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Porównanie gęstości naczyń siatkówki oraz grubości warstw siatkówki za pomocą optycznej koherentnej tomografii u pacjentów z chorobą Alzheimera oraz z jaskrą pierwotnie otwartego kąta

Rozprawa na stopień doktora nauk medycznych

Promotor:

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Składam serdeczne podziękowania mojemu promotorowi

Panu prof. dr. hab. Jakubowi Kałużnemu

za cenne rady, zaangażowanie, opiekę naukową

i życzliwość podczas realizacji niniejszej pracy doktorskiej.

Spis treści

Wykaz skrótów

AD – choroba Alzheimera

AUROC (area under the receiver operating characteristic curve) – pole powierzchni pod wykresem krzywej

Aβ – β-amyloid

DSM-IV – Diagnostic and Statistical Manual of Mental Disorders

DVP (deep vascular plexus) – splot naczyniowy głęboki

FAZ (foveal avascular zone) – dołkowa strefa beznaczyniowa

GCL (ganglion cell layer) – warstwa komórek zwojowych

HC (health controls) – zdrowa grupa kontrolna

ILM (internal limiting membranę) – błona graniczna wewnętrzna

INL (inner nuclear layer) – warstwa jądrzasta wewnętrzna

IPL (inner plexiform layer) – warstwa splotowata wewnętrzna

IRL (inner retinal layer) – warstwy wewnętrzne siatkówki

MMSE (Mini-Mental State Examination) – test Mini-Mental

NIA/AA – National Institute on Aging and the Alzheimer's Association

OCT (optical coherence tomography) – optyczna koherentna tomografia

OCTA (optical coherence tomography angiography) – angiografia optycznej koherentnej tomografii

OPL (outer plexiform layer) –warstwa splotowata zewnętrzna

ORL (outer retinal layer) – warstwy zewnętrzne siatkówki

OUN – ośrodkowy układ nerwowy

PET – pozytonowa tomografia emisyjna

POAG (primary open-angle glaucoma) – jaskra pierwotnie otwartego kąta

PPG (preperimetric glaucoma) – jaskra preperymetryczna

pRNFL (peripapillary retinal nerve fiber layer) – okołotarczowa warstwa włókien nerwowych siatkówki

RGC (retinal ganglion cell) – komórki zwojowe siatkówki

RPC (radial peripapillary capillaries) – warstwa radialna okołotarczowych kapilar

RPE (retinal pigment epithelium) – nabłonek barwnikowy siatkówki

SVP (superficial vascular plexus) – splot naczyniowy powierzchowny

VEGF (vascular endothelial growth factor) – naczyniowo-śródbłonkowy czynnik wzrostu

Nota informacyjna i wykaz publikacji stanowiących rozprawę doktorską

Zgodnie z warunkami ubiegania się o stopień naukowy doktora nauk medycznych, które zostały wyszczególnione w uchwale nr 89 Senatu Uniwersytetu Mikołaja Kopernika w Toruniu z dnia 25 czerwca 2019 roku: „w sprawie postępowania o nadanie stopnia doktora na Uniwersytecie Mikołaja Kopernika w Toruniu", niniejsza rozprawa doktorska: „Porównanie gęstości naczyń siatkówki oraz grubości warstw siatkówki za pomocą optycznej koherentnej tomografii u pacjentów z chorobą Alzheimera oraz z jaskrą pierwotnie otwartego kąta" ma formę spójnego tematycznie zbioru czterech artykułów opublikowanych w czasopismach naukowych wymienionych w części A listy czasopism punktowanych Ministerstwa Nauki i Szkolnictwa Wyższego. Doktorant jest pierwszym autorem każdej z czterech publikacji włączonych do cyklu. Trzy prace są pracami oryginalnymi a jedna jest pracą przeglądową. Łączna wartość współczynnika Impact Factor dla cyklu wynosi 8,628, a łączna wartość punktów ministerialnych według czasopism punktowanych Ministerstwa Nauki i Szkolnictwa Wyższego wynosi 350 punktów.

Lista prac:

1. Diagnosis of Alzheimer's Disease by Assessing Structural and Microvasculature Changes in the Retina Using Optical Coherence Tomography Angiography – a Review of Eye Biomarkers for Alzheimer's Disease.
   Klinika Oczna/Acta Ophthalmologica Polonica, 2020, *121*(4), 238-246.
   Zabel Przemysław, Kałużny J. Jakub, Zabel Katarzyna, Gębska-Tołoczko Martyna, Ołownia Klaudia, Wiłkość-Dębczyńska Monika.
   MNiSW = 40 pkt.
2. Peripapillary retinal nerve fiber layer thickness in patients with Alzheimer's disease: a comparison of eyes of patients with Alzheimer's disease, primary open-angle glaucoma, and preperimetric glaucoma and healthy controls.

Medical Science Monitor: international medical journal of experimental and clinical research, 2019, *25*, 1001.

Zabel Przemysław, Kałużny J. Jakub, Wiłkość-Dębczyńska Monika, Gębska-Tołoczko Martyna, Suwała Karolina, Kucharski Robert, Araszkiewicz Aleksander.

MNiSW = 70 pkt; IF= 1.918

3. Comparison of retinal microvasculature in patients with Alzheimer's disease and primary open-angle glaucoma by optical coherence tomography angiography.

   Investigative Ophthalmology & Visual Science, 2019, *60* (10), 3447-3455.

   Zabel Przemysław, Kałużny J. Jakub, Wiłkość-Dębczyńska Monika, Gębska-Tołoczko Martyna, Suwała Karolina, Zabel Katarzyna, Kucharski Robert, Araszkiewicz Aleksander.

   MNiSW = 140 pkt; IF= 3.470

4. Quantitative assessment of retinal thickness and vessel density using optical coherence tomography angiography in patients with Alzheimer's disease and glaucoma.

   Plos One, 2021, *16* (3), e0248284.

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   MNiSW= 100 pkt; IF= 3.240

Wstęp

Choroba Alzheimera (AD) stanowi najczęstszą przyczynę otępienia u osób starszych. Szacuje się, że na świecie cierpi na nią około 36 milionów ludzi. Istotą choroby jest apoptoza komórek nerwowych i utrata połączeń między neuronami. Proces neurodegeneracyjny rozpoczyna się lata wcześniej, zanim choroba zostanie w pełni wyrażona klinicznie. Główną przyczyną powstania i progresji tego schorzenia jest gromadzenie się nieprawidłowych białek – zewnątrzkomórkowego β-amyloidu (Aβ) oraz tworzącego wewnątrzkomórkowe sploty neurofibrylarne białka tau. Diagnostyka opiera się głównie na ocenie funkcji kognitywnych, a ponieważ badania neuroobrazowe nadal są bardzo drogie i trudno dostępne, dlatego poszukiwanie nowych, nieinwazyjnych oraz tanich biomarkerów stanowi obiecujący obszar badań.

Od lat 90. XX wieku, gdy wprowadzano do użytku optyczną koherentną tomografię (ang. optical coherence tomography – OCT), pomiar grubości okołotarczowej warstwy włókien nerwowych siatkówki (ang. peripapillary retinal nerve fiber layer – pRNFL) stał się parametrem powszechnie stosowanym w diagnostyce oraz monitorowaniu schorzeń siatkówki, nerwu wzrokowego (n. II), a w szczególności jaskry. Jaskra należy do neurodegeneracyjnych chorób oczu, której cechą jest apoptoza komórek zwojowych siatkówki (ang. retinal ganglion cell – RGC) i związany z nią zanik RNFL prowadzący do typowych dla tej choroby zmian w wyglądzie tarczy n. II oraz ubytków w polu widzenia. Zauważono, że uszkodzenie RGC skutkujące obniżeniem grubości pRNFL oraz warstwy komórek zwojowych siatkówki (ang. ganglion cell layer – GCL) można również zaobserwować w innych chorobach neurodegeneracyjnych, takich jak: choroba Parkinsona, stwardnienie rozsiane, otępienie z ciałami Lewy'ego oraz przede wszystkim w AD. Rozszerzenie techniki obrazowania OCT stanowi angiografia oparta na optycznej koherentnej tomografii (OCTA), dająca dodatkowe możliwości w postaci nieinwazyjnej oceny ilościowej i jakościowej stanu mikronaczyń w obrębie plamki i tarczy n. II. Pośmiertne badania pacjentów z AD wykazały, że

apoptozie komórek nerwowych w ośrodkowym układzie nerwowym (OUN) towarzyszą naczyniowe zmiany w postaci angiopatii amyloidowej. Patologiczne zmiany związane z AD dotyczą nie tylko uszkodzenia komórek nerwowych w OUN, ale także układu naczyniowego, dlatego zastosowanie nieinwazyjnej techniki OCTA do obrazowania naczyń wewnątrzgałkowych może stanowić nowy biomarker AD.

N. II, w skład którego wchodzą aksony RGC, nie ma swoistych cech nerwu obwodowego – zasadniczo jest pęczkiem istoty białej mózgowia otoczonej oponami mózgowo-rdzeniowymi. W fazie embriogenezy siatkówka oraz n. II rozwijają się jako bezpośrednie przedłużenie międzymózgowia, dlatego nieprawidłowości zachodzące w mózgu u pacjentów z AD możemy również obserwować na dnie oka. Uważa się, że uszkodzenie RGC w przypadku AD może mieć podobną patogenezę jak jaskra pierwotnie otwartego kąta (ang. primary open-angle glaucoma – POAG). W związku z tym coraz częściej podnosi się temat wspólnych czynników ryzyka oraz mediatorów odpowiedzialnych za ich powstanie i rozwój. McKinnon i wsp. w badaniach na modelu zwierzęcym zasugerowali, że przyczyną śmierci RGC u pacjentów z nadciśnieniem ocznym może być przewlekła neurotoksyczność spowodowana odkładaniem się $A\beta$ indukowanego wzrostem ciśnienia wewnątrzgałkowego oraz zmniejszony poziom naczyniowo-śródbłonkowego czynnika wzrostu (ang. vascular endothelial growth factor – VEGF), co na poziomie molekularnym przypomina AD. Yoneda i wsp. w swoich badaniach nad patogenezą jaskry wykazali znaczący spadek wolnego $A\beta$, co mogło być spowodowane odkładaniem się go w okolicach naczyń siatkówki, a także wzrost poziomu nieprawidłowego białka tau w ciele szklistym.

W 1986 roku Hinton i wsp. jako pierwsi opisali, że zmiany neurodegeneracyjne zachodzące w mózgu pacjentów z AD dotyczą również n. II oraz siatkówki. Zgodnie z wynikami badań pośmiertnych u pacjentów z AD, wykorzystując *in vivo* obrazowanie OCT, potwierdzono znaczny spadek grubości pRNFL, zaobserwowano również zmiany w grubości i objętości siatkówki w plamce, podobnie jak dzieje się to u pacjentów z neuropatią jaskrową. W 2001 roku jako pierwsi Parisi i wsp. przy użyciu Stratus OCT 3

wykazali istotne obniżenie grubości pRNFL w każdym z czterech ocenianych kwadrantów u pacjentów z AD w stosunku do osób zdrowych. W kolejnych latach badania *post mortem* pacjentów z demencją typu Alzheimera wykazały, że choroba powoduje także patologie naczyniowo-mózgowe. Pomimo wiedzy dotyczącej dysfunkcji małych naczyń mózgu, związanej z powstaniem i rozwojem otępienia, mikrokrążenie w OUN nadal pozostaje trudne do zbadania *in vivo*. Dzięki temu, że naczynia krwionośne mózgu i siatkówki mają wspólne pochodzenie embriologiczne i wykazują podobieństwo pod względem cech anatomicznych oraz właściwości fizjologicznych, badanie naczyń siatkówki może być cennym podejściem w dostarczaniu nowych informacji na temat AD. Zidentyfikowano potencjalny związek między odkładaniem się nieprawidłowego $A\beta$ w okolicach naczyń a zaburzeniem przepływu krwi oraz średnicą naczyń w siatkówce u pacjentów z rozpoznaną AD.

Wprowadzenie nowoczesnej i nieinwazyjnej techniki obrazowania mikrokrążenia za pomocą OCTA umożliwia nie tylko badanie jakościowe, lecz także pomiary ilościowe gęstości naczyń na różnych głębokościach siatkówki. Badania, w których wykorzystano OCTA do oceny mikrokrążenia u pacjentów z AD, wykazały, że gęstość naczyń siatkówki jest istotnie obniżona w porównaniu do zdrowej grupy kontrolnej. Bulut i wsp. jako pierwsi zastosowali technikę OCTA do oceny zmian naczyniowych wyłącznie w splocie powierzchownym siatkówki (ang. superficial vascular plexus – SVP) u pacjentów z AD. Stwierdzili, że gęstość naczyń w SVP była u nich mniejsza niż w grupie kontrolnej, co może być związane ze zredukowaną angiogenezą oraz osadzaniem się wewnątrz ścian naczyń krwionośnych złogów amyloidu, prowadząc do okluzji i zmniejszenia przepływu krwi. Kolejne doniesienia naukowe również potwierdziły, że u pacjentów z AD dochodzi do obniżenia gęstości naczyń w każdym splocie siatkówki. Podobne zmiany naczyniowe opisywano w mikrokrążeniu siatkówki u pacjentów z POAG. Do tej pory nie istniały badania porównujące AD z POAG pod względem unaczynienia siatkówki z wykorzystaniem OCTA, co skłoniło nas do pogłębienia wiedzy w tym temacie.

Aktualnie coraz większe nadzieje wiąże się z ocznymi biomarkerami AD. Najbardziej obiecujące wydają się zmiany strukturalnie w siatkówce oraz jej mikrokrążeniu, które mogą mieć bezpośredni związek z odkładaniem się Aβ. Nowe technologie, takie jak OCT oraz OCTA, przyczyniają się do postępu wiedzy w diagnostyce AD. Niestety zmiany strukturalne stwierdzane na dnie oka u pacjentów z AD, obserwowane za pomocą obrazów OCT, mogą być niespecyficzne i wspólne dla innych chorób neurodegeneracyjnych, jak np. dla jaskry czy choroby Parkinsona. Niemniej jednak jednoczesna ocena zmian strukturalnych siatkówki techniką OCT oraz mikrokrążenia w poszczególnych splotach siatkówki z wykorzystaniem techniki OCTA potencjalnie może ujawnić swoiste dla poszczególnych jednostek chorobowych zmiany, co zwiększy zdolności diagnostyczne i będzie cennym podejściem w prognozowaniu AD.

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(38)

Diagnosis of Alzheimer's Disease by Assessing Structural and Microvasculature Changes in the Retina Using Optical Coherence Tomography Angiography – a Review of Eye Biomarkers for Alzheimer's Disease

Diagnoza choroby Alzheimera poprzez ocenę zmian strukturalnych i mikronaczyniowych w siatkówce za pomocą angiografii optycznej koherencyjnej tomografii – przegląd ocznych biomarkerów choroby Alzheimera

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Abstract: Alzheimer's disease is a chronic neurodegenerative disorder that manifests as cognitive decline and memory impairment. Diagnosis is mainly based on the assessment of cognitive functions, while neuroimaging techniques are still very expensive and difficult to access. During the embryogenesis phase, the retina and optic nerve develop as a direct extension of the diencephalon, so that abnormalities occurring in the brain can also be observed in the fundus of the eye. Using optical coherence tomography, a significant decrease in thickness of the retinal nerve fiber layer and a reduction in retinal thickness and volume in the macular area have been demonstrated. In post-mortem studies of patients with Alzheimer's disease, it has been proven that the disease, in addition to nerve cell damage, also has its cerebrovascular pathology. A potential association with the accumulation of abnormal Aβ around vascular walls, impaired blood flow and the diameter of the vessels in the retina have been identified in patients with AD. Using optical coherence tomography angiography to retinal microcirculation imaging showed a reduction in retinal vascular density compared to the control group. Unfortunately, the structural changes in the retina in patients with dementia observed by means of optical coherence tomography images may be non-specific and common to other neurodegenerative diseases, such as reduction in the thickness of the retinal nerve fiber layer in glaucoma. Nevertheless, combined measurements of retinal structural changes and microvasculature assessment in each retinal plexuses using optical coherence tomography angiography potentially increase the diagnostic ability of Alzheimer's disease.

Key words: Alzheimer's disease, retinal microvasculature, peripapillary retinal nerve fiber layer, optical coherence tomography angiography.

Abstrakt: Choroba Alzheimera jest przewlekłym zaburzeniem neurodegeneracyjnym, która objawia się spadkiem funkcji poznawczych i zaburzeniem pamięci. Diagnostyka opiera się głównie na ocenie funkcji poznawczych, a badania neuroobrazowe nadal są bardzo drogie i trudno dostępne. W fazie embriogenezy siatkówka oraz nerw wzrokowy rozwijają się jako bezpośrednie przedłużenie międzymózgowia, dlatego nieprawidłowości zachodzące w mózgu u pacjentów z chorobą Alzheimera możemy również obserwować na dnie oka. Przy zastosowaniu nowoczesnej techniki obrazowania za pomocą optycznej koherentnej tomografii wykazano znaczący spadek grubości warstwy włókien nerwowych siatkówki oraz zmniejszenie grubości i objętości siatkówki w plamce. W badaniach pośmiertnych pacjentów z demencją udowodniono, że choroba Alzheimera poza uszkodzeniem komórek nerwowych cechuje się także patologią naczyniowo-mózgową. Zidentyfikowano potencjalny związek z odkładaniem się nieprawidłowego β-amyloidu w okolicach naczyń a zaburzeniem przepływu krwi oraz średnicą naczyń w siatkówce. Zastosowanie nieinwazyjnej metody obrazowania mikrokrążenia za pomocą angiografii optycznej koherentnej tomografii wykazało zmniejszenie gęstości naczyń siatkówki w porównaniu do grupy kontrolnej. Niestety zmiany strukturalne siatkówki u pacjentów z demencją obserwowane za pomocą obrazów optycznej koherentnej tomografii mogą być niespecyficzne i wspólne dla innych chorób neurodegeneracyjnych, jak np. zmniejszenie grubości warstwy włókien nerwowych siatkówki w jaskrze. Niemniej jednak kombinowane pomiary zmian strukturalnych siatkówki oraz ocena mikrokrążenia w poszczególnych splotach siatkówki z wykorzystaniem techniki angiografii optycznej koherentnej tomografii potencjalnie mogą zwiększać zdolność diagnostyczną choroby Alzheimera.

Słowa kluczowe: choroba Alzheimera, mikrokrążenie siatkówkowe, okolotarczowa warstwa włókien nerwowych siatkówki, angiografia optyczna koherentna tomografia.

Autorzy zgłaszają brak konfliktu interesów w związku z publikowaną pracą/ The authors declare no conflict of interest

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Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder and the most common cause of dementia among the elderly (1). It is estimated that the prevalence of AD is about 36 million people worldwide. Due to the increase in life expectancy and the demographic ageing, the number of patients affected may double every 20 years, reaching the number of 115 million patients in 2050 (2).

Recent studies have shown that AD begins decades before it is fully clinically expressed (3–5). Before the onset of a full-blown disease, cognitive impairment progresses slowly without significant interference in daily activities. This prodromal phase is known as mild cognitive impairment (MCI) (6, 7). It is currently believed that MCI is an intermediate condition between normal aging and early dementia, in which patients may experience impairment of one or even more cognitive domains without compromising daily life performance (8). Although some MCI patients may remain stable over the life course, it is well known that the amnestic and multi-domain phase of MCI increases the risk of progression to AD (9).

Taking into consideration the fact that the diagnosis of AD remains still complicated, especially at the MCI stage, the search for new, non-invasive and inexpensive biomarkers is a promising area of research (10). In clinical practice, the diagnosis of AD is mainly based on the assessment of cognitive function, which may be unreliable in people with high cognitive reserve (11). Additionally used diagnostic techniques that include brain neuroimaging (e.g. magnetic resonance imaging (MR), positron emission tomography (PET)) or molecular measurement of protein levels in cerebrospinal fluid (CSF) (e.g. Tau and Amyloid-β (Aβ)) can be used to confirm AD diagnosis. The disadvantage of these methods are their high costs or invasiveness associated with the collection of CSF. In addition, there are still doubts as to whether they are sensitive and specific enough to allow establishing definitive diagnosis of AD (12, 13).

Since the 1990s, when optical coherence tomography (OCT) was introduced, the measurement of the peripapillary retinal nerve fiber layer (pRNFL) thickness has become a parameter commonly used in the disease diagnosis and monitoring including disorders of retina, optic nerve (CNII) and, in particular, glaucoma (14, 15). It has been noticed that damage to the retinal ganglion cells (RGC) leading to thinning of pRNFL and the retinal ganglion cell layer (GCL) thickness can also be seen in neurodegenerative diseases such as Parkinson's disease (16–18), multiple sclerosis (19, 20), dementia with Lewy bodies (21) and primarily in AD (22, 23). The optical coherence tomography angiography (OCTA) is an extension of the OCT imaging technique, which provides additional capabilities in the form of non-invasive method for the quantitative and qualitative assessment of vascularization status within the macula and optic nerve head (ONH). Post-mortem studies of patients with AD have shown that apoptosis of nerve cells in the central nervous system (CNS) is accompanied by vascular lesions in the form of amyloid angiopathy (24). Pathological changes involve not only the CNS, but also the vascular system, so using the OCTA technique for examining intraocular vessels may become a new AD biomarker (25).

An eye as a "window to the brain"

CNII consisting of RGC axons does not have the specific features of a peripheral nerve – it is basically a bunch of white matter of the brain surrounded by meninges. However, in the embryogenesis phase, the retina and CNII develop as a direct extension of the diencephalon, so we can also observe CNS abnormalities in patients with AD while performing funduscopic examination (26, 27). It is believed that RGC damage in AD may have similar pathogenesis as primary open-angle glaucoma (POAG), therefore the issue of common risk factors and mediators responsible for their emergence and development is increasingly raised (28). Both diseases are characterized by initial changes in neuronal circuits and phosphorylation of mitogen-activated protein kinases (MAPK). Propagation of neurodegenerative processes related to glial reaction, neuroinflammation, mitochondrial abnormalities with the production of reactive oxygen species and oxidative stress, etc., leads to nerve cells apoptosis (29, 30). In animal model studies, McKinnon et al. suggested that the RGC death in patients with ocular hypertension may also be due to chronic neurotoxicity caused by the accumulation of Aβ induced by an increase of intraocular pressure, and a reduced VEGF level which at the molecular level resembles AD (31). Yoneda et al. in their research on the pathogenesis of glaucoma showed a significant decrease in free Aβ which was caused by its accumulation around the retinal vessels, as well as an increase in the level of abnormal tau protein in the vitreous body (32). Similar changes in the amount of these proteins occur in the CSF of people with AD (33).

Pathophysiological changes in AD

AD is a progressive neurodegenerative disease characterized by cognitive decline and memory impairment (34). The essence of the disease is apoptosis of nerve cells and loss of connections between neurons (35). It accounts for 60–80% of dementia cases and remains the main reason for their occurrence over the age of 60 (36). AD may occur sporadically or be genetically conditioned. The incidence of familial cases, with an autosomal dominant pattern of inheritance, is low (5–10%), associated with mutations in three different genes: amyloid precursor protein (APP), presenilin 1 (PSEN-1) and presenilin 2 (PSEN-2). Sporadic cases account for 90–95% of all cases and have multifactorial pathogenesis, consisting of a combination of genetic and environmental factors, with age as a leading risk factor (37). The main gene associated with the sporadic manifestation of the disease is the apolipoprotein E (APOE) gene located on chromosome 21. From among three isoforms, APOE 4 occurs in 50% of patients affected by AD and carries a threefold higher risk of developing the disease (38, 39). The main reason for the emergence and progression of the disease is the accumulation of abnormal proteins: extracellular Aβ arising from the APP and the formation of intracellular neurofibrillary tangles of the tau protein (40, 41). These characteristic changes are well reflected in CSF, which demonstrates abnormal levels of tau protein and Aβ (42).

