Project: Early detection of Graves' Orbitopathy development in patients with thyroid dysfunction and methods for assessing its activity and severity

Call: Junior Grant Application (team of researches <35 years)

Project description:

Introduction:

The Graves' Orbitopathy (GO), alternatively called an Endocrine Orbitopathy (EO), is a serious, progressive eye complication seen in patients with autoimmune thyroid disease. GO does not affect the eye bulb itself but soft tissue of orbit (extraocular muscles and surrounding orbital connective tissue). It develops mostly in patients with Graves' disease (GD), approximately 20% of patients with GD have symptoms of GO at diagnosis, however the eye disease could precede the thyroid affection. GO rarely accompanies also Hashimoto thyroiditis (HT) (Bartalena et al. 2016). Annual incidence of Graves's disease is established as 20-50 per 100 000, annual incidence of Graves's orbitopathy is 16 per 100 000 (Hiromatsu et al. 2014).

Common signs of GO are upper eyelid retraction, redness in the eyes or lids, eyelid swelling and bulging eyes (exophthalmos). The severe cases involve corneal necrosis and sigh-loss due to optic nerve compression. Pictures of affected patients can be found below (fig. 1). Disease phenotype is result of autoimmune process (production of antibodies against self-antigens such as TSH receptor (TSHR) and IGF-1 receptor (IGF-1R)), inflammatory infiltration, and accumulation of glycosaminoglycans (GAG) leading to edematous-infiltrative changes in periocular tissues. However, the GO pathogenesis is still not fully understood (Lacheta et al. 2019).

Early diagnosis and correct timing of immunosuppression therapy are critical steps in management of GO patients. The natural course of the disease is described as Rundle's curve a sample of this curve is depicted as (fig. 2) (Hiromatsu, Eguchi, Tani, Kasaoka and Teshima 2014). Unfortunately, the markers of early detection of GO are missing. Correct diagnosis is usually established by interdisciplinary cooperation of endocrinologist and ophthalmologist and in cases of uncertainty also the radiologist. Unfortunately, significant delays before a diagnosis are reported (Mitchell et al. 2015). In our study cohort we observed the time from symptoms of GO to treatment longer than a year (Schovanek et al. 2018). In order to decrease the interval in between the development of GO and first examination in specialized center or initiation of the treatment, specific early assessment tools were suggested. Those include an early warning questionnaire developed for use in the routine endocrine practice and pocket-sized card designated for patients (Mitchell, Goss, Mathiopoulou, Morris, Vaidya, Dickinson, Quinn, Dayan, McLaren, Hickey, Lazarus, Rose, Foley, MacEwen and Perros 2015; Mitchell et al. 2017).

In order to better evaluate progress of GO, automatic retinal oximetry method seem to be a promising clinical approach deserving future investigation. Retinal oxygen saturation measuring by automatic retinal oximetry (Oxymap ehf. Reykjavík, Iceland) is a new noninvasive method with high reproducibility for measurement of hemoglobin oxygen saturation SaO2 in retinal blood vessels using digital oxygen saturation imaging (Fig. 3). Currently, it is the only experimental method with vast potentials not only in the realm of the possibility of observing eye diseases (diabetic retinopathy, retinal vein occlusion or glaucoma) but also in developing interdisciplinary cooperation. Up to day, only one study investigated the effect of GO on retinal oxygen saturation and/or vessel diameter, and only in inactive patients (Yang et al. 2017).

Substantial changes in patients orbit and potentially also eyes accompany the active phase of GO. Ability to early and precisely diagnose extra-ocular muscles damage would allow better treatment

scheduling and also provide more reliable prognosis to our patients. Ultrasonography is the method of choice and it is highly recommended as the first imaging method. However, it is less accurate than MRI regarding measuring the thickness of the extra-ocular muscles. Magnetic resonance (MRI) is a precise tool for extraocular muscles examination providing information about muscle thickness and signal intensity. Advantage of MRI is its relative operator independency and low inter-reader variability in assessing the extraocular muscles. Thickening of the extraocular muscles in patients with EO has been well documented (Glatt 1996). MRI relaxometry is more precise method than visual assessment of the MRI signal intensity in determination of the extra-ocular muscles' involvement in patients with GO. This comparison could create a platform on which MRI examination should be recommended in patients with the suspected GO. Intravoxel incoherent motion (IVIM) introduced by Denis Le Bihan in the 1980s is a magnetic resonance method based on diffusion weighted imaging (DWI). Conventional DWI uses strong magnetic field gradients to achieve signal loss due to the Brownian motion. On contrary IVIM uses weak magnetic gradients to depict signal loss due to perfusion effect which allows the imaging of a microcirculation (Le Bihan 1990; Le Bihan et al. 1988; Le Bihan et al. 1986). IVIM has found many applications so far (Le Bihan 2019). It might be a promising method in a detection of the active form of GO.

