Macular Perfusion Parameters in Different Angiocube Sizes: Does The Size Matter in Quantitative Optical Coherence Tomography Angiography?

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PURPOSE. To investigate the macular quantitative parameters interchangeability of three different optical coherence tomography angiography (OCT-A) angiocubes (i.e., 3 × 3, 6 × 6, and 12 × 12 mm) on healthy subjects and patients affected by diabetic retinopathy (DR) and to assess the interrater reliability of such indices across the different scan protocols.

METHODS. Retrospective study involving 20 eyes of healthy subjects and 20 eyes with DR. All eyes underwent swept-source OCT-A with 3 × 3, 6 × 6, and 12 × 12-mm angiocubes centered on the fovea. Foveal avascular zone (FAZ) area and vessel density on 3 × 3, 6 × 6, and 12 × 12-mm macular scans were calculated by three independent operators at all retina, superficial, deep, and choriocapillary vascular layers. Interchangeability and interrater reliabilities were estimated using intraclass correlation coefficient (ICC).

RESULTS. Interscan reproducibility of FAZ area was very strong (ICC > 0.85) at every plexus. On the contrary, vessel density values significantly varied across different scan sizes (ICC < 0.51). Intrascan interrater reliability was high for all retina and superficial FAZ areas, while it was satisfactory at deep capillary plexus only for 3 × 3-mm scan.

CONCLUSIONS. FAZ area at all plexuses is a robust parameter even if calculated on angiocubes with different size. However, interrater reliability is higher when measured in smaller scans. Conversely, vessel density results depend on the size of angiocube, although their interrater reliability is extremely high. Studies involving OCT-A should take into consideration that scan size may influence macular perfusion parameters and interrater reliability.

Keywords: optical coherence tomography angiography, quantitative optical coherence tomography angiography, foveal avascular zone, vessel density, ImageJ

Optical coherence tomography angiography (OCT-A) is a relatively new technology, which allows the visualization of retinal and choroidal vascular layers with no need of dye injection.1 Differently from fluorescein angiography, OCT-A permits the evaluation of retinal and choroidal vascular networks in a separate, noninvasive and three-dimensional fashion.1 OCT-A relies on the principle that erythrocytes flowing in blood vessels are the only moving structure across co-registered sequential B-scans and, therefore, they can be used as a motion contrast to differentiate vessels from static tissues.1 Based on this assumption, several algorithms have been developed by many instruments including complex optical microangiography (OMAG) for Zeiss devices (Angioplex and PLEX Elite 9000; Carl Zeiss Meditec, Inc., Dublin, CA, USA),2,3 split spectrum amplitude decorrelation angiography algorithm (SSADA) for RTVue XR Avanti (Optovue Inc., Fremont, CA, USA),4 OCT angiography ratio analyses (OCTARA) for DRI OCT Triton (Topcon, Tokyo, Japan),5 and full-spectrum amplitude decorrelation algorithm (FSADA) for Heidelberg (Spectralis; HRA Heidelberg, Heidelberg, Germany).6 All these machines provide en face angiogram images, which can be postprocessed in order to obtain quantitative parameters, such as foveal avascular zone (FAZ) area and vessel density. Many studies have reported that these parameters have high intra- and interoperator reproducibility both in eyes with and without retinal diseases, for the same scan type and for the same machine.7–13 In a study by Dong and coworkers,14 a good agreement between 3 × 3- and 6 × 6-mm angiocubes was found for choriocapillary vessel density and for FAZ area at all retina vasculatures; conversely, reliability for retinal vessel density was weak. However, scan interchangeability has been only tested for small angiocubes and it remains uncertain for wider size scans (e.g., 12 × 12 mm). One of the advantages of OCT-A compared with fluorescein angiography is the possibility to distinguish between superficial and deep plexuses; however, the effect of different scan sizes on interchangeability and interoperator reliability for each plexus is still unknown. In addition, interchangeability of 3 × 3- and 6 × 6-mm angiocubes has been demonstrated only for one spectral-domain (SD)-OCT-A device (Optovue) using an automatic method and only in healthy Chinese subjects.14 Therefore, it remains unclear whether other instruments or manual measurements may yield different results, particularly when applied to individuals with retinal disorders.
To address these points, we conducted a study, which aimed (1) to investigate the impact of three different angiocubes (i.e., 3 × 3, 6 × 6, and 12 × 12 mm) on quantitative measures in healthy subjects or with diabetic retinopathy (DR), and (2) to assess the interrater reliability of such indices across the different scan protocols.

