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Dziedzina: nauki medyczne i nauki o zdrowiu

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ROZPRAWA DOKTORSKA

Ocena przydatności białek stanu zapalnego w diagnostyce choroby Alzheimera

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Rozprawa doktorska została zrealizowana w ramach kształcenia w Szkole Doktorskiej UMB

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Pracę tę dedykuję wszystkim osobom z chorobą Alzheimera.

Składam najserdeczniejsze podziękowania:

***Mojej promotor, Pani Prof. dr. hab. Barbarze Mroczko** oraz*

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Spis treści

1. Wykaz publikacji będących podstawą rozprawy doktorskiej

1.1 Praca Przeglądowa

P1. Doroszkiewicz J, Mroczko P, Kulczyńska-Przybik A. Inflammation in the CNS: Understanding Various Aspects of the Pathogenesis of Alzheimer's Disease. Curr Alzheimer Res. 2022;19(1):16-31. doi:10.2174/1567205018666211202143935

IF: **2,1** MNiSW: **100**

1.2 Prace Oryginalne

P2. Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Borawska R, Zajkowska M, Słowik A, Mroczko B. Potential Utility of Cerebrospinal Fluid Glycoprotein Nonmetastatic Melanoma Protein B as a Neuroinflammatory Diagnostic Biomarker in Mild Cognitive Impairment and Alzheimer's Disease. *J Clin Med*. 2023;12(14):4689. Published 2023 Jul 14. doi:10.3390/jcm12144689

IF: **3,0** MNiSW: **140**

P3. Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. Associations between microglia and astrocytic proteins and tau biomarkers across the continuum of Alzheimer’s disease. Int J Mol Sci. 2024;25(14):7543. Published 2024 Jul 9. doi:10.3390/ijms25147543

IF: **4,9** MNiSW: **140**

Łączna wartość Impact Factor dla cyklu publikacji: 10

Łączna liczba punktów MNiSW dla cyklu publikacji: 380

2. Zestawienie publikacji

Rodzaj publikacji	**Liczba**	**Impact Factor**	**Punktacja MNiSW**
Prace włączone do rozprawy doktorskiej	3	10	380
Prace, które nie zostały włączone do rozprawy doktorskiej	8	39.008	980
Streszczenia zjazdowe	11		
Razem	**22**	**49.008**	**1500**

3. Wykaz skrótów

AD - choroba Alzheimera

APP - białko prekursorowe amyloidu

Aβ - amyloid beta

CTRL – grupa kontrolna

CV - współczynnik wariancji

ES - Erlangen Score

GFAP - kwaśne białko włókienkowe

GPNMB - Glikoproteina, białko czerniaka nieprzerzutowego - Glycoprotein nonmetastatic melanoma protein B

IFN-β - interferon beta

LCN2 - lipokalina-2 (lipocalin-2)

LPS - lipopolisacharydy

MCI - Łagodne zaburzenia poznawcze (Mild Cognitive Impairment)

MMSE - Mini Mental State Examination

MRI - rezonans magnetyczny

NET - zewnątrzkomórkowe pułapki neutrofili

NGAL - lipokalina związana z żelatynazą neutrofilów (neutrophil gelatinase-associated lipocalin)

NINCDS-ADRDA - Narodowy Instytut Zaburzeń Neurologicznych i Komunikacyjnych oraz Udaru mózgu – Stowarzyszenie Chorób Alzheimera i Pokrewnych Zaburzeń

OUN – ośrodkowy układ nerwowy

PMR - Płyn mózgowo-rdzeniowy

pTau181 - fosforylowana forma tau 181

QAlb - współczynnik albuminowy

TK - tomografia komputerowa

t-tau – całkowite białko tau

4. Wstęp

Choroba Alzheimera (AD – Alzheimer's disease) jest jedną z najczęstszych przyczyn demencji na świecie. To progresywna i nieuleczalna choroba, dotykającą głównie osoby powyżej 65. roku życia. W związku ze starzejącym się społeczeństwem co roku obserwujemy wzrost zachorowań. W 2020 roku szacowało się, że ok. 55 milionów ludzi na świecie jest dotkniętych chorobą Alzheimera. WHO zakłada, że ta liczba będzie się podwajać co 20 lat i w 2050 roku może wynosić prawie 140 milionów osób chorujących na tę chorobę [1]. Zaburzenie to stanowi ogromne obciążenie nie tylko dla osób dotkniętych AD, ale także dotyka ich krewnych, opiekunów i systemy opieki zdrowotnej. Jest to choroba przewlekła, w której procesy patologiczne rozpoczynają się nawet 20 lat przed manifestacją kliniczną choroby [2]. Ze względu na stopień zauważalnych zmian w mózgu oraz objawów choroby Alzheimera można podzielić ją na kilka faz:

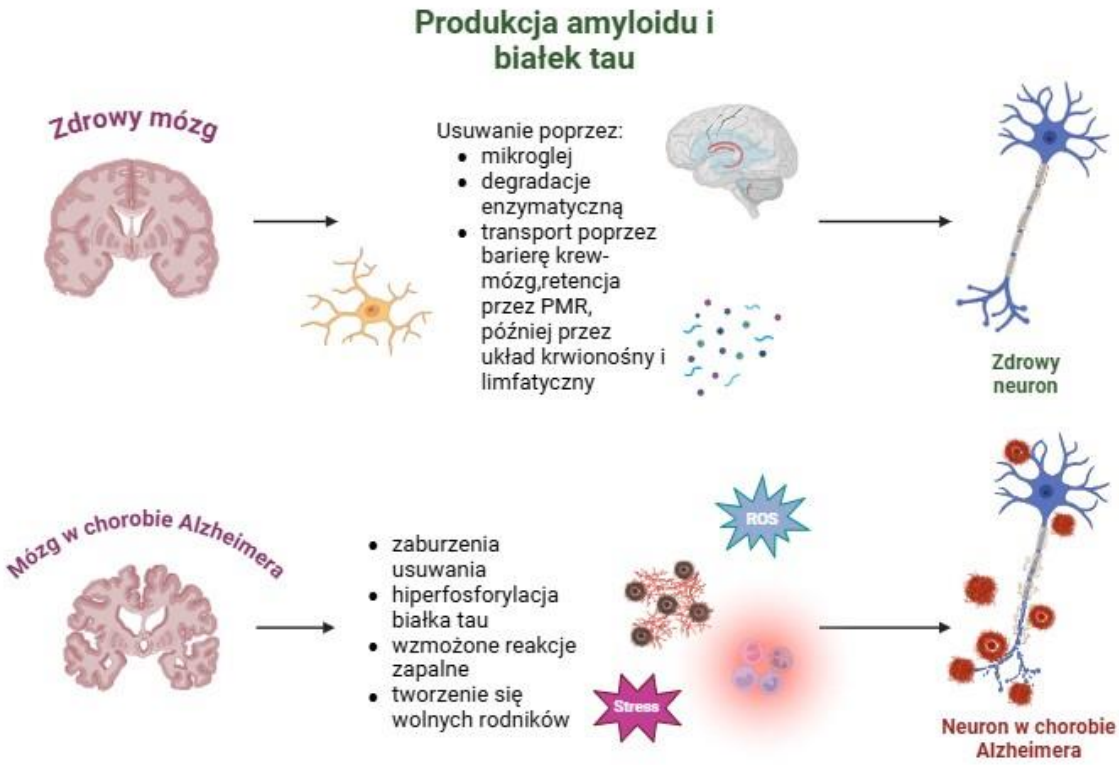
- Faza przedobjawowa (przedkliniczna) – powstawanie pierwszych patologii w obrębie mózgu, przy czym brak jest objawów klinicznych;
- Łagodne zaburzenia poznawcze (MCI - Mild Cognitive Impairment) – występuje pogorszenie procesów poznawczych, jednak bez widocznych oznak otępienia, pacjent zdolny do samodzielnego funkcjonowania;
- Faza demencji – faza objawowa, pacjent często niezdolny do samodzielnego funkcjonowania, spełnia wszystkie kryteria rozpoznania choroby Alzheimera [3].

Pomimo znacznych postępów w diagnostyce chorób neurodegeneracyjnych nadal nie ma specyficznych biomarkerów odzwierciedlających mechanizm molekularny, leżący u podstaw AD. Stąd też niezwykle istotne jest znalezienie substancji, które pomogą wykryć chorobę na jak najwcześniejszym etapie rozwoju i potencjalnie przyczynią się do optymalizacji leczenia przyczynowego.

4.1. Etiologia choroby Alzheimera

Etiologia choroby Alzheimera nie jest do końca poznana, aczkolwiek udało się zidentyfikować kilka kluczowych czynników, które w znacznym stopniu przyczyniają się do jej rozwoju. Uznaje się, że udział w jej powstawaniu mogą mieć czynniki środowiskowe, jak i genetyczne. Jednakże przypadki genetycznej patofizjologii choroby stanowią mniej niż 5% stwierdzonych przypadków AD i dotyczą kilku mutacji

genowych: preseniliny 1, preseniliny 2, białka prekursorowego amyloidu (APP) oraz allelu ε4 genu APOE [4,5]. Większość stwierdzanych przypadków choroby ma charakter sporadyczny i pojawia się po 65. roku życia. Do histopatologicznych cech charakterystycznych AD należą: blaszki pozakomórkowe zbudowane z amyloidu β i zwyrodnienie włóknikowe, których podstawę stanowi hiperfosforylowane białko tau. Oba wstępują również w mózgach osób starszych, bez stwierdzonej demencji, jednak są to dużo mniejsze ilości niż u pacjentów z AD [6]. Najbardziej toksycznymi cząsteczkami, które biorą udział w patologii AD, są nierozpuszczalne peptydy amyloidu beta (Aβ-42). Układ immunologiczny może je rozpoznać jako nieznane i wywołać reakcję zapalną w celu ich usunięcia. Powstają one na skutek proteolitycznego cięcia białka prekursorowego amyloidu poprzez kilka sekretaz [7]. Odkładanie się i akumulacja nieprawidłowo powstałych białek (w tym przypadku Aβ-42) zapoczątkowuje kaskadę procesów, między innymi neurozapalenia, które prowadzi do uszkodzenia i niszczenia komórek ośrodkowego układu nerwowego (OUN) [8,9]. Organizm człowieka jest wyposażony w mechanizmy dążące do usunięcia złogów amyloidu z mózgu. Są nimi procesy eliminacyjne występujące w mikrogleju, degradacja enzymatyczna, transport poprzez barierę krew-mózg oraz retencja przez płyn mózgowo-rdzeniowy (PMR) oraz usunięcie poprzez układ krwionośny i limfatyczny [10]. Zaburzenia tych procesów prowadzą do wzmożonego odkładania się amyloidu w blaszkach. Amyloid beta może również mieć wpływ na białko tau powodując jego hiperfosforylację poprzez modyfikowanie aktywności kinaz i fosfataz. Różnice pomiędzy prawidłowymi procesami a tymi zachodzącymi u pacjentów z AD zostały przedstawione na rycinie 1.



Rycina 1. – Procesy odpowiedzialne za usuwanie tworzących się białek amyloidu i tau oraz zaburzenia tych procesów u pacjentów z chorobą Alzheimera. Stworzone w Biorender.com

Kolejną cechą charakterystyczną choroby Alzheimera jest występowanie zwyrodnienia włóknikowego związanego z hipersfosforylowanym białkiem tau, które jest odpowiedzialne za utrzymanie struktury mikrotubul w neuronach [11]. Obniżenie powiązania, a co za tym idzie łączenia się tau z mikrotubulami, związane jest z późniejszą dysfunkcją synaps. Co więcej, tau podlega wielu modyfikacjom potranslacyjnym takim jak acetylacja, glikacja, nitracja, czy też najważniejsza w przebiegu AD - hiperfosforylacja [12]. Zaburzona równowaga pomiędzy kinazą a fosfatazą tau powoduje zwiększoną fosforylację tego białka i odkładanie się w postaci zwyrodnienia włóknikowego u pacjentów z chorobą Alzheimera. W wyniku tych zmian dochodzi do osłabienia plastyczności synaptycznej, co prowadzi do uszkodzenia i śmierci komórek nerwowych [13,14].

4.2. Rola neurozapalenia w rozwoju choroby Alzheimera

Aspektem, na który w ostatnich latach mocno zwraca się uwagę w kontekście patologii choroby Alzheimera, jest występujące w jej przebiegu neurozapalenie.

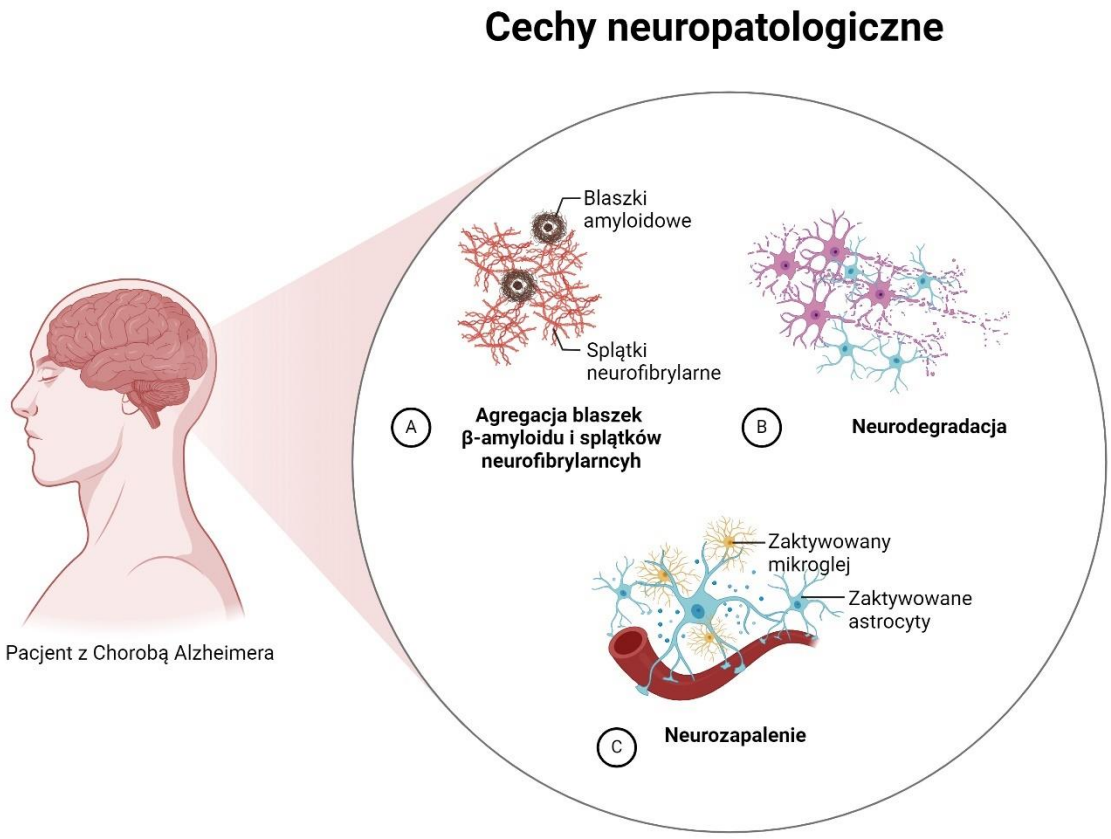
Powodujące ten stan mechanizmy ciągle nie są w pełni poznane, mimo że temat jest badany już od ponad 20 lat. Liczne analizy genetyczne i immunologiczne wykazały istotny związek pomiędzy stanem zapalnym a patologią AD [15].

Mikroglej są to makrofagi tkanki mózgowej, które odgrywają rolę głównie ochronną [16]. Podczas rozwoju płodowego odpowiedzialny jest on za usuwanie nadmiaru połączeń synaptycznych w mózgu, podczas gdy w pełni rozwiniętym mózgu odpowiada za remodelowanie obwodów neuronalnych [17]. Mikroglej może przejść do swojej aktywnej formy poprzez szereg różnych czynników wyzwalających, takich jak: uraz, cytokiny prozapalne czy utrata homeostazy jonowej. Po aktywacji jest on zdolny do uwalniania czynników cytotoksycznych, wolnych rodników, ale też cytokin prozapalnych, na przykład TNF-α [18]. Aczkolwiek należy pamiętać, że nie tylko w ten jeden sposób manifestuje się aktywacja mikrogleju. Można ją wstępnie podzielić na dwa rodzaje: M1 – klasyczny i M2 – alternatywny. Klasyczna aktywacja jest powodowana między innymi przez lipopolisacharydy (LPS), TNF-α czy IFN-γ, które powodują udział mikrogleju M1 w mechanizmach obronnych przed patogenami poprzez wydzielanie cytokin prozapalnych np. IL-1β, TNF-α czy IL-6. Z kolei fenotyp M2 jest indukowany poprzez IL-4 i IL-13, co powoduje uwalnianie molekuł neuroprotekcyjnych, np. TGF-β, IL-10 i IGF-1 [19]. Poprzez balansowanie pomiędzy stanami M1 i M2 mózg jest w stanie usprawnić przebudowę i naprawę tkanki mózgowej. Zmiana stanów z M1 na M2 może być bardzo szybka i niespodziewana [20,21]. Z drugiej strony, istnieją publikacje wykazujące wpływ zaktywowanego mikrogleju na rozwój choroby Alzheimera. Aktywny mikroglej bierze udział w wiązaniu się z płytkami Aβ poprzez kilka receptorów, takich jak CD14, CD36 i receptory Toll-podobne, TLR4 i TLR2, które aktywują mikroglej i stymulują wzmożone uwalnianie przez niego czynników prozapalnych [15,22]. Co więcej, aktywowany mikroglej kumulujący się wokół płytek starczych, wytwarza środowisko prozapalne, co sprzyja tworzeniu i powiększaniu się blaszek amyloidowych [23,24].

Podobnie do mikrogleju, również astrocyty mogą wejść w stan aktywacji, podczas którego przechodzą szereg zmian morfologicznych i funkcjonalnych. W swojej aktywnej formie charakteryzują się one zwiększoną objętością komórki, jak i nadmiernym wydzielaniem neurotoksycznych substancji. Wydzielają również w zwiększonej ilości wimetynę, która powoduje powiększanie komórek astrocytów, i kwaśne białko włókienkowe (GFAP). Pomimo swojej funkcji neuroprotekcyjnej, mogą również tworzyć

środowisko zapalne poprzez uwalnianie szeregu cytokin prozapalnych, takich jak TNF-α, IL-6 czy IL-12 [25,26]. Badania sugerują, że astrocyty mogą gromadzić się w pobliżu blaszek amyloidowych, co sprzyja ich aktywacji przez amyloid. Zostało to potwierdzone przy użyciu modeli zwierzęcych AD, jak i pośmiertnych badań mózgów pacjentów chorujących na tę chorobę [25,27]. Prawdopodobnie istnieje kilka mechanizmów udziału astrocytów w neurozapaleniu. Jednym z nich może być aktywacja astrocytów przez NFκB, uwalniający białko dopełniacza C3, które wiąże neuronalny C3aR. NFκB kontroluje funkcję i trwałość neuronów, co dowodzi roli astrogleju w uszkodzeniu neuronów [33, 34]. Jednak może on też odgrywać rolę neuroprotekcyjną. Poprzez kumulowanie się wokół blaszek starczych, w celu ograniczenia szkód przez nie spowodowanych, tworzy swojego rodzaju barierę nazywaną blizną glejową i inflitruje blaszkę Aβ. Co więcej, badania przeprowadzone in situ i in vitro wykazały, że reaktywne astrocyty wykorzystują fagocytozę do eliminacji lub zmniejszenia złogów Aβ [28,29].

Neutrofile to białe krwinki, które działają na pierwszej linii wczesnej odporności wrodzonej poprzez fagocytozę, uwalnianie zewnątrzkomórkowych pułapek neutrofili (NET) i wytwarzanie reaktywnych form tlenu [30]. W AD występują zaburzenia w działaniu neutrofili. Badania na mysich modelach AD wykazały obecność neutrofili wokół blaszek amyloidowych, ale też ich nadmierną aktywność spowodowaną amyloidem β. Co więcej, inhibicja neutrofili poprzez blokowanie integryny LFA-1 we wczesnych stadiach demencji poprawiła pamięć u mysich modeli AD [31]. Z kolei inne badania pokazały, że białka wydzielane przez te komórki mogą mieć funkcje neurotoksyczne, jak i neuroprotekcyjne. CAP37, katepsyna G i elastaza neutrofilowa, uznawane za neuroprotekcyjne, wykazały pozytywny wpływ na dezintegrację i późniejsze usuwanie blaszek amyloidowych [32]. Procesy neuropatologiczne, występujące w AD, związane z neurozapeleniem, zostały przedstawione na rycinie 2. Bardziej szczegółowy opis neurozapalenia i jego mechanizmów oraz białek z nim związanych został opisany w pracy przeglądowej (P1), stanowiącej część rozprawy doktorskiej.



Rycina 2. – Procesy neuropatologiczne w przebiegu choroby Alzheimera. Stworzone w Biorender.com

4.3 Diagnostyka choroby Alzheimera

Rozpoznanie choroby Alzheimera stawia się na podstawie wywiadu lekarskiego, badania fizykalnego, neurologicznego, ale również na podstawie obrazowania mózgu i badań laboratoryjnych. Najpopularniejszym testem psychologicznym wykorzystywanym w ocenie zaburzeń funkcji poznawczych jest Mini Mental State Examination (MMSE) [33]. Ważna jest również diagnostyka różnicowa AD z innymi zaburzeniami neurologicznymi, stąd też istotne jest wykonanie badań biochemicznych, tj. stężenia witaminy B12, kwasu foliowy czy hormonów tarczycy, ale również obrazowania mózgu przy użyciu rezonansu magnetycznego, tomografii komputerowej czy też pozytonowej tomografii emisyjnej. W codziennej praktyce klinicznej rozpoznanie stawia się na podstawie kryteriów diagnostycznych m.in. Narodowego Instytutu Zaburzeń Neurologicznych i Komunikacyjnych oraz Udaru mózgu – Stowarzyszenia Chorób Alzheimera i Pokrewnych Zaburzeń (NINCDS-ADRDA), Podręcznika Diagnostycznego

i Statystycznego Zaburzeń Psychicznych (DSM-IV) oraz Międzynarodowej Klasyfikacji Chorób (ICD-10) [34–36]. Według tych kryteriów pacjentów charakteryzują postępujące zaburzenie funkcji poznawczych takich jak uczenie się z współwystępującymi zaburzeniami pamięci świeżej, czyli dotyczącej niedawnych zdarzeń [37]. Identyfikacja tych podstawowych symptomów w procesie diagnostycznym powinna być połączona z biomarkerami biochemicznymi albo obrazowymi. Kryteria oceny biochemicznej chorych na AD zostały określone na podstawie zaleceń National Institute on Aging Alzheimer's Association (NIA-AA), które uznają stężenia biomarkerów oznaczanych w PMR [38]. Ciągły rozwój biomarkerów biochemicznych może pozwolić na dokładniejszą diagnostykę, a nawet przewidywanie choroby na wiele lat przed jej wystąpieniem, a także monitorowanie postępu choroby.

4.4. Markery biochemiczne

Zgodnie z definicją biomarker powinien wskazywać obiektywne i wymierne cechy procesów biologicznych, które mogą powiedzieć nam o stanie pacjenta lub przewidywać występowanie skutków toczących się procesów albo chorób [39]. Aby usprawnić proces diagnostyczny choroby Alzheimera do praktyki klinicznej wprowadzono biomarkery oznaczane w PMR, który przez swoją lokalizacje najlepiej ukazują procesy występujące w mózgu. W rutynowej diagnostyce stosuje się obecnie oznaczenia stężeń Aβ1-42, współczynnik Aβ1-42/ 1-40, całkowitego Tau (t-tau) i fosforylowanej formy tau 181 (pTau181). Podstawowe biomarkery AD w płynie mózgowo-rdzeniowym zwiększają dokładność diagnostyczną rozpoznawania tej choroby, szczególnie w innych przypadkach, jak na przykład prodromalna faza choroby. Co więcej, biomarkery pozwalają na rozróżnienie AD od innych zaburzeń psychicznych. W związku z tym ciągle poszukuje się nowych, dokładniejszych i jak najwcześniej występujących w przebiegu choroby biomarkerów. Dlatego też wczesna diagnostyka AD w oparciu o biomarkery może również znaleźć zastosowanie w opracowywaniu nowych metod leczenia przyczynowego. Dane literaturowe wskazują, że ocena biomarkerów neurozapalenia może być bardzo pomocna we wczesnym diagnozowaniu AD [15,40].

