• Clinical Research •

Retinal pigment epithelium–Bruch's membrane volume in grading of age-related macular degeneration

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Abstract

● **AIM:** To assess the agreement of optical coherence tomography (OCT) algorithm-based retinal pigment epithelium –Bruch's membrane complex volume (RBV) with fundus photograph-based age-related macular degeneration (AMD) grading.

● **METHODS:** Digital color fundus photographs (CFPs) and spectral domain OCT images were acquired from 96 elderly subjects. CFPs were graded according to Age-Related Eye Disease Study (AREDS) classification. OCT image segmentation and RBV data calculation were done with Orion™ software. Univariate and multivariate analyses were performed to find out whether AMD lesion features associated with higher RBVs.

● **RESULTS:** RBV correlated with AMD grading (r_s=0.338, P=0.001), the correlation was slightly stronger in early AMD (n=52; r_s=0.432, P=0.001). RBV was higher in subjects with early AMD compared with those with no AMD lesions evident in fundus photographs (1.05±0.20 *vs* 0.96±0.13 mm^3, P=0.023). In multivariate analysis higher RBVs were associated significantly with higher total drusen (β=0.388, P=0.027) and pigmentation areas (β=0.319, P=0.020) in fundus photographs, whereas depigmentation area (β=-0.295, P=0.015) associated with lower RBV.

● **CONCLUSION:** RBV correlate with AMD grading status, with a stronger association in patients with moderate, non-late AMD grades. This effect is driven mostly by lesions with drusen or pigmentation. Lesions with depigmentation tend to have lower values. RBV is more comprehensive measurement of the key area of AMD pathogenesis, compared to sole drusen volume analysis. RBV measurements are independent on grader variations and offer a possibility to quantify early and middle grade AMD lesions in a research setting, but may not substitute fundus photograph-based grading in the whole range of AMD spectrum.

● **KEYWORDS:** age-related macular degeneration; drusen; optical coherence tomography; Bruch's membrane
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INTRODUCTION

Age-related macular degeneration (AMD) is the most important cause of visual impairment in elderly in high-income countries[1]. The current hypothesis on the pathogenesis of AMD is that early changes in the Bruch's membrane (BrM) such as linear and laminary deposits precede and modulate the disposal of cellular debris originating from the visual cycle[2-4]. The accumulation of such debris results in the formation of drusen [globular elevations of the retinal pigment epithelium (RPE)], chronic low-grade inflammation, RPE migration and subretinal neovascularization or atrophy of the retina[5-6]. All of these processes occur in the RPE-BrM complex and all of them except for atrophy, include thickening of the RPE-BrM complex. The severity of early/intermediate AMD predicts the risk of developing neovascular AMD or geographic atrophy, the forms of the disease responsible for most of the visual handicap attributable to AMD[7]. The classification of early AMD has traditionally been based on qualitative/semiqualitative classification of color fundus photographs (CFPs) by trained graders. This approach has proved successful in many seminal studies on AMD pathogenesis[8-9]. However, AMD grading parameters from fundus photographs are two-dimensional and subject to loss of sensitivity due to color photograph image quality. The advances in optical coherence tomography (OCT) imaging have made it possible to observe the retina-RPE-BrM complex in significantly greater detail than

available from CFPs. Moreover 3-dimensional models of the AMD lesion can be produced from the transverse sections. The OCT based gradings of early AMD have mostly employed an algorithm delineating drusen, the key lesions in early AMD[10]. Drusen volume analyses have correlated with progression and genetic risk factors for late AMD[11-12]. However, in these studies the drusen volume was zero in a significant proportion of eyes with early AMD by fundus photograph grading[13-17]. Since drusen is only one manifestation of early AMD tissue changes, using algorithms delineating and measuring drusen only, may overlook other early AMD lesions resulting in reduced sensitivity. We hypothesize that an algorithm measuring the volume of the whole RPE-BrM complex may be more sensitive to reflect any AMD related pathology in OCT images.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the local Medical Ethics Committee, approval number HUS/2241/2016. Signed informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Subjects Information regarding age, smoking status and other eye pathology were collected from the study questionnaire. All subjects were Caucasian Finns. The right eye of each subject was used for the analysis.