In 1986, Hinton et al. were the first to describe that neurodegenerative changes in the brain of AD patients also affect CNII and retina (43). Further reports on this issue have also shown a loss of RGC leading to GCL and RNFL thinning in AD (44–46).

Koronyo-Hamaoui et al. identified post mortem Aβ deposits in the retina of AD patients. In another study, by using a modified scanning laser ophthalmoscope, they demonstrated in vivo increase in retinal fluorescence after curcumin supplementation in AD patients, which hypothetically reflected Aβ deposits. As in the case of the brain, these deposits were accumulated mainly around the blood vessels (47, 48). A potential association with the accumulation of abnormal Aβ around the retinal vessels was observed, namely impaired blood flow, increased vascular permeability and their diameter in the retina in patients diagnosed with AD (49, 50). As other researchers have not confirmed the presence of abnormal protein deposits, the incidence of Aβ in the retina of AD patients is still controversial and provides the basis for further studies in this direction (51, 52).

Visual symptoms in patients with AD

Visual symptoms are often one of the earliest manifestations in people with AD, with visual impairment affecting most patients, contributing to reduced quality of life.

Pathological changes in the visual function mainly concern disturbances in the visual field (most often the lower hemisphere is damaged), abnormal electrophysiological tests, incorrect perception of colours and contrast sensitivity, disturbances in perception of depth and movement sensitivity (53, 54). More complex symptoms such as visual memory deficits and visual hallucinations may appear earlier, even before other AD symptoms arise (55, 56). In addition, abnormalities in eye movements were observed in patients with AD and MCI. Smooth pursuit and saccadic movements become slower and less precise as a result of the disease (57, 58).

Visual symptoms in AD are not only caused by damage to the associative visual areas, but there is more and more evidence that the involvement of retina and CNII is also a contributing factor of developing visual symptoms, which raises the interest of ophthalmologists in the search for new AD biomarkers in the retina (59–62).

The appearance of the retina and optic disc in AD patients

Morphometric studies in vivo using non-invasive diagnostic techniques have provided evidence of the involvement of retina, CNII and choroid in neurodegenerative processes occurring in patients with AD.

Fundus photography is the oldest, classic technique for documenting and imaging the retina and optic disc. Examination of patients with AD on the basis of fundus photographs showed abnormalities in the RNFL (images using a green filter), as well as changes in the appearance of optic disc. It has been found that in patients with AD there is an increase in the volume of optic nerve disc cup, an increased cup-to-disc (C/D) ratio and decreased area of the neuroretinal rim, which may correlate with the duration of the disease (23, 63).

In recent years, some efforts have been made to use fundus photographs to analyze changes in the retinal blood vessels. By measuring qualitatively and quantitatively microcirculation parameters, the fractal dimension, caliber, as well as the tortuosity and branching patterns of the retinal vessels have been analyzed. Studies have shown that patients with AD have an altered retinal microvascular network (narrower veins, increasing variability of vessel width, attenuate complexity of branching characteristic, reduced optimality of the branching geometry and less tortuous venules) compared to the control group. These changes in retinal microvasculature may reflect similar pathophysiological processes in brain microvasculature in AD patients. (64–67).

Application of OCT in AD diagnostics

OCT is a high-resolution, non-invasive imaging technique for retina based on the principle of low coherence interferometry, which is commonly used to assess morphological changes in the retina of the macular and optic disc area (14).

According to post-mortem studies in AD patients, a significant decrease in pRNFL thickness was confirmed using in vivo OCT imaging, as well as changes in retinal thickness and volume of the macula. In 2001 Parisi et al. are the first using the Stratus OCT 3 examined people with diagnosed AD disease. They found a significant reduction in pRNFL thickness in each of the 4 quadrants evaluated (68). Some analyzes have shown that statistically significant changes occur only in the superior (69–71) or both superior and inferior quadrants (72, 73). It has been suggested that the probable reason for the differences in the results of the above tests may be the severity of the disease assessed on the basis of the Mini-Mental State Examination (MMSE) test. Kelser et al. have shown a correlation between MMSE results and a reduction in pRNFL thickness, which suggests that retinal nerve fiber degeneration and CNS damage happen simultaneously (73). In studies, where patients obtained low results in the MMSE, statistically significant reduction in pRNFL thickness involved all quadrants (68, 74). In contrast, in the trials conducted by Berish et al. and Paquet et al., in which patients with AD obtained high MMSE results, there was a reduction in pRNFL thickness only in the superior quadrants, postulating that this is the area of the earliest RGC axonal damage in AD patients (75, 76).

Recently, the OCT technique has evolved from older generations, i.e. time domain OCT (TD-OCT) to spectral domain OCT (SD-OCT) and swept source OCT (SS-OCT), which have faster scanning speeds, higher axial resolution and smaller measurement variation (77). Thanks to the progress in OCT technology, it is possible to precisely assess the individual retinal layers in which RGCs are located (78). Macular lesions in the form of damage to the ganglion cell complex (GCC) consisting of three inner layers of the retina: RNFL, GCL and the inner plexiform layer (IPL) are also sensitive markers of neurodegenerative disease (79). Macula contains more than 50% of all RGCs, whose cell body size is 10 to 20 times the diameter of their axons, therefore lowering the thickness of GCL and IPL may be more sensitive to pathological changes in AD than lowering the thickness of pRNFL (80). In addition, individual variability has less effect on GCL and IPL thickness compared to pRNFL thickness (81). In studies where the thickness of individual retinal layers in the macula was assessed in patients with AD, it was confirmed that lowering the thickness of GCL-IPL complex may be a new marker for detecting nerve cell damage in MCI and AD which is associated with the process of neurodegeneration (80, 82–84).

SD-OCT with the use of enhanced depth imaging (EDI SD--OCT) is a technique that allows visualization of deeper struc-

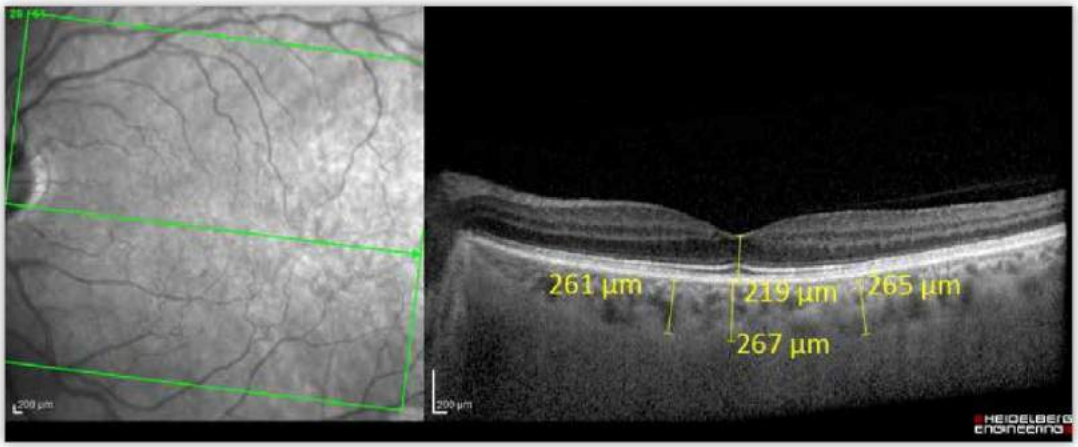

Fig. 1. Measurements of retinal thickness in the fovea and choroidal thickness in the subfoveal, nasal and temporal regions as assessed with enhanced depth imaging optical coherence tomography in a patient with Alzheimer's disease (authors' archives).

Ryc. 1. Pomiary grubości siatkówki w dołeczku oraz naczyniówki w okolicy poddołeczkowej, w nosowej i skroniowej za pomocą optycznej koherencji tomografii o zwiększonej głębokości obrazowania u pacjenta z chorobą Alzheimera (materiał własny).

tures of the eye such as choroid. The thickness of the choroid (CT) varies depending on the location of the examination – the thickest is under the fovea, and the thinnest in the nasal part of the retina (85). The results obtained with EDI SD-OCT confirmed that CT is thinning with age. In addition, it has been shown that CT is significantly reduced in all regions in patients with AD (fig. 1) (86–88).

Interpreting the result obtained using by OCT should always be aware of other neurodegenerative diseases, which can also lead to damage of the RGC. In the study in which we were analyzing the thickness of pRNFL, we found that OCT may be an auxiliary technique in the diagnosis of AD. Analysis of pRNFL thickness showed that AD patients had significantly reduced pRNFL thickness compared to healthy subjects, but this thickness was higher than for POAG patients. However, we found no difference in pRNFL thickness between the group of patients with AD and preperimetric glaucoma, i.e. in which there were no changes in the Visual field (fig. 2) (89).

The use of OCTA in the diagnosis of AD

Post-mortem studies of patients with Alzheimer's dementia have shown that the disease has cerebrovascular pathology. Although small brain vessel abnormalities are involved in the creation and development of MCI and AD, microvasculature in the CNS remains difficult to investigate in vivo (90). Brain and retinal blood vessels share a common embryological origin and show similarity in anatomical features and physiological properties, therefore retinal vascular examination may be valuable in providing new information on AD (91). The study conducted by Berish et al. with the use of laser Doppler has shown that AD patients have narrower retinal veins and lower venous blood flow in comparison to healthy controls (75). A potential association with the accumulation of abnormal Aβ around vascular walls, impaired blood flow and the diameter of the vessels in the retina have been identified in patients with AD (92–94).

The introduction of a modern and non-invasive imaging technique OCTA to visualisation of the vascular network of the retina enables qualitative and quantitative measurements of vessels at various retinal depths. OCTA is a technique that uses motion contrast for imaging, generating high-resolution angiographic images in a few seconds. OCTA compares the decorrelation signal (differences in the intensity or amplitude of the backscattered OCT signal) between successive b-scans made in exactly the same cross-section to create a blood flow map (95, 96). The first commercially used software versions of OCTA devices did not allow the measurement of capillary network parameters in the optic disc scan, therefore researchers determined the total number of black pixels which corresponded to the total area of capillaries (97).

Some studies using OCTA to assess microvascular network in AD patients have shown that retinal vascular density is significantly reduced in comparison to a healthy control group. Bulut et al. were the first to use the OCTA technique to analyze vascular lesions only in the superficial vascular plexus (SVP) of the retina in AD patients. They found that the density of vessels in SVP was lower in patients with AD than in the control group, which correlated with the results obtained in MMSE, while the area of the foveal avascular zone (FAZ) increased. They suggested that this may have been associated with reduced angiogenesis due to VEGF binding and blocking by Aβ deposits (98). In addition, Aβ deposits, which build up inside the walls of blood vessels, probably lead to occlusion and reduce blood flow, which was also emphasized in previous reports (99). Further scientific reports have also confirmed that in patients with AD there is a decrease in the density of vessels in each retinal plexus. Analysis of vascular lesions in the white matter of the brain expressed with the Fazekas scale has shown a significant correlation with reduced vascular network density in the superficial retinal OCT angiogram. However, no significant relationship were found between the retinal microcrovasculature density and the level of Aβ, tau protein or MMSE result (100). In turn, Jiang et al. with the use of OCTA, by means of fractal analysis (box counting, Dbox) to assess the density of vascular network in SVP and deep vascular plexus (DVP), examined the relationship between microvascula-

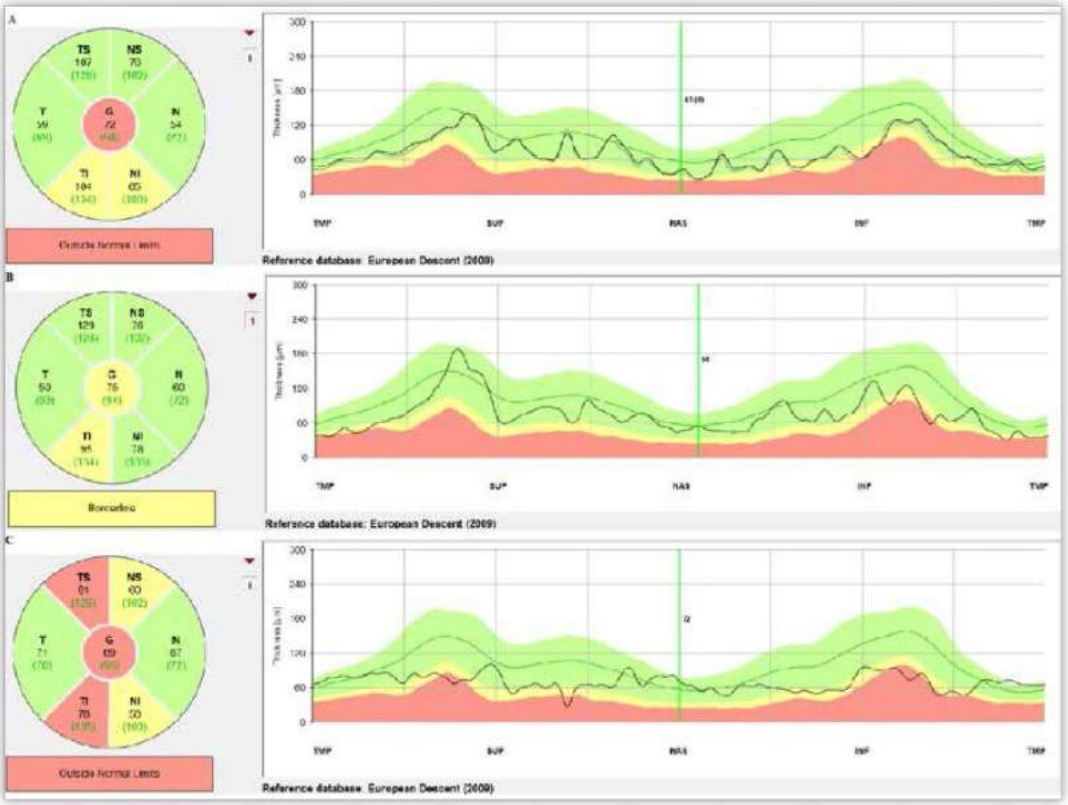

Fig. 2. Measurements of the peripapillary retinal nerve fiber layer thickness in patients with Alzheimer's disease (A), preperimetric glaucoma (B) and primary open-angle glaucoma (C) using optical coherence tomography. Reduction of the peripapillary retinal nerve fiber layer thickness to the borderline in a patient with Alzheimer's disease in the inferior sectors (A) while in a patient with preperimetric glaucoma, only mild reduction of the thickness in the peripapillary retinal nerve fiber layer in inferior temporal sector compared to the normative database is visible. Significant reduction of the preipapillary retinal nerve fiber layer thickness in superior and inferior sectors of a patient with primary open-angle glaucoma where red color indicates significant difference in respect to normative database (C) (authors' archives).

Ryc. 2. Badanie grubości okołotarczowej warstwy włókien nerwowych siatkówki u pacjentów z chorobą Alzheimera (A), jaskrą preperymetryczną (B) oraz jaskrą pierwotnie otwartego kąta (C) przy pomocy optycznej koherentnej tomografii. Zmniejszenie grubości warstwy włókien nerwowych siatkówki u pacjenta z chorobą Alzheimera do wartości granicznej w sektorach dolnych (A) natomiast u pacjenta z jaskrą preperymetryczną widoczne jest jedynie niewielkie zmniejszenie grubości okołotarczowej warstwy włókien nerwowych siatkówki w sektorze dolno-skroniowym w porównaniu z normatywną bazą danych. Znaczące zmniejszenie grubości okołotarczowej warstwy włókien nerwowych siatkówki w sektorach górnych i dolnych u pacjenta z rozpoznaniem jaskry pierwotnej otwartego kąta gdzie kolor czerwony wskazuje znaczącą różnicę w stosunku do normatywnej bazy danych(C) (materiał własny).

ture and GCL-IPL thickness in patients with AD and MCI. They found a reduction in vascular density in each retinal plexus in AD patients, with a significant correlation between density in DVP and retinal thickness of GCL-IPL (101).

In years 2017–2018, our study group conducted the research in which retinal microvasculature in the macular area and optic disc was compared in patients with AD, POAG and a healthy control group. Using the OCTA technique, we revealed that the density of vessels in each retinal plexuses between the examined groups showed significant differences. Patients with AD had a significantly reduced density in DVP and an enlarged area of FAZ in comparison to the other groups. In addition, we observed that the SVP also occur slight decrease in vessel density in comparison to healthy controls, while injury of this plexus was significantly lower than in patients with POAG. In POAG, the reduction in vascular density affected all retinal vascular plexuses, but significant changes, unlike the results obtained in patients with AD, occurred only in the RPC layer and in SVP, which correlated with the loss of pRNFL thickness. We found no correlation between MMSE result, pRNFL thickness, and retinal vascular density in AD patients (fig. 3) (102).

Summary

The discovery of AD biomarkers, in PET imaging or analysis of CSF composition, has enabled a better understanding of the disease. These biomarkers are crucial for monitoring and recruiting AD patients for clinical trials, however, their widespread implementation remains a challenge due to their difficult availability, high costs and invasiveness in regard to CSF sample collection.

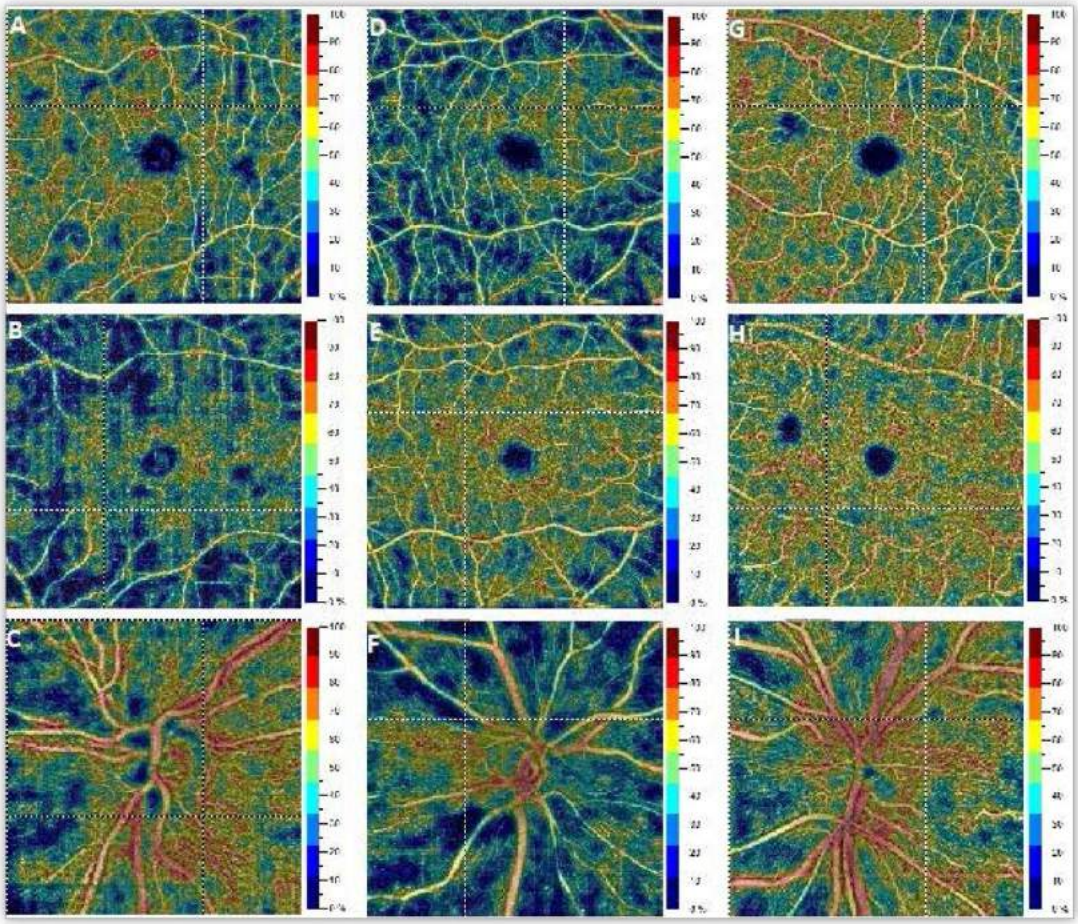

Fig. 3. Comparison of vessels density in angiograms (6 x 6 mm²) of patient with Alzheimer's disease (A, B, C), primary open-angle glaucoma (D, E, F) and healthy control (G, H, I) in the superficial retinal vascular plexuses (A, D, G), deep retinal vascular plexuses (B, E, H) and in the radial peripapillary capillary (C, F, I). Patient with Alzheimer's disease shows a significant reduction in the density of vessels in the deep vascular plexus (B) compared to patient with primary open-angle glaucoma (E) and control group (H). Patient with primary open-angle glaucoma has a reduced vascular density in the superficial vascular plexus (D) and in the radial peripapillary capillary (I) compared to other groups (authors' archives).

Ryc. 3. Angiogramy (6 x 6 mm²) pacjenta z chorobą Alzheimera (A, B, C), jaskrą pierwotną otwartego kąta (D, E, F) oraz osoby zdrowej (G, H, I) porównujące gęstość naczyń w powierzchownych splotach naczyniowych siatkówki (A, D, G), splotach naczyniowych głębokich siatkówki (B, E, H) oraz w warstwie radialnej okołotarczowych kapilar (C, F, I). Pacjenci z chorobą Alzheimera wykazują znaczne zmniejszenie gęstości naczyń w splocie głębokim (B) w stosunku do pacjentów z jaskrą pierwotnie otwartego kąta (E) oraz grupy kontrolnej (H). Pacjenci z jaskrą pierwotnie otwartego kąta mają zmniejszoną gęstość naczyń w splocie naczyniowym powierzchownym (D) oraz w warstwie radialnej okołotarczowych kapilar (I) w porównaniu do pozostałych grup (materiał własny).

Currently, growing high hopes are attached to AD eye biomarkers. The most promising appear to be structurally changes in the retina and its microvasculature that can be directly related to the deposition of Aβ. New technologies such as OCT and OCTA contribute to the progress of knowledge in AD diagnostics. Unfortunately, structural lesions found at optic disc in patients with AD assessed with OCT images may be non-specific and common to other neurodegenerative diseases, such as reduced pRNFL thickness in glaucoma. Nevertheless, combined measurements of retinal structural changes using the OCT technique and microvasculature assessment in each retinal plexus using the OCTA technique can potentially increase diagnostic ability and be a valuable approach in predicting development of AD. Further research addressing this issue is required so that these methods can become sensitive and specific enough to be useful in everyday practice.

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Cele pracy doktorskiej

Wyniki dotychczasowych badań wskazują, że zarówno AD jak i POAG należą do chorób neurodegeneracyjnych, w których zmiany w budowie siatkówki mogą być podobne, co zostało dokładniej omówione w artykule 1. włączonym do cyklu niniejszej pracy doktorskiej. Do tej pory nie istniały badania w których bezpośrednio porównywano AD z POAG pod względem struktury oraz unaczynienia siatkówki z wykorzystaniem OCT i OCTA, co skłoniło nas do pogłębienia wiedzy w tym temacie.

Cele prowadzonych badań:

1) Porównawcza analiza grubości warstw siatkówki w biegunie tylnym gałki ocznej u pacjentów z AD oraz POAG za pomocą OCT.
2) Ilościowa ocena gęstości oraz jakościowa analiza sieci mikronaczyń siatkówki u pacjentów z AD oraz POAG za pomocą OCTA.
3) Bezpośrednia ewaluacja procentowej utraty gęstości naczyń siatkówki oraz grubości wewnętrznych i zewnętrznych warstw siatkówki w tych samych obszarach plamki u pacjentów z AD i POAG.