4.4.1. GPNMB

Glikoproteina, białko czerniaka nie przerzutowego - Glycoprotein nonmetastatic melanoma protein B (GPNMB; znana również pod nazwą osteoaktywina) jest białkiem związanym z zapaleniem, łączonym z chorobą Alzheimera. Po raz pierwszy została

zidentyfikowana w liniach komórkowych czerniaka o niskim stopniu przerzutów w roku 1995 [41]. Pomimo swojej nazwy, wiele badań wskazywało na związek tej glikoproteiny z zapaleniem, co skierowało wielu badaczy tego zagadnienia do przestudiowania jej właściwości w tym właśnie stanie. Jedno z badań wykazało, że nadekspresja GPNMB w makrofagach zmniejszała wytwarzanie cytokin prozapalnych in vitro [42]. Ponadto, sugeruje się, że czynniki prozapalne mogą mieć wpływ na ekspresje GPNMB w mózgu [43]. Dowiedziono, że podwyższone stężenie tej glikoproteiny występuje w mózgach u osób z różnymi chorobami neurodegeneracyjnymi, między innymi w AD, chorobie Parkinsona czy stwardnieniu zanikowym bocznym (ALS) [44–46]. Niemniej jednak wpływ wzrostu stężenia GPNMB na patofizjologię tych chorób nie został jeszcze dokładnie wyjaśniony. Według dostępnych danych, GPNMB wydaje się odgrywać rolę neuroprotekcyjną. Jednak ten mechanizm nadal nie jest w pełni poznany. Ostatnie publikacje wykazały, że GPNMB promuje polaryzację makrofagów do typu „M2", który określa się jako bardziej ochronny ze względu na uwalnianie cytokin przeciwzapalnych, takich jak IL-10 i TGF-β [44,47,48]. Zatem GPNMB może prawdopodobnie działać jako czynnik wpływający na pamięć i plastyczność synaptyczną lub działać jako środek neuroprotekcyjny w podobny sposób do karotenoidów [49], flawonoidów [50], koenzymu Q10 [51] czy luteiny [52].

4.4.2. YKL-40

YKL-40 to lektyna wiążąca chitynę należąca do rodziny hydrolaz glikozylowych 18, znana również jako białko 1 podobne do chitynazy 3 (CHI3L1) lub ludzka glikoproteina chrząstki 39 (HC-gp39) [53]. Wiele typów komórek, w tym makrofagi, chondrocyty, neutrofile i fibroblasty maziowe, wykazują ekspresję białka YKL-40 [54]. Jest ona związana z odpowiedzią zapalną i bierze udział w przebudowie macierzy zewnątrzkomórkowej [54]. Ponadto, w stanach neurozapalenia w astrocytach występuje obfita ekspresja białka YKL-40. Transkrypcja tego białka może być indukowana przez cytokiny uwalniane przez makrofagi, co prowadzi do zmian morfologicznych oraz zmienionej ruchliwości astrocytów [53]. Wykazano również, że zwyrodnienie włóknikowe i blaszki amyloidowe są powiązane ze zwiększoną syntezą YKL-40 przez aktywowane astrocyty i/lub mikroglej [55].

4.4.3 NGAL

NGAL, czyli lipokalina związana z żelatynazą neutrofilów (neutrophil gelatinase-associated lipocalin) znana również jako lipokalina-2 (lipocalin-2, LCN2) albo syderocalina, pochodzi głównie z neutrofili, ale jej ekspresję odkryto też m.in. w komórkach kanalikowych nerek, serca, płuc i komórkach dendrytycznych. Główne zastosowanie znalazła w ocenie funkcjonowania różnych zaburzeń nerek [56]. Na podstawie danych literaturowych wykazano, że jest ona wydzielana poprzez aktywowany mikroglej i astrocyty u pacjentów z AD [57]. Wyniki badań wskazują, że lipokalina może wpływać na procesy neurobiologiczne, w tym stan zapalny, sygnalizację śmierci i przeżycia komórek, a także metabolizm żelaza. W OUN lipokalina indukuje insulinooporność, aktywuje gliozę i nasila śmierć neuronów [58–60]. NGAL może także ułatwiać przedostawanie się neutrofili i makrofagów do mózgu oraz stymulować prozapalną aktywację komórek glejowych [61]. Dekens et al. opisali podwyższone poziomy NGAL w hipokampie i ciele migdałowatym u pacjentów z AD. Ponadto autorzy wykazali kolokalizację NGAL z mikroglejem i neuronami [62]. Dodatkowo, oznaczenie stężenia tego białka pozwala z dużą dokładnością na odróżnienie otępienia naczyniowego od AD [63].

4.4.4. CXCL-11

Kolejnym białkiem, wydzielanym przez aktywowany mikroglej, jest mała cząsteczka rodziny chemokin CXC – CXCL11. Po raz pierwszy odkryto go w astrocytach myszy leczonych interferonem beta (IFN-β). Wykazano, że ludzkie astrocyty i płodowy mikroglej mogą być stymulowane do wytwarzania białka CXCL11 przez sam IFN-Y lub w połączeniu z interleukiną (IL)-1 [64]. Jednakże, ta chemokina cechuje się również wysoką ekspresją w elementach układu pokarmowego, takich jak wątroba, trzustka, a w mniejszych ilościach w jelicie cienkim [65]. Podwyższony poziom CXCL11 stwierdzono w zakażonych podskórnie mózgach myszy i płynie mózgowo-rdzeniowym pacjentów z chorobami neurozapalnymi, takimi jak bakteryjne zapalenie opon mózgowo-rdzeniowych i wirusowe zapalenie opon mózgowo-rdzeniowych [66,67].

4.4.5. sTREM1 i sTREM2

TREM1 czyli triggering receptor expressed on myeloid cells-1, należy do nadrodziny immunoglobulin i jest wydzielany głównie przez mikroglej, komórki mieloidalne, w tym neutrofile, makrofagi i monocyty [68]. TREM1 odgrywa znaczącą

rolę w wywoływaniu i zaostrzaniu reakcji zapalnych, także w OUN. Niektóre badania wykazały, że bierze on udział w rozwoju wielu chorób zakaźnych, jak i chorób autoimmunologicznych, nowotworów złośliwych i chorób neurodegeneracyjnych [69,70], jednak wciąż niewiele wiadomo na temat jego roli w AD. Celem określenia stężeń tego receptora oznacza się jego rozpuszczalną formę czyli sTREM1 w płynach biologicznych, co zostało przedstawione w publikacji będącej częścią niniejszej rozprawy.

Podczas gdy wiele publikacji opisuje TREM1 jako wskaźnik prozapalny, TREM2 jest znany jako inhibitor zapalenia i uważany za czynnik neuroprotekcyjny w OUN [70]. Podobnie jak TREM1, jest wydzielany przez mikroglej. Badania wykazały, że rzadki wariant genu TREM2 zwiększa podatność na zachorowanie na chorobę Alzheimera porównywalnie do allelu ε4 genu APOE [71]. Ponadto wykazano, że sTREM2 może zwiększać syntezę białka TREM2 i przeżycie mikrogleju poprzez stymulację syntezy wrodzonych składników odporności [72]. TREM2 jest niezbędny, aby mikroglej mógł wykrywać sygnały neurodegeneracyjne i reagować na nie [71].

5. Cel pracy

Pomimo istniejących w literaturze danych dotyczących roli neurozapalenia w patogenezie AD, brakuje jak dotychczas prac oceniających zależność stężenia białek stanu zapalnego w kontinuum patologii amyloidu beta, czy też białka Tau oraz analizy zmian stężeń wyżej wymienionych białek w zależności od stadium rozwoju choroby. Stąd też celem niniejszej rozprawy było:

1. Zbadanie wybranych białek związanych z procesem zapalnym (pro- i przeciwzapalnych) tj. NGAL, YKL-40, CXCL11, sTREM1, sTREM2 i GPNMB u pacjentów z zaburzeniami funkcji poznawczych (w tym osób chorych na chorobę Alzheimera (AD) i łagodne zaburzenia funkcji poznawczych (MCI)) w odniesieniu do stężeń tych białek w grupie osób bez zaburzeń poznawczych.

2. Porównanie stężeń badanych białek ze wskaźnikami nasilenia zaburzeń funkcji poznawczych oraz stężeniami klasycznych biomarkerów AD tj. amyloidu Aβ1-42, współczynnika Aβ1-42/ 1-40, całkowitego białka tau i jego fosforylowanej formy (pTau 181) w różnych stadiach choroby.

3. Analiza przydatności klinicznej badanych białek jako potencjalnych biomarkerów mogących mieć zastosowanie w diagnozowaniu oraz ocenie progresji AD.

6. Materiał i metody

6.1. Materiał

Pacjenci byli diagnozowani i rekrutowani w Katedrze Neurologii Uniwersytetu Jagiellońskiego w Krakowie. Badaniem objęto 80 osób (55 kobiet i 25 mężczyzn), tj.: 42 pacjentów z AD (33 kobiet i 9 mężczyzn), 18 z MCI (11 kobiet i 7 mężczyzn) i 20 osób bez zaburzeń poznawczych (12 kobiet i 8 mężczyzn). Płyn mózgowo-rdzeniowy pobierany był jednorazowo podczas rutynowych badań diagnostycznych. Po pobraniu PMR został odwirowany, podzielony do eppendorfów i zamrożony w temperaturze -80°C do czasu przeprowadzenia badań. Badania przeprowadzano w Zakładzie Diagnostyki Chorób Neurozwyrodnieniowych po uzyskaniu zgody Komisji Bioetycznej No. R-I-002/103/2019.

U wszystkich pacjentów włączonych do badania przeprowadzono badania neurologiczne, obrazowe (tj. tomografii komputerowej (TK) albo rezonansu magnetycznego (MRI) mózgu), rutynowe badania diagnostyczne we krwi i PMR, jak również oceniono zaburzenia funkcji poznawczych za pomocą testów neuropsychologicznych. Diagnozę AD i MCI postawiono na podstawie kryteriów National Institute on Aging oraz Alzheimer's Association (NIA-AA) [34]. W celu dokładniejszej stratyfikacji klinicznej pacjentów z AD i MCI badania neuroobrazowe i neuropsychologiczne połączono z wynikami badań biochemicznych (ocena stężeń Aβ1-42, Tau i pTau181 oraz współczynnika Aβ1-42/Aβ1-40). Do interpretacji biomarkerów w płynie mózgowo-rdzeniowym zastosowano algorytm Erlangen (Erlangen Score-ES). Do grupy badanej AD włączono wyłącznie pacjentów z 4 punktami w skali Erlangen. Pacjenci z 2 lub 3 punktami w ES zostali sklasyfikowani jako MCI. Kryteria wykluczenia z grupy badanej stanowiły: podejrzenie choroby naczyń mózgowych, podwyższony wynik współczynnika albuminowego (QAlb), sugerujący dysfunkcję bariery krew-płyn mózgowo-rdzeniowy lub zmiany w badaniach obrazowych tj. TK/MRI. Charakterystyka biochemiczna uczestników badania na podstawie stężeń klasycznych biomarkerów choroby Alzheimera i parametrów płynu mózgowo-rdzeniowego została przedstawiona w sekcji materiały i metody lub w tabelach każdego opublikowanego artykułu (P2-3). Za pomocą testu MMSE oceniono ciężkość zaburzeń funkcji poznawczych. Grupę kontrolną stanowili pacjenci z nawracającymi bólami głowy oraz osoby które nie miały subiektywnych zaburzeń pamięci i niespełniały kryteriów MCI. Dokładne badanie osób

kontrolnych, ze szczegółową analizą płynu mózgowo-rdzeniowego, pozwoliło wykluczyć organiczne tło objawów. Pacjenci z grupy kontrolnej nie wykazywali żadnych istotnych zmian w klasycznych biomarkerach choroby Alzheimera (tj. stężeniu Aβ1-42, Tau, pTau181 i współczynniku Aβ1-42/Aβ1-40). Wszyscy pacjenci zakwalifikowani do grupy kontrolnej uzyskali 0 punktów w skali Erlangen.

6.2. Metody

Klasyczne biomarkery AD zostały ocenione metodą immunoenzymatyczną ELISA, która jest metodą immunologiczną i polega na pomiarze badanego analitu za pomocą kompleksu antygen-przeciwciało. Zestawy, którymi badano stężenia białek, wykorzystywały kanapkowy wariant testu ELISA, oparty na dwóch przeciwciałach (wychwytujących i wykrywających).

Stężenia biomarkerów AD mierzono w płynie mózgowo-rdzeniowym przy użyciu komercyjnych zestawów firmy IBL do oznaczania Aβ1–42 i Aβ1–40 (RE59661, RE59651, Hamburg, Niemcy) oraz zestawów firmy Fujirebio (81572, 81574, Gent, Belgia) do oznaczania białek tau i pTau181. Wszystkie etapy oznaczania i analizy przeprowadzono zgodnie z instrukcjami producentów zestawów. Intensywność kolorymetryczną reakcji dla każdego białka mierzono przy użyciu czytnika mikropłytek (Diasorin EtiMax 3000 i Synergy 2 BioTek). Stężenia badanych białek odczytano przy pomocy krzywej standardowej. Wszystkie próbki i standardy analizowano w duplikatach ze współczynnikiem wariancji (CV) <20%. Próbki z wyższym CV niż limit zostały wyłączone z badania.

Badanie stężeń białka NGAL przeprowadzono za pomocą zestawu ELISA, firmy R&D Systems, Abingdon, Wielka Brytania. Do oznaczeń YKL-40 wykorzystano także test immunoenzymatyczny MicroVue, firmy Quidel (San Diego, CA, USA). Z kolei ocenę stężeń białek CXCL11, sTREM1, sTREM2 i GPNMB przeprowadzono przy pomocy techniki multipleksingu na analizatorze Luminex 200 przy użyciu zestawu Luminex Human Discovery z firmy R&D Systems (Abingdon, UK). Metoda multipleksingu polega na wykorzystaniu mikrokulek, na których tworzą się kompleksy immunologiczne. Każda kulka jest znakowana wewnętrznie barwnikami fluorescencyjnymi o innym stężeniu, dzięki czemu posiada indywidualne widmo światła specyficznego tylko dla niej. W ten sposób jest zlokalizowana w jednym ze 100 regionów zaprogramowanych w analizatorze. Kompleksy immunologiczne na powierzchni kulki są znakowane innymi barwnikami fluorescencyjnymi, co pozwala na ocenę ilościową badanej substancji.

Analizę statystyczną przeprowadzono przy użyciu programów: RStudio (Version 1.4.1106, Boston, MA, USA) i Statistica 13.3 (StatSoft Polska, Krakow, Poland).

7. Wyniki

Wyniki badań, które stanowią podstawę mojej rozprawy doktorskiej, zostały szczegółowo opisane w poniższych publikacjach:

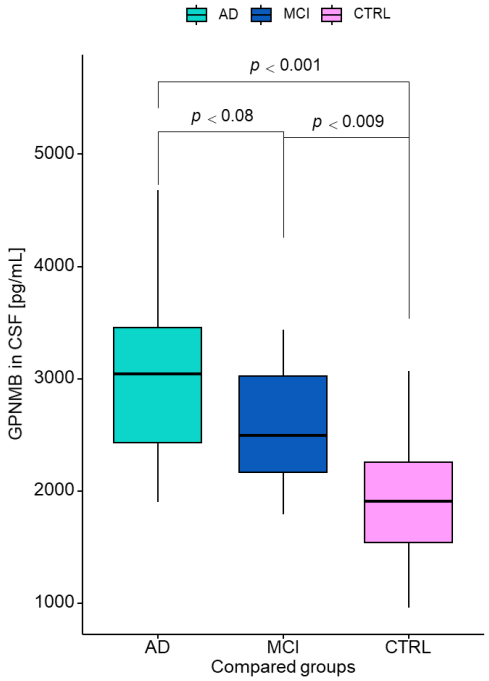
P2. Doroszkiewicz J., Kulczyńska-Przybik A., Dulewicz M., Borawska R., Zajkowska M., Słowik A., Mroczko B. Potential utility of cerebrospinal fluid glycoprotein nonmetastatic melanoma protein B as a neuroinflammatory diagnostic biomarker in mild cognitive impairment and Alzheimer's disease. Journal of Clinical Medicine, 2023, 12(14):4689. doi:10.3390/jcm12144689.

P3. Doroszkiewicz J., Kulczyńska-Przybik A., Dulewicz M., Mroczko J., Borawska R., Słowik A., Zetterberg H., Hanrieder J., Blennow K., Mroczko B. Associations between microglia and astrocytic proteins and tau biomarkers across the continuum of Alzheimer's disease. International Journal of Molecular Sciences 2024, 25(14), 7543; https://doi.org/10.3390/ijms25147543

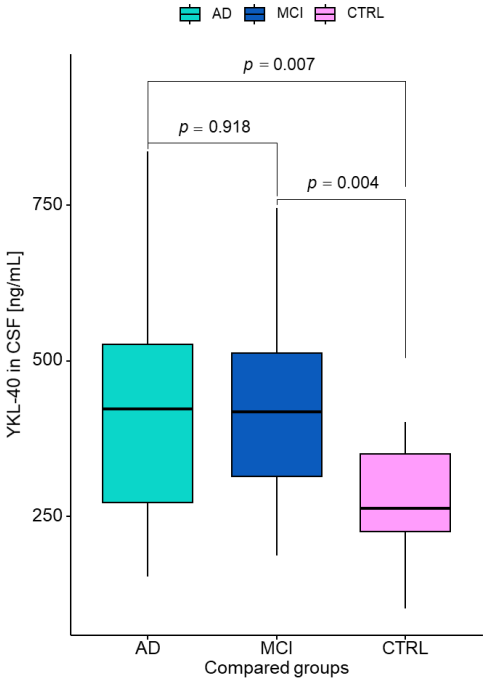
7.1. Potencjalna użyteczność GPNMB ocenianej w płynie mózgowo-rdzeniowym jako neurozapalnego markera diagnostycznego w łagodnych zaburzeniach poznawczych i chorobie Alzheimera.

Pierwsza publikacja badawcza skupia się na ocenie stężeń GPNMB i YKL-40 w płynie mózgowo-rdzeniowym pacjentów z chorobą Alzheimera i MCI w porównaniu do osób bez demencji (grupa kontrolna - CTRL). Oba białka związane są ze stanem zapalnym toczącym się w mózgach pacjentów z zaburzeniami poznawczymi. Neurozapalenie jest jednym z procesów prowadzących do progresji choroby. GPNMB może działać jako regulator neurozapalenia, poprzez swoje działania przeciwzapalne tj. zmniejszanie wytwarzania cytokin prozapalnych i polaryzację makrofagów do „ochronnego" typu „M2", który ma charakter neuroprotekcyjny. Natomiast YKL-40 jest białkiem związanym z negatywnym wpływem na procesy zapalne toczące się w mózgu.

W opublikowanej pracy wykazaliśmy istotnie statystycznie wyższe stężenia GPNMB, jak i YKL-40 u pacjentów w pełni rozwiniętej chorobie Alzheimera, ale również u tych z łagodnymi zaburzeniami poznawczymi w porównaniu do grupy kontrolnej pacjentów bez zaburzeń poznawczych. Wyniki przedstawione są na rycinach 3 i 4.



Rycina 3 – Porównanie stężeń GPNMB w PMR w grupach AD, MCI i CTRL



Rycina 4 – Porównanie stężeń YKL-40 w w PMR w grupach AD, MCI i CTRL

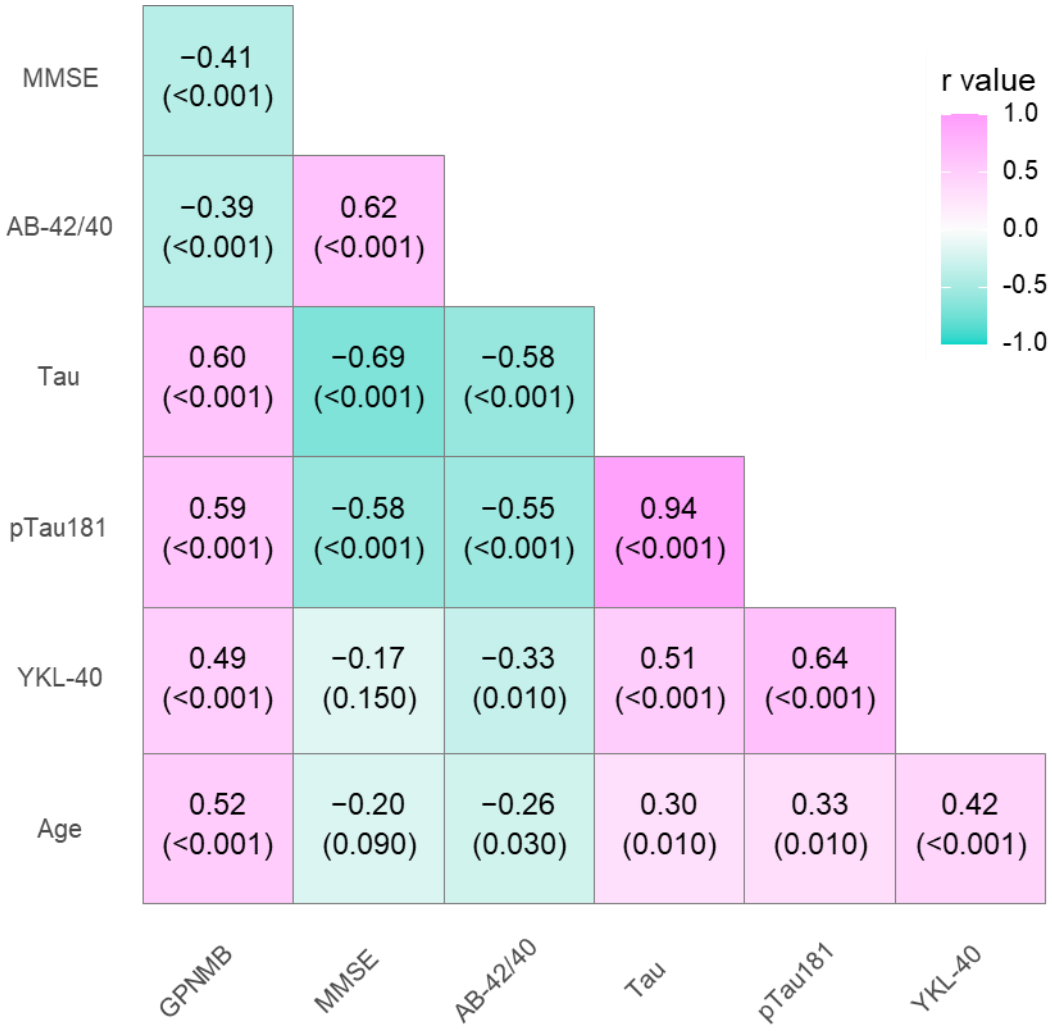
Dodatkowo, nasze grupy badane (AD i MCI) podzieliliśmy na podgrupy Aβ1-42(+) (AD = 17; MCI = 12) i Aβ1-42(−) (AD = 18; MCI = 6) względem stężeń Aβ1-42, zgodnie z punktem odcięcia 538 pg/ml. Grupa AD Aβ(+) wykazała istotnie wyższe stężenia GPNMB niż grupa AD Aβ(−), co przedstawiono w Tabeli 1. Stężenie GPNMB

w grupie MCI Aβ(+) było również wyższe niż w grupie MCI Aβ(−), chociaż różnica nie była istotna statystycznie.

	n	**GPNMB Mediana**	**Zakres pomiędzy kwartylami**	***p*-Value (* *p* < 0.05)**
AD	Aβ(+) = 17	3328	2830–3842	0.028 *
	Aβ(−) = 18	2559	2276–3397	
MCI	Aβ(+) = 12	2658	2366–3328	0.122
	Aβ(−) = 6	2174	1923–2950	

Tabela 1 – Stężenia GPNMB u pacjentów z chorobą Alzheimera i łagodnymi zaburzeniami poznawczymi (MCI) podzielone na podstawie statusu amyloidu Aβ. (* p < 0,05)

Analiza korelacji rang Spearmana została użyta do oceny zależności pomiędzy badanymi białkami. W całej badanej populacji zaobserwowano istotnie dodatnie korelacje między stężeniami GPNMB i Tau (R = 0,6, p < 0,001), pTau (R = 0,59, p < 0,001), YKL-40 (R = 0,49, p < 0,001) i wiekiem (R = 0,52, p < 0,001) w płynie mózgowo-rdzeniowym. Zaobserwowano ujemną korelację pomiędzy GPNMB a współczynnikiem Aβ1-42/1-40 (R = −0,39, p < 0,001) i MMSE (R = −0,41, p < 0,001). Dodatkowo, poziom YKL-40 w płynie mózgowo-rdzeniowym w całej populacji korelował dodatnio z Tau (R = 0,51, p < 0,001) i pTau (R = 0,64, p < 0,001) oraz ujemnie ze współczynnikiem Aβ1-42/1-40 (R = 0,33, p = 0,01) (ryc.5).

