Comparison of histometric data obtained by optical coherence tomography and routine histology

Thilo Gambichler
Stefanie Boms
Markus Stücker
Alexander Kreuter
Michael Sand
Georg Moussa
Peter Altmeyer
Klaus Hoffmann
Ruhr-University Bochum
Department of Dermatology
Gudrunstr. 56
44791 Bochum, Germany

Abstract. There is a lack of systematic investigations comparing optical coherence tomography (OCT) with histology. OCT assessments were performed on the upper back of 16 healthy subjects. Epidermis thickness (ET) was assessed using three methods: first, peak-to-valley analysis of the A-scan (ET-OCT-V); second, manual measurements in the OCT images (ET-OCT-M); third, light microscopic determination using routine histology (ET-Histo). The relationship between the different methods was assessed by means of the Pearson correlation procedure and Bland and Altman plots. We observed a strong correlation between ET-Histo (79.4±21.9 μm) and ET-OCT-V (79.2±15.5 μm, r=0.77) and ET-OCT-M (82.9±15.8 μm, r=0.75), respectively. Bland and Altman plots revealed a bias of −0.19 μm (95% limits of agreement: −27.94 μm to 27.56 μm) for ET-OCT-V versus ET-Histo and a bias of 3.44 μm (95% limits of agreement: −24.9 μm to 31.78 μm) for ET-OCT-M versus ET-Histo. Despite the strong correlation and low bias observed, the 95% limits of agreement demonstrated an unsatisfactory numerical agreement between the two OCT methods and routine histology indicating that these methods cannot be employed interchangeably. Regarding practicability, precision, and indication spectrum, ET-OCT-V and ET-OCT-M are of different clinical value. [DOI: 10.1117/1.2039086]

Keywords: epidermal thickness; interferometry; bioengineering; noninvasive skin imaging; histopathology.

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1 Introduction

Various optical techniques including confocal microscopy, high-frequency ultrasound, and optical coherence tomography (OCT) have recently been developed to section human skin with high resolution and contrast.[1] OCT is a noninvasive technique capable of generating cross-sectional images of tissue microstructure. Analogous to ultrasound, OCT uses infrared light instead of sound waves. OCT employs low-coherence interferometry to produce a two-dimensional image of optical scattering from internal tissue microstructures. Interference fringes are formed when the optical path length of light reflected from the sample matches that reflected from the reference arm within the coherence length of the light source. An axial depth scan (A-scan) is obtained by scanning the reference arm length, resulting in localized interference fringes with amplitudes related to sample reflectivity. The fringe intensities in adjacent A-scans are combined to form a two-dimensional image (B-scan). The source coherence length and the spot size of the beam focus on the sample determine the depth resolution and lateral image resolution, respectively. New systems with ultrahigh resolution (1 μm × 3 μm) have recently been developed, however, resolution on the order of about 10 μm is more typical. Doppler and polarization-sensitive functions provide distinct, complementary information to conventional structural OCT. Moreover, real-time multifunctional OCT represents a further advance in OCT imaging.[2–5] For comprehensive reviews we refer the reader to those papers of Fujimoto[6] and Drexel[7] that deal with theory and applications of OCT.

Apart from medical disciplines such as ophthalmology and internal medicine, OCT is increasingly used in dermatologic research. OCT enables imaging of skin layers as deep as about 1 mm. Hence it is particularly capable of presenting morphological features of the epidermis and papillary dermis. OCT can provide cross-sectional ultrahigh-resolution images of structures below the tissue surface in analogy to histology. In previous studies, skin appendages including hair follicles and eccrine ducts were identified by conventional OCT. Correlation of OCT images with histology confirmed observation of morphologic changes such as blistering, tumor tissue, and inflammatory conditions including psoriasis and contact dermatitis.[1,2,8,9] However there is a lack of systematic investigations comparing OCT with histology, which is still considered the "gold standard" for investigation of skin morphology and histometric assessments.[10,11] Knowledge of the skin thickness is of great significance in several areas of medicine such

Address all correspondence to Dr. T. Gambichler. Tel: +49-234-5090; Fax: +49-234-5093409; E-mail: t.gambichler@klinikum-bochum.de

