Altered plasma cytokine levels in acute and chronic central serous chorioretinopathy

Izabella Karska-Basta,1 Weronika Pociej-Marcia,1 Michał Chrząszcz,1 Agnieszka Kubicka-Trzaska,1 Bożena Romanowska-Dixon1 and Marek Sanak2

1Faculty of Medicine, Department of Ophthalmology, Clinic of Ophthalmology and Ocular Oncology, Jagiellonian University Medical College, Kraków, Poland
2Faculty of Medicine, Department of Internal Medicine, Molecular Biology and Clinical Genetics Unit, Jagiellonian University Medical College, Kraków, Poland

ABSTRACT.

Purpose: To evaluate plasma levels of selected cytokines and investigate their correlation with choroidal thickness (CT) in patients with acute and chronic central serous chorioretinopathy (CSC).

Methods: We enrolled 30 patients with acute CSC, 30 patients with chronic CSC and 20 controls. Plasma concentrations of 12 cytokines, interleukins IL-8, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10 and IL-12 p70, granulocyte-macrophage colony-stimulating factor, interferon-γ, tumour necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF), were measured using multiplex immunoassays. Differences in cytokine levels between groups were assessed. We also investigated correlations between cytokine levels and CT using swept-source optical coherence tomography, as well as an association between plasma cytokine profile and systemic hypertension.

Results: We noted differences in IL-6 (p = 0.005), IL-10 (p = 0.03), IL-12 p70 (p = 0.028) and VEGF (p = 0.029) levels between groups. Pro-inflammatory IL-12 p70 and multidirectional IL-10 cytokines were upregulated, while pro-angiogenic VEGF was downregulated in chronic CSC as compared with controls (p = 0.005, p = 0.025 and p = 0.027, respectively). Interleukin-6 (IL-6) was upregulated in acute and chronic CSC (p = 0.030 and p = 0.005, respectively). Interleukin-5 (IL-5), IL-6 and IL-12 levels correlated with mean CT in acute CSC (p = 0.008, p = 0.003 and p = 0.044, respectively), while IL-8, IL-6 and TNF-α plasma levels correlated with hypertension in chronic CSC (p = 0.005, p = 0.033 and p = 0.001, respectively).

Conclusion: We provided new evidence for the possible role of plasma cytokines in the pathogenesis of CSC. Our results suggest that IL-6 may be important in the pathophysiology of acute and chronic CSC. The association between inflammatory response and hypertension in patients with CSC was also confirmed.

Key words: central serous chorioretinopathy – choroidal thickness – cytokines – hypertension – inflammation – interleukins – VEGF

Introduction

Central serous chorioretinopathy (CSC) is the fourth most common retinopathy after age-related macular degeneration (AMD), diabetic retinopathy and retinal vein occlusion (Wang et al. 2008). Progression of CSC over time may lead to vision disorder and deteriorated quality of life (Karska-Basta et al. 2020). There are two types of CSC: acute and chronic, with a threshold of between 4 and 6 months adopted in most published reports to distinguish between both entities (Daruiach et al. 2015). The disease is characterized by the presence of serous subretinal fluid, retinal pigment epithelial damage, as well as dilated choroidal vessels (so-called pachyvessels) and their hyperpermeability on indocyanine green angiography (Jirarattanasopa et al. 2012; Cheung et al. 2019; van Haalen et al. 2020).

As the pathogenesis of CSC has not been fully elucidated (Daruiach et al. 2015), there is currently no effective treatment (Pociej-Marcia et al. 2016; Lee et al. 2019). Numerous studies have emphasized the key role of choroidal thickness (CT) in the pathogenesis of CSC, which is nowadays considered as one of the pachychoroid diseases (Kim et al. 2011; Sakurada et al. 2018).

Central serous chorioretinopathy (CSC) was reported to be a potential risk factor for coronary artery disease in men as well as an independent risk...
factor for ischaemic stroke, which could indicate an association with other cardiovascular diseases (Tsai et al. 2013; Chen et al. 2014). Moreover, persistently elevated levels of plasma cytokines have been reported in patients with a history of vascular dysfunction and cardiovascular diseases (Sprague & Khalil 2009). However, data on the links between the upregulation of plasma cytokine levels and CSC are inconsistent (Lim et al. 2010; Terao et al. 2018). There have been only few studies indicating that dysregulation of aqueous humour (AH) cytokine levels may be associated with CSC (Shin & Lim 2011; Terao et al. 2018). To date, only one report describing the lack of an association between plasma cytokine levels [vascular endothelial growth factor (VEGF) and interleukin IL-8] and CSC has been published (Lim et al. 2010).

The aim of this study was to analyse the potential role of plasma cytokines: interleukins IL-8, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10 and IL-12 p70, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ, tumour necrosis factor-α (TNF-α) and VEGF in the pathogenesis of acute and chronic CSC.

Materials and Methods

Study population

We screened 87 white patients over the age of 18 years with a diagnosis of CSC, of whom 49 men and 11 women were finally included in this case-control study. The research was conducted at the Clinic of Ophthalmology and Ocular Oncology in Kraków, Poland, from November 2017 to the end of May 2018. The diagnosis of CSC was based on characteristic fundus findings, fluorescein angiography (FA), fundus autofluorescence and swept-source optical coherence tomography (SS-OCT). General exclusion criteria were as follows: any acute illness, C-reactive protein levels higher than 10 mg/l, renal or hepatic dysfunction, cancer, acute myocardial infarction or stroke, anticoagulation and corticosteroid treatment. Ocular exclusion criteria included uveitis, choroidal neovascularization (CNV), retinal vasculitis, polypoidal choroidal vasculopathy or other maculopathies causing macular exudation. We excluded 27 patients due to comorbidities. The control group comprised 20 individuals from the general population sample, who were matched for age, sex, smoking and hypertension. This study was conducted in accordance with the tenets of the Declaration of Helsinki. The study was approved by Jagiellonian University Bioethical Committee (no. 122.6120.266.2016), and all patients provided written informed consent to participate in the study.

Clinical examination

All patients and controls underwent a complete ophthalmological examination, including best-corrected visual acuity (BCVA) assessment, fundus biomicroscopy and SS-OCT (DRI OCT Atlantis, Topcon, Japan). Fluorescein angiography (FA) was performed only in the CSC group (SPECTRALIS, Heidelberg Engineering, Germany).

