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Uniwersytet Medyczny w Białymstoku

Patryk Jan Sidorczuk

Ocena parametrów siatkówki i naczyniówki z uwzględnieniem naczyniówkowego wskaźnika naczyniowego u pacjentów z cukrzycą

Rozprawa doktorska w dziedzinie nauk medycznych i nauk o zdrowiu

dyscyplina: nauki medyczne

Promotor

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Spis treści

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WYKSZTAŁCENIE

- 2017-2022r. szkolenie specjalizacyjne z okulistyki, Uniwersytecki Szpital Kliniczny w Białymstoku
- 2009-2015r. Uniwersytet Medyczny w Białymstoku, Wydział Lekarski

DOŚWIADCZENIE ZAWODOWE I ZATRUDNIENIE

- 2022r. uzyskanie tytułu specjalisty w dziedzinie okulistyka
- 2022r członek zespołu badawczego projektu naukowego finansowanego z subwencji Uniwersytetu Medycznego w Białymstoku pt: „ Kompleksowa ocena okulistyczna, anatomiczna oraz funkcjonalna, pacjentów z twardziną układową”.
- 2020- 2022r. członek zespołu badawczego projektu naukowego pt: „Ocena naczyniówki i siatkówki u pacjentów z cukrzycą”. Uniwersytet Medyczny w Białymstoku
- 2017-2022r. rezydent, Klinika Okulistyki, Uniwersytecki Szpital Kliniczny w Białymstoku
- 2019-2022r. członek zespołu raportującego standardowe procedury operacyjne (SOP).
- 2015-2016r staż podyplomowy, Uniwersytecki Szpital Kliniczny w Białymstoku

PUBLIKACJE

- **Sidorczuk, P**.; Obuchowska, I.; Konopinska, J.; Dmuchowska, D.A. Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy. *J. Clin. Med.* **2022**, *11*, 3882. https://doi.org/10.3390/jcm11133882. IF 4.964; MEiN 140

- Dmuchowska DA, **Sidorczuk P**, Pieklarz B, Konopińska J, Mariak Z, Obuchowska I. Quantitative Assessment of Choroidal Parameters in Patients with Various Types of Diabetic Macular Oedema: A Single-Centre Cross-Sectional Analysis. Biology (Basel). 2021 Jul 29;10(8):725. doi: 10.3390/biology10080725. PMID: 34439957; PMCID: PMC8389323. IF 5.168; MEiN 100

- **Sidorczuk P**, Pieklarz B, Konopinska J, Saeed E, Mariak Z, Dmuchowska D. Foveal Avascular Zone Does Not Correspond to Choroidal Characteristics in Patients with Diabetic Retinopathy: A Single-Center Cross-Sectional Analysis. Diabetes Metab Syndr Obes. 2021 Jun 28;14:2893-2903. doi: 10.2147/DMSO.S318860. PMID: 34234487; PMCID: PMC8254029. IF 3.249; MEiN 100

- Pieklarz B, Grochowski ET, Saeed E, **Sidorczuk P**, Mariak Z, Dmuchowska DA. Iridoschisis-A Systematic Review. J Clin Med. 2020 Oct 16;9(10):3324. doi: 10.3390/jcm9103324. PMID: 33081187; PMCID: PMC7602847. IF 4.242; MEiN 140

- Pieklarz B, Grochowski ET, Dmuchowska DA, Saeed E, **Sidorczuk P**, Mariak Z. Iris-Claw Lens Implantation in a Patient with Iridoschisis. Am J Case Rep. 2020 Aug 28;21:e925234. doi: 10.12659/AJCR.925234. PMID: 32857754; PMCID: PMC7483544. MEiN 70

CZŁONKOSTWO W TOWARZYSTWACH NAUKOWYCH

- Od 2017r. – Polskie Towarzystwo Okulistyczne, Oddział w Białymstoku

Rozdział 2. Wykaz publikacji stanowiących rozprawę doktorską

1. **Sidorczuk, P**.; Obuchowska, I.; Konopinska, J.; Dmuchowska, D.A. Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy. *J. Clin. Med.* **2022**, *11*, 3882. https://doi.org/10.3390/jcm11133882 IF 4.964; MEiN 140 (praca oryginalna)

2. **Sidorczuk P**, Pieklarz B, Konopinska J, Saeed E, Mariak Z, Dmuchowska D. Foveal Avascular Zone Does Not Correspond to Choroidal Characteristics in Patients with Diabetic Retinopathy: A Single-Center Cross-Sectional Analysis. Diabetes Metab Syndr Obes. 2021 Jun 28;14:2893-2903. doi: 10.2147/DMSO.S318860. PMID: 34234487; PMCID: PMC8254029. IF 3.249; MEiN 100 (praca oryginalna)

Zestawienie publikacji doktoranta

Rodzaj Publikacji	**Liczba pozycji**	**Impact Factor**	**Punktacja MEiN**
Publikacje włączone do rozprawy doktorskiej	2	8.213	240
Publikacje niewłączone do rozprawy doktorskiej	3	9.41	310
Komunikaty zjazdowe	-	-	-
Razem	**5**	**17.623**	**550**

Rozdział 3. Wstęp

3.1. Retinopatia i choroidopatia cukrzycowa

Retinopatia cukrzycowa (*diabetic retinopathy DR*) i cukrzycowy obrzęk plamki (*diabetic macular edema DME*) mogą znacznie upośledzać widzenie, a nawet prowadzić do ślepoty. Zrozumienie patogenezy tego schorzenia jest niezbędne celem opracowania skutecznych metod prewencji i leczenia. W związku z dynamicznym rozwojem metod leczenia (m.in. różne preparaty anty-VEGF *vascular endothelial growth factor*) identyfikacja czynników predykcyjnych wspomogłaby odpowiednią kwalifikację pacjentów i optymalne wykorzystanie tych metod. Przełożyłoby się to na poprawę efektów leczenia przy prawdopodobnej redukcji nakładów finansowych.

Unaczynienie siatkówki pochodzi z dwóch niezależnych źródeł. Wewnętrzne warstwy siatkówki są zaopatrywane przez tętnicę środkową siatkówki, a zewnętrzne przez naczyniówkę. Cukrzyca wpływa na krążenie krwi w obrębie siatkówki i naczyniówki. Naczyniówka stanowi jedyne źródło wymiany metabolicznej dla strefy beznaczyniowej dołka (*foveal avascular zone FAZ*), który jest obszarem największej gęstości czopków i związanej z tym aktywności metabolicznej. W porównaniu do wielkości FAZ, która jest osobniczo zmienna, zarys FAZ obrazuje utratę kapilar i stopień niedokrwienia wewnętrznych warstw siatkówki. FAZ można obrazować m.in. za pomocą angiografii fluoresceinowej (*fluorescein angiography AF*). Choroidopatia jest jednym z elementów, o niecałkowicie zdefiniowanej roli, złożonej patogenezy retinopatii cukrzycowej i cukrzycowego obrzęku plamki oraz klinicznie znamiennego obrzęku plamki *(clinically significant macular edema CSME*). Nie jest jednoznaczne, czy poprzedza, towarzyszy, czy następuje po rozwoju zmian siatkówkowych, oraz czy jest od nich zależna. Ta luka w wiedzy dotyczącej patogenezy retinopatii i choroidopatii cukrzycowej stała się inspiracją niniejszej pracy.

3.2. Optyczna koherentna tomografia

Optyczna koherentna tomografia (*optical coherence tomography OCT*) umożliwia obrazowanie zmian w siatkówce i naczyniówce. Dzięki tej nowoczesnej technice możliwa jest dokładna ocena grubości naczyniówki, zależnej m.in. od morfologii jej naczyń. Wprowadzenie tego badania na stałe do

praktyki klinicznej u pacjentów leczonych z powodu DR i DME poprawiło diagnostykę, monitorowanie i prognozowanie. Przyczynia się również do lepszego zrozumienia patogenezy tej choroby i umożliwia zoptymalizowanie jej leczenia.

3.3. Naczyniówkowy wskaźnik naczyniowy

Z rozwojem technologii OCT możliwe stało się dokładniejsze obrazowanie naczyniówki. Oprócz najczęściej stosowanego parametru jakim jest grubość i objętość naczyniówki, w 2016 roku wprowadzono ilościowy wskaźnik odzwierciedlający stosunek komponenty naczyniowej naczyniówki do jej całej powierzchni – naczyniówkowy wskaźnik naczyniowy (*choroidal vascularity index CVI*). W odróżnieniu od grubości naczyniówki, CVI jest parametrem bardziej niezależnym od czynników ogólnoustrojowych. CVI jest wykorzystywany jako marker wczesnych zmian i monitorowania progresji u pacjentów z różnymi chorobami siatkówki i naczyniówki oraz schorzeniami ogólnoustrojowymi. CVI jest coraz powszechniej stosowanym parametrem charakteryzującym naczyniówkę, co znajduje odzwierciedlenie w rosnącej z roku na rok liczbie publikacji z wykorzystaniem tego współczynnika. Dotychczasowe prace nie mają jeszcze bezpośredniego zastosowania praktycznego, lecz służą badaniom naukowym. Aktualnie trwają prace nad zautomatyzowaniem metody pomiaru CVI.

Rozdział 4. Omówienie prac składających się na rozprawę doktorską

4.1 Cel badań

Za główny cel prac przyjęto określenie powiązania choroidopatii i retinopatii u pacjentów z cukrzycą. Stwierdzenie i scharakteryzowanie takiego związku mogłoby znaleźć praktyczne zastosowanie we wczesnej diagnostyce okulistycznej oraz zindywidualizowanej kwalifikacji pacjentów do najbardziej odpowiednich metod terapeutycznych. Wykorzystanie innowacyjnego wskaźnika CVI do charakterystyki naczyniówki w poniższych pracach wypełnia lukę w literaturze w tym zakresie. W porównaniu do dotychczas stosowanej oceny grubości, CVI umożliwia dokładniejszą charakterystykę uwzględniając dwie składowe: naczynia i zrąb. Ponadto przeprowadzona ocena objętości a nie tylko grubości naczyniówki uwzględnia nieregularność granicy naczyniówkowo-twardówkowej.

Do tej pory w literaturze brak było tak szczegółowych jak nasze (i na tak licznej grupie pacjentów) doniesień dotyczących analizy związku naczyniówki i zaopatrywanych przez nią zewnętrznych warstw siatkówki u pacjentów z DR. Brak było również informacji dotyczących zależności krążenia w obrębie FAZ scharakteryzowanych za pomocą jej stopnia uszkodzenia i w obrębie naczyniówki u pacjentów z cukrzycą.

Cele szczegółowe:

1. Ocena zależności parametrów naczyniówkowych (grubości i CVI) i zewnętrznych warstw siatkówki u pacjentów z DR z lub bez DME oraz w grupie porównawczej.

2. Ocena potencjalnego związku między stopniem uszkodzenia FAZ a parametrami naczyniówkowymi (grubością, objętością i CVI) u pacjentów z DR.

4.2 Materiał i metodologia

4.2.1 Projekt badania

Było to jednoośrodkowe retrospektywne badanie przekrojowe. Analizie poddano dokumentację medyczną za okres 28.02.2017r. – 20.02.2021r.

Badanie uzyskało zgodę Komisji Bioetycznej Uniwersytetu Medycznego w Białymstoku (uchwała nr APK 002.216.2020) i było zgodne z założeniami Deklaracji Helsińskiej.

4.2.2 Grupa badana i porównawcza

Badaniem objęto pacjentów z cukrzycą typu 1 i 2, którzy zostali skierowani do Kliniki Okulistyki Uniwersyteckiego Szpitala Klinicznego w Białymstoku celem wykonania angiografii fluoresceinowej i OCT.

Kryteria włączenia pacjentów do projektu badawczego:

- obecność retinopatii cukrzycowej (w grupie badanej);
- wiek > 18 r.ż.;

Kryteria wyłączenia dotyczące grupy badanej i porównawczej:

- uprzednia operacja tylnego odcinka oka;
- iniekcje do ciała szklistego;
- fotokoagulacja laserowa plamki;
- zmiany plamki wynikające z innych chorób oczu;
- jaskra;
- ametropia ≥3,0 dioptrii;
- znana patologia oka lub układowa, która mogłaby mieć wpływ na naczyniówkę;
- niedostateczna jakość badania OCT i/lub AF.

W pracy dotyczącej korelacji parametrów zewnętrznych warstw siatkówki z parametrami naczyniówki oceniono 286 oczu u 191 pacjentów (139 z DR i 52 z grupy porównawczej). Grupa DR została podzielona na dwie podgrupy na podstawie obecności DME (DR+DME+, n=76, 61,5±11,9 lat, 51,0% kobiety) lub jego braku (DR+DME-, n=134, 60,0±13,2 lat, 52,2% kobiety). Grupę porównawczą (52 osoby, 76 oczu) stanowili

pacjenci zakwalifikowani do rutynowego badania okulistycznego w Klinice Okulistyki Uniwersyteckiego Szpitala Klinicznego w Białymstoku. Średnia wieku wynosiła 55,7±17,8 lat, 53,8% kobiety. Pacjenci z DR i z grupy porównawczej nie różnili się istotnie pod względem wieku, płci i ekwiwalentu sferycznego ($p>0.05$).

Natomiast w badaniu oceniającym zależność pomiędzy FAZ a parametrami naczyniówki wzięto pod uwagę 210 oczu 152 pacjentów z retinopatią cukrzycową (średni wiek 60,7±12,4 lat, 49,3% kobiety). Na podstawie AF pacjentów przydzielono do poszczególnych grup zgodnie z zarysem i rozmiarem FAZ oraz obecnością lub nie klinicznie znamiennego obrzęku w plamce (CSME).