The GO disease activity is assessed by the CAS score (Clinical Activity Score) and estimates the level of orbit's soft tissue inflammation. The disease severity is also evaluated either by NOSPECS of EUGOGO (newer) assessment score (Bartalena, Baldeschi, Boboridis, Eckstein, Kahaly, Marcocci, Perros, Salvi and Wiersinga 2016).

The typical course of GO in patients receiving no specific treatment, other than that to control thyroid dysfunction, was first described by Rundle, the curve is now known as Rundle's curve (fig. 2) (Wiersinga and Kahaly 2017). This curve could be used to show the two major features of GO, its activity and severity and their relationship to potential treatment strategies (Hiromatsu, Eguchi, Tani, Kasaoka and Teshima 2014).

The correct evaluation is the key point for timely and correct treatment because after the progression of disease into fibrotic phase, the immunosuppression therapy is not effective and patients develop irreversible changes. Corticosteroids are used in treatment of GO due to their anti-inflammatory and anti-edematous effect. However, there are limited therapeutic options available to individuals who respond inadequately to glucocorticoids, whose GO progresses despite glucocorticoids or who cannot tolerate glucocorticoids. Several other immunosuppressive treatment strategies were evaluated. Currently none of them is listed as preferred second choice in current guidelines (Bartalena, Baldeschi, Boboridis, Eckstein, Kahaly, Marcocci, Perros, Salvi and Wiersinga 2016). Our team and others reached satisfactory results with rituximab (monoclonal chimeric mice/human antibody) which targets the CD20 transmembrane protein expressed on the surface of B lymphocytes (Du Pasquier-Fediaevsky et al. 2019; Karasek et al. 2017). Widely discussed at top forums is adding Mycophenolate to corticosteroid treatment (MINGO study) (Kahaly et al. 2018). Recently a pharma company sponsored study was published using the insulin-like growth factor I receptor inhibitor teprotumumab (Douglas et al. 2020).

Precise knowledge of the disease progression based on combination of imaging and immunological parameters together with expected disease natural course will help us to better stratify patients and select the treatment approach i.e. glucocorticoid treatment, radiotherapy and thyroidectomy.

Project hypothesis

1) The early diagnosis and correct timing of therapy are critical steps in management of GO patients in order to prevent irreversible changes developed in fibrotic phase of GO. Currently used laboratory markers e.g. autoantibodies against thyroid stimulating hormone receptor (TSHR) are not helpful in

establishing an early and reliably diagnosis of GO, disease activity and the subsequent risk of its progression. The reliable immunological marker(s) that would predict risk of GO's development and progression is missing. We propose that immune and inflammatory characterization of serum and immune cells may nominate novel diagnostic and prognostic GO markers.

2) To better evaluate clinical activity and severity of GO, we propose to compare orbital ultrasonography with MRI relaxometry and automatic retinal oximetry. We expect that this comparison could better define the role of MRI examination in GO diagnosis and treatment. We also suggest the retinal oximetry method as a promising clinical approach to GO severity evaluation.

3) Precise knowledge of the disease progression based on combination of imaging and immunological parameters together with expected disease course may contribute to early diagnosis, better timing of treatment and evaluation of treatment response.

4) Currently, no early warning program for GO patients is established in the Czech Republic yet. We propose that establishment of early warning program for clinical endocrinologist and patients in the Czech Republic could significantly accelerate the diagnostic process.

Objectives

The aims of the proposed project:

Ad 1] To screen for candidate diagnostic and prognostic markers by advanced sensitive immune and inflammatory marker analysis. We will measure serum levels of panel of selected proteins associated with inflammation (such as cytokines, soluble receptors) by sensitive and specific methods. We will complement the proteomic analysis with immunophenotyping of peripheral blood immune cells by flow cytometry in order to investigate candidate diagnostic cell-surface immune markers.

Ad 2] To evaluate the benefits of both MRI tissue relaxometry and diffusion weighted sequences including a novel technique IVIM (intravoxel incoherent motion) in assessment of EO activity and compare the results of orbital ultrasonography. To test whether an investigation of a microcirculation of the extraocular muscles with IVIM MRI would yield an applicable data for distinguishing the active phase of GO from the non-active phase. Next, to evaluate the hemoglobin oxygen saturation (SaO2) in retinal blood vessels by (Oxymap T1) in patient with GO in active and non-active phase. Results correlation with disease activity and severity.

Ad 3] To study the association of laboratory, imaging and clinical parameters, and their combinations with the GO clinical course.

Ad 4] To develop and validate GO early warning program for clinical endocrinologist and patients.