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Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Alzheimer's Disease: A Comparison of Eyes of Patients with Alzheimer's Disease, Primary Open-Angle Glaucoma, and Preperimetric Glaucoma and Healthy Controls

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this study was to assess and compare peripapillary retinal nerve fiber layer (RNFL) thickness in patients with Alzheimer's disease (AD), primary open-angle glaucoma (POAG), preperimetric glaucoma (PPG), and healthy controls with the use of Spectral Domain Optical Coherence Tomography (SD-OCT).

Material/Methods: Thirty patients with AD, 30 patients with POAG, 30 patients with PPG, and 30 healthy controls were enrolled in this cross-sectional study. Only 1 randomly selected eye of each patient was analyzed. Every subject underwent a thorough ophthalmological examination and OCT of the optic disc. The peripapillary RNFL thickness in each of the 6 sectors and globally was analyzed.

Results: The RNFL was thinnest in patients with POAG. The mean RNFL thickness value was 60.97±12.97 µm and it was significantly lower than in healthy controls (106.30±8.95 µm), patients with PPG (93.20±12.04 µm), and AD patients (95.73±13.52 µm). Mean RNFL thickness in patients with AD was significantly lower when compared to healthy controls, and was higher compared to eyes with POAG, while there were no significant differences compared to patients with PPG.

Conclusions: Neuronal damage in the central nervous system (CNS) also affects to retinal axons. A major problem is to distinguish the cause for a moderate decrease in the RNFL thickness. This is particularly true for patients with glaucoma who have not been diagnosed with changes in the visual field. It is not possible to distinguish the cause of a mild decrease in the RNFL thickness based on the SD-OCT. This may result in misdiagnosis of glaucoma, unnecessary use of anti-glaucoma eye drops, and a delayed diagnosis of AD.

MeSH Keywords: Alzheimer Disease • Glaucoma, Open-Angle • Tomography, Optical Coherence

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Background

Alzheimer's disease (AD) is the most frequent cause of dementia across the world, and with the aging of population the prevalence of the disease is rising. In 2006, 26.6 million people were diagnosed with AD. It is estimated that by 2050 there will be a 4-fold increase in the incidence of the disease [1,2].

In AD there is a gradual dying-back of nerve fibers, which is preceded by the formation of extracellular β-amyloid (Aβ) deposits and intracellular neurofibrillary tangles (NFTs) of tau protein [3,4]. The disease is characterized by a progressive decline in cognitive function, resulting in change of behavior. A diagnosis by exclusion is used to detect AD. This diagnostic method allows the clinician to identify the disease entity's specific neuropsychological and neurological characteristics and at the same time to eliminate other possible causes of dementia. A final diagnosis can be reached following a histopathological examination of the brain tissue attesting to the presence of the amyloid deposits [5,6]. New diagnostic methods are constantly being developed with the use of brain imaging, such as, for example PET, SPECT, and fMRI [7]. However, these diagnostic technics are costly and not usually available, and there are doubts about whether they are sensitive enough to enable final diagnosis of AD.

In 1986, Hinton et al. were the first to report that neurodegenerative changes in the brain also involve the optic nerve and retina [8]. Examinations carried out with optical coherence tomography (OCT) showed that the peripapillary retinal nerve fiber layer (RNFL) thickness is significantly reduced in patients with AD comparing to healthy controls. The difference in the OCT examination is from 16.64 µm to 8.25 µm and is the biggest in the superior quadrant [9]. The correlation between the clinical stage of AD and the average RNFL thickness is not clear. Most research papers deny a correlation between the Mini-Mental State Examination (MMSE) results and the RNFL thickness [10–14]. Changes in the RNFL thickness are not only due to AD, but can also be linked to other neurodegenerative diseases of the central nervous system (CNS), most importantly, to glaucoma. Primary open-angle glaucoma (POAG) is the most common cause of blindness and the incidence of the disease in people over 40 years of age is 1%. It could be presumed that due to their prevalence, AD and POAG are often coexisting medical conditions, making the diagnosis of both conditions even more difficult. Accordingly, a comparison of the 2 populations of patients and an attempt to find differences regarding the extent and topography of the RNFL damage could be of clinical importance.

The aim of this study was to assess and compare the RNFL thickness in the eyes of patients with AD, POAG, preperymetric glaucoma (PPG), and healthy controls in the individual segments around the optic nerve disc.

Material and Methods

This cross-sectional study, conducted in the years 2016–2018, included patients from the Oftalmika Eye Hospital, Bydgoszcz, Poland. Each patient was examined by MMSE screening for cognitive impairment in order to exclude them if they did not belong to the AD group, and underwent eye examination by an ophthalmologist, including the measurement of visual acuity, tonometry (Icare TAO1i, USA), assessment of the fundus with the Volk lens, and biomicroscopy with anterior angle assessment and also SD-OCT (Spectralis OCT, Heidelberg Engineering, Germany). The common inclusion criteria for the project were the best corrected visual acuity (BCVA) ≥0.6, refractive error between ±3.0 Dsph, and age ≥50 years old. The exclusion criteria were any previous surgical eye procedures (except for uncomplicated cataract surgery), advanced stage of cataract, health condition after head injuries, and other eye and neurological conditions. This study followed the tenets of the Declaration of Helsinki. Informed written consent was obtained from all subjects after explaining the nature and possible consequences of the study. The research was approved by the Bioethics Committee of Nicolaus Copernicus University in Toruń at Collegium Medicum in Bydgoszcz.

A group of patients with POAG was defined based on the diagnostic characteristics of glaucomatous optic neuropathy (focal or diffuse neuroretinal rim thinning, hemorrhage on the edge of the optic nerve disc abnormal cup-to-disc (c/d ratio >0.6, the c/d asymmetry between the 2 eyes 0.2) accompanied by a decreased peripapillary RNFL thickness corresponding to a loss of visual field in perimetry with an open-angle. Glaucomatous losses in the visual field were identified with a computerized threshold perimetry (SITA Standard 24-2, Humphrey Field Analyzer II; Carl Zeiss Meditec). Only reliable visual field tests (fixation loss ≤20%, false-positive rate ≤15% and false-negative rate ≤33%) were included. One of the following changes observed in the 2 consecutive visual field tests were used as a criterion for glaucomatous damage, namely a cluster of 3 or more adjacent points in a typical localization for glaucoma, with p<5% in PSD, and for one of them with p<1% in PSD and/or glaucoma hemifield test (GHT) outside normal limits and/or average PSD value calculated for the entire tested area found in less than 5% of healthy eyes. Patients in moderate stage of glaucoma were included in the study [15,16].

A sample of individuals with PPG included patients with glaucomatous defects of the optic disc detected in ophthalmoscopy, a decreased peripapillary RNFL thickness detected in

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Table 1. Demographic and clinical data of the groups.

	AD	POAG	PPG	Controls	p-Value*
Number of participants	30	30	30	30	–
Women/Men	18/12	15/15	17/13	17/13	–
Age**	69.97±7.07	69.20±8.58	69.10±5.69	69.70±7.48	0.962
Best corrected visual acuity** (Snellen)	0.94±0.12	0.91±0.13	0.97±0.07	0.96±0.07	0.174
Intraocular pressure** (mmHg)	16.97±2.49	19.07±3.87	17.81±2.52	16.09±2.31	0.001

AD – Alzheimer's disease; POAG – primary open-angle glaucoma; PPG – preperymetric glaucoma. * ANOVA test, P-value <0.05 was considered to be statistically significant; ** M ±SD.

SD-OCT, without characteristic glaucoma scotoma detected in the visual field testing.

A sample of individuals with AD included patients from Department of Psychiatry, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland. A diagnosis of AD was made by a psychiatrist base on the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) and the criteria of the National Institute on Aging and the Alzheimer's Association (NIA/AA) confirmed by a positive result from a PET scan [16]. Patients with mild dementia (with the Mini-Mental State Examination score ranging from 11 to 18 points) were qualified for the study. Additional inclusion criteria for the participants were intraocular pressure (IOP) <21 mmHg, absence of changes in the optic nerve head that would suggest glaucoma. Visual field testing requires cooperation, significant concentration and it may be impossible, to perform in patients with AD [17]. Trick et al. performed a study where evaluated visual field test in 61 people with AD at various stages of the disease. The reliable result was obtained only in 55.7% of people [18]. Therefore, we decided that, due to the low reliability of the visual field test, we will not perform in the AD group.

Healthy controls had IOP less than 21 mmHg, healthy optic disc without asymmetry, the RNFL thickness within normal limits, and no abnormalities in visual exam.

The peripapillary RNFL thickness was measured using SD-OCT with a 3.46 mm in diameter scan circle centered on the optic disc (Spectralis, Heidelberg, Germany). The Spectralis OCT combines confocal Scanning Laser Ophthalmoscopy (cSLO) that allows for tracking eye movement and averaging of several OCT B-scans formed in the same retinal location in real time. Spectralis provides peripapillary RNFL thickness values for 4 quadrants (N – nasal, T – temporal, S – superior and I – inferior), 6 sectors (N – nasal, NS – nasal-superior, T – temporal, TS – temporal-superior, NI – nasal-inferior), and global mean values (360 degrees).

Only 1 randomly selected eye of each patient was analyzed.

Statistical calculations were conducted with Statistica 12. The results obtained in the studied groups and in the control group were analyzed using one-way analysis of variance ANOVA or in the absence of normal distribution the Kruskal-Wallis test by ranks. For the remaining variables, the *t* test was used or in the absence of normal distribution of the compared parameters the Mann-Whitney U test was administered. Unless otherwise indicated, the data are given are ± mean values and standard deviation (SD) with the p-value threshold for statistical significance of less than 0.05.

Results

The study groups consisted of 30 patients with AD, 30 patients with POAG, 30 patients with PPG, and 30 healthy controls. The groups were well matched by age and sex distribution. Demographic data are presented in Table 1.

Age differences were not statistically significant (p>0.05). BCVA of the study subjects was not statistically different between the groups (p>0.05), while intraocular pressure values, despite the treatment used in individuals with glaucoma, were higher in the POAG group despite the treatment. Figure 1 shows examples of peripapillary RNFL thickness exam in eyes from subsequent groups.

The thinnest peripapillary RNFL was observed in patients with POAG. The global mean value for all quadrants was 60.97±12.97 µm and was significantly lower when compared with healthy eyes (106.30±8.95 µm), eyes with PPG (93.20±12.04 µm) and eyes of patients with AD (95.73±13.52 µm). Comparison of global peripapillary RNFL thickness is presented in Figure 2.

The average global peripapillary RNFL thickness value in patients with AD was significantly lower when compared with healthy controls and significantly higher than in patients with POAG. However, the differences were statistically insignificant compared with eyes of patient with PPG. Comparison of peripapillary RNFL thickness in each sector is presented in Figure 3.

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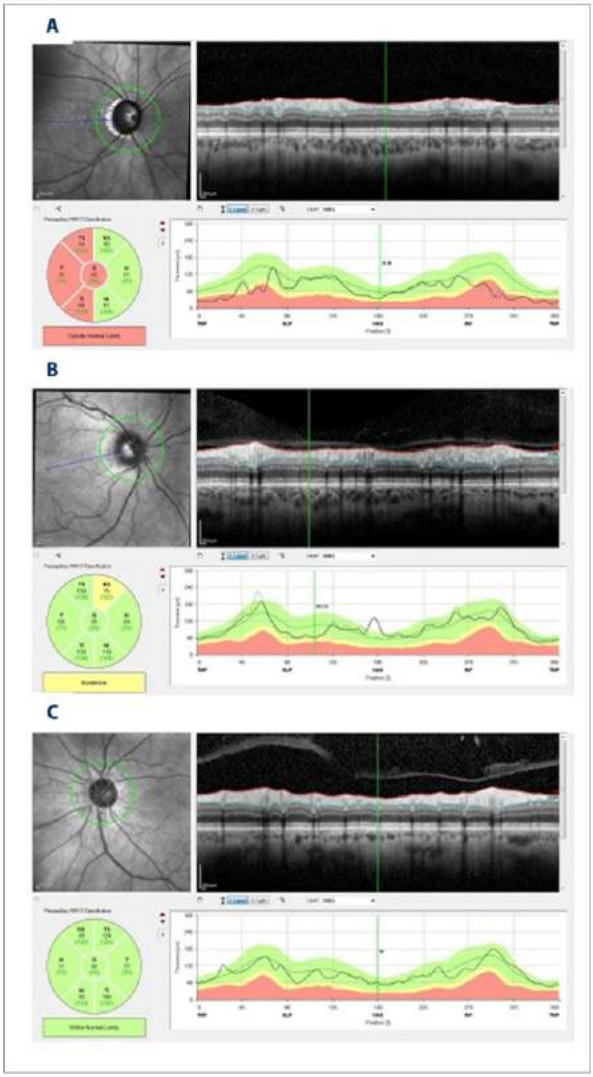

Figure 1. Examples of peripapillary RNFL measurement in the 3 examined groups: (**A**) Significant reduction of RNFL thickness in all temporal sectors in patient with diagnosis of POAG. The red color indicates significant difference in respect to normative database. (**B**) In eye with diagnosis of PPG, only mild reduction of RNFL thickness compared to normative database in nasal temporal sector is visible. (**C**) In the eye of patient with AD, the peripapillary RNFL thickness is within normal limits.

In all the studied groups, the RNFL thickness value was highest in the temporal-inferior and temporal-superior quadrants. The lowest value, regardless of the reason for RNFL thinning, was reported in the nasal and temporal quadrants. The differences between AD and POAG group regarding average peripapillary RNFL thickness were significant in all sectors and between AD and controls in all sectors except for the nasal-superior. Comparison of average peripapillary RNFL thickness within sectors between AD and PPG did not reveal significant changes in all sectors; however, in the temporal sector this difference was close to the statistical significance. The values of RNFL thickness within sector in subsequent groups are presented in Table 2.

Discussion

Our study confirmed the differences in the peripapillary RNFL thickness among the groups of healthy controls, patients with

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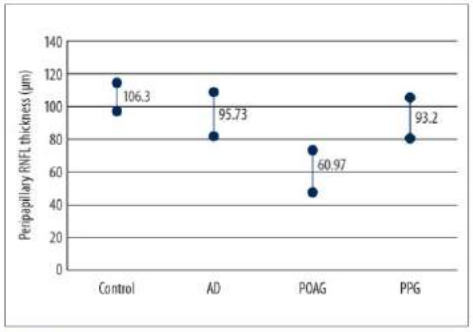

Figure 2. Comparison of the global average peripapillary RNFL thickness. The values in AD groups were significantly lower compared to normal control and higher than in eyes from the POAG group. The differences between AD and PPG were not statistically significant. AD – Alzheimer's disease; POAG – primary open-angle glaucoma; PPG – preperymetric glaucoma.

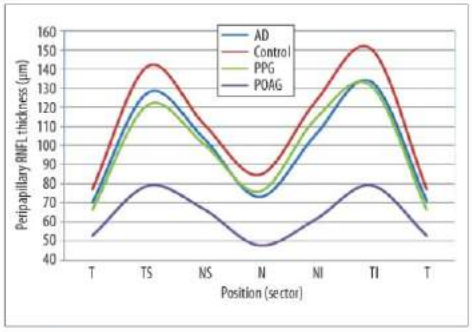

Figure 3. Graphical presentation demonstrates reduction of average peripapillary RNFL in all sectors in POAG, PPG, and AD compared to normal controls. AD – Alzheimer's disease; POAG – primary open-angle glaucoma; PPG – preperymetric glaucoma; T – temporal sector; TS – temporal-superior sector; NS – nasal-superior sector; N – nasal sector; NI – nasal-inferior sector; TI – temporal-inferior sector.

Table 2. Comparison of average peripapillary RNFL thickness in subsequent sectors and globally between AD patients and POAG, PPG and healthy controls.

	AD	POAG	p-Value	AD	PPG	p-Value	AD	Controls	p-Value*
RNFL T (µm)	71.23± 10.1	52.93± 22.53	**<0.001**	71.23± 10.1	66.20± 12.75	0.051	71.23± 10.1	77.17± 14.59	**0.039**
RNFL TS (µm)	127.57± 23,86	78.97± 22.39	**<0.001**	127.57± 23,86	121.30± 20.61	0.144	127.57± 23,86	141.17± 15.85	**0.006**
RNFL NS (µm)	103.67± 23.84	66.83± 24.41	**<0.001**	103.67± 23.84	100.70± 18.38	0.299	103.67± 23.84	111.27± 16.44	**0.081**
RNFL N (µm)	73.27± 18.01	47.77± 16.49	**<0.001**	73.27± 18.01	75.77± 14.69	0.283	73.27± 18.01	84.60± 16.19	**0.007**
RNFL NI (µm)	105.30± 29.28	61.33± 24.74	**<0.001**	105.30± 29.28	113.73± 29.28	0.117	105.30± 29.28	122.77± 26.55	**0.025**
RNFL TI (µm)	133.27± 22.29	79.23± 33.35	**<0.001**	133.27± 22.29	131.33± 24.59	0.309	133.27± 22.29	150.63± 15.95	**<0.001**
RNFL G (µm)	95.73± 13.52	60.97± 12.97	**<0.001**	95.73± 13.52	93.20± 12.04	0.184	95.73± 13.52	106.30± 8.95	**<0.001**

AD – Alzheimer's disease; POAG – primary open-angle glaucoma; PPG – preperymetric glaucoma; T – temporal sector; TS – temporal-superior sector; NS – nasal-superior sector; N – nasal sector; NI – nasal-inferior sector; TI – temporal-inferior sector; G – global.
* Independent t-test, P-value < 0.05 was considered to be statistically significant.

POAG, and patients with AD. Previous research regarding the changes in RNFL in patients with AD suggested that that RNFL thickness analysis may be considered a diagnostic biomarker of this disease [19–21]. In 2001, Parisi et al. used Stratus OCT 3 to study individuals with a diagnosed AD. They compared the results obtained in the study with the values obtained in healthy controls. The analysis showed a decrease in peripapillary RNFL thickness (P<0.01) in all 4 quadrants [13]. Some analyses showed that statistically significant differences occur in the superior quadrants only [22–24] or in the superior and inferior ones [10,25]. It was suggested that the likely reason for the variation in the above results was the stage of the disease assessed with the Mini-Mental State Examination (MMSE) test. Kelsey et al. showed a correlation between the MMSE results and the decreased peripapillary RNFL thickness, suggesting that retinal nerve fiber degeneration and

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the damage to the CNS are simultaneous [10]. In studies in which patients scored low on the MMSE test (Parisini et al. and Iseri el al.), statistically significant decreases in RNFL thickness were observed in all the quadrants [13,26]. Studies by Berisha et al. and Paquet et al., in which patients with AD scored high on the MMSE test, reported decreased RNFL thickness only in the superior quadrants, postulating this to be the location of early retinal damage in patients with AD [14,27].

AD and POAG are multifactorial, chronic, and age-related neurodegenerative diseases. It is important to note that the optic nerve and retina develop as a direct extension of the diencephalon during the embryonic development stage; therefore, abnormalities that occur in the CNS in the case of AD can also be observed at the fundus of the eye [28]. It is believed that nerve cell damage may have a common pathogenesis, so the topics of common risk factors and mediators responsible for the emergence and development of AD and POAG are increasingly raised [29]. Yoneda et al. [30], in their investigation of the pathogenesis of glaucoma, identified a significant decrease in Aβ as well as an increase in the abnormal tau protein in the vitreous humor. Similar changes in the amount of these proteins occur in the cerebrospinal fluid (CSF) of people with AD [31]. McKinnon et al., in an animal models study, suggested that the cause of retinal ganglion cells (RGC) death in patients with ocular hypertension may be chronic neurotoxicity due to Aβ induced by increased of IOP, which at the molecular level resembles AD [32]. These results support the hypothesis that neurodegenerative changes in the eye with glaucoma may have the same pathogenesis as in the case of AD. In addition to specific neuropathological changes caused by abnormal proteins, impairment of vascular are also strictly associated neurodegenerative diseases [33]. The most important modifiable risk factor contributing to the development and progression of AD and POAG is a high-level of blood pressure variability (BPV) [34–37]. Lattanzi et al. conducted a study that evaluated visit-to-visit BPV in a cohort of patients with AD and healthy controls, finding that AD patients had significantly higher-level BPV compared with age-matched cognitive normal controls, which also confirms the influence of BPV on the pathogenesis and progression of AD [38]. In a postmortem study of 291 brains, significantly fewer neuritic plaques and neurofibrillary tangles were found in the group of people with treated hypertension than in the nonhypertensive group, suggesting that antihypertensive agents may interfere with AD-associated neuropathology [39]. Research results also suggest that reducing BPV by antihypertensive therapy can effectively decrease the risk and delay the development of cognitive impairment [40,41]. Similarly, obstructive sleep apnea syndrome and impairment of cerebrovascular reactivity are other equally important risk factors for the occurrence and progression of glaucomatous neuropathy and dementia [42–46]. BPV, obstructive sleep apnea syndrome, and impaired cerebrovascular reactivity are vascular risk factors resulting in impaired blood flow in the form of ischemia-reperfusion injury. Death of nerve cells via apoptosis occurs as a result of metabolic disturbances, impaired retrograde transport of neurotrophins, increased vascular permeability, oxidative stress, and increased inflammatory cytokine synthesis [47–50].

In AD and POAG, we deal with axonal damage in the retina. A review of publications available in PubMed showed that only Eraslan et al. compared peripapillary RNFL thickness in normal tension glaucoma (NTG) patients with AD patients and the control group [51]. Contrary to our analysis, the result of that study did not show statistically significant differences in RNFL thickness of patients diagnosed with glaucoma and those with AD ($p>0.05$). Research to date has not assessed the correlation of changes in RNFL thickness in patients with AD and individuals diagnosed with glaucoma despite the absence of losses in the visual field. Our data indicate that there are no significant differences in BCVA, IOP, and peripapillary RNFL thickness among the groups of AD patients and patients with PPG. We believe that this may lead to the misdiagnosis of PPG in patients who in fact suffer from dementia.

Thinning of RNFL is also observed in other neurological diseases, such as Parkinson's disease [52–54], multiple sclerosis, dementia with Lewy bodies [55], inflammation of the optic nerve [56], and migraines [57]. Further research is necessary to find a biomarker specific for Alzheimer's disease. *In vivo* imaging of extracellular amyloid deposits in retina layers is emerging as the most appropriate way to achieve this. Maya Koronyo-Hamaoui et al. identified post mortem Aβ deposits in the retina of patients with suspected or diagnosed AD, and the image corresponded with the histological brain examination [58].

Conclusions

RNFL thickness measured with OCT can be an additional diagnostic tool for AD. Analyses of RNFL thickness prove that neural damage to the CNS also involve axonal damage of the cells in the retina. A major difficulty is to distinguish the cause of mild reduction in RNFL thickness. This is particularly true for glaucoma patients with no changes in the visual field. This may result in misdiagnosis of glaucoma, unnecessary use of anti-glaucoma eye drops, and a delayed diagnosis of AD. In cases of decreased RNFL thickness, it seems particularly important to pay attention to symptom suggesting dementia.

Conflicft of interests

None.

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Comparison of Retinal Microvasculature in Patients With Alzheimer's Disease and Primary Open-Angle Glaucoma by Optical Coherence Tomography Angiography

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Purpose. Comparison of retinal microvasculature within the macula and the optic nerve head in the eyes of patients with Alzheimer's disease (AD), primary open-angle glaucoma (POAG), and in a healthy control (HC) group, using optical coherence tomography angiography (OCTA).

Methods. In this cross-sectional study, 27 patients with AD, 27 with POAG, and 27 healthy controls were enrolled. The Mini-Mental State Examination test was used to assess cognitive function. Ophthalmic examination included OCTA, which was used for the imaging of vascular flow within the layer of radial peripapillary capillaries (RPCs), and also in the superficial vascular plexus (SVP) and deep vascular plexus (DVP) of the retina.

