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Investigations in functional thyroid diseases: Autoimmune activity of endocrine orbitopathy detected by imaging technique and the role of thyroid function in the treatment of lipid disorders

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1. Introduction

The autoimmune thyroid diseases (AITD) are typical representatives of organ-specific autoimmune syndromes. The development of AITD occur as consequences of multifactorial causes. Genetical predisposition, endocrine factors and environmental trigger-mechanisms together lead to the loss of immune tolerance. According to our present knowledge we are not able to predict who will be affected by AITD. The most important AITDs are Graves' disease (GD) and Hashimoto's thyreoiditis (HT), both of which are characterized by the lymphocyte infiltration pattern of the thyroid parenchyma. The clinical appearance is due to the disturbed or disregulated hormone-producing function of the thyroid tissue: thyrotoxicosis (GD) and hypothyroidism (HT).

The Graves' disease is the most common cause of thyrotoxicosis, characterized by 5:1 female dominance. GD can occur in any age, however, the incidence is the highest between the second and fourth decade. The constant presence of thyroid receptor antibodies (TRAb) against the thyroid hormone receptors (TSHR) will lead to hyperthyroidism with elevated free thyroxine (fT4) and triiodothyronine (fT3) along with suppressed TSH. The overall result of the above changes will lead to clinical signs as tachycardia, tremor, weight loss, anxiousness and goiter.

In the process of GD treatment reaching euthyreoidism is principal, which can be done by administering antithyroid drugs (methimazol, propylthiouracil or carbimazol), ^{131}I radioiodine treatment or performing thyroidectomy.

Graves' orbitopathy (GO) is the most common and the most important extrathyreoideal manifestation of Graves' disease, which is present in 25-50% of GD patients. In the development of GO multiple factors are contributing, namely genetic predisposition, gender, age, and environmental factors like smoking. Other underlying factors are subsequent of the treatment of GD, hypo- or hyperthyroidism, the effects of radioiodine treatment and the presence of TRAb. The development of GO may develop earlier, coincide with or appear after the onset of hyperthyroidism. GD can be present without eye symptoms or can be accompanied with a mild or severe form of GO. In 3-5% of all cases a sight-threatening form of orbitopathy may occur.

The most common complaints of GD patients affected by GO are eye dryness, eye burning sensation, light sensitivity, enhanced tear production, double vision or retrobulbar pain. The most common sings are retraction of the upper eye lid, edema and erythema of the periorbital tissue and conjunctiva and the appearance of proptosis.

The sight-threatening severe form are accompanied by massive pain and inflammatory signs. The presence of cornear ulceration and optical nerve compression may even lead to the loss of sight.

The characteristic findings of GO are consequences of complex cellular and molecular mechanisms which due to their interactions and the edema of the eye muscle with enhancement of the orbital tissue lead to elevated intraorbital pressure, direct physical effects and trauma. These changes together form the background of GO.

A critical step in the development of GO is the T-lymphocyte mediated autoreactive response against the thyroidal and orbital antigens (presumably thyroid-stimulatory hormone receptor, TSHR and insulin-like growth factor 1, IGF-1) expressed by the orbital fibroblasts and adipocytes.

According to our present knowledge the autoimmune reaction against the TSHR represents a trigger mechanism in the development of GO, while the response against the IGF-1 contributes to the maintenance of the autoimmune process. Due to cytokine action the orbital fibroblasts are secreting high amounts of hyaluronic acid and will differenciate into adipocytes with elevated TSHR on their surface.

During the natural course of the disease the introductory inflammation (active phase) is followed by the stability phase (plateau) and finally by remission (burned-out or inactive phase). Therefore the accurate determination of the severity and activity of the disease is crucial for establishing the optimal modality and time of treatment.

The establishing of clinical activity and severity in GO can be done using CAS or imaging methods. The NOSPECS criteria gives precise information about the severity of the disease, however, it is inaccurate in establishing the presence of autoimmune activity. The standard procedure to define the activity of GO in the everyday clinical work is the clinical acitivity score (CAS) method. CAS is established using 7 parameters related to the inflammation (pain during eye movements, retrobulbar pressure, conjunctival hyperemia, eyelid hyperemia, eyelid edema, caruncula swelling, chemosis, proptosis, vision impairment.) A score of 0 (no activity) to 7 (maximal activity) describes the severity of the underlying autoimmune disease which is calculated adding 1-1 point according to their presence or worsening compared to the initial status.

In case of CAS $\geq$3 GO is considered to be active and a higher CAS value is predictive of a good therapeutic answer to glucocorticoid therapy. For the establishment of the orbital activity the CAS alone is not enough adequate, confirmed by the results of the first investigation on its clinical usefulness, which has not confirmed that all patients with CAS<3 had inactive GO, in contrary, in a significant number of patients the used immunosuppressive therapy led to therapeutic response.

