib improved progression free survival (PFS) comparing to placebo.

**Objective**

Our aim is to report the efficacy and safety of lenvatinib in our population with aggressive DTC.

**Methods**

We retrospectively reviewed the clinical records of 25 patients with advanced well-DTC who started treatment with lenvatinib in our center between January 2016 and January 2022. Patients with poorly differentiated or anaplastic thyroid carcinomas were excluded. Response evaluation was made according to the RECIST version 1.1 criteria. PFS and median overall survival (OS), best overall response (BOR), disease-control rate (DCR), response rate (RR) and clinical benefit rate (CBR) were also evaluated as efficacy measures. Additionally, the change of the sum of target lesion's greatest diameters from baseline to nadir and tumor volume doubling times (TVDT) before and after therapy were also calculated.

**Results**

A total of 25 patients with well-DTC treated with lenvatinib were analyzed. Mean age at the initiation of treatment was 67.6 ± 1.8 and 64% of patients were female. Twenty-four (96%) had metastasis (M1): 91.7% in the lung; 62.5% in bone and 62.5% of patients had M1 in ≥ 2 locations. Median duration of treatment with

**PS3-14-131****Somatic BRAF V600E mutation in a patient with medullary thyroid carcinoma**Eliska Vaclavikova<sup>1</sup>, Barbora Pekova<sup>1</sup>, Vlasta Sykorova<sup>1</sup>, Jiřka Moravcova<sup>1</sup>, Karolina Mastnikova<sup>1</sup>, Zdenek Novak<sup>2</sup>,Jana Drozenova<sup>3</sup>, Martin Chovanec<sup>4</sup>, Josef Vcelak<sup>1</sup> & Bela Bendlova<sup>1</sup>  
<sup>1</sup>Institute of Endocrinology, Department of Molecular Endocrinology, Prague, Czech Republic; <sup>2</sup>Institute of Endocrinology, Department of Clinical Endocrinology, Prague, Czech Republic; <sup>3</sup>3rd Faculty of Medicine, Charles University, Royal Vinohrady Teaching Hospital, Department of Pathology, Prague, Czech Republic; <sup>4</sup>3rd Faculty of Medicine, Charles University, Royal Vinohrady Teaching Hospital, Department of Otorhinolaryngology and Head and Neck Surgery, Prague, Czech Republic**Introduction**

Medullary thyroid carcinoma (MTC) is a calcitonin-producing tumor that predominantly occurs in a sporadic form (75%) and less commonly in an inherited form. Besides activating germline mutations of the *RET* proto-oncogene in hereditary syndromes of MEN2, somatic *RET* mutations are detectable in about 50% of sporadic MTC. Further, also *RAS* mutations have been discovered in 30%

lenvatinib was 9.1 months and mean daily dose was 16.7 mg. The BOR was complete response in 1 patient (4%), partial response in 10 (40%), stable disease in 9 (36%) and progressive disease in 2 patients (12%). Response was not evaluable in 3 patients. RR was 44%; DCR 80% and CBR 68%. Median PFS was 25.6 (95% confidence interval (CI): 5.5-60.4) and OS was 29.6 (95% CI 25-34.1). The mean change of the sum of target lesion's greatest diameters from baseline to nadir was -32.7% ( $\pm 6.9$ ). Median TVDT pre-levatinib was 10 months and median TVDT post-levatinib was -3.5 months. Our data showed that lenvatinib results in prolongation of TVDT in 86.7% of patients. AE were reported in 96% of patients, resulting in interruption and dose reduction in 68% and 52% respectively. The most frequent AE was hypertension in 85%. One patient had a grade 5 AE (rectum-vaginal fistulae with sepsis) and she had history of previous pelvic irradiation.

#### Discussion

Our results are in line with other real-life data and show that the clinical benefit can be obtained with lower doses. In addition, in our series lenvatinib showed an increased benefit in rapidly progressive disease. AE were frequent and serious AE related with wound healing may be potentiated by previous radiotherapy.

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## Thyroid Cancer Diagnosis & Treatment

### PS3-15-133

#### European thyroid association guideline on the management of pediatric thyroid nodules and thyroid carcinoma

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#### Objectives

At present no European recommendations for the management of pediatric thyroid nodules and differentiated thyroid carcinoma (DTC) exist. Differences in clinical, molecular, and pathological characteristics between pediatric and adult DTC emphasize the need for specific recommendations for the pediatric population.

