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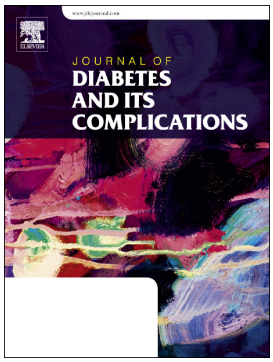

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SEROLOGICAL INFLAMMATORY FACTORS AS BIOMARKERS FOR ANATOMIC RESPONSE IN DIABETIC MACULAR EDEMA TREATED WITH ANTI-VEGF

Systemic biomarkers in diabetic macular edema

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Abstract

Purpose: To study the relationship between systemic pro-inflammatory factors and macular structural response to intravitreal bevacizumab for diabetic macular edema (DME)

Methods: Prospective study including 30 cases with DME, treated with bevacizumab and a minimum follow-up of 6 months. All cases underwent baseline laboratory testing for cardiovascular risk (high sensitivity C-reactive protein (hsCRP), homocystein), dyslipidemia, renal dysfunction and glucose control. Serum levels of VEGF, soluble ICAM-1, MCP-1 and TNF-α were assessed by enzyme-linked immunosorbent assay kits. Significant associations between systemic factors and quantitative and qualitative spectral-domain optical coherence macular features were analyzed

Results: A mean of 4.82±0.56 intravitreal injections was performed, resulting in significant improvement of central foveal thickness (CFT) ($p<0.001$). A significant association with third month CFT decrease < 10% was found for hsCRP (3.33±2.01 vs 1.39±1.15 mg/L, $p=0.007$) and ICAM1 (975.54±265.49 vs 727.07±336.09 pg/ml, $p=0.012$). ROC curve analysis indicated hsCRP and ICAM1 as significant biomarkers for 3rd month reduced anatomic response (area under the curve (AUC)=0.807, $p=0.009$ for hsCRP; AUC=0.788, $p=0.014$ for ICAM1). ROC curve analysis revealed hsCRP as a significant biomarker for 6th month CFT decrease < 10% (AUC=0.903, $p<0.001$, cutoff value=1.81 mg/L). A significant association with 6th month CFT decrease ≥ 25% was found for serum MCP1 (244.69±49.34 pg/ml vs 319.24±94.88 pg/ml, $p=0.017$) and serum VEGF (90.84±37.33 vs 58.28±25.19, $p=0.027$). The combined model of serum VEGF and LDL-cholesterol was found to be predictive of 6th month hard exsudate severity ($p=0.001$, $r2=0.463$)

Conclusions: Increased levels of hsCRP and ICAM1 were found to be significant biomarkers for early reduced anatomic response to anti-VEGF treatment. Cases with higher serum levels of such factors had increased CFT values, despite treatment, suggesting inner blood-retinal barrier breakdown that is not adequately responsive to anti-VEGF monotherapy.

Keywords

Anti-VEGF, Bevacizumab, C-Reactive Protein, Diabetic Macular Edema, Inflammatory biomarkers

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Introduction

Diabetic macular edema (DME) is a major cause of visual impairment in industrialized countries due to increasing prevalence of Diabetes Mellitus[1]. Pivotal trials, revealed that monthly intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors, allowed DME cases to achieve significant gains in VA[2, 3], therefore such agents have become the gold standard treatment for DME. However, a significant percentage of patients won't achieve a sustained response to anti-VEGF injections[4, 5]. Additionally, there is a wide variability in the frequency of injections and duration of treatment required to achieve clinical stability[6, 7]. Therefore, there is increased interest in finding significant predictors of clinical response. While the pathogenesis of DME is not fully understood, it is known that prolonged hyperglycemia leads to activation of pro-inflammatory cascades that in turn cause structural damage to the capillary endothelium[8]. The end result is capillary obliteration leading to retinal ischemia[9] and inner blood-retina-barrier (iBRB) breakdown causing intraretinal fluid accumulation[10]. Indeed, the pioneering studies DCCT[11] and UKPDS[12] identified the importance of metabolic control in delaying DR. More recently, attention shifted to identifying the molecular agents underlying the pathogenesis of DR. It is now well known that VEGF plays a crucial role in retinal angiogenesis and vasopermeability[13, 14]. Additionally, there seems to be increasing evidence that insulin resistance occurring in DM2 is associated with a subclinical pro-inflammatory state identified by increased levels of C-reactive protein and Interleukin-6 (IL6)[15-17]. In the case of diabetic eye disease, there is evidence of increased levels of VEGF, IL6[18], Intracellular Adhesion Molecule 1 (ICAM1)[19] and Monocyte chemoattractant protein-1 (MCP1)[20] in the aqueous or vitreous of patients with advanced DR. Despite, the increasing evidence of pro-inflammatory activity occurring in DR, there is little research on how such biochemical factors can potentially interfere with macular response to current intravitreal treatments. Considering DME is a local manifestation of a complex systemic disease, it is possible that different metabolic and pro-inflammatory profiles portend different diabetic eye disease patterns. In this regard, we evaluated several systemic factors encompassing metabolic, renal, cardiovascular and inflammatory functions known to be associated with the pathogenesis of DM and/or DR, in order to study the possible associations with tomographic features of DME, and to assess their value as possible biomarkers for anatomic response to anti-VEGF treatment.

