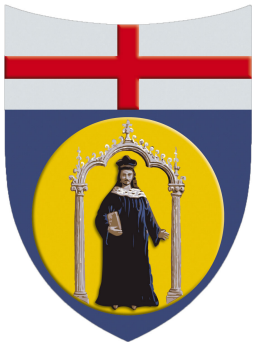

Università degli studi di Genova

Dottorato di Ricerca in

Biotecnologie in Medicina Traslazionale

CICLO XXXIV

Coordinatore: Prof. Paolo Malatesta

Evaluation of retinal, choroidal and peripapillary vascular flow in patients with Thyroid Associated Ophthalmopathy (TAO) using Optical Coherence Tomography Angiography (OCT-A)

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Introduction

Thyroid-associated orbitopathy (TAO) is an organ-specific immunemediated inflammatory disease that may be both sight-threatening and disfiguring. It is characterized by an uncontrolled proliferation of orbital fibroblasts and by an increased production of glycosaminoglycans and collagen that cause an expansion of extraocular muscles and intraorbital fat [1]. While most patients with TAO have clinical and biochemical evidence of hyperthyroidism or hypothyroidism, some are euthyroid; at least at the time of presentation [2]. Thyroid dysfunction may precede, be coincident with or follow TAO. It is clinically relevant in approximately 30-50% of patients with Graves' disease, severe forms affecting 3–5% of patients. The age-adjusted annual incidence of clinically relevant TAO is 16 per 100,000 population in women and 2.9 in men. TAO is most commonly associated with Graves' disease (90%) but ay occur in 3% of Hashimoto thyroiditis [3]. The most evident histological characteristic of TAO is the presence of a marked infiltration of immunocompetent cells, T lymphocytes, and macrophages and a lower quantity of B lymphocytes [4]. In addition, a perimysial fibroblast proliferation has been described [5]. Orbital tissues became extremely hydrophilic due to high osmotic pressure and retain water causing edema. Several clinical manifestations of TAO are caused by an increase in the

volume of soft orbital tissues leading to an increase in pressure within the orbital bone cavity [1]. TAO can be classify into type 1 (predominant orbital fat expansion) and type 2 (predominant extraocular muscles expansion and restrictive myopathy), with the latter been found in an older age group. The European Group on Graves' Ophthalmopathy classifies TAO as: sight threatening (disthyroid optic neuropathy and corneal breakdown); moderate to severe (no sight-threatening but with a strong impact on quality of life to justify immunosoppressive therapy or surgery); mild (only a minor impact on daily life) [6]. Recent clinical studies have shown that spectral domain optical coherence tomography (SD-OCT) can be a fast and safe diagnostic tool for the evaluation of TAO activity [7, 8]. The SD-OCT was recently developed to quantify the choroidal thickness and the degree of vascularization of the choroid using Angio-OCT technology [9]. The choroid is a vascular structure that supplies oxygen and nutrients to the retina. Choroidal veins are drained into ophthalmic veins: this implies that systemic conditions that influence blood flow in ophthalmic veins may influence choroidal thickening [10]. The aim of our preliminar study was to evaluate changes in choroidal thickness and any changes in macular choroidal vascular flow by the use of OCT and Angio-OCT in patients with TAO and their relationship with clinical features and disease activity.

Treatment options for TAO generally depend on disease activity and severity. For active and mild disease, ocular lubricants, sunglasses, and prisms are used for supportive management, for moderate-to-severe disease immunosoppressive therapy strategies are used, while in sight-threatening disease surgical intervention is often needed [11]. Glucocorticoids are used as a method of medical decompression due to their anti-inflammatory and immunosuppressive properties in patients with severe TAO to prevent disease progression [12]. Pulse therapy is based on intermittent intravenous infusion of corticosteroids. The standard dosage is the infusion of methylprednisolone 500 mg IV per week for 6 weeks followed by 250 mg IV per week to taper off. Methylprednisolone should be used with caution in patients with diabetes mellitus, hypertension and liver disease. It is absolutely contraindicated in patients with fungal and bacterial infections [13]. Prosecution of our preliminary study was to analyse the effects of intravenous high-dose methylprednisolone therapy in patients with TAO and the relationship between changes in macular vascular blood flow investigated by Angio-OCT technology.

Our previous studies identified changes in retinal and choroidal blood flow in patients with TAO using OCT and angio-OCT and changes correlate with the severity of the disease. The main site of TAO involvement are periorbital and orbital tissues.

Interstitial oedema, fat hyperplasia and swollen of extraocular muscles can lead to expansion of the orbital structures and subsequent compression of the optic nerve, proptosis of the eye, and exposure of the corneal surface that may ultimately lead to a compromise in visual integrity [14]. Orbital swelling at the apex can cause pressure on the optic nerve leading to dysthyroid optic neuropathy (DON). DON is only seen in severe disease with crowding of the orbit apex by enlarged extraocular muscles and it affects only 5% of clinical TAO patients [15].

Our latest study focused on the analysis of the peripapillary vascular blood flow in patient with TAO in order to identify any hemodynamic changes correlated with optic nerve compression.

Evaluation of choroidal thickness and choroidal vascular blood flow in patients with thyroid-associated orbitopathy (TAO) using SD-OCT and Angio-OCT.