The currently available laboratory assays for determining the thyroid-receptor antibodies (TRAb) were not predictive in the evaluation of the later appearance of GO according to the terapeutic response to the immunsupressive treatment.

Out of the conventional imaging methods the ultrasound of the eyes (US), computed tomography (CT) and magnetic resonance (MRI) investigations are useful for evaluating GO. US gives information about the diameter and reflectability of the eye muscles, however, the retrobulbar area can not be examined with this method. The CT investigation for the follow-up of the orbits is not useful because of the significant radiation exposure, besides, it gives little information about the edema of the eye muscles, and as a result, about the activity of the ongoing inflammation. The MRI is useful for the establishment of the edema of the eye muscles in addition to the morphological information obtained.

Considering the nuclear medicine methods, the ^{111}Indium-labelled somatostatin analogue penreotide binds to the lymphocyte surface somatostatin receptors and is accumulated on the site of inflammation both in the thyroid and the orbits.

The ^{111}Indium-labelled penreotide SPECT investigation was found to be a sensitive method with positive predictive value during the selection of patients to give a good response to immunsupressive therapy, and also for further monitoring of the treatment.

Our previous studies proved that ^{99m}Technecium-dietilenetriaminpentaacetate acid (DTPA) single-photon emission computer tomography (SPECT) is a sensitive method for the evaluation of GO immune activity, the method's sensitivity is comparable to that of octreotide SPECT method.

The DTPA injected into the periferal venous system can detect any inflammation site in the body, as the substance penetrates through the damaged vascular wall and binds to the polypeptides of extracellular fluid and so the site of inflammation can be detected due to it's enhanced vascularization. As a result with this simple method both qualitative and quantitative information about the GO activity can be obtained. The orbital DTPA uptake is predictive for the immunsupressive therapy responsiveness.

The treatment of Graves' orbitopathy is a mutidisciplinary task that involves the constant cooperation of endocrinologist, radiologist, ophthalmologist and nuclear medicine specialists. The thyroid function has to be adjusted within the euthyroid range without even transitory hypothyroidism. Besides, cessation of smoking is indispensable. However, the treatment of GO continues to be challenging, as the most common treatment modalities used in the active phase of the disease (glucocorticoids, orbital irradiation) in most of the cases do not grant full recovery.

Lately several new molecules, potential future drugs were discovered and tested in vitro on thyrocyte and orbital fibroblast cell cultures for the treatment of GD and GO acting on the immune modulation pathway. Among TNF-inhibitors the effect of rituximab was evaluated during several studies involving moderately severe and severe, immunologically active GO patients and was found to be efficient as first line therapy or after insufficient results with previous glucocorticoid administration. In the inactive phase of GO ophthalmologic surgeries can be performed for rehabilitation purposes to diminish the residual symptoms (decompression surgery for the correction of exophthalmos, surgery of the eye muscles for treating strabism, the accompanying diplopia and eye lid correction).

The most common cause of primary hypothyroidism is Hashimoto's thyreoditis, which is also the most frequent autoimmune disorder affecting 5-10% of the population. It is 5-10 times more common in women and it's prevalence increases with age, the most patients are diagnosed between 45-65 years. In Hashimoto's thyroiditis the subclinical or overt hypothyroidism occurs due to the lymphocytic and IgG4-positive plasma cell infiltration and consequential fibrosis of the thyroid gland tissue. The symptoms of hypothyroidims are diverse, among the non-specific features are the impaired stroke volume and bradycardia followed by decreased cardiac output. Due to the increased periferal vascular resistency about 20% of patients will suffer from hypertension, which, together with the common dyslipidaemia will increase the risk of atherosclerosis.

The dyslipidemia involves elevated total and LDL-cholesterol levels due to the decreased number of cell-surface LDL-receptors and consequential LDL-cholesterol clearance impairement. The levels of Lp(a), apolipoprotein-B, homocystein and C-reactive protein (CRP) can increase. As a consequence of the increased cardiovascular risk the correction of both dyslipidemia and hypothyroidism is of particular importance, and also the evaluation of the safety of the most commonly used statins in the treatment of dyslipidemia is crucial.

Data from large scale epidemiologic studies showed positive correlation between TSH and dyslipidemia, the common subclinical hypothyroidism (elevated TSH levels with free hormone levels in the normal range) was characterized as transitorial impairment.