Methodology

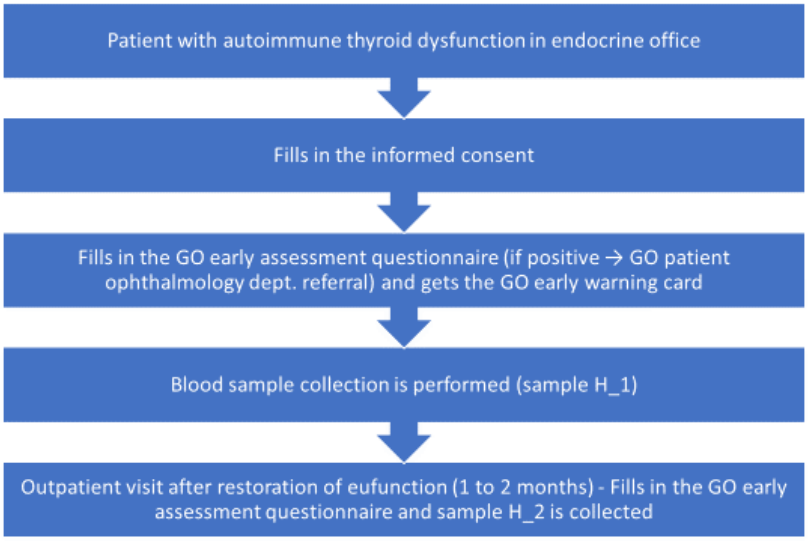
Subjects:

After the signing an informed consent the participants will be enrolled in the study. During the project total of approximately 150 participants will be enrolled. Exclusion criteria for participation in the study will be as followed: a) Hyperthyroidism other than primary autoimmune (e.g. thyroid tumor, biologic treatment). b) Previous orbital irradiation or surgery for GO. c) Decreasing visual acuity or a visual-field or color vision defect from optic nerve involvement within the previous 6 months. d) Glucocorticoid use (cumulative dose equivalent to ≥1 g of methylprednisolone for the treatment of thyroid eye disease). e) Previous treatment with rituximab or tocilizumab. Patients for this study will be enrolled either at the endocrinology outpatient clinic or at the ophthalmologic GO specialized outpatient clinic. The enrolment is further specified at the designated paragraph.

Clinical endocrine examination

At our endocrine outpatient clinic, we will enroll patients referred to us for diagnosis of primary hyperthyroidism of autoimmune origin. Most commonly patients with Graves' disease however we will not exclude patients with Hashitoxicosis. After signing the informed consent patient will receive the newly developed Czech version of GO early warning questionnaire and early warning pocket card. If they score positive for potential GO, they will be evaluated by ophthalmologist. The process is further described at Diagram 1.

Diagram 1: Clinical endocrine examination, H: blood sample collection of patient with autoimmune thyroid dysfunction.



Clinical ophthalmologist examination

At our GO outpatient clinic, we will enroll into the study patients who are referred usually by primary ophthalmologist or endocrinologist to confirm the diagnosis and initiate treatment. We also serve as tertiary center of excellence for patients with already established diagnosis but complicated course of the disease. After signing of informed consent, clinical assessment will include a full ophthalmic examination: visual acuity, intraocular pressure measurement, evaluation of the adnexa and eyelids, slit-lamp evaluation of the anterior segment, and fundus evaluation by indirect ophthalmoscopy. Eye motility will be also evaluated. Proptosis will be measured by Hertel exophthalmometer. All patients will be asked about symptoms. The Clinical Activity Score (CAS) and severity (EUGOGO) will be determined according to the clinical findings.

All patients will be examined with B-scan ultrasound. Hemoglobin oxygen saturation in retinal blood vessels (Oxymap T1) will be measured in all patients with active GO (CAS ≥ 3). Following check-ups will be planned according to the Diagram 2.