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Introduction

Thyroid-associated orbitopathy (TAO) is an immunemediated inflammatory disease. It is characterized by an uncontrolled proliferation of orbital fibroblasts and by an increased production of glycosaminoglycans and collagen that cause an expansion of extraocular muscles and intraorbital fat. This condition usually occurs in patients with hyperthyroidism but can sometimes occur in patients with euthyroidism or hypothyroidism [1]. TAO is clinically relevant in approximately 50% of patients with Graves' disease, severe forms affecting 3–5% of patients. The age-adjusted annual incidence of clinically relevant TAO is 16 per 100,000 population in women and 2.9 in men. The most evident histological characteristic of TAO is the presence of a marked infiltration of immunocompetent cells, T lymphocytes, and macrophages and a lower quantity of B lymphocytes [4]. In addition, a perimysial fibroblast proliferation has been described. Orbital tissues became extremely hydrophilic due to high osmotic pressure and retain water causing edema. Several clinical manifestations of TAO are caused by an increase in the volume of soft orbital tissues leading to an increase in pressure within the orbital bone cavity. Recent clinical studies have shown that spectral domain optical coherence tomography (SD-OCT) can be a fast and safe diagnostic tool for the evaluation of TAO activity [7, 8]. The SD-OCT was recently

developed to quantify the choroidal thickness and the degree of vascularization of the choroid using Angio-OCT technology [9]. The choroid is a vascular structure that supplies oxygen and nutrients to the retina. Choroidal veins are drained into ophthalmic veins: this implies that systemic conditions that influence blood flow in ophthalmic veins may influence choroidal thickening [10]. The aim of this study is to evaluate changes in choroidal thickness and any changes in macular choroidal vascular flow by the use of OCT and Angio-OCT in patients with TAO and their relationship with clinical features and disease activity.

Methods

The study was conducted in accordance with the tenets of the Helsinki Declaration. This prospective study was conducted at the University Eye Clinic of Genoa, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), IRCCS Ospedale Policlinico S. Martino, Genova, Italy. All participants gave their informed consent. Patients with a clinical diagnosis of TAO and healthy control subjects matched for age and sex were examined. TAO was defined as a combination of any of the following: eyelid retraction, lagophthalmos, conjunctival injection, chemosis, eyelid swelling, proptosis, or myopathy as a

sequelae of thyroid gland dysfunction. All subjects diagnosed with TAO were previously seen by the endocrinology team, and the laboratory diagnosis of Graves–Basedow disease was based on the finding of serum TSH and on the presence of elevated thyroid hormone levels in the blood, associated with the presence of circulating antibodies (TRAb). Exclusion criteria were other systemic diseases, previous orbital surgery or radiotherapy, myopia greater than − 3 diopters, IOP > 18 mmHg, and ocular diseases that affect choroidal thickness such as glaucoma, uveitis, retinal, and choroidal diseases. All patients and control subjects underwent a complete eye examination including best-corrected visual acuity and biomicroscopic examination with the use of vital stains to evaluate the ocular surface; intraocular pressure (IOP) measurement, indirect ophthalmoscopy, and Hertel exophthalmometry; subjective diplopia evaluation using the Gorman score (0: none diplopia, 1: intermittent diplopia, 2: inconstant diplopia, 3: constant diplopia), SD-OCT, and Angio-OCT (OCT Topcon ImageNet 6; DRI OCT Triton, Topcon Corporation); and perimeter examination Humphrey 30-2 (Carl Zeiss Meditec, 2003). TAO activity was assessed through the Clinical Activity Score (CAS). According to EUGOGO (European Guidelines for European Basedow ophthalmopathy), patients were divided into non-active TAO (CAS < 3) and active TAO (CAS ≥ 3). This score

was also used to divide the patients into mild, moderate, or severe TAO. After pupillary dilation, all patients and controls included in the study were evaluated with SD-OCT and Angio-OCT scans using OCT Topcon ImageNet 6 (DRI OCT Triton, Topcon Corporation). Each subject underwent horizontal and vertical line scans (12 mm and 128 averaging), 12 radial line (12 mm), and three-dimensional (3D) macula acquisition protocol centered on the fovea. Choroidal thickness was defined as the distance between the Bruch's membrane and the chorioscleral interface. The integrated software of the Topcon ImageNet 6 automatically measured the choroidal thickness in the macular region. OCT swept-source angiography scans were obtained from an area of 4.5 × 4.5 mm2 centered on the fovea. The images of the choriocapillary (CC) were generated automatically by the software that is able to move a segmentation line on the Bruch membrane level. We also manually obtained other angiographic scans at 3 different consecutive levels (L1, L2, and L3) below the choriocapillary, shifting the segmentation lines to 59.6 and 80.6 lm, 80.6 and 119.6 lm, and 119.6 and 137.8 lm from the Bruch membrane, respectively. These images have been studied in order to analyze the deeper layers of the choroid, in particular, the Sattler and Haller layers [16]. Vascular flow was measured assuming that the percentage of white or black pixels of the obtained images represented an indirect

measure of vascular flow. The flow was therefore calculated as a percentage of the portion of white or black pixels present in the entire scan. The averages of the values obtained for both the eyes of the healthy subjects and those of the patients with TAO were finally calculated.

Statistical analysis

The data are reported as mean values ± standard deviation. Parameters of the two groups were compared using Student's t test for continuous variables and chi-square test for categorical variables. Choroidal measurements of patients with TAO and control eyes were compared using the independent sample t test. The significance of association between choroidal thickness and clinical activity score according to the EUGOGO guidelines was determined by Pearson's correlation coefficient test. The values with $p < 0.05$ were considered statistically significant, and all pvalues were based on two-tailed tests.

Results

Thirty-six eyes of 18 patients, 4 (22%) male and 14 (78%) female aged between 19 and 74 years (mean age 26.5 ± 4.9 years) with diagnosis of TAO, were compared with 36 eyes of 18 patients, 7 (39%) male and 11 (61%) female matched for age

(mean age 26.5 ± 3.5 years), and were included in this study. There were no significant differences in age (t test, p = 0, 81) and gender (chi-square, p = 0.72). Two of 18 patients (11.1%) referred diplopia. Hertel exophthalmometry measurements ranged from 18 to 29 mm (mean ± SD, 19.5 ± 0.7 mm). According to the severity assessment, 10 of 18 (55.5%) patients were classified with mild TAO, 7 patients (39%) were classified with moderate TAO, and one patient (5.5%) had a severe form of TAO. No patient showed the presence of relative afferent pupillary defect or an optic neuropathy related to orbital pathology. The CAS score was < 3 in 10 patients and ≥ 3 in the remaining 8 patients. The subfoveal choroid was significantly thicker in TAO patients than the control eyes (285.6275 ± 32.5 μm vs. 135.89 ± 19.8 μm, respectively, p = 0.0089) as shown in Figs. 1 and 2. The correlation analysis in the TAO group shown a significant correlation between the choroidal thickness and EUGOGO clinical score (Pearson, r = 0.84, p = 8.44032E-07). In patients with mild TAO, choroidal thickness was 228.172 ± 86.53 μm, in those with moderate TAO was 296.146 ± 27.54 μm, whereas in the patient with severe TAO was 661.415 μm (Table 1). Regarding the vascular flow data, statistically significant differences were found in the levels of choriocapillary and L3 compared with healthy subjects. In fact, vascular flow of choriocapillary was markedly reduced in subjects with TAO

compared with healthy subjects (49.78 ± 4.5 vs. 53.36 ± 1.07, respectively, p = 2.5105E-07). On the contrary, vascular flow of the deeper layer L3 resulted higher in subjects with TAO compared with healthy subjects (46.9 ± 20.23 vs. 41.475 ± 3.06, respectively, p = 0.01168). No significant differences were observed for vascular flow values in L1 and L2. Clinical features and demographic data are summarized in Table 2.