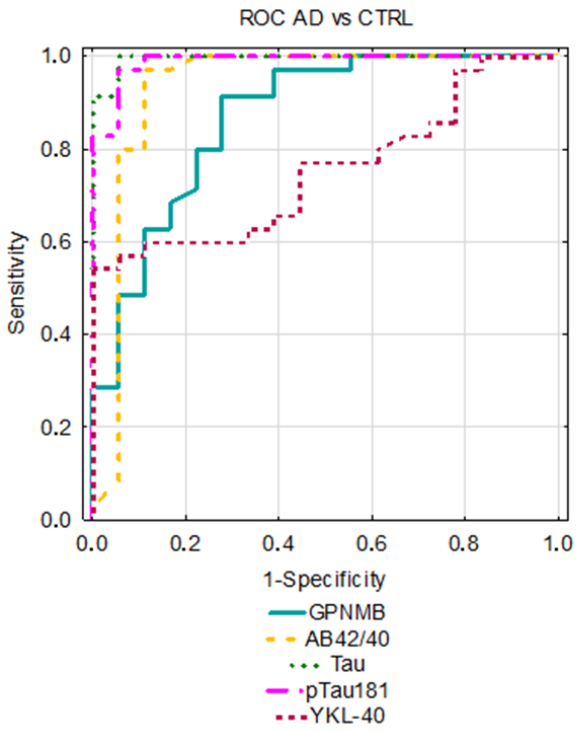


Rycina 5 – Korelacje rang Spearmana pomiędzy klasycznymi biomarkerami a testowanymi białkami w całej badanej populacji

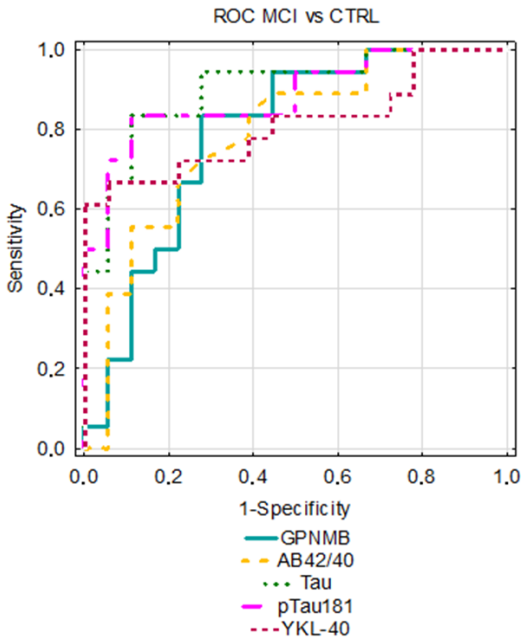
U pacjentów z chorobą Alzheimera wyższe stężenie GPNMB w płynie mózgowo-rdzeniowym było istotnie związane ze stężeniem YKL-40 (R = 0,5, p < 0,001) i wiekiem (R = 0,62, p = 0,001). Aby określić wpływ niższej mediany wieku w grupie CTRL, wybraliśmy dopasowane do siebie wiekowo grupy tj. pacjentów z chorobą Alzheimera, łagodnymi zaburzeniami poznawczymi i CTRL (mediana wieku: odpowiednio 73, 74 i 73 lata) oraz porównaliśmy stężenia GPNMB w grupach. W wyniku tej analizy uzyskaliśmy podobne trendy statystyczne, jak przedstawiono wcześniej (AD vs. CTRL p = 0,0029). Słabszą korelację zaobserwowano między poziomami YKL-40 a Tau (R = 0,38, p = 0,02), ale silniejszą zależność stwierdzono między stężeniami YKL-40 a pTau (R = 0,6, p > 0,001).

W grupie MCI nie wykazano istotnych korelacji pomiędzy GPNMB a innymi parametrami. Jednakże stężenia YKL-40 korelowały dodatnio z poziomami Tau ($R = 0{,}78$, $p < 0{,}001$), pTau ($R = 0{,}86$, $p < 0{,}001$) i Aβ1-42 ($R = 0{,}56$, $p = 0{,}016$).

W celu określenia przydatności diagnostycznej badanych białek wyznaczono pole powierzchni pod krzywą ROC dla pacjentów z AD i MCI w porównaniu do grupy kontrolnej. W różnicowaniu osób chorych na chorobę Alzheimera i pacjentów bez zaburzeń poznawczych, wyższą wartość AUC wykazano dla GPNMB w porównaniu do oceny stężeń Aβ1-42. W przypadku pacjentów z AD najniższą przydatność diagnostyczną wykazywały stężenia YKL-40, które przedstawiono na rycinie 6. Podobne wyniki dla GPNMB i YKL-40 obserwowano u pacjentów z MCI w porównaniu z grupą kontrolną; ich wartości AUC były wyższe niż dla Aβ1-42 i współczynnika Aβ1-42/1-40, a niższe niż dla białek Tau (ryc. 7).



Rycina 6 - Porównanie pola pod krzywymi ROC (AUC) dla GPNMB, YKL-40 i klasycznych biomarkerów w chorobie Alzheimera pomiędzy grupami AD i CTRL.

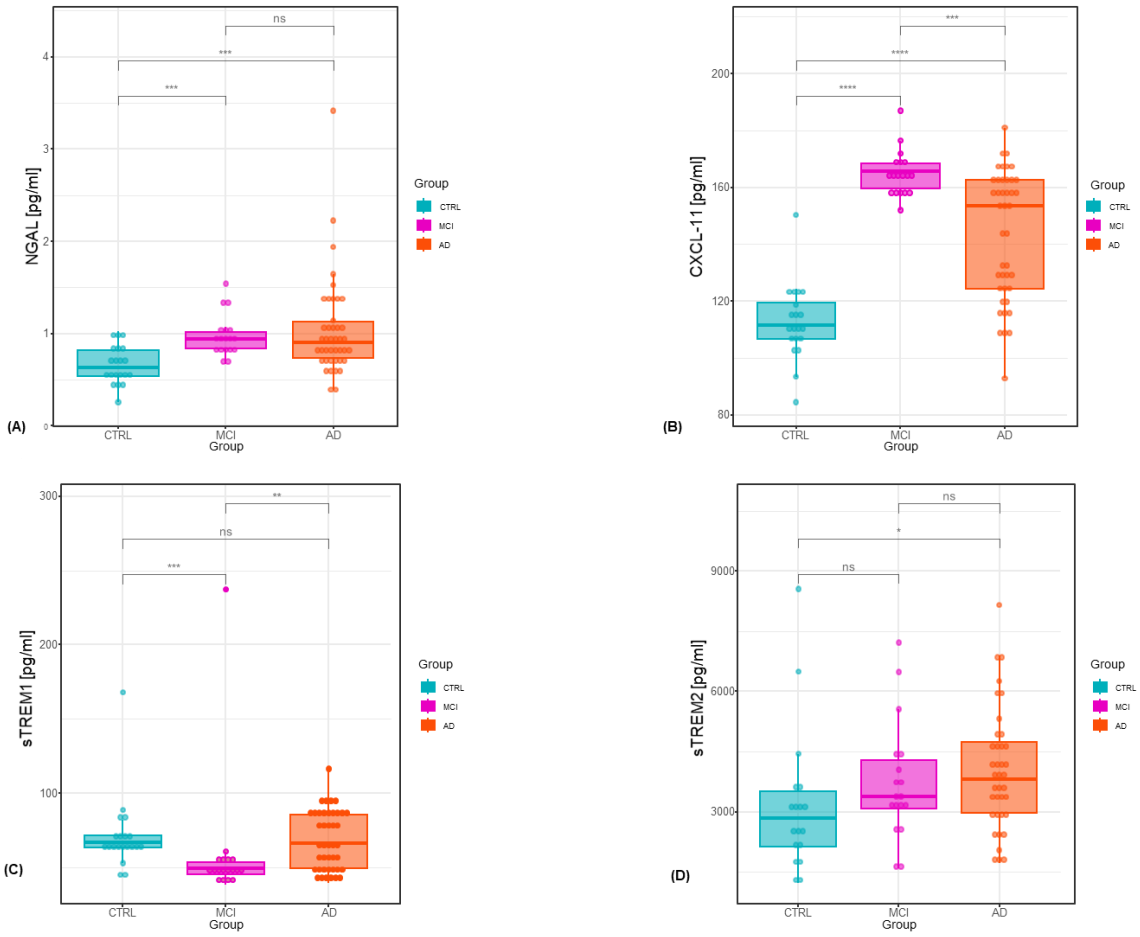


Rycina 7 - Porównanie pola pod krzywymi ROC (AUC) dla GPNMB, YKL-40 i klasycznych biomarkerów w chorobie Alzheimera pomiędzy grupami MCI i CTRL.

7.2. Analiza białek astrocytarnych i mikroglejowych o aktywności pro- i przeciwzapalnej w kontinuum choroby Alzheimera

W celu poszerzenia badań dotyczących neurozapalenia, postanowiono oznaczyć kolejne białka związane z tym stanem w chorobie Alzheimera. Głównym celem tego badania była ocena wybranych białek pro- i przeciwzapalnych, odzwierciedlających aktywację mikrogleju i astrocytów oraz porównanie ich poziomów z upośledzeniem funkcji poznawczych u pacjentów z chorobą Alzheimera i łagodnymi zaburzeniami poznawczymi. Oznaczono stężenia białek NGAL, CXCL-11, sTREM1 i sTREM2 w płynie mózgowo-rdzeniowym. W dostępnej literaturze brakuje danych dotyczących stężeń w płynie mózgowo-rdzeniowym sTREM1 i CXCL-11 – białek zapalnych uwalnianych z neutrofili, mikrogleju i astrocytów w procesach neuropatologicznych związanych z chorobą Alzheimera. W publikacji wykazaliśmy, że białka prozapalne NGAL i CXCL-11 najwyższe wartości uzyskały u pacjentów z łagodnymi zaburzeniami poznawczymi, rozróżniając ich od pacjentów z w pełni rozwinietą chorobą Alzheimiera. Natomiast wysokie stężenia białka wykazującego aktywność przeciwzapalną, czyli sTREM2, u pacjentów z AD pozwoliły na odróżnienie ich od grupy kontrolnej. Wyniki naszych badań mogą wskazywać, że niektóre mechanizmy ochronne w neurozapaleniu

są aktywowane w bardziej zaawansowanych stadiach. W tych stadiach demencji nasilona aktywność mechanizmów ochronnych w mózgu może odzwierciedlać podwyższone stężenia białka przeciwzapalnego sTREM2 w AD. Wyniki stężeń wszystkich białek przedstawiono na rycinie 8.

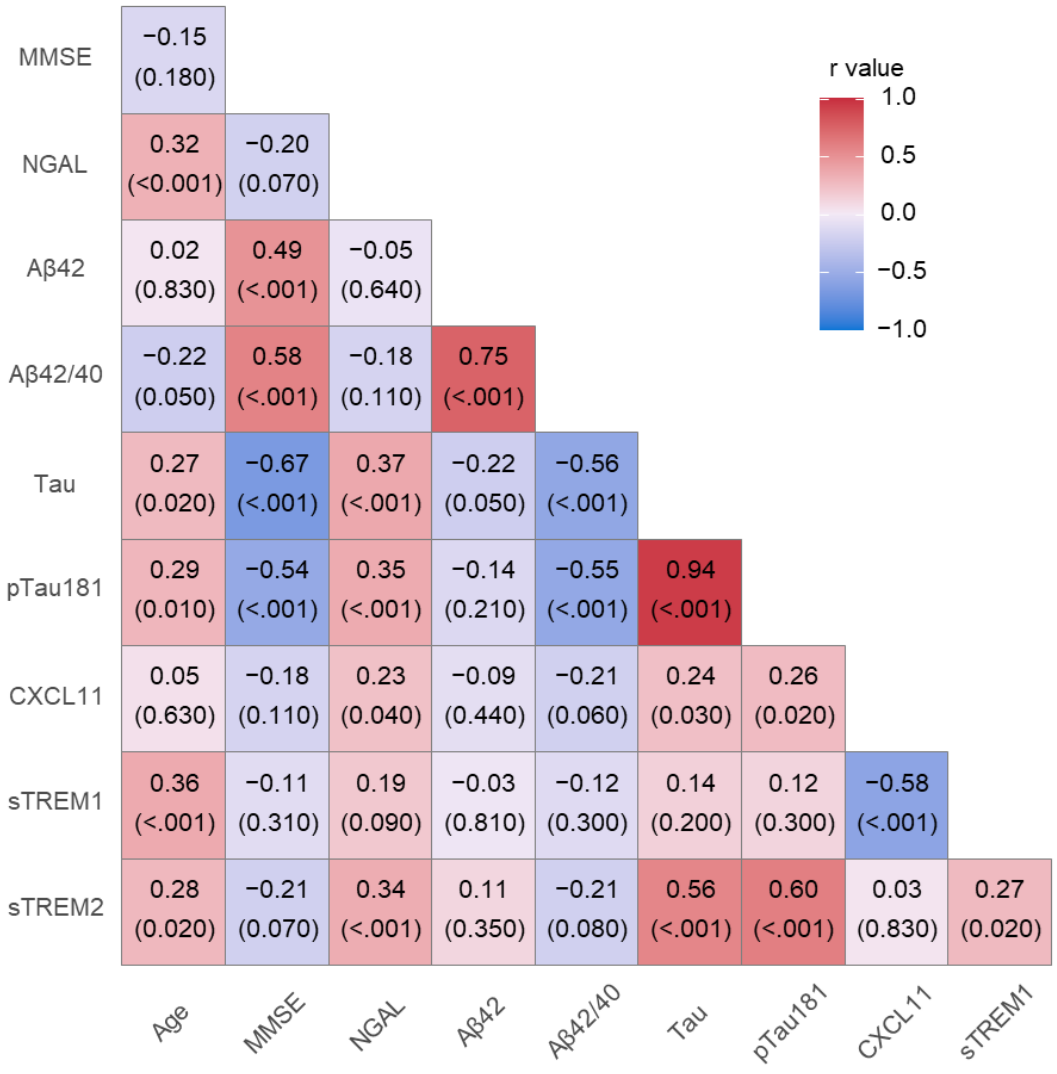


Rycina 8 - Stężenia badanych białek w płynie mózgowo-rdzeniowym pacjentów z AD, MCI oraz osób bez zaburzeń poznawczych

(A) NAGL - Lipokalina związana z żelatynazą neutrofilową. (B) CXCL-11 – Chemokina 11, (C) sTREM1 - rozpuszczalny receptor wyzwalający w płynie mózgowo-rdzeniowym wyrażonego na komórkach mieloidalnych – 1, D) sTREM2 - rozpuszczalny receptor wyzwalający w płynie mózgowo-rdzeniowym wyrażonego na komórkach mieloidalnych – 2;

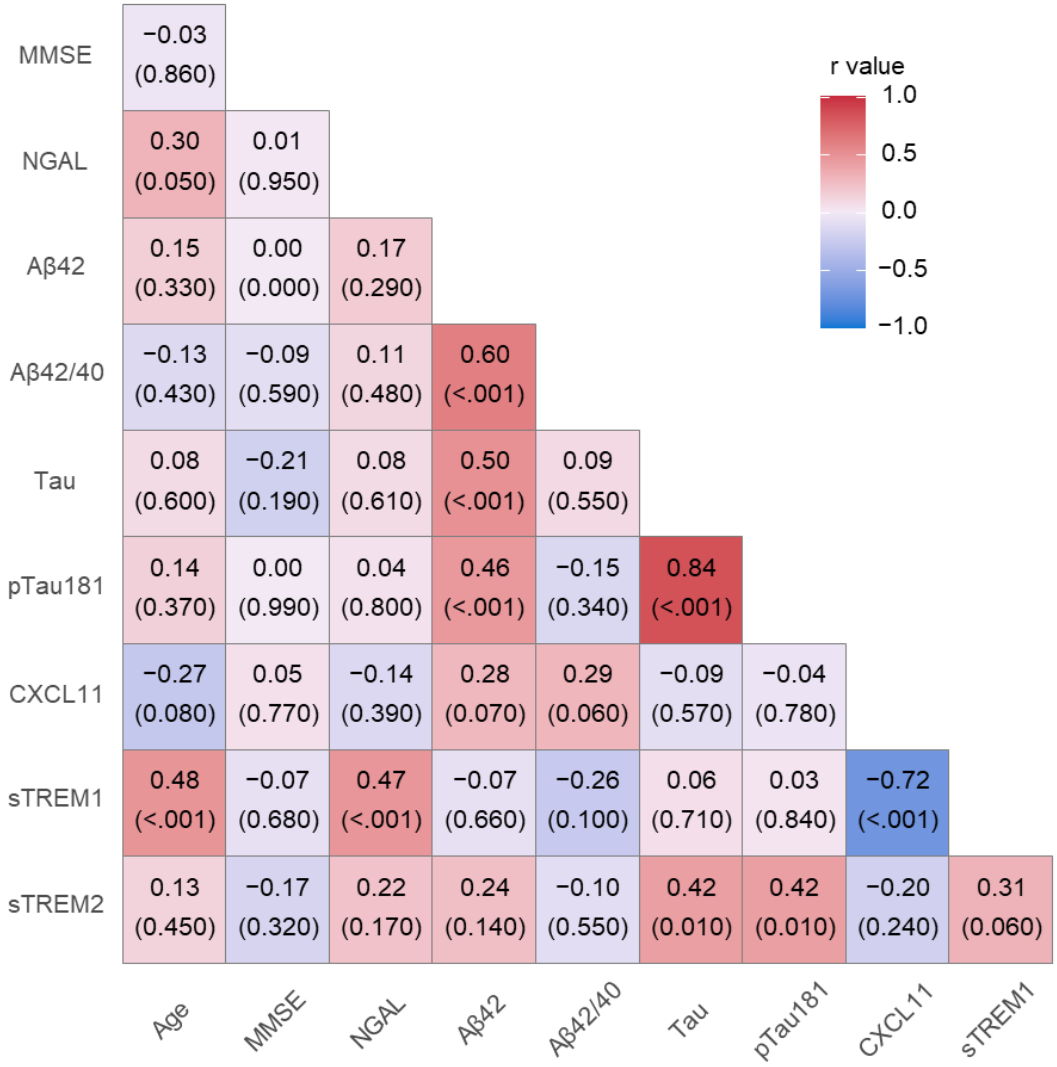
W całej grupie badanej, dzięki analizie rang Spearmana, zaobserwowaliśmy istotne statystycznie dodatnie korelacje między stężeniami NGAL i Tau w płynie mózgowo-rdzeniowym ($R = 0,37$, $p < 0,001$), pTau ($R = 0,35$, $p < 0,001$), wiekiem ($R = 0,32$, $p < 0,001$), CXCL11 ($R = 0,23$, $p = 0,04$) i sTREM2 ($R = 0,34$, $p < 0,001$). Poziomy sTREM2 korelowały dodatnio z sTREM1 ($R = 0,27$, $p = 0,02$), tau ($R = 0,56$, $p < 0,001$),

pTau (R = 0,6, p < 0,001) oraz wiekiem (R = 0,28, p = 0,02), podczas gdy sTREM1 wykazał dodatnią istotną korelację z wiekiem (R = 0,36, p < 0,001) i ujemną korelację z CXCL11 (R = −0,58, p < 0,001). Podobnie CXCL-11 i tau (R = 0,26, p = 0,02) wykazały dodatnią korelację, co przedstawiono na ryc. 9.



Rycina 9 – Korelacje pomiędzy białkami pro- i przeciwzapalnymi oraz biomarkerami AD w płynie mózgowo-rdzeniowym w całej grupie badanej.

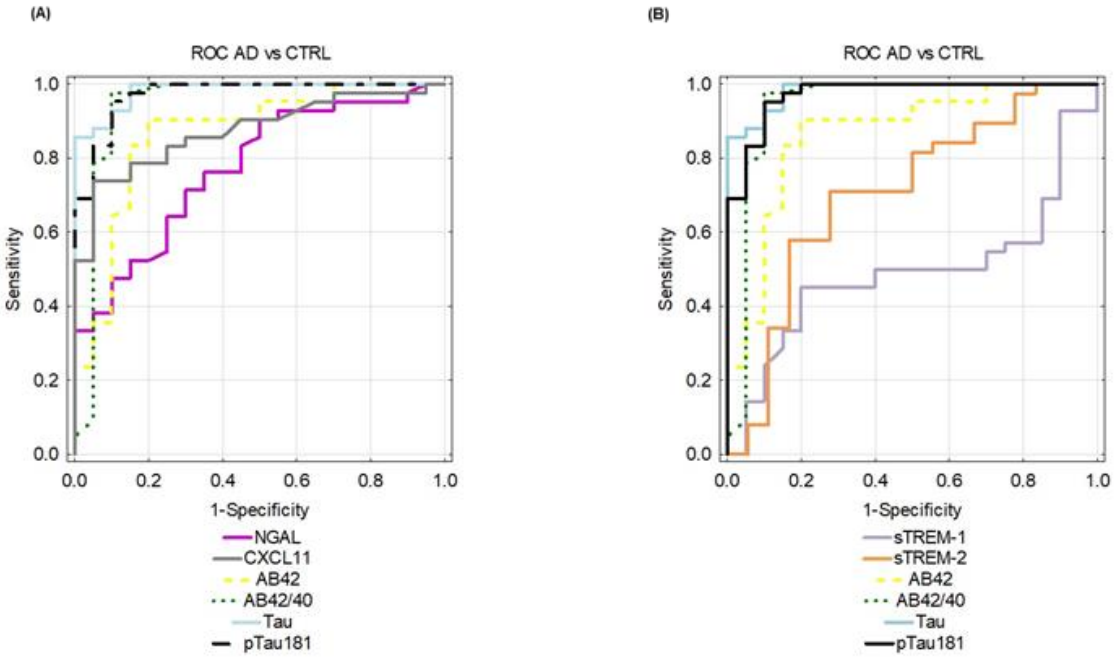
U pacjentów z chorobą Alzheimera poziom NGAL w płynie mózgowo-rdzeniowym był istotnie związany ze stężeniem sTREM1 (R = 0,47, p < 0,001) i wiekiem (R = 0,30, p = 0,05). Ponadto sTREM2 korelował dodatnio z tau (R = 0,42, p = 0,01) i pTau (R = 0,42, p = 0,01). Dodatkowo sTREM1 korelował z wiekiem (R = 0,48, p < 0,001) (ryc. 10).



Rycina 10 – Korelacje pomiędzy białkami pro- i przeciwzapalnymi oraz biomarkerami AD w płynie mózgowo-rdzeniowym w grupie pacjentów z AD.

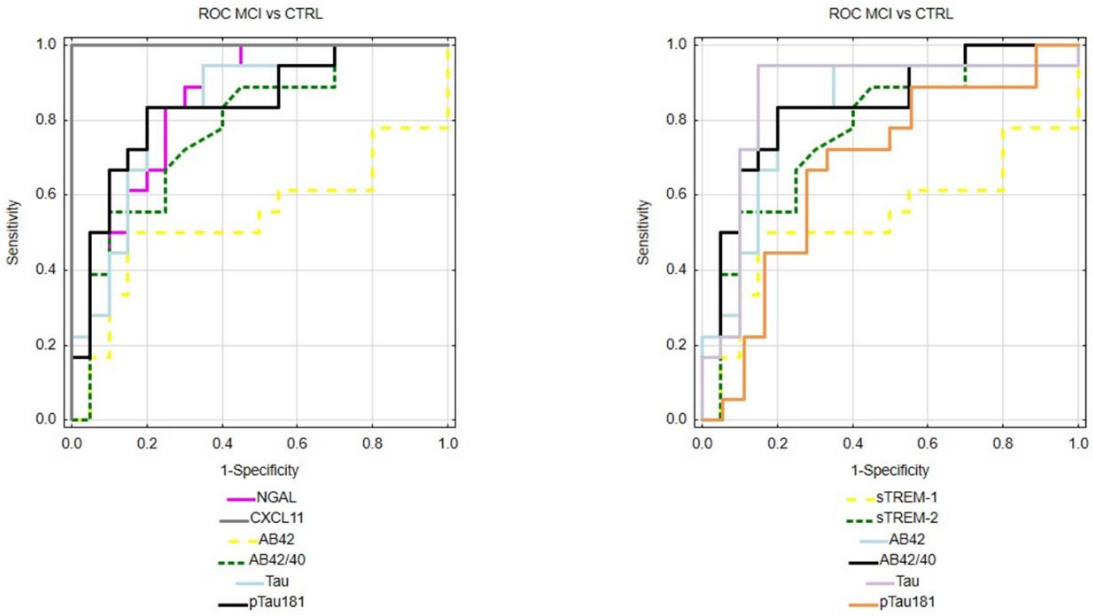
W grupie MCI nie zaobserwowano istotnych korelacji pomiędzy NGAL a innymi białkami. Jednakże istniała silna, istotna korelacja między sTREM2 i Tau ($R = 0,71$, $p < 0,001$) oraz pTau ($R = 0,77$, $p < 0,001$).

