

Effects of vitreous angiopoietin-2 levels in rhegmatogenous retinal detachment on the macular microvascular bed

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Background: Our previous optical coherent tomography angiography (OCTA) study of rhegmatogenous retinal detachment (RRD) treatment has found an impaired retinal microvascular bed over a 12-month postoperative follow-up and warranted for the study of vitreous angiopoietin (Ang)-2 levels as a vascular destabilizing factor in patients treated for RRD.

Purpose: To assess vitreous Ang-2 levels in patients with primary RRD and their association with OCTA-based changes in the macular microvascular bed in patients with RRD.

Material and Methods: Eighty-seven patients with primary RRD were involved in the study and divided into two groups depending of the state of the macula: patients that underwent surgery for macular-on RRD (group 1) and those that underwent surgery for macular-off RRD (group 2). All patients had their vitreous samples taken and underwent subtotal posterior vitrectomy. Concentrations of Ang-2 in these samples were measured by an enzyme-linked immunosorbent assay (ELISA) kit (Human Angiopoietin-2 ELISA Kit, Thermo Fisher SCIENTIFIC, Rockford, IL, USA) in accordance with the manufacturer's protocol. We assessed correlations of vitreous Ang-2 levels and OCTA-based parameters in patients.

Results: At the preoperative vitreous Ang-2 level of 129.7 ± 51.99 pg/ml in group 1, there was a strong correlation of preoperative OCTA-based foveal avascular zone (FAZ) area, parafoveal deep capillary plexus density (PDCPD) and foveal deep capillary plexus density (FDCPD) with vitreous Ang-2 levels over the study period for group 1. At the preoperative vitreous Ang-2 level of 693.8 ± 634.7 pg/ml in group 2, there was a strong correlation of OCTA-based FAZ area, FDCPD, and PDCPD with vitreous Ang-2 levels at baseline and 12 months, and a moderate direct correlation of OCTA-based FAZ area, FDCPD, and PDCPD with vitreous Ang-2 levels at months 3 and 6.

Conclusion: Vitreous Ang-2 levels were significantly lower in macula-on RRD eyes than in macula-off RRD eyes (129.7 ± 51.99 pg/ml versus 693.8 ± 634.7 pg/ml, $p < 0.001$). There was a strong direct correlation ($p < 0.05$) of Ang-2 with OCTA-based characteristics of the retinal microvascular bed (FAZ area, PDCPD and FDCPD) for both groups, which indirectly indicates the effect of the vascular destabilizing factor on the postoperative best-corrected visual acuity.

Keywords:

angiopoietin-2, rhegmatogenous
retinal detachment, OCT
angiography, macula

Introduction

Although minimally invasive surgery is a current approach to the treatment of rhegmatogenous retinal detachment (RRD), its clinical success rates are still unsatisfactory [1]. Our optical coherent tomography angiography (OCTA) study [2] of RRD treatment found an impaired retinal microvascular bed over a 12-month follow-up warranted for the study of vitreous angiopoietin (Ang)-2 levels as a vascular destabilizing factor and the relationship of this characteristic with impaired OCTA parameters in patients treated for RRD.

Angiopoietins are a family of growth factors that control signal pathways in endothelial cells, vascular permeability and reorganization and development of blood vessels. This is directly correlated with the growth of new blood vessels or angiogenesis [3]. Protein Ang-1 is a natural stimulator of the tyrosine kinase receptor (TIE2) [4].

Ang-1 is beneficial for the activity of TIE2, whereas Ang-2 impairs hypoxia- and ischemia-induced TIE2 activity, resulting in inactivation of TIE2 [5]. The destabilization of the ocular vascular network occurs when Ang-1 is inhibited, which results in increased susceptibility to vascular endothelial growth factor (VEGF) and other proinflammatory cytokines, leading to increased permeability of the vitreoretinal barrier [6, 7]. These findings have been confirmed by others [8, 9].

The purpose of the study was to assess vitreous Ang-2 levels in patients with primary RRD and their association with OCTA-based changes in the macular microvascular bed in patients with RRD.

