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## CONTRIBUTION OF BLOOD VESSELS TO RETINAL NERVE FIBER LAYER THICKNESS IN NMOSD

### OPEN

Neuromyelitis optica spectrum disorders (NMOSDs) are relapsing inflammatory demyelinating disorders with optic neuritis (ON) as the hallmark. ON causes neuroaxonal damage to the optic nerve and retina, regularly leading to severely impaired visual acuity (VA).<sup>1</sup>

Peripapillary retinal nerve fiber layer (pRNFL) thickness measured by optical coherence tomography (OCT) has been increasingly recognized as a marker for neuroaxonal damage and correlate of visual dysfunction.<sup>1</sup> As such, pRNFL is implemented as an outcome in clinical trials of ON-associated disorders. Blood vessels (BVs) running within the pRNFL contribute approximately 13% to an average RNFL thickness<sup>2</sup> and could present an important confounder when tracking small pRNFL changes or in diseases with severe thinning such as NMOSD.<sup>1</sup> Against this background, the objective of this study was to investigate the influence of retinal BVs on pRNFL measurements in an NMOSD cohort.

**Methods.** Forty patients from a prospective observational cohort study at the NCRC at Charité–Universitätsmedizin Berlin were enrolled (women/men: 39/1, age:  $44.7 \pm 15.4$  years, 42 ON eyes). Inclusion criteria were a minimum age of 18 years and diagnosis of NMOSD according to the 2015 IPND criteria<sup>3</sup> ( $n = 37$ , aquaporin-4 antibody seropositive  $n = 28$ ) or myelin oligodendrocyte glycoprotein-IgG-associated encephalomyelitis ( $n = 3$ ).<sup>4</sup> Exclusion criteria were any other diseases which could influence OCT results.

All patients were examined with a Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) using automatic real time (ART) function for image averaging. pRNFL was measured with a 3.4 mm ring scan around the optic nerve head ( $12^\circ$ , 1536 A scans  $16 \leq \text{ART} \leq 100$ ) and segmented semiautomatically (Eye Explorer 1.9.10.0 with viewing module 6.0.9.0) and manually corrected by an experienced grader. BV positions were automatically detected by OCTSEG<sup>5</sup> (figure, A and B) and manually corrected. Three eyes were

excluded because of insufficient image quality based on OSCAR-IB criteria.

High-contrast VA was examined monocularly under habitual correction and photopic conditions with ETDRS charts at a simulated 20 ft distance using the Optec 6500 P System (Stereo Optical, Chicago, IL).

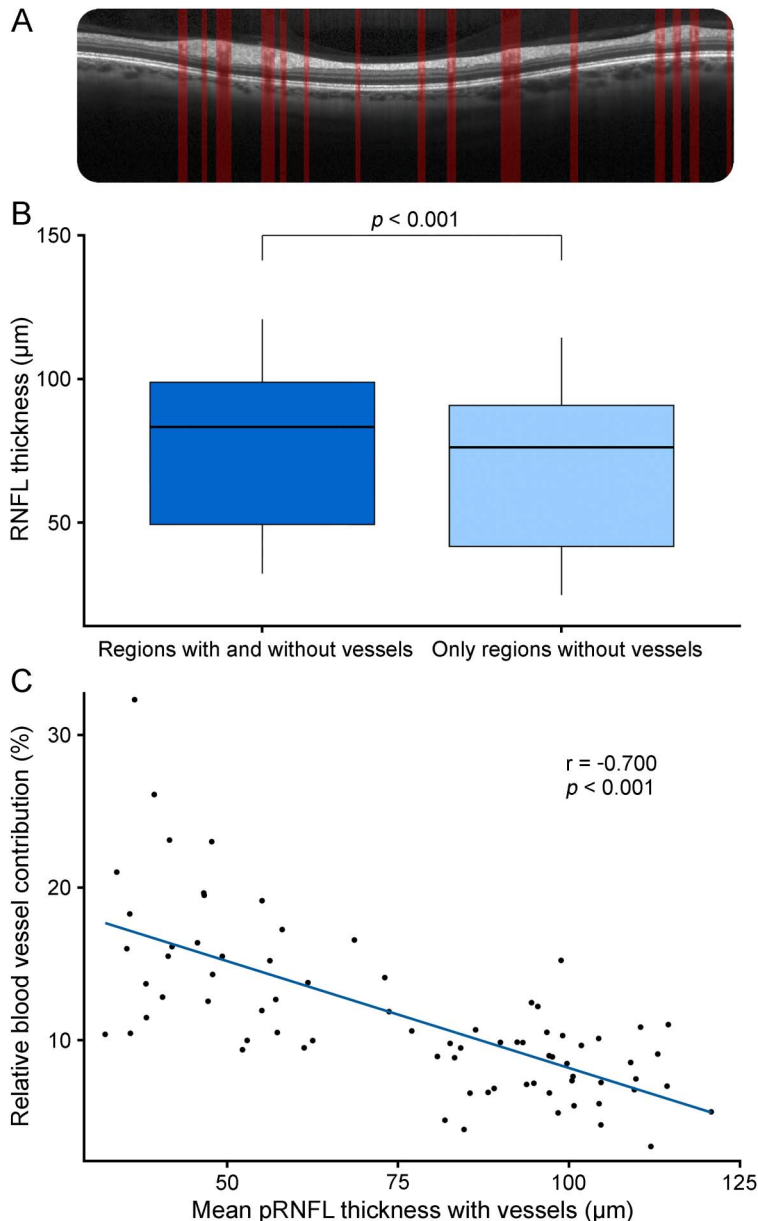
pRNFL without vessels was calculated as mean thickness of all ring scan positions not marked as part of vessels and was compared with pRNFL with vessels using paired *t* tests. We performed Pearson correlation analyses for evaluation of the relationship between pRNFL and VA and Fisher z test for correlation comparison. The relative BV contribution in percentage was calculated as  $([\text{pRNFL}_{\text{with vessels}} - \text{pRNFL}_{\text{without vessels}}] / \text{pRNFL}_{\text{with vessels}} \times 100\%)$ . All statistical tests were performed using R 3.1 with significance established at  $p < 0.05$ . The study was approved by the local Ethics Committee at Charité–Universitätsmedizin Berlin and was conducted in accordance with the Declaration of Helsinki.