Worldwide cardiovascular diseases are considered the most important causes of mortality. Treating atherosclerosis as one of the most important etiological factor the cardiovascular risk can be reduced. Statins are diminishing hyperlipidemia by inhibiting the 3-hydroxi-metylglutaryl-coenzim A reductase (HMG-CoA) enzyme and have an important role in the prevention of cardiovascular diseases, however, their expected clinical benefit is not achieved due to their known side-effect profile.

In case of patients suffering from both hypothyroidism caused by autoimmune thyroid disorder and simultaneous dyslipidemia the evaluation of the relationship between the side-effect profile of statins and the thyroid function is essential. Hypothyroidism is often accompanied by complaints of myopathy which can be also among the first symptoms, may be present with proximal muscle weakness and muscle-related serum enzyme elevation together with polymyositis-like syndromes. A known positive correlation exists in overt and subclinical hypothyroidism between the serum creatinine-kinase (CK) and thyroid stimulating hormone (TSH) levels. In case of statin therapy with concomitant hypothyroidism (which alone can cause muscle involvement) the risk of developing myopathy is increased. The symptoms of myopathy caused by statins and hypothyroidism are similar, therefore laboratory measurement of TSH is advisable before starting statin therapy to exclude the presence of unknown AITD. Also, it is recommended in case of all statin-treated patients presenting statin-induced side-effects. Beside hypothyroidism hyperthyroidism can also cause myopathies in the proximal and distal skeletal muscles.

In patients receiving medical treatment for simultaneous presence of dyslipidaemia and hypothyroidism the evaluation of drug metabolism and drug interactions to avoid statin-induced side-effects (hepatopathy, myopathy, gastrointestinal side-effects) is important.

Beside gender, age, co-morbidities and eating habits a person's drug metabolism is influenced by other concurrent drug administrations as well. It is well known that interference in the activity of drug enzymes and transporters involved in drug metabolism leads to drug interactions. In case of blocking these agents the levels of drugs metabolized with their help rises, while in case of enzyme induction the amount of drug molecules and metabolites decreases. As a result the serum levels and therapeutic effects of concomitantly used drugs differs from the levels during sole usage.

These days the increasingly robust investigations and clinical trials on health outcome are drawing a particular attention to the co-morbid, multimormid patient population affected by chronic diseases, aware that their multiple drug adminstration enhances the risk of drug interactions. The majority of phase I reactions occur on the cytochrome P450 (CYP450) enzyme superfamily pathway. The induction or inhibition of different CYP metabolizing subunits by food, drugs and inflammatory factors can seriously influence the farmacological and toxicological effects of the formed substrates (xenobiotics and drugs).

The primary metabolism of the majority of pharmacological agents occur with the help of six members of the P450 enzyme superfamily: CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6 és CYP2E1 (142). Cytochrom P450 2D6 (CYP2D6) is responsible for the biodegradation of more than 25% of commonly used drugs, while CYP3A4 meabolizes more than half of medications. As a result CYP3A4 and CYP2D6 together are responsible for the occuring drug interactions and unexpected side

effects. The antilipid agents simvastatin, atorvastatin and lovastatin are metabolized on CYP3A4, fluvastatin on CYP2C9, while rosuvastatin on CYP2C9 and 2C19. The metabolism of levothyroxine occurs with the help of CYP3A4 isoform, as a result the risk of developing statin-induced side-effects increases in case of concomitantly administered thyroid hormone substitution and lipid lowering therapy.

2. Objectives

2.a Evaluating autoimmune activity of newly diagnosed Graves' disease patients, whom are not affected of Graves' orbitopathy at the time of presentation with DTPA-SPECT method

- *Evaluation of the autoimmune activity using DTPA-SPECT method in newly diagnosed patients with Graves' disease without orbitopathy*

- *Evaluation of immune activity detectable via DTPA SPECT in Graves' disease patients whom are not affected by Graves' orbitopathy during follow-up*

2.b Testing the relation between correction of hypothyroidism and statin treatment

- *Evaluation of the ratio of patients with thyroid dysfunction and euthyreoid patients among hyperlipidemic patients referred because statin induced adverse effects*

- *Evaluation the association between the occurence of statin-induced advers effect and concomitant medication.*

- *Evaluation the association between the occurence of statin-induced advers effect and thyroid dysfunction.*

3. Patients and methods

Our studies were conducted among patients referred to the Endocrine and the Metabolism Departments of Internal Medicine Institute of Clinical Center of University of Debrecen. All participating patients signed the informed consent and the study got the approval of the Ethics Committee of the University of Debrecen.