#### Methods and results

An expert panel was instituted by the executive committee of the European Thyroid Association (ETA) including an international community of experts from a variety of disciplines including pediatric and adult endocrinology, pathology, endocrine surgery, nuclear medicine, clinical genetics, and oncology. The American Thyroid Association (ATA) pediatric guideline 2015 was used as framework for the present guideline. Areas of discordance were identified, clinical questions were formulated and literature searches were performed. The expert panel members discussed the evidence and formulated recommendations based upon the latest evidence and expert opinion.

#### Conclusions

Children with a thyroid nodule or DTC require expert care in an experienced center. The present guideline provides guidance for healthcare professionals to make well-considered decisions together with patients and parents regarding diagnostics, treatment and follow-up of pediatric thyroid nodules and DTC.

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### PS3-15-134

#### Patient-reported outcomes (PROs) from patients with RET-mutant medullary thyroid cancer (MTC) and ret fusion-positive TC treated with pralsetinib in the arrow trial

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#### Objectives

RET alterations are targetable oncogenic drivers in TC. Patients with TC, especially those with MTC treated with the multikinase inhibitors cabozantinib and/or vandetanib (C/V), often experience significant treatment-related side effects. Pralsetinib, a selective RET tyrosine kinase inhibitor, showed efficacy in patients with RET-altered TC from the phase 1/2 ARROW trial (NCT0307385). We present the impact of pralsetinib on PROs in patients with RET-mutant MTC and RET fusion-positive TC, including quality of life (QoL) and disease-related symptoms.

#### Methods

PROs (exploratory endpoint since protocol v4.1) were evaluated in adults with RET-altered, non-resectable advanced TC from ARROW (pralsetinib 400 mg QD) who completed the European Organization for Research and Treatment of Cancer (EORTC) QoL questionnaire (QLQ-C30) before treatment initiation (baseline). Patients then completed the QLQ-C30 every 4 weeks until treatment discontinuation. Score changes > 10 points from baseline were considered clinically meaningful. Data cut-off: 18 October 2021.

#### Results

Of 100 efficacy-evaluable patients with RET-mutant MTC enrolled from protocol v4.1, 98 (98%) completed a baseline PRO assessment. They reported moderate baseline Global Health Status (GHS)/QoL (mean score: 67/100) and high baseline functioning scores (mean scores: > 79/100), with no clinically meaningful changes from baseline to Week 44 ( $\geq 25\%$  of patients still enrolled). Mean score for diarrhoea improved from baseline to Week 44 (-25.9). In the prior C/V RET-mutant MTC subcohort ( $n = 39$ ), baseline mean GHS/QoL score was 59/100 and functioning scores were > 68/100. Clinically meaningful increases in mean scores from baseline were observed throughout Weeks 8–32 for GHS/QoL, Weeks 24–32 for physical functioning and Weeks 12–44 for role functioning. At Week 44, disease-related symptoms including diarrhoea (mean score change from baseline: -20.6), fatigue (-14.8), appetite loss (-12.7) and insomnia (-12.7) were improved. Patients with treatment-naïve RET-mutant MTC ( $n = 54$ ) had higher baseline GHS/QoL (mean score: 71/100) and functioning scores (mean scores: > 82/100) than those who had received prior C/V, and their PROs remained stable following pralsetinib treatment. In patients with RET fusion-positive TC enrolled from protocol v4.1 ( $n = 24$ ), baseline PRO questionnaire completion rate was 100%. At baseline, these patients had moderate GHS/QoL (mean score: 59/100), and moderate-to-high functioning scores (mean scores: > 70/100); episodic clinically meaningful improvements were seen for GHS/QoL and role functioning, and symptom burden remained low throughout.

#### Conclusions

Following pralsetinib treatment, patients with RET-altered TC, especially those with RET-mutant MTC treated with prior C/V, reported improved or stable GHS/QoL and functioning scores, and a reduced symptom burden from baseline.