Methods

This was a prospective interventional study including cases diagnosed and treated for DME at the Ophthalmology Department of Hospital de Braga, Braga, Portugal. Study protocols were submitted to and approved by the Hospital Ethics Committee. The research procedures followed the tenets of the declaration of Helsinki and all patients provided written informed consent for the inclusion in the study. The recruited cases had Diabetes Mellitus type 2 (DM2) and nonproliferative diabetic retinopathy (NPDR) with central-involved diabetic macular edema (DME). All cases underwent complete ophthalmological examination and diagnosis was confirmed by fluorescein angiography and spectral-domain-optical coherence tomography (SD-OCT). The severity of NPDR and foveal involvement was graded based on fluorescein

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angiography findings, according to the international clinical diabetic retinopathy and diabetic macular edema disease severity scales[21]. The following inclusion criteria were considered: cases with DM2, NPDR and central DME with CFT > 330 µm and intraretinal cysts in the foveal area. The exclusion criteria included: history of any other vision impairing ocular disease, history of retinal laser treatment or intravitreous injection, as well as those previously submitted to vitreoretinal surgery were excluded. Regarding systemic history, the could be no record of cardiovascular events in the preceding year, no known history of chronic infectious, inflammatory or malignant disease, and no surgical procedures or hospital admissions of any kind in the preceding 6 months.

All recruited cases underwent treatment with intravitreous bevacizumab (BVZ) injections (1.25 mg/0.05 cc) following the strategy of 3 monthly injections plus *pro re nata* treatment according to tomographic criteria, namely persistent central subfield thickness > 330 µm, with identifiable intraretinal cystic lesions and / or subretinal fluid.

Blood samples were taken at baseline to evaluate the following systemic markers: cardiovascular risk (high sensitivity C-reactive protein, serum homocysteine), renal dysfunction (blood urea nitrogen (BUN), serum creatinine), hypercholesterolemia (low-density lipoprotein (LDL), high-density lipoprotein (HDL) and total cholesterol (T.Chol)) and diabetic profile (glycated hemoglobin, blood glucose level). Additionally, for each case a blood sample was obtained and centrifuged at 1000G to isolate the serum fraction which was then immediately stored at -80ºC, for posterior dosing of the following pro-inflammatory cytokines: VEGF, sICAM-1, MCP-1 and TNF-α, by enzyme-linked immunosorbent assay (ELISA) using the specific ELISA kits, procured from Sigma-Aldrich®. All procedures were performed according to the manufacturer's protocol. Samples were diluted accordingly, to comply with the detection range of the relevant assay. Color intensities were determined using a microplate reader. Duplicate samples were used in all assays. The level of each factor in serum was within the detection range of the relevant assay.

SD-OCT images were obtained with the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) using the following acquisition protocol: a 30º horizontal foveal scan resulting from the averaging of 100 frames, and a 20x20º macular square consisting of 25 individual horizontal scans, resulting from the averaging of a minimum of 20 frames. The value obtained in the 1mm central ring of the macular thickness map was considered the central foveal thickness (CFT). In cases with bilateral DME, the eye with highest CFT was chosen for this study.

The main outcome measures were: change from baseline in CFT (µm) and macular volume (mm^3), obtaining a CFT < 330 µm, achieving a CFT decrease ≥ 25% or a CFT decrease < 10% of baseline CFT. Also, regarding OCT analysis, three of the authors (PB, JC, RS) blinded to clinical records, registered and/or graded the following qualitative findings in the 5 central scans: subretinal fluid, largest intraretinal cyst (IRC) height (Figure 1), disruption of the ellipsoid zone (EZ) and presence of hyperreflective spots (HRS). The presence of HRS was graded based on the most common tomographic patterns, according to the following criteria: Grade 1 – few small clearly spaced HRS; Grade 2 – larger rounded HRS clumped closer together, some could cause faint shadowing in the underlying retinal layers; Grade 3 – even larger rounded HRS forming plaque like hyperreflective lesions with significant shadowing of the underlying retinal layers (Figure 2). Disruption of the EZ band was classified as grade 0 if there was continuous reflectivity in the 5 central scans; grade 1 if minor disruption (≤200 µm) was present; and grade 2 if at least one large (>200 µm) disruption of the EZ was found[22].