Discussion

SD-OCT is a non-invasive method used for a morphological evaluation of the retina. In this study, we demonstrated that subfoveal choroidal thickness in patients with TAO is significantly higher than that of healthy subjects matched for age. One of the primary characteristics of patients with TAO is the increased volume of extraocular muscles and intraorbital volume determined by the increase in connective tissue, extracellular matrix, and intraorbital fat. This pathogenic mechanism leads to an increased pressure within the orbit that causes a great obstacle to venous outflow [17]. Higher values of episcleral and retrobulbar pressure due to a reduction in venous drainage have been already reported in other studies in patients with TAO [18, 19]. Our study shown that choroidal thickness was thicker in patients affected by

TAO. Similar results were found by Odrobina et al. [20] and Yu et al. [21]. In fact, the well-known increase in intraorbital pressure in patients with TAO may lead to congestion and edema of the episclera and conjunctiva affecting the vascular drainage. The relative stagnation of venous blood on the episclera and conjunctive has been described as the major reason for the increase in choroidal thickness [20, 21]. According to Özkan et al. [8], we believe that the increase in choroidal thickness in patients with TAO is due to an impeded venous outflow caused by the high congestion of ophthalmic veins which causes a reduced choroidal drainage. In our study, we also found a strong correlation between choroidal thickness and TAO clinical activity according to the EUGOGO guidelines. This observation is in contrast with Yu et al. [21] that found a poor relationship between choroidal thickness and CAS, exophthalmos, and thyroid function tests. This may be due to a difference assessment of both severity and activity of the disease. In fact, EUGOGO classification is not limited to a CAS evaluation but included other clinical parameters that allow a proper severity assessment of patients affected by TAO disease [6]. Some previous studies have examined orbital blood flow in patients with TAO through the use of eco-colored Doppler imaging. These studies demonstrated an important venous stasis and an inverse correlation between blood flow and disease

activity [22]. It is known that the orbital Doppler imaging method is characterized by various limitations that may affect the accuracy of the results, such as difficulty in detecting retrobulbar vessels, variation in flow velocity influenced by the pressure applied by the probe on the globe, and the movements of the eyes that can cause artificiality in background noise [22, 23] . In contrast, OCT is a non-invasive, non-contacting technique that could potentially exceed the color limits mentioned above. In our study, it was possible to highlight how important variations of vascular flow are present in the various choroidal levels, in particular, in the choriocapillary layer and in the deeper chorioscleral level in patients with TAO. In the choriocapillary layer, there is a reduction in vascular flow compared with healthy subjects, while in the deeper layer, we can see an increase in vascular flow in subjects with TAO compared with healthy subjects. To the best of our knowledge, this is the first report describing choroidal vascular flow in TAO patients. These data can be explained on the basis of the concept of a hindered venous return at the level of ophthalmic veins that would support the hypothesis of a greater flow at the level of the deeper choroidal layer (L3) and a reduction in the more superficial layer. Given the results obtained, we can state that it is reasonable to use the choroidal thickness and the value of macular choroidal blood flow as a new parameter in the evaluation of

patients with TAO to estimate the degree of orbital congestion, especially in those patients with subclinical and early TAO manifestations. We recognize that this study has limitations as a small sample size and the data are not matched for gender. Further investigations are needed to establish the diagnostic and prognostic role of SD-OCT analysis of choroidal thickness and choroidal vascular flow to establish its role in appropriate long-term follow-up in a larger sample of patients with TAO.

Figures and Tables

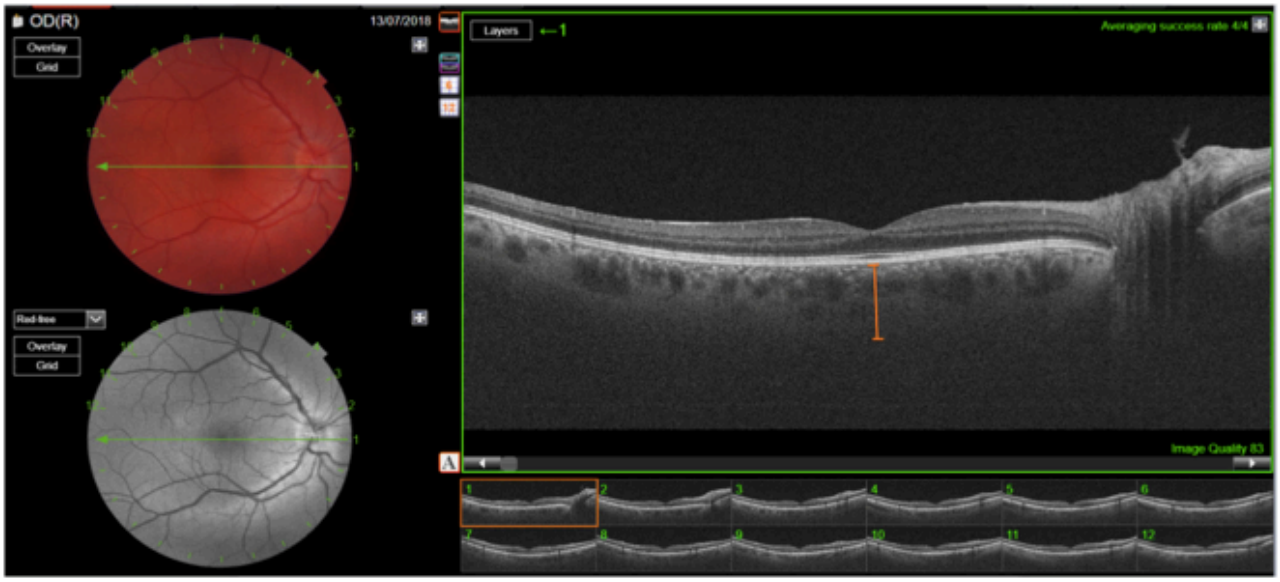

Fig. 1 Spectral domain optical coherence tomography (SD-OCT) (DRI OCT Triton, Topcon Corporation). Horizontal optical coherence tomography shows subfoveal choroidal thickness in a patient with thyroid-associated orbitopathy (TAO)