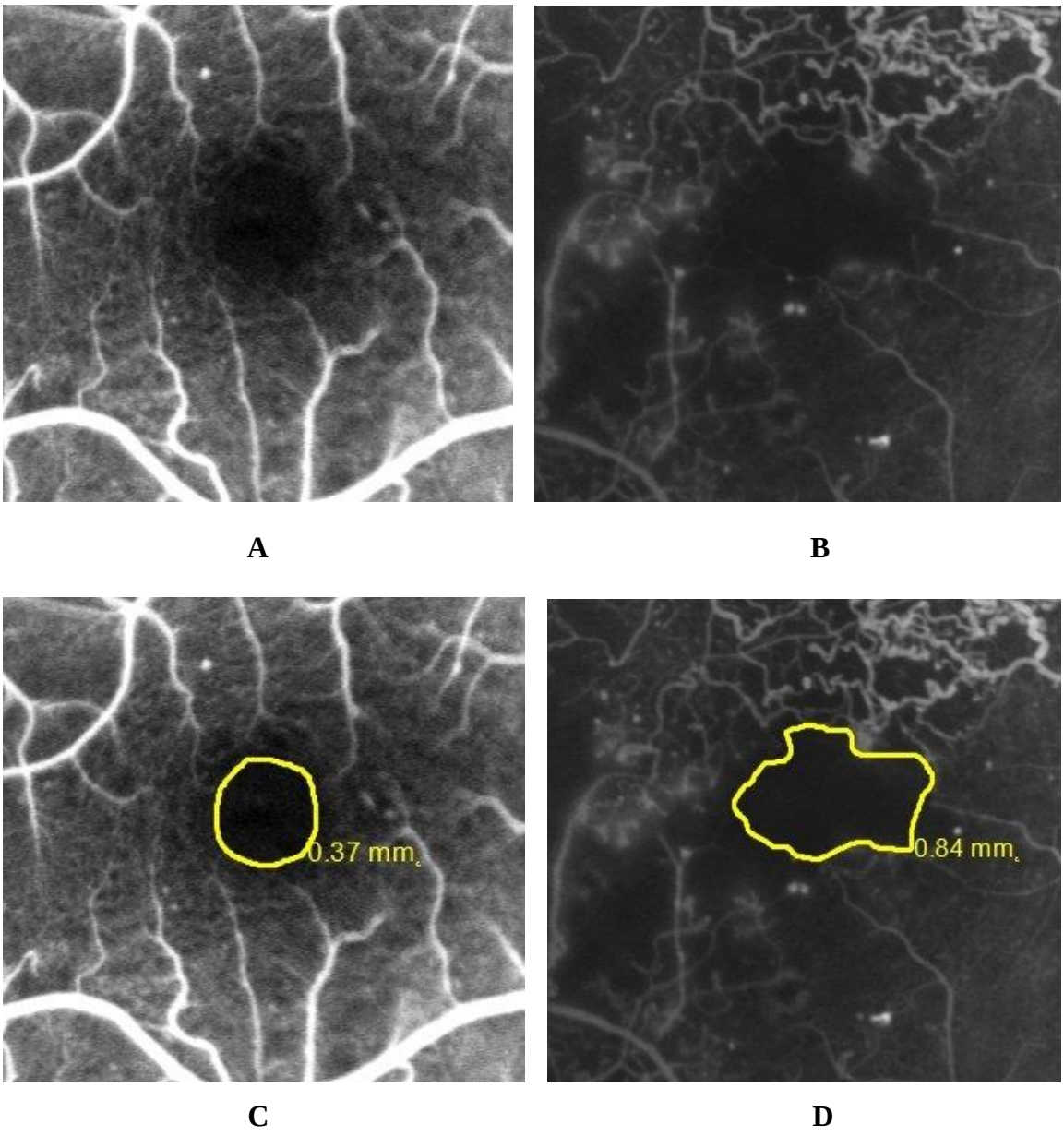
4.2.3 Metody – uwagi ogólne

U pacjentów w grupie badanej wykonano spektralną OCT oraz angiografię fluoresceinową (AF) za pomocą Spectralis HRA + OCT (Heidelberg Engineering, Heidelberg, Niemcy). W grupie porównawczej zostało wykonane OCT.

Wyniki zostały poddane analizie statystycznej za pomocą programu R software wersja 3.5.1. Za poziom istotności statystycznej przyjęto współczynnik $p<0.05$.

4.2.4 Zasady opracowania obrazów AF

Angiografia fluoresceinowa została wykorzystana do oceny zaawansowania DR, wykrycia CSME i scharakteryzowania FAZ zgodnie z kryteriami grupy badawczej Early Treatment Diabetic Retinopathy Study Research Group (ETDRS). W przypadku pomiarów FAZ, zaznaczano manualnie obszar (ryc. 1.). Oceniono wielkość i zarys FAZ. Zarys FAZ jest określany jako miara utraty naczyń włosowatych spowodowana procesami niedokrwiennymi. Zgodnie z Raportem ETDRS nr 11 stopień 1 i 2 dotyczy uszkodzenia < 50% obwodu FAZ, a stopień 3 i 4 ≥50%. Grupę badaną podzielono na 2 grupy przyjmując uszkodzenie 50% obwodu FAZ jako poziom odcięcia.



Ryc. 1.

Obrazy angiografii fluoresceinowej z zaznaczeniem FAZ. **(A)** stopień uszkodzenia <50% obwodu. **(B)** stopień uszkodzenia FAZ >50% zgodnie z Raportem ETRDS nr 11. Te same angiogramy z zaznaczonym zarysem granicy i wielkości FAZ **(C)** i **(D)**.

4.2.5 Zasady opracowania obrazów OCT

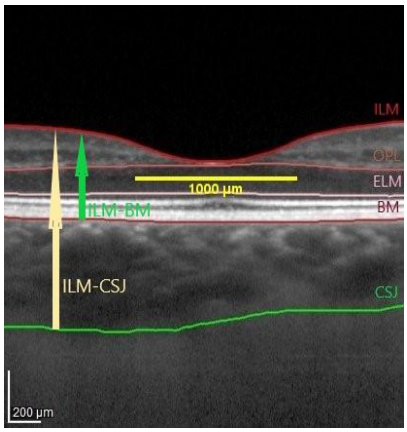
Protokół obrazowania OCT składał się z 25 poziomych skanów rastrowych (20x20°) i liniowego B-skanu przez dołek. W poszczególnych polach ETDRS zostały oznaczone grubości warstw siatkówki oraz grubości i objętości naczyniówki. Pomiar centralnej plamkowej grubości siatkówki pozwolił na stwierdzenie obecności DME przy wartościach powyżej 300 mikrometrów. Dwie składowe zewnętrznej siatkówki, które są zaopatrywane przez naczyniówkę, definiowano jako przyjęte standardowo w automatycznym oprogramowaniu Spectralis: warstwę siatkówkową zewnętrzną (*outer retinal layer ORL*) i warstwę jądrzastą zewnętrzną (*outer nuclear layer ONL).* Ich granice wyznaczały odpowiednio błona Brucha-błona graniczna zewnętrzna (*Bruch's membrane BM, external limiting membrane ELM*) oraz ELM-zewnętrzna granica warstwy splotowatej zewnętrznej (*outer plexiform layer OPL*).

Celem obliczenia CVI przezdołkowy skan OCT został poddany binaryzacji i segmentacji zgodnie z protokołem pomiarów zaproponowanym przez Sonoda et al. (PMID: 24894395) i Agrawal et al. (PMID: 26751702) z modyfikacjami. CVI zostało obliczone jako stosunek powierzchni światła naczyń naczyniówki do całkowitej powierzchni naczyniówki. Ze względu na jeszcze nie w pełni zautomatyzowany i złożony sposób obliczania CVI, poniżej przedstawiono skrót sposobu.

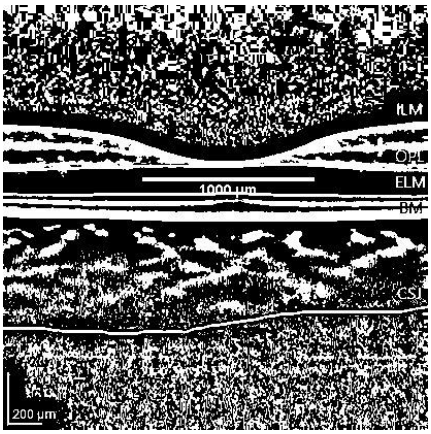
W trakcie analizy skanów OCT w pierwszej kolejności został wybrany przezdołkowy skan, który następnie poddawany był obróbce komputerowej.

Dany region siatkówki i naczyniówki był zaznaczany i analizowany za pomocą oprogramowania ImageJ (http://imagej.nih.gov/ij, accessed on 5 May 2021, version 1.49, U. S. National Institutes of Health, Bethesda, MD, USA).

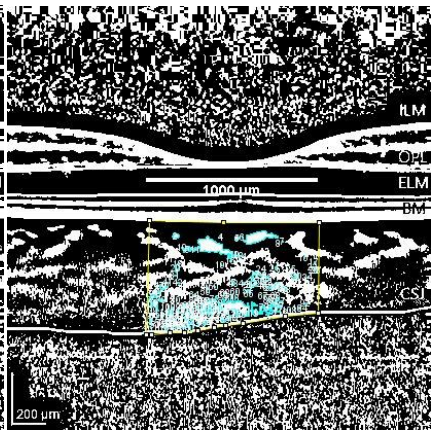
Segmentacja warstw siatkówki została przeprowadzona automatycznie za pomocą oprogramowania Spectralis (wersja 6.7, Heidelberg Engineering, Heidelberg, Niemcy). Błona graniczna wewnętrzna (ILM), OPL, ELM i BM zostały zaznaczone automatycznie, a połączenie naczyniówkowo-twardówkowe (*choroidoscleral junction CSJ*) zaznaczono manualnie na każdym skanie, przesuwając linię BM do połączenia naczyniówkowo-twardówkowego (ryc. 2.).



Ryc. 2.



Ryc. 3.



Ryc. 4.

W kolejnym etapie stosowano binaryzację skanu OCT metodą Niblack. Jasne piksele reprezentowały obszar zrębu (*stromal area SA*), a czarne i ciemne piksele reprezentowały obszar światła (*luminal area LA*). (ryc. 3.).

Po kolejnych konwersjach, ostatnim etapem pracy było określenie naczyniówkowego wskaźnika naczyniowego (CVI). Dalszym analizom poddano poddołkowy obszar o długości 1000µm. Zmierzono powierzchnię światła naczyniowego (LA) oraz całkowitą powierzchnię naczyniówki *(total choroidal area TCA*). Obliczono powierzchnię zrębu (SA), a CVI określono jako stosunek LA do TCA (ryc. 4.).

4.3 Zbiorcze omówienie wyników

W badaniach nad powiązaniem między parametrami naczyniówki a zewnętrznymi warstwami siatkówki wykazano, że pacjenci z grupy kontrolnej mieli znacznie grubsze naczyniówki i wyższe wartości CVI niż pacjenci z DR. W porównaniu z grupą porównawczą, pacjenci z grupy badanej bez obrzęku w plamce (DR+DME-) mieli mniejszą grubość obu składowych zewnętrznej siatkówki, ORL i ONL. Tymczasem wartości obu tych parametrów u pacjentów z grupy z retinopatią cukrzycową ze współistniejącym obrzękiem w plamce (DR+DME+) były znamiennie wyższe niż w grupie kontrolnej. Przeanalizowano korelacje między zewnętrzną siatkówką (ONL i ORL) a parametrami naczyniówki (grubość naczyniówki, CVI, LA, SA i TCA) w każdej z trzech grup. Nie wykazano istotnych korelacji między parametrami naczyniówki i zewnętrznej siatkówki w grupie kontrolnej i grupie DR+DME+. Natomiast, co

warto podkreślić, w grupie DR+DME- ORL korelowało dodatnio z podplamkową grubością naczyniówki, CVI i LA. Dodatkowo stwierdzono dodatnią korelację między ONL a CVI.

Podsumowując, obecność DR (z towarzyszącym DME lub bez) wiązała się ze ścieńczeniem naczyniówki oraz obniżeniem wartości wskaźnika CVI. W porównaniu do grupy porównawczej stwierdzono ścieńczenie poszczególnych składowych zewnętrznej siatkówki w grupie pacjentów z retinopatią cukrzycową bez DME, natomiast ich pogrubienie u pacjentów z retinopatią cukrzycową z DME. Wykazano korelacje pomiędzy parametrami naczyniówkowymi a grubościami warstw zewnętrznej siatkówki u pacjentów z DR bez DME. Korelacje takie nie zostały wykazane w grupie porównawczej i u pacjentów z DR i DME.

W pracy oceniającej zależność pomiędzy parametrami naczyniówki a FAZ stwierdzono, że grubość naczyniówki, objętość i inne parametry naczyniówki nie wykazują istotnych różnic w analizowanych grupach: ≤2 i ≥3 stopnia uszkodzenia zarysu FAZ wg ETDRS. Obie grupy nie różniły się również istotnie, gdy w modelu uwzględniono potencjalne czynniki zakłócające, takie jak płeć, wiek, CSME, zaawansowanie DR i wcześniejszą fotokoagulację siatkówki. W trakcie badań oceniano również powierzchnię FAZ. W całej grupie pacjentów z DR powierzchnia FAZ nie korelowała istotnie z grubością, objętością i innymi parametrami naczyniówki. Biorąc pod uwagę istotną różnicę w obszarze FAZ u pacjentów z CSME i bez, przeprowadzono również analizę podgrup. Analiza nie wykazała istotnych korelacji między obszarem FAZ a parametrami naczyniówkowymi innymi niż CVI u pacjentów z CSME. W dalszych analizach, pacjentów z DR podzielono na 2 grupy zależnie od pola powierzchni FAZ, z medianą 0,355 mm^2 jako poziomem odcięcia. Grupy nie różniły się grubością naczyniówki, objętością i innymi parametrami. Podobnie nie stwierdzono istotnych różnic między grupami, kiedy w modelu uwzględniono również czynniki zakłócające jak wyżej.

Podsumowując, u pacjentów z DR, wielkość i zarys FAZ nie korelowały z parametrami naczyniówki (grubością i objętością w poszczególnych polach ETDRS oraz z CVI).

Lepszy wgląd w patogenezę DR i DME może być jednym z elementów na drodze do dokładniejszej oceny rokowania u pacjentów oraz kwalifikacji do spersonalizowanego leczenia w zależności od potencjalnego ryzyka progresji choroby i przewidywalnej skuteczności planowanego leczenia. W kontekście

poszerzenia wiedzy dotyczącej patofizjologii, niewątpliwą zaletą badań jest zastosowana metodologia (CVI, objętość naczyniówki) i włączenie pacjentów z DME, którzy nie byli dotychczas leczeni preparatami anty-VEGF. Dzięki wprowadzonemu w Polsce w 2021 roku Programowi Lekowemu dostępność tego leczenia jest dobra, natomiast byłby to niewątpliwie czynnik zakłócający w prawidłowej ocenie naturalnego przebiegu choroby. Dodatkowo, dotychczas wielu badaczy oceniało związek CVI z grubością całej siatkówki. Niewątpliwym atutem naszej pracy było wyodrębnienie zewnętrznej warstwy siatkówki, która jest zaopatrywana w krew przez naczyniówkę a w której są obecne fotoreceptory. Wykazują one wysokie potrzeby metaboliczne i są wrażliwe na zaburzenia w dopływie krwi, które występują w retinopatii cukrzycowej.

4.4 Wnioski

1. Obecności retinopatii cukrzycowej towarzyszą zmiany parametrów naczyniówki i zewnętrznych warstw siatkówki oraz FAZ.
2. U pacjentów z retinopatią cukrzycową bez DME wykazano zależność pomiędzy naczyniówką a zaopatrywanymi przez nią zewnętrznymi warstwami siatkówki. Korelacji takich nie wykazano w grupie porównawczej i u pacjentów z DR i DME. Sugeruje to bardziej złożony patomechanizm zmian w obrębie zewnętrznej siatkówki u pacjentów z DR i DME z wpływem dodatkowych czynników.
3. Nie stwierdzono związku między uszkodzeniem naczyń siatkówki i naczyniówki w obrębie plamki u pacjentów z DR. W konsekwencji, te dwa procesy wydają się być równoległe, ale niezależne.

Rozdział 5. Publikacje składające się na rozprawę doktorską

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ORIGINAL RESEARCH

Foveal Avascular Zone Does Not Correspond to Choroidal Characteristics in Patients with Diabetic Retinopathy: A Single-Center Cross-Sectional Analysis

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Purpose: The aim of the study was to compare two non-overlapping blood supply systems of the retina to obtain a better insight into the relation between diabetic macular retinopathy and choroidopathy. Specifically, the study focused on the relationships between (1) retinal vascular changes around the fovea in fluorescein angiography (FA) and (2) choroidal thickness, volume and other parameters assessed by optical coherence tomography (OCT).