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The systemic blood/serum concentrations of measured metabolic and pro-inflammatory factors were assessed for predictive associations with the previously listed anatomic response parameters at the 3rd month and 6th month of follow-up. Such time points were chosen because they correspond respectively to the moment in which all cases had received the mandatory loading dose of BVZ (3rd month) and the moment at which persistent DME is considered to be poorly responding to anti-VEGF treatment (6th month)[4, 23]. All recorded results were subject to statistical analysis using the IBM SPSS Statistics software (SPSS version 23, IBM Corporation). Numerical values are expressed as mean±standard deviation. The study of possible interactions between variables was performed by Spearman 2-tailed correlations. Predictive associations were studied by ordinary least squares regression and bivariate logistic regression. The value of significant systemic factors as biomarkers was further studied by ROC curve analysis. Categorical variables were assessed using chi-square test and numerical variables were compared by Mann-Whitney-U or Kruskal Wallis tests. Statistical significance was considered for $p < 0.05$.

Results

The study included 30 consecutive patients diagnosed with DME requiring treatment with intravitreal anti-VEGF injections. Mean patient age was 66.76±9.36 years and mean duration of DM2 was 17.20±6.85 years. Thirteen patients were on insulin therapy for a mean of 7.23±6.91 years. All cases had NPDR, classified as moderate in 17 cases and severe in 13 cases. There was a significant association between NPDR severity and serum VEGF level; cases with severe NPDR had higher circulating VEGF than those with moderate NPDR (92.25±37.70 vs 59.42±25.87 pg/ml, p=0.014) (Table 1). The mean baseline CFT value was 519.63±119.26 µm, improving to 401.53±167.40 µm at the 6th month (p<0.001) (Figure 3). The mean CFT change from baseline was -135.31±139.76 at the 3rd month and -113.50±131.11 at the 6th month. Mean macular volume followed a similar improvement profile (11.08±2.22 mm^3 at baseline improving to 9.83±2.00 mm^3 at the 6th month, p<0.001). Between the 3rd and 6th month visit, 8 cases had an increase in CFT, but the difference did not reach statistical significance (p=0.089 for CFT and p=0.061 for MV). A favorable anatomic response (≥ 25% decrease in CFT) was found in 13 cases (43.3%) at the 6th month. On the other end, a limited anatomic response (< 10% decrease in CFT) was found in 10 cases (33.3%) at the 6th month. Finally a CFT < 330 µm was verified in 10 cases at the 6th month (33.3%). Such results were obtained with a mean of 4.82±0.56 intravitreal bevacizumab injections.

Systemic factors and quantitative macular outcomes

No significant associations were found between systemic factors and baseline CFT or MV. At the third month of follow-up significant associations were found between both baseline hsCRP (3.33±2.01 vs 1.39±1.15 mg/L, p=0.007) and ICAM1 (975.54±265.49 vs 727.07±336.09 pg/ml, p=0.012) with obtaining a CFT decrease < 10% (Table 2). Logistic regression revealed a predictive association between hsCRP and CFT decrease < 10% (p=0.014, R^2=0.249, odds ratio=2.17, confidence interval 95%, 1.17-4.04) and such association was independent of DM related variables such as duration of disease, blood glucose level and HbA1c percentage. On the other end of the anatomic response spectrum, a lower serum MCP1 level was significantly associated with a CFT decrease ≥ 25% (242.42±48.96 vs 315.93±93.14, p=0.015). ROC curve

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analysis indicated hsCRP and ICAM1 as significant biomarkers for 3rd month reduced anatomic response (area under the curve (AUC)=0.807, p=0.009, cutoff value=1,54 mg/L for a sensitivity of 78.0% and a specificity of 61.9% for hsCRP; AUC=0.788, p=0.014, cutoff value=900.08pg/ml for sensitivity of 78.0% and specificity of 85.7% for ICAM1).

At the sixth month of follow-up (Table 3), significant Spearman correlations with 6th month CFT were found for hsCRP (p=0.02, 0.423) and MCP1 (p=0.024, r=0.440). Mean 6th month CFT change correlated with hsCRP (p=0.007, r=0.468), MCP1 (p=0.015, r=0.472) and VEGF (p=0.034, r= - 0.416). Further exploring these results, we verified a significant association between higher hsCRP level and obtaining a CFT decrease < 10% (3.36±1.65 mg/L vs 1.28±1.24 mg/L, p<0.001). Such association was found to be predictive by logistic regression analysis (p=0.009, R^2=0.312, odds ratio=2.59, confidence interval 95%, 1.26-5.28) and such result was again independent of DM related variables (DM duration, blood glucose level and HbA1c percentage). ROC curve analysis of hsCRP as a biomarker for 6th month reduced anatomic response, indicated an AUC of 0.903, p<0.001, and a value of 1.81 mg/L for a sensitivity of 90% and specificity of 85%. A significant association with 6th month CFT decrease ≥25% was found for lower serum MCP1 (244.69±49.34 pg/ml vs 319.24±94.88 pg/ml, p=0.017) and higher serum VEGF (90.84±37.33 vs 58.28±25.19, p=0.027). By logistic regression serum VEGF was predictive of a 6th month significant anatomic response (p=0.029, R^2=0.219, odds ratio=1.035, CI95%, 1.00-1.06) and such association was independent from DM related variables. Finally, a significant association was found between obtaining a 6th month CFT < 330 µm and hsCRP (1.10±0.96 vs2.48±1.83 mg/L, p=0.021) and MCP1 (238.96±46.47 vs 318.47±91.81 pg/ml, p=0.004), the latter was found to be a negative predictor of such outcome (logistic regression, p=0.035, r^2=0.305, odds ratio=0.97).