Material and Methods

Eighty-seven patients with primary RRD were involved in the study. Patients were divided into two groups depending of the state of the macula: patients that underwent surgery for macular-on RRD (group 1) and patients that underwent surgery for macular-off RRD (group 2). All patients underwent vitrectomy with gas endotamponade. Exclusion criteria included previous history of retinal detachment surgery, uveitis, diabetic retinopathy, diabetes mellitus, renal insufficiency or age-related macular degeneration.

Immediately after scleral ports were made, 0.5-0.7 ml undiluted vitreous samples were obtained using a vitreous cutter, placed in sterile Eppendorf tubes and rapidly frozen at -80°C until analyzed. Concentrations of Ang2 in these samples were measured by an enzyme-linked immunosorbent assay (ELISA) kit (Human Angiopoietin-2 ELISA Kit, Thermo Fisher SCIENTIFIC, Rockford, IL, USA) in accordance with the manufacturer's protocol. Ang2 levels are presented as mean \pm standard deviation (pg/ml).

OCTA-based retinal vasculature parameters (foveal avascular zone (FAZ) area, parafoveal deep capillary plexus density (PDCPD) and foveal deep capillary plexus density (FDCPD) were assessed. Follow-up examinations were conducted 1, 3, 6, and 12 months after surgery. OCTA images of retinal microvascular bed (3 x 3 mm) were acquired using the Angio Vue OCTA system (RTVue XR OCT Avanti, Optovue, Inc.) which utilizes split-spectrum amplitude-decorrelation angiography (SSADA). OCTA images were obtained using the 3 x 3 mm scanning protocol in the Angio Retina mode to allow for improved image resolution and reduce the probability of eye motion artefacts and segmentation errors.

All procedures performed in the study were in accordance with the ethical standards of the Helsinki declaration. Prior to treatment, all patients signed informed consent to participate in the study and to undergo surgery. The study was approved by a local bioethics committee.

Statistical analyses were carried out with GraphPad Prism 8.0.1 (244) software (GraphPad Peism, San Diego, CA). The Kruskal-Wallis test was used to compare groups in terms of vitreous Ang-2 levels. A multifactorial correlation regression analysis was applied to evaluate the impact of OCTA data on vitreous Ang-2 levels in groups over the study period. The multidimensional linear function $\hat{Y} = a_0 + a_1X_1 + a_2X_2 + \dots + a_nX_n$ was used for this purpose. FDCPD (X1), FAZ (X2), and PDCPD (X3) were used as model factors, and Ang-2 was used as a characteristic of Yy function. A mild correlation was defined as $0.1 < r < 0.3$; a moderate correlation was defined as $0.3 < r < 0.7$; a strong correlation was defined as $0.7 < r < 1.0$; a perfect positive correlation was defined as $r = 1$, and a perfect negative correlation was defined as $r = -1$. The level of significance $p \leq 0.05$ was assumed.

Results

The mean vitreous Ang-2 levels in patients of groups 1 and 2 were 129.7 ± 51.99 pg/ml (range, 84.50 to 268.3 pg/ml) and 693.8 ± 634.7 pg/ml (range, 103.7 to 2065 pg/ml), respectively. Vitreous Ang-2 levels were significantly lower in eyes with macula-on than in eyes with macula-off ($p < 0.001$).

In both groups, vitreous Ang-2 levels did not depend on the age or gender ($p > 0.05$).

Actual preoperative and statistically significant predicted postoperative vitreous Ang-2 values in groups 1 and 2 are presented in Tables 1 and 2, respectively.

The results of the multiple regression model (Table 1) showed that, in group 1, the predicted vitreous Ang-2 level at 1 month after surgery was twice as high as the preoperative vitreous Ang-2 level, with no substantial change in the predicted vitreous Ang-2 level at 3, 6 and 12 months.

The results of the multiple regression model (Table 2) showed that, in group 2, the predicted vitreous Ang-2 level at 1 month after surgery was 1.1 times as high as the preoperative vitreous Ang-2 level (and this difference was statistically significant), with a gradual reduction in the predicted vitreous Ang-2 level at 3, 6 and 12 months.

At the preoperative vitreous Ang-2 level of 129.7 ± 51.99 pg/ml in group 1, there was a strong correlation of preoperative OCTA-based FAZ area, FDCPD, PDCPD with vitreous Ang-2 levels at baseline, at 1 month, 3, 6 and 12 months for group 1 (Table 3).