**Results.** pRNFL measurements were thinner without including BVs ( $76.1 \pm 26.6 \mu\text{m}$  with,  $68.3 \pm 26.2 \mu\text{m}$  without,  $p < 2e^{-16}$ ; figure, C). Relative BV contribution increased with lower pRNFL ( $r = -0.700$ ,  $p = 1e^{-12}$ ) (figure, D). When only considering eyes with pRNFL thickness below  $60 \mu\text{m}$ , the mean relative BV contribution was significantly higher with  $16\% \pm 5\%$  compared with  $9\% \pm 3\%$  in eyes with RNFL  $>60 \mu\text{m}$  ( $p = 8e^{-8}$ ).

VA ( $36 \pm 19$  ETDRS letters) was associated with pRNFL including BV ( $r = 0.621$ ,  $p = 2e^{-9}$ ) and without BV ( $r = 0.618$ ,  $p = 2e^{-9}$ ). In eyes with pRNFL measurements below  $60 \mu\text{m}$ , pRNFL-VA correlation was numerically higher for pRNFL excluding BV ( $r = 0.495$ ,  $p = 0.007$ ) than pRNFL including BV ( $r = 0.482$ ,  $p = 0.009$ ), but the difference was not significant ( $p = 0.476$ ). There were no influences of antibody status, disease duration and therapy on pRNFL, relative BV contribution, or enlargement of BV areas with pRNFL thinning (data not shown).<sup>6</sup>

**Discussion.** BV contribution to average pRNFL measurements is higher in thin compared with normal/high pRNFL measurements.

**Figure** Contribution of blood vessels to retinal nerve fiber layer thickness



(A) pRNFL scanned by optical coherence tomography, segmented with vessel detection. (B) Mean pRNFL thickness in  $\mu\text{m}$ ; all regions including vessels and only regions without vessels. (C) Correlation of the relative blood vessel contribution to the pRNFL including vessels. pRNFL = peripapillary retinal nerve fiber layer.

A previous study reported an average BV contribution of 13% to pRNFL measurements.<sup>2</sup> Our study expands these findings by showing that BV contribution is increased in low pRNFL measurements like the ones regularly found in NMOSD patients with severe ON.

A relevant contribution of BV artifacts to measurement noise has been reported.<sup>7</sup> Although our results did not show a structure–function correlation improvement for vessel-corrected measurements, they suggest a downgrade in pRNFL measurement sensitivity. In NMOSD cohorts, a wide range of pRNFL

thickness measurements are seen, including those lower than  $60 \mu\text{m}$ .<sup>1</sup> Typically, pRNFL differences of only a few micrometers are used to evaluate drug efficacy in ON trials.<sup>8</sup> Thus, in longitudinal studies, vessel artifacts potentially interfere with the comparability of an absolute thickness change because the relative vessel contribution increases with thinner pRNFL.

We propose analyzing OCT data in studies including NMOSD and other conditions with low pRNFL measurements in addition to vessel correction. Further studies of retrospective and prospective data and larger cohorts are required to confirm and specify BV influence and to identify reliable surrogates for tracking ON-related damage in NMOSD.

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