Systemic factors and qualitative macular outcomes

The mean baseline intraretinal cyst height was 350.04±144.00 µm, improving to 221.68±211.80 µm at the 6th month (p=0.002). A significant correlation was found between hsCRP and 6th month IRC height (p=0.004, r=0.588). Regarding HRS severity, at presentation 14 cases were classified as grade 3, while 7 cases had grade 1 small HRS. The classification of HRS improved significantly at the 6th month (p=0.008, Wilcoxon signed rank) with grade 3 HRS persisting in only 8 cases. Stepwise linear regression revealed that only VEGF was predictive of baseline HRS grade (p=0.002, r^2=0.340, beta=0.583). Regarding 6th month HRS grade, the statistical model including LDL-cholesterol and serum VEGF level, was found to be the most significantly predictive (p=0.001, r^2=0.463) (Table 4). Cases with improved HE severity at the 6th month were significantly younger (58.88 vs 67.23 years-old, p=0.019), but no significant association was found between systemic factor levels and improvement of HE severity. Subretinal fluid (SRF) was present at baseline in 10 cases. Cases with SRF had significantly higher serum homocysteine levels (16.58 vs 13.24 µmol/L, p=0.032). There was a significant improvement in SRF at the 6th month, persisting but with reduced vertical height in only 3 cases (p=0.02, Wilcoxon signed-rank).

Finally, grade 2 EZ disruption was found at baseline in 17 cases, while at the 6th month such defects persisted in 14 cases (p=0.096). No significant associations were found for baseline EZ disruption grade, but cases with persistent grade 2 EZ band disruption had significantly higher serum VEGF when comparing with cases with continuous EZ band (50.42±23.30 vs 82.65±31.39, p=0.031).

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Discussion

Our results revealed that systemic inflammatory factors such as hsCRP, ICAM1 and MCP1 may have an important role in the identification of anti-VEGF nonresponders. In fact, higher hsCRP levels were consistently associated with more severe cystoid macular edema (higher 6th month CFT, lower CFT change and larger intraretinal cysts), both hsCRP and ICAM1 were associated with early reduced anatomic response (CFT change < 10%) and both hsCRP and MCP1 were significantly lower in cases which obtained a sixth month favorable anatomical outcome (CFT < 330 µm). These results suggest a prominent role for pro-inflammatory factors causing persistent cystoid edema despite treatment with anti-VEGF. In this regard, it is interesting to note that hsCRP, an inflammatory protein associated with cellular apoptosis, was found by ROC curve analysis, to be a statistically significant biomarker of reduced foveal response to BVZ, particularly at the 6th month of follow-up when for suitable cases treatment was administered according to *pro ne nata* strategy, which resulted in a small increase in CFT in 8 cases. Such result suggests that despite the anatomic response to the initial anti-VEGF loading dose, cases with higher hsCRP values, may benefit from a more prolonged monthly treatment dosing. Alternatively, considering the inhibitory effect of corticosteroids in leukocyte migration[24], if OCT imaging reveals severe macular edema with large intraretinal cystic spaces after the loading dose of anti-VEGF, the patient could benefit from early switching to a corticosteroid implant. Such point is particularly important as it is well known that chronic cystoid macular edema may lead to permanent neuronal retinal damage thereby limiting the potential for visual acuity recovery[25]. It is therefore of the utmost importance to identify as early as possible those cases that exhibit limited anatomic response to anti-VEGF treatment in order to promptly optimize treatment strategies. A possible interpretation is that in cases with higher circulating pro-inflammatory proteins, the cystoid edema is probably more related to cellular iBRB breakdown rather than increased vasopermeability from hypoxia-induced VEGF expression. Indeed, leukostasis is considered to have a prominent role in the pathogenesis leading to iBRB breakdown, with upregulated ICAM1 playing a role in increased leukocyte adhesion to the capillary endothelium, while MCP1 seems to be an important biomarker for increased leukocyte mobilization to the retina[26]. Previous studies had demonstrated a role for elevated CRP in DR and DME[27-30], but to our knowledge, this is the first study identifying a relation between hsCRP level and macular outcomes in DME cases having completed a treatment course of at least 4 BVZ injections.