3.a Evaluation of the autoimmune activity using DTPA-SPECT method in newly diagnosed patients with Graves' disease without orbitopathy

In our study evaluating the predictibility of the development of GO in the course of GD we included 27 patients from our endocrinology outpatient clinic. Our patient group included 23 women and 4 men, their mean age was 38.6 ± 10.4 years, their demographic charactheristic are concordant with the population's 5:1 female dominance and the typical age occurance of 20-40 years. To get certain that GO was not present at the time of enrollment, each patient had to fulfill the following 3 criteria: no signs and symptoms of GO were detected at the first check-up at the endocrinology clinic, and GO signs based on the NOSPECS classification were not present (NOSPECS ≤2a, 3 0, 4 0, 5 0, 6 0) and CAS did not exceed 2. Patients earlier treated because of hyperthyroidism in medical history or using ophthalmic measures before or at the time of diagnosis were excluded from participation.

The treatment of our GD patients was in accordance with the clinical guidelines, the hyperthyroidism characteristics of GD were corrected by administering antithyroid drugs in 16 patients, radioiodine therapy was used in 3 patients and subtotal thyroidectomy in 8 patients. The follow-up period time was two years.

20 patients' DTPA-SPECT orbit-images were used as controls, who underwent the same imaging in the process of evaluating Raynaud-syndrome and had no thyroid or eye disease at the time of the intervention, their mean age was 41.4 ± 13.7 years.

^{99m}Tc-labelled diethylenetriamine pentaacetic acid SPECTs (DTPA SPECT) were performed within seven days after the first endocrine check-up after the recruitment, and also at the end of the follow-up period. In the case of detecting GO signs or symptoms during the follow-up period, an additional DTPA SPECT imaging was performed.

The patients' eye signs had been evaluated by the same ophthalmologist once in every three months, however the ophthalmologist was not aware of the results of the previously performed DTPA SPECT. An additional visit was inserted if the patient had GO symptoms or clinical signs of GO were detected by the endocrinologist.

According to the previously described and published DTPA-SPECT protocol 7 MBq/kg DTPA (PromptCarry, Szeged, Hungary) injection was used iv. Later the imaging tecnique was obtained by a 4-headed X-Ring (Mediso, Budapest, Hungary). During the procedure the sum of the 128 frames was obtained. After the correction of absorption was performed, a 30^{o} orientation to the base of skull was set. Sagittal and coronal sets of slides were reconstructed encompassing the whole orbit.

Both right and left orbits were considered by outlining the areas of interest in order to obtain a qunatified bulbar DTPA accumulation.

The computerized results were obtained by summing the axial slice results that contained the whole section of the orbits. The SPECT's sensitivity was calibrated before the procedure in order to obtain a quantitative evaluation of DTPA uptake.

To obtain propper correction, the difference between the activity of the radiofarmacon before and after the intravenous administration was calculated. The final DTPA SPECT uptake was calculated upon the amount of the intravenous dose that was accumulated in the preselected orbital area and recorded as „units of volume".

For statistical analysis the IBM Statistical Package for the Social Sciences (SPSS) program was used. For testing the normal distirbution we used the Kolmogorov-Smirnov test. The differentiation of the analyzed groups the Newman-Keuls test was used with ANOVA statistical program. We considered a p value of < 0.05 as statistically significant The obtained results were registered as mean ± standard deviation (SD).

3.b Testing the relation between correction of hypothyroidism and statin treatment

A total of 101 patients with hyperlipidemia (age 61.3±9.9 years) with adverse reactions due to statin treatment pariticipated, all treated at outpatient clinics of the Department of Medicine, University of Debrecen. The patients enrolled in this study were treated with simvastatin, atorvastatin, fluvastatin, rosuvastatin or pravastatin. In 56 cases statin-induced myopathy (myalgia with or without creatinine-kinase elevation), in 39 cases statin-induced liver enzyme elevation (hepatopathy) and in 24 cases statin-induced severe gastrointestinal symptoms were observed, in additional 18 cases patients were present with the combination of two types of adverse effects. Myopathy was detected in 47% of cases, hepatopathy occurred in 26%, gastrointestinal symptoms in 9%, and the combination of the previous types in a total of 18 %. Combination of hepatopathy+GI adverse effect was observed in 10 %, myopathy and GI adverse effect in 5%, myopathy and hepatopathy in 3 % of all cases had been observed.

Patients with concomitant alcohol or drug dependence, malignant diseases, pregnancy or lactation, as well as patients receiving anticoagulant therapy were excluded.

At baseline after a 12 hours fasting time, a 10 ml venous blood sample was obtbained in the morning between 07:30 and 08:00. Lipid markers, serum cholesterol (LDL-cholesterol and HDL-C) and triglyceride levels were registered. Apolipoprotein (ApoA and ApoB) examination was also

performed. Creatinine-kinase activities and C-reactive protein (CRP) levels were determined. We investigated markers of thyroid function, free thyroid hormones (triiodothyronine (T3) and thyroxine (T4) and thyroid stimulating hormone (TSH).