Ninety-six elderly subjects undergoing other studies were recruited for fundus imaging between March 2018 and December 2019 at the Department of Ophthalmology at Helsinki University Hospital. The visit included dilated 35-degree digital CFPs and spectral domain OCT imaging (Heidelberg Spectralis, 20×20 degree field centered on the fovea, 521 scans) and a structured study questionnaire.

The dilated CFPs were graded according to the Age-Related Eye Disease Study (AREDS) classification modified for digital photographs[18]. To reduce the number of categories the features of largest drusen, drusen area, pigmentation, depigmentation and age were further combined into four groups as follows: Largest drusen size–no drusen (none or questionable), small drusen (diameter <63 μm), intermediate drusen (diameter 63-124 μm) and large drusen (diameter >125 μm). Drusen area - group 1 [<circle C-1=circle diameter 0.083 disc diameters (DD)], group 2 (≥C-1, <I-2=circle diameter 0.241 DD), group 3 [≥I-2, <1/2 disc area (DA)] and group 4 (≥1/2 DA). Depigmentation area–group 1 (none or questionable), group 2 (<O2=circle diameter 0.439 DD), group 3 (≥O2, <1 DA) and group 4 (≥1 DA). Pigmentation area–group 1 (none or questionable), group 2 (<C-1), group 3 (≥C-1, <O2) and group 4 (≥O2). Age was divided into quartiles.

OCT images were acquired with Heidelberg Spectralis OCT using a 521×128 scan pattern on a 20×20-degree field centered on fovea. OCT image segmentation and volumetric data

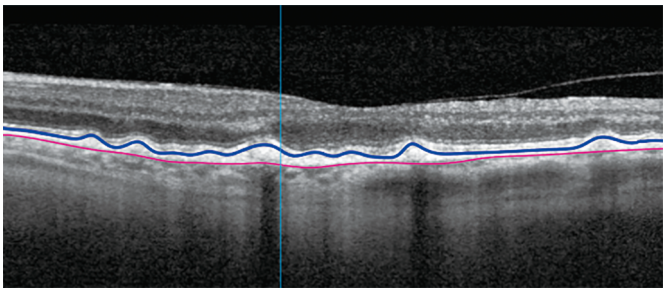

Figure 1 RPE (blue line) and BrM (red line) segmentation by Orion™
RPE: Retinal pigment epithelium; BrM: Bruch's membrane.

calculation of the RPE-BrM complex was done with Orion™ (Voxeleron, Pleasanton, CA, Software version 2.1.6362). Software was used to segment each individual B-scan in to 8 structural layers (inner limiting membrane, retinal nerve fiber layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, RPE and BrM). Data on RPE and BrM segmentation and the inter-layer space in between were used for our study (Figure 1). An automated algorithm in the Orion™ software was used for volumetric calculation of retinal pigment epithelium-Bruch's membrane complex volume (RBV) of the entire OCT volume of each study subject. Each segmented B-scan was controlled and manually corrected if needed. Segmentation analysis of one eye failed due to technical reasons and was excluded from the analyses.

Statistical Analysis All means are presented with standard deviation (SD). Spearman's rho correlation was used to test correlations between RBV and AREDS classification, largest drusen size, drusen area, depigmentation, pigmentation, age and smoking. Mann-Whitney *U* test was used for testing differences in distribution of RBV with and without AMD. ANOVA was used for comparing differences in RBV between groups within AMD level, largest drusen size, drusen area, depigmentation, pigmentation, age quartiles and smoking. Multiple comparisons were accounted for with Tukey HSD. Multiple linear regression was used for multivariate analysis. The statistical analyses were done in SPSS (IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp., USA).