METHODS

Study Design and Population
The present study was a retrospective analysis of clinical data acquired from healthy subjects and patients with DR presented at the Medical Retina & Imaging Unit of the Department of Ophthalmology, University Vita-Salute, San Raffaele Hospital, Milan, Italy. In our clinic, all patients referred for OCT examination undergo a standard imaging protocol, which includes SD-OCT (Spectralis; HRA Heidelberg) and OCT-A (PLEX Elite 9000; Carl Zeiss Meditec, Inc.).

Electronic clinical records, SD-OCT and swept source (SS)-OCT-A images from both healthy subjects and patients diagnosed with DR were reviewed. For each group, we included the first 20 consecutive eligible eyes starting from April 4, 2017 and going backward. Inclusion criteria were: age greater than or equal to 18 years old; refractive error between −6 and +3 diopters; availability of all 3 × 3, 6 × 6, and 12 × 12-mm OCT-A scans with a signal strength greater than or equal to 7 and centered on the fovea. Exclusion criteria were: presence of any retinal disease other than DR; presence of any optic nerve disease; previous ocular surgery other than uncomplicated cataract extraction and IOL implantation performed less than or equal to 6 months before enrollment; artifacts on OCT-A images.

Structural SD-OCT Measurements
The structural SD-OCT acquisition protocol included 19 horizontal raster linear B-scans, each composed by nine averaged OCT B-scans (1024 A-scans per line) at 240-μm intervals, covering an area of 20° × 15°. The central macular thickness (CMT) in the central 1-mm diameter circle of the Early Treatment Diabetic Retinopathy Study (ETDRS) thickness map was recorded with the Spectralis Software.

SS-OCTA Device and Scanning Protocol
The SS-OCTA instrument (PLEX Elite 9000) uses a swept laser source with a central wavelength of 1040 to 1060 nm (980–1120 nm full bandwidth) and operates at 100,000 A-scans per second. The tissutal axial and transverse resolutions are approximately 6 μm and 20 μm, respectively. In order to generate an OCT-A image, datasets are processed by means of the OMAG algorithm, which uses variations in both the intensity and phase information between sequential B-scans acquired at the same position.5,15 The 3 × 3-mm acquisition is made of 300 × 300 A-scans, while the others (i.e., 6 × 6, 12 × 12 mm) are acquired with 500 × 500 A-scans.

All 3 × 3, 6 × 6, and 12 × 12 mm employ a 1024 × 1024-pixel array with 2.9, 5.9, and 11.7 μm-spacing between pixels, respectively.

All scans were centered on the fovea and automated segmentations of full-thickness (internal limiting membrane to Bruch’s membrane) retinal vasculatures, superficial, deep, and choriocapillary vascular layers were reviewed and manual corrections were carried out as necessary to ensure an accurate segmentation. Instrument projections removal algorithm was applied to images at deep capillary plexus and choriocapillaris.

Quantitative Analysis of OCT-A Images
All images were exported to Joint Photographic Expert Group (jpeg) format and analyzed using ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) by three independent operators (AR, FG, AM), as illustrated in Figure 1. The FAZ area was manually measured at all retina, superficial, and deep vascular layers using a previously published method.16,17 Briefly, the FAZ area was encircled using the polygon selection tool and its area was recorded in squared millimeters.

The vessel density was calculated on binarized images using a threshold algorithm, similarly to other studies.18,19 The ImageJ ‘mean threshold’ algorithm, which automatically computes the threshold value as the mean of the local grayscale distribution, was applied to all images. Binarized 8-bit images were converted to red-green-blue (RGB) color model. A region of interest of 512 × 512 and 256 × 256 pixels centered on the fovea was applied, respectively, to the 6 × 6- and 12 × 12-mm images and pixels outside that perimeter were cleared to pure blue color, in order to select the area corresponding to the 3 × 3-mm scan. FAZ area was restored to each corresponding image and it was colored to pure blue. White pixels were considered as vessel, black pixels as background, and blue pixels were automatically excluded from the analysis in order to prevent the inclusion of FAZ area and areas outside the 3 × 3-mm perimeter during the vessel density calculation. Vessel density was calculated as the ratio between the number of white pixels and the total number of pixels after excluding blue pixels.

Statistical Analysis
The variables included in the analysis were as follows: demographic data; best corrected visual acuity (BCVA); DR severity, CMT; FAZ area at full-thickness, superficial and deep layers, and vessel density at full thickness, superficial, deep, and choriocapillary vascular layers. Interobserver reliability among the three operators was assessed using intraclass correlation coefficient (ICC) for all patients, healthy subjects and patients with DR. Macular perfusion parameters values calculated by the three raters for each plexus were averaged and their consistency across the 3 different scans (i.e., 3 × 3, 6 × 6, and 12 × 12 mm) was assessed by means of ICC for all patients, healthy subjects and patients with DR. All results are reported as mean ± SD values. All statistics were performed using GraphPad Prism software 6.0 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS software 21 (SPSS, Inc., Chicago, IL, USA).

