Assessment of retinal vascular calibers as a biomarker of disease activity in 

Birdshot chorioretinopathy

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Vasculitis

Summary statement: This study describes vascular involvement in BCR and suggests vascular caliber measurements as a potential tool for BCR staging and activity.
Abstract

Purpose: Birdshot chorioretinopathy (BCR) is a potentially blinding ocular disorder involving the retinal vasculature and choroid without any systemic manifestations. The objective of the study was to describe vascular caliber changes in BCR and analyze the possibility of this optical biomarker for staging and monitoring disease activity in BCR.

Methods: This retrospective case-control study at a tertiary referral eye center in the UK included 33 eyes from 21 patients with BCR and equal number of eyes from control subjects. Diagnosis of BCR was confirmed on fundus fluorescein and indocyanine green angiography. Vascular calibers were measured using validated semi-automated software. Covariates adjusted propensity score was calculated.

Results: BCR patients had smaller retinal venular calibers (CRVE) than controls (211.32 µm versus 227.93 µm, p = 0.008). After adjusting for variables, the difference between the two groups for CRVE remained statistically significant based on two different analysis methods. CRVE was lower at the six months follow up visit (206.25 µm versus 213.84 µm, p value=0.03) and arteriole to venule ratio (AVR) was larger (0.74 vs. 0.71, p=0.04). Arteriolar caliber (CRAE) remained the same.

Conclusion: This study provides novel insight into the pattern of vascular involvement in BCR. CRVE and AVR could potentially be used as non-invasive markers for disease prognosis and as a follow up parameter. More studies are needed to correlate this data with visual function and to validate the findings.
Introduction:

Birdshot chorioretinopathy (BCR) is a well-known, yet poorly understood form of posterior uveitis, characterized by multiple distinctive hypo-pigmented choroidal lesions, and strongly associated with human leukocyte antigen (HLA)-A29. (Levinson et al. 2006) Early inflammatory signs of the disease may be detected on fundus fluorescein angiography (FFA) and/or indocyanine green angiography (ICGA) without clinical evidence of the disease. (Reddy et al. 2015, Shah et al. 2005) Early cases may be missed absence of characteristic fundus lesions proposed by the revised diagnostic criteria by Levinson in 2006. (Levinson et al. 2006)

Gass has reported diffuse narrowing of retinal vessels and less frequently vascular tortuosity, perivascular hemorrhages and optic disc swelling among patients with BCR. (Gass 1981) However, the vascular caliber changes in BCR and the pattern and extent of these vascular changes during the disease progression has not yet been characterized. Currently FFA, ICGA, electroretinogram (ERG) and visual fields are the investigative tools available for both diagnosis and monitoring of BCR. (Arya et al. 2015, Gordon et al. 2007, Tzekov & Madow 2015) However, while FFA and ICGA are invasive in nature, ERG is resource intensive and these tests hence have hence inherent limitations.

Recent advances in imaging including autofluorescence have provided greater insights into ocular inflammatory disorders, and may provide better insight into prognosis and potentially treatment response. (Rothova et al. 2004, Piffer et al. 2014) Previous studies have shown that retinal vascular caliber is altered in systemic diseases such as hypertension and diabetes, which may reflect early
microcirculatory alterations prior to the onset of clinically significant complications such as retinopathy, nephropathy, stroke and myocardial infarction. (Crosby-Nwaobi, Heng & Sivaprasad 2012, Ding et al. 2012, Grauslund et al. 2009, Islam et al. 2009, Klein et al. 2006, Sun et al. 2009) Retinal image analysis offers substantial promise as a novel non-invasive measurement of early changes in the microvasculature, not detectable on routine clinical examination, to identify individuals at risk of developing cardio-metabolic diseases. (Ding et al. 2012) Furthermore, studies have shown retinal vascular caliber changes in inflammatory conditions such as systemic vasculitis including Kawasaki disease and auto-immune disorders such as rheumatoid arthritis. (Liew et al. 2015, Van Doornum et al. 2011) However, there are no reported studies in the literature regarding retinal vascular caliber changes for ocular diseases such as BCR without any systemic involvement.

Our current study aims to characterize the retinal vascular caliber in patients with BCR in comparison to healthy controls. The objective of this pilot study was to understand the nature of vasculopathy in BCR and ascertain whether retinal vascular imaging could be utilised as a potential tool for diagnosis, staging and monitoring disease activity in BCR.