Acute CSC was diagnosed based on the presence of typical clinical features and symptoms lasting less than 6 months. In the acute form, FA detected one or several leakage points at the level of the retinal pigment epithelium, while SS-OCT revealed pigmented epithelial detachment or subretinal fluid (or both) as well as increased CT. When persistent subretinal fluid on SS-OCT was observed for longer than 6 months and widespread areas of fluorescein leakage from the sites of retinal pigment epithelial damage were seen on FA, chronic CSC was diagnosed.

The assessment of CT was based on the method previously described by Branchini et al. (2013). We performed a horizontal SS-OCT B-scan, and CT was measured from the Bruch’s membrane to the inner wall of the sclera at three points: beneath the fovea and then 750 µm temporally and 750 µm nasally from the fovea (Fig. 1). The average of these three measurements was considered as mean CT.

Sample collection

Blood samples in all participants were obtained from the antecubital vein in the morning into Vacutainer (BD Life Sciences, Franklin Lakes, NJ, USA). The Magnetic Luminex Performance Assay kit for human high-sensitivity cytokines (FCSTM09-12; R&D Systems, Bio-Technne, Minneapolis, MN, USA) was used to measure the concentrations of 12 different cytokines in blood plasma. Samples obtained from all participants were tested. The multiplex immunoassay contains premixed fluorogenic beads with monoclonal antibodies against GM-CSF, interferon-γ, IL-8, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p70, TNF-α and VEGF. The measurements were done according to the manufacturer’s protocol using 1:2 diluted plasma and the xMAP analyser (Luminex Corporation, Austin, TX, USA). Bead-trapped cytokines were detected by biotin-
The demo-
tension were noted between patients
smoking status or prevalence of hyper-
micropulse laser, oral mineralocorti-
photodynamic therapy, subthreshold
form, depending on indications. Treat-
ment modalities included half-dose
photodynamic therapy, subthreshold
micropulse laser, oral microbial-in-
fection, monoclonal antibody or topical
nonsteroidal anti-inflammatory drugs.
No differences in sex distribution, age,
smoking status or prevalence of hyper-
tension were noted between patients
with CSC and controls. The demo-
graphic and clinical characteristics as
well as data on comorbidities in the
study groups are presented in Table 1.
There were no patients with diabetes,
ulcerative colitis or obesity. We exclud-
27 patients with comorbidities that
might have affected the results
(e.g., acute inflammatory disorders or
any conditions associated with elevated
C-reactive protein levels). Any comor-
bidities that did not constitute an
exclusion criterion are presented in
Table 1. We did not observe any asso-
ciation between those underlying con-
ditions and the obtained results.
We noted significant differences in
BCVA and mean CT between the three
groups (Table 1). Among the 12
cytokines analysed, there were signif-
icant differences in plasma levels of IL-6
(p = 0.005), IL-10 (p = 0.030), IL-12
p70 (p = 0.028) and VEGF (p = 0.029)
between groups. The plasma levels of
cytokines in CSC groups and controls
are shown in Table 2. Plasma IL-10
levels were significantly higher in
patients with chronic CSC than in the
control group (Fig. 2). Similarly, IL-12
p70 levels were significantly higher in
chronic CSC than in controls (Fig. 3).
Interestingly, VEGF levels were signif-
icantly lower in patients with chronic
CSC than in controls (Fig. 4). Finally,
patients with chronic and acute CSC had
significantly higher plasma IL-6 levels
than the control group (Fig. 5). There were no significant differences in
plasma cytokine levels between patients
with acute and chronic CSC (data not
shown).
Correlations between mean CT and
cytokine plasma levels in patients with
acute CSC, chronic CSC and controls
are summarized in Table 3. Of note,
positive correlations were observed
only in patients with acute CSC: for
mean CT and plasma IL-5, IL-6 and
IL-12 p70 levels. In patients with acute
and chronic CSC, we noted negative
but nonsignificant correlations between
mean CT and plasma VEGF levels.
This was in contrast to the control
group, which showed a positive corre-
lation between these variables.
Interestingly, only in the chronic
CSC group, individuals with systemic
hypertension had higher median (IQR)
plasma levels of IL-8 [2.95 pg/ml
(2.53–3.47) versus 1.56 pg/ml
(1.34–
2.06); p = 0.005], IL-6 [3.6 pg/ml
(3.1–
4.5) versus 2.1 pg/ml (1.4–3.0);
p = 0.033] and TNF-α [4.09 pg/ml
(4.09–4.25) versus 3.33 pg/ml
(2.95–
3.79); p = 0.001], as compared with
those without hypertension. There were
no differences in the median (IQR)
plasma levels of cytokines between
patients with and without hypertension
in the acute CSC group [IL-8: 1.69 pg/
ml (1.29–3.09) and 1.67 pg/ml (1.19–
1.99), p = 0.830, respectively; IL-6:
2.2 pg/ml (2.00–3.1) and 2.2 pg/ml
(1.5–2.6), p = 0.635, respectively;

Table 1. Demographic and clinical characteristics of patients with acute and chronic central serous chorioretinopathy and controls.

| Parameter | Acute CSC (n = 30) | Chronic CSC (n = 30) | Control (n = 20) | p-value |
|-----------|------------------|-------------------|----------------|----------|
| Female sex, n (%) | 5 (17) | 6 (20) | 9 (45) | 0.056 |
| Age, years | 42.7 ± 9.9 | 44.5 ± 6.1 | 39.2 ± 7.4 | 0.078 |
| Hypertension, n (%) | 11 (37) | 6 (20) | 4 (20) | 0.260 |
| Hashimoto’s thyroiditis, n (%) | 1 (3) | 2 (6) | 0 (0) | 0.781 |
| Helicobacter pylori infection, n (%) | 0 (0) | 6 (20) | 0 (0) | 0.007 |
| Gout, n (%) | 1 (3) | 2 (6) | 0 (0) | 0.781 |
| Ischaemic heart disease, n (%) | 1 (3) | 1 (3) | 0 (0) | 1.000 |
| Smoking, n (%) | 7 (23) | 8 (27) | 7 (35) | 0.658 |
| (current, former) | | | | |
| CT, µm | 421.5 ± 85.3 | 406.1 ± 88.1 | 317.4 ± 61.4 | < 0.001 |
| Affecte eye, n (%) | | | | |
| Right | 14 (47) | 10 (33) | – | 0.225 |
| Left | 13 (43) | 12 (40) | – | |
| Both eyes | 3 (10) | 8 (27) | – | |
| BCVA, n (%) | | | | |
| 0.5±<5<1.0 | 20 (67) | 23 (77) | 20 (100) | 0.017 |
| 0.1±<5<0.5 | 10 (33) | 7 (23) | 0 | |