W celu określenia przydatności diagnostycznej badanych białek wyznaczono pole powierzchni pod krzywą ROC dla pacjentów z AD i MCI w porównaniu do grupy kontrolnej. Białka prozapalne wykazały następujące wyniki: NGAL (AUC = 0,773, $p < 0,001$), CXCL-11 (AUC = 0,875, $p < 0,001$) podczas różnicowania grup AD i CTRL. Natomiast AUC dla białka przeciwzapalnego sTREM2 wyniosło AUC = 0,705 ($p = 0,01$) w różnicowaniu grup AD i CTRL (ryc. 11).



Rycina 11 - Porównanie pola powierzchni pod krzywymi ROC (AUC) dla stężeń białek (A) prozapalnych (B) przeciwzapalnych oraz klasycznych biomarkerów AD w płynie mózgowo-rdzeniowym w grupach AD i CTRL.

W grupie MCI skuteczność diagnostyczna sTREM1 (AUC = 0,858, p < 0,001) i NGAL (AUC = 0,844, p < 0,001) była lepsza niż uznanych biomarkerów (ryc.12).



Rycina 12 - Porównanie pola powierzchni pod krzywymi ROC (AUC) dla stężeń białek (A) prozapalnych (B) przeciwzapalnych oraz klasycznych biomarkerów AD w płynie mózgowo-rdzeniowym w grupach AD i MCI.

8. Wnioski

1. Rozwijający się stan zapalny, a wraz z nim wzrost stężenia takich białek jak GPNMB, NGAL i CXCL11 już we wczesnych okresach choroby (etap MCI), może być jednym z kluczowych mechanizmów sprzyjających progresji choroby.

2. Zaobserwowana we wczesnych stadiach rozwoju AD lepsza przydatność diagnostyczna GPNMB (wartość AUC) w porównaniu do białka amyloidu oraz podwyższone stężenie GPNMB w grupie Aβ (+) wskazują na potencjalną przydatność diagnostyczną tego białka w AD.

3. Śledzenie dynamiki zmian stężeń białek stanu zapalnego uwalnianych z neutrofili (NGAL, CXCL11) w połączeniu z oceną klasycznych biomarkerów u pacjentów z MCI może pozwolić na poprawę wczesnej diagnostyki choroby Alzheimera.

4. W zaawansowanym stadium choroby aktywacja białek przeciwzapalnych (tj. GPNMB, sTREM2) wydaje się być kluczowym mechanizmem ochronnym przed rozwojem patologii białka Tau.

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10. Streszczenie w języku polskim

Choroba Alzheimera (AD) jest jedną z najczęstszych przyczyn demencji w Polsce i na świecie. Pomimo znaczących postępów w diagnostyce chorób neurodegeneracyjnych wciąż brakuje specyficznych biomarkerów, które odzwierciedlałyby molekularne mechanizmy tej choroby. Choć etiologia choroby Alzheimera nie została jeszcze w pełni wyjaśniona, zidentyfikowano kilka kluczowych czynników, które znacząco wpływają na jej rozwój. Do charakterystycznych cech histopatologicznych AD zalicza się pozakomórkowe blaszki amyloidowe zbudowane z amyloidu β oraz zwyrodnienie włókienkowe, którego podstawą jest hiperfosforylowane białko tau.

Pomimo istniejących w literaturze danych dotyczących roli neurozapalenia w patogenezie AD, brakuje jak dotychczas prac oceniających zależność stężenia białek stanu zapalnego w kontinuum patologii amyloidu beta, czy też białka Tau oraz analizy zmian stężeń wyżej wymienionych białek w zależności od stadium rozwoju choroby. Stąd też celem niniejszej rozprawy było zbadanie wybranych białek związanych z procesem zapalnym (pro- i przeciwzapalnych) u pacjentów z zaburzeniami funkcji poznawczych (w tym osób chorych na chorobę Alzheimera (AD) i łagodne zaburzenia funkcji poznawczych (MCI)) w odniesieniu do stężeń tych białek w grupie osób bez zaburzeń poznawczych, a także porównanie stężeń badanych białek ze wskaźnikami nasilenia zaburzeń funkcji poznawczych oraz stężeniami klasycznych biomarkerów AD w różnych stadiach choroby, jak również analiza przydatności klinicznej badanych białek jako potencjalnych biomarkerów mogących mieć zastosowanie w diagnozowaniu oraz ocenie progresji AD.

Glikoproteina, białko czerniaka nieprzerzutowego B (GPNMB), YKL-40, lipokalina związana z żelatynazą neutrofilów (NGAL), CXCL-11, sTREM1 i sTREM2 zostały ocenione metodami immunologicznymi (tj. klasyczną metodą ELISA oraz technologią multiplexingu xMAP na platformie Luminex 200) w płynie mózgowo-rdzeniowym (PMR) pacjentów z MCI, AD oraz osób z grupy kontrolnej bez zaburzeń poznawczych.

W wyniku przeprowadzonych badań wykazano, iż rozwijający się stan zapalny, a wraz z nim wzrost stężenia takich białek jak GPNMB, NGAL i CXCL11 już we wczesnych okresach choroby (etap MCI), może być jednym z kluczowych mechanizmów sprzyjających progresji choroby. Ponadto zaobserwowana we wczesnych stadiach

rozwoju AD lepsza przydatność diagnostyczna GPNMB (wartość AUC) w porównaniu do białka amyloidu oraz podwyższone stężenie GPNMB w grupie Aβ (+) wskazują na potencjalną przydatność diagnostyczną tego białka w AD. Śledzenie dynamiki zmian stężeń białek stanu zapalnego uwalnianych z neutrofili (NGAL, CXCL11) w połączeniu z oceną klasycznych biomarkerów u pacjentów z MCI może pozwolić na poprawę wczesnej diagnostyki choroby Alzheimera, przy czym w zaawansowanym stadium choroby aktywacja białek przeciwzapalnych (tj. GPNMB, sTREM2) wydaje się być kluczowym mechanizmem ochronnym przed rozwojem patologii białka Tau.

11. Streszczenie w języku angielskim

Alzheimer's disease (AD) is one of the most common causes of dementia in Poland and worldwide. Despite significant progress in the diagnosis of neurodegenerative diseases, there is still a lack of specific biomarkers that would reflect the molecular mechanisms of this disease. Although the etiology of Alzheimer's disease has not yet been fully explained, several key factors have been identified that significantly affect its development. The characteristic histopathological features of AD include extracellular amyloid plaques composed of amyloid β and fibrillary tangles, the basis of which is hyperphosphorylated tau protein.

Despite existing data in the literature on the role of neuroinflammation in the pathogenesis of AD, there are currently no studies assessing the relationship between the concentration of inflammatory proteins in the continuum of amyloid beta pathology or Tau protein, and analysis of changes in the concentrations of the above-mentioned proteins depending on the stage of disease development. Hence, the aim of this dissertation was to investigate selected proteins associated with the inflammatory process (pro- and anti-inflammatory) in patients with cognitive impairment (including Alzheimer's disease (AD) and mild cognitive impairment (MCI)) in relation to the concentrations of these proteins in the group of people without cognitive impairment, as well as to compare the concentrations of the studied proteins with indicators of the severity of cognitive impairment and concentrations of classic AD biomarkers in different stages of the disease, as well as to analyze the clinical usefulness of the studied proteins as potential biomarkers that may be used in the diagnosis and assessment of AD progression.

Glycoprotein, non-metastatic melanoma protein B (GPNMB), YKL-40, neutrophil gelatinase-associated lipocalin (NGAL), CXCL-11, sTREM1 and sTREM2 were assessed using immunological methods (i.e. classical ELISA method and xMAP multiplexing technology on the Luminex 200 platform) in the cerebrospinal fluid (CSF) of patients with MCI, AD and control subjects without cognitive impairment.

The conducted studies have shown that the developing inflammation and the associated increase in the concentration of proteins such as GPNMB, NGAL and CXCL11 already in the early stages of the disease (MCI stage) may be one of the key mechanisms promoting disease progression. Moreover, the better diagnostic utility of

GPNMB (AUC value) observed in the early stages of AD development compared to amyloid protein and the increased concentration of GPNMB in the Aβ (+) group indicate the potential diagnostic utility of this protein in AD. Monitoring the dynamics of changes in the concentrations of inflammatory proteins released from neutrophils (NGAL, CXCL11) combined with the assessment of classic biomarkers in patients with MCI may allow for the improvement of early diagnosis of Alzheimer's disease, while in the advanced stage of the disease, the activation of anti-inflammatory proteins (i.e. GPNMB, sTREM2) seems to be a key protective mechanism against the development of Tau protein pathology.

12. Publikacje wchodzące w skład rozprawy doktorskiej

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REVIEW ARTICLE

Inflammation in the CNS: Understanding Various Aspects of the Pathogenesis of Alzheimer's Disease

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Abstract: Alzheimer's disease is a progressive and deadly neurodegenerative disorder and one of the most common causes of dementia globally. Current, insufficiently sensitive and specific methods of early diagnosing and monitoring this disease prompt a search for new tools. Numerous literature data have indicated that the pathogenesis of Alzheimer's disease (AD) is not limited to the neuronal compartment but involves various immunological mechanisms. Neuroinflammation has been recognized as a very important process in AD pathology. It seems to play pleiotropic roles, both neuroprotective and neurodegenerative, in the development of cognitive impairment depending on the stage of the disease. Mounting evidence demonstrates that inflammatory proteins could be considered biomarkers of disease progression. Therefore, the present review summarizes the role of some inflammatory molecules and their potential utility in detecting and monitoring dementia severity. This paper also provides a valuable insight into new mechanisms leading to the development of dementia, which might be useful in discovering possible anti-inflammatory treatment.

Keywords: Neuroinflammation, Alzheimer's disease, dementia disorders, biomarkers, neurodegeneration, chemokines, interleukins.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the world. It is an untreatable and progressive neurodegenerative disease. AD develops mainly in individuals over the age of 65. Every year, we observe an increasing number of new cases correlated with the aging society [1]. It is estimated that 50 million people worldwide suffer from dementia, and this number will triple by 2050 [2]. The highest number of cases are reported in North America and Western Europe. In the USA, AD is the sixth leading cause of death [3]. The disorder imposes an enormous burden not only on affected individuals but also on their relatives, caregivers, and healthcare systems. Despite significant advances in the diagnosis of neurodegenerative disorders, there are still no specific biomarkers that reflect the molecular mechanism underlying AD. Therefore, it is crucial to find new biomarkers that can help detect AD earlier in the course of the disease and potentially contribute to the discovery of treatment.

AD is a chronic disorder in which pathological processes begin at least 20 years prior to disease onset [3]. The etiology of AD is heterogeneous and not fully understood. It is assumed that genetic and environmental factors may play a role in the pathogenesis of the disease. However, genetic cases constitute less than 5% of AD cases overall, with several gene mutations associated with the disease. In early-onset Alzheimer's disease (EOAD), we can identify mutations in three genes: presenilin 1, presenilin 2, and amyloid precursor protein (APP) [4], whereas sporadic, late-onset AD (LOAD) is associated with the APOE ε4 gene [5]. The histopathological hallmarks of AD include the gradual formation of extracellular plaques composed of amyloid β and neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau. They cause neuronal and synaptic loss [6]. The most toxic molecules involved in AD pathology are insoluble peptides of amyloid-beta 1-42 (Aβ-42), which can be recognized as unfamiliar by the immune system, triggering the inflammatory response. They are formed by proteolytic processing of amyloid precursor protein by several secretases [7]. The accumulation and aggregation of Aβ-42 initiate a cascade of pathological processes, including neuroinflammation, which leads to CNS (Central Nervous System) cell damage [8, 9]. However, several mechanisms are involved in Aβ removal from the brain, such as transport through the blood-brain barrier (BBB), microglia, enzymatic degradation, and retention by CSF into the circulatory and lymphatic system [10]. Disturbances in these processes can result in increased amyloid accumulation. Amyloid-beta may also be implicated in tau hyperphosphorylation by modulating the activity of kinases or phosphatases.

Another hallmark of AD is neurofibrillary tangles connected with hyperphosphorylated tau [11]. Tau is a protein responsible for stabilizing microtubules in neurons. A decline in tau attachment to microtubules results in synaptic dysfunction. Moreover, tau is a subject of many post-translational modifications, such as acetylation, glycation, nitration,

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and phosphorylation, particularly important in AD pathology [12]. The imbalance between tau kinase and phosphatase activity results in increased tau phosphorylation and intracellular tau aggregation, leading to the creation of NFTs in AD patients. Finally, formed NFTs cause the weakening of synaptic plasticity [13, 14], leading to the damage of neuronal cells.

In the current review, we summarize recent studies investigating the role of various inflammatory molecules in AD pathology. Furthermore, advances in understanding potentially novel mechanisms leading to AD and key genetic polymorphisms of inflammatory molecules associated with enhanced AD risk are described.

2. MECHANISMS OF NEUROINFLAMMATION

Mechanisms underlying AD neuroinflammation are still not fully understood, although they have been investigated for over 20 years. Numerous genetic and immunological analyses have shown a significant connection between inflammation and AD pathology (Fig. **1**) [15].

Krstic *et al.* presented a new perspective on inflammation related to late-onset AD, which illustrates the evolution of AD pathology. According to Krstic, a healthy brain reacts with elevated APP synthesis in normal conditions following exposure to inflammation. This results in the aggregation of its products. Moreover, in LOAD, tau hyperphosphorylation and mislocalization are observed. Typically, microglia and astrocytes are responsible for eliminating unnecessary aggregates [16, 17]. Pathological processes in the brain may lead to microglia overactivity and enhanced synthesis of pro-inflammatory cytokines without protecting normal microglia. Consequently, neurons are more prone to neurotoxic degradation and formation of plaques, thus increasing the pro-inflammatory response [17]. It is suggested that one of the mechanisms of neuroinflammation may be related to the presence of antigen CD14 on microglia and LPS (Lipopolysaccharide), which coordinate with toll-like receptor 4 (TLR-4). This receptor is an important signaling protein capable of inducing microglial activation, which initiates the production of proinflammatory cytokines [17]. Furthermore, LPS may be associated with enhanced β- and γ-secretase activity, resulting in increased accumulation of $A\beta_{1\text{-}42}$. These relationships indicate a link between amyloidogenesis and neuroinflammation, although exact mechanisms behind this process are still not fully known [18, 19]. As mentioned above, the key contributors of the neuroinflammatory reaction are microglia and astrocytes, which, when activated, release inflammatory molecules, such as cytokines and chemokines, and regulate the intensity and duration of the immune response.

Microglia are macrophages of brain tissue that play a primarily protective function [20]. During brain development, they are responsible for eliminating the surplus of synaptic connections, while in a fully-developed, adult brain, they engage in synaptic plasticity regulation and remodeling of neuronal circuits [21]. Microglia can be transformed into their activated form by a number of triggers, such as injury, pro-inflammatory cytokines, or loss of ion homeostasis. In this state, microglia are capable of releasing cytotoxic factors, free radicals, and proinflammatory cytokines, such as TNF-α [22]. Two activation states are generally distinguished: M1: classical activation state and M2: alternative phenotype. Lipopolysaccharide (LPS), IFN-γ, or TNF-α result in classical activation, which is involved in defense mechanisms against pathogens by secreting pro-inflammatory factors, such as IL-1β, TNF-α, IL-6, and reactive oxygen species [23]. On the contrary, the M2 phenotype is induced by IL-4 and IL-13, resulting in the release of neuroprotective factors, *e.g.,* TGF-β, IL-10, and IGF-1 [23]. Through balancing inflammation, M2 microglia are capable of improving the remodeling and repairing of brain tissue. The M1-to-M2 switch can be very rapid [24, 25]. On the other hand, microglia are also implicated in the development of some pathological states. They are involved in binding to Aβ plaques via several receptors, such as CD14, CD36, and toll-like receptors, TLR4 and TLR2, which activate microglia and stimulate the enhanced release of proinflammatory factors by microglia [15, 26]. Moreover, the activated microglia around senile plaques, which generate the proinflammatory environment, promote senile plaque formation [27, 28].

Similar to microglia, astrocytes can also be activated, which are expressed by increased release of glial fibrillary acidic protein (GFAP). Despite their neuroprotective function, they can also create an inflammatory environment by releasing a number of proinflammatory cytokines, such as TNF-α, IL-6, or IL-12 [29, 30]. It is suggested that astrocytes may accumulate in close proximity to plaques and amyloid β, thus promoting astrocytes activation [31]. Studies have confirmed the presence of activated astrocytes in the brains of animal models and AD patients [29, 32]. There are possibly a few mechanisms of astrocyte involvement in neuroinflammation. One of them may be astrocyte activation by NFκB releasing complement protein C3, which binds neuronal C3aR. NFκB controls neuronal function and durability, thus proving the role of astroglia in neuronal damage [33, 34].

Neutrophils are white blood cells that act at the first line of early innate immunity throughout phagocytosis, releasing neutrophil extracellular traps (NETs) and producing reactive oxygen species [35]. Studies have revealed abnormal reactions of neutrophils in AD [36]. Research conducted on mouse models of AD has demonstrated the presence of neutrophils near amyloid plaques and the overactivity of neutrophils caused by amyloid. Moreover, inhibiting neutrophils through blocking LFA-1 integrin in the early stages of dementia improved memory in mice [37]. These discoveries may suggest that the overactivity of neutrophils has a detrimental effect on the brain in AD. Stock *et al.* revealed that neutrophil granulate proteins might act as either neuroprotective or neurotoxic molecules. Considering their protective functions, CAP37, cathepsin G, and neutrophil elastase may help in Aβ cleavage, resulting in plaque removal [38].

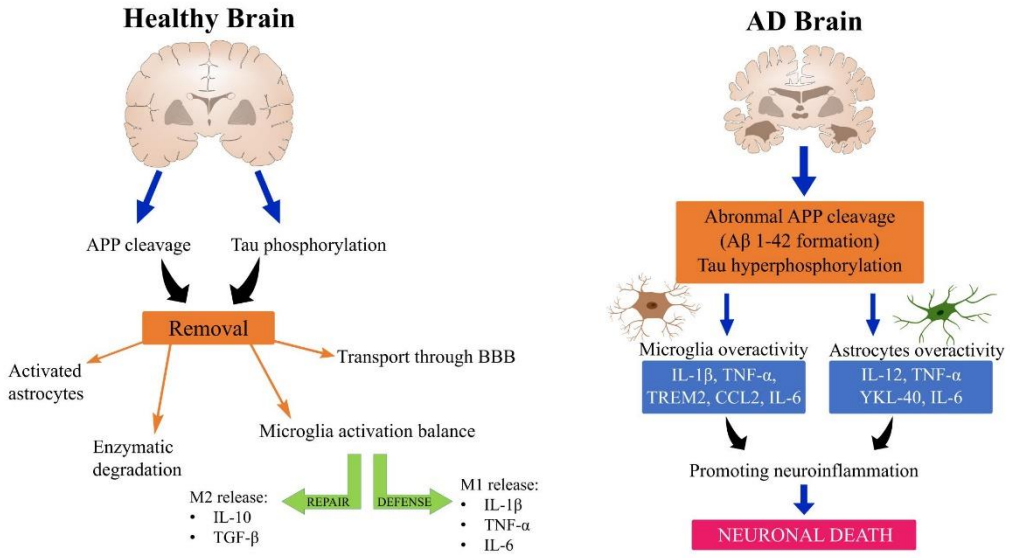

Fig. (1). The mechanism of neuroinflammation in healthy and AD brains. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3. RELATIONSHIP BETWEEN THE GUT MICROBIOTA AND AD

A new perspective on neuroinflammation is offered by exploring the connection between gut microbiota and the nervous system. The so-called "brain-gut axis" is discussed in a large number of new studies exploring its contribution to AD pathology [39]. Research on rodent AD models and AD patients has demonstrated different compositions of the gut microbiota in AD patients in comparison to healthy controls, which is associated with the loss of epithelial barrier integrity and chronic systemic and intestinal inflammation [40, 41]. Moreover, the gut microbiota synthesizes LPS, bacterial amyloid, and other toxins, which can alter physiological barriers and can be associated with systemic inflammation. Despite differences in structure between CNS and gut amyloid, the latter can also cause enhanced immune responses, resulting in neuronal amyloid aggregation [39, 42, 43]. Furthermore, LPS release from the gut microbiota, in particular, can result in microglial activation, thus disturbing amyloid removal and neurotoxicity [39]. A study by Kim *et al.* demonstrated that transplanting healthy mice microbiota into AD models alleviated glial activation and formation of Aβ plaques and NFTs [40]. Furthermore, the abundant presence of several bacterial, pro-inflammatory taxa, belonging to Escherichia/Shigella, positively correlated with elevated blood levels of pro-inflammatory cytokines, such as cytokines IL-1β, NLRP3, and CXCL2 in amyloid-positive patients, and negatively correlated with anti-inflammatory E.rectale [44]. Interestingly, probiotic treatment resulted in a reduction in the levels of IL1α, IL1β, IL2, IL12, IFNγ, and TNFα, and an increase in the levels of IL4 and IL6, which are anti-inflammatory cytokines [45].

4. POTENTIAL DIAGNOSTIC USEFULNESS OF INFLAMMATORY PROTEINS IN AD PATHOLOGY

Inflammation plays a pivotal role in the development and progression of AD. Comprehensive brain imaging studies have supported the relationship between potentiated neuroinflammation and progression of MCI (Mild Cognitive Impairment) and AD [46]. However, little is known about inflammation during the early stages of cognitive decline or whether it differs in different neurodegenerative diseases. Identifying inflammatory profiles in the cerebrospinal fluid (CSF) and blood of patients may help find novel biomarkers reflecting AD pathology and explore possible therapeutic targets.

Many proteomic studies have analyzed the direction of changes, significance, and diagnostic usefulness of specific inflammatory biomarkers in the pathology and progression of AD [47]. CSF levels of selected inflammatory proteins allow for the discrimination of patients with dementia (MCI and AD) from subjects without cognitive decline. Furthermore, CSF levels can be associated with amyloid and/or tau pathology. A recent study has revealed that although the correlations between neuropsychological performance and in-

flammatory markers were weak, they were most apparent in AD and cognitive tests [48]. It is indicated that a panel of specific proteins reflecting different pathological mechanisms could be a valuable and potent tool for a more definitive diagnosis of AD and a more precise classification of patients suffering from the disorder.

Current data have suggested the utility of machine learning tools to investigate novel biomarkers for AD. It is an artificial intelligence algorithm that chooses the best model to decode available data [49]. It has been revealed that the specificity and sensitivity of diagnosis may be significantly enhanced by combining machine learning, numerous variables, and novel biomarkers [50]. A recent comprehensive meta-analysis has demonstrated that naive Bayes and random forest models seem to be the best models for determining susceptibility to MCI and AD [50]. Abate *et al.* revealed that machine learning models use plasma levels of unfolded p53 (U-p532D3A8+), MMSE scale, and apolipoprotein E epsilon-4 (APOEε4), allowing AD prediction with nearly 87% agreement with clinical diagnosis. Moreover, plasma levels of unfolded p53 (U-p532D3A8+) could be a promising potential blood biomarker for AD [51]. Another group of researchers found that combining different methods of deep-learning convolutional neural networks (CNNs) with an automatic image interpretation system of FDG and florbetapir tau PETcould accurately predict future cognitive decline in patients with MCI with significantly higher prediction accuracy (84.2%) for conversion to AD in comparison to conventional methods [52]. Studies have demonstrated encouraging results concerning machine learning techniques and novel biomarkers as a promising approach for AD prediction [50].

4.1. Changes in the Concentrations of Proinflammatory Proteins in Dementia

Growing evidence from comprehensive proteomic studies has suggested that increased concentrations of inflammatory proteins could be a useful biological marker of cognitive decline severity. Measuring changes in the levels of selected inflammatory proteins in CSF and blood may allow for monitoring of disease progression (Table **1**).