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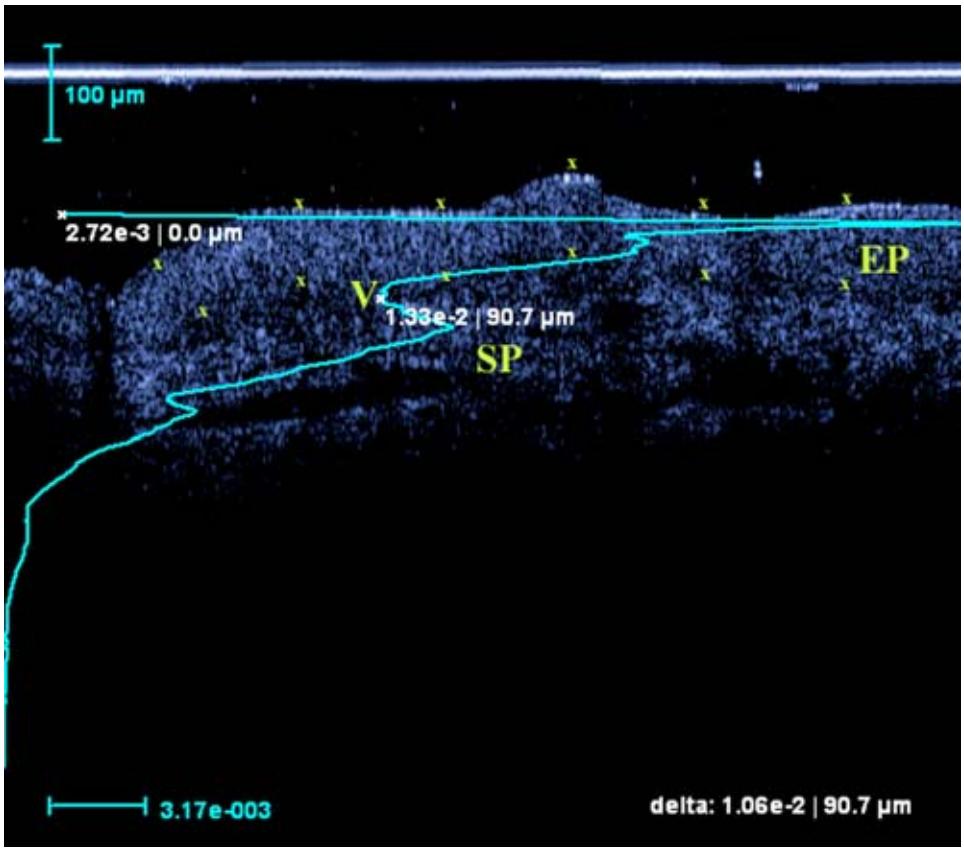

Fig. 1 OCT image including averaged A-scan and different measurement sites for determination of ET: valley (V) prior to the second peak (SP) for determination of ET-OCT-V (90.7 μm); five predefined manual measurement sites (*x* to *x*) for determination of mean ET-OCT-M (75 μm). Corresponding histology revealed ET-Histo of 95 μm.

as dermatology, plastic surgery, and pharmacology. It is useful to measure epidermal thickness (ET), since the protection against harmful agents, such as chemicals and ultraviolet radiation among other factors, depends on this parameter. Moreover transepidermal drug delivery may significantly depend on ET. For example, ET may serve as a parameter to study photoadaptive processes or to monitor over time the effects of drugs and surgical procedures.[1,2,4,12–14] Since ET can be quantified by means of histology as well as OCT, it might be a suitable parameter for method comparison studies.

In clinical and experimental medicine we often indirectly assess biological quantities. When a new method is proposed we can measure its value by comparison only with other established techniques rather than with the "true" quantity being assessed. We cannot be certain that either method gives us an unequivocally correct measurement and we try to assess the degree of agreement between them. Some lack of agreement between different methods is inevitable. We want to know by how much the new method is likely to differ from the old, so that if this is not enough to cause problems in clinical interpretation we can replace the old method by the new, or even use the two interchangeably.[15] In a recent pilot study we observed a poor relationship between routine histology and OCT assessments that were based, as previously suggested by Welzel and co-workers,[9,14,16] on distance calculation between the entrance peak and second peak of the A-scan.[17] In the present systematic comparison study, we aimed to investigate the relationship between routine histology and OCT using two different OCT algorithms for the quantification of ET.