Normally distributed variables are shown as mean ± SD.
BCVA = best-corrected visual acuity, CSC = central serous chorioretinopathy, CT = choroidal thickness.
Table 2. Plasma cytokine levels in patients with acute and chronic central serous chorioretinopathy and controls.

| Cytokine         | Acute CSC (n = 30) | Chronic CSC (n = 30) | Control (n = 20) | p-value |
|------------------|-------------------|----------------------|-----------------|---------|
| TNF-α, pg/ml     | 3.11 (2.57–3.79)  | 3.48 (3.03–3.94)    | 3.26 (2.65–3.79)   | 0.429   |
| VEGF, pg/ml      | 7.74 (4.23–17.87) | 7.70 (3.58–12.46)  | 17.26 (8.59–27.10) | 0.029   |
| IL-1β, pg/ml     | 0.27 (0.20–0.36)  | 0.28 (0.18–0.41)    | 0.23 (0.19–0.35)   | 0.955   |
| IL-2, pg/ml      | 0.93 (0.55–1.33)  | 0.85 (0.70–1.50)    | 0.70 (0.55–0.93)   | 0.150   |
| IL-4, pg/ml      | 3.69 (0.51–5.31)  | 4.23 (2.06–7.43)    | 2.06 (1.01–5.31)   | 0.319   |
| IL-5, pg/ml      | 0.25 (0.20–0.30)  | 0.25 (0.20–0.35)    | 0.19 (0.15–0.27)   | 0.064   |
| IL-6, pg/ml      | 2.18 (1.73–2.86)  | 2.29 (1.52–3.56)    | 1.52 (1.10–1.95)   | 0.005   |
| IL-8, pg/ml      | 1.68 (1.29–2.12)  | 1.67 (1.36–2.68)    | 2.49 (1.68–3.28)   | 0.103   |
| IL-10, pg/ml     | 0.37 (0.30–0.43)  | 0.37 (0.30–0.51)    | 0.30 (0.23–0.39)   | 0.030   |
| IL-12 p70, pg/ml | 2.73 (2.06–3.46)  | 2.73 (2.39–3.46)    | 2.23 (1.91–2.73)   | 0.028   |
| GM-CSF, pg/ml    | 0.25 (0.19–0.32)  | 0.30 (0.23–0.36)    | 0.32 (0.25–0.49)   | 0.114   |
| Interferon-γ, pg/ml | 2.62 (1.52–3.84)  | 2.06 (1.52–3.43)    | 1.87 (1.52–2.24)   | 0.238   |

Data are shown as median (interquartile range). Significant correlations are presented in bold. CSC = central serous chorioretinopathy, GM-CSF = granulocyte-macrophage colony-stimulating factor, IL = interleukin, TNF-α = tumour necrosis factor-α, VEGF = vascular endothelial growth factor.

Fig. 2. Box-and-whisker plot of plasma interleukin-10 levels in patients with acute and chronic central serous chorioretinopathy and controls.

Fig. 3. Box-and-whisker plot of plasma interleukin-12 p70 levels in patients with acute and chronic central serous chorioretinopathy and controls.

TNF-α: 3.18 pg/ml (2.57–4.09) and 3.03 pg/ml (2.57–3.79), p = 0.846, respectively. Similarly, there were no differences in the median (IQR) levels of cytokines between patients with and without hypertension in the control group [IL-8: 2.00 pg/ml (1.46–2.65) and 2.60 pg/ml (1.68–3.74), p = 0.385, respectively; IL-6: 1.9 pg/ml (1.5–2.6) and 1.4 pg/ml (1.1–1.7), p = 0.064, respectively; TNF-α: 3.03 pg/ml (2.29–3.33) and 3.33 pg/ml (2.73–3.94), p = 0.249, respectively]. To eliminate bias, an additional analysis to compare IL-6, IL-8 and TNF-α levels in patients with hypertension between the groups with acute CSC, chronic CSC and controls was performed. The cytokine levels were highest in patients with chronic CSC. There were significant differences in the levels of IL-6 and TNF-α between groups (p = 0.042 and p = 0.005, respectively), while differences in IL-8 had borderline significance (p = 0.053). The results are presented in Table 4.

Most positive correlations between ILs were similar in patients with acute and chronic CSC. Interestingly, the control group showed the lowest number of significant correlations between the cytokines, and these correlations were also weaker than those in patients with CSC. The correlations between the plasma levels of all analysed cytokines in patients with acute and chronic CSC and controls are shown in Table 5.

Discussion

The role of cytokines in CSC and their potential mechanism of action are still not well established. Cytokines were suggested to be involved in ocular diseases that contribute to choroidal abnormalities (Schellevis et al. 2018; Weinstein & Pepple 2018). To our knowledge, our study is the first comprehensive analysis of various cytokines in the plasma of patients with acute and chronic CSC as compared with healthy individuals.

We noted differences in plasma levels of IL-6, IL-10, IL-12 p70 and VEGF between the three study groups. Some of the analysed cytokines are involved in retinal and choroidal hyperpermeability, while the role of hyperpermeability of the thickened choroid in the pathogenesis of CSC has been emphasized in several recent
studies (Kim et al. 2011; Chung et al. 2016; Sakurada et al. 2018). Interleukin-6 (IL-6) and VEGF alter the junction integrity and downregulate occludin and zonula occludens-1 (Behzadian et al. 2003; Murakami et al. 2012; Liu et al. 2016; Yun et al. 2017). Moreover, IL-6 activates VEGF production by the STAT3 protein (Yun et al. 2017), which causes increased vascular permeability and angiogenesis (Mesquida et al. 2014). Interleukin-12 (IL-12) regulates angiogenesis (Zhou et al. 2016) and induces T-helper type 1 (Th1)-specific immune responses (Mantelli et al. 1993).

Interleukin-10 (IL-10) is an essential anti-inflammatory cytokine responsible for negative regulation of immune responses to microbial antigens (Rutz & Ouyang 2016). It is known to exert multidirectional and complex actions, including the immune regulation of autoimmune diseases (Jung et al. 2019). It is generally classified as an anti-inflammatory cytokine; however, it is important to note that its pro-inflammatory activity in vivo has also been reported (Sharif et al. 2004; Mühl 2013).