Also of note was the finding of a significant correlation between systemic VEGF level and the stage of NPDR. Previous studies demonstrated a correlation between serum VEGF and occurrence of DR[31-33] and DME[34], but the results are not unanimous. In fact, a recent meta-analysis reviewed the literature on circulating biomarkers of diabetic retinopathy and verified that VEGF was not consistently elevated comparing to diabetic patients without DR[35]. The previous referred studies did not provide detail on the composition of the NPDR group, also the immunoassay technique varied among studies, which adding to the inherent complexity of studying a systemic disease such as DM may at least partly explain the different reports. In order to minimize the effect of unrelated systemic conditions, we did not include any cases with any other known infectious, inflammatory or malignant disease, additionally we excluded all cases with hospital admissions due to cardiovascular events in the prior 12 months. Also, we included two biomarkers currently in use to assess cardiovascular risk (hsCRP and homocystein), which provided and objective indicator to guide the review of systemic history records. Nevertheless, experimental models reveal that VEGF expression increases in the hypoxic retina[36]. Considering VEGF is a mediator of angiogenesis[37] it is theoretically feasible

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that as RD progresses there is increased stimulus for retinal VEGF expression. Prolonged DM increases the risk of vascular damage affecting the eyes, kidneys and peripheral nerves and is also a major risk factor for acute ischemic events affecting the brain and the heart, we could therefore hypothesize that the correlation of serum VEGF to NPDR stage suggests either a tendency of ocular disease to mirror the overall systemic vascular state or that ocular expression of VEGF is reliably detected in the systemic circulation. In this regard, it is worth noticing that a significant association was found between higher serum VEGF levels and persistent photoreceptor EZ disruption. Such result suggests that in this study population the higher serum VEGF could indicate a more advanced hypoxic ocular state leading to impaired photoreceptor metabolism and decreased reflectivity of the EZ. Also of interest in our study was the relation of serum VEGF with hard exudate grade on OCT imaging. It is known that VEGF, a potent mediator of vascular permeability, is secreted by the retinal Muller cells under hypoxic conditions[38], facilitating leakage of plasma protein and lipid into the retinal space. It is therefore possible that patients with higher VEGF levels may be more prone to pronounced exudation as the increased capillary permeability may supersede the capacity of the retinal pigment epithelium to remove the accumulating plasmatic molecules. In agreement with the ETDRS report[39], we also found dyslipidemia to be an important factor for HRS severity, namely patients with higher LDL-cholesterol had significantly more severe HRS. In fact both VEGF and LDL- cholesterol were associated with HE severity after 6 months of treatment, meaning that while BVZ is effective at significantly reducing macular thickness, the complete reabsorption of lipoproteinaceous material may take much longer to occur if at all. Such results suggest that in patients with DME and pronounced hard exudates visible on fundoscopy, optimum control of blood lipids may be beneficial in order to mitigate the leakage of potentially inflammatory lipoproteins[34, 40].

Finally, it is also important to notice that despite the importance of glycemic control in delaying the progress of DR, in this study glycemic variables (blood glucose level and HbA1c) had no correlation with either baseline or 6th month macular features. A previous study evaluated the role of metabolic factors on the clinical response to BVZ[41]. The authors verified that cases with better glycemic control (HbA1C <7%) had more significant improvements in central subfield thickness (CST), but there was no significant difference in final CST between groups. In this regard, a study by Bressler et al[42] reviewed several systemic and ocular variables in cases with DME treated with ranibizumab, but after 1 year of follow-up, no significant difference in CST was found according to HbA1c value (<7.5% vs >7.5%). While glycemic control is a crucial factor in delaying the onset of clinical DR, glucose parameters may not be reliable markers for DME clinical response. In fact, glycated hemoglobin translates the mean plasmatic glucose concentration of the preceding 3 months, such value may rapidly change with dietary adjustment and oral antidiabetics or insulin treatment. Considering the risk of DR increases with duration of diabetes[43], the most recent HbA1c value may not reflect the longstanding hyperglycemia that led to ocular disease. The largest studies evaluating the role of systemic factors on clinical response to DME treatment are the post hoc analysis of the RIDE/RISE trials[44, 45]. In such studies, no systemic variables were found to be correlated with macular outcomes, but components of systemic state such a cardiovascular status were based on medical records, and not on reliable laboratory markers, additionally no serum or ocular cytokines were studied, which limits the insight on the evaluation of inflammatory status in the pathogenesis of DME. Our study has limitations, namely the fact that the study population is small and systemic biomarkers where only measured at baseline. Nevertheless we believe our results are relevant as in addition to a rigorous effort to exclude possible confounding factors, we performed an extensive study of all major systemic variables related to DM by

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quantification of corresponding biochemical factors, with particular emphasis on pro-inflammatory status (hsCRP, VEGF, ICAM1, MCP1 and TNFα). Additionally, the results provided relevant insight regarding the possible effect of systemic inflammation in the pathogenesis of persistent DME and may lead to improvement of current clinical practices, namely in guiding implementation of DME treatment strategies according to inflammatory profiles.