Patients and Methods: The retrospective cross-sectional single-center study included 210 eyes from 152 patients with diabetic retinopathy (mean age 60.7±12.4 years, 49.3% of women; foveal avascular zone [FAZ] outline: 44.3% grade ≤2, 55.7% grade ≥3). The outline of FAZ, a measure of capillary loss due to ischemic processes, was analyzed on FA according to the Early Treatment Diabetic Retinopathy Study Research Group (ETDRS) standards. The eyes were stratified according to the FAZ outline and size and the presence of clinically significant diabetic macular edema (CSME). Then, resultant groups were compared in terms of the spectral domain OCT parameters: choroidal thickness and volume (within ETDRS subfields), luminal, stromal and total choroidal areas and choroidal vascularity index (based on the foveal scan). Statistical analysis was based on univariate models with the choroidal parameters as independent variables, and age, sex, panretinal photocoagulation, the severity of diabetic retinopathy and CSME as covariates.

Results: No significant relationships were found between the FAZ outline and area and choroidal characteristics of patients with diabetic retinopathy. In patients without CSME, no correlation was observed between the FAZ area and choroidal characteristics. In patients with CSME, no correlation was found between the FAZ area and choroidal characteristics other than the choroidal vascularity index.

Conclusion: In patients with diabetic retinopathy, damage to the macular retinal vasculature (FAZ) does not seem to be associated with changes in the choroidal vasculature, and these two processes appear to occur independently.

Keywords: choroid, choroidal thickness, choroidal volume, choroidal vascularity index, OCT

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Introduction

Retina receives blood from two independent systems. The outer retina is supplied via diffusion from the choriocapillaris, whereas the inner retina receives blood from the central retinal artery. The outer plexiform layer constitutes the border. Foveal avascular zone (FAZ), best visualized on fluorescein angiography (FA) or optical

coherence tomography (OCT) angiography, is a capillary-free area in the central macula with high photoreceptor density and high metabolic activity.[1] There is no retinal circulation in the foveola (avascular zone). Choriocapillaris circulation is the major source of oxygen and nutrients for the outer retina and the only source of blood for the avascular fovea.[2]

FAZ has regular boundaries in healthy persons. In pathologic conditions associated with retinal capillary dropout, such as diabetes mellitus, FAZ margins can be enlarged and irregular (Figure 1).[1] Diabetic macular ischemia (DMI) is characterized by the occlusion and loss of the macular capillary network or capillary dropout.[3,4] Diabetes may also lead to choroidal abnormalities, similar to those observed in the retina, such as microaneurysms, dilatation and obstruction of the choriocapillaris, vascular remodeling with increased vascular tortuosity, vascular dropout, focal vascular non-perfusion and choroidal vascularization.[5–7] Hence, diabetes affects both retinal and choroidal vasculature. However, it is unclear if the choroidal changes observed in diabetes are predictive, modulatory, causative, or independent for diabetic retinopathy.[6,8–10]

Unlike commercially available OCT angiography that visualizes only the choriocapillaris layer of the choroid, enhanced depth imaging (EDI) and swept-source (SS)-OCT are suitable for the evaluation of the entire cross-sectional image of the choroid. Published data about choroidal thickness in diabetic retinopathy are inconclusive, and this parameter seems to be also influenced by diabetic macular edema (DME).[6] To address this problem, the choroidal vascular index (CVI), an OCT-based choroidal quantitative parameter, was introduced. CVI specifically analyzes the vascular component of the choroid, including all choroidal vessel layers, ie choriocapillaris, Sattler's and Haller's layer. CVI is defined as the ratio of the luminal area (LA) to the total choroidal area (TCA). Compared

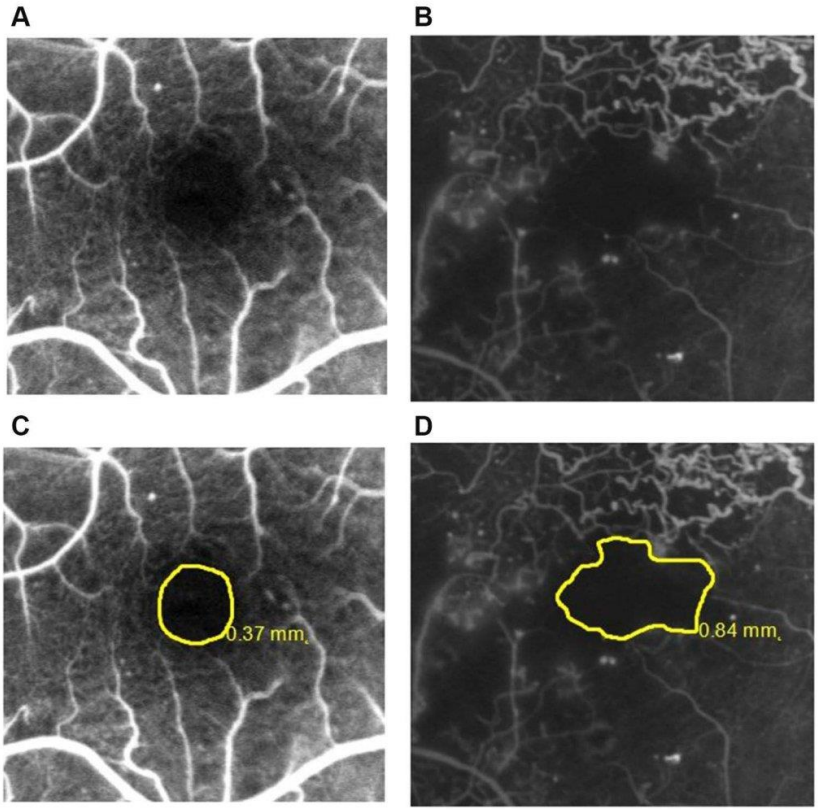

Figure 1 Representative fluorescein angiograms with FAZ outlines: (**A**) grade ≤2; (**B**) grade ≥3 according to the ETDRS report number 11.[15] The same angiograms with FAZ area boundaries and sizes: (**C** and **D**).

with choroidal thickness, this parameter appears to be less dependent on various confounding factors.[11,12] CVI proved to be useful in the early diagnosis of various retinal and choroidal diseases, as well as in the monitoring of their progression and patient stratification.[11] In the present study, CVI was calculated complementarily to the evaluation of the choroidal thickness and volume.

Little is known about the temporal relationship between diabetic retinopathy (DR) and diabetic choroidopathy. A better insight into the relationship between retinal and choroidal vasculature would shed new light on the pathogenesis of these two conditions and improve patient stratification in terms of the disease progression or treatment responses. Our present study centered primarily on the evaluation of the macula. Macular changes do not reflect the severity of DR; for example, DME may develop at any stage of the DR.[13] Thus, in this study, we focused specifically on the macular retinal and choroidal vasculatures. Aside from the severity of DR, we also analyzed FAZ to estimate the degree of macular ischemia. The aim of this study was to analyze a unique relationship between the FA-assessed degree of DMI and OCT-based choroidal characteristics in patients with DR.

Materials and Methods

Study Design, Participants, Eligibility Criteria and Ethics

This retrospective single-center cross-sectional study included 210 eyes from 152 patients with type 1 and 2 diabetes mellitus, who underwent same-day FA and OCT at the Department of Ophthalmology, University Teaching Hospital of Bialystok (Poland) between March 22, 2017, and November 13, 2020. All patients presented with DR. The patients' eyes were stratified according to the FAZ outline and size, with clinically significant diabetic macular edema (CSME) taken into account.

Patients were not eligible for the study if they had a history of prior posterior segment surgery or intravitreal injections, macular laser photocoagulation, ametropia ≥3.0 diopters, macular changes resulting from other ocular diseases, glaucoma, known ocular or systemic pathology potentially able to affect choroidal vasculature and insufficient quality of fluorescein angiograms or OCT images. Controls were recruited among non-diabetic patients scheduled for routine ocular examination.

The protocol of the study followed the provisions of the Declaration of Helsinki and was approved by the Local Bioethics Committee at the Medical University of Bialystok (decision no. APK.002.216.2020). Written informed consent was sought from all patients involved in the study.

Fluorescein Angiograms Acquisition and Analysis

OCT and FA images were acquired one after another in mydriasis, between 8 am and 11 am. The images were independently assessed by two investigators (D.A.D. and P.S.), blinded to the clinical characteristics of the examined eyes.

FA remains the gold standard in the evaluation of retinal perfusion status and potential leakage and in the detection of macular ischemia in patients with DR. FA was performed with Spectralis HRA+OCT imaging device (Heidelberg Engineering, Heidelberg, Germany) according to the standard procedure. The FA images were used to assess the severity of DR, to detect CSME and to characterize FAZ according to the Early Treatment Diabetic Retinopathy Study Research Group (ETDRS) criteria.[14–17]

An early phase frame (after 20–30 seconds) and a late frame (after 3–5 minutes) were selected for further analysis. CSME was defined as the dye leakage within 500 µm radius from the fovea or 1500 µm radius in cases with hard exudate in this area. The severity of perifoveal capillary occlusion was characterized based on the FAZ outline graded according to the ETDRS report number 11 (grade 0 = normal; grade 1 = questionable, outline not smoothly round or oval, but visible irregularities not definitely abnormal; grade 2 = outline definitely destroyed in less than one half of the original circumference; grade 3 = outline definitely destroyed for one half or more of the original circumference, but some remnants remain; grade 4 = capillary outline completely destroyed; grade 8 = cannot be graded). Angiograms with grade 8 FAZ outline were excluded from the analysis.[15] Representative images of FAZ outlines are shown in Figure 1. The examined eyes were divided into two groups according to the severity of damage to the FAZ outline: grade ≤2 and grade ≥3. FAZ boundaries were delineated manually, and the area was measured automatically.

Optical Coherence Tomography Images Acquisition and Analysis

SD-OCT was carried out with a Spectralis HRA+OCT imaging device (Heidelberg Engineering, Heidelberg, Germany). The protocol of SD-OCT imaging comprised

of 25 horizontal raster scans (20 x 20°) and a linear B-scan centered at the fovea, as shown in Figure 2.

The internal limiting membrane (ILM) and Bruch's membrane (BM) were detected automatically, and the choroidal-scleral junction was marked manually on each scan by the same grader. The retinal parameters were calculated from the ILM to the BM while the choroidal parameters from the BM to the choroidal-scleral junction.

Averaged thickness and volume maps were created automatically according to the conventional ETDRS grid with nine subfields: central macular subfield (a central field within a 500 µm radius), four inner subfields (within a 500–1500 µm radius) and four outer subfields (within a 1500–3000 µm radius).[14] There were separate maps for the retinal thickness/volume as well as for the sum of the retinal and choroidal thickness/volume. The values of the choroidal parameters were calculated by subtracting retinal parameters from the summed retinal and choroidal parameters.

Subfoveal choroidal thickness (SFCT) was defined as the distance between the BM and the choroidal-scleral junction at the fovea.

CVI quantifies the vascular status of the choroid.[11,18] To calculate CVI, choroidal areas on the OCT scans were binarized with a modified Niblack method, as described by Sonoda et al.[12,19] Briefly, the entire horizontal scan (6 mm) across the fovea was assessed, with the BM as an upper margin and the choroidal-scleral junction as the lower margin. Binarization and segmentation of the images were done with ImageJ software (http://imagej.nih.gov/ij, version 1.49). TCA, LA and stromal area (SA) were calculated, and CVI was determined as the LA to TCA ratio.

Statistical Analysis

Statistical analyses were carried out with R software, version 3.5.1 (http://cran.r-project.org). Descriptive statistics included numbers (% of each group) for nominal variables and means ± standard deviations (SD) or medians with lower and upper quartiles (Q1; Q3) for continuous variables, depending on the data distribution. The normality of the distribution was verified with the Shapiro–Wilk test, on the basis of skewness and kurtosis values, as well as based on visual inspection of histograms. Between-group comparisons were carried out with a chi-square test for nominal variables and *t*-test or Mann–Whitney *U*-test for continuous variables, whichever appropriate. Linear mixed-effects models were created for the comparisons of FAZ outlines (grade ≤2 vs grade ≥3) and FAZ areas (<0.355 vs >0.355 mm^2 based on median), with random effects for the correlation of two eyes from the same patient. A range of univariate models with choroidal parameters as independent variables and age, sex, panretinal photocoagulation (PRP), DR severity and CSME as covariates was developed as well. Finally, the relationships between pairs of continuous variables were verified on Spearman correlation analysis. All tests were two-tailed with $\alpha = 0.05$.

Results

Baseline Characteristics

Demographic and clinical characteristics of the study patients are shown in Table 1. The groups selected according to the FAZ outline and CSME were age-, sex- and refractive error-matched. The groups identified based on

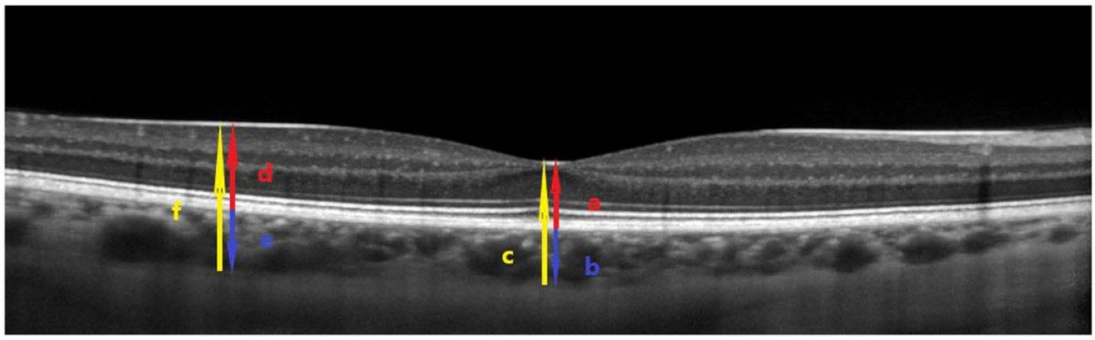

Figure 2 Representative OCT scan across the fovea. ILM and BM were detected automatically, while the choroidal-scleral junction was marked manually. SFCT was defined as the distance between the BM and the choroidal-scleral junction at the fovea (blue arrow b). The SFCT was obtained as a difference between the total thickness (retinal + choroidal thickness, from the ILM to the choroidal-scleral junction) (yellow arrow c) and the central foveal thickness (red arrow a). Analogically, the choroidal thickness outside the fovea (blue arrow e) was calculated by subtracting the retinal thickness (red arrow d) from the total thickness (retinal + choroidal thickness, from the ILM to the choroidal-scleral junction) (yellow arrow f).