Some investigators showed that serum IL-1β, IL-6, IL-8, IL-10, IL-17, IL-22, IL-23 and TNF-α play an important role in uveitis, and their function is related to pathologic Th17 cells (Zelazowska-Rutkowska et al. 2017; Weinstein & Pepple 2018). Experimental studies have reported Th17 cells to be the key mediators of inflammatory eye disease (Weinstein & Pepple 2018). A relationship between the systemic upregulation of cytokines in patients with neovascular AMD has also been postulated (Zehetner et al. 2014; Faber et al. 2015; Nassar et al. 2015), but the results of recent studies indicate that AMD is related to dysregulation of immune intraocular factors, whereas plasma cytokine levels are not elevated, as compared with controls (Fauser et al. 2015; Agrawal et al. 2019). Moreover, the role of inflammation and the involvement of intraocular cytokines in macular oedema have been postulated (Daruich et al. 2018).

We identified only one report in the literature describing the involvement of plasma cytokines (VEGF and IL-8) in the pathophysiology of CSC (Lim et al. 2010). Lim et al. revealed no differences in plasma IL-8 and VEGF levels between CSC and controls (Lim et al. 2010), but our study showed a significant upregulation of plasma IL-6, IL-10 and IL-12 p70 levels in patients with chronic CSC. Contrary to Lim et al. (2010), our results support the association between plasma cytokine dysregulation and CSC.

More data are available on AH than on plasma cytokine levels in CSC, but the results are inconsistent. Terao et al. (2018) noted that AH IL-6 and IL-8 levels were upregulated in patients with chronic CSC. Some investigators did not find any difference in IL-6, IL-8, monocyte chemo-attractant protein-1 and VEGF levels in AH between patients with CSC and controls (Lim et al. 2010; Shin & Lim 2011). However, it may be due to the fact that AH in control groups in those studies was obtained mostly from young patients with cataract, which can indicate the coexistence of some other general or eye diseases (Lim et al. 2010; Shin & Lim 2011). In line with the study by Terao et al. (2018), we hypothesize that intraocular and systemic upregulation of IL-6 levels may be involved in the pathophysiology of CSC.

Surprisingly, we noted a lower plasma VEGF concentration in patients with chronic CSC, as compared with healthy individuals. The cytokine was downregulated only in chronic CSC, which is consistent with the findings reported by Shin & Lim (2011). However, unlike in our study, they analysed the cytokine profile in AH samples. No differences in plasma VEGF levels between patients with CSC and controls were reported by Lim et al., but the sample size was small (Lim et al. 2010). The VEGF
Table 3. Correlations between mean choroidal thickness and plasma cytokine levels in patients with acute and chronic central serous chorioretinopathy and controls.

| Cytokine       | Mean CT                  | Acute CSC (n = 30) | Chronic CSC (n = 30) | Control (n = 20) | p-value |
|----------------|--------------------------|--------------------|----------------------|------------------|--------|
| TNF-α          | r = 0.004                | r = −0.245         | r = −0.075           |                  |        |
|                | p = 0.982                | p = 0.192          | p = 0.752            |                  |        |
| VEGF           | r = −0.035               | r = −0.299         | r = 0.173            |                  |        |
|                | p = 0.857                | p = 0.108          | p = 0.466            |                  |        |
| IL-1β          | r = 0.093                | r = −0.136         | r = −0.155           |                  |        |
|                | p = 0.630                | p = 0.472          | p = 0.515            |                  |        |
| IL-2           | r = 0.453                | r = −0.154         | r = −0.116           |                  |        |
|                | p = 0.114                | p = 0.415          | p = 0.627            |                  |        |
| IL-4           | r = 0.283                | r = −0.079         | r = −0.024           |                  |        |
|                | p = 0.137                | p = 0.677          | p = 0.920            |                  |        |
| IL-5           | r = 0.488                | r = −0.228         | r = −0.087           |                  |        |
|                | p = 0.007                | p = 0.227          | p = 0.716            |                  |        |
| IL-6           | r = 0.532                | r = −0.295         | r = −0.110           |                  |        |
|                | p = 0.003                | p = 0.114          | p = 0.644            |                  |        |
| IL-8           | r = 0.235                | r = −0.261         | r = 0.229            |                  |        |
|                | p = 0.220                | p = 0.164          | p = 0.331            |                  |        |
| IL-10          | r = 0.297                | r = −0.150         | r = −0.018           |                  |        |
|                | p = 0.118                | p = 0.429          | p = 0.939            |                  |        |
| IL-12 p70      | r = 0.376                | r = 0.122          | r = 0.188            |                  |        |
|                | p = 0.044                | p = 0.522          | p = 0.428            |                  |        |
| GM-CSF         | r = 0.218                | r = −0.154         | r = 0.119            |                  |        |
|                | p = 0.256                | p = 0.417          | p = 0.618            |                  |        |
| Interferon-γ   | r = 0.250                | r = −0.141         | r = −0.188           |                  |        |
|                | p = 0.191                | p = 0.456          | p = 0.427            |                  |        |

Data are presented as Spearman rho correlation coefficient; p < 0.05 was considered significant (bold).

CSC = central serous chorioretinopathy, GM-CSF = granulocyte-macrophage colony-stimulating factor, IL = interleukin, TNF-α = tumour necrosis factor-α; VEGF = vascular endothelial growth factor.

Table 4. Plasma cytokine levels in patients with systemic hypertension according to the study group.

| Cytokine       | Mean CT                  | Acute CSC (n = 11) | Chronic CSC (n = 6) | Controls (n = 4) | p-value |
|----------------|--------------------------|--------------------|---------------------|------------------|--------|
| IL-6           | 2.2 (2.0–3.1)            | 3.6 (3.1–4.5)      | 1.9 (1.5–2.6)       |                  | 0.042* |
|                |                          |                    |                     |                  | 1.009† |
|                |                          |                    |                     |                  | 0.144‡ |
|                |                          |                    |                     |                  | 0.111§ |
| IL-8           | 1.69 (1.29–3.09)         | 2.95 (2.53–3.47)   | 2.0 (1.46–2.65)     |                  | 0.053* |
|                |                          |                    |                     |                  | 1.000‡ |
|                |                          |                    |                     |                  | 0.015§ |
|                |                          |                    |                     |                  | 0.018§ |
| TNF-α          | 3.18 (2.57–4.09)         | 4.09 (4.09–4.25)   | 3.03 (2.29–3.33)    |                  | 0.005* |

Data are shown as median (interquartile range). A p-value of <0.05 was considered significant. The p-value for pairwise comparison was adjusted using the Bonferroni correction.