Conclusions

In conclusion, our study revealed a significant effect of systemic inflammatory status on DME treatment, verified in a real-world practice setting. Indeed, the levels of serum VEGF, LDL-cholesterol, MCP-1 and hsCRP revealed to be significantly associated with tomographic features of DME. More importantly, we verified that elevated systemic inflammatory factors such as hsCRP and MCP1, were associated with increased CFT, six months after commencing BVZ treatment, indicating a possible role for identification of anti-VEGF nonresponders. A longer follow-up and larger study population will be crucial to identifying the true role of such systemic biomarkers as prognostic factors for clinical response in DME, and eventually lead to the optimization of current treatment guidelines.

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Tables

Table 1 – demographic and laboratory data according to diabetic retinopathy type

	Moderate NPDR	Severe NPDR	P value
Age	63.00±10.09	66.71±17.52	0.476
DM2 years	18.50±8.59	21.92±17.73	0.497
Glucose	177.50±58.11	178.64±34.59	0.072
HbA1c	8.10±1.07	7.80±1.08	0.660
Creatinin	1.18±1.04	0.89±0.20	0.448
B.U.N	38.81±14.95	53.21±17.16	**0.019**
LDL	106.87±37.36	107.68±27.11	0.702
HDL	48.60±12.05	53.71±21.25	0.790
Total-Chol	184.18±51.52	185.92±25.33	0.111
hsCRP	1.94±1.81	2.00±1.60	0.886
Homocystein	13.91±3.46	15.07±4.71	0.257
VEGF	59.42±25.87	92.25±37.70	**0.027**
MCP1	308.71±102.86	272.27±33.77	0.164
ICAM1	821.61±384.29	778.76±274.97	0.984
TNFα	0.078±0.075	0.058±0.012	0.237

Table 2 – Mean value of systemic factors and macular outcomes at the third month of follow-up

Systemic factors	CFT decrease < 10%		P Value	CFT decrease ≥ 25%		P Value	CFT < 330 µm		P Value
	Yes	No		Yes	No		Yes	No	
Glucose	184,60	174,75	0.321	189,16	170,61	0.152	177,77	178,14	0.705
HbA1c %	8,51	7,79	0.921	8,02	7,92	0.310	8,17	7,87	0.395
Creatinine	0,95	1,09	0.657	1,21	,89	0.518	1,35	,91	0.722
B. U. N	53,66	44,04	0.328	44,00	46,55	0.545	44,00	46,19	0.563
LDL-Chol	93,71	113,05	0.720	115,66	101,64	0.153	113,60	104,53	0.060
HDL-Chol	48,62	52,00	0.625	48,50	52,64	0.884	50,33	51,26	0.790
Total-Chol	162,11	194,80	0.235	191,66	180,55	0.207	192,00	182,00	0.226

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Homocyst.	15,80	13,88	0.413	14,45	14,46	0.819	12,98	15,09	0.397
hsCRP	3,33	1,39	**0.007**	1,55	2,25	0.368	1,60	2,13	0.372
VEGF	60,66	77,97	0.188	82,44	66,62	0.281	74,01	72,94	0.868
MCP1	347,71	261,66	0.094	242,42	315,93	**0.015**	266,57	294,50	0.597
ICAM1	975,54	727,07	**0.012**	687,08	877,97	0.172	717,98	837,49	0.533
TNFa	0.051	0.076	0.866	,055	,080	0.919	,10	,054	0.874

Table 3 – Mean value of systemic factors and macular outcomes at the sixth month of follow-up

Systemic factors	**CFT decrease < 10%**		**P Value**	**CFT decrease ≥ 25%**		**P Value**	**CFT < 330 µm**		**P Value**
	Yes	**No**		**Yes**	**No**		**Yes**	**No**	
Glucose	184,60	174,75	0.350	182,92	174,29	0.711	178,63	177,68	0.933
HbA1c %	8,51	7,79	0.053	7,78	8,10	0.385	7,86	8,02	0.641
Creatinine	1,01	1,07	0.120	1,18	,95	0.408	1,22	,95	0.350
B. U. N	54,90	40,85	0.120	38,23	51,12	0.059	36,45	46,79	0.062
LDL-Chol	102,36	109,70	0.713	119,80	97,65	0.094	114,74	102,91	0.145
HDL-Chol	58,80	47,08	0.131	48,15	53,15	0.805	51,45	50,71	0.420
Total-Chol	187,60	183,70	0.713	198,15	174,94	0.300	194,18	179,68	0.268
Homocyst.	16,41	13,48	0.074	13,15	15,45	0.103	13,02	15,29	0.094
hsCRP	3,36	1,28	**<0.001**	1,44	2,38	0.072	1,10	2,48	**0.021**
VEGF	53,95	80,44	0.094	90,84	58,28	**0.027**	77,61	70,16	0.646
MCP1	291,33	282,44	0.427	244,69	319,24	**0.017**	238,96	318,47	**0.004**
ICAM1	952,87	725,98	0.183	746,47	843,78	0.742	665,02	880,69	0.102
TNFa	0.052	0.076	0.988	,079	,062	0.311	,0942	,052	0.919