Table 1 Baseline Characteristics of Patients with Diabetic Retinopathy

	Overall	FAZ Outline		p	CSME		p
		Grade ≤2	Grade ≥3		Absent	Present	
Number of patients	152	63	89		60	92	
Number of eyes	210	93	117		76	134	
Age, years, mean ± SD	60.73±12.40	62.18±12.06	59.71±12.61	0.225	60.80±13.01	59.95±12.73	0.704
Sex, female, n (%)	75 (49.3)	28 (44.4)	47 (52.8)	0.395	30 (50.0)	45 (48.9)	>0.999
Spherical equivalent, mean ± SD	0.30±1.02	0.42±0.95	0.20±1.07	0.107	0.14±0.83	0.39±1.11	0.076
DR severity, n (%) NPDR PDR	 135 (64.3) 75 (35.7)	 78 (83.9) 15 (16.1)	 57 (48.7) 60 (51.3)	 <0.001	 49 (64.5) 27 (35.5)	 86 (64.2) 48 (35.8)	 >0.999
CSME, n (%) Present Absent	 134 (63.8) 76 (36.2)	 55 (59.1) 38 (40.9)	 79 (67.5) 38 (32.5)	 0.267	 – –	 – –	 –
PRP, n (%) No Yes	 138 (65.7) 72 (34.3)	 72 (77.4) 21 (22.6)	 66 (56.4) 51 (43.6)	 0.002	 51 (67.1) 25 (32.9)	 87 (64.9) 47 (35.1)	 0.866
FAZ area, mm^2, median (Q1; Q3)	0.36 (0.25;0.51)	0.32 (0.10;0.75)	0.41 (0.28;0.59)	<0.001	0.42 (0.29;0.53)	0.35 (0.24;0.49)	0.034
FAZ outline, n, (%) Grade ≤2 Grade ≥3	 93 (44.3) 117 (55.7)	 – –	 – –	 –	 38 (50.0) 38 (50.0)	 55 (41.0) 79 (59.0)	 0.267

Notes: Groups compared with the chi-square test for nominal variables and with *t*-test or Mann–Whitney *U*-test (FAZ area) for continuous variables.
Abbreviations: FAZ, foveal avascular zone; CSME, clinically significant macular edema; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; PRP, panretinal photocoagulation.

the FAZ area was age- and sex-matched (see Supplementary Material, Table S1).

FAZ Outline and Choroidal Characteristics of Patients with Diabetic Retinopathy

Choroidal thickness, volume and other choroidal parameters, stratified according to the FAZ outline and size, are presented in Table 2. No significant differences in the analyzed parameters were found between the eyes with grade ≤2 and grade ≥3 FAZ outline. The two groups did not also differ significantly when potential confounders, such as sex, age, CSME, DR severity, and PRP, were included in the model (Table 3).

FAZ Area and Choroidal Characteristics in Patients with Diabetic Retinopathy

In the entire group of patients with DR, FAZ area did not correlate significantly with choroidal thickness, volume and other choroidal parameters (Tables 2 and 4). Given a significant difference in the FAZ area in patients with CSME and without (Table 1), a subgroup analysis was carried out as well. The analysis did not demonstrate significant correlations between the FAZ area and the choroidal parameters other than CVI in patients with CSME (Table 4).

The study patients were further stratified according to their FAZ area, with the median of $0.355mm^2$ as a cut-off level. The groups did not differ in terms of the choroidal thickness, volume and other parameters. Similarly, no significant between-group differences were found when potentially confounding factors such as sex, age, CSME, DR severity and PRP were included in the model (Table 5).

Discussion

In the present study, the angiographically-identified status of the FAZ was compared with the OCT-based choroidal characteristics in patients with DR. In general, no significant associations were found between the FAZ outline/area and choroidal parameters, such as thickness and volume (within the ETDRS subfields), LA, SA, TCA and CVI (based on the foveal scan). This observation is consistent with the results published by Gerendas et al.[20] and Adhi

Table 2 Choroidal Parameters in Patients with Diabetic Retinopathy

Characteristic	Total Group	FAZ Outline		FAZ Area (mm^2)	
		Grade ≤2	Grade ≥3	<0.355	>0.355
Choroidal thickness (μm):					
Outer T	246.64±50.99	240.82±56.25	249.76±47.86	251.89±53.55	241.35±47.95
Inner T	256.75±55.06	251.82±62.94	259.37±50.41	261.07±57.61	252.43±52.30
Central macular	260.06±56.19	252.47±64.44	264.11±51.04	262.47±57.14	257.66±55.38
Inner N	250.23±61.33	240.81±65.74	255.25±58.48	252.22±62.24	248.24±60.64
Outer N	222.36±63.89	213.93±65.66	226.85±62.70	224.11±64.34	220.60±63.68
Outer S	263.91±53.76	259.72±59.59	266.14±50.48	267.10±54.62	260.69±52.96
Inner S	265.41±53.13	261.34±62.42	267.58±47.56	267.04±55.08	263.78±51.33
Inner I	254.22±60.01	244.66±66.34	259.32±55.93	256.78±60.65	251.67±59.54
Outer I	243.82±61.14	238.15±64.00	246.91±59.55	248.01±62.11	239.59±60.16
SFCT	260.07±58.57	251.11±64.85	264.85±54.58	263.10±58.59	257.05±58.67
Choroidal volume (mm^3):					
Outer T	1.31±0.27	1.28±0.30	1.32±0.25	1.33±0.28	1.28±0.25
Inner T	0.41±0.09	0.40±0.10	0.41±0.08	0.41±0.09	0.40±0.09
Central macular	0.20±0.04	0.20±0.05	0.21±0.04	0.21±0.04	0.20±0.04
Inner N	0.39±0.10	0.38±0.11	0.40±0.09	0.40±0.10	0.39±0.10
Outer N	1.17±0.34	1.13±0.34	1.20±0.33	1.18±0.34	1.16±0.34
Outer S	1.40±0.28	1.38±0.31	1.41±0.27	1.42±0.29	1.38±0.28
Inner S	0.42±0.08	0.41±0.10	0.42±0.07	0.42±0.09	0.41±0.08
Inner I	0.40±0.09	0.38±0.10	0.41±0.09	0.40±0.10	0.40±0.09
Outer I	1.29±0.32	1.26±0.34	1.30±0.31	1.31±0.33	1.26±0.31
Total	6.99±1.49	6.81±1.66	7.09±1.39	7.09±1.52	6.89±1.46
Other choroidal parameters:					
CVI	0.59±0.06	0.59±0.06	0.59±0.06	0.59±0.06	0.59±0.05
LA (mm^2)	1.35±0.40	1.27±0.40	1.37±0.38	0.13±0.42	1.34±0.37
SA (mm^2)	0.91±0.23	0.87±0.23	0.94±0.22	0.93±0.24	0.90±0.22
TCA (mm^2)	2.25±0.56	2.13±0.56	2.31±0.55	2.27±0.59	2.23±0.53

Note: Data presented as means ± SD.

Abbreviations: FAZ, foveal avascular zone; T, temporal; I, inferior; N, nasal; S, superior; SFCT, subfoveal choroidal thickness; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; conventional ETDRS grid with nine subfields, central macular field (central field within a 500 μm radius), four inner subfields (within a 500–1500 μm radius) and four outer subfields (within a 1500–3000 μm radius).

et al.[21] according to whom choroidal changes did not correlate with the level of retinal pathology in patients with DR; however, it needs to be stressed that those authors used different methodological approach than in the present study.

We analyzed two FAZ parameters, outline and size. FAZ outline might be a better indicator of ischemia given the heterogeneity in the size of FAZ in healthy persons (as reviewed by Sun et al.[7]). Johannesen et al postulated to determine FAZ area as a part of the diagnostic process but emphasized that this parameter might be unsuitable if used alone.[22] Therefore, our study was mainly focused on the FAZ outline; this parameter reflects capillary loss and ischemic processes and seems to be a more reliable measure of potential impairment of blood supply to the inner retina than the FAZ area. Choroidal characteristics analyzed in this study included not only the thickness and volume but also a novel parameter, CVI. The latter quantifies the vascular component of the choroid. In previous studies, CVI was determined not only in ocular pathologies but also in systemic disorders, including inflammatory conditions, whereby it was implicated as a biomarker of disease activity.[11] Choroidal thickness depends on various physiological and pathological factors, including age, ethnicity, sex, refraction, axial length and time of the day.[23] In contrast, CVI is modulated solely by SFCT.[24] According to Campos et al, choroidal thickness (and consequently volume) may be affected by age, sex, CSME, DR severity and PRP. Thus, we included all

Table 3 Univariate Models to Analyze Relationships Between Choroidal Parameters and FAZ Outline in Patients with Diabetic Retinopathy

Characteristic	Univariate Models			Univariate models (Sex, Age, CSME, PRP, DR Severity as Covariates)		
	β	SE	p	β	SE	p
Choroidal thickness (μm):						
Outer T	5.17	6.93	0.456	−1.79	6.75	0.790
Inner T	2.86	8.04	0.723	−3.92	7.94	0.622
Central macular	9.29	8.01	0.248	2.67	7.93	0.644
Inner N	12.30	8.52	0.150	5.97	8.53	0.485
Outer N	13.38	8.53	0.118	7.97	8.56	0.353
Outer S	2.85	7.56	0.706	−2.54	7.36	0.730
Inner S	7.69	7.50	0.307	1.21	7.46	0.871
Inner I	11.75	8.44	0.165	6.27	8.39	0.456
Outer I	4.20	8.36	0.616	−0.56	8.16	0.945
SFCT	7.41	8.66	0.394	1.94	8.51	0.820
Choroidal volume (mm^3):						
Outer T	0.03	0.04	0.422	−0.01	0.04	0.829
Inner T	0.008	0.01	0.522	−0.004	0.01	0.782
Central macular	0.007	0.006	0.264	0.003	0.006	0.638
Inner N	0.02	0.01	0.206	0.01	0.01	0.332
Outer N	0.08	0.04	0.082	0.05	0.04	0.246
Outer S	0.02	0.04	0.705	−0.01	0.04	0.748
Inner S	0.01	0.01	0.367	0.001	0.01	0.908
Inner I	0.02	0.02	0.172	0.009	0.01	0.467
Outer I	0.02	0.04	0.601	−0.002	0.04	0.962
Total	0.25	0.19	0.182	0.10	0.19	0.593
Other choroidal parameters:						
CVI	0.003	0.009	0.682	0.004	0.009	0.595
LA (mm^2)	0.04	0.06	0.522	0.006	0.06	0.912
SA (mm^2)	0.02	0.03	0.614	−0.009	0.04	0.810
TCA (mm^2)	0.05	0.08	0.553	−0.008	0.08	0.924

Notes: Only patients with DR were included, 210 eyes (93 eyes with FAZ grade ≤2 and 117 eyes with FAZ grade ≥3). Covariates: age in years, sex (male/female), CSME (absent/present), PRP (no/yes), DR severity (NPDR/PDR).

Abbreviations: β, coefficient from regression model; SE, standard error; CSME, clinically significant macular edema; PRP, panretinal photocoagulation; T, temporal; I, inferior; N, nasal; S, superior; SFCT, subfoveal choroidal thickness; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; conventional ETDRS grid with nine subfields, central macular field (central field within a 500 μm radius), four inner subfields (within a 500–1500 μm radius) and four outer subfields (within a 1500–3000 μm radius).

Table 4 Correlations Between FAZ Area and Choroidal Parameters in Patients with Diabetic Retinopathy Overall and in Patients with CSME and Without

Characteristic	Overall		CSME Absent		CSME Present	
	rho	p	rho	p	rho	p
Choroidal thickness (μm):						
Outer T	−0.10	0.273	−0.14	0.389	−0.10	0.380
Inner T	−0.11	0.260	−0.17	0.301	−0.07	0.531
Central macular	−0.05	0.618	−0.16	0.331	−0.03	0.815
Inner N	−0.05	0.598	−0.14	0.399	0.01	0.956
Outer N	−0.01	0.876	−0.11	0.487	0.06	0.590
Outer S	−0.06	0.481	−0.14	0.398	−0.01	0.913
Inner S	−0.04	0.699	−0.12	0.454	0.02	0.896
Inner I	−0.04	0.641	−0.14	0.388	0.02	0.893
Outer I	−0.10	0.295	−0.16	0.316	−0.04	0.719
SFCT	−0.04	0.681	−0.09	0.571	−0.02	0.841
Choroidal volume (mm^3):						
Outer T	−0.10	0.283	−0.15	0.371	−0.10	0.402
Inner T	−0.07	0.440	−0.11	0.488	−0.05	0.649
Central macular	−0.05	0.559	−0.15	0.341	−0.03	0.784
Inner N	−0.04	0.708	−0.09	0.575	0.01	0.972
Outer N	−0.04	0.695	−0.11	0.482	0.03	0.810
Outer S	−0.06	0.491	−0.14	0.391	−0.01	0.918
Inner S	−0.07	0.435	−0.13	0.438	−0.04	0.717
Inner I	−0.03	0.748	−0.11	0.516	0.02	0.886
Outer I	−0.10	0.295	−0.16	0.318	−0.04	0.727
Total	−0.09	0.316	−0.16	0.345	−0.05	0.680
Other choroidal parameters:						
CVI	0.16	0.087	−0.12	0.466	0.27	0.020
LA (mm^2)	0.05	0.622	−0.12	0.478	0.12	0.298
SA (mm^2)	−0.13	0.180	−0.01	0.932	−0.16	0.164
TCA (mm^2)	−0.01	0.908	−0.06	0.721	0.03	0.772

Notes: Only patients with DR were included, solely single eyes, n=116. Since the data set included a single eye from each patient, there was no violation of the independence assumption between observations for correlation analysis.

Abbreviations: rho, Spearman correlation coefficient; CSME, clinically significant macular edema; T, temporal; I, inferior; N, nasal; S, superior; SFCT, subfoveal choroidal thickness; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; conventional ETDRS grid with nine subfields, central macular subfield (central field within a 500 μm radius), four inner subfields (within a 500–1500 μm radius) and four outer subfields (within a 1500–3000 μm radius).

these variables as potential confounding factors in linear mixed-effects models of FAZ.[25]

One previous study analyzed a relation between the FAZ and choriocapillaris visualized with OCT angiography.[9] Associations of DR with choroidal thickness[6,23,25] and CVI[26–28] have been studied as well, but the results are inconclusive. To the best of our knowledge, the present study was the first to analyze the relation between FAZ and choroidal parameters, not only the thickness and volume but also other vascular indices, in patients with DR.

