Impact of Choriocapillaris Flow on Multifocal Electroretinography in Intermediate Age-Related Macular Degeneration Eyes

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METHODS. In this prospective, observational, cross-sectional study, macular optical coherence tomography angiography images and multifocal electroretinograms were obtained in 20 eyes with intermediate age-related macular degeneration from 20 patients. The main outcome measures were (1) the percent nonperfused choriocapillaris area (PNPCA), which represents a measure of the total area of CC vascular dropout, and (2) the average size of the CC signal voids, which represent contiguous regions of CC dropout. Furthermore, amplitude and implicit time of multifocal electroretinograms N1 and P1 waves in the two central rings (R1 and R2) were included in the analysis.

RESULTS. Of the 20 patients enrolled, only 17 eyes from 17 patients (13 women) were included in this analysis. Three patients were excluded because of poor scan quality. Mean ± SD age was 75.1 ± 7.9 years (range, 62–89 years). The best corrected visual acuity was 0.17 ± 0.13 logarithm of the minimum angle of resolution. In univariate analysis, both the PNPCA and average signal void size were found to have a significant direct relationship with N1 implicit time in the R2 ring (P = 0.006 and P = 0.035, respectively). Neither PNPCA nor the average signal void size was associated with P1 or N1 implicit times in R1.

CONCLUSIONS. In intermediate age-related macular degeneration eyes, PNPCA and average signal void size are related to N1 multifocal electroretinogram implicit times, which suggests an association between CC perfusion and photoreceptor function.

Keywords: age-related macular degeneration, optical coherence tomography, electroretinogram
Previous studies used the best corrected VA (BCVA) to evaluate the 
macular function in iAMD eyes.\textsuperscript{15} Nevertheless, the introduc-
tion of the multifocal electroretinogram (mfERG) has allowed 
clinicians to test the whole macular function and to study morpho-
functional correlations in eyes affected by AMD.\textsuperscript{16-18} The 
aim of this study was to explore the relationship between the CC perfusion and macular function in iAMD eyes as assessed by mfERG.

METHODS

Study Participants
In this prospective, observational, cross-sectional study, patients 50 years of age and older with iAMD in at least one eye were enrolled at the ophthalmology clinic of University G. 
d'Annunzio, Chieti-Pescara, Italy. The study was approved by 
the institutional review board and adhered to the tenets of the 
Declaration of Helsinki. An informed consent approved by the 
institutional review board was obtained from all patients.

All patients enrolled were imaged with the RTVue XR Avanti 
AngioVue OCTA (Optovue, Inc., Fremont, CA, USA) between 
October 2016 and June 2017. Moreover, all patients received a 
complete ophthalmologic examination, which included the 
measurement of Snellen BCVA, IOP, and dilated ophthalmoscopy.

The inclusion criteria for iAMD eyes included the presence 
of drusen >125 μm in diameter with or without pigmentary 
abnormalities as assessed by clinical examination and con-
firmed by dense volume optical coherence tomography (OCT) 
(pigment abnormalities on OCT manifesting as intraretinal 
hyper-reflective features).\textsuperscript{1} Exclusion criteria for iAMD eyes were 
(1) pseudodrusen on the OCT scan, because of their 
presence have been shown to influence both CC perfusion\textsuperscript{15} 
and mfERG values;\textsuperscript{3,6} (2) previous ocular surgery or history of 
antivascular endothelial growth factor therapy; (3) any 
maculopathy secondary to causes other than AMD (including 
the presence of an epiretinal membrane or vitreomacular 
tractions syndrome); (4) the presence of significant media 
opacities; (5) myopia greater than –3.00 diopters; and (6) any 
eopic neuropathy, including glaucoma. Furthermore, images 
with a strength index less than 40, with either significant 
motion artifact (seen as large dark lines on the en face 
angiogram) or incorrect segmentation at the level of the CC 
were excluded from the analysis.

Imaging
Patients underwent OCTA imaging using the RTVue XR Avanti 
spectral-domain OCT device with AngioVue software (version 
2016.1.0.26; Optovue, Inc.), which is based on a split-spectrum 
amplitude-decorrelation angiography algorithm. Flow is detected 
as a variation of amplitude over time in the speckle pattern formed 
by the interference of light scattered from red blood cells and 
adjacent tissue structures.\textsuperscript{20} For each patient, a 3×3-mm cube 
scan was acquired containing 304×304 A-scans.