RESULTS

The mean age of the study participants was 76.7±2.7y and 46 (47.9%) were female. Six (6.3%) were current smokers, 31 (32.3%) had smoked regularly in the past and 2 (2%) did not answer the question (Table 1). Of the 96 eyes included in the study, one eye had both ungradable fundus photo and OCT examination and in one segmentation analysis failed due to technical reasons. Both eyes were excluded from the analysis. Totally 53 (55.3%) had early AMD defined as AMD level 2 or 3 according to AREDS classification, whereas 2 (2%) had late AMD defined as AMD level 4, both with neovascular

Table 1 The main characteristics of the study subjects

Characteristics	*n* (%)	Mean±SD	Range
Eyes (non-gradable CFP)	96 (100)		
Female	46 (47.9)		
Age (y)		76.7±2.7	65.5-81.6
Smoking status			
Never	57 (59.4)		
Ex-smoker	31 (32.3)		
Current	6 (6.3)		
Data missing	2 (2.1)		
CFP AMD status			
No AMD	40 (41.7)		
Early AMD	30 (31.3)		
Intermediate AMD	23 (24)		
Late AMD	2 (2)		
Non-gradable	1 (1)		
Neovascular AMD	2 (2)		
RBV (mm^3)		1.01±0.18	0.72-1.80

CFP: Color fundus photographs; AMD: Age-related macular degeneration; RBV: Retinal pigment epithelium–Bruch's membrane complex volume; SD: Standard deviation.

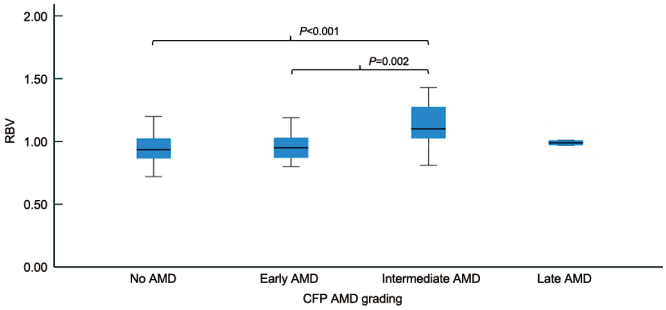

Figure 2 RBV (mm^3) according to AMD level RBV: Retinal pigment epithelium–Bruch's membrane complex volume; CFP: Color fundus photograph; AMD: Age-related macular degeneration.

AMD. Mean RBV was 1.01±0.18 mm^3 (range 0.72-1.80). There was a significant difference in RBV between subjects with (1.05±0.20 mm^3) and without (0.96±0.13 mm^3) early AMD (U=1329, P=0.023). RBV showed a moderate positive correlation with AMD grading (r_s=0.338, P=0.001) that was slightly stronger in eyes with early AMD (n=52; r_s=0.432, P=0.001).

Significant differences in RBV within groups of AMD level, largest drusen size, drusen area, depigmentation, pigmentation, age and smoking are shown in Figures 2-8.

The proportion of eyes with an RBV equal or above the median was significantly higher in AMD level 3-4 (80.0%) eyes than AMD level 1-2 (39.1%, P<0.001; Figure 9).

Multiple regression was used to test if largest drusen size, drusen area, depigmentation and pigmentation significantly predicted RBV. The overall regression was statistically significant [R^2=0.497, F(4, 89)=7.299, P<0.001]. Drusen area

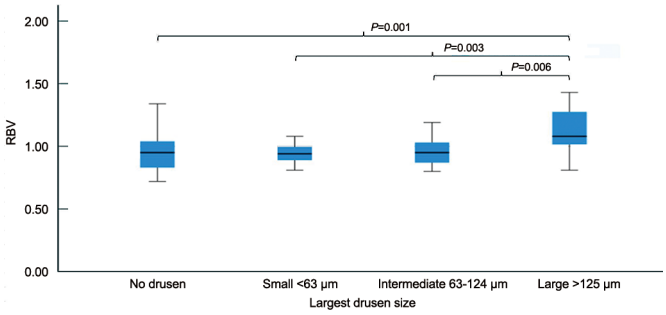

Figure 3 RBV (mm^3) according to largest drusen size RBV: Retinal pigment epithelium–Bruch's membrane complex volume.