Results. In the AD group, the density of vessels in DVP was significantly reduced and the foveal avascular zone was increased when compared to POAG and HC groups ($P < 0.001$). Patients with POAG had a significantly reduced vessel density in RPCs and SVP as compared to AD and HC groups ($P < 0.001$). The average thickness of peripapillary retinal nerve fiber layer was correlated with the vessel density in SVP in patients with POAG (Pearson's $r = 0.66$; $P = 0.0002$) and was significantly lower in POAG and AD groups than in the HC group ($P < 0.001$).

Conclusions. AD and POAG are neurodegenerative diseases associated with apoptosis of nerve cells and impairment of microvasculature. Despite the fact that in both diseases there are abnormalities of the entire retinal vascular system, significant microcirculatory impairment in POAG patients affects superficial vessels, whereas in AD patients it affects vessels located in the deeper retinal layers.

Keywords: Alzheimer's disease, primary open-angle glaucoma, retinal microvasculature, peripapillary retinal nerve fiber layer, optical coherence tomography angiography

Alzheimer's disease (AD) is the most frequent cause of dementia worldwide, accounting for approximately 60% to 70% of all cases. Its incidence increases with age and it is estimated that the number of patients globally will quadruple by 2050.[1,2]

AD is a progressive, irreversible impairment of cognitive function due to apoptosis of nerve cells and brain atrophy.[3] The main cause of development and progression of dementia is extracellular β-amyloid (Aβ) plaque formation, a consequence of amyloid precursor protein proteolysis in the vicinity of synapses, and the formation of intracellular neurofibrillary tangles composed of tau-protein (p-tau).[4,5] There is evidence that abnormalities in the central nervous system (CNS) manifest up to 20 years before clinical symptoms appear, emphasizing the importance of identifying a biomarker for early diagnosis.[6]

The retina is considered to be a CNS nodule connected to the CNS through the optic nerve (also known as cranial nerve II, CNII). In 1986, Hinton et al.[7] were the first to describe how CNII and the retina are affected by neurodegenerative changes in patients with AD. Previous studies have shown that the peripapillary retinal nerve fiber layer (pRNFL) is significantly thinner in AD patients than healthy subjects. In spectral-domain optical coherence tomography (SD-OCT), the difference is from 16.64 µm to 8.25 µm and is highest in the superior quadrant.[8] However, changes in the thickness of pRNFL occur not only in AD but also in other neurodegenerative diseases, especially glaucoma.[9,10] Recently, Aβ deposits have been identified on histopathologic examination in the retina of patients with AD. Similarly to neurological tissue, these deposits accumulate primarily in the vicinity of blood vessels.[11] A potential relationship between deposition of abnormal Aβ in the vicinity

of vessels and the disturbance of the blood flow, as well as the diameter of retinal vessels, has been identified in AD patients.[12–14]

The pathologic mechanism underlying primary open-angle glaucoma (POAG) involves apoptosis of retinal ganglion cells (RGCs) and gradual decrease in thicknesses of both pRNFL and the retinal ganglion cell layer (GCL).[15] In addition to increased intraocular pressure (IOP), vascular mechanisms play an important role in this process. It has been observed that patients with glaucoma have reduced blood flow in retina and choroid, which contributes to neurodegenerative processes in the eye.[16,17]

Both AD and POAG affect similar populations of older people and both diseases occur more frequently with age. Previous studies have confirmed that in patients with AD, POAG, and even with preperimetry glaucoma, similar retinal changes exist, manifesting as thinning of the pRNFL.[18] However, there are no studies that have attempted to directly compare these diseases with respect to retinal vascular changes, which would make it possible to identify new, more specific markers. OCT angiography (OCTA) is a new, noninvasive method for the quantitative and qualitative assessment of vascularization status within the macula and optic nerve head (ONH). A direct comparison of OCTA imaging results carried out in AD patients and POAG patients could make a significant contribution to understanding the pathophysiology of these diseases and may help elucidate the underlying cause of damage to the pRNFL.

The aim of our study was to compare the changes in retinal microvasculature in patients with AD and POAG and to find a biomarker that will distinguish these diseases. We used OCTA for this purpose. It is important to note that AD is a neurodegenerative disease in which abnormal proteins are deposited in the CNS, as well as in the retina and its vessels, whereas in POAG, pathologies are found mainly in the inner layers of the retina. We hypothesized that patients with AD have a decreased microvascular density in both deep and superficial vascular plexus (DVP and SVP), whereas patients with POAG have microvascular damage mainly in the SVP.

Methods

Study Design and Patient Recruitment

This cross-sectional study was carried out between September 2017 and December 2018 in the Oftalmika Eye Hospital in Bydgoszcz, Poland. The study protocol was approved by the local bioethics committee and each subject signed a consent for participation. The study was conducted in accordance with the principles of the Helsinki Declaration and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. Each patient enrolled in the study had been examined by a psychologist to assess cognitive function with the Mini-Mental State Examination (MMSE) screening test. All patients underwent ophthalmologic examination including measurement of visual acuity using a Snellen chart, tonometry (Icare TAO1 i, Findland Oy, Vantaa, Finland), evaluation of the fundus of the eye using Volk lens, biomicroscopy and assessment of the iridocorneal angle, measurement of the pRNFL thickness with SD-OCT, and measurement of retinal vascular density with OCTA. These examinations were carried out over 1 day by a single ophthalmologist.

The group of AD patients was referred from the Department of Psychiatry, Collegium Medicum, Nicolaus Copernicus University in Bydgoszcz and the Center of Psychoneurology of the Elderly in Bydgoszcz. AD was diagnosed by a psychiatrist-physician on the basis of DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) and criteria of the National Institute on Ageing and the Alzheimer's Association, confirmed by neuroimaging for the presence of fibrillar brain amyloid using positron emission tomography (PET) imaging with Florbetapir (18 F) radioligand. AD subjects were classified as amyloid positive if their neocortex standardized uptake value ratio was >1.5.[19] Patients with mild and moderate dementia (MMSE 10–23 points) qualified for study entry. Additional inclusion criteria were a normal IOP (<21 mm Hg) and the absence of the ocular fundus changes suggesting glaucoma. Owing to the low reliability of static perimetry test in patients with AD, this examination was not performed in this group of patients.[20,21]

The group with perimetric POAG comprised patients who have been treated for this reason at the Oftalmika Eye Hospital with a normal MMSE score (≥27 points), and with glaucomatous features of optic nerve neuropathy. These features were diffuse or focal thinning of the neuroretinal rim, hemorrhages on the edge of ONH, abnormal cup/disc (C/D) ratio >0.6, asymmetry of the two eyes (C/D ratios exceeding 0.2). The neuropathy was accompanied by a decrease in the pRNFL thickness corresponding to the loss of the visual field in perimetry with an open iridocorneal angle. Glaucomatous losses in the visual field were identified by static perimetry in a threshold approach (SITA Standard 24-2, Humphrey Field Analyser II; Carl Zeiss Meditec, Inc., North Ryde, NSW, Australia). Only reliable visual field tests (fixation loss ≤20%, false-positive and false-negative rate ≤20%) were included. One of the following changes observed in two consecutive visual field tests were used as a criterion for glaucomatous damage: a cluster of three or more adjacent points in a typical localization for glaucoma, with P value <5% in PSD, and for one of them with P value <1% in pattern standard deviation (PSD), and/or glaucoma hemifield test outside normal limits and/or average PSD value calculated for the entire tested area found in less than 5% of healthy eyes.[22] All participants (100%) in the POAG group were treated with at least one type of ocular antihypertensive drops. Beta blockers were used most often (59%), carbonic anhydrase inhibitors in 56%, prostaglandin analogues in 51%, and α2-adrenergic receptor agonist in 22% of cases. The average number of antihypertensive eye drops was 2.0 ± 0.83.

Participants in the healthy control (HC) group were members of the Senior Club in Bydgoszcz and after consenting, were subjected to an ophthalmologic and psychological examination. The criteria for inclusion in the control group were a normal score on MMSE (≥27 points), the absence of glaucoma or any other eye disease, normal appearance of the ONH and normal thickness of pRNFL, IOP below 21.0 mm Hg, and no losses characteristic for glaucoma in visual field testing.

Exclusion criteria were subjects younger than 55 years and older than 85 years, best corrected visual acuity (BCVA) ≤ 0.6, refraction abnormality above +3.0 Dsph and below −3.0 Dsph, and history of eye surgery except for uncomplicated cataract phacoemulsification. The study also excluded persons with diabetes, unregulated arterial hypertension (>145/85 mm Hg), a body mass index ≥30 kg/m^2, damage to CNII with an etiology other than glaucoma, and the presence of any disease of the macula and neurodegenerative diseases other than AD.

OCTA Imaging

OCTA imaging was carried out with an Avanti RTVue XR (Optovue, Inc., Fremont, CA, USA) capable of scanning at 70.000 A-scans/s, permitting measurements with an axial resolution of 5 µm using a source of light with a wavelength of 840 ± 10 µm. We used a newly developed system (software

Table 1. Demographic Data and Clinical Characteristics of Patients

Parameter	AD	POAG	HC	*P* Value*
Number of eyes	27	27	27	
Age, y†	74.11 ± 5.87	71.85 ± 7.06	74.26 ± 7.66	0.361
Sex, female:male	21:6	17:10	19:8	0.491‡
BCVA, Snellen†	0.93 ± 0.09	0.97 ± 0.07	0.95 ± 0.09	0.423
IOP, mm Hg†	16.73 ± 3.67	19.22 ± 2.27	17.07 ± 2.17	0.003
MMSE, points†	20.55 ± 5.46	28.92 ± 1.14	28.39 ± 1.04	<0.0001

* ANOVA test, *P* value <0.05 was considered to be statistically significant.
† Mean ± SD.
‡ χ^2 test, *P* value <0.05 was considered to be statistically significant.

version 2017.1.0.151) equipped with three-dimensional Projection Artifact Removal (3D PAR); therefore, the projection artifacts are reduced in all the deeper layers while maintaining their authentic layout, and the foveal avascular zone (FAZ) parameters have been improved.[23,24] Both eyes of all patients were examined on the same day between 8.00 AM and 12.00 AM after their pupils were dilated. The macula was analyzed by using B-scans covering an area of 6 × 6 mm^2 repeated horizontally and vertically. Each of the B-scans consisted of 400 A-scans (versus traditional 304 A-scans) centered on the fixation point. On an area of 4.5 × 4.5 mm^2 centered on the ONH, the peripapillary vessels were analyzed. The images consisted of two sets of B-scans repeated horizontally and vertically, each consisting of 400 A-scans. Only measurements of good technical quality with a signal quality (SQ) of 6 or more on a 10-degree scale, with which a commercial camera is equipped, qualified for further analysis. Measurements with motion artifacts present on the en face images (irregular patterns of vessels or a blurred boundary of the ONH) were also rejected. The data were analyzed with commercially available software consisting of automatic segmentation of the SVP and DVP, and then automatic measurement of the density of vessels in both these plexuses as well as in the FAZ in the macular area. The 3D PAR algorithm developed by Optovue reduces projection artifacts from the entire OCTA volume on a per voxel basis, using information from the OCT and OCTA volume to distinguish the OCTA signal in situ from projection artifacts, based on parameters acquired from the OCTA and OCT intensity profiles around the voxel of interest.[25,26] The scan covering the ONH was used to measure the density of vessels throughout the en face image with dimensions of 4.5 × 4.5 mm^2 and the density of vessels in the peripapillary area extending between the 2- and 4-mm-diameter elliptical contour lines around the disc margin. The density of the radial peripapillary capillaries (RPCs) was defined as extending from the internal limiting membrane to the posterior boundary of the RNFL. Macular vessel density was analyzed throughout the examined area of 6.0 × 6.0 mm^2, the area of fovea within the annulus of 1 mm in diameter, the parafoveal area between the annuli at 1 mm and 3 mm from the center of the fovea, and the perifoveal area between the annuli at 3 mm and 6 mm from the center of the fovea.

SD-OCT Imaging

All patients underwent SD-OCT imaging with the objective of measuring the thickness of pRNFL using the Spectralis OCT device (Heidelberg Engineering, Heidelberg, Germany). The pRNFL was measured within a circular scan that consisted of 768 A-scans. The scanned circle was 3.46 mm in diameter and was cocentered with the ONH. The global thickness of the pRNFL was analyzed over 360°. The Avanti RTVue XR system (Optovue) was used to perform SD-OCT measurements and to analyze ONH parameters such as disc area, rim area, C/D area, and cup volume.

Statistical Analysis

One eye of each patient was included in the analysis. In cases where both eyes met the assumed criteria, the eye was selected according to the higher SQ score of the macula in OCTA imaging (when the SQ of OCTA images was the same in both eyes, the quality of ONH images was decisive). Scores and variability, arithmetic mean, and the coefficient of variation (%) were calculated. Among the groups, OCTA and SD-OCT results were compared by using multifactor analysis of variance (ANOVA), allowing analysis of the influence of several factors interfering with OCTA results on a dependent variable. To reduce error variance during the analysis, the total variability of the examined feature was divided into variability caused by the influence of sex, age, and SQ. Tukey's post hoc test was used to determine any significant differences between the groups. The independence between the variables was analyzed with χ^2 test. The surface area under the receiver operating characteristic curve (AROC) was used to determine diagnostic accuracy of the analyzed parameters discriminating between AD, POAG, and HC patients. An AROC of 1.0 represents perfect discrimination, whereas an AROC of 0.5 represents accidental discrimination. Pearson's correlation was used to determine the effect of MMSE and pRNFL on the measurements of vessel density in individual retinal plexuses.

Results

Initially, 86 patients were enrolled in the study. Owing to the poor quality of OCTA images of both eyes (movement artifacts, segmentation errors, SQ <6, a significant amount of floaters), three people from the AD group and two from the POAG group were excluded. As a result, 81 people qualified for the analysis and were assigned to three groups. Twenty-seven patients with AD, 27 patients with POAG, and 27 healthy subjects, a control group, were enrolled in the study. There was no significant difference in age or sex between the study groups. The AD group was characterized by a significantly lower MMSE score (20.56 ± 5.46) than the other groups ($P < 0.001$). In all eyes of the patients with glaucoma, the disease was of a perimetric nature and medium degree of severity (MD: −8.77 ± 7.85 dB), whereas patients in the control group had normal results on visual field testing (MD: −0.34 ± 1.47). The mean IOP was significantly higher in the group of patients with glaucoma (Table 1).

The OCTA cross sections of the macula with visible flow signals both through the center of the fovea and in the circumferential part of the macula were similar in all groups. En face angiograms were also similar; however, in patients with AD, the flows in SVP were narrower and more interrupted than

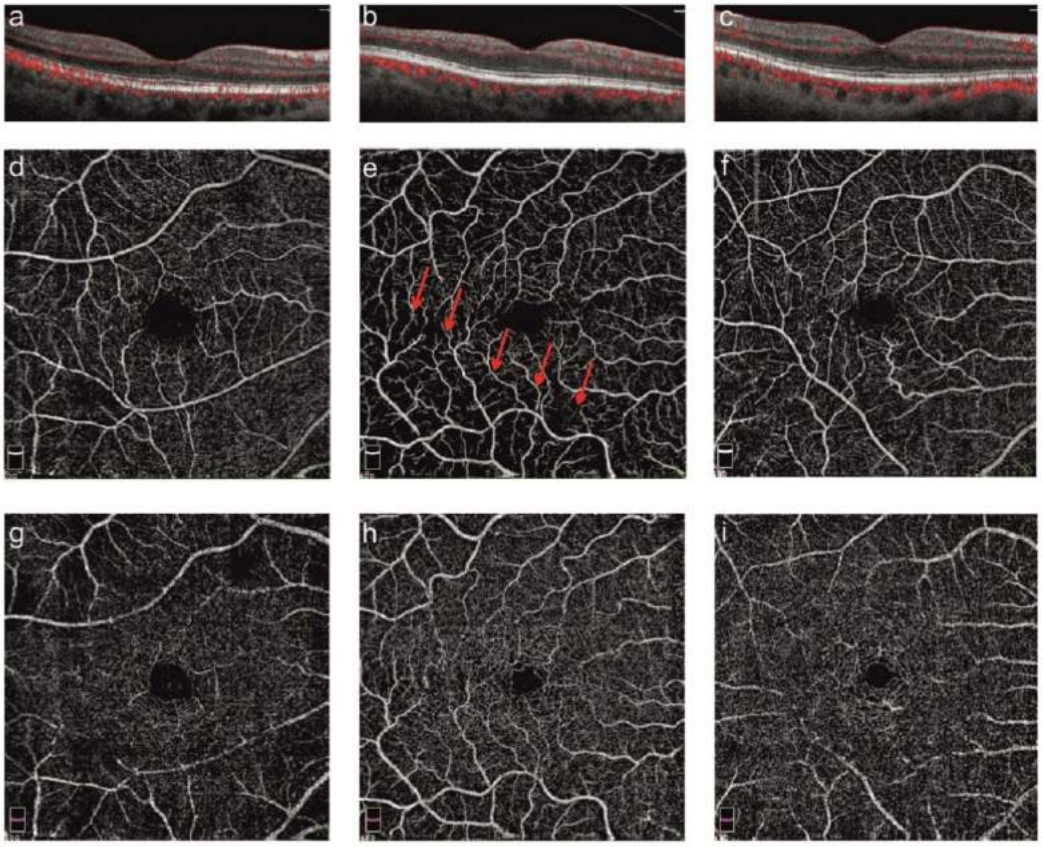

FIGURE 1. Vascular flows within the superficial retinal vascular plexuses and deep retinal vascular plexuses in the sample of optical coherence tomography angiography imaging (6×6 mm^2). (**a**) The central cross section with focal flows marked in the Alzheimer's disease patient has a similar appearance as in the eyes with primary open-angle glaucoma (**b**) and in the control group with healthy eyes (**c**). (**d**) En face optical coherence tomography angiography of the Alzheimer's disease patient shows thinner capillaries in the superficial retinal vascular plexuses than for the other groups. (**e**) Area of capillary drop out (*red arrows*) within superficial retinal vascular plexuses in primary open-angle glaucoma patient. (**f**) Normal superficial retinal vascular plexuses with noticeable flows in the patient from the healthy control group. (**g**) Capillary system and width of the deep retinal vascular plexuses in Alzheimer's disease group is similar to that observed in the primary open-angle glaucoma group (**h**) and in the healthy control group (**i**).

in other groups. In 13 eyes with glaucoma, areas with reduced flow within SVP were visible. On en face images, DVP was similar in all groups (Fig. 1).

Comparison of mean vessel density is presented in Table 2. In the macula the lowest density in SVP occurred in patients with POAG compared to patients with AD and to the HC group ($P < 0.001$). Patients with AD had the lowest vessel density in DVP, compared to other groups (Fig. 2). Differences in vessel density within SVP and DVP persisted in both parafovea and perifovea (Fig. 3). The largest difference in vessel density in DVP between the group of patients with AD and other groups was in the perifoveal area. The ratio of DVP to SVP whole density was 0.93 in the AD group and differs significantly from that of the POAG and HC groups where this ratio gained values of 1.20 and 1.03, respectively ($P < 0.001$). Patients with AD had the largest mean FAZ area and that was significantly different from that of other groups ($P < 0.001$). Patients with POAG also had a significantly increased mean FAZ area in comparison to the control group ($P = 0.015$) but smaller in comparison to the AD group ($P = 0.012$). Analysis of vessel density in the RPC layer on the entire surface of the en face image and in the peripapillary area revealed that patients with glaucoma had a significantly reduced capillary network density ($P < 0.001$). The density in this area did not differ significantly between patients with AD and healthy subjects.

The thickness of pRNFL was significantly reduced among patients with POAG in comparison to the other groups ($P < 0.001$). However, in patients with AD, pRNFL was also significantly thinner than in the control group ($P < 0.05$). In the eyes of patients with glaucoma, the parameters characterizing the ONH such as rim area, C/D area, and cup volume differed significantly as compared to the two other groups (Table 3).

TABLE 2. Differences in the Density of Retinal Vessels in OCTA Imaging Between the Studied Groups

Parameter	AD	POAG	HC	*P* Value*
SVD whole, %†	47.42 ± 3.04	39.72 ± 4.97	48.15 ± 3.03	<0.0001
DVP whole, %†	43.95 ± 5.15	47.44 ± 6.07	49.46 ± 4.27	0.0006
Peripapillary RPCs, %†	51.54 ± 3.08	38.7 ± 8.32	50.49 ± 2.48	<0.0001
Whole RPCs, %†	49.10 ± 4.45	38.49 ± 6.88	47.46 ± 2.41	<0.0001
FAZ, mm^2†	0.32 ± 0.09	0.26 ± 0.08	0.21 ± 0.07	<0.0001
Signal quality†	7.35 ± 0.84	7.04 ± 1.2	7.78 ± 0.89	0.0025

DVP, deep retinal vascular plexus; SVD, superficial retinal vascular plexus.
* ANOVA test, *P* value <0.05 was considered to be statistically significant.
† Mean ± SD.

No significant correlation was found between the MMSE score and vessel density in SVP or DVP and the FAZ area in patients with AD. In the group of patients with glaucoma, there was a significant correlation between vessel density in SVP and the thickness of pRNFL (Pearson's $r = 0.66$; $P = 0.0002$). This relationship was not found in the DVP. Table 4 shows that the AROC was used to reflect the diagnostic accuracy for each parameter to provide distinction between AD and other examined patients (AD and POAG, AD and HC group, AD and combined POAG with HC group). Satisfactory AROC results were obtained only for two single parameters: the density of the peripapillary RPCs (0.96) and SVP whole (0.92) to differentiate between AD and POAG. The ratio of DVP to SVP whole density creates a parameter that has a relatively high AROC of 0.86 for distinguishing AD from other surveyed participants. The use of a logistic regression model with three parameters—the density of the peripapillary RPCs, DVP whole, and area of the FAZ—allowed us to obtain an AROC of 0.93 to separate AD patients from combined groups of POAG patients and HCs.

DISCUSSION

AD and POAG are multifactorial neurodegenerative diseases associated with aging. During embryogenesis, the CNII and retina develop as a direct extension of the diencephalon, so that abnormalities occurring in the CNS can also be observed in the fundus of the eye in the case of AD.[27] It is believed that nerve cell damage can have a common pathogenesis for both AD and POAG, and there is increasing discussion about common risk factors and mediators responsible for the onset

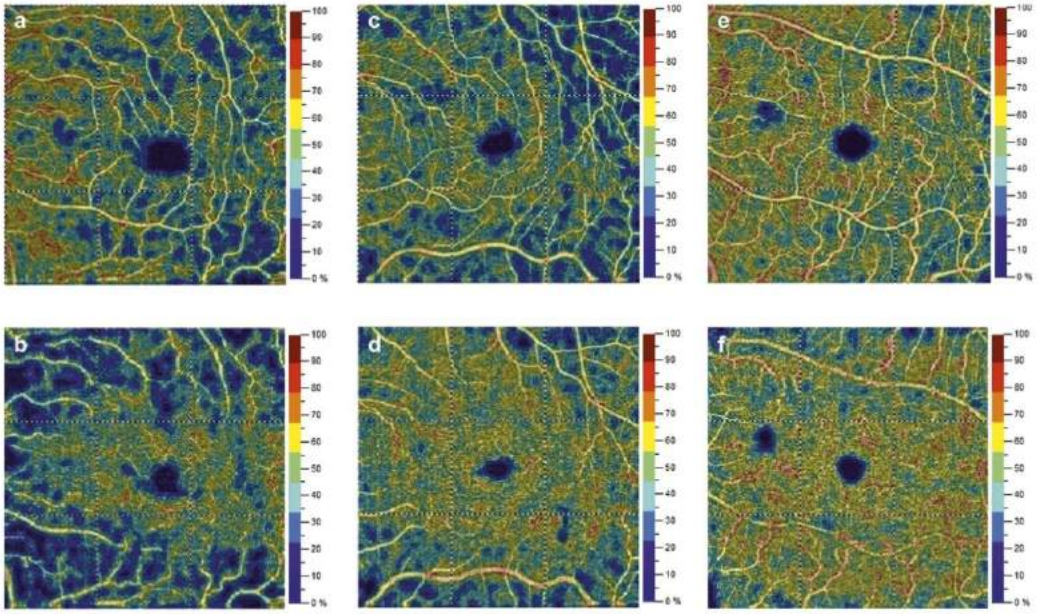

FIGURE 2. The graphical representation of vessel density within the superficial retinal vascular plexuses and deep retinal vascular plexuses in sample optical coherence tomography angiography images in the groups studied. *Warm colors* indicate areas with high vessel density and *cold colors* indicate areas with low density. (**a**) Density in the superficial retinal vascular plexuses in a patient with Alzheimer's disease. (**b**) Density in the deep retinal vascular plexuses in a patient with Alzheimer's disease. (**c**) Density in the superficial retinal vascular plexuses in a patient with primary open-angle glaucoma. (**d**) Density in the deep retinal vascular plexuses in a patient with primary open-angle glaucoma. (**e**) Density in the superficial retinal vascular plexuses in a healthy control group. (**f**) Vascular density in the deep retinal vascular plexuses of a healthy control group. Cloudiness in the vitreous body causes an artifact visible as the dark blue area of the reduced vascular density in the upper nasal part of the macula.