Our study confirmed that the FAZ area's enlargement was associated with DR progression[3,7,22] and that the FAZ

Table 5 Univariate Mixed-Effect Models to Compare Choroidal Parameters in Patients with Diabetic Retinopathy and FAZ Area >0.355 and <0.355mm^2

Characteristic	Univariate Models			Univariate Models (Sex, Age, CSME, PRP, DR Severity as Covariates)		
	β	SE	p	β	SE	p
Choroidal thickness (μm):						
Outer T	−4.30	5.71	0.453	−2.27	5.41	0.675
Inner T	−4.11	6.97	0.556	−0.92	6.62	0.889
Central macular	−2.52	6.75	0.709	0.83	6.44	0.897
Inner N	−5.51	6.99	0.432	−2.52	6.75	0.709
Outer N	0.19	6.88	0.977	2.16	6.63	0.745
Outer S	−2.28	6.25	0.716	0.83	5.91	0.888
Inner S	0.14	6.27	0.983	2.98	6.00	0.620
Inner I	−1.12	6.98	0.872	1.95	6.69	0.771
Outer I	−0.60	6.72	0.929	1.74	6.42	0.787
SFCT	−6.35	7.57	0.403	−2.03	7.16	0.777
Choroidal volume (mm^3):						
Outer T	−0.02	0.03	0.504	−0.01	0.03	0.740
Inner T	−0.003	0.01	0.779	0.002	0.01	0.841
Central macular	−0.002	0.005	0.775	0.001	0.005	0.823
Inner N	−0.009	0.01	0.441	−0.004	0.001	0.750
Outer N	0.001	0.03	0.986	0.01	0.03	0.797
Outer S	−0.01	0.03	0.741	0.005	0.03	0.870
Inner S	−0.001	0.01	0.924	0.003	0.009	0.740
Inner I	−0.002	0.01	0.836	0.003	0.01	0.789
Outer I	−0.004	0.03	0.904	0.005	0.03	0.869
Total	−0.03	0.15	0.829	0.02	0.14	0.903
Other choroidal parameters:						
CVI	0.006	0.007	0.432	0.009	0.007	0.220
LA (mm^2)	0.02	0.05	0.589	0.04	0.04	0.375
SA (mm^2)	−0.005	0.03	0.878	−0.001	0.03	0.963
TCA (mm^2)	0.03	0.07	0.687	0.05	0.07	0.512

Notes: Only patients with DR were included, 210 eyes (105 eyes with FAZ area <0.355mm^2 and 105 eyes with FAZ area >0.355mm^2). Covariates: age in years, sex (male/female), CSME (absent/present), PRP (no/yes), DR severity (NPDR/PDR).
Abbreviations: β, coefficient from regression model; SE, standard error; CSME, clinically significant macular edema; PRP, panretinal photocoagulation; T, temporal; I, inferior; N, nasal; S, superior; SFCT, subfoveal choroidal thickness; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; conventional ETDRS grid with nine subfields, central macular subfield (central field within a 500 μm radius), four inner subfields (within a 500–1500 μm radius) and four outer subfields (within a 1500–3000 μm radius).

area was significantly larger in eyes with higher FAZ outline grades.[15,29]

Similar to Sim et al.[30] we found no association between the FAZ outline and choroidal thickness. Also, no significant correlation was observed between the FAZ enlargement and choroidal thickness, which is consistent with the findings published by Lee et al.[31] We also found no significant relationships between choroidal volume and FAZ outline/area. Furthermore, no associations between the FAZ area and other choroidal parameters, such as LA, SA, TCA and CVI, were observed, other than a correlation between the FAZ area and CVI in patients with CSME (rho=0.27, p=0.02). This could be either due to luminal area increase and/or due to a decrease in TCA. However, the latter two parameters did not correlate significantly with the FAZ area in patients with CSME and hence, this finding should be interpreted with caution. Unlike Oh et al, we did not find an inverse correlation between CVI with FAZ area.[32] This discrepancy might be associated with the fact that those authors examined a group of healthy persons, whereas our study included patients with already established diabetes-related vascular damage.

We speculate that the unparallel rate of macular vascular damage to the retina and choroid might reflect the multifactorial impact of diabetes on these vessels. First, the rate of development and progression of microangiopathy may vary, and the changes within the choroid seem to occur at early stages.[8,33] Second, not only the vascular mechanism should be considered, as autonomic neuropathy also seems to be implicated in choroidopathy.[34,35] Choroidal vascular tone does not depend solely on local regulation but autonomic innervation as well. Third, some other factors, such as VEGF and inflammation,[5] might affect both vascular systems differently. Those factors might also contribute to changes in morphometric choroidal parameters analyzed in the present study. Fourth, macular changes may occur independently of peripheral changes in DR.[13] Similarly, submacular choroidal changes might not necessarily correspond to the peripheral ones. Some location-based differences in the choriocapillaris structure are probably related to the different vulnerability of these vessels to diabetes, with the mid-peripheral vessels being affected first.[36,37] These factors could explain the variable rate of diabetes-related damage in different vascular regions. Fifth, unlike the relatively stable FAZ patterns, the choroidal patterns might vary considerably even in healthy persons. Examples of such individual variability are the irregularities of the choroidal-scleral junction and the differences in the number and distribution of the posterior ciliary arteries.[38] Also, this factor might contribute to interpersonal and regional variability in choroidal characteristics and their relation to FAZ.

This study has some strengths, among them the inclusion of DME treatment-naïve patients and the analysis of age-, sex- and refractive error-matched groups. All measurements were taken between 8 am and 11 am to avoid diurnal variations. Only patients with spherical equivalent refractive error <3.0 diopters were included. Compared with previous studies, we included a larger number of patients with proliferative DR, often underrepresented in clinical research. We also conducted a subgroup analysis with the presence of CSME as a grouping factor. Importantly, we analyzed not only the FAZ area but the FAZ outline as well. Further, we considered not only the choroidal thickness but also the choroidal volume over a 6 mm diameter in the macula to obtain a better insight into the characteristics of the choroid. According to Singh et al, even an accurate estimate of choroidal thickness at a few sampling points could be inadequate in assessing choroidal involvement due to the irregularities in the choroidal-scleral junction. Hence, volumetric analysis of the choroid is preferable.[23] To evaluate the choroid even further, we focused on its luminal and stromal components too.

We are well aware of the potential limitations of this study. Due to its retrospective design, the data on the type of diabetes mellitus, duration of the disease, glycated hemoglobin (HbA1C) and fasting blood glucose levels were not available. However, according to Agrawal et al, none of these parameters correlates with CVI.[11] Regarding retinal imaging, OCT angiography would provide more detailed information about the retinal circulation as it visualizes both superficial and deep capillary plexus. However, obtaining OCT angiographic images in patients with DME could be technically challenging as the presence of cystic spaces might be detrimental to the image quality. This problem does not refer to the fluorescein angiograms, though. Additionally, OCT angiography and FA provide comparable results in terms of the FAZ area measurements.[3] In the present study, we determined CVI based on a single foveal scan; this is a relatively common practice given that CVI is similar across all the ETDRS subfields.[23]

FA visualizes the superficial capillary plexus. In future studies involving OCT angiography, it would be interesting to compare FAZ at the deep capillary plexus level with the choroidal parameters. The deep capillary plexus seems to be affected first in the course of diabetes.[26,39] However, technical problems related to fluid accumulation that may interfere with the imaging and segmentation capabilities of OCT angiography in patients with DME would need to be taken into account. Furthermore, the evaluation of choroidal vasculature across all layers, not merely the choriocapillaris, would contribute to a better understanding of the pathogenesis of diabetic choroidopathy. Unfortunately, none of the currently commercially available OCT angiography devices is suitable for this purpose. Another direction of future research could be the application of the wide-field spectral-domain OCT. As suggested by Ferrara et al, a standard cross-sectional OCT limited to the macular area may be insufficient to capture the full spectrum of manifestations in diabetes, especially considering the topographical differences.[40] Regarding future improvements of CVI assessment, a fully automated CVI algorithm integrated into the OCT device might help to standardize this valuable parameter, as highlighted by Agrawal et al.[41]

Conclusion

This study demonstrated that in patients with DR:

1. No relation exists between the FAZ outline/area and choroidal characteristics, such as thickness and volume (within the ETDRS subfields), LA, SA and TCA, and CVI (based on foveal scan).
2. In patients without CSME, no association exists between the FAZ area and the choroidal parameters.
3. In patients with CSME, the FAZ area does not correlate with the choroidal characteristics except for the CVI.

To summarize, this study did not identify a link between macular retinal and choroidal vasculature damage in patients with DR. Hence, these two processes seem to occur independently from one another.

Data Sharing Statement

All the materials and information will be available upon an e-mail request to the corresponding author. Names and exact data of the participants of the study may not be available owing to patient confidentiality and privacy policy.

Ethics Approval and Informed Consent

The protocol of the study followed the provisions of the Declaration of Helsinki and was approved by the Local Bioethics Committee at the Medical University of

Bialystok (decision no. APK.002.216.2020). Written informed consent was sought from all patients involved in the study.

Consent for Publication

The participants have consented for the submission of results of the study to the journal.

Author Contributions

PS and DD worked on the conception, study design, execution, acquisition of data, and main text with figures and tables. BP, JK, ES, ZM worked on the main text. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy

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Abstract: The choroid supplies blood to the outer retina. We quantified outer retinal and choroidal parameters to understand better the pathogenesis of diabetic retinopathy (DR) and diabetic macular edema (DME). The retrospective cross-sectional single-center study included 210 eyes from 139 diabetic patients and 76 eyes from 52 healthy controls. Spectral-domain optical coherence tomography (OCT) was carried out with a Spectralis HRA + OCT imaging device. The outer retinal layer (ORL), outer nuclear layer (ONL), and choroidal thicknesses were assessed along with the choroidal vascularity index (CVI). The presence of DR, whether with DME or without, was associated with choroidal thinning ($p < 0.001$). Compared with the controls, patients with DR without DME presented with lower ORL and ONL thickness ($p < 0.001$), whereas those with DR and DME had higher values of both parameters ($p < 0.001$). Significant correlations between outer retinal and choroidal parameters were found only in patients with DR without DME (ORL with choroidal thickness: $p = 0.003$, rho = 0.34; ORL with CVI: $p < 0.001$, rho = 0.49, ONL with CVI: $p < 0.027$, rho = 0.25). No correlations between choroidal and outer retinal parameters were observed in the controls and patients with DR and concomitant DME. Aside from diabetic choroidopathy, other pathogenic mechanisms seem to predominate in the latter group.

Keywords: choroid; choroidal thickness; choroidal vascularity index; diabetic macular edema; OCT; outer retina; outer retinal layer; outer nuclear layer

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1. Introduction

The macula is supplied with blood from two independent sources. The inner retina, located between the internal limiting membrane (ILM) and the outer plexiform layer (OPL), is perfused by the central retinal artery, whereas the outer retina, spreading between the outer border of the OPL and the Bruch's membrane (BM), is supplied mainly by the choroid [1,2]. Since no retinal vasculature exists in the foveal region, compromised choroidal blood flow may lead to photoreceptor dysfunction [3–6]. Many previous studies demonstrated that in patients with diabetic macular edema (DME), the integrity of the ellipsoid zone and external limiting membrane (ELM) is closely associated with visual function [7–10]. Furthermore, the thickness of some outer retinal layers, e.g., total outer retina [11–13], photoreceptor outer segments [14,15], retinal pigment epithelium (RPE) [16,17], and retinal tissue between the plexiform layers [18], was shown to correlate with visual acuity.

Diabetes may lead to choroidal abnormalities similar to those observed in the retina, such as microaneurysms, dilatation and obstruction of the vessels, vascular remodeling with increased vascular tortuosity, vascular dropout, focal vascular non-perfusion, atrophy of Sattler's layer and Haller's layer, and choroidal vascularization [19–22]. Choroidopathy may trigger the development of retinopathy due to retinal tissue hypoxia and overexpression of vascular endothelial growth factor (VEGF); this contributes to further retinal damage and DME [5,20]. However, it is still unclear whether choroidopathy precedes, accompanies, or follows the retinal changes [3,22–24]. Moreover, the exact contribution of diabetic

choroidopathy to the diabetes-associated damage of the neuroretina and occurrence of DME remains poorly understood [22].

DME, the major cause of severe vision loss in patients with diabetes, can occur at any stage of diabetic retinopathy (DR) and affects both the outer and inner retina [25,26]. DME is defined as an abnormal increase in intra- and extracellular fluid volume in the macula. This multifactorial condition involves many complex mechanisms, including the breakdown of the inner- and outer blood-retinal barrier (BRB) [27,28]. Other underlying pathomechanisms of DME include ischemia, neurodegeneration, and edema [29].

Optical coherence tomography (OCT) is the primary tool to visualize the retina and choroid in healthy persons and diabetic patients with DME or without. To assess the relationship between the OCT-based characteristics of the choroid and outer retina, we divided the latter into the outer retinal layer (ORL) and outer nuclear layer (ONL), with the ELM as a border separating the two. ELM is a marker of photoreceptor integrity, and its disruption is associated with visual impairment in DME [7–10]. ELM is an intercellular junction between the Müller cells and photoreceptor cells, constituting a barrier for macromolecules [30]; a disruption of ELM may result in the migration of blood components into the outer retinal layers and resultant exacerbation of photoreceptor damage. ELM can be altered due to hyperglycemia [24]. ORL includes outer and inner segments of photoreceptors and RPE. The outer segments contain discs filled with opsin, responsible for absorbing photons for later signal transduction, whereas the inner segments are a reservoir of mitochondria needed for energy supply. Consequently, both inner and outer segments have an essential function in the visual pathway [11]. RPE constitutes the outer BRB. It removes the waste that remained after the phagocytosis of photoreceptors' outer segments, provides nutrients for photoreceptors, absorbs light, pumps the fluid towards choriocapillaris, and controls retinal oxidative stress [24].

In the present study, we focused on choroidopathy, a component of DR and DME pathogenesis [3,5,20,22–24]. We aimed to explain the relationship between outer retinal and choroidal parameters. Aside from determining the choroidal thickness, we also calculated the choroidal vascularity index (CVI). CVI is a novel, OCT-based choroidal quantitative parameter providing more detailed information about the vascular component of the choroid across all its layers, i.e., choriocapillaris, Sattler's layer, and Haller's layer [31,32]. CVI has been proposed as a marker for early diagnosis, progression monitoring, and stratification of patients with various retinal and choroidal diseases and systemic conditions, including those of vascular or inflammatory origin [31]. Unlike the choroidal thickness, which depends on multiple physiological and pathological factors [31–35], CVI is considered a relatively stable parameter to evaluate changes in choroidal vasculature [36].

A number of previous studies analyzed either choroidal [6,17,34,37–43] or retinal characteristics [11–18,44] in diabetic patients with DR with concomitant DME or without. However, to the best of our knowledge, this is the first study to explore the link between the choroidal parameters (CVI and choroidal thickness) and the parameters of outer retinal thickness in such patients. A better insight into the pathogenesis of DR and DME may facilitate the stratification of patients in terms of prognosis and their qualification for novel treatments from the spectrum of personalized medicine.

2. Materials and Methods

The retrospective single-center cross-sectional study included 286 eyes from 191 patients (139 with DR and 52 controls). Medical records were analyzed for the period between 28 February 2017 and 20 February 2021. In 210 eyes from patients with diabetes, DR was confirmed by fluorescein angiography. The DR group was divided into two subgroups based on the presence of DME (DR + DME+) or lack thereof (DR + DME−). The control group (76 eyes) consisted of patients scheduled for routine ocular examination at the Department of Ophthalmology, University Teaching Hospital of Bialystok.

All patients underwent spectral-domain OCT examination. DME was diagnosed whenever the retinal thickness in the central macular subfield (1 mm in diameter) of

the Early Treatment Diabetic Retinopathy Study (ETDRS) grid sector was ≥300 µm and excluded if the thickness was <300 µm [45].

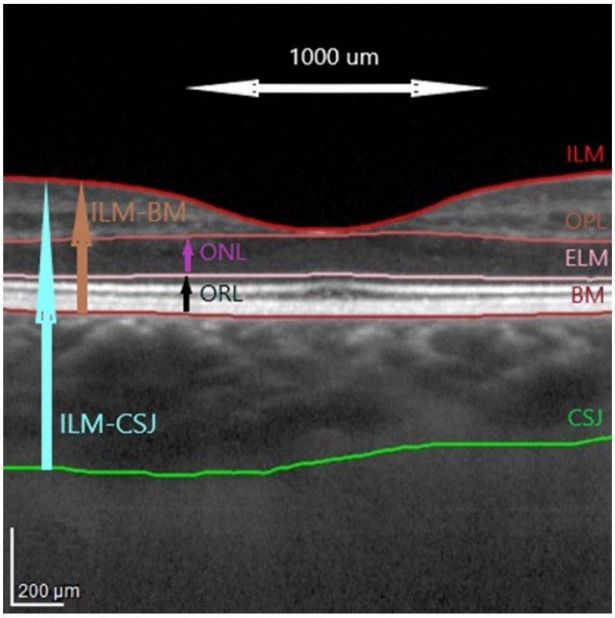
The exclusion criteria of the study were: prior posterior segment surgery or intravitreal injections, macular laser photocoagulation, ametropia ≥ 3.0 diopters, macular changes resulting from other ocular diseases, glaucoma, known ocular or systemic pathology potentially able to affect the choroidal vasculature, and insufficient quality of OCT images.