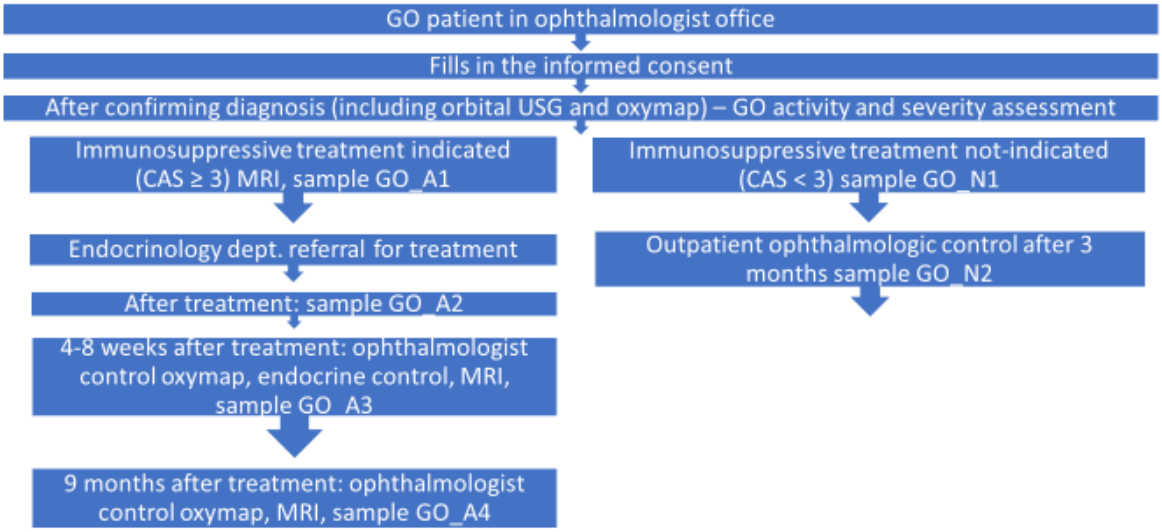
MRI examination

MRI examination will be performed in patients with an active form of the GO as well as in patients with an inactive or mild form of the disease. Initial MRI examination of patients with the active form of the EO will be performed in prior to a treatment initiation. MRI will be performed no later than 10 days from the ultrasound examination. Initial examination will provide a baseline data for future reference. Patients being considered as having the active form of the GO will undergo a follow-up examination three months after the end of the treatment which corresponds approximately to 6 months interval from the initial examination. Patients being considered as having the inactive or mild form will undergo a follow-up examination after the same period of 6 months. The second follow-up examination will be performed 12 months after the initial MRI in both groups. The assessment of the

extraocular muscles will be performed by a radiologist who will be blinded to patient status - activity of the disease.

Acquired data on muscle thickness and signal quality will be finally correlated with findings of other examination techniques. Results in both groups (active and inactive/mild form) will be compared to measurements acquired in healthy individuals from a pilot study data.

Diagram 2: Clinical ophthalmologist and radiologist examination, GO_A: blood sample collection of active GO patient; GO_N: blood sample collection of non-active GO patient



Laboratory parameters assessment

The screening for candidate diagnostic and prognostic GO markers will be performed by advanced sensitive immune and inflammatory marker analysis. We will measure serum levels of panel of selected proteins associated with inflammation (such as cytokines, soluble receptors) by Proximity Extension Assay (PEA; Olink Bioscience, Sweden) and ELISA. PEA is innovative, highly sensitive and specific technique based on utility of two non-identical antibodies against each analyte. The spectrum of molecules of interest will include mediators for which the association with GD in the specific populations has been already published, such as osteopontin, CCL11 and B-cell activating factor (BAFF) (Cheng et al. 2019; Cheng et al. 2020), CXCL9, CXCL10, CXCL11 and other inflammatory and profibrotic mediators. Our laboratory (Molecular Immunology, OLGEN, www.olgen.cz) has already expertise in PEA, ELISA (Dyskova et al. 2019; Mrazek et al. 2016; Petrackova et al. 2017; Schneiderova et al. 2017).

Next, we will complement the proteomic analysis with immunophenotyping of peripheral blood cells by flow cytometry in order to investigate associated cell-surface immune markers. Panel including major and minor immune cell subpopulations, their activation markers and candidate chemokine receptors (CXCR1-CXCR5, CCR4, CCR6 and CCR7) are already established in the lab. Our laboratory (Molecular Immunology, OLGEN) has expertise in flow cytometry and established suitable panels for analysis of immune cells in peripheral blood (Gabcova et al. 2019; Kriegova et al. 2018; Manukyan et al. 2020; Manukyan et al. 2017; Manukyan et al. 2018; Starostka et al. 2018). For candidate molecules, the immunogenetic analysis of functional polymorphisms, will be performed. We have expertise in MassArray and next-generation sequencing technologies (Kuba et al. 2020; Margaryan et al. 2020; Obr et al. 2018; Petrackova et al. 2020b; Petrackova et al. 2019).

Pilot data

Our group has previously published study focused on treatment of GO with focus on its side effects and we noted there had been the unnecessary long delay between the first symptoms of GO and

beginning of its treatment (Schovanek, Cibickova, Karhanova, Kovarova, Frysak and Karasek 2018). With this study we would like to decrease this time by establishing the early warning program in the Czech Republic.

Our pilot laboratory experiment showed a decrease in CXCL10 serum levels but not CCL2, IL-10 and IL-4 in three of the four patients when comparing serum levels at the treatment initiation and after the end of the corticoid pulse therapy. Next, we also detected a higher expression of CXCR3 (receptor for CXCL10) in B and T cell population in patient in active phase of GO when compared to patient in non-active phase.