Cytokines are a large family of proteins, including interleukins and chemokines, which are released following activation by infection, inflammation, or damage of brain cells. They are a heterogeneous group of proteins that can act as pro-and anti-inflammatory factors [70]. One of the largest groups of proinflammatory proteins modulating inflammatory response in the CNS is interleukins. A growing body of evidence indicates that assessing cytokine profiles in the CSF and serum of patients with dementia could be a useful diagnostic tool for monitoring disease progression. Studies on mice and humans have identified several interleukins, including IL-1, IL-6, TNF-α, which contribute to the progression of AD.

Table 1. Direction of changes in the concentrations of proinflammatory proteins in patients with dementia.

Protein	Patients	Controls	CSF	Serum	Plasma	Whole Blood	Refs.
IL-1β	AD=60	94	-	↑	-	-	[53]
IL-1β	AD=58	31	-	↑	-	-	[54]
IL-1β	AD=11	12	↑	-	-	-	[55]
IL-6	AD=2295	2498	-	-	-	↑	[56]
TNF-α	AD=2433	1984	-	-	-	↑	[56]
TNF-α	EOAD=22	50	-	↑	-	-	[57]
TNF-α	LOAD=54	50	-	↑	-	-	[57]
TNF-α	AD=32	17	↑	-	-	-	[58]
CCL2	AD=310	120	-	-	↑	-	[59]
CCL2	MCI=66	120	-	-	↑	-	[59]
CX3CL1	AD=42	20	↑	-	-	↑	[60]
CX3CL1	MCI=18	20	↑	-	-	↑	[60]
IL-15	AD=17	32	↑	-	-	-	[61]
IL-15	AD=57	508	↑	-	-	-	[62]
IL-15	MCI=256	508	↑	-	-	-	[62]
IL-4	MCI=21	20	-	-	↑	-	[63]
IL-10	AD=27	18	-	↑	-	-	[64]
IL-10	MCI=21	20	-	-	↑	-	[63]
CXCL-12	AD=30	30	-	-	↓	-	[65]
sTREM2	AD=66	100	↑	-	-	-	[66]
sTREM2	MCI=184	100	↑	-	-	-	[66]
YKL-40	AD=11	35	↑	-	-	-	[67]
HMGB1	MCI=12	12	-	↑	-	-	[68]
GPNMB	AD=10	10	↑	-	-	-	[69]

The contribution of IL-1, particularly IL-1β isoform, to the pathogenesis of AD is well described in the literature [18, 23, 58]. During inflammation in the CNS, interleukin 1 is released by activated microglial cells. This is confirmed by a study conducted by Griffin *et al.*, in which increased levels of IL-1 in the brain tissue, CSF, and serum of patients with AD were found [71]. Studies by Griffin *et al.* revealed that elevated expression of IL-1 is associated with tau phosphorylation and NFT formation through kinase MAPK-P38 activation and IL-1-driven cascades [71, 72]. A comparable tendency in IL-1β has been described in patients with MCI [54, 73]. However, CSF IL-1β levels are decreased in amnestic MCI patients [74].

Mounting evidence indicates the significance of IL-6 in the development of inflammation in the brains of AD patients [75-77]. IL-6 is a major proinflammatory cytokine, the expression of which is increased in the brains of AD patients [76]. This interleukin is released during microglia activation caused by APP amino acids and is found near amyloid plaques [78]. Various cellular models have demonstrated that IL-6 has a pleiotropic effect. By way of illustration, IL-6 contributed to the activation of APP expression in primary rat cortical neurons, whereas no effect was observed on glial cells [79, 80]. It is suggested that higher levels of IL-6 and IL-1β mRNA are associated with different expressions. The involvement of IL-6 in neuroinflammation was confirmed by treating hippocampal neurons with IL-6, which resulted in the elevation of hyperphosphorylated tau [81]. In the Mayo Clinic Study of Aging, a greater likelihood of diagnosing MCI was connected with enhanced IL-6 levels [82]. Moreover, it seems that soluble receptor IL-6Rα may also be a marker for early pathology in AD. Wang *et al.* assessed a panel of 43 inflammatory proteins in the CSF of patients with AD and subjective cognitive decline (SCI). The authors reported that elevated levels of IL-6Rα in the SCI of patients may be related to an undiagnosed psychological stress reaction and may lead to the development of future cognitive decline [83].

IL-15 is a pleiotropic cytokine expressed in astrocytes and activated microglia. In a pro-inflammatory state, this cytokine is responsible for T and B cell proliferation. Nevertheless, IL-15 may also act as a neuroprotective protein. It promotes T cell recruitment in an injured brain, which is associated with neuroprotection [84, 85], but its specific role in neurodegeneration is still not fully understood. However, studies have demonstrated a close connection between serum IL-15 levels and dementia [84]. Research on the brains of AD patients has revealed markedly elevated IL-15 levels in the advanced stages of the disease [86]. Moreover, analyses of the CSF of AD patients also revealed elevated levels of IL-15 [58, 62, 83], which were correlated with age of onset [61].

Anti-inflammatory cytokines are responsible for neutralizing and preventing inflammation. IL-4 has been described as a cytokine, which inhibits the release of TNF-α, IL-1β, and IL-6 by activated monocytes [87]. In AD, IL-4 induces M2 microglia activation, in which microglia release anti-inflammatory cytokines, such as IL-10. Higher IL-4 levels were observed in MCI and AD patients, although they were negatively correlated with disease severity [63]. However, Leung *et al.* discovered that in patients with AD showing rapid cognitive decline, IL-4 levels were significantly elevated compared to patients with slow cognitive decline [88]. As acetylcholinesterase is linked to amyloid deposits, studies involving treatment with Acetylcholinesterase inhibitors (AChEI) were conducted. It was found that the therapy caused upregulation of IL-4 in AD patients [89, 90]. Moreover, treating mice with IL-4 was associated with both a decline in Aβ aggregation and a reduction in tau phosphorylation [91, 92].

Il-10 is another anti-inflammatory cytokine found in the M2 microglia activation state [23]. This cytokine is predominantly regarded as an inhibitor of the expression of several inflammatory cytokines, such as IL-1β and TNF-α, as well as an inhibitor of antigen presentation through inhibiting MHC II and CD80 on cells [93]. In mouse models of AD, improvement in neurogenesis was linked with the upregulation of IL-10 [94]. Research on gene polymorphism in the IL-10 gene, which relates it to AD pathology, is currently underway [95]. Studies examining the CSF and blood of AD patients have demonstrated elevated levels of this cytokine in comparison to non-demented controls [58, 63]. Moreover, IL-10 plasma levels were negatively correlated with ventricular and brain volume obtained using neuroimaging MRI tests [88]. Additionally, CSF levels of Aβ42 and Aβ42/p/tau ratio were negatively correlated with IL-10 serum levels [64].

The physiological role of TNF-α (Tumor Necrosis Factor-α) in the CNS is extensive, which includes promoting cell migration and proliferation, impacting synaptic plasticity, and maintaining ion homeostasis. However, TNF-α is also involved in neuropathology as it stimulates the activation of microglia and astrocytes and causes synaptic loss [96]. Activated microglia and astrocytes are the main structures releasing this protein [22]. Increased expression of TNF-α in the brains of AD patients [97] and elevated levels of TNF-α in body fluids of AD patients compared to healthy controls have been evaluated in recent articles [56, 58, 98]. It has been suggested that TNF-α contributes to AD pathology through modulation of Aβ plaque formation and regulation of the glial response by recruiting peripheral monocytes into the brain [99, 100]. Furthermore, some studies have indicated a relationship between elevated CSF and serum concentrations of TNF-α in EOAD and LOAD compared to non-demented controls [57, 58].

Physiologically, chemokines expressed within the CNS may play a role in initiating progenitor cell and neuronal migration during brain development and may function as trophic factors for neurons. A close relationship between the expression of selected chemokines (*e.g.,* CX3CL1, CXCL12, CCL2) and the influx of inflammatory cells into the CNS has been demonstrated. Chemokines expressed in the CNS, *i.e.,* CX3CL1 and CXCL12, seem to be key players in the development of inflammation present in the brains of AD pa-

tients. Fractalkine (CX3CL1) seems to be a pivotal factor in the development of dementing disorders, particularly in the early stages of the disease [60]. Fractalkine and its CX3CR1 receptor are particularly interesting in the context of an association between AD and inflammation. CX3CL1 is engaged in the communication between neurons and microglia cells expressing CX3CR1. It has been demonstrated that the CX3CR1/CX3CL1 complex modulates neuronal survival, plaque load, and cognition [15]. Therefore, dysregulation of the CXC3CL1 pathway exerts harmful effects on neurogenesis, synaptic plasticity as well as cognition [101, 102]. Disturbance in the CX3CL1 signaling axis has been described in several neurodegenerative diseases, including AD, although its specific role has not been fully elucidated to date [103-106]. Findings from investigations involving animal models of AD confirmed that CX3CR1/CX3CL1 interaction participates in the clearance of amyloid plaques and may play a role in the intraneuronal accumulation of hyperphosphorylated tau [60]. CX3CL1 may be useful as an early biomarker in the diagnosis of cognitive disorders, particularly MCI and AD. Furthermore, it is suggested that increased levels of CX3CL1 may predict the risk of future dementia in individuals who are cognitively normal. The inclusion of CX3CL1 in the biomarker panel may improve the diagnostic performance of established biomarkers (Aβ1-42, tau, pTau), particularly in patients with mild cognitive decline [60].

A very important chemokine in the process of neuroregeneration seems to be CXCL12, also known as stromal cell-derived factor 1 (SDF1). Studies have revealed that mice without CXCL12 died during the embryonic period due to serious defects in nervous and hematopoietic systems [107]. Furthermore, Trousse *et al.* demonstrated that CXCL12 knockdown mice models have disturbed memory and impaired ability to learn [108]. This chemokine binds to the CXCR4 receptor, being the only chemokine ligand that binds to this receptor [109]. It is not only expressed in peripheral blood but also in microglia, astrocytes, and neurons [110]. Studies on transgenic mice have demonstrated altered expression of CXCR4 near NFT [111]. Moreover, it has been found that the brains of both AD patients and mice expressed decreased levels of CXCL12 in comparison to controls [109, 112]. Furthermore, Janssens *et al.* reported that injection of CXCL12 into brain ventricles of mice was associated with a decreased number of amyloid aggregates [109]. This may contribute to reduced plasma and CSF levels of CXCL12 in patients with mild AD [65]. Interestingly, treating mouse models of AD with SDF1 resulted in a decrease in the number and range of AB plaques [113], indicating a protective role of this cytokine.

As CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), is responsible for macrophage and monocyte migration [114] and microglia activation during inflammation, it is linked to aggregation of Aβ plaques during AD pathology [115]. Research on AD brains has demonstrated the overexpression of CCL2, particularly in microglia, astrocytes, and near Aβ plaques [59, 115, 116]. Interestingly, MCP-1 expression is also regulated by amyloid 1-42, as proven in a study on cell cultures [117] and AD brains. The expression could be mediated by the JNK-AP1 signaling pathway [118]. The axis between CCL2 and its receptor, CCR2, is linked to AD development through provoking the inflammatory cascade, facilitating the migration of macrophages to the brain, and regulating other cytokines linked to inflammation [119]. Recent data have shown that plasma and CSF levels of CCL2 are elevated in AD and MCI compared to controls, higher in AD patients than individuals with MCI, and correlated with disease progression [59, 120, 121]. Furthermore, rapidity of cognitive decline was described in prodromal AD patients in combination with CCL2 levels [122].

CXCR3 is a chemokine receptor expressed not only in astrocytes and microglia, both in the activated and resting states, but also in neurons [123, 124]. Studies have demonstrated that lowering the expression of CXCR3 in transgenic mice reduced the number of amyloid plaques and decreased Aβ protein levels [125]. As CXCR3 binds to a group of chemokines, including CXCL9, CXCL10, and CXCL11, scientists have also decided to examine them. Elevated levels of CXCL10 were found in MCI and AD patients compared to controls. Furthermore, the chemokine was positively correlated with MMSE [126, 127]. In a study by Corrêa *et al.*, the same observations were made between CXCL10 and Aβ in AD patients, although the levels of this chemokine did not differ between the groups [128].

4.2. Genetic Polymorphisms of Inflammatory Proteins and Risk of Dementia

Studies have demonstrated that not only monitoring of changes in the concentrations of proteins associated with inflammation but also polymorphism in inflammation-related genes may be important in the assessment of cognitive impairment development. Advanced technologies for the analysis of genetic polymorphism have identified numerous genetic loci that affect AD. Moreover, it has been observed that both pro- and anti-inflammatory molecules, particularly IL-1A, TNF-α, VEGF, IL-4, IL-10, and TGF-β1, are associated with a higher risk of AD [129]. Interestingly, the analysis of numerous polymorphisms in different ethnic groups from different regions showed that the same genotype might either increase or exert no impact on AD risk, depending on the population. By way of illustration, *IL-1A* 889 C/T polymorphism seems to be a risk factor for Caucasians but not Asians [130, 131]. It is suggested that polymorphism may influence the expression of cytokines in AD, leading to increased gene transcriptional activity and abundant proinflammatory cytokine release by microglia after stimulation [132, 133]. Investigations of the relationship between polymorphisms in inflammatory molecules and AD risk could provide a deeper insight into the biological mechanisms of the disease and may contribute to finding effective therapies.

Knowing that cytokines play an important role in neuroinflammation, researchers have started to investigate the influence of gene polymorphisms on overall AD risk [129]. It was found that the IL-1 beta -511 C/T genotype was linked to the risk of developing AD in the Caucasian popula-

tion independent of the presence or absence of APOE4 allele [134], similar to IL1B +3953 TT polymorphism, which was also associated with a higher risk of AD [135]. TNF-α -308 A/G gene alterations, similar to IL-1, were found to be associated with an increased risk of AD but only in the East Asian population, with no such association found in European or Middle Eastern subjects [136]. IL-4 polymorphism was also studied, and it was revealed that −590 C/T and −1098 T/G alterations were associated with altered IL-4 transcription, causing an increased risk of AD [137, 138].

Although gene alterations are commonly negatively correlated with disease, some of them act as protective agents. A meta-analysis by Hua *et al.* demonstrated that −174 G/C polymorphism occurring in IL-6 could display a protective ability in the Asian population [130]. Studies on IL-10 -1082A genotype in the Brazilian population revealed a decreased risk of AD compared to other examined genotypes [139].

Recent genome-wide association studies (GWAS) have established that TREM2 triggering receptors expressed in myeloid cells-2 and CD33, which have been proven to participate in innate immune activation, are genes related to the risk of AD. Recent investigations have linked TREM2 and antigenCD33 dysfunction to the promotion of inflammation, which leads to Aβ production, although further research is needed. To confirm GWAS findings, the expression of CD33 mRNA was tested, and the results showed its elevated expression in microglia [140]. Furthermore, it was found to be positively correlated with the number of plaques and Aβ1-42 levels [141]. In turn, TREM2 variants were linked to accelerated brain atrophy compared to non-carriers [140]. Furthermore, these variants may alter TREM2 receptors, probably affecting Aβ clearance and, as a result, promoting neurodegeneration [140, 142]. Mouse models of AD with TREM2 and CD33 deletions confirmed this hypothesis by associating them with a microglial impairment that causes Aβ production [143, 144].

4.3. Biomarkers of Glial Activation

Glial cells are non-neuronal cells located within the whole nervous system (central and peripheral NS), providing structural and metabolic support for the neurons, maintaining synaptic homeostasis, involving in neuronal repair, and eliminating pathogens and dead CNS cells. Glial activation occurs as part of an altered immune response characterized by an enhanced inflammatory response during AD progression. Notably, a longitudinal TSPO-PET study revealed diminished microglial activation over time in patients at the MCI stage and increased activation in patients at the AD stage of dementia [145]. These results suggest that at the early onset of dementia, microglial activation has beneficial effects, whereas it is rather detrimental in the later stages of the disease. Well-known markers of glial activation seem to be TREM2 and chitinase-3-like protein 1 (CHI3L1), also known as YKL-40.

TREM2, when expressed in microglia, is thought to positively regulate neuroinflammation through plaque phagocytosis. On the other hand, it can exert a negative effect in the later stages of the disease by inducing inflammatory responses. It has been established that TREM2 is overexpressed in the brains of AD patients [146]. Moreover, significantly higher concentrations of soluble TREM2 have been observed in the CSF of patients with AD. sTREM releases into CSF through ADAM10 and ADAM17 cleavage. Additionally, significantly elevated levels of TREM2 have been observed in the CSF of early symptomatic AD patients [147-149]. A recent study has found that CSF levels of sTREM2 are positively correlated with T-tau and P-tau concentrations, suggesting a role of this protein in the neurodegenerative process of AD [147-150].

A crucial marker of glial activation is YKL-40, a glycoprotein found in the astrocytes of healthy and AD patients' brain tissues [151]. The overall role of YKL-40 in the physiological state remains unknown. However, based on a number of studies, we can assume that in AD, pro-inflammatory cytokines, such as TNF-α and IL-1β, may trigger YKL-40 expression from astrocytes [152]. Furthermore, based on studies using knockout mice, we can hypothesize the neuroprotective role of this cytokine. These studies have revealed more severe neuropathology and noticeable gliosis in knockout mice than wild-type controls [153, 154]. YKL-40 has a few homologs, such as chitinase-3 like 3 (CHI3L3) and chitinase-3 like 2 (CHI3L2), which are shown to increase mRNA expression, and are found in the brains of AD mouse models as well as the human AD brain [155]. Increased concentrations of YKL-40 have been observed in fully developed AD and, importantly, in the very early stages of the disease (preclinical AD, very mild, and mild dementia) [67, 156]. The correlation with elevated levels of total and phosphorylated tau may indicate a pivotal role of this protein in AD pathology. Notably, it appears that YKL-40 may also be a prognostic marker for monitoring disease progression in individuals with and without cognitive impairment symptoms. Interestingly, a recent study has demonstrated that higher levels of YKL-40 are present only in AD with ongoing dementia and correlated with increased astrogliosis [157]. Furthermore, elevated concentrations of YKL-40 predicted progression from MCI to symptomatic AD and other types of dementia, as measured by an annual assessment of MMSE during the follow-up. Elevated YKL-40 concentrations in patients without dementia are found to be associated with a higher risk of developing AD dementia [62]. These findings indicate the prognostic value of YKL-40 as a potential biomarker for AD.

4.4. Novel Biomarkers

High mobility group box-1 (HMGB1), a novel protein, is considered a potential biomarker for AD. It is a nonhistone, nuclear protein, part of the damage-associated molecular pattern (DAMP) expressed in many types of cells, including the brain, microglia, and neurons [158]. It has been proven that HMGB1 induces inflammation through binding to RAGE and TLRs, which results in the production of proinflammatory cytokines [159]. Research on AD has revealed that HMGB1 is involved in maintaining amyloid β 1-42 oli-

gomer stability. Studies on brain tissue have demonstrated that AD patients have elevated levels of this protein than controls. Moreover, a study used immunostaining to detect HMGB1 in Aβ plaques, suggesting a tendency for this protein to accumulate in plaques [160]. Elevated serum levels of HMGB1 in AD patients in comparison to controls were found. Moreover, subjects with MCI showed even higher concentrations in comparison to patients with AD [68], which may indicate its crucial role in the early onset of the disease.

Another factor of potential significance in AD pathology, which is also considered a possible biomarker, may be glycoprotein non-metastatic melanoma protein B (GPNMB). It is a type I transmembrane protein, firstly isolated from a poorly metastatic melanoma cell line [161]. GPNMB is involved in osteoclast differentiation and deterioration, and T--cell activation. It acts as a negative regulator of macrophage inflammatory responses [162, 163]. Studies using transgenic mice have demonstrated GPNMB expression in neurons and astrocytes. Nonetheless, its role in the brain is still not fully known. Overexpression of GPNMB has been detected in some neurodegenerative disorders, such as Amyotrophic Lateral Sclerosis (ALS) [164]. Moreover, Murata *et al.* showed its potential neuroprotective function by improving memory in overexpressed mice [165]. Satoh *et al.* demonstrated that activated microglia accumulating around senile plaques caused elevated levels of this protein in AD brains [166]. Recent studies have associated GPNMB only with activated microglia occurring during AD. Furthermore, analysis of immortalized microglia cell lines produced data, associating soluble Aβ with GPNMB expression. The same study confirmed these results as elevated levels of this protein were not only found in the brains but also in the CSF of AD patients in comparison to controls [69]. However, a study conducted by Aichholzer *et al.* did not confirm these findings [167].

The proteins described in this paper play an important role not only in the pathogenesis of neurodegenerative diseases but may also be useful for diagnostic purposes. Determination of their concentrations in CSF requires a lumbar puncture, which has certain medical and legal limitations. Since it is more invasive than drawing blood, it requires additional consent from the patient, which is regulated differently in every country in which the patient is treated [168]. Various branches of law generally contain provisions that regulate specific areas of social relations and protect the rights of the parties to these relations. This also applies to the norms of medical law, which, among other things, defines the relationship between medical professionals and their patients. The regulation of these relations is manifested, inter alia, in the indication of the rights of the patient and the corresponding duties of the doctor or another individual performing medical procedures. Therefore, it seems that the possibility of determining some of the proteins described in this work in the blood is very important not only for medical reasons but is also associated with fewer legal restrictions. Considering the fact that the person's welfare can be endangered in various medical activities, they are often subjected to detailed regulation not only by medical but also by criminal law. This is particularly evident in the case of AD patients. They are not always able to consent to additional medical procedures concerning them, including a lumbar puncture. Therefore, the issues addressed in this paper regarding the significance of inflammatory markers in the pathogenesis of AD, and the possibility of their determination in blood, which is less complicated to obtain than a lumbar puncture, encompasses medical, economic as well as legal issues.

4.5. Other Molecules Associated with AD

Recent studies have suggested that it is not only proteins that can be used in diagnosing and monitoring the disease. Iron, copper, and zinc are important metals participating in healthy brain processes. However, their accumulation and homeostatic imbalance may act as a trigger of the disease and facilitate its progression. A recent analysis of the brains of AD patients has revealed significant aggregation of these metal ions [169]. The aforementioned molecules tend to accumulate near senile plaques and NFTs [170]. Interestingly, they also have a significant association with the molecular basis of AD. An excessive amount of copper is known to enhance APP expression and production of Aβ [171]. Moreover, iron and copper modulate APP levels by controlling transcription and translation of APP genes [172]. Therefore, a recent study has analyzed metal levels in the CSF of AD patients. It was demonstrated that ferritin concentrations are correlated with lower levels of Aβ 1-42. The discovery demonstrated that iron might be linked to amyloid deposition and, as a result, stimulate AD progression [173]. Another study revealed a correlation between higher iron and copper levels, and elevated concentrations of the established markers of the disease, such as Aβ 1-42 and *p*-Tau [174]. These findings confirmed the potential utility of metal ions as novel biomarkers for AD or even as a therapeutic strategy [172].