2 Methods

2.1 *Subjects and OCT Assessments*

Sixteen healthy subjects (mean age 52.4 years) were enrolled into the study who gave their informed consent prior to the beginning of the study. Prior to OCT assessment, a waterproof mark 4 mm in diameter was drawn on the center of a skin site in the lateral scapular region of each subject. A commercial OCT scanner (SkinDex 300, ISIS optronics GmbH, Mannheim, Germany) was used in this study.[18] The performance of this system regarding spatial resolution and field of view is as follows. A bandwidth $\Delta\lambda = 70$ nm and a center wavelength of $\lambda_0 = 1300$ nm is utilized. Under the assumption of an average refractive index of the sample medium $n_{med} = n_{obj} = 1.43$ this results in a coherence length for depth resolution $A\text{-FWHM}_{Int} = 7.4$ μm. The numerical aperture of the focusing lens is $NA = 0.19$. Thus the diffraction limited lateral resolution yields $A\text{-FWHM}_{Foc} = 4.5$ μm. The architecture of the system with eight parallel scanning channels allows fast scans. Within 2 s a number of 512 scans is acquired along the length of 1 mm in lateral direction and an axial range of 0.9 mm. Echo signals are digitized with 14 bits amplitude resolution. An integrated CCD camera with a field of view of

Table 1 Data (means±SD) of epidermis thickness (ET) measurements* (μm) by means of routine histology and OCT. Data of repeatability# and agreement§ between methods is presented (n+16).

Methods	ET*	RC#	OCT agreement with histology§		
ET-Histo	79.4±21.9	20.1	bias§	95% limits of agreement§	
				lower (95% CI)	upper (95% CI)
ET-OCT-V	79.2±15.5	7.23	−0.19 (−7.7 to 7.4)	−27.94 (−39.8 to −16.1)	27.56 (15.7 to 39.4)
ET-OCT-M	82.9±15.8	19.3	3.63 (−4.3 to 11.1)	−24.90 (−37 to −12.8)	31.78 (19.7 to 43.9)

95% CI=95% confidence interval; RC=repeatability coefficient based on pair of datasets of two repeated measurements for each method performed on day 1 and day 30.

4.5 mm^2 delivers optical images of the skin surface. With the aid of these images it was possible to perform OCT assessments exactly on the skin site previously marked. The 3-D measurement modus of the SkinDex 300 with 5 μm interplane distance was utilized to generate 15 smoothed 2-D images. In order to investigate the ET we used two OCT algorithms. First, the distance between the entrance peak and the valley prior to the second intensity peak of the averaged A-scans (ET-OCT-V) was semi-automatically calculated using the cursor and integrated OCT software. The OCT images were displayed on the computer screen and distances in axial and lateral directions could be measured by mouse clicks. Briefly, ET was calculated by taking the top surface as a reference, setting the lateral points mathematically along one line. This put the lower part of the epidermis on a "wavy line." Averaging in the lateral direction provided then a number for the average ET (Fig. 1). Second, we determined ET in the same OCT image on the computer screen using the integrated measure tool (ruler). For this purpose, we manually measured on five predefined places in the OCT image from the skin surface reflection (entrance echo) to the first well-demarcated change of reflectance intensity with clear echo-poor zone (ET-OCT-M). To minimize interobserver variability all OCT measurements were performed by the same investigator (T.G.).

2.2 *Histology*

Immediately after OCT assessment, 4-mm punch biopsies were taken from the previously marked sites under local anesthesia (1% lidocaine subcutaneously). The excised tissue was fixed in 35% formalin solution and embedded in paraffin. Histological slices of 5 μm were performed for routine hematoxylin and eosin staining. For further evaluation, we selected one histology slice of each patient showing the best quality with regard to preservation of the horny layer and absence of artifacts. The thickness of the maximum epidermis (ET-Histo) defined by the valleys of the papillae was measured by the same investigator (S. B.) on five random chosen places in the histological preparation at magnification 40×. The mean value of the five measurements was calculated for each subject.