The study was conducted in line with the provisions of the Declaration of Helsinki and approved by the Ethics Committee at the Medical University of Bialystok (approval number APK.002.216.2020). Written informed consent was provided by all patients involved in the study.

2.1. Optical Coherence Tomography Images Acquisition and Analysis

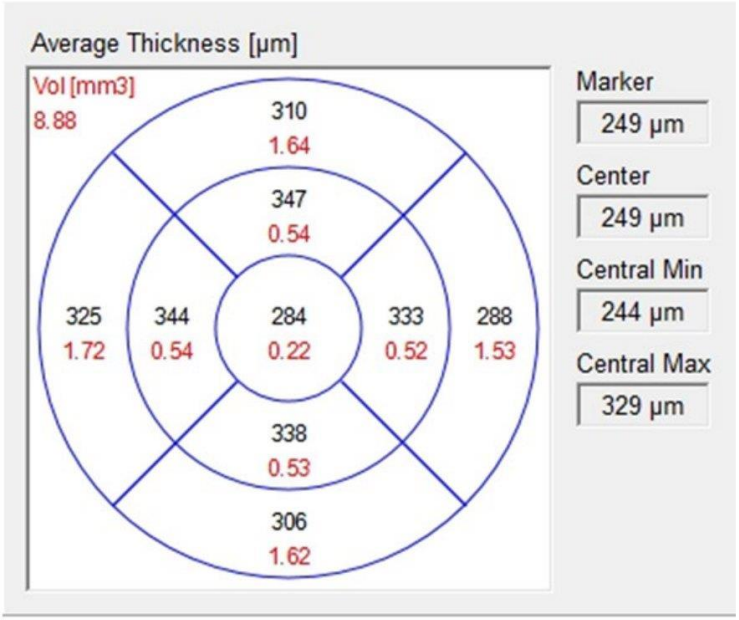
The protocol of the study was described elsewhere [46]. The OCT images were taken in mydriasis between 8 a.m. and 11 a.m. to avoid diurnal variation in the choroidal thickness. The images were independently assessed by two investigators (P.S. and D.A.D.) blinded to the clinical characteristics of examined eyes.

Spectral-domain OCT was carried out with a Spectralis HRA + OCT imaging device with eye tracking (Heidelberg Engineering, Heidelberg, Germany). The protocol of the OCT imaging comprised of 25 horizontal raster scans (20 × 20°) and a linear B-scan centered at the fovea. The segmentation of the retinal layers was carried out automatically with the Spectralis software (version 6.7, Heidelberg Engineering, Heidelberg, Germany), as shown in Figure 1. The internal limiting membrane (ILM), outer plexiform layer (OPL), external limiting membrane (ELM), and Bruch's membrane (BM) were detected automatically, and the choroidal–scleral junction was marked manually on each scan by shifting the BM line to the choroidal–scleral junction, as described previously [46]. Manual measurements were reviewed by the authors, and disagreements were resolved through discussion.



(**A**)

Figure 1. *Cont.*



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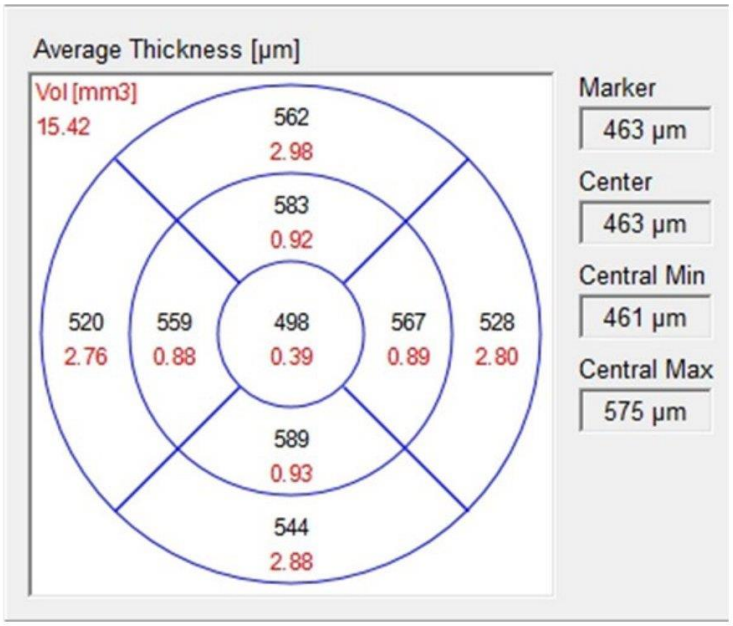

(**C**)

Figure 1. (**A**) Retinal layer segmentation and marking of the choroidal–scleral junction. (**B**) An ETDRS macular map showing retinal thickness (ILM-BM) and volume in 10 subfields. (**C**) An ETDRS macular map showing total thickness (ILM-CSJ) and volume in 10 subfields. Abbreviations: ILM, internal limiting membrane; OPL, outer plexiform layer; ELM, external limiting membrane; BM, Bruch's membrane; CSJ, choroidal–scleral junction; ONL, outer nuclear layer; ORL, outer retinal layer; ETDRS, Early Treatment Diabetic Retinopathy Study.

Based on ETDRS macular maps the values of choroidal parameters were obtained by subtracting retinal parameters (calculated automatically from the ILM to the BM, Figure 1A,B) from total parameters (calculated automatically from the ILM to the manually marked choroidal–scleral junction, Figure 1C). The fovea was checked and, if necessary, manually replotted. The outer retina was defined as the ONL and the ORL sum. The ONL and the ORL were defined according to the Heidelberg Spectralis HRA-OCT software, with the ONL as an area between the outer border of the OPL and the ELM and the outer retinal layer (ORL) as an area between the ELM and the BM (Figure 1A). The choroid was defined as an area between the BM and the choroidal–scleral junction.

The values for the central 1 mm ring (within a 500 µm radius from the center of the macula) were extracted from the ETDRS macular map. Average central macular thickness at the ONL and ORL was calculated by the OCT software. Choroidal central macular thickness was calculated by subtraction, as described above.

2.2. Binarization of Subfoveal Choroidal Images

Binarization and segmentation of the images were done with ImageJ software (http://imagej.nih.gov/ij, accessed on 5 May 2021, version 1.49, U. S. National Institutes of Health, Bethesda, MD, USA), using the protocol proposed by Sonoda [32,47]. Briefly, the area within a 500 µm distance nasally and temporally from the fovea was analyzed on the horizontal scan across the fovea using the polygon selection tool, with the BM as the upper margin and the choroidal–scleral junction as the lower margin. Luminal area (LA) and total choroidal area (TCA) were measured. Stromal area (SA) was calculated, and CVI was determined as the LA to TCA ratio [31,32] (Supplementary Figure S1A–D). The inter-grader reliability was measured by the absolute agreement model of the intraclass correlation coefficient (ICC). ICC values for choroidal parameters were greater than 0.8, which indicated good agreement. With Bland–Altman plot analyses, the fixed and proportional bias were excluded.

2.3. Fluorescein Angiograms Acquisition and Analysis

Fluorescein angiography was performed with a Spectralis HRA + OCT imaging device (Heidelberg Engineering, Heidelberg, Germany) according to the standard procedure. The images were used to assess the severity of DR according to the ETDRS criteria [48,49].

2.4. Statistical Analysis

Statistical analyses were carried out with R software (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics included numbers (percentages within each group) for nominal variables and means ± standard deviations (SD) for continuous variables. The normality of the distribution was verified with the Shapiro–Wilk test, on the basis of skewness and kurtosis values, as well as based on visual inspection of histograms. Between-group comparisons were carried out with a chi-square test or Fisher exact test for nominal variables and one-way ANOVA for continuous variables, whichever appropriate. Whenever the result of the ANOVA was statistically significant, Tukey post hoc test was applied. The relationships between pairs of continuous variables were verified based on Spearman's correlation analysis. Linear univariate and multivariate regression analysis was carried out to assess relationship between choroidopathy and outer retinal thickness. Multivariate models were based on stepwise approach with AIC criterium, and independent variables with $p < 0.157$ in univariate analysis were included into multivariate models [50]. Both univariate and multivariate models were adjusted for covariates: age, sex, DR severity, and PRP. All tests were two-tailed with $\alpha = 0.05$.

3. Results

3.1. Baseline Characteristics

The study included a total of 191 patients (286 eyes). Patients with DR with concomitant DME (DR + DME+) or without (DR + DME−) and the controls did not differ

significantly in terms of age, sex, and spherical equivalent. Baseline characteristics of both groups of patients with DR and the controls are presented in Table 1.

Table 1. Baseline characteristics of patients with DR with concomitant DME or without and healthy controls.

	Overall	Group			*p*
		DR + DME+	DR + DME−	Controls	
Number of patients	191	49	90	52	
Number of eyes	286	76	134	76	
Age, years, mean ± SD	59.24 ± 14.43	61.50 ± 11.92	60.03 ± 13.19	55.73 ± 17.87	0.103
Sex, female, *n* (%)	100 (52.4)	25 (51.0)	47 (52.2)	28 (53.8)	0.960
Spherical equivalent, mean ± SD	0.32 ± 1.10	0.43 ± 1.07	0.23 ± 0.99	0.39 ± 1.28	0.371
DR severity, *n* (%)					
NPDR	135 (47.2)	50 (65.8)	85 (63.4)	-	0.766 [1]
PDR	75 (26.2)	26 (34.2)	49 (36.6)	-	
PRP, *n* (%)					
No	138 (48.3)	48 (63.2)	90 (67.2)	-	0.650 [1]
Yes	72 (25.2)	28 (36.8)	44 (32.8)	-	

Notes: Groups compared with chi-square test or Fisher exact test [1] for nominal variables and with ANOVA for continuous variables. DME defined as present (DME+) when central macular subfield retinal thickness ≥ 300 µm and absent (DME−) when central macular subfield retinal thickness < 300 µm. Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; PRP, panretinal photocoagulation.

3.2. Between-Group Comparison of Outer Retinal and Choroidal Parameters

Healthy controls presented with significantly higher choroidal thickness and CVI values than patients with DR (Table 2). However, the three groups did not differ significantly in terms of other choroidal parameters, i.e., luminal area (LA), stromal area (SA), and total choroidal area (TCA).

Table 2. Choroidal and outer retinal parameters in patients with DR with concomitant DME or without and healthy controls.

	DR + DME+	DR + DME−	Controls	*p*	Post Hoc		
					DR + DME+ vs. DR + DME−	DR + DME− vs. Controls	DR + DME+ vs. Controls
Choroidal parameters							
CVI	0.62 ± 0.05	0.63 ± 0.05	0.65 ± 0.05	0.001	0.576	0.089	**<0.001**
LA (mm^2)	0.24 ± 0.05	0.25 ± 0.08	0.25 ± 0.06	0.266			
SCA (mm^2)	0.15 ± 0.03	0.14 ± 0.04	0.14 ± 0.03	0.204			
TCA (mm^2)	0.39 ± 0.08	0.40 ± 0.01	0.39 ± 0.08	0.773			
Choroid (µm)	250.75 ± 45.61	265.34 ± 60.91	303.64 ± 72.77	<0.001	0.218	**<0.001**	**<0.001**
Central macular thickness (1 mm diameter)							
ORL (µm)	92.58 ± 24.10	84.20 ± 4.97	87.64 ± 3.52	<0.001	**<0.001**	0.157	0.052
ONL (µm)	141.70 ± 85.70	82.38 ± 18.03	92.13 ± 10.81	<0.001	**<0.001**	0.306	**<0.001**

Notes: Data presented as mean ± SD. Groups compared with ANOVA. In cases of statistically significant differences, Tukey post hoc test was applied. Significant *p* values in bold. Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; ORL, outer retinal layer; ONL, outer nuclear layer.

Compared with healthy controls, patients from the DR + DME− group presented with a lower thickness of both components of the outer retina, ORL and ONL. Meanwhile, the values of both these parameters in patients from the DR + DME+ group were significantly higher than in the control group.

3.3. Intra-Group Correlations and Regressions between Outer Retinal and Choroidal Parameters

We analyzed correlations between the outer retinal (ONL and ORL) and choroidal parameters (choroidal thickness, CVI, LA, SA, and TCA) within each of the three groups

(Table 3). No significant correlations between the choroidal and outer retinal parameters were found in the controls and DR + DME+ group. In DR + DME− group, however, ORL correlated positively with choroidal central macular thickness, CVI, and LA, and a positive correlation was found between ONL and CVI.

Table 3. Correlations between outer retinal and choroidal parameters in patients with DR with concomitant DME or without and healthy controls.

Correlation:	DR + DME+		DR + DME−		Controls	
	Rho	*p*	Rho	*p*	Rho	*p*
ORL central macular thickness (1 mm diameter)						
Choroidal central macular thickness (µm)	0.23	0.160	**0.34**	**0.003**	0.27	0.069
CVI	−0.01	0.953	**0.49**	**<0.001**	0.12	0.426
LA (mm^2)	0.23	0.171	**0.41**	**<0.001**	0.19	0.191
SA (mm^2)	0.17	0.314	0.12	0.293	0.05	0.719
TCA (mm^2)	0.18	0.285	0.32	0.005	0.17	0.242
ONL central macular thickness (1 mm diameter)						
Choroidal central macular thickness (µm)	−0.07	0.683	0.01	0.932	−0.27	0.058
CVI	0.35	0.031	**0.25**	**0.027**	0.14	0.336
LA (mm^2)	0.08	0.641	0.09	0.430	−0.02	0.871
SA (mm^2)	−0.22	0.191	−0.20	0.077	−0.16	0.289
TCA (mm^2)	−0.03	0.856	−0.01	0.981	−0.09	0.558

Notes: rho, Spearman's correlation coefficient. Only the results for a single eye from each patient were considered during the analysis, $n = 191$. Since the dataset included the results for a single eye from each patient, there was no violation of the independence assumption between observations for correlation analysis. Significant p values with rho values in bold. Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; ORL, outer retinal layer; ONL, outer nuclear layer.

Based on univariate regression analysis (Table 4), ORL central macular thickness (1-mm diameter) was associated with choroidal central macular thickness ($\beta = 0.03$, $p = 0.019$), CVI ($\beta = 43.70$, $p < 0.001$), LA ($\beta = 18.73$, $p = 0.004$), and TCA ($\beta = 10.20$, $p = 0.021$). Multivariate model for ORL indicated that CVI alone ($\beta = 43.70$, $p < 0.001$) was the best determinant of ORL with $R^2 = 0.26$ (R^2 adj. = 0.21). Inclusion of other parameters did not increase the quality of model. Low value of R^2 indicated presence of other factors additional to this analysis impacting the thickness of ORL.

Table 4. Univariate linear regression for ORL and ONL in patients with DR without concomitant DME.