Table 4 – mean total cholesterol and serum VEGF levels according to hyperreflective (HRS) spots grade at the 6th month of follow-up

6.Month HRS grade		**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	**p value**
Chol. Total	1,00	9	169,11	40,15	99,00	228,00	0.052
	2,00	13	182,53	33,16	131,00	240,00	
	3,00	8	206,87	47,68	132,00	304,00	
	Total	30	181,3000	40,73391	99,00	304,00	
VEGF	1,00	7	45,57	17,51	23,94	78,70	0.032
	2,00	12	75,63	35,97	20,91	153,41	
	3,00	7	97,07	28,72	50,34	132,01	
	Total	26	73,86	36,67764	3,94	153,41	

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Figure Legends

Figure 1

Intraretinal cyst height was measured using the caliper in the Heidelberg Eye Explorer software (HeyEX), by placing the cursor in the superior limit of the cyst hyporeflectivity and dragging along the shape of the cyst until the lower hyporreflective limit

Figure 2

The presence of hyperrreflective spots (HRS) corresponding to hard exsudates was classified according to severity in 3 grades. A – only a few, small, clearly spaced HRS are identifiable (grade 1); B - larger rounded HRS clumped closer together (grade 2); C - larger rounded HRS, some of which become coalescent forming hyper reflective plaque-like deposits with significant shadowing effect of the underlying retinal layers (grade 3).

Figure 3

The boxplot graph represents the mean central foveal thickness during follow-up. The numeric values correspond to the the mean 50^{th} percentile for CFT at each time point, indicating a significant change from baseline to the third month and a nonsignificant increase at the 6^{th} month

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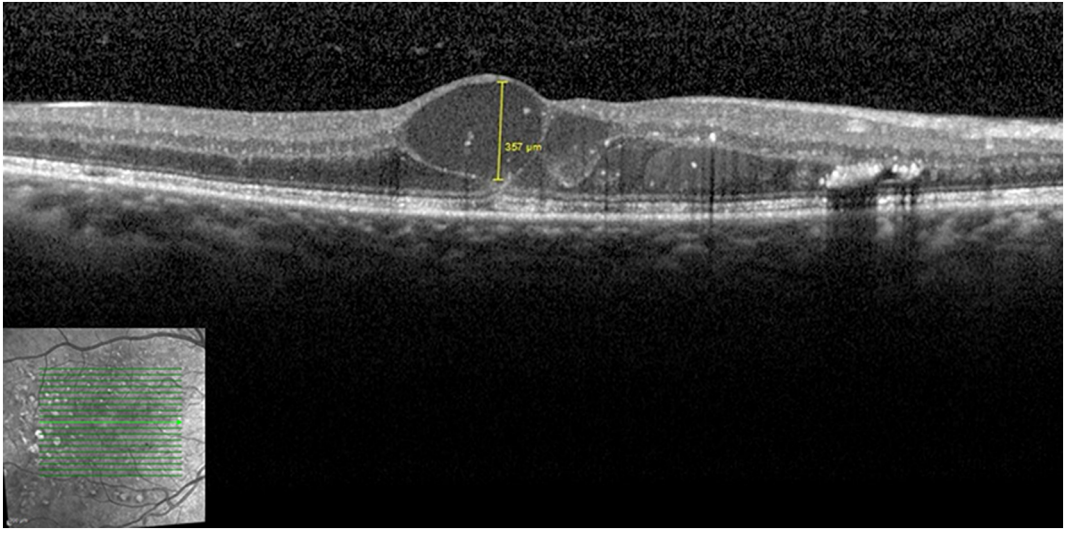