2.3 *Statistics*

Statistical analysis was performed using SPSS.11 for Microsoft (Microsoft, USA) as well as Microsoft Excel with Analyse-it Statistical Add-on for Excel (Analyse-it Software Ltd., UK). All measurement values of skin thickness (μm) were expressed as means±SD. Normal distribution of data was confirmed by the Kolmogorov-Smirnov test (KS test). Correlations between the different methods were calculated using the Pearson correlation procedure including correlation coefficient (r) and the two-tailed t-test for independent samples. Further agreement between histology and OCT was analyzed using the Bland and Altman plots.[19] In brief, agreement between two methods of clinical measurement can be quantified using the differences between observations made using the two methods on the same subject. The 95% limits of agreement, estimated by mean difference ±1.96 SD of the differences, provide an interval within 95% of differences between measurements where the two methods are expected to lie. The calculation of the 95% limits of agreement is based on normal distribution and insignificant difference between replicate measurements. We compared pairs of means from two replicate measurements of each method (interval between two measurements: 30 days). The within-subject standard deviation of replicate assessments was analyzed using the one-way analysis of variance. Measurement error was presented as repeatability of the methods. The difference between two measurements for the same subject is expected to be less than 2.77 × SD for 95% of pairs of observations.[20] The repeatability coefficients are given in Table 1. There was no significant difference between repeated measurements for each of the methods employed. Differences were considered significant when $P<0.05$, very significant when $P<0.01$, and highly significant when $P<0.001$.

3 Results

ET-Histo (79.4±21.9 μm, including stratum corneum thickness of 20±12.1 μm), very significantly correlated (Pearson procedure; t-test) with ET-OCT-V (79.2±15.5 μm, $r=0.77$, $P=0.001$) and ET-OCT-M (82.9±15.8 μm, $r=0.75$, $P=0.001$). Bland and Altman[19] plots of Figs. 2 and 3 display the mean difference (bias) between methods, which was very

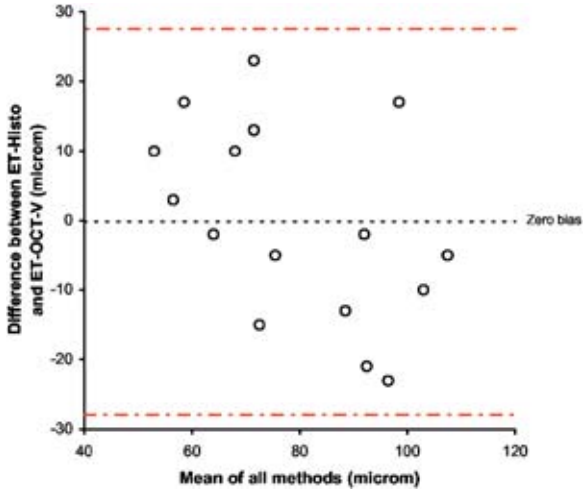

Fig. 2 Bland and Altman[19] plot displaying considerable scatter around the mean difference. Despite the very low bias (dashed line on the zero bias line) there was lack of agreement between ET-Histo and ET-OCT-V with discrepancies ranging from −27.94 μm to 27.56 μm (lower and upper 95% limits of agreement, irregularly dashed lines).

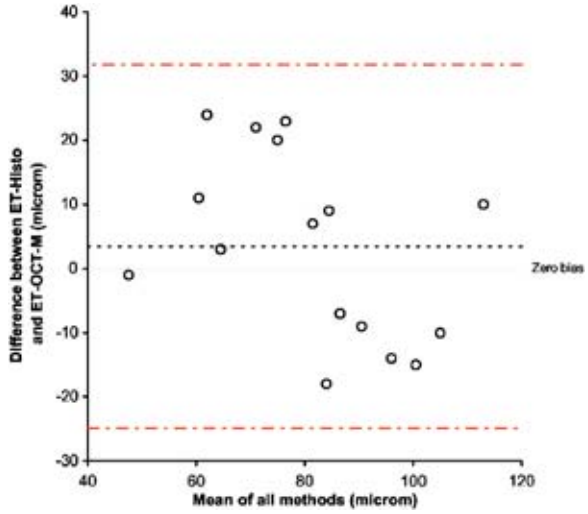

Fig. 3 Bland and Altman[19] plot displaying considerable scatter around the mean difference. Apart from slight bias (dashed line above the zero bias line) of 3.63 μm the plot indicates lack of agreement between ET-Histo and ET-OCT-V with discrepancies ranging from −24.90 μm to 31.78 μm (lower and upper 95% limits of agreement, irregularly dashed lines).