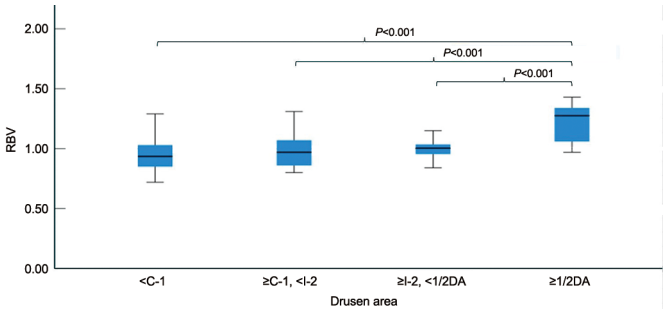

Figure 4 RBV (mm^3) according to drusen area RBV: Retinal pigment epithelium–Bruch's membrane complex volume.

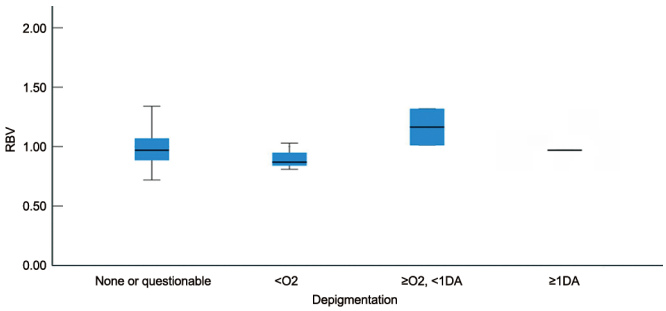

Figure 5 RBV (mm^3) according to depigmentation RBV: Retinal pigment epithelium–Bruch's membrane complex volume.

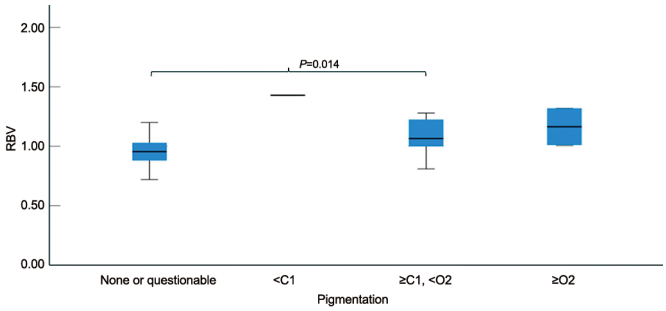

Figure 6 RBV (mm^3) according to pigmentation RBV: Retinal pigment epithelium–Bruch's membrane complex volume.

(β=0.388, P=0.027), depigmentation area (β=-0.295, P=0.015) and pigmentation area (β=0.319, P=0.020) significantly predicted RBV. Largest drusen size did not significantly predict RBV (β=-0.47, P=0.768).

DISCUSSION

In this study we found a correlation between RBV and CFP-based AMD grading. In a multiple regression model drusen

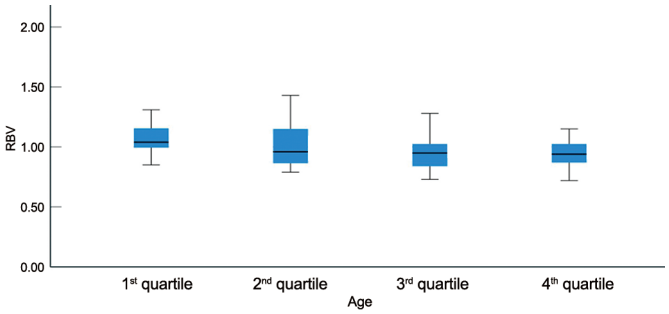

Figure 7 RBV (mm^3) according to age quartile RBV: Retinal pigment epithelium–Bruch's membrane complex volume.

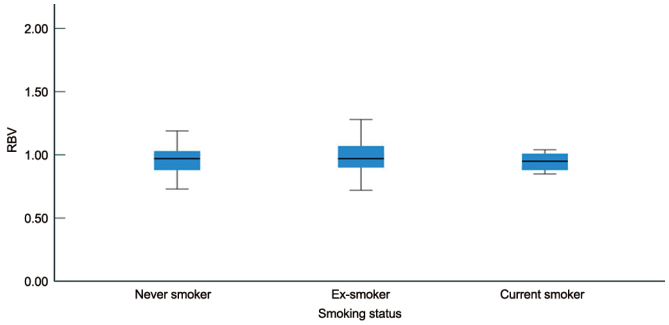

Figure 8 RBV (mm^3) according to smoking status RBV: Retinal pigment epithelium–Bruch's membrane complex volume.