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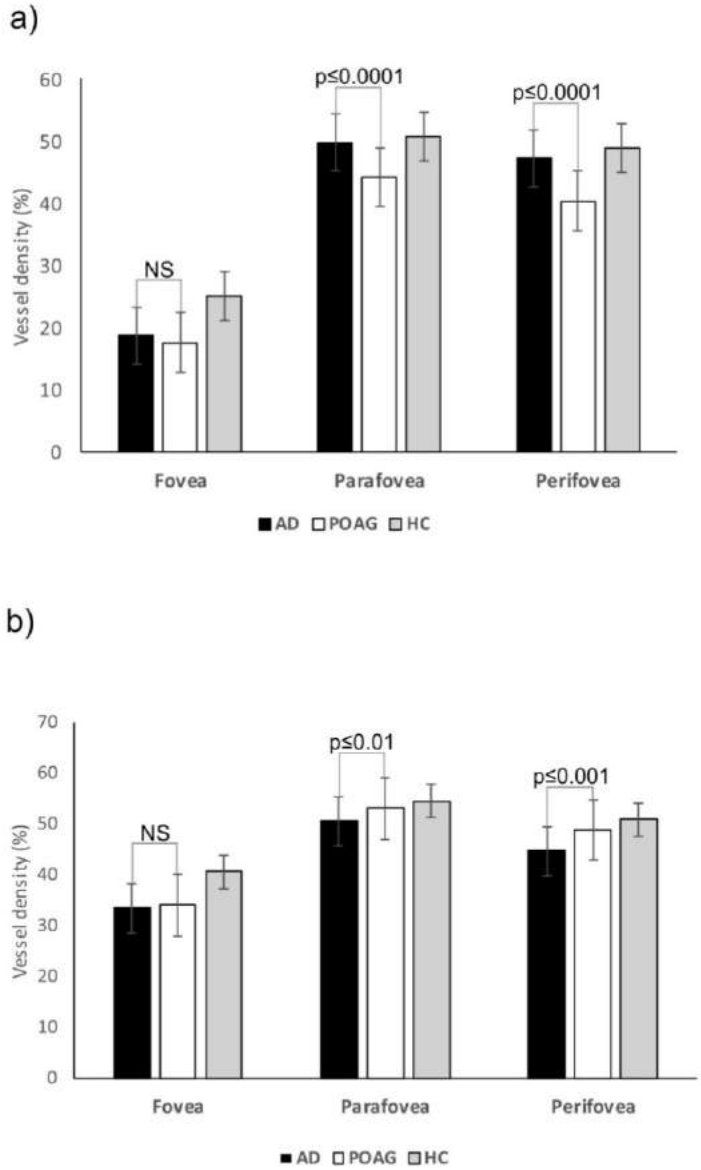

Figure 3. Comparison of vascular density in the foveal, parafoveal, and perifoveal areas in the studied groups within the superficial retinal vascular plexuses (**a**) and deep retinal vascular plexuses (**b**). The average density in the parafoveal and perifoveal part of the macula was significantly different between Alzheimer's disease and primary open-angle glaucoma groups.

and progression of both diseases.[28] Yoneda et al.[29] in their studies on the pathogenesis of glaucoma have shown a significant decrease in Aβ as well as an increase in the level of the tau protein in the vitreous body. Similar changes in the amount of these proteins occur in the cerebrospinal fluid of subjects with AD.[30] McKinnon[31] in animal model studies has suggested that the cause of death of RGCs in patients with ocular hypertension may be the chronic neurotoxicity caused by deposition of Aβ induced by an increase in IOP, as well as reduction in vascular endothelial growth factor (VEGF), which resembles AD at the molecular level. These results confirm the hypothesis that degenerative changes in the eye with glaucoma may have the same pathogenesis as in the case of AD. In addition to specific neuropathologic changes caused by

Table 3. Differences Between the Groups in Morphologic Parameters of the ONH and the Macula in SD-OCT Imaging

Parameter	AD	POAG	HC	*P* Value*
Disc area, mm^2†	1.96 ± 0.52	1.97 ± 0.51	1.96 ± 0.39	0.8443
Rim area, mm^2†	1.71 ± 0.37	1.16 ± 0.45	1.79 ± 0.39	<0.0001
Cup/disc area ratio†	0.17 ± 0.11	0.39 ± 0.23	0.12 ± 0.25	<0.0001
Cup volume, mm^3†	0.04 ± 0.04	0.18 ± 0.17	0.02 ± 0.04	<0.0001
pRNFL, μm†	98.74 ± 6.58	66.11 ± 16.79	102.85 ± 8.87	<0.0001

* ANOVA test, *P* value <0.05 was considered to be statistically significant.
† Mean ± SD.

abnormal proteins, AD and POAG are associated with vascular changes.[32,33]

In our study, we used OCTA technology to assess the retinal microvasculature of the macula and ONH in patients with AD, POAG, and in HCs. We compared the results of the groups, analyzed to each other, in order to find distinctive differences. We found that the density of vessels in individual retinal plexuses differed significantly between the study groups. We showed that patients with AD have a significantly lower vascular density in DVP and enlarged FAZ area in comparison to the other groups. In addition, we observed that SVP also exhibits a reduction in vessel density as compared to the HC group, but this difference was not statistically significant ($P = 0.189$). For the POAG group, a decrease in vessel density could also be observed in all retinal vascular plexuses. However, statistically significant changes occurred only in the RPC layer and in SVP, which correlated with the loss of the pRNFL thickness. No correlation between the MMSE score and the retinal vascular density was demonstrated.

To date, only three studies[34–36] have been published in which OCTA has been used to evaluate the microvasculature in patients with AD. Bulut et al.[34] were the first to use OCTA to evaluate retinal vascular changes in AD. They have found that vessel density in SVP is significantly reduced in the AD group ($P < 0.05$), which correlates with MMSE scores, while the FAZ area is enlarged ($P < 0.001$) compared to the HC group. The positive correlation between vessel density in SVP and FAZ area with MMSE may be due to the fact that the mean MMSE score (16.97 ± 7.39) in AD patients is significantly lower than in our research, which could affect the disclosure of this correlation. A lower degree of dementia is associated with a less advanced stage of the disease in which degenerative changes may be less pronounced. The authors[34] also suggest that vascular impairment may be related to the reduced angiogenesis caused by VEGF's being bound and blocked by Aβ. In addition, as the Aβ deposits settle inside the walls of blood vessels, they are likely to lead to occlusion and reduced blood flow, which has also been reported in previous work.[37,38] The authors have not analyze the density of vessels in DVP probably because the software available to them was in its early version.

Lahme et al.[35] have used OCTA to evaluate vascular density of the macula and ONH in patients with AD. Their results demonstrate a decrease in vascular flow density in each retinal plexus, yet significant changes are found in SVP ($P < 0.001$) and RPCs ($P < 0.05$), which in patients with AD, correlate with the Fazekas scale for white substance changes of vascular origin. Vascular brain damage was associated with reduced flow density in the superficial OCT angiogram of the retina. These authors have not observed significant changes in the FAZ area and in the deep retinal OCT angiogram of the macula ($P = 0.09$) in AD patients compared to HC group. Moreover, they have not found a correlation between the density of vascular flow and the level of Aβ, tau protein, or MMSE score.[35] This can be explained by the fact that the authors examined a macular area of 3×3 mm^2, while the vascular changes in the population under study are most visible in the peripheral part of macula. Moreover, the patients included in their study have a lower degree of dementia (MMSE score 22.32 ± 4.45) than the group analyzed in our investigation.

In turn, Jiang et al.,[36] using OCTA and fractal analysis (box counting, Dbox) for the assessment of vessel network density in SVP and DVP, have investigated the relationship between microvasculature and the thickness of GCL-inner plexiform layer (IPL) in patients with AD and MCI. Their findings are similar to ours, namely, the vessel density decreases in DVP and SVP in the AD group, whereas the GCL-IPL thickness is only correlated to DVP.

There are many more reports on POAG in the literature in which OCTA parameters have been evaluated. Most studies to date have shown that the disease affects all vessels and a decrease in density can be observed in each plexus, and statistical significance has been demonstrated in SVP, RPC, or in full-thickness scans.[39–43] This is consistent with our results. In two other published works,[44,45] a significant decrease in retinal vessel density has been found both in SVP and DVP. The difference in the results obtained in each of these studies may be related to the quality of the images obtained, the artifacts casting shadows especially on the deeper layers of the retina, as well as a difference in the software used in OCTA, which has a particularly large impact on DVP.

The results of this study confirm that AD and POAG are neurodegenerative diseases that are associated with retinal

Table 4. Diagnostic Accuracy of OCTA Parameters in Discriminating Between Patients With AD, POAG, and HC

Parameter	AROC (95% CI)		
	AD vs. POAG	AD vs. HC	AD vs. POAG + HC
Peripapillary RPCs	0.9609 (0.919–1.00)	0.6193 (0.462–0.777)	0.7901 (0.689–0.891)
SVP whole	0.9150 (0.845–0.985)	0.5912 (0.435–0.748)	0.6619 (0.545–0.779)
DVP whole	0.6886 (0.539–0.838)	0.7791 (0.655–0.904)	0.7339 (0.616–0.852)
FAZ	0.6934 (0.549–0.837)	0.8422 (0.739–0.945)	0.7678 (0.659–0.876)
DVP whole/SVP whole	0.9369 (0.877–0.997)	0.7874 (0.660–0.915)	0.8621 (0.778–0.946)

CI, confidence interval.

microvasculature changes, and the degree and level of vascular damage depend on the disease. In the case of POAG, the disease process involves RGCs in the GCL and RNFL, hence vascular damage is more selective and affects SVP and RPC. AD is the disease with the greatest degree of vascular damage in DVP, which is significantly correlated with the decrease in the thickness of GCL-IPL.[46] This is probably due to the larger diameter of vessels within SVP, which are less sensitive to disease progression than within DVP, where vessels are thinner and have a smaller cross section, which was also confirmed by our results in the AD group.[46]

Our study had several limitations. It was a cross-sectional study, which makes it impossible to evaluate the changing retinal microvascular parameters as a function of time and with disease progression. Another limitation was the relatively small number of subjects. The OCTA imaging technique requires that the patient concentrate and cooperate, which makes some of the images obtained unsuitable for analysis. There is a certain probability of selection error because PET neuroimaging was performed only in the group of AD patients. Despite the fact that the screening of cognitive functions with MMSE was done in each patient, it cannot be ruled out with certainty that among the other groups are amyloid-positive people. PET imaging is too expensive to be routinely used in screening tests in our country. Moreover, the MMSE was the only measure of cognitive impairment. We used it first of all because it is a standard procedure that needs to be applied in all Polish National Health Service-based institutions for dementia patients; secondly, it is the only cognitive screening tool that is standardized in the Polish population; and finally, it has been widely applied in other studies. We assume that an extended neuropsychological diagnosis would provide a much clearer picture of the relation between changes in retinal microvasculature and cognitive impairment in AD. To avoid bias of the results and minimize selection error, the observer analyzing the data did not know which group the patient belonged to, and if both eyes met the criteria, the eye was selected according to the higher SQ score in OCTA imaging. Another important problem that should be mentioned concerns projection artifacts caused by superficial vessels projecting shadows onto deeper layers of the retina, which may affect the obtained results. Despite the fact that the latest version of the software was equipped with the AngioVue 3D PAR algorithm, remaining projection artifacts in the deeper retinal layers were noticeable in the perifoveal area, which may have the same effect on the results obtained in each group. We realize that the ability to remove projection artifacts in the 6×6 mm^2 scanning area is less than 3×3 mm^2. Despite this, we decided to explore a larger area of the retina because we have reason to believe that in AD most alterations localize in the peripheral parts of the retina.[47] It is also important to note that, during the experiment, patients with POAG used antihypertensive eye drops, which may have a potential confounding effect on the hemodynamics of ocular blood flow and retinal vascular autoregulation. To eliminate the potential effect of antihypertensive eye drops on the result of the study, they should be discontinued from 1 to 4 weeks before the OCTA examination is performed, but for ethical and medical concerns, the glaucomatous patients in the current study did not stop using antiglaucoma eye drops at the time of the examination.[48]

In summary, this is the first study comparing OCTA angiograms in patients with AD, POAG, and in HCs. The results showed that AD and POAG are associated with retinal microvasculature dysfunction, which can be effectively evaluated with OCTA. Depending on the disease, significant vascular damage can affect different retinal plexuses. Despite the fact that in both diseases there are abnormalities in the entire retinal vascular system, the microvasculature impairment in POAG affects superficial vessels to the greatest extent, whereas in AD, it affects vessels located in the deeper layers of the retina. The ratio of DVP to SVP whole density suggests a different vascular phenotype in AD than in POAG. The results are promising, and further study is warranted because this can be a useful method for diagnosing neurodegenerative diseases. We consider that the use of OCTA may help to distinguish the cause of pRNFL damage and can be used in the future as a new biomarker in the early diagnosis of AD and POAG.

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RESEARCH ARTICLE

Quantitative assessment of retinal thickness and vessel density using optical coherence tomography angiography in patients with Alzheimer's disease and glaucoma

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Abstract

Purpose

Assessment and a direct comparison of retinal vessel density with the thickness of inner retinal layer (IRL) and outer retinal layer (ORL) in the same regions of the macula in subjects with Alzheimer's disease (AD) and primary open-angle glaucoma (POAG).

Methods

We analyzed data from 48 eyes of healthy control (HC) participants, 71 eyes with POAG, and 49 eyes of AD patients. Ophthalmic examination included optical coherence tomography (OCT) imaging to measure IRL and ORL thickness and OCT angiography (OCTA) in the same region for the imaging of vessel density in the superficial vascular plexus (SVP) and deep vascular plexus (DVP) of the retina. A direct comparison of vessel density and retinal layers thickness, which different dynamic ranges, was obtained by normalizing values as percentage losses.

Results

Patients with AD presented significantly greater losses of vascular density in the DVP and ORL thickness compared to POAG ($p < 0.001$), but percentage losses of vessel density in SVP and IRL thickness were considerable in POAG compared to AD eyes ($p<0.001$). Positive associations among presence of AD were observed primarily in outer retina where a 1% decrease of ORL thickness was associated with about 24–29% increase in odds of the presence of AD. According to OCTA measurements, a 1% decrease of vessel density in DVP was positively associated with a 4–9% increase in odds of the presence of AD. In POAG positive associations among presence of disease were observed only in inner retina where

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1% loss of IRL thickness and a 1% loss of vessel density in the SVP were positively associated with a 13–23% increase in risk of presence of the disease.

Conclusions

Analysis of ORL thickness and vessel density in DVP could potentially improve diagnostic capabilities and may provide a valuable approach for predicting of AD.

Introduction

The leading cause of dementia is Alzheimer's disease (AD), characterized by chronic inflammation, glial disorders, and synaptic loss in the central nervous system which begin decades before the disease is fully clinically expressed [1]. In 2018, the National Institute on Aging and the Alzheimer's Association (NIA-AA) proposed a new research framework for AD as a means of diagnosing and staging AD in living people. Biomarkers are grouped into those related to amyloid-β (Aβ) deposition, pathological aggregation of phosphorylated Tau (pTau), and neurodegeneration (ATN). This classification system groups and evaluates various biomarkers using neuroimaging, e.g., structural magnetic resonance imaging (MRI), positron emission tomography (PET), or molecular measurement of protein levels in cerebrospinal fluid (CSF) [2]. However, existing modalities for diagnosing AD present several disadvantages, such as a lack of standardization and invasiveness in the case of CSF markers and high costs and currently limited availability of PET imaging. In addition, there are still doubts as to whether current methods are sensitive and specific enough to establish a definitive diagnosis of AD [3,4].

During embryogenesis, the retina develops as a direct extension of the diencephalon and cranial nerve (CN) II. Retinal ganglion cell (RGC) axons do not have specific features of peripheral nerves and are essentially white matter surrounded by meninges [5]. The first histological studies more than 30 years ago reported changes in the CNII and retina as a result of neurodegenerative changes in the brain of AD patients [6]. Anatomical alterations such as loss of RGC leading to a reduction in thickness of ganglion cell layer (GCL) and retinal nerve fiber layer (RNFL) in eyes of patients with AD were confirmed in subsequent reports [7,8]. However, RGC apoptosis occurs not only in AD but in other neurodegenerative diseases, particularly in glaucoma [9,10].

Since the 1990s when optical coherence tomography (OCT) was introduced, measurements of RNFL and GCL thickness have become parameters commonly used in the diagnosis and monitoring of glaucoma [11]. It is notable, that recent studies using OCT found that values of RNFL, and also GCL thickness were reduced in patients with AD when compared with healthy subjects, but that thickness was even more decreased in eyes with primary open-angle glaucoma (POAG) [12–15]. Therefore, it seems that the thickness measurements of the RNFL and the inner retinal layers (IRL) of the macula, which have been previously proposed as surrogate markers for the identification and monitoring of AD, are not specific enough to be used in everyday practice.

Post-mortem histopathological brain examinations of patients with AD have shown that the disease also causes cerebrovascular pathology, however, changes in the microvasculature of the CNS remain difficult to investigate *in vivo* [16]. Blood vessels of the retina and brain share a common embryological origin and display similar anatomical and physiological properties, thus retinal vascular examination may provide new, valuable information on AD [17]. With

the introduction of OCT angiography (OCTA), a modern technique for non-invasive imaging of retinal blood vessels *in vivo*, it has been demonstrated that retinal vessel density is significantly reduced in patients with AD, likely due to abnormal Aβ deposits that build up inside blood vessel walls [18–21]. Latest studies demonstrated significant correlations between retinal vessel density and cognitive function domains [22,23], apolipoprotein E genotype AD [24], and cerebral volumetric changes [25]. However, OCTA imaging has also provided evidence of microvascular impairment owing to reduced vessel density within the peripapillary area and the macula in POAG [26].

Although new imaging technologies, such as OCT and OCTA, have advanced our understanding of the pathophysiology of AD, identifying which biomarkers of the eye are most useful in the diagnosis of AD remains challenging, moreover, it is difficult to distinguish AD from other neurodegenerative diseases, primarily POAG, with sufficient accuracy.

The purpose of the present study was to characterize and perform a direct comparison of retinal vessel density with the thickness of IRL and outer retinal layer (ORL) in the same regions of the macula in subjects with AD and POAG. In addition, we used spectral-domain OCT (SD-OCT) and OCTA to determine the associations of changes in vessel density and retinal layers thickness with the presence of AD and POAG.

Materials and methods

Study design and subjects

This was a cross-sectional study carried out between January 2018 and March 2019 in the Oftalmika Eye Hospital in Bydgoszcz, Poland. The research was conducted in accordance with the principles of the Helsinki Declaration. The protocol of the study was approved by the Bioethical Commission of Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz (approval number: 473/2017). Written informed consent was obtained from all participants. Each participant enrolled in the study was examined by a psychologist and cognitive function was assessed using the Mini-Mental State Examination (MMSE) screening test. An interview with each subject was conducted by a physician to obtain demographic information, medical and neurologic history, and a risk factor profile. Patients with AD remained under the care of their psychiatrist, who determined that the participants are able to express informed consent. All patients included in the study underwent a detailed ophthalmological examination which included: best-corrected visual acuity (BCVA) assessment, tonometry (Icare TAO1i, USA), slit-lamp biomicroscopy to assess iridocorneal angle, and dilated fundus examination. Thickness of the peripapillary RNFL (pRNFL) and retinal macular region were measured using SD-OCT. Retinal vessel density was assessed in the same region using OCTA. Examinations were carried out over one day by a single ophthalmologist.

The group of AD patients were referred from the Psychoneurology of the Elderly Center in Bydgoszcz. Each of them remained under the care of this center for at least a year, where, in addition to cognitive therapy, they received drugs. In the mild stage of the disease, these were acetylcholinesterase inhibitors, while in the moderate stage, they were NMDA receptor antagonists or combination therapy. AD was diagnosed by a psychiatrist physician according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and the NIA–AA criteria [27]. To confirm the presence of fibrillar brain amyloid, PET imaging with florbetapir F 18 radioligand was performed. Images were constructed using the standard uptake value ratio (SUVr) based on 90–110 min of acquired data. Global values were computed based on the volume weighted average of frontal (superior, middle, and inferior frontal gyrus), parietal (posterior cingulate, superior parietal gyrus, postcentral gyrus, and inferolateral remainder of parietal lobe), and temporal (parahippocampal gyrus, hippocampus, medial temporal lobe,

superior, middle, and inferior temporal gyrus) regions. All SUVr images were visually read by an experienced nuclear physician. If SUVr was > 1.5, AD subjects were classified as amyloid-positive [28]. Patients with mild to moderate dementia (MMSE score, 10–23) qualified for entry into the study. Additional inclusion criteria were a normal intraocular pressure (IOP; < 21 mmHg) and the absence of ocular fundus changes suggestive of glaucoma. Due to poor cooperation affects to the low reliability of static perimetry test in patients with AD, the examination was not performed in this group of patients.

Patients with POAG and cognitively normal according to neuropsychological assessment were enrolled in the study based on the presence of features of glaucoma optic neuropathy, accompanied by a decrease in pRNFL thickness corresponding to loss of visual field based on standard automated perimetry (SITA Standard 24–2, Humphrey Field Analyser II, Carl Zeiss Meditec). Glaucomatous visual filed damage was defined as a glaucoma hemifield test (GHT) outside normal limits and a pattern standard deviation (PSD) outside the 95% normal limit, confirmed by at least two consecutive reliable tests (fixation losses $\leq$ 33%, false-positives, and false-negatives $\leq$ 20%). Patients in stage 1 or 2 glaucomatous damage were included in the study [29]. Each eye (100%) in the POAG group was treated with at least one type of ocular antihypertensive drops. The mean number of antihypertensive eye drops was 1.6 ± 0.7. The most commonly used were beta-blockers (59.2%), followed by prostaglandin analogues in 36.6%, carbonic anhydrase inhibitors in 33.8%, and alpha2-adrenergic receptor agonists in 30.9%.

Participants in the healthy control group had an IOP of less than 21 mmHg, normal optical nerve head (ONH) images without asymmetry, pRNFL thickness within normal limits, normal results in visual field examination, defined as a PSD within the 95% confidence interval (CI), and a GHT result within normal limits. Control subjects were ascertained to be cognitively normal according to neuropsychological assessment.