CSC = central serous chorioretinopathy, IL = interleukin, TNF-α = tumour necrosis factor-α.

* Acute CSC versus chronic CSC versus controls.
† Acute CSC versus controls.
‡ Chronic CSC versus controls.
§ Deute CSC versus chronic CSC.

The family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PIGF). Among all factors, the overexpression of PIGF and VEGF-A levels increases vascular permeability (Witmer et al. 2003). In our opinion, altered ocular levels of pro-inflammatory cytokines and growth factors may have significant implications for increased vascular permeability and development of CNV in the course of CSC. However, in our study, patients with chronic CSC, which is associated with a higher incidence of CNV, had lower VEGF plasma levels than controls. This may indicate an imbalance between the levels of VEGF and other growth factors, but such a hypothesis requires further research. Nonetheless, our results are in line with the study by Spaide (2015) and Sacconi et al. (2019). They hypothesized that CNV in CSC is due to proliferation of new vessels during arteriogenesis, which is characterized by dilatation of the existing vascular channels and is independent of VEGF (unlike angiogenesis, which is highly VEGF dependent) (Schierling et al. 2009; Wu et al. 2010).

Based on OCT angiography, Shiragami et al. (2018) reported CNV in 15.6% of cases with acute CSC and in 21.8% of those with chronic CSC. Bousquet et al. (2018) revealed the prevalence of CNV in 35.6% of patients with chronic CSC, with flat irregular pigment epithelial detachment. Sacconi et al. (2019) considered arteriogenesis to be the central driving force of CNV development in the course of CSC, because despite noting some clinical response after anti-VEGF therapy, the density of CNV vessels did not change on OCT angiography. Downregulated plasma VEGF levels in CSC observed in our study may explain the unsatisfactory effect of intravitreal anti-VEGF treatment in patients with CSC, which was reported in meta-analyses (Chung et al. 2013; Ji et al. 2017). In neovascular AMD, the same patients may also show a weak or no reaction to treatment due to nonresponsiveness or tachyphylaxis (Zuber-Lskawiec et al. 2019).

Interestingly, in our study, IL-6 was the only cytokine upregulated in acute and chronic CSC. Multifunctional IL-6 is involved both in the normal acute inflammatory response and in the detrimental low-grade systemic chronic inflammatory process (Morieri et al. 2017). Apart from pleiotropic activity and the key role in host defence against environmental stress, IL-6 also increases vascular permeability (Mesquida et al. 2014). Rochfort et al. noted that this cytokine decreases the expression of claudin 5, occludin and vascular endothelial cadherin in human brain microvascular endothelial cells. They
Table 5. Correlations between plasma cytokine levels in patients with acute and chronic central serous chorioretinopathy and in controls.

| Group/Parameter | IL-1β | IL-2 | IL-4 | IL-5 | IL-6 | IL-8 | IL-10 | IL-12 p70 | TNF-α | VEGF | GM-CSF | Interferon-γ |
|----------------|-------|------|------|------|------|------|-------|-----------|-------|-------|--------|-------------|
| Acute CSC      |       |      |      |      |      |      |       |           |       |       |        |             |
| IL-1β          | 1.000 |      |      |      |      |      |       |           |       |       |        |             |
| IL-2           | 0.359 | 1.000|      |      |      |      |       |           |       |       |        |             |
| IL-4           | 0.383 | 0.852** | 1.000|      |      |      |       |           |       |       |        |             |
| IL-5           | 0.315 | 0.858** | 0.794** | 1.000|      |      |       |           |       |       |        |             |
| IL-6           | 0.336 | 0.789** | 0.750** | 0.715** | 1.000|      |       |           |       |       |        |             |
| IL-8           | 0.369 | 0.311 | 0.238 | 0.216 | 0.530** | 1.000|      |           |       |       |        |             |
| IL-10          | 0.406 | 0.815** | 0.913** | 0.747** | 0.682** | 0.179 | 1.000|           |       |       |        |             |
| IL-12 p70      | 0.365 | 0.657** | 0.675** | 0.750** | 0.445 | 0.074 | 0.591** | 1.000|       |        |        |             |
| TNF-α          | 0.464** | 0.276 | 0.167 | 0.256 | 0.252 | 0.294 | 0.195 | 0.180 | 1.000|       |        |        |             |
| VEGF           | 0.507** | 0.042 | 0.135 | 0.041 | 0.207 | 0.309 | 0.123 | 0.116 | 0.407** | 1.000|       |        |             |
| GM-CSF         | 0.478** | 0.501** | 0.414** | 0.494** | 0.364 | 0.150 | 0.317 | 0.549** | 0.701** | 0.476** | 1.000|       |             |
| Interferon-γ   | 0.429 | 0.625** | 0.619** | 0.498** | 0.663** | 0.521** | 0.542** | 0.436 | 0.404** | 0.425 | 0.555** | 1.000|       |             |
| Chronic CSC    |       |      |      |      |      |      |       |           |       |       |        |             |
| IL-1β          | 1.000 |      |      |      |      |      |       |           |       |       |        |             |
| IL-2           | 0.245 | 1.000|      |      |      |      |       |           |       |       |        |             |
| IL-4           | 0.389 | 0.879** | 1.000|      |      |      |       |           |       |       |        |             |
| IL-5           | 0.224 | 0.919** | 0.821** | 1.000|      |      |       |           |       |       |        |             |
| IL-6           | 0.328 | 0.901** | 0.811** | 0.857** | 1.000|      |       |           |       |       |        |             |
| IL-8           | 0.341 | 0.170 | 0.210 | 0.071 | 0.224 | 1.000|      |           |       |       |        |             |
| IL-10          | 0.365 | 0.825** | 0.824** | 0.754** | 0.821** | 0.273 | 1.000|           |       |       |        |             |
| IL-12 p70      | 0.305 | 0.630** | 0.738** | 0.543** | 0.582** | 0.216 | 0.698** | 1.000|       |        |        |             |
| TNF-α          | 0.166 | 0.511** | 0.498** | 0.400** | 0.588** | 0.454 | 0.465 | 0.500** | 1.000|       |        |        |             |
| VEGF           | 0.242 | −0.148 | −0.121 | −0.156 | −0.129 | 0.351 | −0.005 | −0.065 | 0.205 | 1.000|       |        |             |
| GM-CSF         | 0.104 | 0.103 | 0.222 | 0.055 | −0.111 | 0.089 | 0.233 | 0.296 | 0.038 | 0.310 | 1.000|       |             |
| Interferon-γ   | 0.265 | 0.848** | 0.734** | 0.839** | 0.871** | 0.119 | 0.839** | 0.552** | 0.365 | −0.096 | −0.062 | 1.000|       |             |
| Controls       |       |      |      |      |      |      |       |           |       |       |        |             |
| IL-1β          | 1.000 |      |      |      |      |      |       |           |       |       |        |             |
| IL-2           | 0.138 | 1.000|      |      |      |      |       |           |       |       |        |             |
| IL-4           | 0.217 | 0.578** | 1.000|      |      |      |       |           |       |       |        |             |
| IL-5           | 0.303 | 0.798** | 0.546** | 1.000|      |      |       |           |       |       |        |             |
| IL-6           | −0.016 | 0.858** | 0.688** | 0.790** | 1.000|      |       |           |       |       |        |             |
| IL-8           | −0.057 | −0.239 | −0.136 | −0.075 | −0.201 | 1.000|      |           |       |       |        |             |
| IL-10          | 0.183 | 0.738** | 0.201 | 0.576** | 0.469** | −0.284 | 1.000|           |       |       |        |             |
| IL-12 p70      | 0.115 | 0.279 | 0.204 | 0.333 | 0.214 | 0.202 | 0.268 | 1.000|       |        |        |             |
| TNF-α          | 0.180 | 0.139 | 0.298 | 0.035 | 0.363 | 0.013 | 0.266 | 1.000|       |        |        |             |
| VEGF           | 0.075 | −0.193 | −0.149 | −0.164 | −0.242 | 0.524 | −0.152 | −0.142 | 0.206 | 1.000|       |        |             |
| GM-CSF         | −0.037 | 0.601** | 0.111 | 0.390 | 0.399 | 0.086 | 0.606** | 0.204 | 0.264 | −0.034 | 1.000|       |             |
| Interferon-γ   | 0.180 | 0.741** | 0.423 | 0.810** | 0.755** | −0.207 | 0.403 | 0.252 | −0.073 | −0.364 | 0.300 | 1.000|       |             |