Statistical analyses

Statistics will be performed using the statistical packages GenEx (MultiD, Sweden) and GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Nonparametric tests (Mann-Whitney, Kruskal-Wallis U-test, Friedman´s Two-way ANOVA by Ranks, as appropriate) will be used to compare protein expression data between groups. Spearman´s rank correlation will be used for assessment of a relationship between expression levels and clinical course and/or other parameters. P-values of less than 0.05 will be considered significant. The multivariate analyses (e.g. Principal Component Analysis, Self Organizing Maps, hierarchical clustering, patient similarity network) will be used for evaluation of candidate markers. Our groups (Molecular immunology, OLGEN) has expertise in advanced data mining analysis (Petrackova et al. 2020a; Petrackova, Minarik, Sedlarikova, Libigerova, Hamplova, Krhovska, Balcarkova, Pika, Papajik and Kriegova 2020b; Turcsanyi et al. 2019).

Timetable for the project

During the project total of approximately 150 participants (25-50-50-25/per year) will be investigated. We expect inclusion of larger number of patient (n = 100) with hyperthyroidism in the endocrine office who will be screened for signs of GO. Samples of those patients will be also used as negative controls to patients with symptoms of GO (n= 50). We plan to enroll smaller number of patients in the first and the last year, since at the beginning we need to prepare the above-mentioned early warning documents and at the end of the grant period we plan to focus on publishing results. Laboratory parameters will be measured regularly during the four years. Preliminary result will be published at domestic and international events during the whole duration of the project.

Table 1: Project timeline

Year																
Quarter of the year	x	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sampling of patients with hyperthyroidism and GO																
Collection of clinical data																
Serum protein analysis																
Flow cytometry analysis																
Establishment of early warning program																
Validation of early warning program																
MRI tissue relaxometry analysis																
Retinal oximetry analysis																
Immunogenetic analysis																
Results evaluation, validation and data analysis																
Data presentation on conferences																
Manuscript preparation and revision experiments																
Annual/final reports																

Expected results and relevance of the project

We expect that our results will improve today's clinical practice by introducing clinical and laboratory approach to better identify patients in risk of GO development and progression. The impact of the project can be seen especially in the direct improvement of the care of the patients and identification of those, who can benefit mostly from the immunosuppressive therapies in order to avoid severe complication of disease. The project would also bring a lot of new theoretical insights into the development of severe autoimmune damage and it will support the interdisciplinary research collaboration between endocrinologists, ophthalmologists, immunologists, and radiologists.

Expected outcome: 2 articles listed in database WOS, 2 articles listed in database Scopus and 6 abstracts.

Cooperation and readiness of the participating departments, research team:

The research team will consist of young researches (<35 years), who fulfill the requirements of the current call for junior grants. Faculty of Medicine and Dentistry of Palacky University Olomouc and Faculty Hospital Olomouc tightly cooperate in many fields. The cooperating departments: Department of Internal Medicine III - Nephrology, Rheumatology and Endocrinology; Department of Immunology; Department of Ophthalmology; Department of Radiology. All departments are fully equipped to perform above mentioned research tasks. Team will include two nurses.

Team Members:

Jan Schovánek, M.D, Ph.D. will be responsible for implementation and management of the project. He will coordinate the project and prepare the early warning card system. As project leader will be responsible for results publication.
Anna Petráčková, MSc. (PhD student – study ends 2020) will be responsible for implementation and laboratory analysis of immune and inflammatory markers and for results publication.
Jakub Čivrný, M.D. (PhD student) will be responsible for MRI examination and interpretation.
Petra Hubnerová, M.D. (PhD student) will be responsible for complex ophthalmological examination, for orbital ultrasound and Oxymap examination.
Marta Karhanová, M.D, Ph.D., FEBO will be responsible for complex ophthalmological examination, for orbital ultrasound and Oxymap examination. She will collect data and prepare their publication.
Michal Křupka, Ph.D. will be responsible for implementation and laboratory analysis of immune and inflammatory markers.
Markéta Trajerová, MSc. (PhD student) will be responsible for flow cytometry testing and data collection.
Dorota Koníčková, MSc. will be responsible for immunogenetic analysis.
Tereza Libigerová, MSc. will be responsible for laboratory analysis and data collection.

Agreed foreign collaboration

In frame of this project, collaboration with institutes with high reputation in the field of endocrinology and thyroid gland autoimmunity is established, with main focus on the exchange of knowledge and consulting activities. The communication will be realized via Skype, personal visits and email.

Yasin I. Tayem, M.D., Ph.D.
Associate professor of pharmacology and therapeutics, College of Medicine and Medical Sciences, Arabian Gulf University in Bahrain, yasiniyt@agu.edu.bh

Paula Bargi de Souza, PhD
Assistant Professor, Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil, bargisouzap@ufmg.br

Figures

Figure 1.: On the left patient with very severe GO and dysthyroid neuropathy in active stage on the right eye. On the right a patient with inactive GO with extraocular muscle involvement. Personal archive of applicant, with consent of patient.