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**PS3-15-135****Clinical relevance of lower titer thyroglobulin (TG) autoantibodies in patients with differentiated thyroid carcinoma**

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**Objectives**

Thyroglobulin (Tg) is an established tumor marker for differentiated thyroid carcinoma (DTC) patients. However, Tg immunoassays can be subject to autoantibody (TgAb) interference resulting in incorrect Tg values. Tg measurement with liquid chromatography-tandem mass spectrometry (LC-MS/MS) could be promising in patients with TgAbs. In this study, we compared a Tg immunoradiometric assay (Tg-IRMA) and a Tg-LC-MS/MS analytically in the presence of TgAbs. Furthermore, we evaluated the clinical concordance between both assays in DTC patients with lower TgAbs titers (<10 U/ml) TgAbs during <sup>131</sup>I ablation therapy.

**Methods**

118 DTC patients diagnosed between 2006 and 2014 in a University Medical Center were followed up with the Tg-IRMA (Thermo Fischer Scientific) and ARCHITECT Anti-Tg (Abbott Laboratories) assays. TgAbs  $\geq 10$  U/ml were defined as potentially interfering. We re-analyzed their samples with a sensitive Tg-LC-MS/MS method (Labcorp, North Carolina, USA, limit of quantification of 0.02 ng/ml). Passing-Bablok regression analysis was performed on samples obtained during <sup>131</sup>I ablation therapy and follow-up.

**Results**

In 304 samples with lower titer TgAb titers, good agreement was found between both Tg assays (slope of 1.09 (95% CI 1.05 - 1.16)). Fifty-five samples with potentially interfering TgAbs showed higher Tg-LC-MS/MS values than Tg-IRMA (slope of 1.45 (95% CI 1.12-> > 100)). For patients ( $n = 91$ ) with lower TgAb titers at the time of <sup>131</sup>I ablation therapy, the clinical concordance of both Tg assays was 91.2%.

**Conclusions**

In DTC patients with lower titer TgAbs, Tg-IRMA is a reliable and useful tumor marker. In DTC patients with potentially interfering TgAbs, Tg-IRMA values are decreased due to TgAb interference.

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**PS3-15-136****The role of core needle biopsy in the diagnosis of primary thyroid lymphoma and anaplastic thyroid carcinoma: a systematic review and meta-analysis**

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**Objective**

Anaplastic thyroid carcinoma (ATC) and primary thyroid lymphoma (PTL) are two highly aggressive malignancies of the thyroid, both leading to a rapidly enlarging neck mass. Fine-needle aspiration cytology (FNAC) is generally

performed as the primary examination for diagnosis in thyroid pathology but shows low sensitivity in diagnosing ATC or PTL. Non-diagnostic FNACs are usually followed by core-needle biopsy (CNB) or diagnostic surgery. As sensitivities of up to 100% have been described, we investigate whether executing CNB primarily is more desirable than FNAC in the diagnosis of ATC and PTL. We want to determine the diagnostic value of CNB by combining all published data on the reliability of CNB in diagnosing ATC and PTL in a systematic review and meta-analysis.

**Methods**

A search was performed on June 23<sup>rd</sup>, 2021 on PubMed, EmBase, Web of Science and Cochrane. Population of interest were patients who underwent CNB due to clinical or ultrasonographical suspicious features of ATC or PTL or patients with final diagnosis of ATC or PTL after CNB or after surgery following CNB.

**Results**

A total of 166 patients were included of which 136 patients were diagnosed as PTL and 14 patients as ATC after CNB. CNB proves to be superior to FNAC with a sensitivity and PPV of respectively 93.8% and 100% for PTL and 82.4% and 100% for ATC. Furthermore, rate of diagnostic surgery after CNB was only 6.2% for PTL and 17.6% for ATC.

**Conclusions**

We conclude that CNB could be a more appropriate intervention for diagnosis of ATC and PTL than FNAC.

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**PS3-15-137****Tendency of progression of antithyroglobulin antibody as a predictor of prognosis in patients with papillary thyroid carcinoma**

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**Objectives**

The clinical significance of antithyroglobulin antibody (TgAb) levels verified concomitantly with undetectable thyroglobulin (Tg) during the follow-up of patients with differentiated thyroid carcinoma remains under debate. The present study aimed to evaluate the presence and prognostic predictive value of TgAb during the follow-up of patients with differentiated thyroid carcinoma.

