Aqueous flare and cells in eyes with retinal vein occlusion – correlation with retinal fluorescein angiographic findings

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Abstract

The laser flare cell meter (LFCM) was used to evaluate alterations of the blood-aqueous barrier in eyes with retinal vein occlusion (RVO). In 42 eyes of 42 patients with RVO (30 branch vein occlusions, 12 central vein occlusions) aqueous flare and aqueous cells were determined and retinal fluorescein angiography was performed. Flare and cell values were compared with 59 normal age and sex-matched control eyes and correlated with fluorescein angiographic findings. Both aqueous flare (12.321 (SD 6.717) photon counts/ms) and aqueous cells (mean 3.37 cells/ $0.075 \times 10^6/l$) were significantly increased in eyes with RVO in comparison with the normal control group (flare 4.288 (SD 1.144) photon counts/ms, cells 0.54 cells/ $0.075 \times 10^6/l$, $p < 0.0001$ and $p < 0.005$). Flare values in eyes with central RVO (19.517 (SD 7.762) photon counts/ms) were significantly higher than in eyes with branch RVO (9.443 (SD 3.307) photon counts/ms, $p < 0.0001$). Significant correlations were found between flare values and area of RVO ($r = 0.70$, $p < 0.0001$) and between flare values and area of retinal non-perfusion ($r = 0.76$, $p < 0.0001$). There were also significant correlations between flare values and number of cotton wool spots ($r = 0.54$, $p < 0.0002$) and between cell counts and degree of cystoid macular oedema ($r = 0.46$, $p < 0.006$). Our findings show that aqueous protein concentration, as indicated by flare, and aqueous cells are increased in RVO and that changes of aqueous flare correlate with fluorescein angiographic findings that reflect the severity of RVO. Measurements with LFCM may be useful non-invasively to quantify changes of the ocular barrier functions in RVO.

(*Br J Ophthalmol* 1993; 77: 280–283)

The laser flare cell meter (LFCM) was developed to quantify aqueous flare and aqueous cells by analysing anterior chamber laser beam scatter in vivo.¹ Clinical studies with this instrument have so far mainly focused on diseases and changes of the anterior segment such as intraocular inflammations,^{2,3} postoperative changes,⁴ diabetic retinopathy,^{5,6} and pseudoexfoliation.⁷ Lately there have been reports that aqueous flare is also increased in alterations of the posterior segment such as retinal vein occlusion (RVO),^{8,9} retinal detachment,¹⁰ and choroidal malignant melanoma.¹¹ It was the aim of this study to determine whether flare and cell values are related to clinical and fluorescein angiographic findings in RVO.

Methods and patients

Aqueous flare and aqueous cell were measured with the LFCM (FC-1000, Kowa Co, Ltd, Tokyo, Japan). The apparatus and techniques have been described previously.^{1,2} The sensitivity and reproducibility of the method have been confirmed by a number of groups in a series of studies.^{1,12–16}

For this study, flare values were indicated as photon counts per millisecond (photon counts/ms). A total of 69 eyes of 69 patients with RVO were examined. Twenty seven patients were excluded because they had already undergone argon laser treatment. Thus, 42 patients (30 branch retinal vein occlusion (BVRO), 12 central retinal vein occlusion (CRVO)) were included in this study within 2 months of onset of their symptoms (22 men, 20 women, mean age 58.3 (SD 12.6) years, age range 25–84 years). Measurements with the LFCM and fluorescein angiography were performed within 1 week. As a control group, 59 randomly selected age and sex-matched normal eyes of 59 subjects (31 men, 28 women, mean age 58.6 (SD 11.6) years, age range 25–84 years) were examined.

In both groups, exclusion criteria were presence of diabetes, pseudoexfoliation, and previous ocular surgery. Also excluded were patients with iris neovascularisation visible at slit-lamp examination. All patients with RVO included in this study had at least one fluorescein angiogram available for review. All of the fluorescein angiograms were reviewed by the authors. In each angiogram, the area of venous outflow obstruction and the area of retinal non-perfusion were estimated in disc areas. In addition, the number of cotton wool spots were counted and degree of retinal haemorrhages and cystoid macular oedema (CMO) were determined semiquantitatively (grade 0–3).

Flare values and cell counts were measured 30 minutes after pupillary dilatation with 0.5% tropicamide and 5% phenylephrine hydrochloride by a total of three examiners. From each eye five measurements were taken and averaged, and care was taken to exclude all measurements spoiled by artefacts. The data were processed using personal computers and statistically analysed with the non-parametric Mann-Whitney test and regression analysis.

Results

Both flare values and cell counts of 42 eyes with RVO (flare 12.321 (SD 6.717) photon counts/ms, cell 3.37, range 0–19.0 cells/ $0.075 \times 10^6/l$) were significantly higher than in normal control eyes (flare 4.288 (SD 1.144) photon counts/ms,

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Accepted for publication
9 December 1992

cell 0.54, range 0–3.6 cells/ $0.075 \times 10^6/l$, $p < 0.0001$ and $p < 0.005$).