Concomitant drug intake was evaluated and recorded in every single case. Cytochrome P450 metabolism was evaluated using local database. Statistical analysis was performed via Windows 7 and IBM Statistical Package for the Social Sciences (SPSS). Normal distribution was assessed by the usage of Kolmogorov-Smirnov test. The differences between the groups were also analyzed with ANOVA computer progrram and „t" test. A p value of < 0.05 was considered as statistically significant. All obtained results were then expressed as mean ± standard deviation (SD).

4. Results

4.a Evaluation of the autoimmune activity using DTPA-SPECT method in newly diagnosed patients with Graves' disease without orbitopathy

All of the enrolled 27 patients participated in the study during the 2 year-long follow-up, of whom in 6 cases (22% of all cases) Graves' orbitopathy occurred during the follow-up. We couldn't detect significant difference between initial free thyroid hormone levels and TRAb levels indicating the immune activity of GD among patients later developing GO and patients unaffected by GO at the and of the follow-up.

In our 6 patients who later developed Graves' orbitopathy the mean initial DTPA-uptake was 10,45±1,72 MBq/cm^3 (mean±SD) in their 12 orbits. The difference between this result and the mean initial DTPA-uptake of the 21 patients (42 orbits) whom were not diagnosed with GO using the traditional methods during the 2 year long follow-up period (9,18±1,18 MBq/cm^3, mean±SD), was not statistically significant.

Both GD patient group had elevated initial DTPA uptake results compared to the controls initial DTPA uptakes of 7.72±2.44 MBq/cm^3 (mean±SD.) This difference was statistically significant compared to patients unaffected by GO during the two year-long follow-up ($p=0.011$), and in patients diagnosed with GO ($p=0.031$.)

At the end of our study patients who didn't develop GO during the follow-up underwent a final DTPA imaging, their mean DTPA uptake was 9.63±3.30 MBq/cm^3 (mean±SD.) This obtained data was statistically different ($p=0,001$) compared to the mean uptakes of the patients diagnosed during the

follow-up with GO, whom underwent their second DTPA imaging at the time of GO establishment. The latter patient group's mean DTPA uptake was 12,1±4,41 MBq/cm^3 (mean±SD) at the time of diagnosis of GO.

4.b Testing the relation between correction of hypothyroidism and statin treatment

We found thyroid hormone levels outside the normal range in five patients (4.95%) in our investigation; manifest hypothyroidism had been established in two and hyperthyroidism in three cases. Eleven patients with no functional thyroid abnormality had a history of hypothyroidism (10.9%). Among the patients currently having thyroid dysfunction, myopathy occurred in one patient with hypothyroidism and in two patients diagnosed with hyperthyroidism. We did not find significant differences in the functional thyroid markers TSH, fT4 and fT3 among patients with statin-induced myopathies nor those patients who presented other adverse reactions.

In our study 77.2% out of the total of78 patients were treated with drugs that ondergo first-pass metabolism on CYP isoforms biotranformating statins (Table 3), mainly anti-hypertensive and anti-glycemic agents. The co-administered drugs metabolized by CYP3A4 were found in 66 patients (65.3%), by CYP2C9 in 67 cases (66.3%) and by 2C19 in 54 patients (53.5%). Patients who developed myopathies were on more medications that are metabolized by the cytochrome 3A4 subunit compared to those who presented other adverse reactions ($p<0.05$).

We found more patients with myopathies among those subjects who were taking simvastatin as lipid lowering agent (52% vs. 38%, ns.). At the same time in the subgroup of patients treated with fluvastatin were significantly less number of myopathy cases (13% vs. 33%, $p<0.05$) in comparison to the group which presented other adverse reactions due to statin treatment.

5. Discussion

Autoimmune thyroid diseases, Hashimoto-thyreoiditis, Graves' disease and its most important complication, Graves' orbitopathy are syndromes of autoimmune origin. GO developes in 25-50% of GD patients due to genetic predisposition, environmental factors, and effects of GD treatment. Graves' orbitopathy is characterized by the enlargement of orbital muscles and indrease of orbital connective tissue. In the process of orbitopathy development dysregulation of orbital fibroblasts consequently autoimmune mechanism is a key momentum, followed by fibroblast proliferation, excess of adipose tissue and oedema and swelling of orbital muscles. These changes lead to the development and presence of clinical symptoms as exophthalmos, connective tissue enlargement, diplopia, the pathomechanism of

which is still not entirely known. Altough our knowledge of humoral and cellular mechanisms of Graves' orbitopathy has been constantly growing in the latest years, the course of GO is still not clearly described. Predicting the development and the severity of a later developing GO is a challenging task for the endocrinologist.