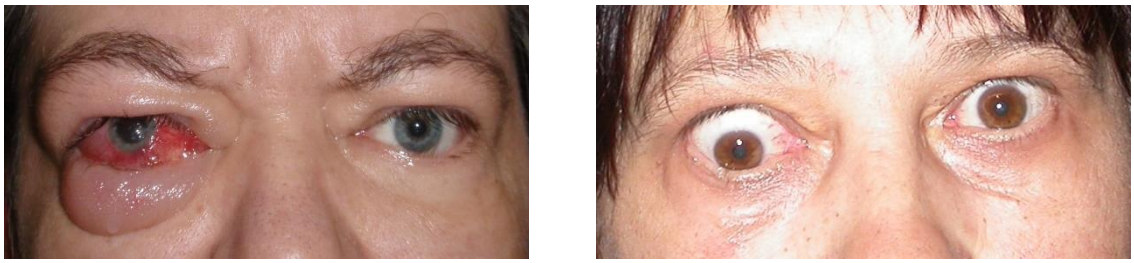


Figure 2.: Natural course of GO and possibilities of correct therapy timing (Hiromatsu, Eguchi, Tani, Kasaoka and Teshima 2014).

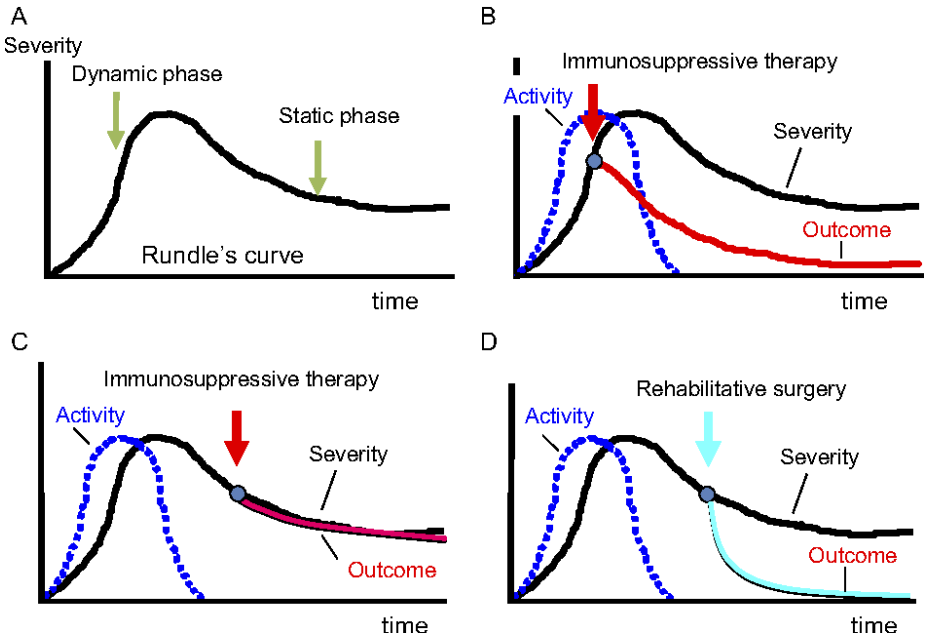
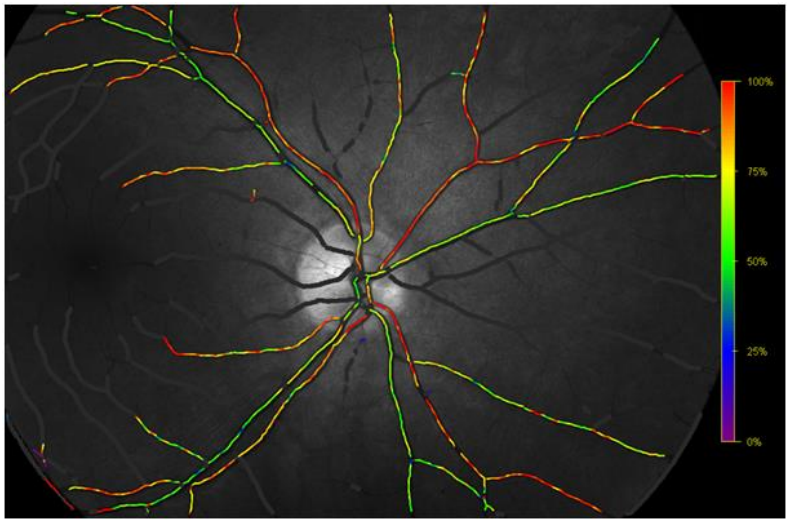


Figure 3.: A sample of retinal oximetry image by Oxymap ehf. Reykjavík, Iceland.