Image Processing

The main outcome measures were (1) the percent non-
perfused choriocapillaris area (PNPCA), which represents a 
measure of the total area of CC vascular dropout, and (2) the 
average size of the CC signal voids, the latter representing 
contiguous regions of CC dropout.

To evaluate the PNPCA and the average signal void size, we 
analyzed the images as already described.\textsuperscript{15,21,22} In brief, the 
PNPCA was computed as the percentage of pixels in the CC en 
face image (slab 30-μm thick starting 31 μm posterior to the 
RPE reference) below a “non-perfusion” (or noise) threshold, 
which was calculated with ImageJ software version 1.50 
(National Institutes of Health, Bethesda, MD, USA; http://rsb. 
info.nih.gov/ij/index.html) as the mean of all the pixel values in 
the outer avascular retina. The PNPCA was thus calculated as 
the number of pixels falling below the threshold (the total area 
with signal voids) divided by the total number of pixels in the 
analyzed area of CC. Furthermore, the “Analyze Particles” command, 
which measured and counted all thresholded areas 
greater or equal to 1 pixel where there was a lack of flow 
information, furnished us the average size of the signal voids.

Notably, the CC directly beneath drusen, as well as under 
superficial retinal vessels, was excluded from the analysis to 
avoid shadowing or projection artifacts from confounding the 
analysis, as already shown (Fig. 1).\textsuperscript{5,12} In brief, the drusen area 
was identified on the “RPE elevation” map elaborated by the 
AngioVue software. This image was imported into ImageJ, and 
the “Split Channels” function was carried on to obtain the 
“green channel” image. In the latter image, the RPE elevation 
area appears darker than the surrounding area, and the “MaxEntropy” threshold was therefore applied to “binarize” 
the obtained image. The superficial capillary plexus (SCP) en 
face OCTA image was segmented with an inner boundary 5 μm 
below the internal limiting membrane and an outer boundary 
set at 15 μm below the inner plexiform layer. The en face SCP 
image was opened in ImageJ, and the “MaxEntropy” threshold 
was applied to visualize only the greater superficial retinal 
vessels (causing shadowing and artifacts). The three obtained 
images (CC en face image and images identifying superficial 
vessels and drusen regions) were merged to mask those 
regions beneath drusen and/or superficial vessels.

Multifocal Electroretinogram (mfERG)
mfERG (Retimax CSO, Florence, Italy) was recorded for each 
patient according to the International Society for Clinical 
electrophysiology of Vision protocol that was updated in 
2011.\textsuperscript{23} Pupils were dilated with 1% tropicamide and recording 
began only when the pupils were dilated to at least 7 mm. 
Because retinal adaptation may affect mfERG results, the 
patients were exposed at the same preexposure light and the 
illumination in the examination room was moderate and the 
same for all patients, as previously suggested.\textsuperscript{23} At each mfERG 
examination, patients positively reported that he/she could 
clearly perceive the fixation target. Moreover, the eye’s 
position was monitored by a video system. During the mfERG 
examination, the ocular fundus was segmented by an array of 
61 hexagons. The amplitudes and implicit times of N1 (first 
negative component) and P1 (first positive component) of the 
first-order kernel were calculated for five regional ring groups 
(R1, R2, R3, R4, and R5). The amplitude of N1 was measured 
from the baseline to the first negative peak. The P1’s amplitude 
was measured from the first negative peak to the first positive 
peak. The latencies were defined as the time period from the 
stimulus onset to the peak of N1 and P1 responses.

The responses from the central two rings were included in 
the analysis, where rings R1 and R2 cover areas approximately 
0° to 5° and 6° to 10°, respectively, from the fovea. This choice 
was made because these two rings overlay a 2.8-mm-diameter 
circle area centered on the fovea, similar to the filed covered by 
the OCTA scan (Figs. 2, 3).

Statistical Analysis

Statistical calculations were performed using Statistical Package 
for Social Sciences (version 20.0, SPSS, Inc., Chicago, IL, USA). 
To detect departures from normality distribution, Shapiro-
Wilk’s test was performed for all variables. All quantitative 
variables were presented as mean and SD in the results.
Univariate regression analyses of potential associations between the OCTA and functional parameters were performed. For each analysis, a standardized $\beta$ coefficient was calculated. This compares the strength of the effect of each individual independent variable to the dependent variable. The higher the absolute value of the standardized $\beta$ coefficient (values between 0 and $\pm 1$), the stronger the effect.