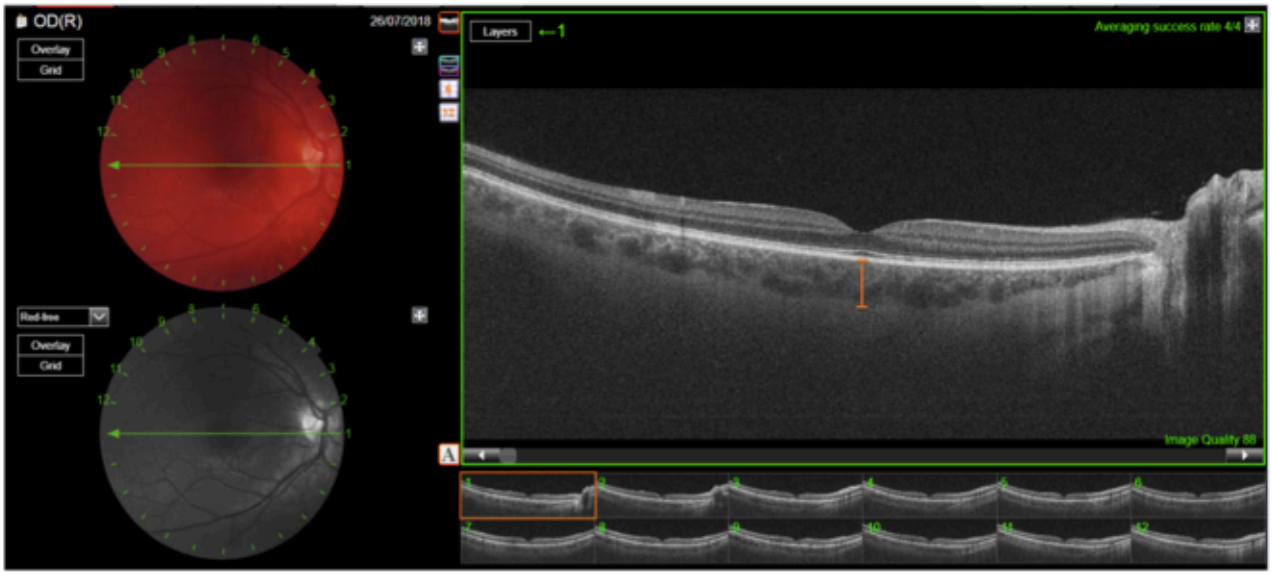

Fig. 2 Spectral domain optical coherence tomography (SD-OCT) (DRI OCT Triton, Topcon Corporation). Horizontal optical coherence tomography shows subfoveal choroidal thickness in a control healthy patient

Table 1 Pearson correlation analysis shows a significant correlation between choroidal thickness in TAO group and EUGOGO clinical score

Severity of TAO	Choroidal thickness (mean ± SD)
Mild TAO	228.17 ± 86.5
Moderate TAO	296.15 ± 27.5
Severe TAO	661.42 ± 0

Table 2 Demographic data and clinical features in TAO patients and healthy subjects

	TAO patients	Healthy patients
Gender: female (%)	14 (78%)	10 (56%)
Gender: male (%)	4 (22%)	8 (44%)
Mean age (years ± SD)	26.5 ± 4.94	26.5 ± 3.53
Visual acuity (logMAR)	0.03 ± 0.65	0.009 ± 1.09
CC choroidal vascular blood flow (mean ± SD)	49.78 ± 4.49*	53.36 ± 1.07
L1 choroidal vascular blood flow (mean ± SD)	44.82 ± 10.52	39.99 ± 0.98
L2 choroidal vascular blood flow (mean ± SD)	46.51 ± 11.87	54.31 ± 16.25
L3 choroidal vascular blood flow (mean ± SD)	46.97 ± 20.23*	41.48 ± 3.06
Choroidal thickness (mean ± SD)	285.63 ± 32.5*	135.89 ± 19.8

CC, choriocapillary; *L1*, choroid layer obtained positioning the segmentation line at 59.6 and 80.6 μm from the Bruch's membrane; *L2*, choroid layer obtained positioning the segmentation line at 80.6 and 119.6 μm from the Bruch's membrane; *L3*, choroid layer obtained positioning the segmentation line at 119.6 and 137.8 μm from the Bruch's membrane

*$p < 0.001$

Evaluation of macular blood flow after intermittent intravenous infusion of high-dose corticosteroids (pulse therapy) in patients with thyroid-associated orbitopathy (TAO) using angio-OCT.