Citations:

BARTALENA, L., L. BALDESCHI, K. BOBORIDIS, A. ECKSTEIN, et al. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy Guidelines for the Management of Graves' Orbitopathy. Eur Thyroid J, Mar 2016, 5(1), 9-26.
DOUGLAS, R. S., G. J. KAHALY, A. PATEL, S. SILE, et al. Teprotumumab for the Treatment of Active Thyroid Eye Disease. N Engl J Med, Jan 23 2020, 382(4), 341-352.
DU PASQUIER-FEDIAEVSKY, L., S. ANDREI, M. BERCHE, L. LEENHARDT, et al. Low-Dose Rituximab for Active Moderate to Severe Graves' Orbitopathy Resistant to Conventional Treatment. Ocul Immunol Inflamm, 2019, 27(5), 844-850.
DYSKOVA, T., E. KRIEGOVA, Z. SLOBODOVA, S. ZEHNALOVA, et al. Inflammation time-axis in aseptic loosening of total knee arthroplasty: A preliminary study. PLoS One, 2019, 14(8), e0221056.
GABCOVA, G., P. HORAK, Z. MIKULKOVA, M. SKACELOVA, et al. Modulatory Effect of the Euro-Lupus Low-Dose Intravenous Cyclophosphamide Regimen on Circulating Immune Cells in Systemic Lupus Erythematosus. Arch Immunol Ther Exp (Warsz), Dec 2019, 67(6), 415-425.
GLATT, H. J. Optic nerve dysfunction in thyroid eye disease: a clinician's perspective. Radiology, Jul 1996, 200(1), 26-27.
HIROMATSU, Y., H. EGUCHI, J. TANI, M. KASAOKA, et al. Graves' Ophthalmopathy: Epidemiology and Natural History. Internal Medicine, 2014, 53(5), 353-360.
CHENG, C. W., K. T. TANG, W. F. FANG AND J. D. LIN Synchronized expressions of serum osteopontin and B cell-activating factor in autoimmune thyroid disease. Eur J Clin Invest, Jul 2019, 49(7), e13122.
CHENG, C. W., C. Z. WU, K. T. TANG, W. F. FANG, et al. Simultaneous measurement of twenty-nine circulating cytokines and growth factors in female patients with overt autoimmune thyroid diseases. Autoimmunity, Apr 25 2020, 1-9.
KAHALY, G. J., M. RIEDL, J. KONIG, S. PITZ, et al. Mycophenolate plus methylprednisolone versus methylprednisolone alone in active, moderate-to-severe Graves' orbitopathy (MINGO): a randomised, observer-masked, multicentre trial. Lancet Diabetes Endocrinol, Apr 2018, 6(4), 287-298.
KARASEK, D., L. CIBICKOVA, M. KARHANOVA, J. KALITOVA, et al. Clinical and immunological changes in patients with active moderate-to-severe Graves' orbitopathy treated with very low-dose rituximab. Endokrynol Pol, 2017, 68(5), 498-504.
KRIEGOVA, E., G. MANUKYAN, Z. MIKULKOVA, G. GABCOVA, et al. Gender-related differences observed among immune cells in synovial fluid in knee osteoarthritis. Osteoarthritis Cartilage, Sep 2018, 26(9), 1247-1256.
KUBA, A., L. RAIDA, F. MRAZEK, P. SCHNEIDEROVA, et al. NFKB1 gene single-nucleotide polymorphisms: implications for graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. Ann Hematol, Mar 2020, 99(3), 609-618.
LACHETA, D., P. MISKIEWICZ, A. GLUSZKO, G. NOWICKA, et al. Immunological Aspects of Graves' Ophthalmopathy. Biomed Res Int, 2019, 2019, 7453260.
LE BIHAN, D. Magnetic resonance imaging of perfusion. Magn Reson Med, May 1990, 14(2), 283-292.
LE BIHAN, D. What can we see with IVIM MRI? Neuroimage, Feb 15 2019, 187, 56-67.
LE BIHAN, D., E. BRETON, D. LALLEMAND, M. L. AUBIN, et al. Separation of diffusion and perfusion in intravoxel incoherent motion MR imaging. Radiology, Aug 1988, 168(2), 497-505.
LE BIHAN, D., E. BRETON, D. LALLEMAND, P. GRENIER, et al. MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. Radiology, Nov 1986, 161(2), 401-407.
MANUKYAN, G., A. MARTIROSYAN, L. SLAVIK, S. MARGARYAN, et al. Anti-domain 1 beta2 glycoprotein antibodies increase expression of tissue factor on monocytes and activate NK Cells and CD8+ cells in vitro. Auto Immun Highlights, Mar 2 2020, 11(1), 5.
MANUKYAN, G., T. PAPAJIK, P. GAJDOS, Z. MIKULKOVA, et al. Neutrophils in chronic lymphocytic leukemia are permanently activated and have functional defects. Oncotarget, Oct 17 2017, 8(49), 84889-84901.