Flare values in eyes with CRVO were 19.517 (SD 7.762) photon counts/ms, and in eyes with BRVO were 9.443 (SD 3.307) photon counts/ms. Statistically significant differences in flare were found between eyes with CRVO and normal control eyes ($p < 0.0001$), and between eyes with BRVO and normal eyes ($p < 0.0001$). Flare values were significantly higher in eyes with CRVO than in BRVO ($p < 0.0001$) (Table 1).

Cell counts in all groups showed considerable variation. In eyes with CRVO, cell counts were 3.87 cells/ $0.075 \times 10^6/l$ (range 0–15.6). The group of eyes with BRVO had cell counts of 3.16 cells/ $0.075 \times 10^6/l$ (range 0–19.0). Statistically significant cell count differences were found between CRVO eyes and normal eyes ($p < 0.005$), and between BRVO eyes and normal eyes ($p < 0.005$). No significant differences were found between eyes with CRVO and eyes with BRVO ($p > 0.5$) (Table 2).

Regression analysis showed correlation between flare and the area of RVO ($r = 0.70$, $p < 0.0001$) (Fig 1) and between flare and the area of retinal non-perfusion ($r = 0.76$, $p < 0.0001$) (Fig 2). Flare values also showed some correlation to number of cotton wool spots ($r = 0.54$, $p < 0.0002$). No significant correlations were observed between flare and extent of retinal haemorrhages, and between flare and degree of CMO ($p > 0.5$) (Table 3). There appeared to be some correlation between cell counts in eyes with

Figure 1 Linear regression between flare values and area of retinal vein occlusion (RVO) ($y = 5.216 + 0.341 \times x$, $r = 0.70$, $p < 0.0001$).

Figure 2 Linear regression between flare values and area of retinal non-perfusion ($y = 8.524 + 0.548 \times x$, $r = 0.76$, $p < 0.0001$).

Table 1 Flare values in normal eyes and eyes with retinal vein occlusion and significance of difference between groups (Mann-Whitney test)

	Number of eyes	Flare values (photon counts/ms) Mean (SD)	Normal (p Value)	CRVO (p Value)
Normal	59	4.288 (1.144)		
All RVO	42	12.321 (6.717)	< 0.0001	
BRVO	30	9.443 (3.307)	< 0.0001	< 0.0001
CRVO	12	19.517 (7.762)	< 0.0001	

RVO=retinal vein occlusion, CRVO=central retinal vein occlusion, BRVO=branch retinal vein occlusion.

Table 2 Cell counts in normal eyes and eyes with retinal vein occlusion and significance of difference between groups (Mann-Whitney test)

	Number of eyes	Cell count (cells/ $0.075 \times 10^6/l$) Mean (range)	Normal (p Value)	CRVO (p Value)
Normal	59	0.54 (0–3.6)		
All RVO	42	3.37 (0–19.0)	< 0.005	
BRVO	30	3.16 (0–19.0)	< 0.005	> 0.5
CRVO	12	3.87 (0–15.6)	< 0.005	

RVO=retinal vein occlusion, CRVO=central retinal vein occlusion, BRVO=branch retinal vein occlusion.

Table 3 Correlations between flare and cell values and retinal fluorescein angiographic parameters in eyes with retinal vein occlusion (linear regression analysis)

	Flare values		Cell counts	
	r	p Value	r	p Value
Area of RVO	0.70	< 0.0001	–0.11	≥ 0.5
Area of retinal non-perfusion	0.76	< 0.0001	0.04	> 0.5
Number of cotton wool spots	0.54	< 0.0002	0.24	> 0.1
Degree of retinal haemorrhages	–0.004	> 0.5	0.19	> 0.2
Degree of CMO	–0.002	> 0.5	0.46	< 0.006

r=correlation coefficient.

RVO and degree of CMO ($r = 0.46$, $p < 0.006$) whereas no such correlation was seen between cell counts and any of the rest of the above mentioned angiographic parameters (Table 3).

Discussion

The results of our study clearly indicate that aqueous protein concentration, as determined by aqueous flare, is increased in eyes with RVO. This is in accordance with previous studies. Zirm, who used anterior chamber tap and biochemical analysis, found increased albumin concentration in the aqueous of eyes with CRVO.^{17 18} Oshika⁸ as well as Miyake *et al*⁹ measured aqueous flare with the LFCM in eyes with RVO and found significantly increased values. Viridi *et al*¹⁹ observed flare in the aqueous humour in cynomolgus monkeys following experimental RVO. Increased protein concentration in the anterior chamber indicates an impairment of the blood-ocular barriers. Accordingly, studies using vitreous fluorophotometry demonstrated significant changes of the blood-retinal barrier permeability to fluorescein in patients with CRVO,^{20 21} and these changes correlated with retinal fluorescein angiographic findings. Our results also showed correlations between flare values and area of RVO, between flare values and area of retinal non-perfusion, and between flare values and the number of cotton wool spots. Miyake *et al*⁹ used the LFCM and fluorophotometry of the aqueous humour and vitreous in eyes with RVO and found significant

antly higher values of aqueous flare and aqueous fluorescein than in normal control eyes.