5. AVAILABLE AND POTENTIAL TREATMENT FOR AD

At the beginning of June 2021, the US Food and Drug Administration (FDA) approved the first drug, Aduhelm (aducanumab), for the treatment of AD. It is a monoclonal antibody targeted amyloid β plaques immunization [175]. Unfortunately, Aduhelm (aducanumab) is not a cure for the disease. Until this year, established drugs are only symptomatic, not capable of halting or slowing neurodegeneration. Donepezil, galantamine, and rivastigmine work as cholinesterase inhibitors, while memantine is an antagonist of the N-methyl-D-aspartate receptor [176]. Knowing how important neuroinflammation is, researchers have started to consider new strategies regarding this issue, particularly using anti-inflammatory drugs [177]. Epidemiological studies conducted in the last decades have demonstrated that the use of non-steroidal anti-inflammatory drugs (NSAIDs) could protect individuals against AD. However, findings from clinical trials concerning the effectiveness of anti-inflammatory drugs in AD therapy are controversial and divergent [178]. However, research on a possible treatment for AD in various fields is still being pursued with a particular focus on non-

pharmacological approaches. Therefore, plant extracts and nutritional approaches to the treatment of the disease are being investigated. Garlic has an established role in the treatment of different diseases, such as cardiovascular and gastrointestinal disorders and cancer [185-187]. Therefore, researchers have started to examine the impact of "Aged Garlic Extract" (AGE) and S-Allyl-L-Cysteine (SAC) *in vitro* on AD models with positive outcomes [179, 180]. A recent study on neuronal cells treated with AGE and SAC stimulated by reactive oxygen species (ROS) has demonstrated that both substances may exert neuroprotective and neurorescue effects, protecting neurons from ROS insults and maintaining pre-synaptic proteins [182]. The same study showed the benefits of pre-treatment with AGE, protecting around 80% of neuronal cells from ROS [179]. Furthermore, another *in vitro* study indicated that both SAC and AGE may affect Aβ, resulting in a decrease in the number of plaques in AD model brains, although the specific mechanism has not yet been fully known [181, 182]. A different substance worth mentioning is curcumin. It is a polyphenol found in turmeric, a spice with a distinct yellow color that is widely used in cooking [183]. Curcumin is known for its anti-inflammatory, antitumor, and antioxidant properties [184]. In Alzheimer's disease, research regarding curcumin in AD has demonstrated that the compound has the ability to inhibit Aβ and tau aggregation *in vivo* [188]. Moreover, a study by Khanna *et al.* revealed that curcumin might have anti-inflammatory and neuroprotective effects [189]. The main pathway by which curcumin work is believed to be through NFkB inhibition by preventing phosphorylation of IkB and consecutive activation of NFkB [190]. Interestingly, this substance is capable of inhibiting enzymes, such as β-secretase (BACE-1), which are responsible for APP cleavage [191]. The limitation of using curcumin is its poor bioavailability due to its solubility in water. Thus, a new form of the substance, a polymeric nanoparticle encapsulated curcumin (NanoCurc™), has been developed [192]. A study by Ray *et al.* demonstrated that the use of NanoCurc™ protected neuronally differentiated human cells from oxidative stress. Furthermore, cell cultures treated with NanoCurc™ displayed elevated levels of Neuron Specific Enolase (NSE), which may suggest the protective role of the molecule on the neuronal phenotype. On the other hand, animal models treated with this molecule showed decreased levels of ROS, but also attenuated activity of caspase-3 and caspase-7 in the brain. Interestingly, it also exerted a positive effect on already ROS insulted cells. These findings suggested the therapeutic application of NanoCurc™ in AD patients, particularly considering its ability to cross the BBB [193].

Another interesting theory concerns music therapy as a potential AD agent. Increasing evidence indicates that listening to music may improve aspects of memory in patients with dementia [194]. Although the exact mechanism has not been fully elucidated, it is known that music interacts with brain regions responsible for emotions and decision-making. This has led to the establishment of some possible mechanisms underlying this phenomenon, such as dopaminergic pathway activation or sympathetic arousal [194]. A systematic review conducted by Garcia-Casares *et al.* confirmed the beneficial influence of music therapy on cognition, emotion, and behavior in AD patients [195]. Interestingly, this type of therapy resulted in a marked improvement in memory and orientation and alleviated depression and anxiety in AD patients after only four sessions [196]. However, further research is needed in this regard.

CONCLUSION

A growing number of scientific reports have demonstrated that neuroinflammation seems to be an important factor in Alzheimer's pathology, which influences amyloid deposition, NFT formation, and consequently leads to neuronal death. During this process, activated microglia and astrocytes release pro- and anti-inflammatory cytokines that modulate inflammation in the CNS. In a healthy brain, they are capable of eliminating abnormalities, whereas, in AD, their over-activation cause elevated expression of cytokines, thus inducing neuroinflammation. Investigation of these cytokines released by activated microglia might be helpful in diagnosing the disease in the early stages due to anti-inflammatory M2 type microglia, which are involved in Aβ clearance before they switch to a more damaging M1 phenotype. Studies in recent years have highlighted the relationship between inflammation in the gut and brain and its association to neuroinflammation in AD. Comprehension and recognition of the mechanisms of inflammatory molecule release might establish a panel of proteins that may potentially be used as new biomarkers. Considering that AD is a heterogenic disorder, it seems that a combination of multiple markers reflecting various pathomechanisms and different stages of disease progression could be the most effective strategy. These discoveries would improve the detectability of the disease and enable the discovery of possible anti-inflammatory treatment. In this review, we described pivotal inflammatory molecules with the inclusion of new and potential ones and hypothesized their use as novel biomarkers for AD. However, further studies are needed to fully understand the contribution of neuroinflammation to AD pathology and determine the clinical utility of these inflammatory markers in the diagnosis, prognosis, and management of AD.

LIST OF ABBREVIATIONS

AChEI	=	Acetylcholinesterase Inhibitors
AD	=	Alzheimer's Disease
AGE	=	"Aged Garlic Extract"
ALS	=	Amyotrophic lateral sclerosis
APOEε4	=	Apolipoprotein E epsilon-4
APP	=	Amyloid precursor protein
Aβ	=	Amyloid Beta
BACE-1	=	β-secretase
BBB	=	Blood Brain Barrier
CNNs	=	Convolutional Neural Networks

CNS = Central Nervous System
CSF = Cerebrospinal Fluid
DAMP = Damage-associated Molecular Pattern
EOAD = Early-onset Alzheimer's Disease
FDA = Food and Drug Administration
GFAP = Glial Fibrillary Acidic Protein
GPNMB = Glycoprotein Non-metastatic Melanoma Protein B
GWAS = Genome-wide Association Studies
HMGB1 = High Mobility Group Box-1
LOAD = Late-onset Alzheimer's Disease
LPS = Lipopolysaccharide
MCI = Mild Cognitive Impairment
MCP-1 = Monocyte Chemoattractant Protein-1
NETs = Neutrophil Extracellular Traps
NFT = Neurofibrillary Tangle
NSAIDs = Non-Steroidal Anti-Inflammatory Drugs
NSE = Neuron Specific Enolase
ROS = Reactive Oxygen Species
SAC = S-Allyl-L-Cysteine
SCI = Subjective Cognitive Decline
SDF-1 = Stromal cell-Derived Factor 1
TLR-4 = Toll-Like Receptor 4
TNF = Tumor Necrosis Factor

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P2. *Potential utility of cerebrospinal fluid glycoprotein nonmetastatic melanoma protein B as a neuroinflammatory diagnostic biomarker in mild cognitive impairment and Alzheimer's disease.*

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Potential Utility of Cerebrospinal Fluid Glycoprotein Nonmetastatic Melanoma Protein B as a Neuroinflammatory Diagnostic Biomarker in Mild Cognitive Impairment and Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a very common neurodegenerative disorder characterized by the gradual loss of neurons and extracellular amyloid-peptide buildup. There is compelling evidence that the disease process depends on neuroinflammatory alterations, such as the activation of astrocytes and microglia cells. A transmembrane glycoprotein known as glycoprotein nonmetastatic melanoma protein B (GPNMB) plays a neuroprotective role during the development of neurodegeneration. To the best of our knowledge, this is the first investigation discussing the potential clinical usefulness of this protein in the AD continuum, especially in the MCI (mild cognitive impairment) stage. A total of 71 patients with AD or MCI as well as controls were enrolled in this study. The concentrations of GPNMB, YKL-40, Aβ1-42 (amyloid beta 1-42), Tau, and pTau and the Aβ1-42/1-40 ratio in the CSF (cerebrospinal fluid) were tested using immunological methods. The concentrations of both GPNMB and YKL-40 in the cerebrospinal fluid were significantly higher in patients with AD and MCI compared to the controls. Moreover, both proteins were biochemically associated with classical biomarkers of AD and were especially associated with the Aβ1-42/1-40 ratio and Tau and pTau levels in the whole study group. Elevated concentrations of GPNMB were observed in the Aβ(+) group of AD patients compared to the Aβ(−) subjects. Additionally, the diagnostic performance (AUC value) of GPNMB was higher than that of amyloid β1-42 in MCI patients compared with controls. Our study indicates that GPNMB might be a promising neuroinflammatory biomarker for the early diagnosis and prognosis of the AD continuum, with potential utility as a therapeutic target.

Keywords: Alzheimer's disease; GPNMB; YKL-40; neuroinflammation

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1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and the most frequent cause of dementia [1]. It is a gradual illness, with up to 20 years before the first observable symptoms occur [2]. Memory loss, the inability to learn new things, aphasia, sleep cycle disruption, and serious issues with short- and long-term memory are some of its best-known symptoms [3]. The most prevalent characteristics of AD include the buildup of amyloid (Aβ) fibrils and insoluble plaques, neurofibrillary tangles (NFT) made of hyperphosphorylated Tau, neuronal and synaptic loss, and atrophy of brain regions important for memory [4]. A cascade of pathogenic events, including neuroinflammation and CNS (central nervous system) cell destruction, are triggered by the accumulation and aggregation of Aβ1-42 [5,6].

Despite the fact that neuroinflammation was described over 20 years ago, its role in the development of the disease is still unclear. Recent findings indicate that different Aβ complexes interact with expressed pattern recognition receptors in microglia and astrocytes, triggering innate immunity [7]. Depending on the disease stage, the innate immune response appears to have pleiotropic functions in the onset of cognitive impairment, with both neuroprotective and neurodegenerative outcomes. Activated microglia can be divided into two types: M1, being strictly connected to proinflammatory responses, and M2, described as neuroprotective [8]. In order to protect themselves, microglia change their phenotype. It has been described that, at the site of injury, M1 microglia typically predominate when the disease reaches its terminal stage and M2 microglia's immune resolution and repair mechanisms are inhibited [8]. Without shielding healthy microglia, pathological brain processes can increase pro-inflammatory cytokine production and microglia hyperactivity. As a result, neurons are more susceptible to plaque formation and neurotoxic degradation, which enhances the pro-inflammatory response [9]. In AD, microglial activation is dynamic, and microglia constantly change between phenotypes. The dual role of microglia in AD pathogenesis led Fan et al. to hypothesize that there may be two peaks of microglial activation in AD: an early anti-inflammatory peak in the preclinical stage and a later pro-inflammatory peak in the clinical stage as illness develops after the failure in Aβ clearance [10,11]. A more recent theory suggests that a progressive change from a homeostatic to a disease-associated state is paralleled in microglia with the development of the illness. The downregulation of homeostatic genes and upregulation of known AD-associated genes, such as apolipoprotein E (APOE), triggering receptors expressed on myeloid cells 2 (TREM2), and TYRO protein tyrosine kinase-binding protein (TYROBP), are linked to the transition from normal microglia to disease-associated microglia (DAM) [12]. It should be noted that microglial activation occurs in two steps, the first of which is TREM2-independent, and the second of which is TREM2-dependent, which emphasizes the critical function of TREM2 in the development of neurodegenerative diseases. Transcriptomic research also supports the effects of aging on human microglial phenotypes, which include the upregulation of IL15, CXCR4, VEGF4 (encoding vascular endothelial growth factor 4), and RUNX3 and the downregulation of genes encoding cytoskeleton-associated proteins, cell surface receptors, and adhesion molecules [12,13].

The published data indicate that glycoprotein nonmetastatic melanoma protein B (GPNMB), an inflammatory molecule, is involved in the pathology underlying AD. GPNMB, also known as osteoactivin, is a type 1 transmembrane glycoprotein that was originally identified in low-metastatic melanoma cell lines in 1995 [14]. A significant amount of data also supports the idea that GPNMB functions as a negative regulator of inflammatory processes, which has interested neuroinflammatory researchers. Interestingly, GPNMB overexpression in macrophages decreased in vitro pro-inflammatory cytokine production [15]. Furthermore, it is suggested that GPNMB expression in the brain may be altered by pro-inflammatory stimuli [16]. LPS (lipopolysaccharide) is one of the factors responsible for neuroinflammation and is used as a model for this pathology, with a documented increase in proinflammatory cytokines after its administration in vitro and in vivo [17,18]. Rats that received LPS intraperitoneally (i.p.) had an increase in GPNMB-expressing cells, particularly in the postrema region. The overexpression of GPNMB correlated with OX42 expression, a marker for macrophages and microglia [19]. Furthermore, it was revealed that ADAM10, a metalloproteinase that cleaves APP and is involved in ectodomain shedding, can release GPNMB [20]. An increase in GPNMB levels in the brain has been found in a variety of neurodegenerative disorders, including AD [21], amyotrophic lateral sclerosis (ALS) [22], and Parkinson's disease (PD) [23]. Nonetheless, the influence of GPNMB upregulation on the pathophysiology of these diseases has not yet been adequately explained. According to the available data, GPNMB appears to play a neuroprotective role. However, the mechanism is still not fully known. Recent publications showed that GPNMB promotes the polarization of macrophages to the "M2" type, which is described as more protective due to the release of anti-inflammatory cytokines such as IL-10 and TGF-β [21,24,25]. There-

fore, GPNMB may possibly act as a factor influencing memory and synaptic plasticity, or working as a neuroprotective agent similar to carotenoids [26], flavonoids [27], coenzyme Q10 [28], or lutein [29].

YKL-40 is a chitin-binding lectin belonging to the glycosyl hydrolase family 18 which is also known as chitinase 3-like protein 1 (CHI3L1) or human cartilage glycoprotein 39 (HC-gp39) [30]. Numerous cell types, including macrophages, chondrocytes, neutrophils, and synovial fibroblasts, express the YKL-40 protein [31]. It was established that the higher production of YKL-40 by activated astrocytes and/or microglia is related to amyloid plaques and with NFT pathology [32]. In AD studies, they showed that, when compared to age-matched controls, the expression of mRNA for chitinase-3 like 3 (CHI3L3), a mouse homologue of YKL-40, was significantly higher in the brains of AD-model animals [33]. Remarkably, mRNA expression of TNF-α and YKL-40 was also significantly higher in brain samples obtained from patients with AD in comparison to controls [33].

Despite the mounting evidence of neuroinflammation occurring in AD, additional studies are necessary to clarify how these mechanisms are related to Aβ and tau pathologies and whether these connections are more significant in the beginning or in the later stages of the disease. Therefore, in the present study we compared the dynamic changes in pro- and anti-inflammatory proteins in different stages of AD. Despite the fact that both YKL-40 and GPNMB proteins are related to an inflammatory state in the CNS, they present opposite functions. While GPNMB is considered an anti-inflammatory protein, YKL-40 acts as proinflammatory protein. Combining these two proteins might provide interesting insight into the mechanisms of neuroinflammation in AD. There is still insufficient data regarding the possible diagnostic applicability of GPNMB in patients with mild cognitive impairments (MCI) and AD. To our best knowledge, there is only one publication assessing markers of microglial activity such as GPNMB in the CSF (cerebrospinal fluid) of AD patients and none describing the levels of this protein in the CSF of patients with MCI. Therefore, the present study aimed to assess the potential clinical significance of GPNMB in the AD continuum, especially in patients with MCI, in relation to other neuroinflammatory biomarkers (YKL-40) and classical biomarkers of AD that reflect amyloid and tau pathologies.

2. Materials and Methods

2.1. Material

The study population consisted of 71 subjects (n = 50 women, n = 21 men; median age: 73 (51–89)) from the Department of Neurology, Jagiellonian University Hospital, Krakow, Poland, and included 35 AD patients (n = 28 women, n = 7 men; age: 75 (51–89)), 18 subjects with MCI (n = 11 women, n = 7 men; age: 75.5 (63–81)), and 18 non-demented controls (n = 11 women, n = 7 men; age: 67.5 (53–82)). Standard medical, physical, and neurological examinations, laboratory screening tests, a neurocognitive assessment, and magnetic resonance imaging or computed tomography of the brain were all employed in the clinical diagnosis of the study groups. Alzheimer's disease cases with sporadic occurrences comprised the AD group. No patients involved in this study acknowledged a family history of Alzheimer's disease in their medical interview. The National Institute on Aging and Alzheimer's Association (NIA-AA) criteria were used to establish the AD diagnosis [34,35]. For the most accurate clinical diagnosis of AD, neurochemical data (levels of Aβ1-42, Tau, and pTau181 as well as values of the Aβ1-42/Aβ1-40 ratio) were combined with neuroimaging and neuropsychological tests. The MMSE score (range 0–30) was used to determine dementia severity (AD patients (MMSE: 22 (0–28)), MCI patients (MMSE: 27.5 (26–29)), and controls (MMSE: 28 (25–30)). The study was conducted in the Department of Neurodegeneration Diagnostics at the Medical University of Bialystok according to the guidelines of the Declaration of Helsinki; prior to any procedure, every patient signed an informed consent form, and the Bialystok University study (No. R-I-002/103/2019) received approval from the Ethics Committee. The exclusion criteria for the study included patients with suspected cerebrovascular disorders (such as cerebral hemorrhage, aortic aneurysm,

intracranial aneurysm, stroke, or arteriovenous malformation), elevated albumin quotients (QAlb) indicative of blood–CSF barrier dysfunction, and changes in CT or MRI scans.

The control group comprised individuals who were not experiencing subjective memory impairments and did not meet the MCI criteria but who might experience recurring headaches. None of the patients in this group displayed any meaningful changes in the levels of the recognized biomarkers for AD (Aβ1-42, Tau, and pTau181), which allowed the exclusion of the symptoms' organic background. An Erlangen Score of 0 points across all 18 of the participants in this group supported these findings.

2.2. Biochemical Measurements

Lumbar punctures in the L4/L5 or L3/L4 interspace were used to collect CSF samples into polypropylene tubes. Prior to analysis, all CSF samples were centrifuged, aliquoted, and frozen at −80 °C. The concentrations of analyzed proteins (GPNMB, YKL-40, Aβ1-42, Aβ1-40, Tau, and pTau181) in the CSF were measured in the Department of Neurodegeneration Diagnostics, Medical University of Bialystok, Poland.

For the assessment of concentrations of neurochemical dementia diagnostics (NDD) biomarkers, IBL kits (RE59661, RE59651, Hamburg, Germany) for Aβ1-42 and Aβ1-40 and Fujirebio kits (81572, 81574, Gent, Belgium) for Tau and pTau181 proteins were used.

The analysis of GPNMB was performed using Luminex Human Discovery assay plates, provided by R&D systems, Abingdon, UK, and a Luminex 200 analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (multiplexing, multiparametric, fluorescence laser reading system on microspheres for the simultaneous determination of multiple parameters). Determination of YKL-40 was performed using an ELISA kit provided by MicroVue, Quidel, San Diego, CA, USA. According to the manufacturer's protocols, duplicate measurements were assessed for each standard, control, and sample.

2.3. Statistical Analysis

The PMCMRplus package in the statistical software RStudio (Version 1.4.1106, Boston, MA, USA) and Statistica 13.3 (StatSoft Polska, Krakow, Poland) were used to perform non-parametric tests. The Shapiro–Wilk test demonstrated that the protein concentrations were not distributed normally. The comparisons between the AD, MCI, and control groups were performed using the Kruskal–Wallis test. The post hoc Dwass–Steele–Critchlow–Fligner test was then used to assess significant differences between the levels of the tested groups in order to determine which groups had statistically significant differences. The results are presented as medians and interquartile ranges. Statistical significance was set at $p < 0.05$. In addition, the receiver operating characteristic (ROC) curve and area under curve (AUC) analysis was used to determine the diagnostic usefulness of the tested proteins as potential neuroinflammation-related biomarkers for AD.

3. Results

3.1. Patient Characteristics and CSF Concentrations of GPNMB and YKL-40

A summary of the CSF biomarker values in the examined groups are presented in Table 1. AD biomarkers were marked in all patients' CSF samples. The Kruskal–Wallis test revealed statistically significant differences between all study groups for concentrations of GPNMB ($p < 0.001$, $\chi^2 = 22$) and YKL-40 in the CSF ($p = 0.002$, $\chi^2 = 12.17$), Aβ1-42 ($p < 0.001$, $\chi^2 = 16.5$), Tau ($p < 0.001$, $\chi^2 = 47.1$), and pTau181 ($p < 0.001$, $\chi^2 = 41.7$) as well as Aβ1-42/Aβ1-40 ratio ($p < 0.001$, $\chi^2 = 33.7$). The GPNMB levels in the CSF differed significantly between the patients with AD and the controls and between MCI patients and controls and are presented in Figure 1. These differences were verified by the post hoc Dwass–Steele–Critchlow–Fligner test. The highest CSF concentration of GPNMB was observed in the AD group in comparison to CTRL ($p < 0.001$) and MCI ($p < 0.009$) groups. In MCI patients, the CSF level of GPNMB was also higher than controls, but the difference was not statistically significant ($p = 0.08$). Additionally, we divided the AD and MCI groups into Aβ1-42(+) (AD = 17; MCI = 12) and Aβ1-42(−) (AD = 18; MCI = 6) subgroups

according to a cut-off point of 538 pg/mL. The AD Aβ(+) group showed significantly higher concentrations of GPNMB than the AD Aβ(−) group ($p = 0.028$) (Table 2). The concentration of GPNMB in the MCI Aβ(+) was also higher than that of the MCI Aβ(−) group, although the difference was not significant.

Table 1. The concentrations of tested proteins in the study groups.

Tested Variables	Median (Interquartile Range)			*p* (Kruskal–Wallis Test)	*p* (Dwass–Steele–Critchlow–Flinger Test)		
	AD	MCI	Controls		AD vs. CTRL	AD vs. MCI	MCI vs. CTRL
Aβ1-42 (pg/mL)	520 (197–994)	802 (382–1878)	851 (357–1235)	<0.001	<0.001	0.030	0.947
Aβ1-42/1-40 ratio	0.03 (0.02–0.05)	0.04 (0.03–0.07)	0.07 (0.02–0.09)	<0.001	<0.001	<0.001	0.011
Tau (pg/mL)	710 (357–1722)	389 (209–994)	221 (148–414)	<0.001	<0.001	<0.001	<0.001
pTau181 (pg/mL)	87.9 (46.4–209)	57.2 (34.1–97.1)	36.6 (24.8–55.6)	<0.001	0.001	<0.001	0.002

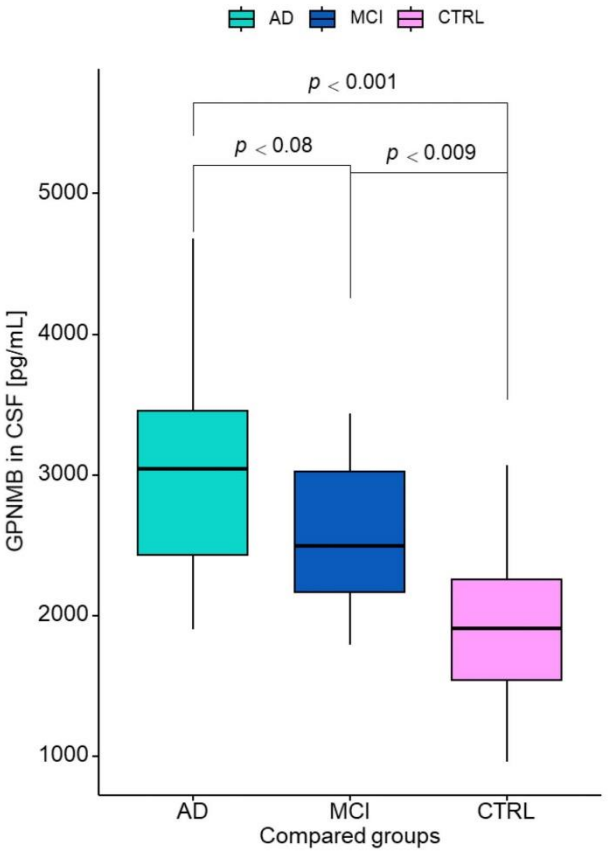

Figure 1. Cerebrospinal fluid levels of GPNMB by group. GPNMB—glycoprotein nonmetastatic melanoma protein B; AD—Alzheimer's disease; MCI—mild cognitive impairment; CTRL—control; CSF—cerebrospinal fluid.

Table 2. The concentrations of GPNMB in AD and MCI patients based on amyloid Aβ status (* $p < 0.05$). GPNMB—glycoprotein nonmetastatic melanoma protein B; AD—Alzheimer's disease MCI—mild cognitive impairment.

	n	GPNMB Median	Interquartile Range	*p*-Value
AD	Aβ(+) = 17 Aβ(−) = 18	3328 2559	2830–3842 2276–3397	0.028 *
MCI	Aβ(+) = 12 Aβ(−) = 6	2658 2174	2366–3328 1923–2950	0.122

The YKL-40 concentrations in the CSF also differed significantly between the patients with AD and the controls as well as between MCI patients and controls. A significantly higher concentration of YKL-40 was found in AD and MCI patients compared to controls ($p = 0.007$; $p = 0.004$). The concentrations in the MCI group were comparable with those of AD group ($p = 0.918$) (Figure 2).