Data are presented as Spearman’s rho correlation coefficient. Significant correlations are presented with *p < 0.005; **p < 0.001.

CSC = central serous chorioretinopathy, GM-CSF = granulocyte-macrophage colony-stimulating factor, IL = interleukin, TNF-α = tumour necrosis factor-α, VEGF = vascular endothelial growth factor.
also demonstrated that IL-6 downregulates the expression of tight junction proteins and interendothelial adherence, thus increasing paracellular permeability (Rochfort et al. 2014). As stated above, hyperpermeability of the choriocapillaris and thickened chorioid plays an essential role in the pathogenesis of CSC (Kim et al. 2011; Sakurada et al. 2018). In our study, a positive correlation was noted between IL-5, IL-6, IL-12 and mean CT only in acute CSC. The mechanism of vasodilation of large choroidal vessels in CSC has not been fully elucidated. Given the association between cardiovascular disease and risk factors for vascular eye diseases, we speculate that increased levels of pro-inflammatory cytokines lead to abnormal endothelium-dependent vasodilation (Sitia et al. 2010; Karska-Basta et al. 2011).

It has been shown that glucocorticoids are involved in the pathogenesis of CSC, and glucocorticoid receptors are expressed both in the choroid and retina (Zhao et al. 2010; Brinks et al. 2018). In rats and humans, corticosterone may cause choroidal thickening, a feature typical for patients with CSC (Zhao et al. 2012). When psychologically stressed (a known risk factor for CSC), the body produces stress hormones such as cortisol, which are able to trigger IL-6 release into the circulation (Clark et al. 2019). Moreover, glucocorticoids were shown to enhance IL-6-dependent expression of pro-inflammatory genes by inhibiting the suppressor of cytokine signalling 3, a physiological mechanism that controls acute inflammatory response (Dittrich et al. 2011). Inflammation is recognized as one of the key factors in the pathogenesis of AMD, with glucocorticoid treatment showing no major benefit (Geltzer et al. 2013). We speculate that a similar mechanism may play a role in CSC (and particularly in chronic CSC, as shown in our study). The disease is associated with increased levels of some pro-inflammatory cytokines, but the use of steroid treatment paradoxically worsens rather than alleviates symptoms. It is not clear whether steroids and cytokines exert their effects independently of or in combination with one another in patients with CSC.

Higher levels of IL-6 and IL-12 with pro-atherogenic properties were seen in patients with CSC and a thicker choroid (Sprague & Khalil 2009). It is difficult to explain the positive correlation between CT and plasma IL-5 levels in patients with acute CSC, because IL-5 suppresses VEGF-induced angiogenesis through STAT5 signalling (Bucher et al. 2018). Usually, a decrease in VEGF in intraocular fluids due to intravitreal anti-VEGF therapy leads to a reduction in CT, for example in patients with diabetic retinopathy (Kniggendorf et al. 2016). However, this phenomenon requires further investigation.

A persistent increase of plasma cytokine levels has been reported not only in patients with autoimmune diseases but also in those with a history of cardiovascular disease (Morieri et al. 2017) and vascular dysfunction (Sprague & Khalil 2009), such as atherosclerosis, abdominal aortic aneurysm, varicose veins and systemic hypertension (Wenzel et al. 2016).

The results of our study confirmed positive correlations between systemic hypertension and plasma IL-8, IL-6 and TNF-α levels in chronic CSC. Growing evidence indicates that the immune response affects the pathogenesis of hypertension (Wenzel et al. 2016). Also, T cells contribute to the development of this condition. The expression of specific transcription factors and the profile of cytokines produced by CD4+ T cells are the basis for classification into 4 major subsets: Th1, Th2, Th17 and regulatory T cells (Wenzel et al. 2016). On the other hand, in our study, controls with hypertension without CSC may we speculate that cytokines that were shown to be elevated in our study are also elevated in the general population of patients with hypertension. The cytokines IL-6 (Zhang et al. 2012), IL-8 (Marek-Trzonkowska et al. 2015) and TNF-α (Puszkar ska et al. 2019) are considered to play an important role in the pathogenesis of hypertension, but their excessive levels were also reported to negatively affect the function of numerous organs. Chae et al. (2001) reported increased blood pressure as a stimulus for inflammatory response and hypothesized this to be a mechanism underlying the role of hypertension as a risk factor for atherosclerotic disease. Our study, which revealed elevated levels of selected cytokines in patients with hypertension and chronic CSC, suggests that hypertension may impact choroidal abnormalities in the course of this long-lasting eye disease.