Del Noce C, Roda M, Ferro Desideri L, Traverso CE, Vagge A.Graefes Arch Clin Exp Ophthalmol. 2022 Feb;260(2):571-576.
doi: 10.1007/s00417-021-05336-4. Epub 2021 Sep 1.PMID: 34468829

Introduction

Graves' disease (GD) is an autoimmune disorder characterized by the excess production of thyroid hormones due to the overstimulation of the thyroid gland by thyrotropin (TSH)-receptor autoantibodies (TRAbs). GD is the most frequent cause of hyperthyroidism, with a prevalence of ~0.5% in the general population, with a female-to-male ratio of ~3:1 [24-26]. Thyroid-associated orbitopathy is an autoimmune disorder related to GD. It occurs in up to 50% of individuals with GD. However, it may also occur without current or prior hyperthyroidism, or in people who are hypothyroid due to chronic autoimmune Hashimoto's thyroiditis [27]. Clinical manifestations of active TAO can vary and may include conjunctival chemosis andinjection, lid swelling, lid retraction, proptosis, strabismus, exposure keratopathy, and optic [28]. Common symptoms may include eye pain, excessive lacrimation, diplopia, photophobia, and blurry vision [29] In assessing the activity level of TAO in a patient, the clinical activity score (CAS) can be used [30]. Treatment options for TAO generally depend on disease activity and severity. Active TAO is typically described as mild, moderate-to-severe, or sight-threatening according to EUGOGO guide lines [31]. For active and mild disease, ocular lubricants, sunglasses, and prisms are used for supportive management, for moderate-

to-severe disease immunosoppressive therapy strategies are used, while in sight-threatening disease surgical intervention is often needed [11]. Glucocorticoids are used as a method of medical decompression due to their anti-inflammatory and immunosuppressive properties in patients with severe TAO to prevent disease progression [30]. Pulse therapy is based on intermittent intravenous infusion of corticosteroids. The standard dosage is the infusion of methylprednisolone 500 mg IV per week for 6 weeks followed by 250 mg IV per week to taper off. Methylprednisolone should be used with caution in patients with diabetes mellitus, hypertension and liver disease. It is absolutely contraindicated in patients with fungal and bacterial infections [12]. The aim of the present study was to analyse the effects of intravenous high-dose methylprednisolone therapy in patients with TAO and the relationship between changes in macular vascular blood flow investigated by Angio-OCT technology.

Methods

This study was a retrospective study. All partecipants gave their informed consent and the study followed the principles of the Declaretion of Helsinki. The study was conducted at the University Eye Clinic of Genoa, Department of Neuroscience,

Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), IRCCS Ospedale oliclinico S. Martino, Genova, Italy. It involved patients affected by moderate to severe TAO disease who underwent pulse therapy. According to EUGOGO classification we included patient with lid retraction ≥ 2 mm, moderate to severe soft tissue involvement, exhophtalmos ≥ 3 mm above normal and constant or incostant diplopia. Exclusion criteria were mild or sight-treatining TAO, previous orbital surgery or radiotheraphy, myopia greater than − 3 diopters, IOP > 18 mm Hg and other disases that affect retinal and choroidal circulation such as glaucoma, uveitis, retinal and choroidal diseases and carotid artery stenosis. Twenty-four right eyes of patients with moderate-to-severe TAO (8 men and 16 women), aged 25-75 years (mean 54,8 ± 15,2), were studied. All subjects diagnosed with TAO were evaluated at baseline (T0) and 2 months after the last infusion of pulse therapy (T1) by the same physician. At T0 and T1 all the patients underwent a complete eye examination including: best-corrected visual acuity, biomicroscopic examination, intraocular pressure (IOP) measurement, Hertel exophthalmometry and indirect ophthalmoscopy. TAO activity was assessed through the Clinical Activity Score (CAS). CAS is composed of a list of seven inflammatory orbital symptoms representing pain, redness, and swelling. A CAS of three points or more indicate the

patient is in an active phase of the disease [13]. After pupillary dilation, all patients included in the study were evaluated with angio- OCT scans using OCT Topcon ImageNet 6 (DRI OCT Triton, Topcon Corporation). OCT angiography scans were obtained from an area of 4.5 X 4.5 mm2 centered on the fovea. We analised macular BFI in four angiographic levels: superficial plexus (SP), deep plexus (DP), external retina (ER) and coriocapillaris (CC). Macular BFI was calculated as the percentage of black and white pixels present in the scan assuming that this percentage represents an indirect measure of vascular flow. The averages of the values obtained were finally calculated.

Statistical analysis

The data are reported as mean values ± standard deviation and inlcude data on the right eyes. Parameters of the two groups were compared using student t-test for continuous variables and chisquared test for categorical variables. Macular vascular blood flow index before and after pulse therapy was compared using the independent sample t test. The values with $p < 0.05$ were considered statistically significant, and all p values were based on two tailed tests.

Results

Macular BFI at T0 was lower in every angiographic level compared to macular BFI at T1. Mean values for DP BFI , ER BFI and CC BFI after pulse therapy (43.6 ± 2.2, 47.9± 1.6, 48.9± 1.7, respectively) were significantly higher (p 0.05) (Table 1). Visual acuity remained stable for twenty patients. In four patients there was an increase in visual acuity at T1 (0.65 ± 0.05) compared to T0 (0.35 ± 0.09, p< 0.05). No correlation was found between visual acuity and macular BFI in every level. Fifteen patients complained diplopia before treatment. After treatment, diplopia disappeared for three patients and remained stable for the remaining twelve. Sixteen patients had corneal involvement before treatment which resolved at the end of therapy for fourteen patients. Mean (±SD) CAS score evaluation was changed from 5,8 ± 0,8 to 3,9 ± 0,9 by pulse therapy (p< 0.01). CAS score, proptosis values and IOP measurements are summarized in Table 2. No serious adverse events occurred in any patients. Six patients (25%) reported mild hypertension and palpitation after pulse therapy.