This indicated a direct impact of vitreous Ang-2 levels on the microvascular bed at all time points. An increase in FAZ area and a decrease in the DCPD under the impact of the vascular destabilizing factor were confirmed by the strong correlation. This deteriorates the blood supply of the macula and affects the best-corrected visual acuity (BCVA). In group 1, the BCVA before surgery and at 12 months was 1.2 ± 0.51 log MAR and 0.08 ± 0.07 log MAR, respectively. In addition, in group 1, 11 patients (24%) had a BCVA in the affected eye of 0.1 ± 0.0 logMAR, which was worse than in the fellow eye. The affected eyes in these patients exhibited lower FDCPD and PDCPD ($33.09 \pm 6.23\%$ and $48.27 \pm 4.23\%$, respectively) and increase in FAZ area (0.275 ± 0.06 m²) at 12 months than the general population in group 1, and their vitreous Ang-2 level was 206.7 ± 31.12 pg/ml.

At the preoperative vitreous Ang-2 level of 693.8 ± 634.7 pg/ml in group 2, there was a strong correlation of OCTA-based FAZ area, FDCPD, and PDCPD with vitreous Ang-2 levels at baseline and 12 months, and a moderate correlation of OCTA-based FAZ area, FDCPD, and PDCPD with vitreous Ang-2 levels at months 3 and 6 (Table 3). These results indicated a strong impact of vitreous Ang-2 levels on OCTA-based microcirculation parameters. The blood vessel density in the outer retina and FAZ area depended on the examined vascular destabilizing factor, which indirectly affected the revival of blood supply in the macula.

Table 1. Preoperative and statistically significant predicted postoperative vitreous Ang-2 levels for group 1

Preoperative vitreous Ang-2		129.73 pg/ml
Statistically significant predicted vitreous Ang-2 level	at 1 month after surgery	282.53 pg/ml
	at 3 months after surgery	280.01 pg/ml
	at 6 months after surgery	284.46 pg/ml
	at 12 months after surgery	260.50 pg/ml

Table 2. Preoperative and statistically significant predicted postoperative vitreous Ang-2 levels for group 2

Preoperative vitreous Ang-2		693.77 pg/ml
Statistically significant predicted vitreous Ang-2 level	at 1 month after surgery	782.32 pg/ml
	at 3 months after surgery	739.78 pg/ml
	at 6 months after surgery	714.32 pg/ml
	at 12 months after surgery	507.58 pg/ml

Table 3. Correlations of OCTA-based morphometric characteristics with the vitreous Ang-2 level for group 1

Time point	Correlation coefficient (r)				
	Preoperative	1 month	3 months	6 months	12 months
FDCPD	0.84	0.98	0.87	0.81	0.86
FAZ area	0.79	0.82	0.94	0.88	0.74
PDCPD	0.99	0.83	0.88	0.94	0.93

Note: FAZ, foveal avascular zone; FDCPD, foveal deep capillary plexus density; PDCPD, parafoveal deep capillary plexus density

In group 2, the BCVA before surgery and at 12 months was 2.61 ± 0.95 log MAR and 0.21 ± 0.19 log MAR, respectively. In addition, in group 2, 10 patients (24%) had a BCVA in the affected eye of 0.24 ± 0.13 logMAR, which was worse than in the fellow eye. The affected eyes in these patients exhibited lower FDCPD and PDCPD ($31.23 \pm 5.37\%$ and $46.58 \pm 4.32\%$, respectively) and increase in FAZ area (0.348 ± 0.062 m²) at 12 months than the general population in group 2, and the vitreous Ang-2 level was 1655.0 ± 295.45 pg/ml.

In groups 1 and 2, the value of the coefficient of determination R² (Table 5) over the study period was close to 1, indicating a statistically significant impact of the

vitreous Ang-2 level on FAZ area, FDCPD and PDCPD. The Fischer's F-ratio demonstrated statistical significance of multidimensional linear models at baseline and at 1, 3, 6 and 12 months. Moreover, the calculated values were higher than the tabulated values, with the exception of the results at 1 month for group 1.

Discussion

Therefore, the results of the statistical analysis of vitreous Ang-2 levels in patients that underwent surgery for macular-on RRD and patients that underwent surgery for macular-off RRD indicated that the level of the examined intravitreal factor was higher in group 2 than in group 1 ($p < 0.001$).