The most important limitations of our pilot study include the fact that the samples were obtained only from plasma and that the study was performed at a single time-point.

In conclusion, we speculate that altered plasma cytokine levels, including downregulation of VEGF and upregulation of IL-6, IL-10 and IL-12 in patients with chronic CSC may reflect an indirect role of these factors in vascular changes observed in the course of this disease. Our results suggest a previously unknown role of plasma cytokines in the pathogenesis of CSC. However, it remains unclear whether abnormalities in intraocular or systemic cytokine levels are more involved in disease development. There is also a possible association between the inflammatory response in CSC and systemic vascular changes. Therefore, further research is needed to confirm this hypothesis.

References
Agrawal R, Balne PK, Wei X et al. (2019): Cytokine profiling in patients with exudative age-related macular degeneration and polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 60: 376–382.
Behzadjan MA, Windsor LJ, Ghaly N, Liu G, Tsai N-T & Caldwell RB (2003): VEGF-induced paracellular permeability in cultured endothelial cells involves urokinase and its receptor. FASEB J 17: 752–754.
Bousquet E, Bonnin S, Mrjen S, Krivosic V, Tadayoni R & Gaudric A (2018): Optical coherence tomography angiography of flat irregular pigment epithelium detachment in chronic central serous chorioretinopathy. Retina 38: 629–638.
Branchini LA, Adhi M, Regatieri CV, Nandakumar N, Liu JJ, Laver N, Fujimoto JG & Duker JS (2013): Analysis of choroidal morphologic features and vasculature in healthy eyes using spectral-domain optical coherence tomography. Ophthalmology 120: 1901–1908.
Brinks J, van Dijk EHC, Habeeb M et al. (2018): The effect of corticosteroids on human choroidal endothelial cells: a model to study central serous chorioretinopathy. Invest Ophthalmol Vis Sci 59: 5682–5692.
Bucher F, Lee J, Shin S, Kim MS, Oh Y-S, Ha S, Zhang H & Yea K (2018): Interleukin-5 suppresses vascular endothelial growth factor-induced angiogenesis through STAXS signaling. Cytokine 110: 397–403.

Chae CU, Lee RT, Rifi RI & Ridker PM (2001): Blood pressure and inflammation in apparently healthy men. Hypertension 38: 399–403.

Chen SN, Chen Y-C & Lian I (2014): Increased risk of coronary heart disease in male patients with central serous chorioretinopathy: results of a population-based cohort study. Br J Ophthalmol 98: 110–114.

Cheung CMG, Lee WK, Koizumi H, Dansingani K, Lai TYY & Freund KB (2019): Pachychoroid disease. Eye (Lond) 33: 14–33.

Chung Y-R, Seo EJ, Lew HM & Lee KH (2016): Role of central serous chorioretinopathy: assessment of Haller and Sattler layers. Retina 36: 1652–1657.

Clark SM, Song Cu, Li X, Keegan AD & Tomelli LH (2019): CD8 T cells promote cytokine responses to stress. Cytokine 113: 256–264.

Daruih A, Matet A, Dirani A, Bousquet E, Zhao M, Farman N, Jaisser F & Behar-Cohen F (2015): Central serous chorioretinopathy: recent findings and new physiopathology hypothesis. Prog Retin Eye Res 48: 82–118.

Daruih A, Matet A, Moulin A et al. (2018): Mechanisms of macular edema: beyond the surface. Prog Retin Eye Res 63: 20–68.

Dittrich A, Khouri C, Dutton-Sacket S et al. (2011): Glucocorticoids increase interleukin-6-dependent gene induction by interfering with the expression of the suppressor of cytokine signaling 3 feedback inhibitor. Hepatology 55: 256–266.

Faber C, Jheh T, Juel HB, Singh A, Falk MK, Sorensen TL & Nissen MH (2015): Early and exudative age-related macular degeneration is associated with increased plasma levels of soluble TNF-receptor II. Acta Ophthalmol 93: 242–247.

Fauser S, Viebahn U & Mueller PS (2015): Intraretinal and systemic inflammation-related cytokines during one year of ranibizumab treatment for neovascular age-related macular degeneration. Acta Ophthalmol 93: 734–738.

Geyer A, Turalba A & Vedula SS (2013): Surgical implantation of steroids with antiangiogenic characteristics for treating neovascular age-related macular degeneration. Cochrane Database Syst Rev 1, CD005022.

van Haalen FM, van Dijk EHC, Savas M et al. (2020): Hair cortisol concentrations in chronic central serous chorioretinopathy. Acta Ophthalmol 98: 390–395.

Ji S, Wei Y, Chen J & Tang S (2017): Clinical efficacy of anti-VEGF medications for central serous chorioretinopathy: a meta-analysis. Int J Clin Pharm 39: 514–521.

Jiraratanasop A, Ooto S, Tsujikawa A, Yamashiro K, Hangai M, Hirata M, Matsumoto A & Yoshimura N (2012): Assessment of macular choroidal thickness by optical coherence tomography and angiographic changes in central serous chorioretinopathy. Ophthalmology 119: 1666–1678.

Jung JH, Song GG, Kim J-H & Choi SJ (2019): The associations between interleukin 10 polymorphisms and susceptibility to autoimmune uveitis – a meta-analysis. Curr Eur J Immunol 44: 246–252.

Karskas-Basta I, Kubicka-Trzaska A, Romanowska-Dixon B & Undas A (2011): Altered fibrin clot properties in patients with retinal vein occlusion. J Thromb Haemost 9: 2513–2515.

Karskas-Basta I, Pojce-Marcik B, Chrzaszcz M, Zubcer-Laskawiec K, Sanak M & Romanowska-Dixon B (2020): Quality of life of patients with central serous chorioretinopathy – a major cause of vision threat among middle-aged individuals. Arch Med Sci [Epub ahead of print].