Discussion

Basedowian ophthalmopathy is a chronic, autoimmune disease that requires a long period to recover. Numerous therapies are available to control the progression of orbital pathology, avoid damage to the cornea and the optic nerve and prevent ocular motility disorders. Although the most effective of the drugs used for immune suppression seem to be steroids, high oral dosage is necessary for a long time, and causes serious adverse effects [33]. Intravenous high-dose steroid pulse therapy is reported to be more effective and adverse effects are less [34]. Moreover, intravenous steroid therapy is more effective and better tolerated by patients than oral administration [35]. However, several adverse events associated with pulse therapy have been documented including severe hepatic insufficiency [36]. Side effects were in relation to dose, treatment regimen, age and patient's comorbidities [37]. Patients should be screened for recent hepatitis, cardiovascular disease, hypertension, liver dysfunction, diabetes and glaucoma. Therefore, careful patient selection is mandatory. It is recommended to maintain a dose range between 6 and 8 grams and to visit the patient monthly during treatment [38]. It has also been reported that tighter monitoring is advised in patients older than 53 years as they have a higher risk of hepatic failure at high doses [39]. However, steroid treatment remains the first

choice therapy for patients with moderate to severe TAO [40]. Several other studies have been conducted to investigate retinal vascular changes in patients with TAO using OCT angiography. Lanchu et al. evaluated retinal and choroidal variation in TAO patients by angio-OCT. They observed that patients with TAO had significant changes in RNFL thickness, choroidal thickness and superficial retinal vessels. Patients with inactive TAO had greater retinal vascular density than healthy controls [41]. In the study by Tehrani et al. the average peripapillary vessel density was significantly lower in patients with active TAO compare to the other groups [42]. Similarly, Dave et al. found that in patients with active TAO peripapillary and macular area vascularization were reduced in patients with active TAO compared to healthy controls [43]. Another study analyzed choriocapillary vascularization using angio-OCT and found that in patients with TAO choriocapillary vascular flow was markedly reduced compared to healthy controls of the same age and sex [44]. Ocular hemodinamic changes in patients with TAO have also been studied by the group of Lei et al. On the contrary, they found an increased superficial retinal layer and deep retinal layer microvascular density in the macular area in patients with TAO [45]. This study found differences in macular vascularity in patients with active TAO treated with pulse therapy. In particular, it has been studied that macular

vascularization increased after pulse therapy in all retinal levels: superficial plexus, deep plexus, external retina and choriocapillary. These results can be explained based on the fact that in active TAO there is an increase of the musculature and of the intraorbital soft tissues that determine an obstacle to the normal retinal vascularization. After therapy with intravenous methylprednisolone, the anti-inflammatory power of cortisone determines a resolution of muscles' edema and intraorbital soft tissues involvement, allowing to resolve vascular flow obstruction and increasing retinal vascular index. We recognise that this study has limitations as it is retrospective and has a small cohort. Further studies are necessary to analyzed pulse therapy effects on retinal vascularity and to establish its role in appropriate long-term follow-up in a larger sample of patients. In conclusion this study demonstrated a change in macular vascular blood flow in patients with active TAO who undergoes pulse therapy treatment. In particular, two months after pulse therapy, all the patients showed an increase in macular vascular blood flow in each angiographic level. According to our results, Angio-OCT values of macular vascular blood flow could be add in the evaluation of patients who undergo pulse therapy.

Figures and Tables

	T0	T1
SP BFI (%±SD)	38.5 ± 3.7	38.9 ± 2.5
DP BFI (%±SD)	42.5 ± 2.2	43.6 ± 2.2*
ER BFI (%±SD)	45.8 ± 1.6	47.9± 1.6*
CC BFI (%±SD)	47.3 ± 2.1	48.9± 1.7*

SP BFI= superficial plexus blood flow index, DP BFI= deep plexus blood flow index, ER BFI= external retina blood flow index, CC BFI= coriocapillaris blood flow index.

%= percentage; SD= standard deviation

- = $p<0.01$ T0 vs. T1

Table 1: Macular blood flow index (BFI) values measured by angio-OCT at four different retinal levels before pulse therapy (T0) and 2 months after pulse therapy (T1).

	T0	T1
CAS (n±SD)	5,8 ± 0,8	3,9 ± 0,9*
Hertel Exophthalmometry (mm±SD)	22,6 ± 2,3	21,2 ± 2,5*
IOP (mmHg±SD)	13.3 ± 2.8	14.3± 2.1*

CAS= clinical activity score, n= number, SD= standard deviation, IOP= intraocular pressure

* = $p<0.01$ T0 vs. T1

Table 2: Clinical Activity Score (CAS), Hertel Exophthalmometry measurements and intraocular pressure (IOP) values before pulse therapy (T0) and 2 months after pulse therapy (T1).

Evaluation of peripapillary vascular flow in patients with Thyroid-Associated Ophthalmopathy (TAO) by OCT Angiography.

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Introduction

Thyroid associated ophthalmopathy (TAO) is a systemic autoimmune disease with a large spectrum of signs and symptoms [46]. Since it generally occurs in people with Graves' disease, it is also called Graves orbitopathy. Ocular and thyroid symptoms often occur simultaneously, however, sometimes ophthalmopathy precedes or follows the onset of hyperthyroidism by many years [1]. Clinic assessment has been made traditionally by the "NO SPECS" mnemonic [Table 1], in order to easily classify the severity of the disease. Nevertheless, it was not reliable in defining the progression of the disease, therefore in 1989 Mouritis et al introduced the Clinical Active Score "CAS" [Table 2], which allows to distinguish the disease in active and inactive form by considering seven different parameters that suggest grade of activity of the disease [47]. The main site of involvement are periorbital tissues, where interstitial oedema, orbital fat hyperplasia and massively swollen of extraocular muscles occur [48]. Other common symptoms are upper eyelid retraction, diplopia, proptosis, or a sensation of pressure behind the eyes, which are usually bilateral and often asymmetric [49].

Haemodynamic changes in these patients have been studied in several different ways, such as Heidelberg retina flowmetry, ocular blood flow tomography, oculodynamometry, and color Doppler imaging (CDI) [50]. CDI is a non-invasive approach, which has been widely used to assess orbital vessels blood flow. Nevertheless, there are several influencing factors in the measuring process, such as the pressure applied on an eyeball, eye movement, sampling volume, and angle [21]. Optical coherence tomography (OCT) is a rapid and non-invasive technique that allows detailed structural visualisation of retina and choroid. Thanks to the development of OCT angiography (OCTA) is possible to study retinal and choroidal vascularisation by producing 3-dimensional microcirculation vascular map without the use dye [51]. Through the en-face presentation of the volumetric angiogram there is the possibility of quantifying features of interest, such as density of vessels in the macula or blood flow in specific areas of the retina [52].