**Methodology:**

**Study population and design:** This was a retrospective hospital-based case-control study of patients with BCR seen at uveitis clinic at a tertiary referral eye care centre in central London over a three year period from 2011-2013. Subjects with no known
ocular or systemic disease (controls) were recruited. Data for control groups were obtained from the same Caucasian population as in the retina vasculitis study published by Liew et al. (Liew et al. 2015) Approval for retrospective review of medical records and fundus image analysis was obtained from the hospital review board and adhered to the tenets set forth in the Declaration of Helsinki.

All cases with BCR who had no past medical history of diabetes or hypertension and were diagnosed by a senior uveitis consultant (CP) based on clinical examination and supported ancillary investigations such as FFA, ICGA and HLA-A29 were recruited for the study. Baseline demographic characteristics, systolic (SBP) and diastolic blood pressure (DBP), visual acuity and intraocular pressure (IOP) were recorded for all the study subjects. Details about the treatment (oral corticosteroids and/or immunosuppressive agents) was recorded in the datasheet. Mean arterial pressure (MAP) was computed using the formula: \( \text{MAP} = \text{DBP} + \frac{1}{3} (\text{SBP} - \text{DBP}) \) and mean ocular perfusion pressure (MOPP) was calculated using the formula: \( \frac{2}{3} (\text{MAP} - \text{IOP}) \).

**Fundus images:** Photographic images were obtained with a Topcon digital retinal camera (Topcon TRC 501X; Topcon Medical Systems Inc., Paramus). Best quality disc centred fundus images were retrieved from the imaging database for all the patients recruited under the study (Figure 1). The fundus images were anonymized and electronically transferred on an encrypted disc to the Center for Vision Research, Sydney, Australia, for retinal vascular caliber assessment by one trained grader. Fundus images with poor image quality were excluded from the study analysis, as variability in camera focus by the photographer can increase apparent vessel calibers.
**Fundus Image Analysis:** A grader (NJ) masked to participant identity and characteristics performed the retinal vessel measurements on right and left eyes of each subject, from each examination. Retinal vessel caliber was estimated using a validated and highly reproducible computer-assisted grading method, (Sherry et al. 2002) used in the previous clinic and population-based studies. (Liew et al. 2008, McGeechan et al. 2009, Wong et al. 2004) Briefly, this semi-automated imaging program (IVAN vessel measurement system, version 1.0, University of Wisconsin, Madison, USA) was used to measure the diameter of all retinal vessels in a zone 0.5 to 1.0 disc diameter away from the optic disc margin (Figure 2). Retinal vascular caliber was measured based on the revised Knudtson-Parr-Hubbard protocol. (Li et al. 2011, Tan et al. 2013) The central retinal arteriolar and venular equivalents were recorded, representing the average arteriolar and venular calibers of each eye. Geometric parameters of the retinal vasculature were measured and characterized from retinal photographs collected from this study population at baseline and at three to six months follow up post treatment. Average values for retinal arteriolar and venular caliber (termed the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively) for each eye were calculated, and the arteriole-to-venule ratio (AVR) was defined as the ratio of mean arteriolar to venular calibers. Similar image analysis was done for the study subjects at follow up visits.

**Statistical analysis:** The comparison of age, IOP, MAP, MOPP between groups was performed using the independent sample t-test, while gender comparison was done using chi-square test. The measurements on vascular calibers CRAE, CRVE and AVR, also referred to as outcome variables, were obtained for all the subjects from
the two groups. For study subjects, in addition to the baseline data before treatment, single point follow-up (at six months) measurement while on treatment with oral corticosteroids with or without immunosuppressive was obtained. Vascular risk factors (diabetes, hypertension) that could be confounders to retinal vascular calibers were taken into consideration. The baseline comparison of crude measurements of vascular calibers was performed between groups using the generalized estimating equation (GEE) model. The paired t-test was used to compare the variables between right eyes for study (n=21) and control (n=21) groups to avoid sampling bias. Moreover, assuming that covariates age, gender, IOP, MOPP are associated with caliber measurements, relative effect estimates that quantify the impact of confounding on outcome due to each covariate were obtained. The adjustment was performed using linear regression (univariate regression) of outcome on covariates. The covariates with large confounding impact were considered in the propensity score (PS) based models to address the bias in baseline profiles of two groups. PS attempts to reduce the bias due to confounding variables such as gender, IOP and age. The score is estimated for each subject independently using logistic regression model with group status regressed on observed baseline characteristics. All analyses were performed using the ‘nonrandom’ library in R (version 3.0.0) programming language, and the statistical significance was evaluated at the 5% level.