Figure 1

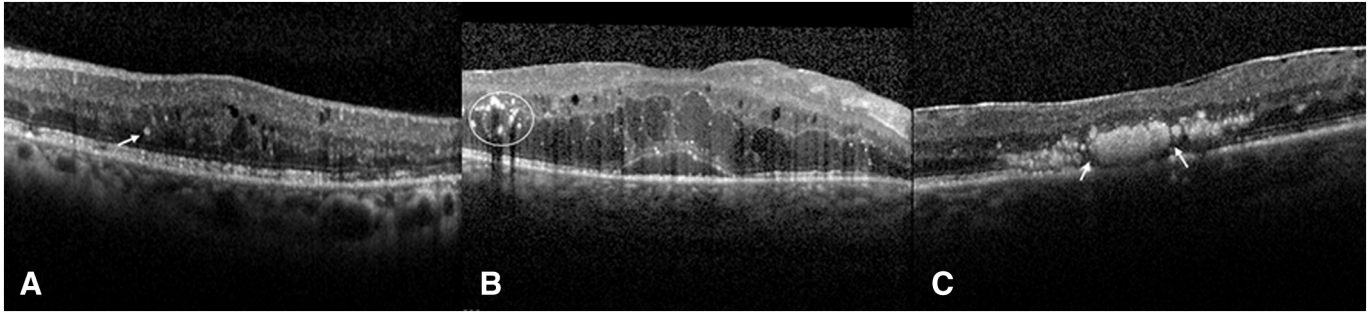

Figure 2

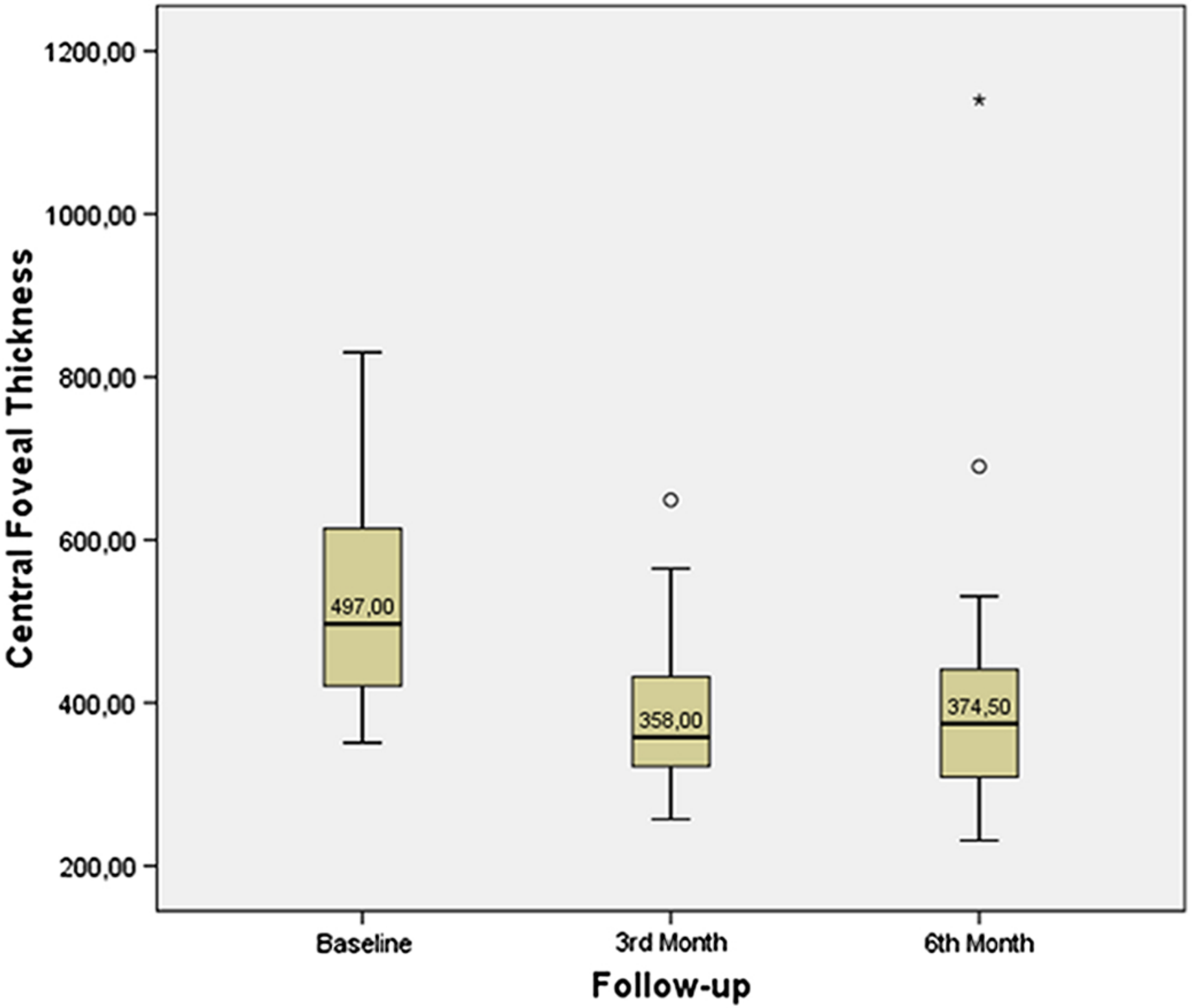
1200,00
1000,00
800,00
600,00
400,00
200,00
Central Foveal Thickness
497,00
358,00
374,50
Baseline
3rd Month
6th Month
Follow-up

Figure 3