The BCVA for each eye was converted to the logarithm of the minimum angle of resolution, as previously described. The chosen level of statistical significance was $P < 0.05$.

**RESULTS**

**Characteristics of Patients Included in the Analysis**

Of the 20 patients enrolled, only 17 eyes from 17 patients (13 women) were included in this analysis. Three patients were excluded because of poor scan quality. Mean ± SD age was 75.1 ± 7.9 years (range, 62–89 years). The BCVA was $0.17 \pm 0.13$ logarithm of the minimum angle of resolution.

mfERG N1 and P1 amplitudes were $-0.38 \pm 0.30$ and $0.50 \pm 0.35 \mu V$, and $-0.22 \pm 0.13$ and $0.49 \pm 0.25 \mu V$ in the R1 and R2 rings, respectively.

mfERG N1 and P1 implicit times were $18.6 \pm 2.4$ milliseconds and $38.2 \pm 2.1$ milliseconds in R1, $16.5 \pm 3.9$ milliseconds and $36.2 \pm 2.3$ milliseconds in R2.

In the OCTA evaluation of the CC layer, the whole PNPCA was $4.1 \pm 2.0\%$, the average signal void size was $281.7 \pm 65.4 \mu m^2$, and the average signal void number was $1225.2 \pm 336.3$, respectively. Topographically, the PNPCA in the 1.4-mm-wide circle centered on the fovea was $5.2 \pm 12.0\%$ of the whole PNPCA in the $3 \times 3$-mm-wide scan.

**Regression Analysis**

In univariate analysis (Table), both the PNPCA and average signal void size were found to have a significant direct relationship with N1 implicit time in the R2 ring ($P = 0.006$ and $P = 0.035$, respectively). In R1, the average signal void size was statistically associated with N1 amplitude ($P = 0.015$). Neither the PNPCA nor the average signal void size was...
associated with P1 or N1 implicit times in R1. OCTA values were not associated with BCVA (P > 0.05 for all univariate analyses).

**DISCUSSION**

In this prospective, cross-sectional study we investigated CC features and macular function in iAMD eyes. Overall, we found that the PNPCA was associated with changes in mfERG implicit time, but not mfERG response amplitudes.

In recent years, OCTA technology has been shown to be useful in studying AMD eyes. Several studies assessed the retinal and choroidal vasculature in eyes with iAMD and showed that iAMD eyes are characterized by a reduced SCP and CC perfusion density, especially in the presence of nascent geographic atrophy or neovascularization in the fellow eye. In the latter study, the authors demonstrated that a selective dysfunction in the cone-driven pathway, as evaluated by mfERG, especially with regard to implicit times, which were shown to be altered beyond the visible drusen area. Interestingly, mfERG implicit times were demonstrated to have a progressive worsening despite stable VA.

Wu et al. investigated the association between macular function by mfERG and photoreceptor damage, which was assessed by analyzing the inner segment/outer segment (IS/OS) junction reflectivity with OCT. The reflection signal arising from IS/OS junction has been suggested to originate from the photoreceptor IS ellipsoids. Because discontinuities in the IS/OS junction are seen as hyporeflective areas on the en face image, it has been demonstrated that the reflectivity of the IS/OS junction might be a surrogate for photoreceptor damage. Wu et al. demonstrated a significant negative association between IS/OS reflectivity and mfERG values, which may suggest that the mfERG responses are influenced by the photoreceptor loss.

The mfERG macular function in early/intermediate AMD eyes was also studied by Parisi et al., who enrolled 27 patients and assessed the influence of short-term carotenoid and antioxidant supplementation on macular function. In this study, the authors demonstrated that a selective dysfunction in the macula may be improved by this supplementation.

<table>
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<tr>
<th>Tested Variables</th>
<th>PNPCA</th>
<th>Average Signal Void Size</th>
<th>Average Signal Void Number</th>
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<tr>
<td></td>
<td>Standardized β Coefficient</td>
<td>P</td>
<td>Standardized β Coefficient</td>
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<tr>
<td>R1N1A</td>
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<tr>
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<td>R2N1L</td>
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<tr>
<td>R2P1L</td>
<td>0.116</td>
<td>0.650</td>
<td>0.347</td>
</tr>
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</table>

Standardized β coefficient comprised between 0 and ±1. The higher the absolute value is, the stronger is the association between the two variables. Significant P values (<0.05) are in bold. OCTA, optical coherence tomography angiography; PNPCA, percent nonperfused choriocapillaris area; R1N1A, first negative component amplitude in the first ring; R1P1A, first positive component amplitude in the first ring; R1N1L, first negative component implicit time in the first ring; R1P1L, first positive component implicit time in the first ring; R2N1A, first negative component amplitude in the second ring; R2P1A, first positive component amplitude in the second ring; R1N1L, first negative component implicit time in the second ring; R1P1L, first positive component implicit time in the second ring.