Kim YT, Kang SW & Bai KH (2011): Choroidal thickness in both eyes of patients with unilaterally active central serous chorioretinopathy. Eye (Lond) 25: 1635–1640.

Kniggeford VF, Novais EA, Kniggeford SL, Xavier C, Cole ED & Regatiere CV (2016): Effect of intravitreal anti-VEGF on choroidal thickness in patients with diabetic macular edema using spectral domain OCT. Arq Bras Oftalmol 79: 155–158.

Lee JH, Lee SC, Kim H & Lee CS (2019): Comparison of short-term efficacy between oral spironolactone treatment and photodynamic therapy for the treatment of nonre solving central serous chorioretinopathy. Retina 39: 1809–1817.

Lim JW, Kim MU & Shin M-C (2010): Aqueous humor and plasma levels of vascular endothelial growth factor and interleukin-8 in patients with central serous chorioretinopathy. Retina 30: 1465–1471.

Liu X, Dreffs A, Diaz-Coraguez M, Runkle EA, Gardner TW, Chioldo VA, Hausworth WW & Antonetti DA (2016): Oxaladin S490 phosphorylation regulates vascular endothelial growth factor-induced retinal neovascularization. Am J Pathol 186: 2486–2499.

Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G & Romagnani S (2001): Role of pro-inflammatory cytokines in arteriogenesis. J Vasc Res 38: 1809–1817.

Marek-Trzonkowska N, Kwieczynska A, Reier-Gostomska M, Kolinski T, Molisz A & Siebert J (2015): Arterial hypertension is characterized by imbalance of pro-angiogenic versus anti-angiogenic factors. PLoS One 10: e0126190.
Sharif MN, Tassiulas I, Hu Y, Mecklenbraucker I, Tarakhovsky A & Ivashkov LB (2004): PF-alpha priming results in a gain of proinflammatory function by IL-10: implications for systemic lupus erythematosus pathogenesis. J Immunol 172: 6476–6481.

Shin MC & Lim JW (2011): Concentration of cytokines in the aqueous humor of patients with central serous chorioretinopathy. Retina 31: 1937–1943.

Shiragami C, Takasago Y, Osaka R, Kobayashi M, Ono A, Yamashita A & Hirooka K (2018): Clinical features of central serous chorioretinopathy with type 1 chorioidal neovascularization. Am J Ophthalmol 193: 80–86.

Sitia S, Tomasoni L, Atzeni F et al. (2010): From endothelial dysfunction to atherosclerosis. Autoimmun Rev 9: 830–834.

Spaide RF (2015): Optical coherence tomography angiography signs of vascular abnormalization with antiangiogenic therapy for choroidal neovascularization. Am J Ophthalmol 160: 6–16.

Sprague AH & Khalil RA (2009): Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol 78: 539–552.

Terao N, Koizumi H, Kojima K et al. (2018): Association of upregulated angiogenic cytokines with choroidal abnormalities in chronic central serous chorioretinopathy. Invest Ophthalmol Vis Sci 59: 5924–5931.

Tsai DC, Chen SJ, Huang CC et al. (2013): Epidemiology of idiopathic central serous chorioretinopathy in Taiwan, 2001–2006: a population-based study. PLoS One 8: e66858.

Wang M, Munch IC, Hasler PW, Printe C & Larsen M (2008): Central serous chorioretinopathy. Acta Ophthalmol 86: 126–145.

Weinstein JE & Pepple KL (2018): Cytokines in uveitis. Curr Opin Ophthalmol 29: 267–274.

Wenzel U, Turner JE, Krebs C, Kurts C, Harrison DG & Ehmke H (2016): Immune mechanisms in arterial hypertension. J Am Soc Nephrol 27: 677–686.

Witmer AN, Vrensen GFJM, Van Noorden CJF & Schlingemann RO (2003): Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 22: 1–29.

Yu S, Wu X, Zhu W, Cai WJ, Schaper J & Schaper W (2010): Immunohistochemical study of the growth factors, aFGF, bFGF, PDGF-AB, VEGF-A and its receptor (Flk-1) during arteriogenesis. Mol Cell Biochem 343: 223–229.

Yun J-H, Park SW, Kim K-J et al. (2017): Endothelial STAT3 activation increases vascular leakage through downregulating tight junction proteins: implications for diabetic retinopathy. J Cell Physiol 232: 1123–1134.

Zehetner C, Kirchmair R, Neururer SB, Kralinger MT, Bechrakis NE & Kieselbach GF (2014): Systemic upregulation of PDGF-B in patients with neovascular AMD. Invest Ophthalmol Vis Sci 55: 337–344.

Zelazowska-Rutkowska B, Mrugacz M & Cylwik B (2017): Comparison of the diagnostic power of serum IL-6, IL-8 and TNF-α for the idiopathic anterior uveitis in children. Clin Lab 63: 1889–1895.

Zhang W, Wang W, Yu H et al. (2012): Interleukin-6 underlies angiostatin H-induced hypertension and chronic renal damage. Hypertension 59: 136–144.

Zhao M, Valamanesh F, Celerier I et al. (2010): The neuroretina is a novel mineralocorticoid target: aldosterone up-regulates ion and water channels in muller glial cells. FASEBJ 24: 3405–3415.

Zhao M, Celerier I, Bousquet E et al. (2012): Mineralocorticoid receptor is involved in rat and human ocular chorioretinopathy. J Clin Invest 122: 2672–2679.

Zhou Y, Yoshida S, Kubo Y et al. (2016): Interleukin-12 inhibits pathological neovascularization in mouse model of oxygen-induced retinopathy. Sci Rep 6: 28140.

Zuber-Laskawiec K, Kubicka-Trząska A, Karska-Basta I, Poejeć-Marciač W & Romanowska-Dixon B (2019): Non-responsive-ness and tachyphylaxis to anti-vascular endothelial growth factor treatment in naive patients with exudative age-related macular degeneration. J Physiol Pharmacol 70: 779–785.

Received on March 21st, 2020. Accepted on June 17th, 2020.

Correspondence:
Izabella Karska-Basta, MD, PhD
Department of Ophthalmology and Ocular Oncology
Division of Ophthalmology
Jagiellonian University Medical College
ul. Kopernika 38, 31-501 Kraków
Poland
Tel: +48-12-4247540
Fax: +48-12-4247563
Email: izabasta@gmail.com

This work was supported by Jagiellonian University Medical College (no. K/ZDS/006283; to I. Karska-Basta).