Alterations of the blood-aqueous barrier similar to the retinal changes may occur in the anterior segment of the eyes with RVO. Using iris fluorescein angiography, iridal neovascularisation can sometimes be detected very early after RVO before it is detectable on slit-lamp examination.²² Virdi *et al*¹⁹ found increased fluorescein in the aqueous and iris vascular leakage on fluorescein angiography in eyes with major branch or CRVO without any evident iris abnormality or rubeosis. Fluorescein leakage from iris vessels was also found in monkeys with experimental RVO before the development of iris neovascularisation, and there was a correlation between the retinal leakage and the development of ocular neovascularisation.¹⁹ Therefore, leakage of protein through altered iris vessels is a likely cause of increased flare values in eyes with RVO. The alterations of iris vessels in RVO are believed to be caused by growth factors that are released by the ischaemic retina into the intraocular fluids to the iris.^{19,23} Another possible mechanism that could be responsible for increased aqueous protein may be passive diffusion of protein from the retina via vitreous cavity and posterior chamber into the anterior chamber, as there is no dense barrier that prevents diffusion from the vitreous into the aqueous.²⁴⁻²⁶ This possible mechanism may correspond with the observations by Amuzi *et al*²⁷ in eyes with diabetic retinopathy, who found that aqueous flare is increased distinctly after vitreous haemorrhage. However, Miyake *et al*,⁹ who used anterior chamber and vitreous fluorophotometry in eyes with RVO, observed that fluorescein concentrations were only increased in the anterior chamber and the posterior vitreous, whereas values in the middle vitreous were low or normal. Therefore they concluded that increased aqueous flare in RVO mainly reflects blood-aqueous barrier disruption.⁹

Although the mechanisms by which aqueous flare is increased in eyes with RVO are not fully understood, our results suggest that the LFCM is a useful tool for quantification of changes in the aqueous in eyes with RVO.

Our measurements also showed increased cell counts in eyes with RVO. These 'cells' may represent leucocytes or even melanin granules. Interestingly, our finding of a correlation of cell counts with degree of CMO might point to pathogenetic mechanisms in the development of CMO such as release of prostaglandins that lead to increased vascular permeability and diapedesis of leucocytes. For clinical evaluation of RVO with the LFCM, flare values seem to be more useful than cell counts as cell counts show high variations both in normal eyes and in eyes with RVO.

Measurements of the aqueous flare with the LFCM can be useful to quantify the extent and severity of the alterations of the blood-ocular barriers in eyes with RVO, as we and others⁹ found a correlation between flare values and both the area of RVO and the area of non-perfused retina. However, as the correlation between flare and area of retinal non-perfusion is stronger,

retinal non-perfusion might possibly be the factor that affects the blood-aqueous barrier more severely. But still, eyes with CRVO without significant retinal non-perfusion may show high flare values (Fig 2), thus indicating that RVO without non-perfusion also alters the blood-aqueous barrier. Aqueous flare may also be of prognostic significance in the development of iris neovascularisation, because the development of iris neovascularisation in eyes with RVO correlates with the extent of retinal non-perfusion²⁰ and with the degree of retinal vascular leakage.¹⁹ Further studies are needed to follow up aqueous changes in eyes with RVO and to correlate these changes with electrophysiological finding, visual outcome, and the development of complications such as iridal neovascularisations and neovascular glaucoma, and to assess the effects of argon laser photocoagulation on aqueous flare and aqueous cells.

Presented in part at the 64th Annual Meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Florida, 6 May, 1992.

This study was supported by Alexander von Humboldt Foundation (Germany) and Marohn Foundation of the University Erlangen-Nürnberg (Germany).

The authors do not have any commercial or proprietary interest in the laser flare cell meter.