small for ET-OCT-M vs ET-Histo (−0.19 μm). However comparison of ET-OCT-M with ET-Histo shows a slight bias (3.44 μm), indicating that ET-OCT-M tends to generate greater values for ET due to a systematic error. The solid lines of the Bland and Altman[19] plots represent the zero bias where mean of difference is zero. Moreover Bland and Altman[19] plots display considerable scatter around the mean difference (Figs. 2 and 3). ET-OCT-V vs ET-Histo revealed a lower 95% limit of agreement of −27.94 μm and an upper 95% limit of agreement of 27.56 μm (Fig. 2). Similarly, ET-OCT-M vs ET-Histo resulted in a lower 95% limit of agreement of −24.90 μm and an upper 95% limit of agreement of 31.78 μm (Fig. 3). As shown in Table 1, the 95% confidence intervals of the 95% limits of agreement were wide, reflecting the small sample size and the great variation of differences. Furthermore the 95% confidence intervals of the 95% limits of agreement indicate that even in the best case scenario there may be differences between the methods of more than 10 μm (Table 1).

4 Discussion

In a recent pilot study on ET,[17] we observed neither correlation (r=0.29, P=0.27) nor agreement (bias 26.63 μm, 95% limits of agreement ranging from −18.03 to 71.28) between routine histology and OCT using the conventional peak-to-peak algorithm for ET determination as previously proposed by Welzel and colleagues.[9,14,16] The considerable bias observed in the aforementioned study clearly indicates a systematic error. Thus the second peak of the A-scan probably does not correspond to the dermo-epidermal junction zone. A typical OCT image of skin on the back shows two bright reflecting layers. The upper layer (entrance peak) is due to scattering from the stratum corneum. Welzel and co-workers[9,14,16] ascribed the second layer (second peak) to the fibrous structure immediately below the basal cell layer, the dermo-epidermal junction. Thus the relationship between the entrance signal and the second intensity peak has been considered a measure for the ET, except for the relatively thick skin on the palms and soles, where it corresponds to the thickness of stratum corneum. The latter can exclusively be determined by OCT on glabrous skin.[21] By contrast Neerken and colleagues[22] recently showed in a comparison study of OCT images with corresponding confocal laser scanning microscopy data that the second bright reflecting band is located much deeper below the epidermal basal layer and can be ascribed to scattering of light at the fibrous structure in the upper dermis. Consequently, we employed two alternative OCT algorithms for the determination of ET, manual calculation on the PC screen using an integrated ruler (ET-OCT-M) and software-based semi-automated calculations of the distance between the entrance peak and the valley prior to the second peak (ET-OCT-V). The valley prior to the second peak of the A-scan may represent the slightly echo-poor zone following the entrance echo (Fig. 1). Hence it seems to correlate with the dermo-epidermal junction and might be a valid measure for determination of *in vivo* ET.

Few systematic studies on *in vivo* measurements of ET exist using high-frequency ultrasound, confocal laser scanning microscopy, and OCT.[23–25] Comparison of these studies however is difficult due to different study methods and differences in study population, sample size, anatomic sites measured, and definition of ET used. Determination of ET is strongly dependent on the definition of markers, for example minimum ET can be defined by the top of the uppermost papillae, and maximum ET by the valleys of the papillae. Thus due to undulation of the dermo-epidermal junction, large differences can be obtained, particularly in young subjects.[10,11] Comparative *in vivo* investigations of ET have recently been performed employing 20-MHz ultrasound versus confocal laser scanning microscopy or the latter versus OCT. The authors observed good correlation between the methods utilized but they did not perform analysis of agreement.[22,24]