General exclusion criteria included: age below 50 and above 85, BCVA $\leq$ 0.6, refractive defect above ± 3.0 Dsph, IOP > 23 mmHg, ocular trauma, vascular or non-vascular retinopathies, non-glaucomatous optic neuropathies, macular disease with a history of eye surgery, except uncomplicated cataract phacoemulsification for all groups and uncomplicated anti-glaucoma surgery only for the POAG group when at least 3 months have passed since surgery. People with neurodegenerative diseases other than AD, a history of alcohol abuse or carbon monoxide poisoning, or other serious chronic medical conditions affect the vascular system, such as diabetes, thyroid disease, uncontrolled arterial hypertension were also excluded from the study.

OCTA and SD-OCT acquisitions

The study used the RTVue XR Avanti (Optovue Inc., Fremont, CA, USA) SD-OCT device with AngioVue software (version 2017.1.0.151), which provides non-invasive visualization of the retinal vascular network using the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm. The system is implemented on an existing commercially available SD-OCT platform that provides both retinal thickness and vessel density measurements. By simultaneously acquiring the OCT and OCTA volume of the AngioVue scan and using automatic segmentation, both the vessel density and thickness can be obtained from the same scan with accurate registration of analyzed areas. The OCTA device has the ability to perform 70,000 A-scans per second and allows measurements with an axial resolution of 5 μm using a light source with a wavelength of 840 ± 10 nm and a bandwidth of 45 nm. To correct motion artifacts, OCTA combines orthogonal fast-scan directions (horizontal and vertical) and is equipped with DualTrac Motion Correction Technology [30]. The software is equipped with a

three-dimensional Projection Artifact Removal algorithm to reduce projection artifacts in deeper layers from the OCTA volume on a per voxel basis using information from the OCT and OCTA volumes to differentiate the OCTA signal from projection artifacts *in situ* [31].

The protocol for macular scanning consisted of B-scans covering a 6x6-mm area repeated horizontally and vertically. Each B-scan contained 400 A-scans with the center located at the fixation point. Scanned images of the 4.5x4.5-mm areas centered on the ONH also consisted of two sets of B-scans repeated horizontally and vertically, each consisting of 400 A-scans. For further analysis, only good technical measurements with a scan quality (SQ) index of 6 or higher on a 10-point scale with which a commercial device was equipped, qualified. Measurements with motion artifacts on en face images (irregular patterns of vessels or a blurred boundary of the ONH) were discarded, as well as those with poor segmentation of individual vascular plexuses.

Vessel density analysis

The study was conducted on all patients between 10:00 and 16:00 following pupil dilation. Data analyses, including automatic segmentation of the superficial vascular plexus (SVP) and deep vascular plexus (DVP) of the macula and the peripapillary radial peripapillary capillary (pRPC) layer in the ONH area, followed by automatic measurements of vessel density, were performed on commercially available software. Vessel density was calculated as the percentage of area occupied by flowing blood vessels in the selected region. For ONH scans, vessel density was analyzed in the peripapillary area, which extends outwards from the ONH border with an elliptical area between 2–4 mm. The RPC layer was defined as extending from the inner limiting membrane (ILM) to the posterior border of the RNFL. In the macula, analyses of vessel density and retinal thickness were performed on the entire surface of 6x6-mm en face images, inner circle of the Early Treatment of Diabetic Retinopathy Study chart (i.e., foveal area, 1-mm diameter circle), parafoveal area (rings between 1 mm and 3 mm from the center of the fovea), perifoveal area (rings between 3 mm and 6 mm from the center of the fovea), and its sectors. The SVP comprised the area between the ILM and the outer boundary of the inner plexiform layer (IPL), while the DVP comprised the area between the outer boundary of the IPL and the outer boundary of the outer plexiform layer (OPL).

Thickness analysis of retinal layers

Thickness of the retinal layers was evaluated using the same 6x6-mm and 4.5x4.5-mm OCTA acquisitions used for the vessel density analysis. Mean retinal thickness in the peripapillary and foveal, parafoveal, and perifoveal areas were output by the software. Furthermore, the software automatically segmented the pRNFL as well as the IRL and ORL of the macula. The IRL includes the RNFL, GCL, and IPL, whereas the ORL includes layers starting from the inner nuclear layer (INL) up to the outer portion of the hyper-reflective line corresponding to the retinal pigment epithelium (RPE) (Fig 1).

Statistical analysis

Summary statistics for normally distributed continuous variables are presented as mean ± one standard deviation (SD) and median with interquartile range (IQR) for non-normally distributed variables. Categorical variables are presented as frequencies. Differences between continuous, normally distributed variables were analyzed using Student's t-test or analysis of variance (ANOVA) with Bonferroni adjustment for multiple tests. Differences among non-normally distributed data were assessed using the Wilcoxon or Kruskal-Wallis test. When multiple patient groups were compared, multiple testing corrections were applied. Differences

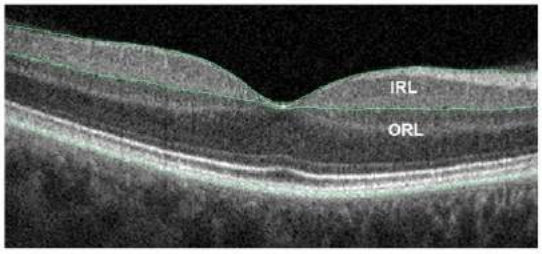

Fig 1. Representative optical coherence tomography image of an eye in a patient with Alzheimer's disease. Cross-sectional image along the horizontal meridian showing the segmentation boundaries of the inner retinal layer (IRL) and outer retinal layer (ORL) (green lines).

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between categorical variables were assessed using the chi-square test or Fisher's exact test for independence. To compare distributions of vessel density and retinal layer thickness in HC, AD, and POAG groups, the linear mixed effect model (LMM) was used, which takes into account the correlation between repeated observations for the same individual (inter-eye correlation).

A direct comparison of vessels density and retinal layers thickness, which different dynamic ranges, was obtained by normalizing values as percentage losses. For patients from the AD and POAG groups, the percentage loss of retinal structure compared to the HC was obtained for the considered variables, i.e., vessel density in the SVP and DVP and thickness of the IRL and ORL calculated using the LMM model [32]. Values of percentage losses were adjusted for inter-eye correlation, age, gender, and SQ, where applicable.

The LMM model was applied to study the association between percentage losses of vessel density and thickness of retinal layers, with thickness as the dependent variable and vessel density, age, gender, and SQ as independent variables. Results are reported as the coefficient of determination (R2), which evaluates the amount of variance in the dependent variable explained by the model. To investigate associations between the percentage losses of considered variables and the presence of AD and POAG, generalized estimating equations (GEE) for correlated multinomial responses were applied [33]. Results are expressed as odds ratios with 95% CI per 1% loss of function.

Results are considered statistically significant when the p-value is less than 0.05. Statistical analyses were performed in the R software (version 3.6.2) using the gls function in the lme4 package, r2glmm package, and multgee package.

Results

This study initially enrolled 112 subjects (179 eyes) who met the inclusion criteria. Due to poor image quality (motion artifacts, vitreous floaters, incorrect segmentation) in OCTA and SD-OCT examinations, two eyes from the HC group, four eyes from the POAG group, and five eyes from the AD group were excluded. Analyses were carried out on data from 31 HC participants (48 eyes), 46 POAG patients (71 eyes), and 31 AD patients (49 eyes). When both eyes of the same patient were included in the study, we controlled for correlation between same-patient eyes.

Demographic and clinical characteristics of study subjects are summarized in Table 1. There were no significant differences among groups in terms of age, gender, BCVA, and SQ

Table 1. Demographics and clinical characteristics of participants.

Parameter	Healthy	POAG	AD	P-Value†
Number of eyes (patients)	48(31)	71(46)	49(31)	
Age (years)	71.4±9.1	72.1±8	74.4±6.1	0.287
Gender (Male/Female)	9/23	23/23	9/22	0.747
MMSE (points)	29(28–29)*	29(28–30)*	20.5(18.5–24.5)*	**<0.001**
BCVA (Snellen)	1(1–1)*	1(1–1)*	1(1–1)*	0.082
IOP (mmHg)	18±2.5	18.2±2.3	16.9±1.8	**0.013**
pRPC (%)	51±2.8	41.6±7.3	50.7±3.8	**<0.001**
pRNFL (μm)	109.2±10.5	78.8±14.5	102.9±13.8	**<0.001**
SQ index Macula	8(7–8)*	7(7–8)*	7(7–8)*	0.063
SQ index Optic Disc	8(8–9)*	8(7–9)*	8(7–9)*	0.062

Significant values appear in boldface.

Mean (standard deviation).

*Median (interquartile range).

†Statistical significance tested by ANOVA or Kruskal-Wallis test (for continuous variables) and by chi-square or Fisher exact test (for categorical variables).

Abbreviations: POAG = primary open-angle glaucoma, AD = Alzheimer's disease, MMSE = Mini-Mental State Examination, BCVA = best corrected visual acuity, IOP = intraocular pressure, pRPC = peripapillary radial peripapillary capillaries, pRNFL = peripapillary retinal nerve fiber layer, SQ = scan quality.

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index ($p>0.05$). The IOP of eyes differed between groups ($p = 0.013$), with the highest scores reported for POAG. In all eyes with POAG, the disease was of a perimetric nature (mean deviation (MD) -4.7 (-8.2–2.4) dB), whereas eyes in the HC had normal results on visual field in standard automated perimetry, (MD -1.1 (-2.2–0.2) dB) ($p<0.001$). The MMSE score was significantly lower in AD patients ($p<0.001$) and median (interquartile range) was 20.5 (18.5–24.5). In the groups of POAG and HC patients, the MMSE score was within the normal range. Thickness of the pRNFL were significantly different among the three groups ($p<0.001$) with the thinnest mean pRNFL measurement in the POAG group and thickest mean measurement in the HC group. Despite decreased pRNFL thickness ($p = 0.038$), patients with AD did not exhibit any differences in vessel density in the pRPC ($p = 0.906$) compared to the HC group, whereas pRNFL thickness was significantly reduced in the POAG group ($p<0.001$ for POAG vs. AD and POAG vs. HC).

Vessel density and retinal layer thickness of the three groups are presented in Table 2. Statistically significant decreases in SVP vessel density and IRL thickness were found in the perifoveal and parafoveal areas in POAG compared to AD and HC, based on whole en face images ($p<0.001$ for all). Compared to POAG and HC, a significant decrease in vessel density was found in the DVP of the AD group as well as thinning of the ORL ($p<0.05$). The most noticeable DVP impairment in AD occurred in the perifoveal region compared to POAG and HC ($p = 0.002$ and $p = 0.004$, respectively), while in the ORL, the most noticeable reduction in thickness applies to the whole en face image ($p<0.001$ and $p = 0.004$, respectively).

The percentage loss of vessel density in the SVP and thickness of the IRL in AD and POAG are summarized in Table 3. Percentage losses of vessel density in SVP and IRL thickness were considerable in eyes with POAG compared to AD ($p<0.001$ for all, except SVP vessel density in the perifoveal area, where $p = 0.003$). The extent of IRL thickness percentage losses were significantly greater than corresponding percentage losses of vessel density in the SVP in the AD and POAG groups ($p<0.001$ for all, except the perifoveal region in AD, where $p = 0.01$).

Table 4 summarizes the calculated percentage losses for vessel density in the DVP and thickness of the ORL in AD and POAG. Significantly greater losses of vascular density in the

Table 2. Vessel density and retinal layers thickness of the studied groups.

Parametr	Healthy	POAG	AD	P-Value† AD vs. POAG	P-Value† AD vs. Healthy	P-Value† POAG vs. Healthy
SVP vessel density (%)						
whole	48.5± 3.4	42.4±5.4	46.8±3.2	**<0.001**	0.766	**<0.001**
fovea	23.9±6.6	18.4±5.7	19.7±6.2	0.45	**0.011**	**<0.001**
parafovea	51.4±4.3	46.7±5.5	49.4±4	**<0.001**	0.68	**<0.001**
perifovea	48.8±3.6	42.7±5.9	46.8±3.3	**<0.001**	0.582	**<0.001**
DVP vessel density (%)						
whole	48.5±5.1	47.6±5.2	45±4.7	**0.014**	**0.032**	0.926
fovea	39.6±5.6	34.7±7.6	34.3±7.3	0.748	**0.005**	**0.006**
parafovea	53.2±3.4	53.5±4.1	51.7±3.6	**0.038**	**0.045**	0.21
perifovea	50±5.3	48.7±5.7	45.4±5.4	**0.002**	**0.004**	0.922
IRL thickness (μm)						
whole	100.3±8.6	81.3±12.1	93.5±7.7	**<0.001**	**0.032**	**<0.001**
fovea	60.8±9.4	49.7±9.2	53.5±7.9	0.077	**0.001**	**<0.001**
parafovea	110±9	89.9±14.8	102.5±9.8	**<0.001**	0.061	**<0.001**
perifovea	100.6±10.5	80.6±11.6	94.5±8.1	**<0.001**	0.066	**<0.001**
ORL thickness (μm)						
whole	202.4±7.2	207.4±8	195.7±7.5	**<0.001**	**0.004**	**0.01**
fovea	221.7±13.9	219.8±12.6	211.7±14.1	**0.023**	**0.005**	0.274
parafovea	215.9±8.1	217.8±8.7	208.9±8.8	**<0.001**	**0.007**	0.468
perifovea	184.2±6.9	184.5±6.8	178.6±6.5	**0.004**	**0.018**	0.748

Significant values appear in boldface.

Mean (standard deviation).

†P-values adjusted for age, inter-eye correlation and SQ (in OCTA), based on Linear Mixed Effects Model.

Abbreviations: POAG = primary open-angle glaucoma, AD = Alzheimer's disease, SVP = superficial vascular plexus, DVP = deep vascular plexus, IRL = inner retinal layers, ORL = outer retinal layers, SQ = scan quality, OCTA = optical coherence tomography angiography.

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DVP and ORL thickness were observed in AD compared to POAG ($p<0.001$ for all, except DVP vessel density in the perifoveal area and whole en face images, where $p<0.05$). In addition, the extent of percentage losses in studied groups were similar ($p>0.1$, except whole en face images in POAG, where $p = 0.005$, and perifoveal area in AD, where $p = 0.001$).

Table 5 shows associations between the presence of AD and POAG based on SD-OCT and OCTA measurements adjusted for age, sex, SQ, and inter-eye correlation using the GEE for multinomial responses. Positive associations among SD-OCT parameters were observed in the presence of AD, primarily loss of ORL thickness in each analyzed area where a 1% decrease of ORL thickness was associated with about 24–29% increase in odds of the presence of AD. According to OCTA measurements, a 1% decrease of vessel density in DVP was positively associated with a 4–9% increase in odds of the presence of AD. In POAG, the loss of pRNFL and IRL thickness measured by SD-OCT and the loss of vessel density in pRPC and SVP measured by OCTA measurements were positively associated with the presence of the disease. Conversely, in POAG, a 1% loss of pRNFL and IRL thickness measured by SD-OCT and a 1% loss of vessel density in the pRPC and SVP measured by OCTA were positively associated with a 13–23% increase in risk of presence of the disease.

Scatterplots presented in Fig 2 illustrate the association between percentage loss of IRL thickness and vessel density in the SVP and percentage loss of ORL thickness and vessel density in the DVP in AD and POAG. The observed correlation between percentage loss of IRL

Table 3. Percentage loss of vessel density in superficial vascular plexus and inner retinal layer thickness in the primary open-angle glaucoma and Alzheimer's disease groups.

Parametr	Percentage loss†		P-Value
	SVP vessel density (%)	IRL thickness (%)	
Whole image			
POAG	11.1 (8.7–13.4)	17.22 (14.4–20.1)	**<0.001**
AD	0.9 (-0.7–2.5)	6.0 (3.7–8.3)	**<0.001**
P-value	**<0.001**	**<0.001**	
Perifoveal			
POAG	10.9 (8.3–13.6)	16.9 (14.0–19.7)	**<0.001**
AD	1.5 (-0.3–3.3)	4.6 (2.1–7.1)	**0.01**
P-value	**0.003**	**<0.001**	
Parafoveal			
POAG	7.6 (5.4–9.8)	17.2 (14.0–20.3)	**<0.001**
AD	1.19 (-0.8–3.2)	6.2 (3.6–8.7)	**<0.001**
P-Value	**<0.001**	**<0.001**	

Significant values appear in boldface.

†Percentage loss, which was calculated with the use of the Linear Mixed Effects Model, are shown in mean (95% confidence interval). The values of the percentage loss have been adjusted for the inter-eye correlation, age, gender and the scan quality (where applicable).

Abbreviations: POAG = primary open-angle glaucoma, AD = Alzheimer's disease, SVP = superficial vascular plexus, IRL = inner retinal layers.

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Table 4. Percentage loss of vessel density in deep vascular plexus and outer retinal layer thickness in the primary open-angle glaucoma and Alzheimer's disease groups.

Parametr	Percentage loss†		P-Value
	DVP vessel density (%)	ORL thickness (%)	
Whole image			
POAG	0.4 (-1.6–2.4)	-2.5 (-3.4- -1.6)	**0.005**
AD	4.9 (2.7–7.3)	3.2 (2.2–4.3)	0.158
P-value	**0.004**	**<0.001**	
Perifoveal			
POAG	0.8 (-1.3–2.9)	-0.7 (-1.6–0.2)	0.149
AD	6.9 (4.5–9.5)	2.4 (1.4–3.4)	**0.001**
P-value	**<0.001**	**<0.001**	
Parafoveal			
POAG	-1.3 (-2.97–0.3)	-0.7 (-1.7–0.2)	0.497
AD	1.8 (-0.1–3.6)	3.4 (2.2–4.6)	0.131
P-value	**0.015**	**<0.001**	

Significant values appear in boldface.

†Percentage loss, which was calculated with the use of the Linear Mixed Effects Model, are shown in mean (95% confidence interval). The values of the percentage loss have been adjusted for the inter-eye correlation, age, gender and the scan quality (where applicable).

Abbreviations: POAG = primary open-angle glaucoma, AD = Alzheimer's disease, DVP = deep vascular plexus, ORL = outer retinal layers.

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Table 5. Multivariable analysis of the associations of 1% loss in vessel density and retinal layers thickness with the presence of Alzheimer's disease and primary open-angle glaucoma.

Parameter	AD		POAG	
	OR per 1% loss	95% CI	OR per 1% loss	95% CI
Optical coherence tomography angiography (vessel density)				
SVP whole	1.03	(0.96–1.11)	**1.23**	**(1.13–1.33)**
SVP perifoveal	1.03	(0.98–1.09)	**1.17**	**(1.09–1.26)**
SVP parafoveal	1.03	(0.96–1.1)	**1.13**	**(1.06–1.2)**
DVP whole	**1.07**	**(1.02–1.13)**	1.01	(0.96–1.06)
DVP perifoveal	**1.09**	**(1.04–1.14)**	1.01	(0.97–1.06)
DVP parafoveal	1.04	(0.98–1.11)	0.97	(0.91–1.04)
pRPC	1.01	(0.93–1.09)	**1.23**	**(1.14–1.32)**
Spectral-domain optical coherence tomography (thickness)				
IRL whole	**1.09**	**(1.03–1.15)**	**1.21**	**(1.14–1.29)**
IRL perifoveal	1.04	(1–1.09)	**1.16**	**(1.1–1.23)**
IRL parafoveal	**1.09**	**(1.03–1.15)**	**1.19**	**(1.13–1.25)**
ORL whole	**1.29**	**(1.12–1.5)**	0.85	(0.74–0.97)
ORL perifoveal	**1.24**	**(1.07–1.43)**	0.97	(0.85–1.1)
ORL parafovea	**1.25**	**(1.09–1.44)**	0.97	(0.87–1.09)
pRNFL	1.04	(0.99–1.09)	**1.21**	**(1.14–1.28)**

Significant values appear in boldface.

Analysis adjusted for age, gender and scan quality.

Abbreviations: AD = Alzheimer's disease, POAG = primary open-angle glaucoma, OR = odds ratio, CI = confidence interval, SVP = superficial vascular plexus, DVP = deep vascular plexus, IRL = inner retinal layers, ORL = outer retinal layers, pRPC = radial peripapillary capillaries, pRNFL = peripapillary retinal nerve fiber layer.

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thickness and vessel density in the SVP (R2 values ranged from 0.43 to 0.63) was stronger than the association between ORL thickness and vessel density in the DVP (R2 values ranged from 0.13 to 0.23), however, all associations were statistically significant (Fig 2).

Discussion

In this study, both ORL thickness and vessel density in DVP were significantly reduced in AD. A direct comparison of the percentage losses of vascular density and retinal thickness revealed a loss of ORL thickness and vessel density in DVP associated with the presence of AD, whereas a loss of IRL and pRNFL thickness and a loss of vessel density in SVP and RPC were associated with the presence of POAG. Analysis of the associations of 1% loss in vessel density and retinal layers thickness with the presence of AD shows positive associations primarily among SD-OCT parameters, where a 1% decrease of ORL thickness was associated with about 24–29% increase in odds of the presence of AD. We also confirmed that changes in retinal vasculature in SVP and DVP were respectively correlated with damage to the IRL and ORL in AD and POAG eyes.

Previous reports have indicated that RGC loss in AD may have a similar pathogenesis to POAG; therefore, the issue of common risk factors and mediators responsible for their emergence and development is increasingly raised [34]. Both diseases are characterized by initial changes in neuronal circuits and phosphorylation of mitogen-activated protein kinases. Propagation of neurodegenerative processes related to glial reaction, neuroinflammation,

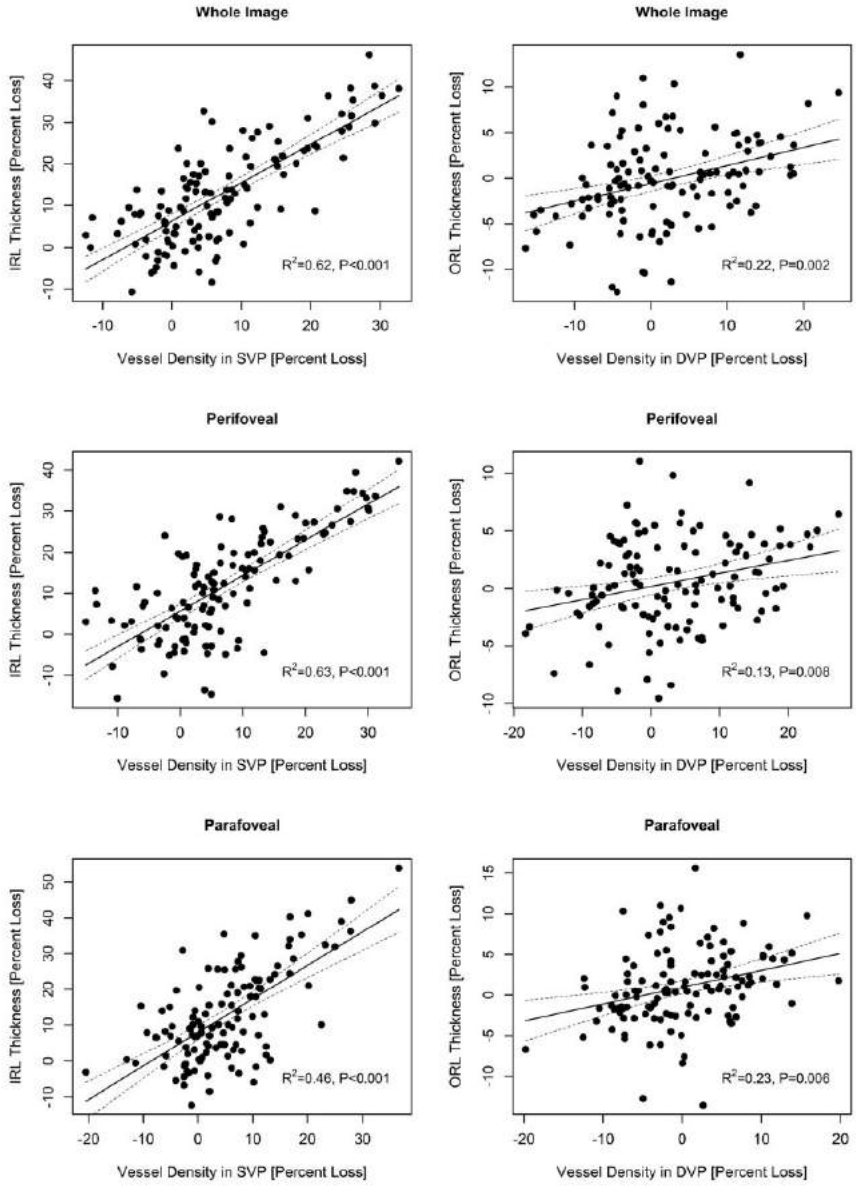

Fig 2. Scatterplots illustrating the correlation between percentage loss of retinal layers thickness and vessel density with linear regression curves in Alzheimer's disease and primary open-angle glaucoma eyes.