- 1 Sawa M, Tsurimaki Y, Tsuru T, Shimizu H. New quantitative method to determine protein concentration and cell number in aqueous in vivo. *Jpn J Ophthalmol* 1988; 32: 132-42.
- 2 Sawa M. Clinical application of laser flare-cell meter. *Jpn J Ophthalmol* 1990; 34: 346-63.
- 3 Ohara K, Okubo A, Miyazawa A, Sasaki H. Aqueous flare and cell meter in iridocyclitis. *Am J Ophthalmol* 1988; 106: 487-8.
- 4 Drews RC. Management of postoperative inflammation: dexamethasone versus flurbiprofen, a quantitative study using the new laser flare cell meter. *Ophthalmic Surg* 1990; 21: 560-2.
- 5 Oshika T, Kato S, Funatsu H. Quantitative assessment of aqueous flare intensity in diabetes. *Graefes Arch Clin Exp Ophthalmol* 1989; 227: 518-20.
- 6 Kuchle M, Schönherr U, Nguyen XN, Steinhäuser B, Naumann GOH. Quantitative measurement of aqueous flare and aqueous 'cells' in eyes with diabetic retinopathy. *German J Ophthalmol* 1992; 1: 164-9.
- 7 Kuchle M, Nguyen XN, Horn F, Naumann GOH. Quantitative assessment of aqueous flare and aqueous 'cells' in pseudoexfoliation syndrome. *Acta Ophthalmol (Kbh)* 1992; 70: 201-8.
- 8 Oshika T. Blood-aqueous barrier function in branch retinal vein occlusion. *Folia Ophthalmol Jpn* 1990; 41: 554-9.
- 9 Miyake K, Miyake T, Kayazawa F. Blood-aqueous barrier in eyes with retinal vein occlusion. *Ophthalmology* 1992; 99: 906-10.
- 10 Oshika T. Aqueous protein concentration in rhegmatogenous retinal detachment. *Jpn J Ophthalmol* 1990; 34: 63-71.
- 11 Kuchle M, Nguyen XN, Naumann GOH. Aqueous flare in eyes with choroidal malignant melanoma. *Am J Ophthalmol* 1992; 113: 207-8.
- 12 Bigar F, Herbort CP, Pittet N. Tyndallométrie de la chambre antérieure avec le laser flare-cell meter Kowa FC-1000. *Klin Monatsbl Augenheilkd* 1991; 198: 396-8.
- 13 Oshika T, Araie M, Masuda K. Diurnal variation of aqueous flare in normal human eyes measured with laser flare-cell meter. *Jpn J Ophthalmol* 1998; 32: 143-50.
- 14 Rimmer S, Holland GN. Studies of the Kowa laser flare-cell meter for determination of aqueous cell and protein concentration. *Invest Ophthalmol Vis Sci* 1991; 32: 674.
- 15 Spalton DJ, Shah SM. A comparative study of the laser flare cell meter and fluorophotometry in the assessment of the blood aqueous barrier in the pathological eye. *Invest Ophthalmol Vis Sci* 1991; 32: 886.
- 16 Yoshitomi T, Wong AS, Daher E, Sears ML. Aqueous flare measurement with laser flare-cell meter. *Jpn J Ophthalmol* 1990; 34: 57-62.
- 17 Zirm M. Protein in aqueous humor. *Adv Ophthalmol* 1980; 40: 100-72.
- 18 Zirm M. Eiweissveränderungen des Kammerwassers ohne Tyndallphänomen. *Ber Dtsch Ophthalmol Ges* 1989; 77: 765-70.
- 19 Virdi PS, Hayreh SS. Ocular neovascularization with retinal vascular occlusion. I. Association with experimental retinal vein occlusion. *Arch Ophthalmol* 1982; 100: 331-41.
- 20 Chahal PS, Chowieńczyk PJ, Kohner EM. Measurement of blood-retinal barrier permeability: a reproducibility study in normal eyes. *Invest Ophthalmol Vis Sci* 1985; 26: 977-82.
- 21 Chahal PS, Fallon TJ, Kohner EM. Measurement of blood-retinal barrier function in central retinal vein occlusion. *Arch Ophthalmol* 1986; 104: 554-7.

- 22 Laatikainen L, Kohner EM, Khoury D, Blach RK. Panretinal photocoagulation in central retinal vein occlusion: a randomised controlled clinical study. *Br J Ophthalmol* 1977; **61**: 741–53.
- 23 Kohner EM, Dollery CT, Shakib M, Henkind P, Paterson JW, de Oliveira LNF, *et al.* Experimental retinal branch vein occlusion. *Am J Ophthalmol* 1970; **69**: 778–825.
- 24 Maurice DM. Protein dynamics in the eye studied with labelled proteins. *Am J Ophthalmol* 1959; **47**: 361–7.
- 25 Cunha-Vaz J, de Abreu JRF, Campos AJ, Figo GM. Early breakdown of the blood-retinal barrier in diabetes. *Br J Ophthalmol* 1975; **59**: 649–56.
- 26 Cunha-Vaz JG. Pathophysiology of diabetic retinopathy. *Br J Ophthalmol* 1978; **62**: 351–5.
- 27 Azumi A, Inoue M, Yamamoto M. Application of laser flare-cell meter in patients with diabetes mellitus. 2. Factors influencing the flare counts. *Folia Ophthalmol Jpn* 1990; **41**: 986–90.