Our histologic data are in accordance with the literature. For example, Sandby-Moller and coworkers[11] observed in their population study a mean thickness of the total epidermis and the stratum corneum of the upper back of 81.3±13.5 μm (stratum corneum thickness: 11±2.2 μm).[5] Interpretation of mean values and correlation coefficients observed in our study apparently indicate a strong relationship between histology and OCT measurements. Nevertheless, even if one observes that the mean values of two methods are equal the differences between the paired measurements can be huge. Correlation data are misleading in that a large *r* value indicates a strong relationship, but measures can be related without having close agreement.[26] The correlation coefficient only shows us whether the measurements go up-and-down together. It can be close to 1 (or equal to 1!) even when there is considerable bias between the two methods. For example, if one method gives measurements that are always 10 units higher than the other method, the correlation will be perfect with a *r*-value of 1 exactly, but the measurements will always be 10 units apart. Hence the magnitude of correlation says nothing about the magnitude of the differences between the paired measurements, which is basically all that really matters. Agreement includes the numerical identity between the test results of two different methods and is suggested as a more useful indication as to whether one method can be a valid substitution for another. Despite the low bias observed in our study the 95% limits of agreement, which showed discrepancies up to almost 30 μm, indicate relatively poor numerical agreement between OCT and routine histology (Fig. 2, Fig. 3, and Table 1). Perfect agreement would have a difference of standard deviation of 0.0 μm, indicating that, for each subject measured, the prediction and criterion methods generated the same ET. However we observed wide 95% confidence intervals of the upper and lower 95% limits of agreement reflecting the small sample size and the great variation of the differences. Furthermore the 95% confidence intervals also demonstrate that even on the most optimistic interpretation there can be considerable discrepancies between routine histology and OCT. Even in the best case scenario there may be differences between the methods of more than 10 μm, which is not satisfactory from the clinical point of view. How far apart measurements can be without leading to problems depends on the use to which the result is put, and is a question of clinical judgment. Therefore, we suggest that the aforementioned methods of ET assessment cannot be employed interchangeably because the degree of numerical agreement is relatively poor. However it should be noted that the weakness of the Bland and Altman[19] plot is using the average of the criterion and prediction measure to represent the "true" measure for each ET. Since the "true" value of each ET is not known, the mean ET from the criterion and prediction method is considered the best estimate of the "true" value.

Routine histology using conventional formalin-paraffin processing frequently distorts the anatomy of the horny layer and may result in artifacts including swelling and/or shrinkage of the tissue. Cryopreparation may prevent crystallization in the water content and minimize changes in the skin structure and ET during the different histologic preparation steps.[10,11] A further important factor that may explain differences between *in vivo* and *ex vivo* measurements per se is the natural shrinkage of the skin occurring after excision, particularly evident for the dermis.[10,11] The aforementioned factors as well as intraobserver bias probably contributed to the lack of repeatability observed for ET-Histo (Table 1). By contrast, ET-OCT-V showed the best performance in terms of measurement precision. Software-based determination of ET-OCT using the A-scan has also previously proved to be of high repeatability.[13,16] The latter is relevant to the study of method comparison because the repeatabilities of the two methods of measurement limit the amount of agreement that is possible at all. Likewise ET-Histo, the manual determination of ET using a ruler, ET-OCT-M, resulted in relatively poor repeatability. Drawbacks of this OCT method include high observer variability and strong dependence on the quality of OCT image evaluated. In comparison to ET-OCT-V, ET-OCT-M is probably the more suitable method for ET determination of pathologically altered skin where a loss of the second peak of the OCT A-scan is frequently observed.[9,16] Weissman and co-workers[27] recently described a novel shaplet-based image processing technique for the automatic determination of ET. Their automated measurements provided average results that were more reliable than A-scan results and comparable to those obtained by human observers.

5 Conclusion

Despite the strong correlation observed there was relatively poor numerical agreement between the two OCT algorithms used and routine histology indicating that these methods cannot be employed interchangeably. Nevertheless this does not imply that OCT is unsuitable for accurate determination of ET *in vivo*. On the contrary, it is rather a matter of whether the *in vivo* or *ex vivo* ET is of particular interest in a certain clinical or experimental problem. In all likelihood, there is a difference per se between *in vivo* ET and *ex vivo* ET. Consequently *in vivo* methods may correlate but not numerically agree with histology. Under certain circumstances, an up- or downward adjustment may be applied for practical use in order to correct a systematic error. Regarding practicability, precision, and indication spectrum, ET-OCT-V and ET-OCT-M are of different clinical value. Nevertheless, comparison studies between OCT, preferably employing ultrahigh-resolution OCT technique, and more accurate histologic techniques such as cryopreparation, special landmark-based registration tools, and automated image analysis are still needed to fully explore the relationship between *in vivo* ET and *ex vivo* ET assessed by OCT and histology, respectively.[2,3,11,17,27]

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