**Methods**

A retrospective cohort study was performed by reviewing the medical records of patients being followed up in a tertiary service, from 2000 to 2020. Measurement of Tg was performed by chemiluminescence (Cobas 601, Roche) and of TgAb by electrochemiluminescence (Liaison, Diasorin).

**Results**

Data from 868 patients diagnosed with papillary thyroid carcinoma were evaluated, including 62 patients (7.1%) who had detectable TgAb during follow-up, either preoperatively or postoperatively. Most are female (85%), white (81%), non-smokers (77%), mean age of 43.18 years, mean follow-up time of 94 months. There was no relationship between preoperative TgAb levels and worse prognostic characteristics at follow-up. A higher percentage of time with detectable TgAb after thyroidectomy was showed as a predictive factor to non-excellent response to therapy ( $P < 0.0001$ , OR 1.089; CL95% 1.045-1.135), and also, it was observed in carcinomas with angiolymphatic invasion ( $P = 0.002$ ), extrathyroidal invasion ( $P = 0.015$ ), lymph node metastases ( $P = 0.008$ ) and distant metastases ( $P = 0.009$ ), as well as in T3 and T4 tumors ( $P = 0.008$ ). Patients with incomplete structural response had a higher percentage of time with detectable TgAb compared to patients with excellent response one year after thyroidectomy ( $P < 0.001$ ) and current response (last assessment during follow-up) ( $P < 0.001$ ). The majority of patients with detectable TgAb had antibody negative throughout the follow-up period (59%), 24.1% remained stable, 9.6% showed a tendency to decrease and 6.4% to increase, and in this last group, 100% of the patients presented incomplete structural response.

**Conclusions**

This study showed that preoperatively detected TgAb are not associate to a worse prognosis in patients with differentiated thyroid carcinoma. We found that not only the appearance or increase of TgAb, but also the presence of stable TgAb levels were indicative of disease persistence or recurrence. In contrast, significant decline in TgAb was associated with disease-free status. In conclusion, when TgAb was detected during follow-up, temporal evaluation was important for defining the trend of TgAb and the relation with the progression of the disease.

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**PS3-15-138****Routine molecular analysis of fine-needle aspiration biopsies of thyroid nodules**

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**Objectives**

Molecular testing of fine-needle aspiration biopsy (FNAB) samples is increasingly used mainly for indeterminate categories of the Bethesda System for Reporting Thyroid Cytopathology. Our aim was to introduce a routine molecular analysis of the main genetic causes of thyroid cancer.

**Methods**

In total, 1354 FNAB samples of thyroid nodules were analyzed. Testing procedures mainly in samples evaluated as Bethesda categories III and above were gradually established. First, DNA for the most common mutation V600E in the *BRAF* gene using allele specific Real Time PCR (LC480, Roche) is analyzed. *BRAF*-positive samples are screened for *TERT* mutations using direct sequencing (CEQ 8000, Beckman Coulter). *BRAF*-negative samples are analyzed by next generation sequencing (MiSeq, Illumina) using the Thyro-ID panel (4bases) examining other 12 genes. The samples negative in the Thyro-ID panel are subjected to detection of 23 fusion genes including *ALK*, *BRAF*, *GLIS3*, *NTRK1*, *NTRK3*, *PPARG*, *RET* genes using Real Time PCR. Samples suspected of medullary thyroid carcinoma (MTC) for *RET* and *RAS* mutations are tested.

**Results**

*BRAF* mutations in 153 patients, *RAS* mutations in 87 patients, *RET* mutations in 4 patients, *TERT* mutations in 24 patients and fusion genes in 52 patients were detected. Genetic variants in other genes (*TP53*, *PTEN*, *PIK3CA*, *KIT*, *TSHR*) were detected in 25 patients. From our cohort, in 430 patients post-surgical histopathological evaluation has been known. Positive predictive values (PPV) of *BRAF*, *TERT*, *KRAS*, *HRAS*, *NRAS* mutations and fusion genes were 99.3%, 94.1%, 73%, 50%, 45.8% and 97.7%, respectively, if borderline tumors were not included in malignancy. PPV for *BRAF*, *TERT* and fusions were almost 100% except for a follicular adenoma with *BRAF* K601E mutation, one case of follicular tumor of uncertain malignant potential with *TERT* and *NRAS* mutations and one case of hyalinizing trabecular tumor (HTT) with *PAX8/GLIS3* fusion that is pathognomonic for HTT.