MANUKYAN, G., P. TURCSANYI, Z. MIKULKOVA, G. GABCOVA, et al. Dynamic changes in HLA-DR expression during short-term and long-term ibrutinib treatment in patients with chronic lymphocytic leukemia. Leuk Res, Sep 2018, 72, 113-119.
MARGARYAN, S., E. KRIEGOVA, R. FILLEROVA, V. SMOTKOVA KRAICZOVA, et al. Hypomethylation of IL1RN and NFKB1 genes is linked to the dysbalance in IL1beta/IL-1Ra axis in female patients with type 2 diabetes mellitus. PLoS One, 2020, 15(5), e0233737.
MITCHELL, A. L., L. GOSS, L. MATHIOPOULOU, M. MORRIS, et al. Diagnosis of Graves' orbitopathy (DiaGO): results of a pilot study to assess the utility of an office tool for practicing endocrinologists. J Clin Endocrinol Metab, Mar 2015, 100(3), E458-462.
MITCHELL, A. L., J. HICKEY, B. VAIDYA, R. MASON, et al. Raising awareness of Graves' orbitopathy with early warning cards. Clin Endocrinol (Oxf), Dec 2017, 87(6), 853-859.
MRAZEK, F., P. SCHNEIDEROVA, E. KRIEGOVA, L. RAIDA, et al. Profile of Inflammation-Associated Proteins in Early Post-Transplant Samples of Patients After Allogeneic Hematopoietic Stem Cell Transplantation: a Preliminary Study. Arch Immunol Ther Exp (Warsz), Dec 2016, 64(Suppl 1), 55-61.
OBR, A., V. PROCHAZKA, A. JIRKUVOVA, H. URBANKOVA, et al. TP53 Mutation and Complex Karyotype Portends a Dismal Prognosis in Patients With Mantle Cell Lymphoma. Clin Lymphoma Myeloma Leuk, Nov 2018, 18(11), 762-768.
PETRACKOVA, A., P. HORAK, M. RADVANSKY, R. FILLEROVA, et al. Revealed heterogeneity in rheumatoid arthritis based on multivariate innate signature analysis. Clin Exp Rheumatol, Mar-Apr 2020a, 38(2), 289-298.
PETRACKOVA, A., J. MINARIK, L. SEDLARIKOVA, T. LIBIGEROVA, et al. Diagnostic deep-targeted next-generation sequencing assessment of TP53 gene mutations in multiple myeloma from the whole bone marrow. Br J Haematol, May 2020b, 189(4), e122-e125.
PETRACKOVA, A., A. SMRZOVA, P. GAJDOS, M. SCHUBERTOVA, et al. Serum protein pattern associated with organ damage and lupus nephritis in systemic lupus erythematosus revealed by PEA immunoassay. Clin Proteomics, 2017, 14, 32.
PETRACKOVA, A., M. VASINEK, L. SEDLARIKOVA, T. DYSKOVA, et al. Standardization of Sequencing Coverage Depth in NGS: Recommendation for Detection of Clonal and Subclonal Mutations in Cancer Diagnostics. Front Oncol, 2019, 9, 851.
SCHNEIDEROVA, P., T. PIKA, P. GAJDOS, R. FILLEROVA, et al. Serum protein fingerprinting by PEA immunoassay coupled with a pattern-recognition algorithms distinguishes MGUS and multiple myeloma. Oncotarget, Sep 19 2017, 8(41), 69408-69421.
SCHOVANEK, J., L. CIBICKOVA, M. KARHANOVA, D. KOVAROVA, et al. Retrospective Analysis of Patients with Graves Orbitopathy Treated by Pulses of Methylprednisolone, with a Focus on Adverse Events. Endocr Pract, Jul 2018, 24(7), 652-657.
STAROSTKA, D., E. KRIEGOVA, M. KUDELKA, P. MIKULA, et al. Quantitative assessment of informative immunophenotypic markers increases the diagnostic value of immunophenotyping in mature CD5-positive B-cell neoplasms. Cytometry B Clin Cytom, Jul 2018, 94(4), 576-587.
TURCSANYI, P., E. KRIEGOVA, M. KUDELKA, M. RADVANSKY, et al. Improving risk-stratification of patients with chronic lymphocytic leukemia using multivariate patient similarity networks. Leuk Res, Apr 2019, 79, 60-68.
WIERSINGA, W. M. AND G. J. KAHALY *Graves' Orbitopathy A Multidisciplinary Approach - Questions and Answers*. Edtion ed.: S. Karger AG, 2017. ISBN 978-3-318-06084-3 978-3-318-06085-0.
YANG, X., D. HUANG, S. AI, X. LIANG, et al. Retinal Vessel Oxygen Saturation and Vessel Diameter in Inactive Graves Ophthalmopathy. Ophthalmic Plast Reconstr Surg, Nov/Dec 2017, 33(6), 459-465.