The macular sensitivity in iAMD eyes was also tested by Gerth and colleagues, who demonstrated functional changes in the cone-driven pathway, as evaluated by mfERG, especially with regard to implicit times, which were shown to be altered beyond the visible drusen area. Interestingly, mfERG implicit times were demonstrated to have a progressive worsening despite stable VA.

Wu et al. investigated the association between macular function by mfERG and photoreceptor damage, which was assessed by analyzing the inner segment/outer segment (IS/OS) junction reflectivity with OCT. The reflection signal arising from IS/OS junction has been suggested to originate from the photoreceptor IS ellipsoids. Because discontinuities in the IS/OS junction are seen as hyporeflective areas on the en face image, it has been demonstrated that the reflectivity of the IS/OS junction might be a surrogate for photoreceptor damage. Furthermore, they found a lack of correlation between these two functional measures, suggesting that mfERG may evaluate unique aspects of macular dysfunction. The mfERG is indeed considered as an electrophysiological measure of suprathreshold postreceptor responses at photopic levels, which mainly originate from the cone photoreceptors, whereas microperimetry is a measure of sensitivity at mesopic levels, which may be mediated by both rod and cone photoreceptor pathways.

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Two previous notable studies have investigated the association between the CC perfusion and VA in iAMD eyes.15,21 Both studies did not find a correlation between these two parameters in iAMD eyes without pseudodrusen, as confirmed in our results. Interestingly, Nesper et al.13 demonstrated that the PNPCA was negatively correlated with VA only in those iAMD eyes with pseudodrusen. However, given that AMD is a disorder that affects regions beyond the foveola, the functional information provided by VA is limited.12 Furthermore, VA is highly dependent on fixation, which may also be compromised in AMD.43

We add to the literature by reporting the association between CC perfusion and macular function. We found that N1 mFERG implicit time was associated with increased PNPCA and average signal void size. Because the N1 wave is thought to generate from the postreceptorial signals after cones (it is indeed mainly shaped by the initial hyperpolarization of the OFF-bipolar cells), whereas the P1 wave is known to be more influenced by the inner retina, we speculate that the CC changes may affect the postphotoreceptor function. Interestingly, we found that this association was significant only in the R2 ring. This aspect may be explained by the topographical features of AMD, which may affect the parafoveal region more extensively in its early or intermediate stages.42 Furthermore, although we demonstrated that the CC dropout was mainly located in the parafoveal region, this aspect may also explain the absence of association in R1.

Furthermore, the association between CC changes and mFERG implicit time, but not response amplitude, suggests an association with neuroretinal functional abnormalities of the photoreceptor-mediated pathway rather than cell loss.17 However, our findings are unable to determine the exact mechanisms responsible for these functional changes. Prospective longitudinal studies will help shed further light on the relation between CC perfusion and macular function.

Our study has some limitations. The study sample was relatively small and we did not enroll healthy individuals for comparison. However, the aim of this study was to assess the presence of an association between CC perfusion and macular function because other studies have already investigated these OCTA parameters in iAMD and healthy eyes. In addition, this study was not large enough to account for confounding factors such as age, diabetes, or smoking, which are known to affect electroretinogram values.34–40 Furthermore, shifts in fixation may have been a limitation in examining the association between the pathological features and functional changes in the central macular region. Nevertheless, the fixation monitoring system used during mFERG recordings allowed us to assess the occurrence of any contamination by blinks or eye movements. Also, the study was not large enough to account for confounding factors such as age, diagnosis of diabetes, smoking status, gender, or drusen volume. Finally, we did not investigate the micro-perimetry or contrast sensitivity in our study cohort.

In conclusion, despite these limitations, this study investigated the relationship between CC perfusion and mFERG macular function in eyes with intermediate AMD. We observed that the PNPCA and average signal void size are related to N1R2 mFERG implicit times, which suggests an association between CC perfusion and postphotoreceptor function rather than cell loss. These findings may help broaden our knowledge regarding the pathophysiology and evolution of AMD.

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References