Table 4. Correlations of OCTA-based morphometric characteristics with the vitreous Ang-2 level for group 2

Time point	Correlation coefficient (r)			
	1 month	3 months	6 months	12 months
FDCPD	0.94	0.99	0.80	0.89
FAZ area	0.98	0.89	0.85	0.84
PDCPD	0.83	0.54	0.69	0.71

Note: FAZ, foveal avascular zone; FDCPD, foveal deep capillary plexus density; PDCPD, parafoveal deep capillary plexus density

Table 5. Results of multifactorial linear regression model for the effects of vitreous Ang-2 on FAZ area, FDCPD and PDCPD

Statistical characteristics	Time point									
	Preoperative		1 month		3 months		6 months		12 months	
	Groups									
	I	II	I	II	I	II	I	II	I	II
R ²	0.92	N/A	0.94	0.83	0.90	0.87	0.95	0.88	0.98	0.92
Calculated F	3.15*	N/A	3.09*	2.60	3.39*	3.38*	3.96*	3.49*	3.97*	3.59*
Tabulated F	2.53	N/A	2.53	2.55	2.53	2.55	2.53	2.55	2.53	2.55

Note: *, significant difference ($p < 0.05$); F, Fisher's test F-value; FAZ, foveal avascular zone; FDCPD, foveal deep capillary plexus density; R², coefficient of determination; N/A, not available; PDCPD, parafoveal deep capillary plexus density

A significant correlation of microcirculatory changes in the macula (OCTA-based FAZ area, FDCPD and PDCPD) with the vitreous Ang-2 level indicates a direct impact of the examined vascular destabilizing factor on the decreased vascular permeability in the deep capillary plexus, leading to a poor blood supply in the retina and longer restoration of the FAZ area.

In addition, given that a direct strong correlation was found for both groups, we may hypothesize that the state of the macular plays a minor role in postoperative functional results, because, in RRD, the vitreous Ang-2 level in any case affects the retinal microvascular bed. Therefore, we may hypothesize that reduced vitreous Ang-2 levels may result in faster regeneration of the blood supply in the macula, which is an agreement with findings by Jaeryung and Jang [5], and will be beneficial for the functional outcome. The results obtained from multidimensional regression models indicate possible preservation of high vitreous Ang-2 levels after surgery, and their effects on the speed of postoperative regeneration of the retinal microvascular bed, with these effects being more pronounced in the retinal detachment in the macula.

It has been reported that, under ischemic conditions, Ang-2 becomes activated and deactivates Tie-2, which results in vascular leakage, loss of pericytes, and an increase in inflammation [10, 11]. Studies have demonstrated that angiopoietin-like proteins may play an important role in cell metabolism. Co-expression of Ang-2 and VEGF-A has been reported to produce accelerated neovascularization in the developing retina and ischemic retina models. These findings confirmed the vascular effects of Ang-1 and Ang-2 [12].

Therefore, we established the association of Ang-2 with the impaired macular microvascular bed in RRD and the effect of Ang-2 on the speed of postoperative regeneration of the retinal microvascular bed; this indicates that the inhibition of Ang-2 or the Ang-2 receptors seems to be promising for faster postoperative regeneration of the blood supply to the macula, thus indirectly effecting an improvement in the visual acuity. The comparison of the two groups in terms of OCTA-based characteristics and vitreous Ang-2 levels demonstrated a tendency to greater

retinal microvascular bed damage in RRD patients with higher Ang-2 levels.

Conclusion

First, vitreous Ang-2 levels were significantly lower in macula-on RRD eyes than in macula-off RRD eyes (129.7 ± 51.99 pg/ml versus 693.8 ± 634.7 pg/ml, $p < 0.001$).

Second, there was a strong direct correlation ($p < 0.05$) of Ang-2 with OCTA-based characteristics of the retinal microvascular bed (FAZ area, PDCPD and FDCPD) for both groups, which indirectly indicates the effect of the vascular destabilizing factor on the postoperative visual outcome (BCVA).

Finally, we found that high vitreous Ang-2 levels may persist as long as 12 months after surgery, thus affecting the regeneration of the retinal microvascular bed.

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Disclosures

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