In moderate to severe cases of GO local ophthalmological treatments and immunsupressive treatment is needed to be administered in the active phase of the disease (oral or intravenous corticosteroids or retrobulbar irradiation or the combination of the previous, for non-responsive patients rituximab treatment is available.) Immunsupressive treatment is ineffective in the inactive phase of GO, in severe inactive cases corrective rehabilitative surgical treatment is needed.

Clinical Activity Score (CAS) is a simple and widely used tool in evaluating the activity of autoimmune disease and predicting the benefits of immunsupressive treatment. NOSPECS gives precise information about the severity of the orbitopathy but carries no information about autoimmune activity, consequently it supports clinical decision making poorly. Imaging techniques play an important role in evaluating GO. Ultrasound of the eyes has an important role in evaluating the thickness and edema of eye muscles, but it's not suitable for controlling the changes of the retrobulbar space. During MRI on T2-weighted images information on immune activity can be gained detecting eye muscle edema. Among nuclear medicine imaging methods DTPA is an useful tool in detecting immune activity of the orbits and the response to immunsupressive therapy.

In our study we tried to predict in patients newly diagnosed with GD without the signs of GO the development of GO with an initially elevated DTPA uptake during DTPA imaging. We ought to prove the presence of subclinical orbital immune activity in GD patients without clinical signs and symptoms of GO.

Among our findings we detected a moderately elevated initial DTPA uptake irrespectively of the later development of GO. During the 24 months of follow-up period in 22% of the patients GO developed, the proportion of GO occurrence was similar to the ratio described in the literature. Altough DTPA failed to predict the development of GO in our newly diagnosed GD patients, who underwent CAS and NOSPECS evaluation at the beginning of the study which established the patients were not affected by GO at the time of diagnosis, we could detect a moderately elevated (subclinical) immune activity irrespective of the later development of GO. In our previous work we could find similar changes in the constitution of tear cytokins among patients with GD and GO, which indicates a uniformly present subclinical involvement of the orbits. However, our results are consonant with an observation confirmed decades ago by a study group evaluating the activity of immune process in the orbits via MRI method. In this study 71 % of untreated GD patients without the signs of GO showed the enlargement of eye muscles.

Consequently, in the early phase of GD there is a detectable immune activity in every patients' orbits, which leads to a progression into clinical orbitopathy in only 25-50% of the cases.

In our study in the case of 21 patients, who didn't develop GO during the 2 years long follow-up the means of initial DTPA uptakes didn't differ significantly from their final DTPA uptakes. In patients who were diagnosed with GO in the follow-up period, DTPA uptake of the orbits were significantly higher at the time of GO establishment compared to the initial values.

Earlier clinical studies did not provide definite data about the development of autoimmune activity in the retrobulbar space in patients newly diagnosed with GD without GO. During our work we could detect a moderate but uniformly present orbital involvement in every GD patient at the time of diagnosis, independently of the later development of GO. However, the question, why GO remains subclinical in the majority of the patients and why subclinical GO progresses into clinically active GO with obvious clinical findings remains unanswered.

The inhibitors of the 3-hydroxi-metylglutaryl-coenzim A reductase (HMG-CoA) (statins) are widely used drugs, however, their safety is affected considerably negatively by their interactions with other drugs.

Thyroid dysorders have an effect on lipid metabolism. In overt hypothyroidism the number of LDL-receptors in the liver decreases, which results in diminishing LDL-cholesterol clearance, elevated total and LDL-cholesterol levels, and also higher apolipoprotein-B levels. Besides the earlier mentioned changes high density lipoprotein (HDL) level is normal or elevated due to the decreased activity of hepatic lipase and cholesterin-esther transferase, which are regulated by the thyroid hormones. Levothyroxin substitution improves hyperlipidemia in a dose which supresses the release of thyreotropine.

During our one year long follow-up we could detect thyroid dysfunction in 5 patients (4.95%) evaluated because of statin-induced adverse effect. In 2 cases hypothyroidism, in 3 cases hyperthyroidism was detected, which corresponds with data from literature about the incidence of thyroid dysorders in normal population well. In 11 cases (10.9%) earlier permanent hypothyroidism was established from medical history. Myopathy was observed in 1 patient with hypothyroidism and in 2 patients with hyperthyroidism. We couldn't detect statistical difference between the serum levels of TSH, fT3 or fT4 of patients affected by statin-induced adverse effect or other adverse effects.