Results:

A total of 66 eyes from 21 controls (33 eyes) and 21 patients (33 eyes) with BCR were analyzed for the study. Table 1 provides the descriptive statistics for baseline characteristics of subjects based on the various possible confounding factors. The
mean age of patients in study group was 56.28 ± 13.78 years, while that of control group was 45 ± 17.83 years (p=0.03). Gender and IOP showed insignificant differences between the two groups. MAP for study group was 97.6±17.02 (82.33-113.33) mm of Hg and MOPP was 54.47±4.99 (46.22-67.55) mm of Hg. The relative effects describing the extent to which these covariates confound the outcome variables (vascular calibers) are shown in the Table 1. It is evident that covariate age has the highest confounding effect on all the three vascular calibers. Also, IOP exerted a considerable effect on CRAE and CRVE. Gender showed relatively smaller confounding effect on these outcome variables. There was confounding effect of MOPP on any of the three vascular calibers. Prior to adjusting the biomarkers with the above confounding covariates, crude estimates for each vascular caliber were obtained for study and control groups and evaluated for statistical significance. Table 2 shows significantly smaller mean CRVE in the study group as compared to control group (p= 0.003).

To adjust for the effect of confounding covariates like age, gender and IOP on the outcome variables, PS analysis was performed. The score was determined for each subject from study and control groups referring to logistic regression. Stratification of propensity scores was performed into three mutually exclusive subsets using cut-offs 0.33 and 0.66. For age, the stratum-wise mean age of subjects for study and control groups has been illustrated in bar chart (Figure 3). It is evident that strata 2 and 3 were nearly balanced for age; however, an imbalance was observed in stratum 1 due to single subject of 48 years, and the mean age of 24.69 years for control group. On similar lines, the balance was achieved with another covariate IOP (Data not shown).
There was good intra-class correlation coefficient (ICC) between right and left eyes of study subjects (ICC for CRAE 0.71, 95% CI, 0.31-0.87, ICC for CRVE 0.83, 95% CI, 0.60-0.92). As there was good ICC between right and left eye in our study cohort, we used GEE model to analyze the outcome variables for only one eye (right eye) of each patient in study and control group to eliminate the sampling bias of two eyes per patient. Table 3 represents the data for 21 control and 21 study eyes (right eye) and shows a statistically significant difference between CRVE in the two groups.

After stratification (using weighted regression), the difference in the PS based adjusted means of vascular calibers was again evaluated for statistical significance. The difference in the mean values, standard error of difference and the 95% CI for the difference were obtained for each outcome variable as shown in Table 4. It shows that the post-stratified differences of mean CRAE between two groups was statistically insignificant, while the mean CRVE indicated significant difference between groups with $p$-value 0.03 ($p < 0.05$) respectively. The third marker, AVR, differed insignificantly between two groups. Thus, only CRVE was significantly different in study group as compared to control group, both without (Table 2) and with adjustment (Table 4) of covariates through stratification.

Linear regression analysis found no significant effect of the MAP, MOPP and small number of patients with vascular risk factors (diabetes and hypertension) on the vascular calibers; hence, these factors were not taken into further statistical analysis.
Furthermore to determine the effect of treatment on study sample, the post-treatment measures on the vascular calibers were obtained through follow up. All the follow-up images were retrieved from the six month follow up visit and all the patients were on treatment with systemic corticosteroids ± immunosuppressive (varying dose) at time of follow up. The observations were available on 23 out of 33 eyes included in the study sample. The means before and after treatment were evaluated for statistical significance of difference using paired t-test with the results shown in Table 5. AVR values increased (0.74 vs 0.71 respectively, p=0.04) as a result of decrease in mean CRVE value to 206.25 ± 29.39 (p=0.03), while for CRAE the changes were statistically insignificant.

Discussion:

Digital retinal photography and new semi-automated image analysis tools have enabled more precise documentation of subtle and early retinal microvascular changes such as retinal arteriolar narrowing.(Liew et al. 2008) Retinal microvasculature is unique in that it reflects the systemic vasculature in health and disease elsewhere in the body and allows for non-invasive assessment of the systemic microcirculation and the study of its structural and pathologic changes. In the present study, we analyzed the vascular caliber changes in patients with localized ocular inflammatory disease with a prototype disease model of BCR without any systemic chronic vascular diseases such as hypertension, diabetes and other diseases. We found a narrower CRVE and a larger AVR in this particular subtype of retinal vascular inflammatory disease. CRVE continue to decline in patients with BCR despite being on treatment.
Retinal vascular caliber measurements may provide an additional tool in the follow-up and management of patients with BCR, and clinic visits may entail a simple image capture of the retinal vasculature to calculate vessel calibers in order for the clinician to monitor progress objectively. However, this needs to be further validated in larger studies with longer follow up and correlated to other accepted monitoring tools including ERG, FFA, ICG and visual fields.