Retinal and choroidal blood flow of patients with Graves' ophthalmopathy have been largely investigated in order to identify changes in foveal and parafoveal microvascular density and retinal vessel caliber [53] or choroidal thickness and choroidal vascular blood flow. Retinal nerve fibre layer (RNFL) thickness, choroidal and macular thickness have been also investigated using OCT in these patients

[21,44]. However, up to date, alterations in peripapillary vascular blood flow in people who suffer from Thyroid Associated Ophthalmopathy (TAO) have not been investigated yet. Therefore, the aim of this study was to evaluate, through OCT Angiography (OCTA) technology, changes in peripapillary vascular blood flow.

Methods

The study was conducted at the University Eye Clinic of Genoa, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), IRCCS Ospedale Policlinico S. Martino, Genova, Italy. 26 participants with the diagnosis of Graves – Basedow disease and thyroid associated ophthalmopathy (TAO) were recruited. The same number of subjects was enrolled to assemble the control healthy group. All the participants gave their informed consent, and the study was conducted in accordance with the tenets of the Helsinki Declaration. Including criteria were age ≥18, Graves' disease diagnosis, refractive error lower than 3 diopters of spherical equivalent, IOP < 18 mmHg (measured with Goldmann applanation tonometer). Patients with ocular diseases that affect choroidal thickness such as glaucoma, uveitis, retinal, and choroidal diseases were excluded, same as for patients who underwent intraocular surgery or who took drugs that can

affect ocular blood flow. All the subjects in TAO group were previously seen by the endocrinology team, and the laboratory diagnosis of Graves–Basedow disease was based on the finding of serum TSH and on the presence of elevated thyroid hormone levels in the blood, associated with the presence of circulating antibodies (TRAb). Each subject underwent a complete examination including anamnesis, best corrected visual acuity (BCVA), slit lamp examination of the anterior segment, indirect ophthalmoscopy, Hertel exophthalmometry, OCT and angio-OCT (OCT Topcon ImageNet 6; DRI OCT Triton, Topcon Corporation). TAO activity was assessed through the Clinical Activity Score (CAS). According to EUGOGO (European Guidelines for European Basedow ophthalmopathy), patients were divided into non-active TAO (CAS < 3) and active TAO (CAS ≥ 3).

OCT swept-source angiography scans were obtained using OCT Topcon ImageNet 6 (DRI OCT Triton, Topcon Corporation) from an area of 4.5 × 4.5 mm^2 centered on the optic nerve. For each patient were considered images of the four examined layers: superficial capillary plexus (SCP), deep capillary plexus (DCP), external retina and choriocapillaris. Each image was used to calculate, through the ImageJ software, the peripapillary region's Vascular Index. Pictures were converted from black and white into binary images. Afterwards, colours channel has been adjusted through "colour

threshold" in order to visualize vascular structures. Eventually, it was possible to obtain peripapillary region by drawing two ellipses around it (270X230 nm and 60X60 nm) and visualize the Vascular Index of the interested area.

Statistical analysis

Normality was tested with Shapiro-Wilk test and parametric test were used.

T-Test was used to compare vascular index between TAO patients and control groups, whereas Pearson's correlation coefficient test was run to evaluate correlations between Hertel exophthalmometry, IOP, CAS score, visual acuity and vascular indices.

One-way ANOVA was run to evaluate the difference between active Tao and inactive TAO for vascular indices. ROC curves were obtained in order to evaluate cut-off value. See Figure 1.

P values < 0.05 were considered statistically significant.

Statistical analysis was performed using IBM Statistical Package for Social Sciences version 26.

Results

Demographic characteristics

Twenty-six patients with TAO (mean age 53,8 ± 15,2) divided in 19 females (mean age 54.7 ± 5.2) and 7 males (mean age 51.4 ± 16.3) were compared with 26 healthy subjects divided in 15 females (mean age 48.2 ± 14.1) and 11 males (mean age 53.1 ± 15.2).

Hertel exophtalmometry ranged from 13 to 26 mm (mean 20,51 mm, SD 3,49), whereas mean intraocular pressure was 14,26 (SD 2,65); at the clinical assessment, mean visual acuity was 1,00 logMar (SD 0,08) and mean CAS was 3,11 (SD 1,36). Eight patients (30,8%) had inactive TAO (CAS<3), whereas 18 patients (69,2%) had active TAO (CAS ≥3).

Values of deep capillary plexus (DCP) were significantly reduced in TAO patients compared to control eyes (28,6 ± 2,1 vs. 29,7 ± 9,3, p=0,002); also, values of choriocapillary (CC) were reduced compared to control group (46,5 ± 1,72 vs. 47,2 ± 1,2, p=0,019). On the other hand, no statistical correlation was encountered in superficial capillary plexus (SCP) and outer retina (OR) between TAO patients and control group. See Table 3.

Comparing peripapillary vascular indices, CAS, IOP, HERTEL and Visual acuity, we observed that CC was associated with elevated values of CAS (ρ=0,462, p=0,018). Also, a significant correlation was observed between Hertel exophthalmometry values and CAS score in TAO patients (r=0.80, p<0,001). On the other hand, no statistical correlations were encountered comparing CAS, IOP, visual acuity and vascular indices. See Table 4.

Regarding CC plexus, there was a statistically significant difference between active and inactive TAO patients as determined by one-way ANOVA (active TAO mean 47,36, SD=1,74; inactive TAO patients mean 45,58, SD=1,21; p=0,016). Roc curve showed that patients with elevated CC vascular indices were correlated with active TAO (CAS >3), as area under curve (AUC) was 0,813, Youden's index was 0,597 and cut off value was 46,87 (p= 0,012). On the other hand, neither SCP, DCP and OR were associated with active TAO.

Discussion

TAO is a common orbital disorder that can lead to dysthyroid optic neuropathy (DON) by compression of the optic nerve by congested tissues and extraocular muscles. The role of vascular blood flow in the pathogenesis of optic neuropathy has been widely investigated with several methods; different studies with Doppler ultrasonography have demonstrated that increase in intra-orbital pressure in TAO patients leads to severe stasis in the superior ophthalmic vein [19,54]; also, other studies observed an increased resistance in ophthalmic artery and in central retinal artery [56] in TAO patients, which decreases after orbital decompression [57]. Some studies with OCTA observed a reduction in macular density in patients with active and inactive TAO [58], other studies observed a greater macular density in inactive TAO patients[4] and other studies showed an increase in macular density in active TAO patients [45]. Also, several studies showed a reduction of fibers in the RNFL layer [59], and recent studies showed that patients with active TAO present with choroidal thickness [8,44,48].