The 77.2 % of our patients were taking drugs concomittantly metabolized with the help of the same CYP isoforms as statins (3A4: 66 patients (65.3%); 2C9: 67 patients (66.3%); 2C19: 54 patients (53.5%)). Among subjects affected by myopathy more patients received drugs that undergo first-pass

metabolism on the CYP3A4 isoform than in case of patients with other types of adverse effects, the difference was statistically significant ($p<0.05$.) In patients recieving simvastatin treatment vs. other statins more myopathy cases had been observed (52% vs. 38%, ns.), presumably due to the interactions between statins and other drugs using CYP3A4. This phenomenon might be explained by the concomittant levothyroxin substitution, which is degraded by CYP 3A4 isoform, an elevated risk for the development of statin-induced adverse effect might be present in the case of statin therapy using CYP 3A4 and levothyroxin substitution administered at the same time.

Besides, we found significantly decreased number of patients who presented myopathies on fluvastatin treatment when comparing to the subgroup of treated individuals having other types of statin induced adverse effect (13% vs. 33%, $p<0.05$).

The presence of thyroid dysfunction with concomittant medication with drugs metabolized on CYP3A, 2C9 or 2C19 pathways is frequent among hyperlipidemic patients evaluated because of statin-induced adverse effects. The development of statin-induced adverse effect might be diminished after correcting thyroid hormone levels and also by administering concomittant medication with precaution with respect of drug metabolism on CYP isoforms.

6. Summary

Among the 27 patients participating in the study six (22%) developed Graves' orbitopathy. There were no significant differences between patients who later presented signs of GO and who did remain free of orbitopathy, when considering the free hormone and TRAb levels assesed at the beginning of the study.

We detected significantly higher DTPA-uptake results in both groups, who did and who did not develop GO during the two-years follow-up compared to the controls.

We confirmed for the first time that in untreated patients newly diagnosed with Graves' disease and hyperthyroidism without Graves' orbitopathy by Clinical Activity Score a subclinical orbital involvement exists, detected by ^{99m}Technecium-dietilenetriaminpentaacetate acid (DTPA) single-photon emission computer tomography (SPECT), irrespective of the later development of orbitopathy.

Altough our knowledge regarding the important cellular and humoral mechanisms leading to the development of Graves' orbitopathy is constantly growing, the exact underlying process is still poorly understood. With the use of our presently available diagnostic methods we can not predict the development of the clinical signs of orbitopathy.

In our study we couldn't predict the development of orbitopathy on the basis of initial DTPA-uptake; the difference between the DTPA-values obtained at the time of diagnosis of Graves' disease of the patient groups with and without orbitopathy was not statistically significant; however the values differed numerically. Our hypothesis ought to be tested on a greater patient group.

Among our 101 hyperlipidemic patients examined because of statin-induced adverse effect we detected the presence of thyroid dysfunction in 5 patients (4.95%), in 2 cases hypothyroidism in 3 cases hyperthyroidism occured, which corresponds well to the incidence of thyroid dysorders in the normal population.

In 11 cases (10.9%) autoimmune hypothyroidism was present in the medical hystory, which is higher than the prevalence in the normal population (2.8-4.5%). The explanation of this discrepancy might be the concomittant levothyroxin substitution, which is metabolized by CYP 3A4 isoform, leading to an elevated risk of statin-induced adverse effects.

In our study 77.2% of our patients with statin-induced adverse effects were taking concomittant medical treatment metabolized on the same CYP isoform as statins (3A4: 66 patients (65,3%); 2C9: 67 patients (66,3%); 2C19: 54 patients (53,5%)). Among the patients with statin-induced myopathy the

number of concomittantly taken drugs using CYP3A4 was significantly higher than in patient groups with other kinds of adverse effects ($p<0.05$). The highest number of myopathy cases occured among patients on simvastatin treatment, a potential explanation might be the competition among statins and other drugs using CYP3A4. The least myopathy cases had taken place among patients taking fluvastatin, metabolized by the CYP2C19 isoform.

According to our results a particular precaution is needed when starting statin therapy in polymorbid patients who have thyroid dysfunction and hyperlipidemia. The chance to develop statin-induced myopathy is particularly high when drugs metabolized on the CYP3A4 isoform are concomittantly administered.