Previously, the main reason for treating patients with BCR was loss of central vision attributable to cystoid macula oedema.(Trinh et al. 2009) An important development in the management of BCR has been the recognition that early initiation of therapy before central vision deterioration is crucial, as peripheral retinal abnormalities may be well advanced by the time visual acuity suffers a decline.(Menezo & Taylor 2013) This change in treatment strategy is important because early and aggressive management may reverse these abnormalities before they become chronic and permanent. Current validated modalities for charting retinal function such as ERG and visual field testing reflects changes in retinal function during the intervening months or years since the last test but do not provide real-time measures of disease activity.(AndreaD. Birnbaum, Amani A. Fawzi, Alfred Rademaker 2015) Piffer et al have documented the application of wide field autofluorescence in patients with BCR. The authors have shown presence of chorioretinal lesion using autofluorescence in 80% of the subjects and have established direct correlation of lesion with the visual status. In addition, they have also presence of macular hypo-autofluorescence lesion in patients with drop in vision (Piffer et al. 2014).

It is possible that retinal vascular changes might potentially serve as additional cost-
effective, useful non-invasive markers of early disease activity, which in our study manifests as decreased CRVE on diagnosis.

The progressively decreasing venular calibers at follow-up is in line with the existing knowledge that retinal function continues to decline, resulting in progressive peripheral retinal dysfunction. (Tomkins-Netzer, Taylor & Lightman 2014) According to immunogenetic studies, patients with BCR exhibit increased levels of IL-17 related cytokines and pathogenic T cells. (‘Birdshot Chorioretinopathy’ 2014, Kuiper et al. 2015) The narrowed retinal venular caliber in patients with BCR, hence can be explained by progressive vascular inflammation, affecting veins more than the arteries. (Talat, Lightman & Tomkins-netzer 2014) Interestingly AVR is larger for patients with BCR, which could be a reason why retinal ischemia and neovascularization is not a feature of BCR. (Talat, Lightman & Tomkins-netzer 2014) In contrast to BCR, in Behcet’s disease there is inflammation of both arteries and veins with narrowing of arteries in late stages as a consequence of occlusive vasculitis, which results in peripheral retinal ischemia and neovascularisation. (Talat, Lightman & Tomkins-netzer 2014) The results from our current study suggest that there is predominant venular involvement in BCR and complements Gass observation about diffuse narrowing of retinal vessels in patients with BCR.\(^9\)

The study was inherently limited by small sample size and due to its retrospective nature we could not re-photograph patients with fundus images of suboptimal quality resulting in an inability to grade 20% of images. Also, the control group for this study was chosen a priory, which was not age matched, further affecting the conclusions from this study. The findings from this study provides a pilot data about the
characteristic vascular caliber changes in BCR and it will be interesting to correlated these changes with HVF and ERG in the subsequent studies. Despite the limitations, the significantly larger AVR over time shows promise for future development of a monitoring system for BCR. Large multicenter prospective studies are needed to determine if retinal vascular caliber measurement can help in the management of patients with ocular inflammation and BCR.

In summary, venular calibers are affected in BCR and our results show significant remained throughout the course of BCR despite treatment. This result suggests vessel caliber can be a potentially useful tool for monitoring patients with BCR. Further studies are needed to validate the findings and link them to retinal function based on ERGs and visual fields.
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Other acknowledgements:

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Authors’ contribution:

RA had written the first draft of the manuscript and was involved in study design, data collection, analysis and intellectual inputs. NJ did the image segmentation using semi-automated software. LJL did critical data analysis and provided intellectual inputs. JL, AA did the editing and the finalizing of the manuscript. DS conceptualized the study design for semi-automated software analysis of the fundus images of patients with BCR. GL, PK, AA & CP provided critical inputs and mentored the author for this study. GL helped in image analysis. All authors reviewed the manuscript and provided their inputs.
References


Legends:

**Figure 1:** Disc centered color fundus photograph of right eye of a patient with Birdshot retinochoroidopathy (BCR) at baseline (A) and at six months follow up (B).

**Figure 2:** Computer-assisted measurement of retinal vessel caliber from a digital retinal photograph with control panel showing image adjustment and vessel measurement tools. The magnified image inset shows numerous bars along each vessel segment between the middle and outer circle of the grading grid (red depicts arterioles and blue depicts venules). Each vessel caliber measurement is derived from an average of widths, measured by the series of bars.