	ORL Central Macular Thickness (1 mm Diameter)				ONL Central Macular Thickness (1 mm Diameter)			
	β	SE	*p*	R^2/R^2 adj.	β	SE	*p*	R^2/R^2 adj.
Choroidal central macular thickness (µm)	0.03	0.01	**0.019**	0.14/0.08	0.03	0.04	0.395	0.23/0.18
CVI	43.70	9.93	**<0.001**	0.26/0.21	67.03	36.32	0.069	0.26/0.21
LA (mm^2)	18.73	6.22	**0.004**	0.17/0.11	7.94	21.92	0.718	0.23/0.17
SA (mm^2)	12.29	12.54	0.330		−34.52	41.74	0.411	0.23/0.18
TCA (mm^2)	10.20	4.32	**0.021**	0.13/0.07	−0.70	14.91	0.963	0.23/0.17

Notes: β, beta estimate; SE, standard error; R^2 adj., adjusted R-squared. Only the results for a single eye from each patient were considered during the analysis, $n = 90$. All models were adjusted for covariates: age, sex, DR severity, and PRP. Significant p vales in bold. Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; CVI, choroidal vascularity index; LA, luminal area; SA, stromal area; TCA, total choroidal area; ORL, outer retinal layer; ONL, outer nuclear layer.

For ONL univariate regression analysis did not identify significant associations.

4. Discussion

As the demand of the outer retina for oxygen and nutrients is primarily covered by diffusion from the choroidal circulation [5], we decided to quantify outer retinal and choroidal parameters in patients with DR. Our study showed that patients with DR had the lower choroidal thickness and CVI than healthy controls. While the thickness of two components of the outer retina, ORL and ONL, in patients from the DR + DME− group was lower than in the controls, patients from the DR + DME+ group presented with higher values of these parameters than persons from the control group. Additionally, in patients from the DR + DME− group, ORL correlated positively with choroidal central macular thickness and LA; furthermore, both ORL and ONL correlated positively with CVI in this group of patients.

Choroidal thickness varies depending on multiple physiological and pathological factors, such as age, sex, refraction, axial length, time of the day, DME, DR severity and PRP, duration, and control of DM [31–35,40]. Our study groups did not differ significantly in terms of age, sex, spherical equivalent, DR severity, and PRP rate, and hence, a confounding effect of these variables was unlikely. CVI is a more independent measure [31,36] and therefore was considered a primary variable analyzed in this study.

In our study, healthy controls presented with significantly higher choroidal thickness than patients with DR, whether with concomitant DME or without. Although published data in this matter are inconclusive, our findings are in line with most previous studies [6,17,34,37–39]. Wang et al. observed increased choroidal thickness at the early stages of DR, followed by a decrease in this parameter with DR progression; meanwhile, DME was not significantly associated with choroidal thickness. Those findings suggest that alterations in choroidal parameters may play a role in the pathogenesis of DR [51]. In our study, the presence of DR was associated with a substantial decrease in CVI, with a statistically significant difference in this parameter observed between the DR + DME+ group and the controls; this observation is consistent with the results of previous studies [40–43]. However, we found no significant between-group differences in other choroidal parameters, LA, SA, and TCA. In our previous study, which centered around the choroid but not the outer retina, patients with various types of DME (cystoid, diffuse, with subretinal fluid) presented with lower CVI and choroidal thickness than the controls [46].

Both components of the outer retina, ORL and ONL, were thinner in the DR + DME− group and thicker in the DR + DME+ group when compared with healthy controls. Our findings are consistent with the results published by Sim et al., who reported outer retinal thinning in eyes with diabetic macular ischemia without macular edema and found outer retinal thickening in eyes with macular edema [12]. According to those authors, the ischemic thinning might be masked by coexisting edema. Our findings seem to support this hypothesis. We found significant differences in ORL and ONL thickness in patients with DR with concomitant DME and without. Thinning of the outer retina reflects neurodegeneration, which might be a reason behind the reduced outer retinal thickness in the DR + DME− group. Reduced choroidal thickness associated with DR may lead to the hypoxia of retinal tissues, resultant impairment of outer BRB, development of DME, and further progression thereof [6]. At a molecular level, hyperglycemia is associated with the activation of various pathways, including polyol pathway, protein kinase C pathway, generation of advanced glycation end-products, inflammation, and oxidative stress [24,52]. Activation of those pathways affects retinal and choroidal vessels and neurons, RPE cells, and glial cells [26,53].

Our findings are partly in agreement with the results published by Wang et al., according to whom DR patients presented with significantly lower choroidal, ORL, and RPE thickness than the controls, without significant differences observed between patients with concomitant DME and without [17]. Damian et al. found thinner ONL and ORL in patients in whom diabetes coexisted with mild DR or lack thereof, without concomitant DME [54]. Other authors reported outer retinal atrophic changes in DME [13] with PROS (photoreceptor outer segment) shortening [13,44].

In the present study, ORL thickness in the DR + DME− group correlated significantly with choroidal central macular thickness, CVI, and LA; a significant correlation between ONL and CVI was found in this group as well. Multivariate model for ORL indicated that CVI alone was the best determinant of ORL. To the best of our knowledge, none of the previous studies documented such correlations in patients with DR and concomitant DME. Damian et al. analyzed correlations between choroidal and outer retinal parameters in diabetic patients with mild DR or lack thereof, without concomitant DME. They found significant correlations between CVI and RPE thickness as well as between choroidal thickness and photoreceptor layer thickness [54]. While the results of that study could be partially consistent with our findings in the DR + DME− group, the analyzed cohort differed considerably, as it included patients with mild or absent DR.

Consistently with previous reports [54,55], we found no significant correlations between choroidal parameters (CVI and choroidal thickness) and outer retinal parameters in healthy controls.

Similarly, no significant correlations between retinal and choroidal parameters were observed in the DR + DME+ group. Gerendas et al. analyzed total retinal thickness and choroidal thickness in patients with DME and also did not find a significant correlation between these parameters [56]. However, in our present study, we considered outer retinal thickness rather than total retinal thickness, and hence, our findings should not necessarily be directly compared with those reported by Gerendas et al. [56] The lack of correlation between outer retinal parameters and choroidal parameters in patients with DME might reflect complex pathogenesis of this condition. All cells maintain internal homeostasis due to the existence of membrane transport systems that control the inflow and outflow of ions from the cell. Many pathological conditions (e.g., ischemia) are not only associated with the disruption of the BRB but may also lead to the damage of membrane ionic channels and resultant cellular swelling. Thus, neuronal and/or glial swelling may often be a component of retinal edema; probably, this refers in particular to the areas of ischemic capillary loss where severe metabolic insult occurs, and no viable capillaries survive to generate extracellular fluid [57]. Consequently, different stages of macular edema, i.e., cytotoxic (intracellular) versus vasogenic (extracellular), need to be considered and analyzed separately [58]. Moreover, patients may differ in terms of the disease pathways involved. Finally, ischemia, neurodegeneration, and edema can occur independently from one another [29,59,60].

In summary, we cautiously hypothesize that the blood supply is more than sufficient in healthy controls even with a variable flow through the choroid. However, this is not the case in patients with DR, as shown by decreased choroidal thickness and lower CVI values. The choroidal atrophy may lead to ischemia as a predominant pathogenetic mechanism of DR at this stage. Hypoxia may, in turn, cause RPE and photoreceptor degeneration, with resultant thinning of the outer retina. Further stages of the disease, when the disruption of the BRB occurs, and DME develops, involve multiple and complex pathogenetic mechanisms, and as a result, the relationship between choroidal circulation and outer retinal thickness is not that evident.

This study has some strengths, among them the inclusion of DME treatment-naïve patients and the analysis of age-, sex-, and refractive error-matched groups. All measurements were taken between 8 a.m. and 11 a.m. to avoid diurnal variations. The study included a homogenous group consisting solely of patients with spherical equivalent refractive error < 3.0 diopters. Compared with previous studies, we analyzed a larger number of patients with proliferative DR, often underrepresented in clinical research. Notably, we considered multiple choroidal parameters rather than only thickness. Further, this is the first published study to analyze a correlation between CVI and outer retinal thickness in such a group of patients.

We are well-aware of the potential limitations of this study. Due to the retrospective design of the study, the information about the type of diabetes, laboratory values, and the time of the onset of DME was unavailable. It also needs to be stressed that CVI was

determined based on a 1 mm single foveal scan; this is a relatively common practice given that CVI is similar across all the ETDRS subfields [35]. Future advances in automated three-dimensional CVI evaluation may address this limitation. Furthermore, we focused solely on anatomical parameters; meanwhile, the analysis of correlations between functional parameters seems to be an interesting direction for future research. Additionally, we analyzed only a single mechanism involved in DR/DME development, i.e., choroidopathy, whereas the pathogenesis of these conditions is complex. We did not consider a recently discovered role of previously underappreciated, deep capillary plexus in satisfying the metabolic requirements of the outer retina [2]. Future studies may address this limitation by introducing OCT angiography in the study design. Unfortunately, the analysis of OCT angiographic images in patients with DME still constitutes a problem nowadays.

5. Conclusions

In conclusion, the presence of DR with concomitant DME or without is associated with changes in choroidal and outer retinal parameters. However, a significant correlation between outer retinal parameters and choroidal parameters was found only in the DR + DME− group, and no link between these parameters was observed in healthy controls and patients from the DR + DME+ group.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11133882/s1, Figure S1. Image binarization of the choroid and calculation of choroidal vascularity index (CVI).

Author Contributions: P.S. and D.A.D. worked on the conception, study design, data analysis, and main text; P.S. worked on execution, acquisition of data, and figures and tables; I.O. and J.K. reviewed the whole article. All authors are accountable for the accuracy of the contents, and will ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and the resolution documented in the literature. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All the materials and information will be available upon an e-mail request to the corresponding author. Names and exact data of the participants of the study may not be available owing to patient confidentiality and privacy policy.

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Supporting information to **"Correlation between choroidal vascularity index and outer retina in patients with diabetic retinopathy"**

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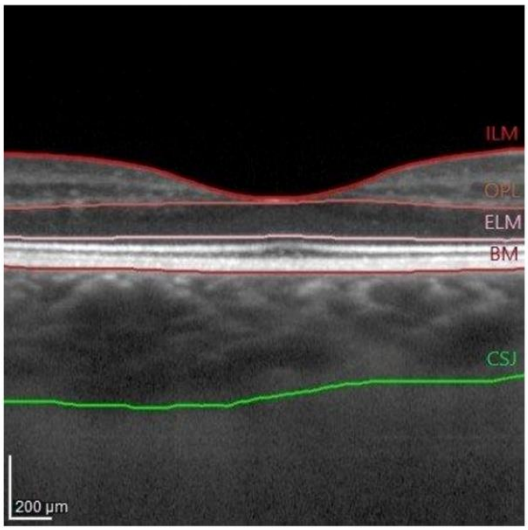
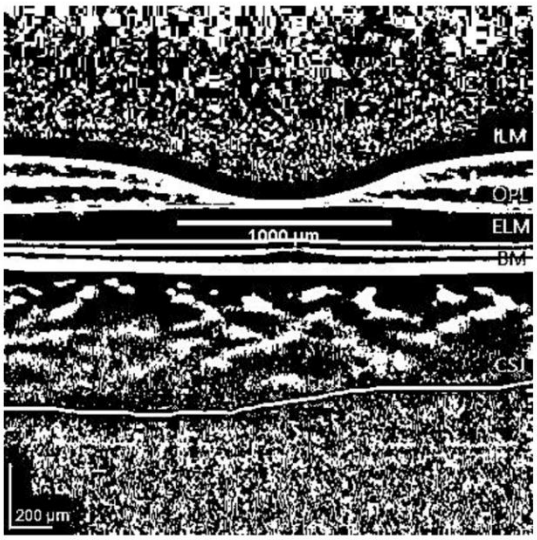
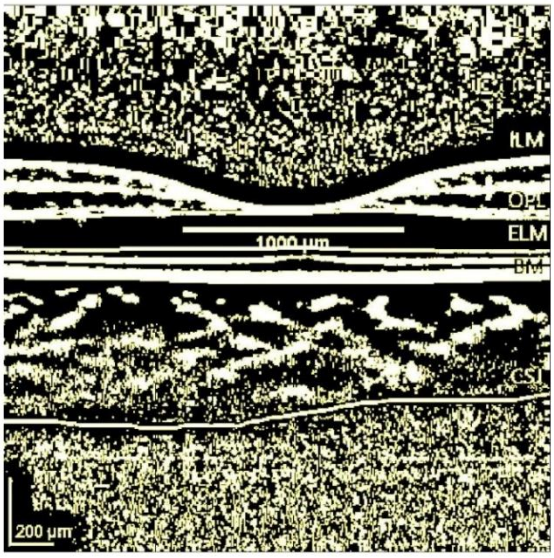
Supplementary Figure S1. Image binarization of the choroid and calculation of choroidal vascularity index (CVI).



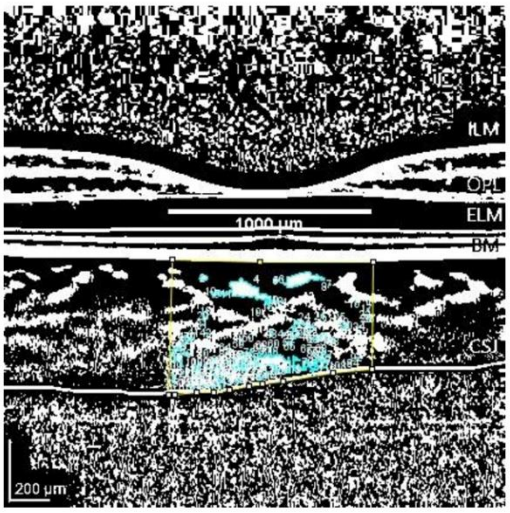
(A) The examined area was determined by Bruch's Membrane (BM, purple line) and choroidoscleral junction (CSJ, green line).



(B) The horizontal macular 1x1µm scan was uploaded in ImageJ software (http://imagej.nih.gov/ij, accessed on May 5, 2021, version1.49) and the image scale was set. Then, the image was converted to 8-bit. A line tool was used to draw the 1000µm line to determine the area beneath the fovea. The image was adjusted by the Niblack auto local threshold tool. The light pixels represented the stromal area (SA) and the black pixels and the dark pixels represented the luminal area (LA).



(C) The binarized image was reconverted to an RGB (red, green, blue) image, and the LA was highlighted using the threshold tool.



(D) The polygon selection tool was used to mark the 1000µm wide subfoveal region of interest (ROI) within the choroid (from BM to CSJ) termed the total choroidal area (TCA). It was selected and added to the ROI manager. The LA was automatically calculated by the ImageJ software. The stromal area (SA) was obtained by subtracting the luminal area (LA) from the TCA. The choroidal vascularity index (CVI) was calculated as the proportion of LA to TCA.

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Białystok, 28.05.2020 r.

Uchwała nr: APK.002.216.2020

Komisja Bioetyczna przy Uniwersytecie Medycznym w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **wyraża zgodę** na prowadzenie tematu badawczego: „Ocena naczyniówki i siatkówki u pacjentów z cukrzycą" przez dr n. med. Dianę A. Dmuchowską wraz z zespołem badawczym z UMB.

Przewodnicząca Komisji Bioetycznej przy UMB

prof. dr hab. Otylia Kowal-Bielecka

Zaświadcza się, że lek. Patryk Sidorczuk jest członkiem zespołu badawczego w/w tematu badawczego.

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Rozdział 6. Streszczenie

Optyczna koherentna tomografia (*optical coherence tomography OCT*) zrewolucjonizowała diagnostykę w okulistyce. Oprócz dotychczas ocenianej grubości naczyniówki, w 2016 roku powstał nowy parametr szczegółowo charakteryzujący naczyniówkę. Naczyniówkowy wskaźnik naczyniowy (*choroidal vascularity index CVI*) odzwierciedla stosunek komponenty naczyniowej do jej całej powierzchni.