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mitochondrial abnormalities associated with production of reactive oxygen species, and oxidative stress, etc., lead to apoptosis of nerve cells [35,36]. In addition, age is a common risk factor for neurodegenerative diseases and the RNFL is believed to naturally decrease with age at a rate of 0.44 μm per year [37]. Meta-analyses show that pRNFL thickness decreases in AD and MCI compared with HC [38,39]. Our results demonstrate a slight decrease in pRNFL thickness compared to HC, but much smaller than that in POAG eyes. Analysis of predictive factors for multinomial responses reveal no association between a decrease of pRNFL thickness and the presence of AD. Our findings are consistent with another large cohort study using advanced OCT that did not report association between dementia or MCI and pRNFL thickness [40].

The macula contains more than 50% of all RGCs, which have a cell body that is 10 to 20 times larger than the diameter of its axon [14]. In addition, structures of the GCL and IPL, containing RGC bodies and their dendrites, respectively, are characterized by less individual variability than the RNFL, which contains axons [41]. This suggests that measurements of retinal thickness in the macular region could be more useful than pRNFL thickness assessments for diagnosing and monitoring neurodegenerative diseases. In 2015, Cheung et al. used SD-OCT to show a decrease in GC-IPL thickness in the macula is more strongly associated with the presence of AD and MCI than a decrease of pRNFL thickness [14]. In other studies where the thickness of specific layers or full macular thickness was assessed, a relationship with the presence of AD was confirmed [40,42–44]. The results of our research reveal similar findings. We found a significant reduction of IRL thickness in whole en face images, but there was no difference in perifoveal and parafoveal areas compared to HC. Comparison of the percentage loss of IRL thickness between AD and POAG groups showed a greater percentage loss in POAG. We have also shown a significant relationship between the decrease of IRL thickness and the presence of AD, however, this association was lower than with the presence of POAG. A greater percentage loss of pRNFL and IRL thickness as well as a stronger relationship with the presence of the disease was observed in POAG compared to AD; therefore, we believe these parameters can be misleading when used to differentiate AD from POAG, and their use as a biomarker for AD is limited.

Previous studies have mainly focused on changes in IRL thickness, whereas few published reports have investigated the outer retinal metrics using SD-OCT. In AD, histological post-mortem studies of humans and animals models have revealed deposition of Aβ plaque in the posterior segment of the eye in various locations including the RNFL, GCL, IPL, OPL, and INL, in the photoreceptor outer segment layer of the retina, and some plaques were also observed in the sclera [45,46]. In addition, Aβ is deposited in the ORL as part of the aging process, where Aβ deposition has been noted in drusen, which can underlie the onset of age-related macular degeneration [47,48]. The extent to which ORL degeneration causes RGC neurotic changes remains unclear. A recent report that outer retinal degeneration may lead to dendritic RGC atrophy as a result of transneuronal changes in mice may explain some of the changes observed in our study [49,50]. We showed that patients with AD exhibit significant thinning of the ORL compared to eyes with POAG and HC, which is consistent with another study [51]. Our multivariable analysis of associations found that reduced ORL thickness is associated with a significant increase in the odds of the presence of AD. Comparison of percentage losses of IRL and ORL thickness demonstrated that the percentage loss of IRL is greater in AD eyes. However, direct comparison between the AD and POAG groups reveals that the percentage loss of IRL thickness is also greater in the POAG group, which suggests this parameter is associated more so with an increase in odds of the presence of POAG than AD. Uchida et al. quantitatively assessed changes in ORL using SD-OCT in various neurodegenerative diseases, including AD. In contrast to our study, they found no identifiable differences in ORL parameters across neurodegenerative disease groups and controls. This could be

explained by several reasons: AD patients did not undergo PET imaging to confirm the presence of Aβ deposits; besides, SD-OCT examination was performed using a different device (Cirrus 4000 HD-OCT) and semi-automatic segmentation on the software platform was performed with manual correction to identify boundaries of interest; finally, thickness of the ORL (between the INL and RPE) was not assessed, but instead, thicknesses were measured from the ONL to ellipsoid zone and from the ellipsoid zone to RPE [52].

Post-mortem studies of patients with dementia have provided evidence that AD involves cerebrovascular pathology. Blood vessels of the retina and brain have common embryological origins and show anatomical and physiological similarities; therefore, retinal vascular examination may be valuable in providing new information on AD [17]. Bulut et al. were probably the first to use OCTA imaging to analyze vascular lesions of the retina in AD patients. They found a reduction in the density of vessels in SVP in the eyes of AD compared to HC [18]. Subsequent research groups confirmed a decrease in retinal vascular density in SVP in patients diagnosed with AD [53–55]. Jiang et al. found a slight decrease in GC-IPL thickness in AD compared with MCI and HC. In addition, they noted a reduction in vascular density in each retinal plexus in AD patients, with a significant correlation between vessel density in the DVP and retinal thickness of the GCL-IPL [56]. There are some doubts about the use of retinal vessel density as a specific biomarker for AD since earlier research on glaucoma confirmed the use of vascular density assessment in the diagnosis and monitoring of POAG. Studies using OCTA in POAG eyes have repeatedly provided evidence of microvascular dropout in the form of a decrease in vessel density within the ONH, the peripapillary retina, and the macula, primarily in the form of a decrease of vascular density in the SVP [20,57,58]. The present study quantitatively compared vascular parameters in the eyes of AD and POAG patients and confirmed previous reports that the density of vessels in the individual retinal plexuses are significantly different among AD and POAG groups. A significant reduction of vessel density in the DVP was observed in AD, whereas a significant decrease of vessel density in the SVP was noted in POAG. Since vessels of the SVP are located in the IRL between the ILM and outer boundary of the IPL, and vessels of the DVP are contained within the outer boundaries of the IPL and OPL, which belong to the ORL. We assessed the relationship between thickness of the retinal layers and density of vessels in their corresponding retinal plexuses and found a correlation between percentage loss of IRL thickness and vessel density in the SVP and between percentage loss of ORL thickness and vessel density in the DVP in both AD and POAG. We believe capillary impairment is associated with AD-mediated neurodegeneration, and it is possible that the retina is highly susceptible to DVP dysfunction in AD, which may indicate disease progression [59]. This is probably due to the diameter of the vessels: DVP vessels are thinner and have a smaller cross-section making them more sensitive to disease progression. Furthermore, Aβ plaques accumulating around the walls of vessels reduce the diameter of vessels leading to blood flow disorders, and also reduced angiogenesis, likely due to binding and blocking of vascular endothelial growth factor by Aβ deposits [18,60].

To compare different parameters with different units and potentially different dynamic ranges, we normalized measurements by calculating the percentage loss of deviation from the mean value of the HC group. By analyzing percentage losses, we were able to directly compare the thickness and density of vessels between groups. In this study, we demonstrated that in POAG eyes there are significant changes in the inner retina, and the percentage loss of IRL thickness was significantly greater than for SVP vessel density. It is different in the deeper layers of the retina, where significant changes are evident only in AD eyes. We found no differences in percentage loss, except in the perifoveal region, where we found a greater percentage loss of vessel density in DVP than of ORL thickness. Therefore, we believe the cause of neurodegeneration in AD may be different to that of POAG. Microvascular and thickness mismatch

in POAG suggest that neurodegeneration may occur sooner and more quickly than vascular damage, which is consistent with another study; whereas, significant changes in eyes of AD patients primarily occur in deeper layers of the retina and the neurodegenerative changes may be secondary to microcirculation disorders where percentage loss of vessel density in DVP is greater than changes in ORL thickness in the perifoveal region [61].

The strength of our study is the fact that AD patients were accurately diagnosed through detailed neurocognitive testing and PET imaging with florbetapir F 18 radioligand analysis, which can readily differentiate participants with normal cognition from those with dementia due to AD. In addition, PET imaging enabled accurate differentiation of AD from dementias with different etiologies.

However, the present study had some limitations. It was a cross-sectional study precluding ability to study patients longitudinally, in addition the case-control design excludes full application in the real clinic population. Another limitation was the relatively small groups of subjects. Therefore, both eyes of some patients were included and the LMM was used which takes into account the correlation between repeated observations from the same individual (with application of the inter-eye correlation). We do not know the time of the disease evolution from its inception to the inclusion of the patient in the study, because most often at the beginning the course of these diseases is asymptomatic. Therefore, we included patients with POAG and AD in mild or moderate stage of disease. We excluded patients in severe stages of the disease that highlight the differences between the study groups. Despite the fact that screening of cognitive function with MMSE was performed for each patient along with a detailed fundus examination to rule out glaucomatous optic neuropathy, selection bias cannot be ruled out. Patients with AD did not undergo visual field testing owing to low reliability of the static perimetry test requiring concentration and cooperation of patients, and PET imaging was performed only in the group of AD patients because it is too expensive to be routinely used in screening tests [62,63]. Patients with POAG did not discontinue ocular hypotensive eye drops, which might affect ocular blood flow [64,65]. The effect of antihypertensive eye drops is likely to persist for 1–4 weeks from the time of withdrawal; therefore, for ethical and medical reasons, patients with POAG involved in the present study did not stop using them [66]. For the same reasons, the use of procognitive drugs in patients with AD has not been discontinued.

Conclusions

New technologies such as SD-OCT and OCTA contribute to progress in the diagnosis of AD and a better understanding of its pathophysiology. Structural changes in the retina and its microcirculation may be directly related to the deposition of Aβ plaques. Unfortunately, structural changes found in the inner retina may be non-specific and are also common in glaucoma. Nevertheless, measurements of deeper retinal layers and analysis of vessel density in DVP could potentially improve diagnostic capabilities and may provide a valuable approach for predicting AD development. More research on a larger group of patients is required to make these methods more sensitive and specific enough to be useful in everyday practice.

Supporting information

S1 Table. Dataset.
(XLSX)

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Podsumowanie

W pracy pt. „Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Alzheimer's Disease: A Comparison of Eyes of Patients with Alzheimer's Disease, Primary Open-Angle Glaucoma, and Preperimetric Glaucoma and Healthy Controls" przeprowadziliśmy analizę porównawczą grubości pRNFL u pacjentów z AD, POAG i jaskrą preperymetryczną (ang. preperimetric glaucoma – PPG) oraz u zdrowej grupy kontrolnej (ang. health controls – HC), wykorzystując w tym celu obrazowanie OCT.

Każdego uczestnika poddano przesiewowym badaniom przez psychologa celem oceny funkcji poznawczych z zastosowaniem testu Mini-Mental (ang. Mini-Mental State Examination – MMSE) oraz szczegółowym badaniom okulistycznym.

Grupę pacjentów z POAG zdefiniowano na podstawie jaskrowej neuropatii n. II z towarzyszącym zmniejszeniem grubości pRNFL, co odpowiadało ubytkom pola widzenia w perymetrii przy otwartym kącie przesączania. Uczestnicy z PPG wykazywali w badaniu cechy jaskrowego uszkodzenia tarczy n. II oraz zmniejszoną grubość pRNFL bez ubytków w badaniu pola widzenia.

Rozpoznanie wśród badanych z AD zostało postawione przez lekarza psychiatrę na podstawie kryteriów DSM-IV (ang. Diagnostic and Statistical Manual of Mental Disorders) oraz NIA/AA (ang. National Institute on Aging and the Alzheimer's Association), potwierdzonych pozytywnym wynikiem obrazowania pozytonowej tomografii emisyjnej (PET).

Do badania ostatecznie zakwalifikowano po 30 osób do AD, POAG oraz HC. Analizie poddano jedno, losowo wybrane oko. Grupy badane nie wykazywały statystycznie istotnych różnic pod względem wieku, rozkładu płci oraz ostrości wzroku ($p > 0,05$), podczas gdy wartości ciśnienia wewnątrzgałkowego, pomimo stosowanego leczenia kroplami przeciwjaskrowymi, były istotnie wyższe w grupie POAG ($p < 0,001$).

Wyniki wykazały najmniejszą grubość pRNFL dla wszystkich kwadrantów wśród pacjentów z POAG (60,97 ± 12,97 µm), która wykazywała

istotne różnice w porównaniu z oczami HC (106,30 ± 8,95 µm), z PPG (93,20 ± 12,04 µm) oraz z AD (95,73 ± 13,52 µm) (p <0,001). Średnia globalna grubość pRNFL u pacjentów z AD była istotnie niższa w porównaniu z uczestnikami HC oraz wyższa niż u pacjentów z POAG (p <0,001). Natomiast różnice te nie były istotne statystycznie w porównaniu z oczami pacjentów z PPG (p= 0,184). Przeprowadzona przez nas analiza porównawcza grubości pRNFL potwierdziła, że uszkodzeniom komórek nerwowych w OUN u pacjentów z AD towarzyszą uszkodzenia aksonów RGC. Badanie grubości pRNFL za pomocą OCT może odgrywać dodatkową rolę w diagnostyce AD. Wydaje się, że podstawowy dylemat dotyczy odróżnienia przyczyny łagodnie zmniejszonej grubości pRNFL, obecnej wśród pacjentów z PPG, u których nie stwierdza się zmian w polu widzenia.

W pracy „Comparison of Retinal Microvasculature in Patients With Alzheimer's Disease and Primary Open-Angle Glaucoma by Optical Coherence Tomography Angiography" wykorzystano OCTA do oceny sieci mikronaczyń siatkówki u pacjentów z AD, POAG oraz w HC.

Każdy włączony do badania uczestnik został zbadany przez psychologa celem oceny funkcji poznawczych za pomocą MMSE. Badanie okulistyczne obejmowało pomiar ostrości wzroku, tonometrię, badanie z użyciem biomikroskopu, pomiar grubości pRNFL za pomocą OCT, a także analizę gęstości naczyń siatkówki z zastosowaniem OCTA.

Diagnoza u pacjentów z AD została postawiona przez lekarza psychiatrę na podstawie kryteriów DSM-IV oraz NIA/AA. Obecność nieprawidłowych złogów Aβ w mózgu potwierdzono za pomocą neuroobrazowania PET. Do badania zakwalifikowano wyłącznie pacjentów z łagodną i umiarkowaną postacią demencji (MMSE 10–23 pkt), z prawidłowym ciśnieniem wewnątrzgałkowym oraz prawidłowym obrazem dna oka bez zmian sugerujących jaskrę.

Grupa uczestników z POAG obejmowała osoby z prawidłowym wynikiem MMSE (≥27 pkt), otwartym kątem przesączania oraz cechami jaskrowej neuropatii n. II, którym towarzyszyło zmniejszenie grubości pRNFL odpowiadające utracie pola widzenia w perymetrii przy otwartym kącie

tęczówkowo-rogówkowym. Wszyscy uczestnicy w grupie POAG leczyli się co najmniej jednym rodzajem kropli przeciwjaskrowych, a średnia liczba stosowanych leków do oczu wynosiła 2,0 ± 0,83.

Kryteriami włączenia do grupy HC były wyniki w MMSE ≥27 pkt, brak jaskry lub jakichkolwiek innych chorób oczu, prawidłowy wygląd tarczy n. II, prawidłowa grubość pRNFL, ciśnienie wewnątrzgałkowe <21 mmHg, a także brak ubytków w badaniu pola widzenia.

Obrazowanie OCTA wykonano aparatem Avanti RTVue XR (Optovue, Inc., Fremont, CA, USA) wykonującym 70 000 A-skanów/s, co pozwala uzyskiwać pomiary z rozdzielczością osiową 5 µm. Wykorzystano najnowszą wersję oprogramowania (2017.1.0.151), wyposażoną w trójwymiarowe usuwanie artefaktów projekcji ze wszystkich głębszych warstwach siatkówki przy zachowaniu autentycznego układu naczyń. Do analizy plamki wykorzystano B-skany o powierzchni $6 \times 6\ mm^2$, powtórzonych w pozycji poziomej i pionowej, natomiast do oceny naczyń okołotarczowych zastosowano protokół badania o powierzchni $4{,}5 \times 4{,}5\ mm^2$, wyśrodkowany na tarczę n. II. Dane jakościowe oraz ilościowe w postaci gęstości sieci naczyń analizowano za pomocą komercyjnego oprogramowania umożliwiającego automatyczną segmentację SVP i splotu głębokiego (ang. deep vascular plexus – DVP). Obliczono również pole powierzchni dołkowej strefy beznaczyniowej (ang. foveal avascular zone – FAZ). Skany obejmujące tarczę n. II wykorzystano do pomiaru gęstości naczyń w warstwie radialnej okołotarczowych kapilar (ang. radial peripapillary capillaries – RPC) na całym obrazie en face o wymiarach $4{,}5 \times 4{,}5\ mm^2$ oraz gęstości naczyń w obszarze okołotarczowym rozciągającym się pomiędzy eliptycznymi liniami konturowymi o średnicy 2 i 4 mm wokół krawędzi tarczy.

Wszystkim uczestnikom za pomocą obrazowania wykonanego urządzeniem Spectralis OCT (Heidelberg Engineering, Heidelberg, Niemcy) zmierzono grubość pRNFL. Do tego celu zastosowano skan kołowy o średnicy 3,46 mm, składający się z 768 A-skanów, wyśrodkowany na centrum tarczy n. II.

Do badania włączono 27 pacjentów z AD, 27 pacjentów z POAG oraz 27 zdrowych osób. Badane kohorty nie różniły się znacząco pod względem wieku oraz płci (p >0,05). Grupa AD charakteryzowała się istotnie niższym wynikiem MMSE niż pozostałe (p <0,001). Średnie ciśnienie wewnątrzgałkowe było istotnie wyższe wśród pacjentów z POAG (p= 0,003).

Porównanie jakościowe angiogramów en face wykazało, że u pacjentów z AD sieci mikronaczyń w SVP były węższe i bardziej poprzerywane niż w innych grupach. W 13 oczach z POAG zaobserwowano obszary o zmniejszonym przepływie w obrębie SVP.

Ilościowa analiza porównawcza mikronaczyń pozwoliła stwierdzić, że oczy pacjentów z POAG wykazują obniżenie średniej gęstości w SVP oraz w RPC, które znacząco różnią się od grup AD i HC (p <0,001). Pacjenci z AD cechowali się najmniejszą gęstością w DVP w porównaniu z innymi grupami (p= 0,0006). Stosunek średniej gęstości w DVP do SVP wyniósł 0,93 w grupie AD i różnił się istotnie od tego w grupach POAG i HC, gdzie stosunek ten zyskał na wartości odpowiednio dla wymienionych grup 1,2 i 1,03 (p <0,001). Pacjenci z AD mieli największą powierzchnię FAZ, która różniła się istotnie od pozostałych badanych grup (p <0,001).

Grubość pRNFL była najmniejsza wśród chorych z POAG (p <0,001), natomiast pacjenci z AD również wykazywali istotne obniżenie grubości pRNFL w porównaniu do grupy HC (p <0,05).

Nie stwierdzono istotnej korelacji między wynikiem MMSE a gęstością naczyń w SVP lub DVP i obszarze FAZ u pacjentów z AD. W grupie pacjentów z POAG wykazano istotną korelację między gęstością w SVP a grubością pRNFL (Pearson's r= 0,66; p= 0,0002).

W celu odzwierciedlenia dokładności diagnostycznej badanego parametru umożliwiającego rozróżnienie pacjentów z AD od pozostałych badanych grup obliczono pole powierzchni pod wykresem krzywej (ang. area under the receiver operating characteristic curve – AUROC). Zadowalające wyniki AUROC w celu odróżnienia AD i POAG uzyskano tylko dla dwóch pojedynczych parametrów: średniej gęstości w pRPC (0,96) oraz średniej całkowitej gęstości SVP (0,92). Stosunek gęstości całkowitej DVP do SVP

tworzy parametr, który ma stosunkowo wysokie AUROC dla odróżnienia AD od pozostałych badanych uczestników (0,86).

Wyniki tego badania wykazały, że AD i POAG są chorobami neurodegeneracyjnymi i wiążą się ze zmianami w mikrokrążeniu siatkówki. Stopień uszkodzenia oraz lokalizacja zmian w naczyniach siatkówki można skutecznie oceniać za pomocą OCTA. Badanie dowodzi, że zastosowanie OCTA stanowi użyteczne narzędzie w rozróżnianiu przyczyn uszkodzenia pRNFL, co pozwoli w przyszłości wykorzystywać je jako nowy biomarker do wczesnej diagnostyki AD i POAG.

Celem pracy pt. „Quantitative assessment of retinal thickness and vessel density using optical coherence tomography angiography in patients with Alzheimer's disease and glaucoma" była ocena i bezpośrednie porównanie gęstości naczyń siatkówki z grubością wewnętrznych i zewnętrznych warstw siatkówki w tych samych obszarach plamki u pacjentów z AD i POAG. Ponadto przy wykorzystaniu OCT i OCTA przeanalizowano związek pomiędzy zmianami w gęstości naczyń i grubości warstw siatkówki a występowaniem AD i POAG.

Uczestnicy włączeni do badania zostali zbadani przez psychologa, a funkcje poznawcze oceniono za pomocą testu przesiewowego MMSE. Następnie poddano ich szczegółowemu badaniu okulistycznemu. Grubości pRNFL i siatkówki w obszarze plamki zmierzono za pomocą obrazowania OCT. Gęstość naczyń siatkówki w poszczególnych splotach oceniano w tym samym regionie za pomocą OCTA.

AD została zdiagnozowana przez lekarza psychiatrę zgodnie z kryteriami NIA–AA oraz DSM-IV. W celu potwierdzenia obecności nieprawidłowych złogów amyloidu w mózgu wykorzystano obrazowanie PET z florbetapirem (F18). Do badania kwalifikowano pacjentów z otępieniem o nasileniu łagodnym do umiarkowanego (10–23 pkt w skali MMSE). Dodatkowymi kryteriami włączenia były prawidłowe ciśnienie wewnątrzgałkowe (<21 mmHg) oraz brak zmian dna oka sugerujących jaskrę.

Pacjentów z POAG, u których wykluczono nieprawidłowości w funkcjach poznawczych, włączono do badania na podstawie obecności cech

jaskrowej neuropatii n. II, któremu towarzyszyło zmniejszenie grubości pRNFL, odpowiadające utracie pola widzenia na podstawie standardowej automatycznej perymetrii (stadium 1 lub 2 zaawansowania).

Pomiary wykonywano urządzeniem Avanti RTVue XR (Optovue, Inc., Fremont, CA, USA). Analizę danych oparto na automatycznej segmentacji naczyń w SVP oraz DVP plamki, a także pRPC w okolicy tarczy n. II. Warstwa pRPC została zdefiniowana jako rozciągająca się od błony granicznej wewnętrznej (ang. internal limiting membrane – ILM) do tylnej granicy RNFL. W plamce dokonano analizy gęstości naczyń i grubości siatkówki na obrazach en face o wymiarach 6×6 mm^2. SVP obejmował obszar między ILM a zewnętrzną granicą warstwy splotowatej wewnętrznej (ang. inner plexiform layer – IPL), podczas gdy DVP – obszar między zewnętrzną granicą IPL a zewnętrzną granicą warstwy splotowatej zewnętrznej (ang. outer plexiform layer – OPL).