RESULTS
In this study, there were 20 eyes of 12 healthy subjects and twenty eyes of 13 patients with DR. Demographic data and main clinical data of the study population are illustrated in Table 1. In one eye in the DR group, quantitative indices of deep capillary plexus at 12 × 12-mm scan were not calculated because FAZ was not recognizable by any of the raters.

The ICCs for FAZ area and vessel density across the three different scan size are shown in Table 2 and Figure 2. With regards to the FAZ area measurement, an excellent (ICC > 0.90) reliability was found at all retina, superficial, and deep capillary plexuses, except for the DR subgroup at the deep capillary plexus, where it was good anyway (ICC 0.852, 95% confidence interval [CI] 0.714–0.935). Not surprisingly, the CIs for the FAZ area were lower in DR patients than in healthy subjects at every plexus.
Conversely, the interscan reliability of vessel density measurements was poor (ICC < 0.50) in every vascular layer, apart from DR patients at the superficial capillary plexus that disclosed by any means a weak reliability (ICC 0.506, 95% CI 0.185–0.753). Noteworthy, the reliability of vessel density assessment was higher in DR subgroup compared with healthy patients at all plexuses. In 2 eyes, the FAZ area at the deep capillary plexus was not entirely contained in the 3 × 3-mm scan.

The interrater reliability for every macular quantitative index at each scan size is shown in Table 3. Regarding the vessel density, the interobserver reliability was excellent (ICC > 0.90) in every vascular plexus at all scan sizes. The FAZ area at all retina and superficial vascular layers disclosed an excellent reliability for all groups at 3 × 3- and 6 × 6-mm scans. For the same plexuses, interrater reliability for FAZ area of the 12 × 12-mm scan was strong (all retina ICC = 0.820, superficial ICC = 0.898) in the whole cohort of patients, although lower than that of smaller scan protocols. Interestingly, reproducibility was higher in healthy subjects than in DR patients.

At the deep capillary plexus, the interrater reliabilities for FAZ area 3 × 3, 6 × 6, and 12 × 12-mm scans were globally strong (ICC = 0.816), moderate (ICC = 0.545), and poor (ICC = 0.290), respectively. In healthy subjects, a good correlation (ICC > 0.70) was appreciated in all three scan protocols. Conversely, interrater reliability in the DR group was strong only for the 3 × 3 mm (ICC = 0.799), while it was poor and very poor for 6 × 6 (ICC = 0.383) and 12 × 12-mm (ICC = 0.113) scans, respectively.

### DISCUSSION

In the present study, we tested whether macular quantitative parameters (i.e., FAZ area, vessel density) are interchangeable when calculated on different angiocubes (i.e., 3 × 3, 6 × 6, and 12 × 12 mm) in subjects with and without retinopathy. In our cohort of patients, the FAZ area, but not vessel density, was a highly reproducible parameter across different angiocubes. Moreover, we investigated if the scan size could affect the inter-rater reliability for all macular quantitative indices at every scan size shown in Table 3. Regarding the vessel density, the interobserver reliability was excellent (ICC > 0.90) in every vascular plexus at all scan sizes. The FAZ area at all retina and superficial vascular layers disclosed an excellent reliability for all groups at 3 × 3- and 6 × 6-mm scans. For the same plexuses, interrater reliability for FAZ area of the 12 × 12-mm scan was strong (all retina ICC = 0.820, superficial ICC = 0.898) in the whole cohort of patients, although lower than that of smaller scan protocols. Interestingly, reproducibility was higher in healthy subjects than in DR patients.

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### Table 3. Demographic and Main Clinical Data of Study Population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Overall</th>
<th>Controls</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N, patients/eyes</td>
<td>25/40</td>
<td>12/20</td>
<td>13/0</td>
</tr>
<tr>
<td>Age y, mean ± SD</td>
<td>57.0 ± 16.1</td>
<td>51.8 ± 17.9</td>
<td>60.8 ± 13.9</td>
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<td>Race, Caucasian</td>
<td>25</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>14/11</td>
<td>7/5</td>
<td>7/6</td>
</tr>
<tr>
<td>Eye, right/left</td>
<td>23/17</td>
<td>13/7</td>
<td>10/10</td>
</tr>
<tr>
<td>BCVA, logmar, mean ± SD</td>
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<td>0.0 ± 0.0</td>
<td>0.29 ± 0.4</td>
</tr>
<tr>
<td>CMT, µm, mean ± SD</td>
<td>299.1 ± 73.8</td>
<td>280.2 ± 25.7</td>
<td>307.6 ± 86.6</td>
</tr>
<tr>
<td>DME, yes/no</td>
<td>9/11</td>
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<td>9/11</td>
</tr>
<tr>
<td>DR severity</td>
<td></td>
<td></td>
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<tr>
<td>Mild</td>
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<tr>
<td>Moderate</td>
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<tr>
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<tr>
<td>Proliferative</td>
<td>2</td>
<td>n/a</td>
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We found a solid reproducibility for both vessel density and FAZ area assessment at the full-thickness retina and superficial vascular layers; conversely, the interrater reliability of FAZ area at the deep capillary plexus was good only for the 3 × 3-mm angiocube.