7. List of publications validated by University and National Library, University of Debrecen

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Registry number: DEENK/94/2018.PL
Subject: PhD Publikációs Lista

Candidate: Eszter Berta
Neptun ID: EFJZMU
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. Berta, E., Bodor, M., Galuska, L., Paragh, G., Erdei, A., Gazdag, A., Ujhelyi, B., Berényi, E., Katkó, M., Gazsó, A., Nagy, E. V.: Early stage Graves' disease is uniformly accompanied by orbital immune activity even in patients who fail to develop orbithopathy during follow-up. Exp. Clin. Endocrinol. Diabet. [Epub ahead of print], 2018.
   DOI: http://dx.doi.org/10.1055/s-0043-125065
   IF: 1.685 (2016)
2. Berta, E., Harangi, M., Zsíros, N., Nagy, E. V., Paragh, G., Bodor, M.: Effect of thyroid hormone status and concomitant medication on statin induced adverse effects in hyperlipidemic patients.
   Pharmazie. 69, 420-423, 2014.
   IF: 1.052

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List of other publications

3. Erdei, A., Steiber, Z., Gazdag, A., Bodor, M., Berta, E., Szász, R., Szántó, A., Ujhelyi, B., Barna, S., Berényi, E., Nagy, E. V.: Az endokrin orbitopathia differenciáldiagnosztikája.
Orvosi Hetilap. 157 (8), 310-315, 2016.
IF: 0.349

4. Csanádiné Galgóczi, E., Jeney, F., Gazdag, A., Erdei, A., Katkó, M., M. Nagy, D., Ujhelyi, B., Steiber, Z., Győry, F., Berta, E., Nagy, E. V.: Cell density dependent stimulation of PAI-1 and hyaluronan synthesis by TGF-[béta] in orbital fibroblasts.
J. Endocrinol. 229 (2), 187-196, 2016.
DOI: http://dx.doi.org/10.1530/JOE-15-0524
IF: 4.706

5. Gazdag, A., Nagy, E. V., Erdei, A., Bodor, M., Berta, E., Szabó, Z., Jenei, Z.: Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer.
J. Endocrinol. Invest. 38 (2), 133-142, 2015.
IF: 1.994

6. Berta, E., Harangi, M., Zsíros, N., Nagy, E. V., Paragh, G., Bodor, M.: A gyógyszerkölcsönhatások és a kóros pajzsmirigyhormon-státusz vizsgálata sztatinnal kezelt hyperlipidaemiás betegekben.
Magyar Belorv. Arch. Suppl. 67, 406-411, 2014.

7. Molnár, Z., Berta, E., Benyó, M., Póka, R., Bazsáné Kassai, Z., Flaskó, T., Jakab, A., Bodor, M.: Fertility of testicular cancer patients after anticancer treatment: experience of 11 years.
Pharmazie. 69 (6), 437-441, 2014.
IF: 1.052

8. Erdei, A., Paragh, G., Kovács, P., Karányi, Z., Berényi, E., Galuska, L., Lenkey, Á., Szabados, L., Győry, F., Ujhelyi, B., Berta, A., Boda, J., Berta, E., Bodor, M., Gazdag, A., Nagy, E. V.: Rapid response to and long-term effectiveness of anti-CD20 antibody in conventional therapy resistant Graves' orbitopathy: a five-year follow-up study.
Autoimmunity. 47 (8), 548-555, 2014.
DOI: http://dx.doi.org/10.3109/08916934.2014.939266
IF: 2.714

9. Erdei, A., Gazdag, A., Bodor, M., Berta, E., Katkó, M., Ujhelyi, B., Steiber, Z., Győry, F., Urbancsek, H., Barna, S., Galuska, L., Nagy, E. V.: Új lehetőségek az endokrin orbitopathia kezelésében.
Orvosi Hetilap. 155 (33), 1295-1300, 2014.
DOI: http://dx.doi.org/10.1556/OH.2014.29963

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10. Berta, E., Erdei, A., Cseke, B., Gazdag, A., Paragh, G., Balla, J., Polgár, P., Nagy, E. V., Bodor, M.: Evaluation of the metabolic changes during hemodialysis by signal averaged ECG. Pharmazie. 67 (5), 380-383, 2012.
DOI: http://dx.doi.org/10.1691/ph.2012.1692
IF: 0.962

11. Berta, E., Sámson, L., Lenkey, Á., Erdei, A., Cseke, B., Jenei, K., Major, T., Jakab, A., Jenei, Z., Paragh, G., Nagy, E. V., Bodor, M.: Evaluation of the thyroid function of healthy pregnant women by five different hormone assays.
Pharmazie. 65 (6), 436-439, 2010.
DOI: http://dx.doi.org/10.1691/ph.2010.0030R
IF: 0.869

12. Bodor, M., Erdei, A., Balla, J., Berta, E., Paragh, G., Polgár, P., Nagy, E. V.: Krónikus hemodialízis programban lévő betegek jelátlagolt EKG-vizsgálata.
É-K. Magyarorsz. Orv. Szle. 6 (1-2), 26-31, 2006.

Total IF of journals (all publications): 15,383
Total IF of journals (publications related to the dissertation): 2,737

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

18 April, 2018

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8. Appendix

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