Celem badań było pogłębienie wiedzy z zakresu jednego z elementów patofizjologii oka w przebiegu cukrzycy, mianowicie związku choroidopatii i retinopatii. Stwierdzenie i scharakteryzowanie takiej zależności mogłoby znaleźć praktyczne zastosowanie we wczesnej diagnostyce okulistycznej oraz zindywidualizowanej kwalifikacji pacjentów do najbardziej odpowiednich metod terapeutycznych. Aktualnie nie jest jednoznaczne czy choroidopatia poprzedza, towarzyszy czy następuje po rozwoju zmian siatkówkowych oraz czy jest od nich zależna.

Były to jednoośrodkowe retrospektywne badanie przekrojowe.

Naczyniówka zapewnia unaczynienie zewnętrznej siatkówki, stąd w pierwszej pracy ocenie poddaliśmy zależność parametrów naczyniówkowych (grubości i CVI) i zewnętrznych warstw siatkówki u pacjentów z retinopatią cukrzycową *(diabetic retinopathy DR*) z lub bez cukrzycowego obrzęku plamki *(diabetic macular edema DME*) oraz w grupie porównawczej. W drugiej pracy oceniliśmy potencjalny związek między stopniem uszkodzenia strefy beznaczyniowej dołka *(foveal avascular zone FAZ*) a parametrami naczyniówkowymi (grubością, objętością i CVI) u pacjentów z DR.

Ocenie parametrów zewnętrznej siatkówki i naczyniówki poddaliśmy 210 oczu u 139 pacjentów z DR. Grupę porównawczą stanowiło 76 oczu u 52 zdrowych osób. FAZ oceniliśmy w 210 oczu u 152 pacjentów z DR. OCT oraz angiografię fluoresceinową (AF) wykonano za pomocą Spectralis HRA + OCT (Heidelberg Engineering, Heidelberg, Niemcy). Angiografia fluoresceinowa została wykorzystana do oceny zaawansowania DR, wykrycia klinicznie znamiennego obrzęku plamki *(clinically significant macular edema CSME*) i scharakteryzowania FAZ zgodnie z kryteriami ETDRS. Oceniliśmy wielkość i zarys FAZ.

Pacjenci z grupy kontrolnej mieli znacznie grubszą naczyniówkę i wartości CVI niż pacjenci z DR z/bez DME. W porównaniu z grupą porównawczą pacjenci z DR+DME- mieli mniejszą grubość obu składowych zewnętrznej

siatkówki: warstwy siatkówkowej zewnętrznej (*outer retinal layer ORL*) i warstwy jądrzastej zewnętrznej (*outer nuclear layer ONL)*. Tymczasem wartości obu tych parametrów u pacjentów z grupy DR+DME+ były znamienne wyższe niż w grupie kontrolnej. Znaczące korelacje między parametrami zewnętrznej siatkówki i naczyniówki stwierdziliśmy wyłącznie w grupie DR+DME-. Grubość ORL korelowała dodatnio z podplamkową grubością naczyniówki i CVI. Grubość ONL korelowała dodatnio z CVI. Korelacji takich nie wykazaliśmy w grupie porównawczej i u pacjentów z DR+DME+.

W pracy oceniającej zależność pomiędzy parametrami naczyniówki a FAZ stwierdziliśmy, że analizowane grupy (≤2 i ≥3 stopnia uszkodzenia zarysu FAZ wg ETDRS) nie różniły się grubością naczyniówki, objętością i innymi parametrami naczyniówki. Różnic takich nie stwierdziliśmy również przy podziale na grupy wg wielkości FAZ z medianą 0,355 mm^2 jako poziomem odcięcia. Podobnie nie stwierdziliśmy istotnych różnic między grupami, kiedy w modelu uwzględniliśmy również czynniki zakłócające, takie jak: płeć, wiek, CSME, zaawansowanie DR i uprzednią panfotokoagulację siatkówki. Powierzchnia FAZ nie korelowała istotnie z grubością, objętością i innymi parametrami naczyniówki. Biorąc pod uwagę istotną różnicę w obszarze FAZ u pacjentów z CSME i bez, przeprowadziliśmy również analizę podgrup. Analiza nie wykazała istotnych korelacji między obszarem FAZ a parametrami naczyniówkowymi innymi niż CVI u pacjentów z CSME. Stąd generalnie u pacjentów z DR, wielkość i zarys FAZ nie korelowały z parametrami naczyniówki (grubością i objętością w poszczególnych polach ETDRS oraz z CVI).

Podsumowując, wykazaliśmy iż obecności retinopatii cukrzycowej towarzyszą zmiany parametrów naczyniówki i zewnętrznych warstw siatkówki oraz FAZ. Stwierdzenie korelacji parametrów zewnętrznej siatkówki i naczyniówki wyłącznie w grupie DR+DME- sugeruje bardziej złożony patomechanizm zmian w obrębie zewnętrznej siatkówki u pacjentów z DR+DME+ z wpływem dodatkowych czynników. Nie stwierdziliśmy związku między uszkodzeniem naczyń siatkówki i naczyniówki w obrębie plamki u pacjentów z DR. W konsekwencji, te dwa procesy wydają się być równoległe, ale niezależne.

Rozdział 7. Summary

Optical coherence tomography (OCT) has revolutionised the diagnostics process in ophthalmology. In addition to the previously assessed choroidal thickness, a novel parameter was developed in 2016 characterising the choroid in detail. The choroidal vascularity index (CVI) is defined as the ratio of the choroidal luminal area to the total choroidal area.

The aim of the study was to broaden our knowledge of one element of eye pathophysiology in diabetes, namely the relationship between choroidopathy and retinopathy. Having found and characterised such a relationship, we could apply it in the ophthalmological diagnostic process and the individualised qualification of patients for the most appropriate therapeutic approaches. Currently, it is not clear whether choroidopathy precedes, accompanies or follows the development of retinal changes, and whether it is dependent on them.

It was a single-centre retrospective cross-sectional study.

The choroid provides blood supply of the outer retinal layer, thus in the first study we assessed the relationship between choroidal parameters (thickness and CVI) and outer retinal layers in patients with diabetic retinopathy *(DR*), with or without diabetic macular oedema *(DME*), and in the control group. In the second study, we assessed the possible relationship between the degree of damage to the foveal avascular zone *(FAZ*) and choroidal parameters (thickness, volume and CVI) in patients with DR.

We assessed the outer retinal and choroidal parameters of 210 eyes in 139 DR patients. The control group consisted of 76 eyes in 52 healthy subjects. We assessed FAZ in 210 eyes in 152 patients with DR. OCT and fluorescein angiography (AF) were performed with Spectralis HRA + OCT (Heidelberg Engineering, Heidelberg, Germany). Fluorescein angiography was used to assess DR progression, detect clinically significant macular oedema (CSME) and characterise FAZ according to ETDRS criteria. We assessed the FAZ size and outline.

Patients in the control group had significantly thicker choroid and CVI values than patients with DR with/without DME. In comparison to the control group, DR+DME- patients had a lower thickness for both components of the outer retina: the outer retinal layer (ORL) and the outer nuclear layer (ONL). The values of both these parameters in the DR+DME+ patients were significantly

higher than in the control group. Significant correlations between outer retinal and choroidal parameters were found only in the DR+DME- group. ORL thickness correlated positively with the subfoveal choroidal thickness and CVI. ONL thickness correlated positively with CVI. Such correlations were not demonstrated in the control group or in the DR+DME+ patients.

In the study evaluating the relationships between choroidal parameters and FAZ, we found that the analysed groups (≤2 and ≥3 grade of disruption of the FAZ outline according to ETDRS) did not differ in terms of choroidal thickness, volume or other choroidal parameters. We also found no such differences after dividing the groups according to the FAZ size, with a median of 0.355 mm^2 as the cut-off level. Similarly, we found no significant differences between the groups after including confounders in the model, such as gender, age, CSME, DR progression and previous retinal panretinal photocoagulation. The FAZ area did not correlate significantly with choroidal thickness, volume or other choroidal parameters. Given the significant difference in the FAZ area in the CSME and non-CSME patients, we also performed a subgroup analysis. The analysis showed no significant correlation between the FAZ area and non-CVI choroidal parameters in CSME patients. In general, the FAZ size and outline in DR patients did not correlate with the choroidal parameters (thickness and volume of individual ETDRS fields and with CVI).

In conclusion, we proved that the presence of diabetic retinopathy is accompanied by changes in the choroidal parameters as well as the outer retinal layers and FAZ. The correlation of outer retinal and choroidal parameters only in the DR+DME- group suggests a more complex pathomechanism of the outer retinal changes in the DR+DME+ patients with the influence of additional factors. We did not find a relationship between retinal and choroidal vascular damage within the macula in the DR patients. Consequently, the two processes appear to be parallel but independent.

Rozdział 8. Piśmiennictwo

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Rozdział 9. Oświadczenia współautorów

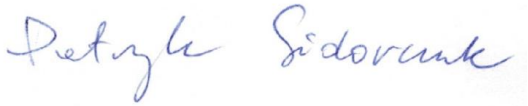
Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu (praca oryginalna)

Sidorczuk, P.; Obuchowska, I.; Konopinska, J.; Dmuchowska, D.A. Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy. *J. Clin. Med.* 2022, *11*, 3882. https://doi.org/10.3390/jcm11133882
IF 4.964; MEiN 140

Imię i nazwisko współautora	*Charakter udziału*	*Procentowy wkład*
doktorant – lek. Patryk Jan Sidorczuk	Koncepcja badania, współudział w zaprojektowaniu planu badań, przeprowadzenie części badań, kwerenda literatury, analiza i interpretacja wyników, przygotowanie i edycja manuskryptu, przygotowanie tabel i rycin wchodzących w skład manuskryptu, autor korespondencyjny.	50%
Dr hab. n. med. Iwona Obuchowska	Recenzja i edycja manuskryptu	5%
Dr hab. n. med. Joanna Konopińska	Recenzja i edycja manuskryptu	5%
Dr hab. n. med. Diana Anna Dmuchowska	Koncepcja badania, współudział w zaprojektowaniu planu badań, przeprowadzenie części badań, kwerenda literatury, analiza i interpretacja wyników, przygotowanie i edycja manuskryptu, autor korespondencyjny.	40%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Patryka Jana Sidorczuka

Podpis doktoranta



Białystok dn. 26.06.2023r

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy autorów Sidorczuk, P.; Obuchowska, I.; Konopinska, J.; Dmuchowska, D.A. opublikowanej w Journal of Clinical Medicine *2022, 11, 3882.*, wchodzącej w skład rozprawy doktorskiej: *Ocena parametrów siatkówki i naczyniówki z uwzględnieniem naczyniówkowego wskaźnika naczyniowego u pacjentów z cukrzycą* wynoszący 5% to recenzja i edycja manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez Patryka Jana Sidorczuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

ADIUNKT KLINIKI OKULISTYKI
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[Podpis]

Dr hab. n. med. Iwona Obuchowska

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Dr hab. n. med. Joanna Konopińska
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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy autorów Sidorczuk, P.; Obuchowska, I.; Konopinska, J.; Dmuchowska, D.A. opublikowanej w Journal of Clinical Medicine *2022, 11, 3882.*, wchodzącej w skład rozprawy doktorskiej: *Ocena parametrów siatkówki i naczyniówki z uwzględnieniem naczyniówkowego wskaźnika naczyniowego u pacjentów z cukrzycą* wynoszący 5% to recenzja i edycja manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez Patryka Jana Sidorczuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

Correlation between Choroidal Vascularity Index and Outer Retina in Patients with Diabetic Retinopathy autorów Sidorczuk, P.; Obuchowska, I.; Konopinska, J.; Dmuchowska, D.A. opublikowanej w Journal of Clinical Medicine *2022, 11, 3882.*, wchodzącej w skład rozprawy doktorskiej: *Ocena parametrów siatkówki i naczyniówki z uwzględnieniem naczyniówkowego wskaźnika naczyniowego u pacjentów z cukrzycą* wynoszący 40% to koncepcja badania, współudział w zaprojektowaniu planu badań, przeprowadzenie części badań, kwerenda literatury, analiza i interpretacja wyników, przygotowanie i edycja manuskryptu, autor korespondencyjny.

Jednocześnie wyrażam zgodę na wykorzystanie przez Patryka Jana Sidorczuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Diana Anna Dmuchowska [Podpis]

Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu

(praca oryginalna)

Sidorczuk P, Pieklarz B, Konopinska J, Saeed E, Mariak Z, Dmuchowska D. Foveal Avascular Zone Does Not Correspond to Choroidal Characteristics in Patients with Diabetic Retinopathy: A Single-Center Cross-Sectional Analysis. Diabetes Metab Syndr Obes. 2021 Jun 28;14:2893-2903. doi: 10.2147/DMSO.S318860. PMID: 34234487; PMCID: PMC8254029. IF 3.249; MEiN 100

Imię i nazwisko współautora	*Charakter udziału*	*Procentowy wkład*
doktorant – lek. Patryk Jan Sidorczuk	Koncepcja badania, współudział w zaprojektowaniu planu badań, wykonanie części badań, prowadzenie bazy danych, kwerenda literatury, analiza i interpretacja wyników, przygotowanie i edycja manuskryptu.	50%
Lek. Barbara Pieklarz	Recenzja i edycja manuskryptu.	1%
Dr hab. n. med. Joanna Konopińska	Recenzja i edycja manuskryptu.	1%
Dr n. med. Emil Saeed	Recenzja i edycja manuskryptu.	1%
Prof. dr hab. n. med. Zofia Mariak	Recenzja i edycja manuskryptu, konsultacja merytoryczna.	2%
Dr hab. n. med. Diana Anna Dmuchowska	Koncepcja badania, współudział w zaprojektowaniu planu badań, przeprowadzenie części badań, kwerenda literatury, analiza i interpretacja wyników, przygotowanie i edycja manuskryptu, autor korespondencyjny.	45%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Patryka Jana Sidorczuk

Podpis doktoranta

Patryk Sidorczuk

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

Foveal avascular zone does not correspond to choroidal characteristics in patients with diabetic retinopathy: a single-center cross-sectional analysis autorów Sidorczuk P, Pieklarz B, Konopinska J, Saeed E, Mariak Z, Dmuchowska D, opublikowanej w Diabetes, Metabolic Syndrome and Obesity 2021, 14:2893-2903, wchodzącej w skład rozprawy doktorskiej: *Ocena parametrów siatkówki i naczyniówki z uwzględnieniem naczyniówkowego wskaźnika naczyniowego u pacjentów z cukrzycą* wynoszący 1% to: recenzja i edycja manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez Patryka Jana Sidorczuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Barbara Pieklarz

[Podpis]

Białystok dn. 26.06.2023r

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

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[Podpis]

Joanna Konopińska

Białystok dn. 26.06.2023r

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15-089 Białystok

Oświadczenie

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Emil Saeed
[Podpis]

Białystok dn. 26.06.2023r

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15-089 Białystok

Oświadczenie

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Jednocześnie wyrażam zgodę na wykorzystanie przez Patryka Jana Sidorczuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

[Podpis]

Białystok dn. 26.06.2023r

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Uniwersytet Medyczny w Białymstoku
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15-089 Białystok

Oświadczenie

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Jednocześnie wyrażam zgodę na wykorzystanie przez Patryka Jana Sidorczuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Diana Anna Dmuchowska
[Podpis]