OCT-A is an extremely attractive tool, which can provide interesting information, such as identification of choroidal neovascularization (CNV) in asymptomatic patients with AMD20 or CNV complicating central serous chorioretinopathy.21 Beyond qualitative assessment, OCT-A angiograms may be post-processed in order to obtain quantitative data. Two popular indices are represented by FAZ area and vessel density. The FAZ is a capillary-free area encircled by interconnecting vessels at its margins, corresponding to the foveola, where capillaries of both superficial and deep plexuses break off.22 Although some devices (i.e., Optovue RTVue XR Avanti, Zeiss Angioplex) allow the automatic quantification of macular perfusion parameters using the built-in software, other instruments require the manual selection of innermost retinal vascular arcades in order to outline the FAZ area and image binarization or skeletonization algorithms to obtain vessel density. Macular quantitative parameters vary across different instruments and techniques (i.e., manual versus automatic assessment); however, several studies demonstrated that they have a good inter- and intraoperator reliability in case of same machine, acquisition and postprocessing protocols.7–9,11,12,23 Measurement at the superficial plexus is highly reliable, while manual demarcation at the deep plexus may be not easy due to less definite contours.12 La Spina and colleagues10 demonstrated that FAZ is a stable parameter, which is not affected by acute changes in retinal and metabolic states. Despite being highly variable even in healthy subjects,24 several conditions (e.g., DR,25 retinal vein occlusion,26 and sickle cell disease)27 may affect the FAZ size due to the occlusion of capillaries surrounding the FAZ. As a result, the FAZ area in these patients is enlarged and more irregular and, thus, its manual quantification may not be straightforward, especially at the deep plexus.

In our cohort of patients, we found a strong reliability for FAZ area measurements in all three scan sizes in both eyes with and without retinopathy; therefore, the FAZ area should be considered as a robust and interchangeable measure even if...
### TABLE 3. Reliability of Quantitative Macular Parameters Among Three Operators

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR</th>
<th>Controls</th>
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<tr>
<td><strong>FAZ (95% CI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Controls</td>
<td>0.982 (0.977–0.987)</td>
<td>0.977 (0.975–0.980)</td>
</tr>
<tr>
<td>DR</td>
<td>0.972 (0.969–0.976)</td>
<td>0.972 (0.969–0.976)</td>
</tr>
<tr>
<td><strong>Vessel density (95% CI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Controls</td>
<td>0.984 (0.979–0.989)</td>
<td>0.979 (0.978–0.987)</td>
</tr>
<tr>
<td>DR</td>
<td>0.984 (0.980–0.990)</td>
<td>0.984 (0.980–0.990)</td>
</tr>
<tr>
<td><strong>SCP (95% CI)</strong></td>
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<tr>
<td>Overall Controls</td>
<td>0.985 (0.980–0.990)</td>
<td>0.982 (0.980–0.988)</td>
</tr>
<tr>
<td>DR</td>
<td>0.984 (0.980–0.990)</td>
<td>0.984 (0.980–0.990)</td>
</tr>
<tr>
<td><strong>DCP (95% CI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Controls</td>
<td>0.986 (0.981–0.991)</td>
<td>0.983 (0.980–0.990)</td>
</tr>
<tr>
<td>DR</td>
<td>0.985 (0.980–0.990)</td>
<td>0.985 (0.980–0.990)</td>
</tr>
</tbody>
</table>

*Note: ICC values are rounded to two decimal places.*
and they should not have been affecting our analysis. Finally, quantitative parameters were manually calculated and only one OCT-A device was used; therefore, our findings may be not applicable to other postprocessing algorithms or OCT-A devices.

In conclusion, we demonstrated that FFA area at all plexuses is a robust parameter even if calculated in different angiocubes. However, its interrater reliability is higher when measured in smaller scans. On the other hand, vessel density results depend on the angiocube size, although their interrater reliability is extremely high. Studies involving OCT-A should take into account that the scan size may affect macular perfusion parameters and interrater reliability.

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