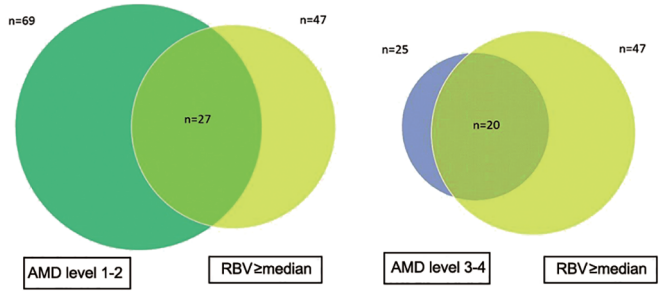

Figure 9 Proportion of subjects with RBV equal or above the median in subjects with no or early AMD and intermediate or late AMD respectively (AREDS levels 1-2 and 3-4) RBV: Retinal pigment epithelium–Bruch's membrane complex volume; AMD: Age-related macular degeneration; AREDS: Age-Related Eye Disease study.

area, depigmentation and pigmentation associated significantly with the RBV, with depigmentation associating with lower RBV. The subjects with a moderate AMD based on CFP had significantly higher RBVs than those without AMD.

The gold standard of AMD grading is manual detection of phenotypic features on CFP. Manual grading is time consuming and face questions about repeatability and interrater agreement. Thus, validated automated algorithms could offer a possibility to effectively assessing very large image datasets.

Automated segmentation of OCT images has been made available in recent years and several studies have investigated the correlation between drusen volume and AMD. Interestingly, in the largest population-based study to date by Cheung *et al*[17], 59% of eyes with AMD based on CFP grading had a drusen volume of 0 mm^3. By measuring drusen volume alone, we fail to find a large portion of eyes that have already developed phenotypical signs of AMD. A possible explanation could be that these algorithms overlook linear and laminary lipid deposition within the RPE-BrM complex that have been shown to precede drusen and AMD. Also pigment deposition in the outer and inner retinas may include space occupying components, but are overlooked in a drusen-focused analysis.

Brandl *et al*[19] analyzed retinal layer thickness (central 1 mm, inner 3 mm and outer 6 mm rings) and its correlation with AMD. Median RPE-BrM complex was significantly thicker in moderate and severe early AMD subjects at the central and inner macular rings. Measuring volume instead of thickness is likely to be more sensitive to measurement errors as small errors in thickness might give large fluctuations in calculated volume. However, volume measurements can take in account alterations in the whole macular area and thus might be more sensitive in reflecting early AMD pathology. A proportion of eyes with a no or only mild AMD grades had elevated RBVs. Whether this reflects the presence of the earliest AMD-related changes in the Bruch's membrane, is a hypothesis that cannot be tested here.

In very large materials, in which manual CFP-based grading is not available, RBV analysis could complement other methods, such as drusen volume and artificial intelligence (AI) based methods. In patients with drusen and pigmentation RBV correlated with drusen and pigmentation areas, but yielded volume data. Thus, in smaller studies with CFP based analysis available, RBV probably gives a more sensitive quantitative measure of the extent of AMD-related pathology, as long as depigmentation or atrophy are not present. Another use, not tested here, would be the quantification of reticular drusen, that are difficult to fit in the classical CFP grading circles.

There are several limitations to our study. The sample size is quite small, making the statistical power of RBV analyses weak. Also, all early AMD subtypes may not have been sufficiently present in the eyes studied. The ETDRS grid and 6×6 mm^2 macular cube on Heidelberg SD-OCT, although close to each other in area, still measure AMD features in slightly different areas and locations. As our study population were all Caucasians the results are not generalizable to more heterogenous populations.

In summary, our data showed a correlation between the current gold standard of CFP based AMD grading and RBV volume derived analysis from OCT images. With the limitations described, RBV-based volumetric measurements could be used as a complementary method in large studies where CFP-based analysis is not practicable, and also in the quantification of AMD lesion burden in eyes with drusen and pigmentation according to manual CFP-based analysis.

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Conflicts of Interest: Kananen F, None; **Immonen I,** None.

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