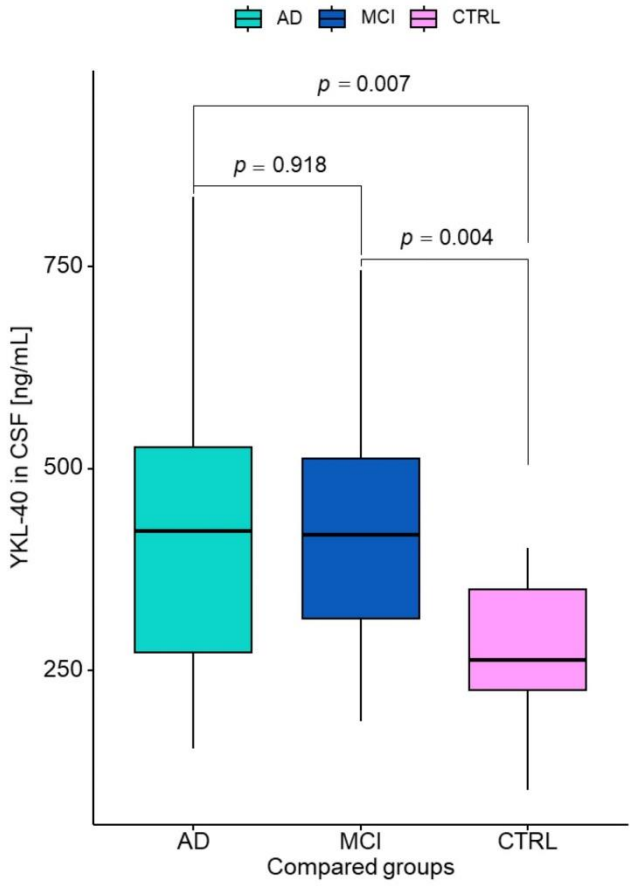

Figure 2. Cerebrospinal fluid levels of YKL-40 in AD, MCI, and CTRL groups. AD—Alzheimer's disease; MCI—mild cognitive impairment; CTRL—control; CSF—cerebrospinal fluid.

3.2. Association between GPNMB, YKL-40, and CSF Biomarkers

The associations between levels of GPNMB, YKL-40, and classical AD biomarkers were analyzed using the Spearman rank correlation test. In the entire study population, significant positive correlations were observed between the CSF levels of GPNMB and Tau (R = 0.6, $p < 0.001$), pTau (R = 0.59, $p < 0.001$), age (R = 0.52, $p < 0.001$), and YKL-40 in the CSF (R = 0.49, $p < 0.001$). A negative correlation was observed between GPNMB and the Aβ1-42/1-40 ratio (R = −0.39, $p < 0.001$) and MMSE (R = −0.41, $p < 0.001$). In addition, CSF YKL-40 levels in the entire population also correlated positively with Tau (R = 0.51, $p < 0.001$) and pTau (R = 0.64, $p < 0.001$) and negatively with the Aβ1-42/1-40 ratio (R = 0.33, $p = 0.01$) (Figure 3).

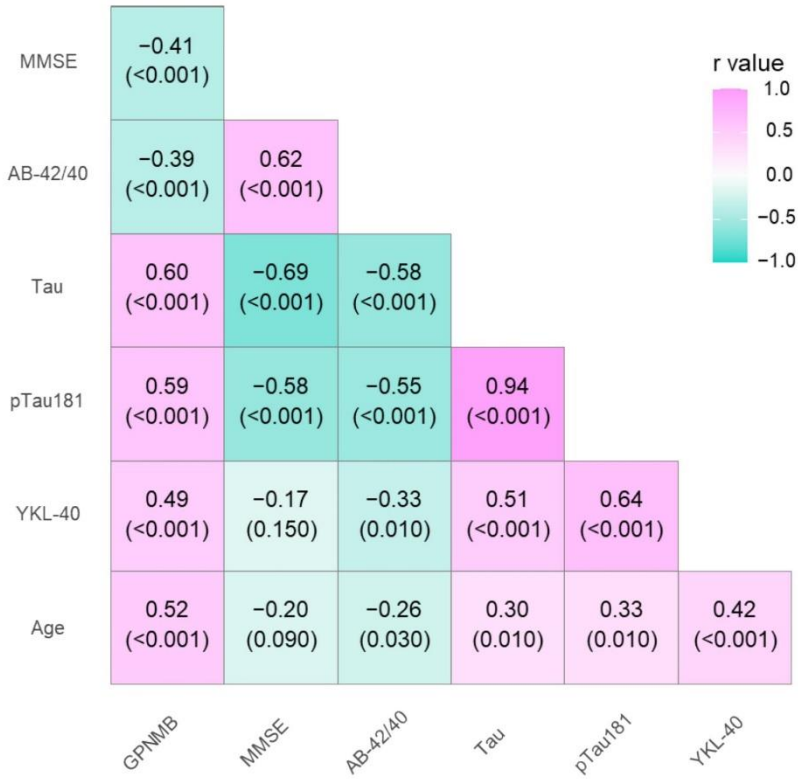

Figure 3. Spearman's correlations between neurochemical biomarkers and tested proteins in the entire study population. MMSE—mini mental state examination score.

In AD patients, the elevated CSF GPNMB level was significantly associated with YKL-40 (R = 0.5, $p < 0.001$) concentrations in the CSF and age (R = 0.62, $p = 0.001$). To determine the impact of the lower median age in the CTRL group, we selected age-matched groups of AD, MCI, and CTRL subjects (median age: 73, 74, and 73, respectively) and compared the concentrations of GPNMB in these groups. As a results of this analysis, we obtained similar statistical trends as were presented earlier (AD vs. CTRL $p = 0.0029$). A weaker correlation was observed between YKL-40 levels and Tau (R = 0.38, $p = 0.02$), but a stronger correlation was found between YKL-40 and pTau concentrations (R = 0.6, $p > 0.001$).

In the MCI group, there were no significant correlations with GPNMB. However, CFS YKL-40 levels correlated positively with Tau (R = 0.78, $p < 0.001$), pTau (R = 0.86, $p < 0.001$), and Aβ1-42 (R = 0.56, $p = 0.016$) levels.

3.3. Diagnostic Usefulness of Candidate Biomarkers

The analysis of the receiver operating characteristic curves (ROCs) was performed for the AD and MCI groups compared to the CTRL group. The AUC for GPNMB was better than that assessing Aβ1-42 alone; however, it was slightly lower in comparison to the Aβ1-42/1-40 ratio and Tau proteins in differentiating between the AD and CTRL groups. In AD, the lowest diagnostic usefulness was presented with the YKL-40 concentrations. Similar results for GPNMB and YKL-40 were observed in MCI patients vs. controls; their AUC values were higher than that of Aβ1-42 and the Aβ1-42/1-40 ratio and lower than that of the Tau proteins. The results are presented in Table 3 and Figures 4 and 5.

Table 3. AUC of tested parameters in compared groups.

Tested Parameters	ROC Criteria in AD Compared to CTRL				ROC Criteria in MCI Compared to CTRL			
	AUC	SE	95% C.I. (AUC)	p (AUC = 0.5)	AUC	SE	95% C.I. (AUC)	p (AUC = 0.5)
GPNMB	0.869	0.054	0.763–0.975	<0.001	0.787	0.078	0.633–0.941	0.003
YKL-40	0.755	0.065	0.627–0.882	0.001	0.812	0.075	0.665–0.959	<0.001
Aβ1-42	0.836	0.063	0.713–0.958	<0.001	0.531	0.102	0.33–0.731	0.763
Aβ1-42/1-40 ratio	0.934	0.052	0.833–0.964	<0.001	0.784	0.078	0.631–0.937	0.003
Tau	0.995	0.006	0.984–1	<0.001	0.901	0.052	0.8–1	<0.001
pTau181	0.989	0.011	0.967–1	<0.001	0.883	0.057	0.77–0.995	<0.001

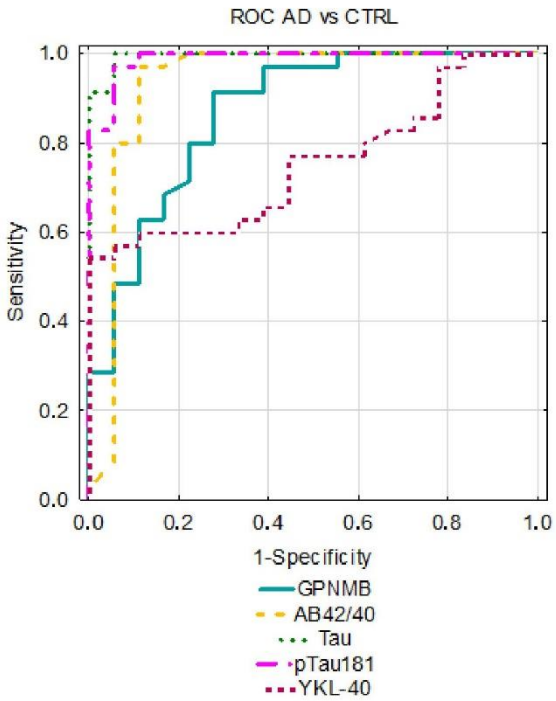

Figure 4. Comparison of area under ROC curves (AUC) for GPNMB, YKL-40, and classical biomarkers in AD between AD and CTRL groups.

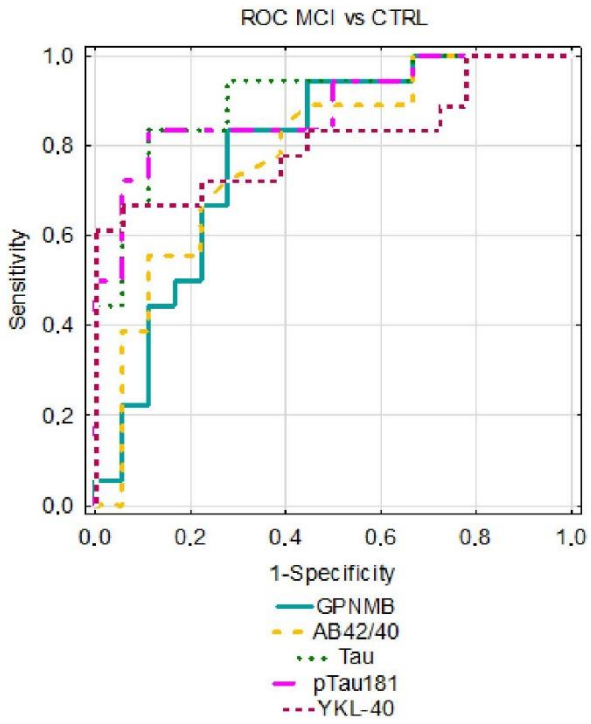

Figure 5. Comparison of area under ROC curves (AUC) for GPNMB, YKL-40, and classical biomarkers between MCI and CTRL groups.

4. Discussion

The course and severity of the disease in AD are influenced by the ongoing activation of microglia and astrocytes in response to misfolded and aggregated proteins causing inflammation [36]. Thus, studying the proteins that represent neuroinflammation as markers of illness progression and the emergence of cognitive impairments seems crucial, especially when we know that targeting amyloid plaques and neurofibrillary tangles as a therapeutic approach is not effective. Taking this information into account, we proposed a potential novel biomarker related to neuroinflammation (GPNMB) to facilitate and improve the early diagnosis of AD. In the APP/PS1KI and 5XFAD mouse models of AD, GPNMB was shown to be significantly elevated in a subpopulation of microglia cells [21,37]. Moreover, another study revealed that GPNMB is able to improve memory in mice, which supports the neuroprotective function of this protein [38].

In our study, we assessed the GPNMB levels in the CSF of AD patients and a control group (i.e., individuals without cognitive decline). The highest GPNMB concentrations were observed in the CSF of AD patients compared to MCI subjects and older people without cognitive decline. Moreover, our findings demonstrated that CSF GPNMB concentrations had already increased in the early clinical stages of cognitive decline (MCI) and continued to rise with disease severity. This may indicate the possible application of this protein as a disease severity biomarker. Our results support the two main hypotheses describing the role of activated microglia in brain disorders. It is suggested that the increased levels of microglia-activated proteins, such as GPNMB, reflect the activation of protective immunological mechanisms in the brain, which leads to an increased clearance of accumulated pathological proteins by phagocytosis. This phenomenon seems to be crucial

for microglia to defend the CNS from damage such as the accumulation of β-amyloid. On the other hand, a number of studies have shown that persistent, progressive microglial activation is detrimental to neurons and, with time, increases the severity and course of the disease. In AD, protective microglial activities are thought to occur in the early stages of the illness despite the fact that the negative effects of microglia activation tend to manifest in later stages of the disease [39]. Our findings are similar to previous studies, where the CSF levels of GPNMB in AD patients were higher than in controls [21]; however, there are conflicting results in the literature [40]. The study by Aichholzer et al. showed no significant increase in the concentrations of this protein, although the authors pointed out limitations that included a lack of information about the inflammatory state of the subjects from the control group [40]. Moreover, the studies by Bai et al. and Wang et al. detected the presence of GPNMB in the cortex, CSF, and serum of AD patients using a multimodal proteomic method, with the elevated CSF levels confirmed by an alternative method (ELISA) [41,42]. The findings from mouse models and human brains demonstrate that GPNMB is largely concentrated in microglial cells around extracellular Aβ deposits in the brain tissues of patients [21,43]. Moreover, the findings of our study showed higher concentrations of GPNMB in AD Aβ(+) patients in comparison to the Aβ(−) group, indicating a connection to amyloid pathology, which may be a result of the neuroprotective activity of GPNMB in the brain areas affected by amyloid plaques. Similar observations were described in Aichholzer et al.'s study [40], in which the AD group was also divided into Aβ-positive and Aβ-negative subgroups through PET imaging, showing higher concentrations of GPNMB in the positive group but without statistical significance [40]. However, GPNMB is not a specific biomarker for AD; overexpression of this protein was also observed in other neurodegenerative diseases, such as ALS and PD. In an ALS study, targeted multiple reaction monitoring mass spectrometry was used to quantify CSF proteins, and individuals with short-lived ALS had higher GPNMB levels [44]. This was confirmed by a newer, autonomous cohort [45]. Furthermore, overexpression and elevated concentrations of GPNMB were observed in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropridine mouse model of PD [46]. Interestingly, examination of recently frozen post-mortem human brain samples from patients with sporadic Parkinson's disease revealed higher GPNMB levels in the substantia nigra of PD patients in comparison to healthy control participants [23].

We also compared the GPNMB levels with another well-known proinflammatory protein—YKL-40. Our study showed significantly higher levels of this protein in the CSF of AD patients compared to MCI subjects and the control group. This is consistent with previous publications and meta-analyses as well as with described trends [47,48]. Moreover, these revelations can confirm the microglia polarization taking place in the early and fully developed disease stages. Furthermore, YKL-40 concentrations correlated with Tau, suggesting an association between tauopathies and AD, which supports earlier research [49]. According to these findings, GPNMB and YKL-40 may help distinguish early stages of dementia (MCI) from cognitively normal subjects, as well as AD dementia from controls without cognitive impairments. Despite both proteins being elevated in the CSF of the cognitively impaired patients, they probably reflect different functions regarding neuroinflammation. YKL-40 is described as a proinflammatory protein released in microglia activation, while GPNMB has anti-inflammatory abilities. A study by Hüttenrauch et al. [21] revealed that GPNMB inhibits the inflammatory response during neurodegenerative processes, which might be protective for neurons by attenuating neuroinflammation. Changes in levels of molecules related to the inflammatory state in the brain demonstrate how the microglia phenotype is constantly changing during the development of the disease. These changes in inflammatory proteins might suggest potential diagnostic utility in using these proteins to monitor disease progression [21].

GPNMB levels correlated positively with Tau proteins in the entire study population, similar to YKL-40. Remarkably, it was demonstrated that Tau can also induce microglia activation in the course of AD progression [50]. Thus, this relationship between GPNMB, YKL-40, and Tau proteins can be interpreted as the influence of neuroinflammation on

accelerated neurodegeneration, which is reflected by the progressive cognitive decline in patients. In addition, in our study, YKL-40 also correlated with the Aβ1-42/1-40 ratio in all groups and in the AD group. Amyloid metabolism disturbances have a positive influence on ongoing inflammatory processes in the diseased brain; therefore, we observed higher levels of inflammatory proteins.

Moreover, we assessed the diagnostic usefulness of the tested proteins based on AUC results. To our knowledge, this paper is the first to assess these parameters regarding GPNMB levels in MCI. GPNMB levels showed better discriminatory capability than Aβ1-42 levels and the Aβ1-42/1-40 ratio in differentiating between the MCI and CTRL groups. Similarly, the AUC value for GPNMB was higher than that of Aβ1-42 but lower than other classical biomarkers (Aβ1-42/1-40 ratio, Tau, pTau) in the AD group compared to the CTRL group. The AUC for YKL-40 was slightly higher than that of GPNMB in MCI patients compared to controls but slightly lower in the AD vs. CTRL group. Our results indicate a comparable clinical utility of both proteins.

Our study has several limitations; therefore, it is important to interpret the results carefully. First, despite the fact that our study focused on a rather small population, we carefully selected the group of patients with cognitive decline (MCI and AD) based on biomarker biochemistry. Moreover, these patients had been diagnosed in the same center and treated within similar time periods. To further prevent the impact of various conditions on the evaluated biomarker levels in our study population, the collection techniques were also standardized.

5. Conclusions

The results from our study suggest a potential clinical application for the GPNMB protective factor as a biomarker of the Alzheimer's disease continuum. We observed a relationship between the altered amyloid metabolism and concentrations of GPNMB, which indicates a role for this protein in the pathogenesis of the disease. Moreover, elevated levels of GPNMB in the Aβ(+) group indicate the potential diagnostic usefulness of this protein. In the early stages of cognitive impairment (MCI group), GPNMB demonstrated better diagnostic performance (AUC value) than amyloid β proteins and was comparable to the Aβ1-42/1-40 ratio. Additionally, the CSF levels of this protein increased with the severity of the disease, suggesting the possible application of this biomarker in monitoring disease progression.

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P3. *Associations between microglia and astrocytic proteins and tau biomarkers across the continuum of Alzheimer's disease.*

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Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease

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Abstract: Recent investigations implicate neuroinflammatory changes, including astrocyte and microglia activation, as crucial in the progression of Alzheimer's disease (AD) Thus, we compared selected proteins reflecting neuroinflammatory processes to establish their connection to AD pathologies. Our study, encompassing 80 subjects with (n = 42) AD, (n = 18) mild cognitive impairment (MCI) and (n = 20) non-demented controls compares the clinical potential of tested molecules. Using antibody-based methods, we assessed concentrations of NGAL, CXCL-11, sTREM1, and sTREM2 in cerebrospinal fluid (CSF). Proinflammatory proteins, NGAL, and CXCL-11 reached a peak in the early stage of the disease and allowed for the identification of patients with MCI. Furthermore, the concentration of the anti-inflammatory molecule sTREM2 was highest in the more advanced stage of the disease and permitted differentiation between AD and non-demented controls. Additionally, sTREM2 was biochemically linked to tau and pTau in the AD group. Notably, NGAL demonstrated superior diagnostic performance compared to classical AD biomarkers in discriminating MCI patients from controls. These findings suggest that proteins secreted mainly through microglia dysfunction might play not only a detrimental but also a protective role in the development of AD pathology.

Keywords: Alzheimer's disease; microglia; astrocytes; NGAL; CXCL-11; sTREM1; sTREM2; neuroinflammation

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1. Introduction

Alzheimer's disease (AD) is a prevalent and debilitating illness that primarlily affects older individuals. It is characterized by memory loss, aphasia, and serious problems with short- and long-term memory [1]. The accumulation of amyloid β (Aβ) fibrils as well as the presence of insoluble plaques, neurofibrillary tangles (NFT) composed of hyperphosphorylated tau, loss of neurons and synapses, and atrophy of memory-related brain areas are the most common features of AD [2]. The buildup and aggregation of Aβ1-42 initiates a series of pathological processes, including neuroinflammation, cytoskeletal

abnormalities, synaptic and neuronal network dysfunction, and ultimately neuronal cell death [3–5]. The illness progresses gradually; it may take up to 15–25 years for symptoms to develop [1].

Inflammation in the peripheral and central nervous systems (CNS) may contribute to AD pathology. Astrocytes and microglia, along with some peripheral immune cells that infiltrate the brain, contribute to neuroinflammation [6,7]. While neuroinflammatory processes can have important neuroprotective roles, persistent and escalating neuroinflammation can have negative consequences on brain function leading to neurological impairment and neurodegeneration [8]. Microglial cells are involved in both innate and adaptive immune responses to pathogens. Moreover, it was established that the type of microglia is crucial for the regulation of myelination and neurogenesis [6,9]. In pathological conditions, microglial cells become activated and secret various inflammatory proteins. Similarly, astrocytes could be activated by pathogenic Aβ, tau species, and proinflammatory cytokines and then release the latter [10,11]. By continuously releasing pro-inflammatory cytokines, chronically activated microglia and astrocytes can cause brain injury by making neurons more susceptible to cell death and encouraging the creation of dangerous protein aggregates [6,12,13]. Some researchers postulate that more intricate interaction between cells of innate immunity and proteinopathy is associated with neurodegeneration. Therefore, studies concerning inflammatory dysregulation mechanisms in neurodegenerative dementias are especially important to deep knowledge about their contribution to the development of the diseases. In our paper, we investigated selected pro- and anti-inflammatory proteins secreted by activated microglia and astrocytes in the continuum of the disease to assess the relationship between tested molecules and the main pathological indicators of dementia disease, particularly tau pathology. Most investigations concerning inflammatory indicators in AD are focused on amyloid pathology; thus, studies referring to relationships between tau pathology and inflammation in the CNS are also needed. The preclinical studies suggest that inflammation may induce tau hyperphosphorylation at both pre- and post-tangles periods [14,15]. Furthermore, recent findings reported that microglia dysfunction could influence tau phosphorylation, synaptic loss, as well as memory impairment even at the very early stages of the disease, before β-amyloid positivity. Considering that findings from similar studies offer a promising candidate therapeutic target to halt cognitive decline associated with aging and AD, further investigations are necessary [16].

The literature evidence indicates that neutrophil gelatinase-associated lipocalin (NGAL) also known as lipocalin-2 (LCN2), or siderocalin is one of the proteins secreted by activated microglia and astrocytes in AD. NGAL is a member of the diverse lipocalin family of carriers of lipophilic/hydrophobic molecules [17]. While NGAL primarily originates from neutrophils, its expression was also discovered in various other cells including tubular cells in the kidney, heart, lung, and dendritic cells [18]. Some research suggests that lipocalin may affect several neurobiological processes, including inflammation, signaling for cell death and survival, as well as iron metabolism. In the CNS, NGAL specifically induces insulin resistance, activates gliosis, and causes neuronal death [19–21]. NGAL may facilitate the infiltration of neutrophils and macrophages into the brain and stimulate pro-inflammatory activation of glial cells [22]. Dekens et al. described elevated levels of NGAL in the hippocampus and amygdala of AD patients. Furthermore, the authors showed colocalization of NGAL with microglia and neurons [23]. The study by Llorens et al. revealed that this protein allows for the discrimination of vascular dementia (VaD) from AD without coexisting vascular changes with high accuracy [24].

Another protein secreted by activated microglia is a small member of the CXC chemokine family—CXCL11. It was first discovered in mouse astrocytes treated with interferon beta (IFN-β). It has been shown that human astrocytes and fetal human microglia may be stimulated to produce the CXCL11 protein in response to IFN-Y alone or in combination with interleukin (IL)-1 [25]. It is also highly expressed by elements of the gastrointestinal system such as the liver, pancreas and in lower amounts expressed in the small intestine [26]. Elevated CXCL11 was found in subcutaneously infected mouse brains

and the cerebrospinal fluid of patients with neuro-inflammatory illnesses such as bacterial meningitis and viral meningitis [27,28]

In the available literature, there is a lack of data concerning CSF levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM1) and CXCL-11, inflammatory proteins released from neutrophils, microglia, and astrocytes in AD-associated neuropathological processes. TREM1 belongs to the immunoglobulin superfamily and is primarily provided by microglia, myeloid cells including neutrophils, macrophages, and monocytes [29]. TREM1 plays a significant role in the induction and exacerbation of inflammatory responses also in the CNS. Some studies reported that it is implicated in the development of numerous infectious and non-infectious diseases, including autoimmune diseases, malignancies, and neurodegenerative diseases [30,31]. However, still little is known about its role in AD.