Grubość warstw siatkówki oceniano przy użyciu tych samych obrazów OCTA 6×6 mm^2 i $4{,}5 \times 4{,}5$ mm^2, które stosowano do analizy gęstości naczyń. Oprogramowanie automatycznie segmentowało pRNFL oraz warstwy wewnętrzne (ang. inner retinal layer – IRL) i zewnętrzne (ang. outer retinal layer – ORL) siatkówki. IRL obejmuje RNFL, GCL i IPL, podczas gdy w skład ORL wchodzą warstwy zaczynające się od warstwy jądrzastej wewnętrznej (ang. inner nuclear layer – INL) do zewnętrznej części hiperrefleksyjnej linii, odpowiadającej nabłonkowi barwnikowemu siatkówki (ang. retinal pigment epithelium – RPE).

Do badania przekrojowego włączono 31 pacjentów z AD (49 oczu), 46 pacjentów z POAG (71 oczu) oraz 31 uczestników z HC (48 oczu).

Bezpośrednie porównanie gęstości naczyń i grubości warstw siatkówki otrzymano, ujednolicając uzyskiwane wartości o różnych zakresach dynamicznych jako procentowe ubytki badanych parametrów. Grubość pRNFL oraz gęstość pRPC wykazywały istotnie różnice między trzema grupami ($p < 0{,}001$) i były najmniejsze w oczach z POAG. Pomimo zmniejszonej grubości pRNFL u pacjentów z AD w stosunku do grupy HC nie wykazano istotnej różnicy w gęstości naczyń w pRPC ($p = 0{,}906$) w porównaniu z grupą HC.

Statystycznie istotne obniżenia gęstości naczyń w SVP oraz grubości IRL stwierdzono w oczach z POAG (p <0,001), a zakres procentowych strat dla całego obrazu en face w grubości IRL był znacznie większy niż odpowiadający im procentowy ubytek gęstości naczyń w SVP (p <0,001). Natomiast w grupie pacjentów z AD wykazano istotnie większe procentowe straty gęstości naczyń w DVP i grubości ORL w porównaniu z POAG (p <0,001), a zakres procentowych strat między DVP a ORL w całościowej analizie obrazu en face był podobny
(p >0,1). Wyłącznie obszar okołodołkowy wykazywał istotne różnice, stwierdzono w nim bowiem większą procentową utratę gęstości naczyń w DVP w porównaniu do utraty grubości ORL.

W analizach wielomianowych ocenie poddano związek między obecnością AD i POAG a ilościowymi zmianami strukturalnymi w obrazach OCT oraz OCTA. Pozytywny związek między zmianą w budowie siatkówki a obecnością AD zaobserwowano głównie w siatkówce zewnętrznej, gdzie 1% spadek grubości ORL wiązał się z 24–29% wzrostem prawdopodobieństwa wystąpienia choroby. Analiza angiogramów wykazała, że 1% spadek gęstości naczyń w DVP był również pozytywnie związany ze wzrostem prawdopodobieństwa wystąpienia AD, które wynosiło 4–9%. W POAG pozytywny związek między 1% utratą grubości siatkówki i gęstości naczyń a prawdopodobieństwem obecności choroby obserwowano tylko w IRL oraz w SVP (odpowiednio 13 i 23%). Analiza porównawcza związków pomiędzy procentową utratą grubości IRL i gęstości naczyń w SVP oraz procentowej utraty grubości ORL i gęstości naczyń w DVP wykazała istotną korelację w obu przypadkach, które były silniejsze w siatkówce wewnętrznej niż zewnętrznej.

Zmiany strukturalne w siatkówce i jej mikrokrążeniu mogą być bezpośrednio związane z odkładaniem się blaszek Aβ. Patologie stwierdzane w wewnętrznej siatkówce nie zawsze są specyficzne i stwierdza się je przede wszystkim w jaskrze co odróżnia ją od AD, gdzie istotne nieprawidłowości stwierdza się głównie w siatkówce zewnętrznej. Podsumowując analiza głębszych warstw siatkówki i gęstości naczyń w DVP może potencjalnie

poprawić możliwości diagnostyczne i stanowić cenne podejście w przewidywaniu rozwoju AD.

Wnioski

„**Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Alzheimer's Disease: A Comparison of Eyes of Patients with Alzheimer's Disease, Primary Open-Angle Glaucoma, and Preperimetric Glaucoma and Healthy Controls**"

1) Ocena pRNFL za pomocą OCT wykazała istotne obniżenie jej grubości wśród pacjentów z AD w stosunku do osób zdrowych, co potwierdza, że uszkodzeniom komórek nerwowych w OUN u pacjentów z AD towarzyszy uszkodzenie aksonów RGC.
2) Najbardziej zaawansowane zmiany w postaci obniżenia grubości pRNFL obserwowano u pacjentów z POAG, natomiast w przypadku osób z PPG nie wykazano istotnych różnic w stosunku do pacjentów z AD.
3) Obserwując łagodne zmniejszenie grubości pRNFL, którym nie towarzyszą odpowiednie zmiany w badaniu pola widzenia, w diagnostyce należy wziąć pod uwagę zarówno PPG, jak i AD.

„**Comparison of Retinal Microvasculature in Patients With Alzheimer's Disease and Primary Open-Angle Glaucoma by Optical Coherence Tomography Angiography"**

1) Badanie dowodzi, że AD i POAG są chorobami neurodegeneracyjnymi, które są związane ze zmianami w mikrokrążeniu siatkówki, lecz poziom i stopień uszkodzenia naczyń zależą od choroby pierwotnej.
2) W przypadku POAG choroba objawia się zmniejszeniem gęstości naczyń w SVP i RPC, natomiast w AD uszkodzenie naczyń w największym stopniu dotyczy DVP, gdzie naczynia są cieńsze, mają mniejszy przekrój, czyniąc je bardziej podatnymi na okluzję przez złogi nieprawidłowego Aβ.
3) Zastosowanie OCTA może okazać się pomocne w rozróżnieniu przyczyny uszkodzenia pRNFL, a z uwagi na brak baz normatywnych dla gęstości naczyń analiza stosunku całkowitej gęstości DVP do SVP jest prostym narzędziem diagnostycznym wskazującym chorobę

podstawową, będącą przyczyną zaburzeń w mikronaczyniach siatkówki.

„Quantitative assessment of retinal thickness and vessel density using optical coherence tomography angiography in patients with Alzheimer's disease and glaucoma"

1) Analiza obrazów OCT oraz OCTA wykazała, że w oczach z POAG występują istotne zmiany w siatkówce wewnętrznej, co objawia się spadkiem grubości IRL oraz obniżeniem gęstości naczyń w SVP, natomiast w oczach z AD patologiczne zmiany są najwyraźniej dostrzegalne w siatkówce zewnętrznej, w której stwierdza się spadek grubości IRL oraz obniżenie gęstości naczyń w DVP.
2) Pacjenci z AD nie wykazywali istotnych różnic w procentowej utracie grubości ORL oraz gęstości naczyń w DVP w całościowej analizie obrazu en face z wyjątkiem obszaru okołodołkowego, gdzie obserwowano istotnie większą procentową utratę gęstości naczyń w DVP, sugerując, że zmiany neurodegeneracyjne mogą być wtórne do zaburzeń mikrokrążenia.
3) W oczach pacjentów z POAG procentowa utrata grubości IRL była istotnie większa niż utrata gęstości naczyń w SVP w badanych obszarach, co może wskazywać, że apoptoza RGC jest procesem pierwotnym, któremu wtórnie towarzyszą zmiany w mikrokrążeniu siatkówki.

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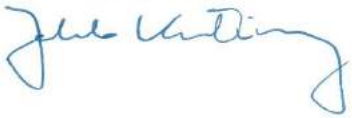

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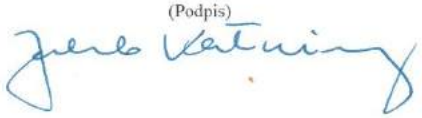

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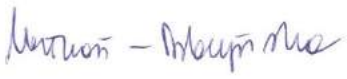

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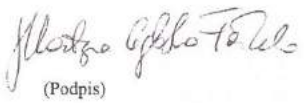

(Podpis)

Katarzyna Zabel
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Zabel P.
(Podpis)

Streszczenie

Rozprawa doktorska stanowi cykl powiązanych tematycznie czterech publikacji skupiających się na analizie porównawczej zmian zachodzących w strukturze i mikrokrążeniu siatkówki u pacjentów z chorobą Alzheimera (ang. Alzheimer's disease - AD) oraz z jaskrą pierwotnie otwartego kąta (ang. primary open-angle glaucoma - POAG). Głównym celem pracy była jakościowa oraz ilościowa ocena grubości poszczególnych warstw siatkówki i gęstości naczyń siatkówki w biegunie tylnym gałki ocznej u pacjentów z AD oraz POAG za pomocą optycznej koherentnej tomografii (OCT) a także angiografii OCT (OCTA).

Pierwsza praca poglądowa „Diagnosis of Alzheimer's Disease by Assessing Structural and Microvasculature Changes in the Retina Using Optical Coherence Tomography Angiography–a Review of Eye Biomarkers for Alzheimer's Disease" (Klinika Oczna/Acta Ophthalmologica Polonica, MNiSW= 40 pkt.) skupia się na przedstawieniu aktualnego stanu wiedzy na temat diagnostyki AD za pomocą nieinwazyjnych badań obrazowych siatkówki. Aktualnie diagnostyka AD opiera się głównie na ocenie funkcji poznawczych, a badania neuroobrazowe nadal są bardzo drogie i trudno dostępne. Wyjaśnia to przyczynę poszukiwania nowych, nieinwazyjnych i tanich biomarkerów. W fazie embriogenezy siatkówka oraz nerw wzrokowy rozwijają się jako bezpośrednie przedłużenie międzymózgowia, dlatego nieprawidłowości zachodzące w mózgu u pacjentów z AD można również obserwować na dnie oka. W badaniach pośmiertnych pacjentów z demencją typu Alzheimera udowodniono, że choroba poza uszkodzeniem komórek nerwowych cechuje się także patologią naczyniowo-mózgową. Stosując nowoczesne techniki obrazowania takie jak OCT a także OCTA wykazano istotne zmiany z strukturze oraz mikronaczyniach siatkówki. Niestety zmiany te u pacjentów z demencją obserwowane w obrazach OCT mogą być niespecyficzne i wspólne dla innych chorób neurodegeneracyjnych, jak np. w jaskrze. Niemniej jednak kombinowane pomiary zmian strukturalnych siatkówki oraz ocena

mikrokrążenia w poszczególnych splotach siatkówki z wykorzystaniem OCT oraz OCTA mogą zwiększać zdolność diagnostyczną AD.

W pracy pt. „ Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Alzheimer's Disease: A Comparison of Eyes of Patients with Alzheimer's Disease, Primary Open-Angle Glaucoma, and Preperimetric Glaucoma and Healthy Controls" (Medical Science Monitor, MNiSW= 70 pkt, IF= 1.918) wykorzystano OCT do oceny oraz porównania grubości okołotarczowej warstwy włókien nerwowych siatkówki (ang. peripapillary retinal nerve fiber layer - pRNFL) u pacjentów z AD, z jaskrą pierwotnie otwartego kąta (ang. primary open-angle glaucoma – POAG), jaskrą preperymetryczną (ang. preperimetric glaucoma – PPG), oraz u zdrowej grupy kontrolnej (ang. healthy controls – HC). Do badania przekrojowego włączono po 30 osób z każdej grupy. Analizie poddano jedno, losowo wybrane oko każdego uczestnika. Średnia grubość pRNFL u pacjentów z POAG wynosiła 60,97 ± 12,97 µm i była istotnie niższa niż w grupie HC (106,30 ± 8,95 µm), w oczach z PPG (93,20 ± 12,04 µm) i u pacjentów z AD (95,73 ± 13,52 µm). Średnia grubość pRNFL u pacjentów z AD była istotnie niższa w porównaniu z grupą HC i wyższa w porównaniu z oczami z POAG, natomiast nie było istotnych różnic w porównaniu z pacjentami z PPG (p >0,05). Badanie dowodzi, że uszkodzeniu komórek nerwowych w ośrodkowym układzie nerwowym (OUN) u pacjentów z AD towarzyszy również uszkodzenie aksonów komórek zwojowych siatkówki. Na podstawie OCT nie jest możliwe rozróżnienie przyczyny łagodnego zmniejszenia grubości pRNFL natomiast wydaje się, że ocena wyników OCT może być dodatkowym narzędziem wykorzystywanym w diagnostyce oraz monitorowaniu AD.

W publikacji pt. „Comparison of Retinal Microvasculature in Patients With Alzheimer's Disease and Primary Open-Angle Glaucoma by Optical Coherence Tomography Angiography" (Investigative Ophthalmology & Visual Science, MNiSW= 140 pkt, IF= 3.812) celem badań była ocena sieci mikronaczyń siatkówki u pacjentów z AD, POAG oraz w grupie HC. Do badania zakwalifikowano po 27 osób w każdej grupie, a ocenie poddano oko, w którm uzyskano lepszą jakość angiogramu. Badanie okulistyczne obejmowało OCTA,

którą wykorzystano do obrazowania sieci mikronaczyń w warstwie radialnej okołotarczowych kapilar (ang. radial peripapillary capillaries - RPC), a także w powierzchownym (ang. superficial vascular plexus - SVP) oraz głębokim splocie naczyniowym siatkówki (ang. deep vascular plexus - DVP). W oczach pacjentów z AD gęstość naczyń w DVP była istotnie zmniejszona, a powierzchnia dołkowej strefy beznaczyniowej uległa zwiększeniu w porównaniu z oczami pacjentów z POAG i w grupie HC ($p < 0,001$). Pacjenci z POAG mieli istotnie zmniejszoną gęstość naczyń w RPC i SVP w porównaniu do pozostałych badanych grup ($p < 0,001$). Średnia grubość pRNFL była skorelowana z gęstością naczyń w SVP u pacjentów z POAG (Pearson's $r = 0,66$; $p = 0,0002$) i była istotnie niższa w grupach POAG i AD niż w grupie HC ($p < 0,001$). AD i POAG to choroby neurodegeneracyjne związane z apoptozą komórek nerwowych i upośledzeniem mikronaczyń siatkówki, co można skutecznie oceniać za pomocą OCTA. Pomimo tego, że w obu chorobach nieprawidłowości stwierdzane sią w całym układzie naczyniowym siatkówki, upośledzenie sieci mikronaczyń w oczach z POAG dotyczy w głównej mierze naczyń powierzchownych, natomiast w AD naczyń położonych w głębszych warstwach siatkówki co może świadczyć o innej etiopatogenezie powstawania tych chorób.

Celem pracy pt. „Quantitative assessment of retinal thickness and vessel density using optical coherence tomography angiography in patients with Alzheimer's disease and glaucoma" (PLOS ONE, MNiSW= 100 pkt, IF= 2.740) była ocena i bezpośrednie porównanie gęstości naczyń siatkówki z grubością wewnętrznych (ang. inner retinal layer - IRL) i zewnętrznych warstw siatkówki (ang. outer retinal layer – ORL) w tych samych obszarach plamki u pacjentów z AD i POAG. Do obadania przekrojowego włączono 49 oczu z AD, 71 oczu z POAG oraz 48 oczu do grupy HC. W badaniu okulistycznym zastosowano OCT do pomiaru grubości IRL oraz ORL, a także OCTA w tym sam obszarze celem analizy gęstości naczyń w SVP i DVP. Pacjenci z AD wykazywali istotnie większą utratę gęstości naczyń w DVP oraz grubości ORL w porównaniu do pacjentów z POAG ($p < 0,001$), u których stwierdzono istotnie większą procentową utratę gęstości naczyń w SVP oraz grubości IRL w

porównaniu do pozostałych grup (p <0,001). Pozytywny związek między obecnością AD obserwowano głównie w siatkówce zewnętrznej, gdzie 1% spadek grubości ORL wiązał się z około 24–29% wzrostem prawdopodobieństwa wystąpienia AD. Analiza angiogramów wykazała, że 1% spadek gęstości naczyń w DVP był dodatnio powiązany z 4–9% wzrostem prawdopodobieństwa wystąpienia AD. W POAG pozytywny związek między obecnością choroby a procentową utratą grubości siatkówki i gęstości naczyń obserwowano tylko w IRL i w SVP. Również wykazano, że zmiany w układzie naczyniowym siatkówki w SVP i DVP były odpowiednio skorelowane z uszkodzeniem warstw IRL i ORL w oczach pacjentów z AD oraz POAG. Patologie stwierdzane w wewnętrznej siatkówce nie zawsze są specyficzne i stwierdza się je przede wszystkim w jaskrze co odróżnia ją od AD, gdzie istotne nieprawidłowości stwierdza się głównie w siatkówce zewnętrznej. Podsumowując analiza głębszych warstw siatkówki i gęstości naczyń w DVP może potencjalnie poprawić możliwości diagnostyczne i stanowić cenne podejście w przewidywaniu rozwoju AD.

W części końcowej zawarto podsumowania oraz najważniejsze wnioski wynikające z przeprowadzonych badań empirycznych. Wskazują również kierunki doskonalenia które w przyszłości mogą przyczynić się w istotny sposób do poprawy metod diagnostycznych chorób neurodegeneracyjnych.

Summary

The doctoral dissertation is a series of four thematically related publications focusing on a comparative analysis of changes in the structure and microcirculation of the retina in patients with Alzheimer's disease (AD) and primary open-angle glaucoma (POAG). The main aim of the study was the qualitative and quantitative assessment of the thickness of individual retinal layers and the density of the retinal vessels in the posterior pole of the eye in AD and POAG patients using optical coherence tomography (OCT) and OCT angiography (OCTA).

The first review paper "Diagnosis of Alzheimer's Disease by Assessing Structural and Microvasculature Changes in the Retina Using Optical Coherence Tomography Angiography – a Review of Eye Biomarkers for Alzheimer's Disease" (Klinika Oczna/Acta Ophthalmologica Polonica, MNiSW = 40 points) focuses on the presentation the current state of knowledge on the diagnosis of AD using non-invasive retinal imaging examinations. Currently, the diagnosis of AD is based mainly on the assessment of cognitive functions, because neuroimaging is still very expensive and difficult to access. This explains the rationale behind the search for new, non-invasive and cheap biomarkers. During the embryogenesis phase, the retina and the optic nerve develop as a direct extension of the diencephalon, so that brain abnormalities in AD patients can also be seen at the fundus. In postmortem studies of patients with Alzheimer's dementia, it has been proven that the disease, apart from damaging nerve cells, is also characterized by cerebrovascular pathology. Using modern imaging techniques such as OCT and OCTA, significant changes in the structure and microvessels of the retina have been demonstrated. Unfortunately, these changes in patients with dementia, observed in OCT images, may be non-specific and common to other neurodegenerative diseases, such as glaucoma. Nevertheless, combined measurements of structural changes in the retina and the assessment of microcirculation in individual retinal plexuses using OCT and OCTA may increase the diagnostic capacity of AD.

In the article "Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Alzheimer's Disease: A Comparison of Eyes of Patients with Alzheimer's Disease, Primary Open-Angle Glaucoma, and Preperimetric Glaucoma and Healthy Controls" (Medical Science Monitor, MNiSW = 70 points, IF = 1.918) OCT was used to assess and compare the thickness of the peripapillary retinal nerve fiber layer (pRNFL) in patients with AD, primary open-angle glaucoma (POAG), preperimetric glaucoma (PPG), and in healthy controls (HC). Thirty participants from each group were enrolled in the cross-sectional study. One randomly selected eye of each participant was analyzed. The mean thickness of pRNFL in patients with POAG was 60.97 ± 12.97 μm and was significantly lower than in the HC group (106.30 ± 8.95 μm), in eyes with PPG (93.20 ± 12.04 μm) and in patients with AD (95.73 ± 13.52 μm). The mean thickness of pRNFL in AD patients was significantly lower compared to the HC group and higher compared to the eyes with POAG, but there were no significant differences compared to the eyes with PPG ($p > 0.05$). The study shows that damage to the nerve cells in the central nervous system (CNS) in AD is also associated with damage to the axons of the retinal ganglion cells. On the basis of OCT, it is not possible to distinguish the cause of a mild reduction in pRNFL thickness, but it seems that the analisis of OCT results may be an additional tool used in the diagnosis and monitoring of AD.

In the publication "Comparison of Retinal Microvasculature in Patients With Alzheimer's Disease and Primary Open-Angle Glaucoma by Optical Coherence Tomography Angiography" (Investigative Ophthalmology & Visual Science, MNiSW = 140 points, IF = 3.812), the aim of the study was to evaluate the retinal microvascular network in patients with AD, POAG and in the HC group. Twenty-seven people in each group were qualified for the study, and the eye with the better quality of the angiogram was assessed. Ophthalmic examination included OCTA, which was used for the imaging of vascular network within the layer of radial peripapillary capillaries (RPC), and also in the superficial vascular plexus (SVP) and deep vascular plexus (DVP) of the retina. In the eyes of patents with AD, the vascular density in DVP was significantly reduced and the area of the foveal avascular zone increased

compared to the eyes of patients with POAG and in the HC group ($p < 0.001$). Patients with POAG had significantly reduced vascular density in RPC and SVP compared to the other studied groups ($p < 0.001$). The mean pRNFL thickness correlated with the vascular density in SVP in POAG (Pearson's $r = 0.66$; $p = 0.0002$) and was significantly lower in the POAG and AD groups than in the HC group ($p < 0.001$). AD and POAG are neurodegenerative diseases associated with apoptosis of nerve cells and impairment of retinal microvessels, which can be effectively assessed by OCTA. Despite the fact that in both diseases the abnormalities are found in the entire vascular system of the retina, the impairment of the microvascular network in the eyes with POAG mainly concerns superficial vessels, while in AD, the vessels located in the deeper layers of the retina, which may indicate a different etiopathogenesis of these diseases.

The purpose of article "Quantitative assessment of retinal thickness and vessel density using optical coherence tomography angiography in patients with Alzheimer's disease and glaucoma" (PLOS ONE, MNiSW = 100 points, IF = 2.740) was the assessment and a direct comparison of retinal vessel density with the thickness of inner retinal layer (IRL) and outer retinal layer (ORL) in the same regions of the macula in subjects with AD and POAG. Fourty-nine eyes with AD, 71 eyes with POAG and 48 eyes with HC were included in the cross-sectional examination. In the ophthalmic examination, OCT was used to measure the thickness of IRL and ORL, as well as OCTA in the same area to analyze the vascular density in SVP and DVP. Patients with AD showed significantly greater loss of vascular density in DVP and ORL thickness compared to patients with POAG ($p < 0.001$), who had significantly greater percent loss of vascular density in SVP and IRL thickness compared to the other groups ($p < 0.001$). A positive relationship between the presence of AD was mainly observed in the outer retina, where a 1% decrease in ORL thickness was associated with an approximately 24–29% increase in the likelihood of AD occurrence. Analysis of angiograms showed that a 1% decrease in vascular density in DVP was positively associated with a 4-9% increase in the likelihood of developing AD. In POAG, a positive association

between disease presence and percent loss of retinal thickness and vascular density was observed only in IRL and SVP. It was also shown that changes in the retinal vasculature in SVP and DVP were correlated appropriately with damage to the IRL and ORL layers in the eyes of AD and POAG patients. The pathologies found in the inner retina are not always specific and are found primarily in glaucoma, which distinguishes it from AD, where significant pathologies are found mainly in the outer retina. Overall, the analysis of deeper retinal layers and vascular density in DVP has the potential to improve diagnostic capabilities and represent a valuable approach in predicting the development of AD.

The final part contains summaries and the most important conclusions coming from the conducted of empirical research. They also indicate the directions of improvement that in the future may significantly contribute to the enhancements of diagnostic methods of neurodegenerative diseases.