The main result of our study is that peripapillary CC is decreased in TAO patients compared to control groups (p=0,019); also, we observed that CC was increased in

active TAO patients compared to inactive TAO (p=0,016), suggesting that CC alterations are firstly involved in the active phase of TAO. Decrease in CC density in TAO patients compared to control group could be secondary to the vascular obstruction determined by extraocular muscles and soft tissues; on the other hand, active TAO is characterized by an hyperdynamic cardiovascular state that could explain elevated values of CC in active TAO patients compared to inactive TAO.

Also, we observed that DCP was significantly decreased in TAO patients compared to control group (p=0,002). However, we observed that changes in peripapillary vessels did not interfere with visual acuity and intraocular pressure, suggesting that optic nerve damage has a complex and multifactorial pathophysiology.

To our knowledge this is the first investigation that studies changes in peripapillary vascularization in TAO patients using OCTA. In accordance to our findings, a recent study by *Wu et al* (2020) showed that both inner intra-retinal layers around the macula, superficial and deep capillary plexus were lower in TAO patients compared to control group, suggesting the idea that retinal alterations occur prior to changes in visual acuity and that OCTA could be used in the early detection of alterations in the optic nerve in TAO [60].

The main limitation of this study was the small number of patients enrolled; secondary, TAO patients were not divided according to the presence of dysthyroid optic neuropathy (DON) in order to quantify the effect of microvascular alterations in accordance with clinical assessment.

Future studies are needed to confirm our findings with the goal of identifying first alterations in optic nerve using OCTA, in order to prevent DON and irreversible loss of visual acuity.

CC and DCS peripapillary vascular plexus were reduced in TAO patients compared to control group. However, when comparing active TAO patients and inactive TAO patients, choriocapillary vascular index was increased in active TAO, suggesting a primary role of peripapillary microvascular alterations in the development of DON.

Figures and Tables

Score	Finding
0	**N**o signs and symptoms
1	**O**nly signs
2	**S**oft tissue involvement with symptoms and signs
3	**P**roptosis (≥20 mm)
4	**E**xtraocular muscle involvement
5	**C**orneal involvement
6	**S**ight loss (VA ≤0.67)

Table 1. NO SPECS classification of clinical assessment

Item	Parameter asses
1	Spontaneous retrobulbar pain
2	Pain on attempted upward or downward gaze
3	Eyelid erythema
4	Eyelid oedema
5	Conjunctival hyperhaemia
6	Conjunctival chemosis
7	Inflammation of caruncle or plica

Table 2. Clinical Activity Score (CAS). One point given for each parameter.

Classified as inactive if CAS is less than three and active if CAS is three or more.

	TAO group	Control group	p value
Superficial capillary plexus, mean (SD)	39,57 (3,09)	40,4812 (1,6149)	p= 0.082
Deep capillary plexus, mean (SD)	28,60 (2,10)	29,7213 (0,93685)	p=0.002
Outer retina plexus, mean (SD)	37,99 (2,94)	37,8758 (1,35873)	p=0.798
Choriocapillary plexus, mean (SD)	46,52 (1,72)	47,221 (1,20444)	p=0.019

Table 3. Values of peripapillary in superficial capillary plexus (SCP), deep capillary plexus (DCP), external retina and choriocapillaris

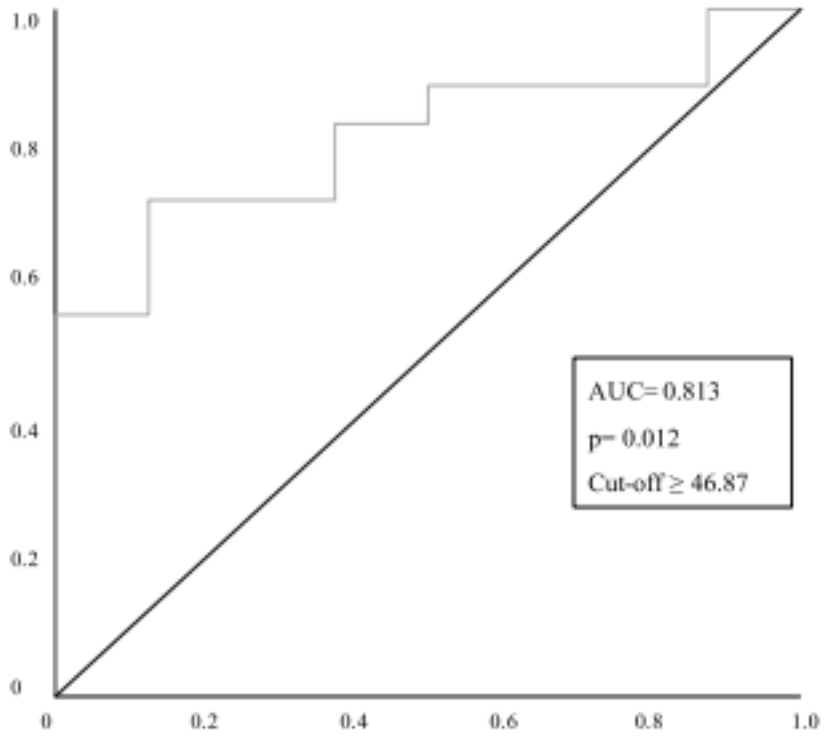

Figure 1. ROC curve analysis was performed to test cutof value of CC vascular fow index sufcient to discriminate between patients with active and inactive TAO. TAO Patients with ≥ 46.87% of CC peripapillary vascular fow index are more likely to present activity of the disease. The area under the curve (AUC), cutof values and p-values are listed in the panel

Conclusions

In summary, our studies propose to identify haemodynamic characteristics in patients with TAO that can be correlated with the stage of the disease in order to prevent complications, implement therapy strategies and preserve vision in TAO patients by the use of non-invasive tools such as OCT and Angio-OCT.

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