**Figure 3:** The stratum-wise mean age of subjects for study and control groups has been depicted through bar charts. It is evident that strata 2 and 3 are nearly balanced for age; however, an imbalance was observed in stratum 1 due to single subject of 48 years, and the mean age of 24.69 years for control group.
Tables:-

Table 1: Baseline characteristics for study and control groups (based on number of patients)

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Study group (n=21)</th>
<th>Control group (n=21)</th>
<th>P-value</th>
<th>Relative effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAE</td>
<td>56.28 ± 13.78</td>
<td>45 ± 17.83</td>
<td>0.03*</td>
<td>12.98</td>
</tr>
<tr>
<td>CRVE</td>
<td>16 (76.19)</td>
<td>15 (71.42)</td>
<td>0.99†</td>
<td>3.90</td>
</tr>
<tr>
<td>IOP [Mean ± SD]</td>
<td>15 ± 2</td>
<td>15 ± 1</td>
<td>0.75</td>
<td>8.27</td>
</tr>
</tbody>
</table>

* t-test for independent samples between study and control groups; †: Chi-square test; S: Significant; NS: Not significant; CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, AVR: arteriole to venule ratio

Table 2: Retinal vascular calibers between study eyes and control eyes at baseline using Generalised Estimating Equation (GEE) model (based on number of eyes)

<table>
<thead>
<tr>
<th>Vascular caliber</th>
<th>Mean ± SD [95% CI]</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAE</td>
<td>152.89 ± 23.63 [144.51-161.27]</td>
<td>0.26</td>
</tr>
<tr>
<td>CRVE</td>
<td>211.32 ± 22.56 [203.32-219.32]</td>
<td>0.003</td>
</tr>
<tr>
<td>AVR</td>
<td>0.72 ± 0.1 [0.68-0.76]</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* t-test for independent samples between study and control groups; CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, AVR: arteriole to venule ratio

Table 3: Retinal vascular calibers between one eye (right eye) per study and control group at baseline (based on number of subjects and one eye per subject)

<table>
<thead>
<tr>
<th>Vascular caliber</th>
<th>Mean ± SD [95% CI]</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAE</td>
<td>151.874 ± 24.02 [140.80-162.67]</td>
<td>0.36</td>
</tr>
<tr>
<td>CRVE</td>
<td>211.41 ± 23.51 [200.71-222.12]</td>
<td>0.04</td>
</tr>
<tr>
<td>AVR</td>
<td>0.72 ± 0.1 [0.67-0.76]</td>
<td>0.42</td>
</tr>
</tbody>
</table>

* t-test for independent samples between study and control groups;

Table 4: Difference in the mean values of vascular calibers between study (n=33 eyes) and control groups (n=33 eyes) after stratification based on propensity scores

<table>
<thead>
<tr>
<th>Vascular caliber</th>
<th>Stratification*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression based estimates†</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>SE(Difference) [95%CI]</td>
</tr>
<tr>
<td>CRAE</td>
<td>-6.05</td>
</tr>
<tr>
<td>CRVE</td>
<td>-14.8</td>
</tr>
<tr>
<td>AVR</td>
<td>0.016</td>
</tr>
</tbody>
</table>

* Based on propensity score considering age, gender and IOP as covariates
† Weighted sum of difference of outcome regressed on covariate age across strata
Table 5: Descriptive statistics for each biomarker before and after treatment (based on number of eyes; n=23 eyes)

<table>
<thead>
<tr>
<th>Vascular caliber</th>
<th>Mean ± SD [95% CI]</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>CRAE</td>
<td>150.92 ± 25.29</td>
<td>153.38 ± 26.56</td>
</tr>
<tr>
<td>CRVE</td>
<td>213.84 ± 24.63</td>
<td>206.25 ± 29.39</td>
</tr>
<tr>
<td>AVR</td>
<td>0.71 ± 0.1</td>
<td>0.74 ± 0.09</td>
</tr>
</tbody>
</table>

†Obtained using paired t test
Disc centered color fundus photograph of right eye of a patient with Birdshot retinochoroidopathy (BCR) at baseline (A) and at six months follow up (B).

142x58mm (300 x 300 DPI)
Computer-assisted measurement of retinal vessel caliber from a digital retinal photograph with control panel showing image adjustment and vessel measurement tools. The magnified image inset shows numerous bars along each vessel segment between the middle and outer circle of the grading grid (red depicts arterioles and blue depicts venules). Each vessel caliber measurement is derived from an average of widths, measured by the series of bars.

160x83mm (250 x 250 DPI)
The stratum-wise mean age of subjects for study and control groups has been depicted through bar charts. It is evident that strata 2 and 3 are nearly balanced for age; however, an imbalance was observed in stratum 1 due to a single subject of 48 years, and the mean age of 24.69 years for the control group.