**Conclusions**

We established molecular testing of thyroid nodules that significantly contributed to clinical management of patients in the Czech Republic. Mutations of *BRAF*, *TERT* and fusion genes are associated with almost 100% risk of malignancy or even worse prognosis, therefore according to ETA guidelines from 2017 and recent publications their carriers are recommended for the total thyroidectomy. The risk of malignancy of *RAS* mutations is lower, clinical interpretation is difficult and thus rather a lobectomy is recommended.

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**PS3-15-139****Thyroid lymphoma - lessons learned when time is of the essence**

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**Introduction**

Primary thyroid lymphoma is rare and accounts for less than 5% of all thyroid malignancies. The risk is almost 60 times higher in those with thyroiditis and is more common in females, with a ratio of 4:1. The most frequent local symptom is a rapidly enlarging thyroid gland along with the resultant obstructive symptoms. Fine needle aspiration is the initial diagnostic procedure for thyroid tissue sampling. The main differential diagnosis is anaplastic thyroid carcinoma by its aggressive behaviour and rapid growth.

**Case Report**

A 69 year old man was referred to our outpatient clinic with a symptomatic, rapidly growing cervical mass with an ultrasound suggestive of thyroid cancer.

The patient primarily complained of progressive dysphagia and, more recently, hoarseness related to the past 4 weeks rapid enlargement of the thyroid gland. Past medical history included multinodular goiter and thyroiditis, having been discharged from our specialized consultation 4 years prior to this event. Fine needle aspiration cytology was performed, with no conclusive result, showing signs of cystic degeneration and chronic thyroiditis. A surgical biopsy was then proposed and undertaken 2 weeks later. Immunohistochemistry study favored a tumor of lymphoid-hematopoietic/histiocytic origin. However, the highly proliferative representation of the tumor, along with conflicting histochemistry marker positivity, determined another inconclusive diagnosis. As time went by, dysphagia got worse and orthopnea ensued. The lacking histologic diagnosis prevented our patient from starting any type of systemic therapy. The previously incised skin became infiltrated with the underlying tumor. Given the imminent airway compromise, the patient was submitted to a percutaneous endoscopic gastrostomy and an attempt at a tracheostomy procedure which could not be done due to significant posterior displacement of the trachea and risk of direct airway invasion. A second incisional biopsy was performed which excluded any malignant epithelial proliferation and confirmed the diagnosis of a T-cell/histiocyte rich large B-cell lymphoma. CT staging found 2 possible secondary lesions in the right lung and involvement of paratracheal nodes. He was started on systemic therapy with R-CHOP protocol and for the moment awaits restaging.

**Conclusion**

Primary thyroid lymphoma remains an extremely rare and life-threatening occurrence. Its high proliferation rate makes the histologic diagnosis both challenging and urgent, given that time is of the essence when a rapidly growing bulky thyroid mass threatens the airway. Bailout procedures should be sought when a definitive airway is not feasible.

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**PS3-15-140****Basal calcitonin as guide for an early and safe thyroid surgery in RET gene carriers**

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**Introduction**

Virtually all familial cases of Medullary Thyroid Carcinoma (MTC) have a *RET* germline mutation. Subjects harboring *RET* germline mutation without unaware of their condition are defined gene carriers (GC). Thyroid surgery timing is decided upon *RET* mutation and calcitonin levels (both basal, bCT, and stimulated, sCT). However, bCT and sCT thresholds for planning thyroid surgery have not been established, yet.

**Methods**

We evaluated 189 GCs by clinical, biochemical (bCT and sCT) and neck US every 6-12 months. Thyroid surgery was planned in case of elevated bCT (i.e., higher than upper limit of normal range) and/or positive stimulation test, or subjects (or parents, if minor) willing. After surgery, all patients were submitted to biochemical analysis (bCT and, if necessary, sCT) and neck ultrasound.

**Results**

92/189 GCs were submitted to thyroid surgery after a median time of 6 months (IQR 2-13). MTC foci (73.3% < 1 cm, 15% between 1 and 2 cm, 11.7% > 2 cm) were present in 71 (77.2%) while CCH in 21 (22.8%) subjects. At last clinical evaluation after surgery (median follow-up 85.5, IQR 35.25-147 months), clinical remission was observed in 71 patients (88.6% of microMTC, 66.6% of MTC between 1 and 2 cm, 28.6% of MTC > 2 cm and 100% of CCH patients) while 18 presented disease persistence (11.4% of microMTC, 33.4% of MTC between 1 and 2 cm and 71.4% of MTC > 2 cm): 13 and 5 MTC patients presented biochemical and structural persistence, respectively. Both presurgical bCT ( $P < 0.001$ ) and sCT ( $P = 0.009$ ) were higher in not cured patients (median 218 and 1326 pg/ml, respectively) compared to cured ones (20 and 178 pg/ml, respectively). Interestingly, presurgical bCT lower than 28.25 pg/ml significantly identified CCH and MTC who will be cured, with a high specificity (93.3%) and good sensitivity (76.2%). Conversely, values of presurgical bCT higher than 12.60 pg/ml and of presurgical sCT higher than 134.0 pg/ml, correlated with the presence of MTC foci with high sensitivity (95.0% and 94.40%, respectively) and good specificity (75.0% and 61.40%, respectively).

**Conclusions**

In a large cohort of consecutive GCs who were submitted to thyroid surgery, presurgical bCT <28.25 pg/ml identified, with high specificity and good

sensitivity, CCH or small MTC that will be cured with thyroidectomy. When presurgical bCT is  $> 12.6$  pg/ml, some cases of MTC may be present but, if the bCT is still  $< 28.25$  pg/ml, they also will be safely cured.

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### PS3-15-141

#### What do different echogenic micro-foci in papillary thyroid carcinoma nodules and metastatic lymph nodes represent in histopathology?

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#### Objective

Ultrasonographic (US) echogenic micro-foci are frequently seen in papillary thyroid carcinoma (PTC). General belief is that microcalcifications represent psammoma bodies. But the subject is debatable and other sonographic intranodular echogenic figures can be seen in PTC. We aimed to examine the nonshadowing echogenic figures and their histopathological correlations in PTC.

#### Methods

We prospectively collected US video records of PTC cases with echogenic micro-foci or metastatic lymph nodes (MLN) and malignant nodules without echogenic foci between 2018 and 2021 in two centers. All video recordings were independently interpreted by three experienced sonographers. Non-shadowing echogenic micro-foci were classified as; microcalcification (punctate echogenic foci  $< 1$  mm), linear ( $> 2$  mm), comet-tail, coarse echogenic foci and unclassifiable. Histopathological evaluation was performed by two experienced pathologists. Data were evaluated by an investigator who is blind to these results.

#### Results

92 nodules and 12 metastatic lymph nodes out of 160 patients, agreed on the type of echogenic foci by at least two of three sonographers, was included in the statistics. While 72 of 92 malignant nodules had any kind of echogenic micro-foci (group 1), 22 did not (group 2). According to histopathological evaluation, number of psammoma bodies, coarse stromal calcifications and papillae are significantly higher in group 1 than in group 2 [(76% vs 5%,  $P < 0.001$ ), (38.6% vs 4.5%,  $P = 0.002$ ), (87% vs 50%  $P = 0.001$ )]. Same parameters were significantly higher in nodules with microcalcifications than in nodules without echogenic foci [(85% vs 4.5%,  $P < 0.001$ ), (29% vs 4.5%,  $P = 0.024$ ), (85% vs 50%,  $P = 0.003$ )]. Coarse stromal calcifications and papillae are significantly higher in nodules with linear echogenic micro-foci than in nodules without echogenic foci [(57% vs 4.5%,  $P = 0.007$ ), (100% vs 50%,  $P = 0.026$ )]. Most common histopathological findings in MLN with echogenic foci were papillae (92%), psammoma (83%) and cystic areas (83%), respectively.

#### Conclusions

Punctate echogenic foci in PTC nodules indicate the presence of psammoma in pathology. Linear echogenic micro-foci are mostly associated with coarse stromal calcifications and papillae. Echogenic foci in metastatic lymph nodes may be associated with microcystic areas as well as psammoma and papillae.

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