While many studies describe that TREM1 promotes neuroinflammation, TREM2 is known as the inhibitor of this state [31]. Triggering receptor expressed on myeloid cells-2 (TREM2) is considered a neuroprotective factor in the CNS. It is produced by microglia cells. Furthermore, it has been proposed that sTREM2 may increase TREM2 protein synthesis and microglial survival by stimulating the synthesis of innate immune components [32]. Even though there is growing evidence that AD is associated with neuroinflammation, more research is needed to understand the relationship between these mechanisms and the pathologies of tau and Aβ, as well as whether these relationships matter more early in the illness or later on.

Therefore, the primary aim of this study was to assess selected pro- and anti-inflammatory proteins reflecting microglial and astrocytic activation and compare their levels with cognitive impairment in patients with AD and MCI. Secondly, the current study determined the association of CSF concentrations of tested biomarkers with the main mechanisms of amyloid and tau pathologies. This is the continuation of our previous works [33,34]. However, it is worth noting that investigations on the clinical potential use of astrocytic and microglial indicators of inflammatory state in the continuum of the disease are particularly crucial not only for diagnostic but also for therapeutic purposes. Additionally, according to our knowledge, this is the first study that investigates the concentrations of CXCL11 in CSF of AD patients, especially in correlation with microglia/astrocytic indicators and classical biomarkers of AD.

2. Results

2.1. Patient Characteristics and Comparison of CSF Concentrations of Tested Proteins Related to Inflammation

The summary of the CSF classical biomarker values in the examined groups was presented in Table 1. AD biomarkers were assesed in CSF samples of all patients. Statistical analysis revealed statistically significant differences between all the study groups for CSF concentrations of NGAL ($p < 0.001$, $\chi^2 = 15.71$), CXCL11 ($p \leq 0.001$, $\chi^2 = 39.26$), sTREM1 ($p = 0.001$, $\chi^2 = 13.20$), sTREM2 ($p = 0.037$), Aβ1-42 ($p < 0.001$, $\chi^2 = 22.94$), Aβ1-42/Aβ1-40 ratio ($p < 0.001$, $\chi^2 = 38.83$), tau ($p < 0.001$, $\chi^2 = 48.97$) and pTau181 ($p < 0.001$, $\chi^2 = 43.25$) (Tables 1 and 2).

Table 1. Concentrations of tested classical biomarkers in the study group.

Tested Variables	Median (Interquartile Range)			*p* (Kruskal–Wallis Test)
	AD	MCI	Controls	
Group size (F/M)	42 (33/9)	18 (11/7)	20 (12/8)	
Age	75.5 (64–80)	75.5 (70.3–78)	68 (63.3–76.8)	
MMSE	22 (19–24)	27.5 (26–29)	28.1 (27–30)	
Aβ1-42 (pg/mL)	502.7 (381–666)	802 (475–1045)	895 (792–1000)	<0.001
Aβ1-42/1-40 ratio	0.033 (0.029–0.04)	0.045 (0.0365–0.058)	0.066 (0.055–0.076)	<0.001
tau (pg/mL)	669 (576–897)	389 (327–495)	223 (192–273)	<0.001
pTau181 (pg/mL)	83.2 (72.7–109)	57.2 (46.9–68.41)	37.5 (33.4–42.2)	<0.001

AD—Alzheimer's disease; MCI—mild cognitive impairment; Aβ—amyloid β; F—Female; M—Male.

The NGAL levels in CSF differed significantly between the patients with AD as well as the controls and also between the MCI patients and controls. The highest CSF concentration of NGAL was observed in the group of patients with AD in comparison to CTRL ($p < 0.01$). sTREM2 concentrations were statistically significantly higher in the AD group vs. CTRL, similar to the MCI group vs. CTRL. The highest levels of CXCL11 were discovered in the MCI group, followed by the AD group, with the lowest in the CTRL group. Interestingly, there was a significant statistical difference between the AD and MCI groups.

A significantly higher concentration of CXCL11 was found in the AD and MCI patients in comparison to controls. CXCL11 concentrations in the MCI group were significantly higher than in AD. sTREM1 concentrations in CSF differed significantly between patients with MCI and controls, as well as also between the MCI patients and the AD subjects (Table 2, Figure 1).

Table 2. Concentrations of tested proteins connected to the neuroinflammation in the study group.

Tested Variables	Median (Interquartile Range)			*p* (Kruskal–Wallis Test)	*p* (Dwass–Steele–Critchlow–Flinger Test)		
	AD	MCI	Controls		AD vs. CTRL	AD vs. MCI	MCI vs. CTRL
NGAL (pg/mL)	0.907 (0.739–1.13)	0.945 (0.841–1.01)	0.629 (0.538–0.822)	<0.001	<0.01	0.908	<0.001
CXCL11 (pg/mL)	154 (124–163)	166 (160–168)	111 (107–120)	<0.001	<0.001	0.002	<0.001
sTREM1 (pg/mL)	66 (49.5–85.2)	50 (45.2–53.3)	67 (63.2–71.4)	0.001	0.989	0.008	<0.001
sTREM2 (pg/mL)	3805 (2968–4732)	3376 (3081–4279)	2835 (2124–3516)	0.037	0.037	0.699	0.179

NGAL—neutrophil gelatinase-associated lipocalin; sTREM1—soluble triggering receptor expressed on myeloid cells-1; sTREM2—soluble triggering receptor expressed on myeloid cells-2; AD—Alzheimer's disease; MCI—mild cognitive impairment; CTRL—control.

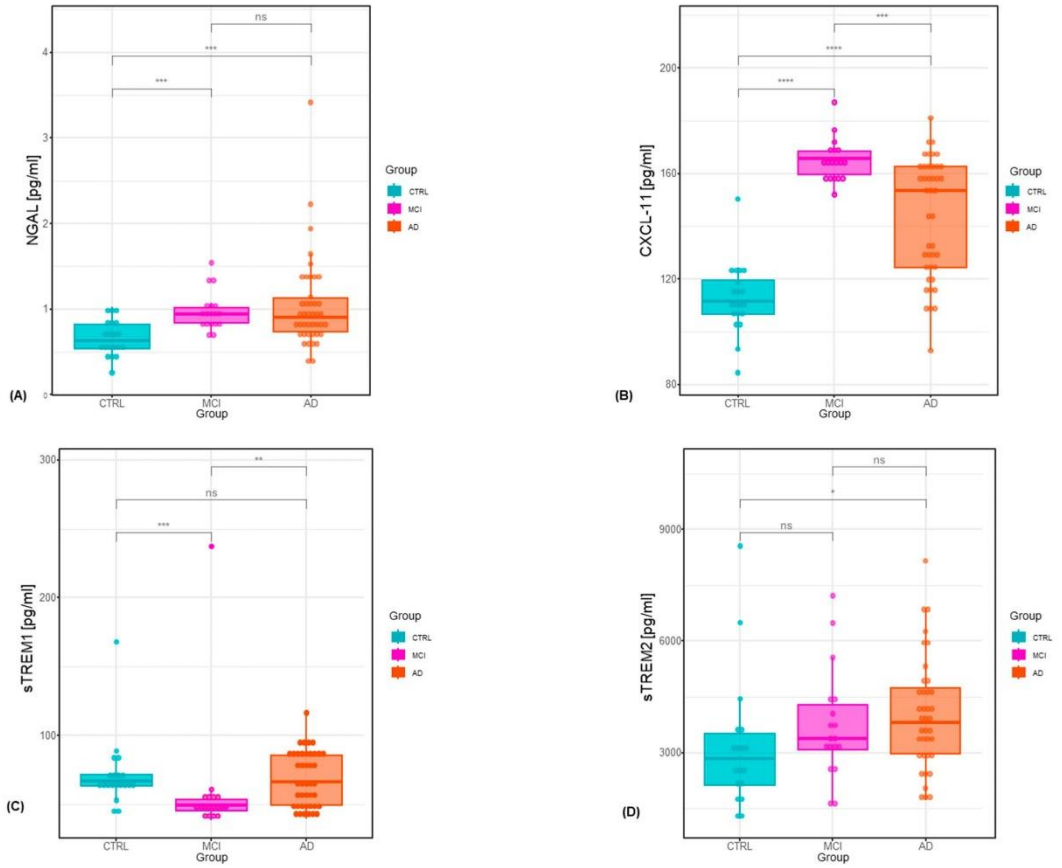

Figure 1. (**A**) Cerebrospinal fluid neutrophil gelatinase-associated lipocalin concentrations by group. (**B**) Cerebrospinal fluid CXCL-11 concentrations by group. (**C**) Cerebrospinal fluid soluble triggering receptor expressed on myeloid cells-1 concentrations by group. (**D**) Cerebrospinal fluid soluble triggering receptor expressed on myeloid cells-2 concentrations by group; CTRL—control; MCI—mild cognitive impairment; AD—Alzheimer's disease. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns—not significant.

2.2. Association between Tested Pro- and Anti-Inflammatory Molecules and Classical Biomarkers

The associations between levels of pro- and anti-inflammatory proteins and classical AD biomarkers were analyzed using the Spearman rank correlation test. In the entire study population, significant positive correlations were observed between CSF levels of NGAL and tau (R = 0.37, $p < 0.001$), pTau (R = 0.35, $p < 0.001$), age (R = 0.32, $p < 0.001$), CXCL11 (R = 0.23, $p = 0.04$) and sTREM2 (R = 0.34, $p < 0.001$) (Figure 2). Levels of sTREM2 correlated positively with sTREM1 (R = 0.27, $p = 0.02$), tau (R = 0.56, $p < 0.001$), pTau (R = 0.6, $p < 0.001$), age (R = 0.26, $p = 0.02$), while sTREM1 showed a positive significant correlation with age (R = 0.36, $p < 0.001$) and a negative correlation with CXCL11 (R = −0.58, $p < 0.001$). CXCL-11 and tau (R = 0.26, $p = 0.02$) also showed a positive correlation.

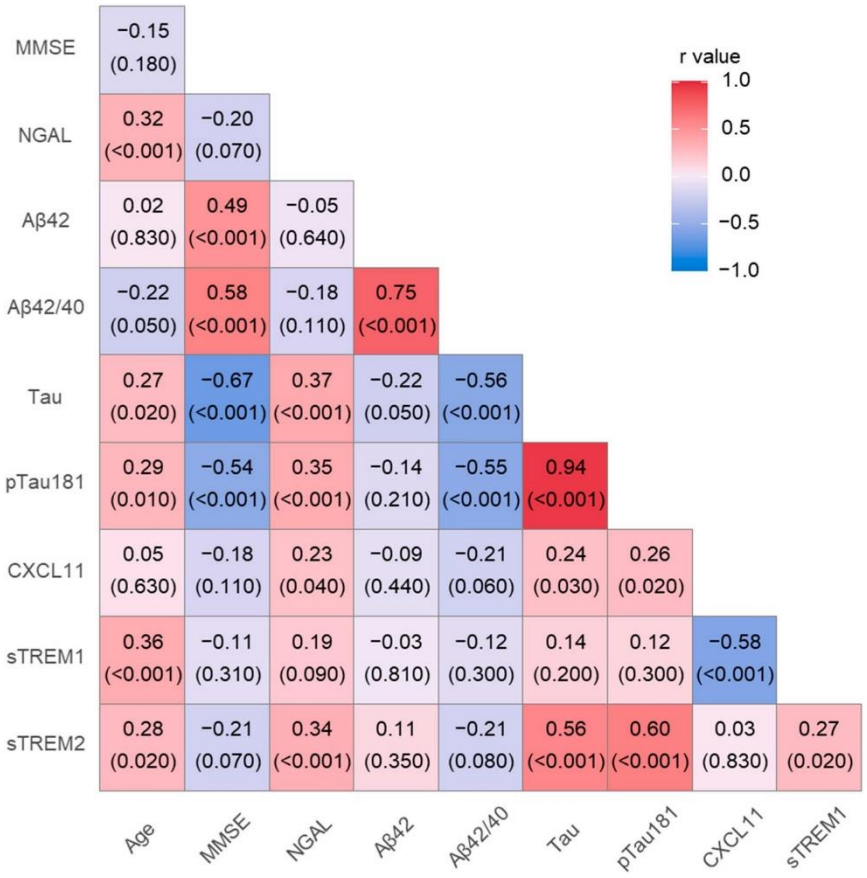

Figure 2. Cerebrospinal fluid levels of pro and anti-inflammatory proteins in the whole study group. NGAL—neutrophil gelatinase-associated lipocalin; sTREM1—soluble triggering receptor expressed on myeloid cells-1; sTREM2—soluble triggering receptor expressed on myeloid cells-2; $p < 0.001$.

In AD patients, the CSF NGAL level was significantly associated with sTREM1 (R = 0.47, $p < 0.001$) concentrations and age (R = 0.32, $p < 0.001$). Additionally, sTREM2 correlated positively with tau (R = 0.42, $p = 0.01$) and pTau (R = 0.42, $p = 0.01$). Moreover, sTREM1 correlated with age (R = 0.48, $p < 0.001$) (Figure 3).

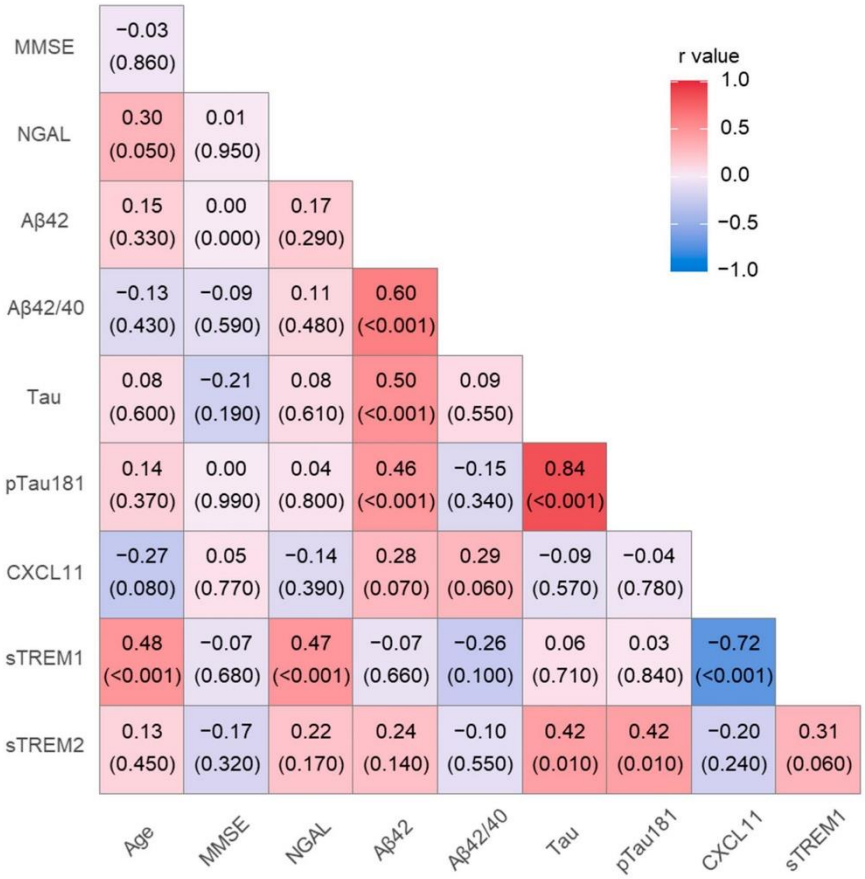

Figure 3. Cerebrospinal fluid levels of pro and anti-inflammatory in AD group. NGAL—neutrophil gelatinase-associated lipocalin; sTREM1—soluble triggering receptor expressed on myeloid cells-1; sTREM2—soluble triggering receptor expressed on myeloid cells-2.

In the MCI group, no significant correlations with NGAL or other proteins were observed. However, there was a strong, significant correlation between sTREM2 and tau ($R = 0.71$, $p < 0.001$) and pTau ($R = 0.77$, $p < 0.001$) (Figure S1 in Supplementary Materials).

2.3. Diagnostic Usefulness of Candidate Biomarkers

The analysis of the receiver operating characteristic curves (ROCs) was performed for the differentiation between the AD and MCI groups and the CTRL group. The proinflammatory proteins showed the following results: NGAL (AUC = 0.773, $p < 0.001$), CXCL-11 (AUC = 0.875, $p < 0.001$) while differentiating between the AD and CTRL groups. On the other hand, anti-inflammatory protein depicted the following data: sTREM2 (AUC = 0.705, $p = 0.01$) in differentiating between the AD and CTRL groups; however, they were not better than classical biomarkers of AD (Figure 4). In the MCI group, diagnostic performance of the sTREM1 (AUC = 0.858, $p < 0.001$) and NGAL (AUC = 0.844, $p < 0.001$) was better than established biomarkers such as Aβ1-42 (AUC = 0.536, $p = 0.723$), Aβ1-42/1-40 ratio (AUC = 0.778, $p < 0.001$), tau (AUC = 0.836, $p < 0.001$), and pTau (AUC = 0.8363, $p < 0.001$)

(Figure 5). The ROC for the AD vs. MCI group can be seen in Supplementary Materials (Figure S2).

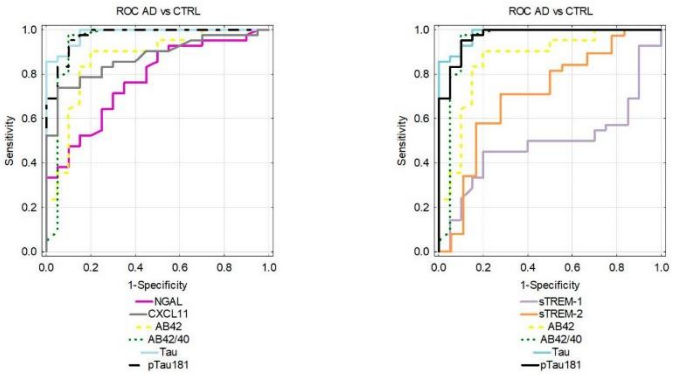

Figure 4. Comparison of area under ROC curves (AUC) for cerebrospinal fluid levels of pro and anti-inflammatory and classical AD biomarkers in AD and CTRL groups. NGAL—neutrophil gelatinase-associated lipocalin; sTREM1—soluble triggering receptor expressed on myeloid cells-1; sTREM2—soluble triggering receptor expressed on myeloid cells-2. AD—Alzheimer's disease; CTRL—control.

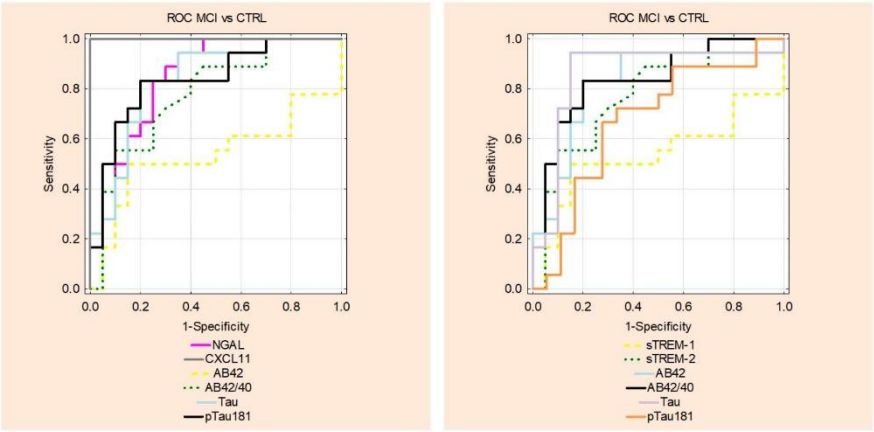

Figure 5. Comparison of area under ROC curves (AUC) for cerebrospinal fluid levels of pro and anti-inflammatory and classical AD biomarkers in MCI and CTRL groups. NGAL—neutrophil gelatinase-associated lipocalin; sTREM1—soluble triggering receptor expressed on myeloid cells-1; sTREM2—soluble triggering receptor expressed on myeloid cells-2; MCI—mild cognitive impairment; CTRL—control.

3. Discussion

The chronic activation of microglia and astrocytes in response to misfolded and aggregated proteins leading to inflammation affects the course and severity of the disease in AD. That state is typical of the injured parts of the central nervous system (CNS). Neurotoxicity and brain damage in AD are primarily caused by gliosis and inflammatory mechanisms, which can start before the onset of the disease or develop alongside degenerative changes (such as neuronal loss, synaptic loss, and neurofibrillary tangle formation) as the disease

advances. Astrogliosis was noted in AD characterized by increased levels of glial fibrillary acidic protein (GFAP) which has been observed in AD, particularly in cases with extended disease duration. [35,36]. Several inflammation-related proteins can be observed in the CSF of AD patients. Recent studies have highlighted the potential connection between proinflammatory cytokine levels and cognitive decline in individuals with mild cognitive impairment (MCI) and AD. Therefore, it is crucial to investigate the proteins that reflect neuroinflammation as potential indicators of the development of cognitive deficits and the course of the illness, particularly in light of the lack of efficacy of therapeutic approaches that target amyloid plaques and neurofibrillary tangles. Considering the aforementioned facts, this study aimed to evaluate the levels of selected pro and anti-inflammatory proteins secreted by activated astrocytes and/or microglia in AD continuum patients and individuals without cognitive decline to investigate their relationship with amyloid and tau pathology in different stages of the dementia process.

Our findings are consistent with other studies and support the upregulation of both proinflammatory and anti-inflammatory molecules released by microglia and astrocytes into the CSF of patients with varying degrees of dementia. The present study specifically found that among all tested proteins, the highest concentration in the earliest phases of the disease, in the MCI patients, were detected for CXCL11 and NGAL. Interestingly, in more severe stages, the level of CXCL11 was slightly lower. According to our knowledge, this is the first study that investigates the concentrations of CXCL11 in CSF of AD patients. However, elevated levels of CXCL11 in cerebrospinal fluid have been shown in patients with some neuro-inflammatory diseases such as bacterial and viral meningitis [28]. Additionally, studies on animal models revealed increased expression of CXCL11 after ischemic damage in the cortex. The authors postulated that activated microglia is a primary source of CXCL11 expression [37]. Our findings indicate that increased levels of CXCL11 in patients with MCI could be a part of the initial immunological response activated in microglia and become an inflammatory process in the brain. At more advanced stages of the disease, this process seems to be alleviated which reflects lower levels of the protein in fully developed AD. However, extensive research is still needed.

In accordance with our findings, a recent study by das Neves et al. showed higher concentrations of CSF NGAL in patients with AD and MCI [38]. Overexpression of NGAL was also found in the brains of AD patients in comparison to controls in areas of the brain that are affected by AD, such as the pre-frontal cortex, amygdala, and hippocampal regions. However, there are also conflicting studies that describe lower concentrations of NGAL in AD vs. CTRL [23,39], and MCI vs. CTRL [39,40]. What is more, a recent meta-analysis showed no differences in AD vs. CTRL but also MCI vs. CTRL [41]. In our opinion, higher concentrations of NGAL in CSF during disease progression may be associated with the increasing activity of astrocytes and neutrophils participating in the inflammatory response. Additionally, given that the blood–CSF barrier in our patients was achieved, our results suggest that higher CSF NGAL levels may reflect pathological processes in the CNS, not the systemic ones. There is an agreement that astrocytes are thought to be the primary producers of NGAL in the brain. This potential function for NGAL in maintaining neuronal homeostasis relates to iron transport, by being able to deliver iron through a transferrin-independent mechanism [42–44]. Additionally, NGAL tends to drive the upregulation of iron-related and proinflammatory genes in astrocytes in response to Aβ1-42, which favors the neurotoxic phenotype [45,46]. Interestingly, NGAL is secreted also by neutrophils at sites of infection and choroid plexus (in which most of the CSF is produced and secreted) epithelial cells, where it acts as an acute phase protein [47,48]. Moreover, recent studies show that NGAL is also linked to changes in overall behavior, cognitive functions, and depression [49,50]. However, Ferreira et al. described the potential neuroprotective roles of the NGAL as regulating the balance between pro and anti-inflammatory responses [44]. As a component of the acute-phase response, LCN2 functions in the initial stages of antimicrobial defense to sequester bacterial siderophores—bacterial compounds that have a greater affinity for iron than the host's iron-binding proteins [51]. Other authors suggested

that increased CSF NGAL levels in AD patients might be connected with the altered secretory activity of damaged choroid plexus in patients with advanced stages of AD [52,53]. Furthermore, our study revealed that tested proinflammatory proteins showed positive correlations only in the advanced stages of dementia. In the AD group, NGAL and sTREM1 showed positive correlations with themselves.

Findings from our investigation suggest that some protective mechanisms in more advanced stages are activated. In more advanced stages of dementia, the accelerated activity of protective mechanisms in the brain could reflect elevated levels of the anti-inflammatory protein sTREM2. Our results are in agreement with other studies that described increased concentrations of sTREM2 in the CSF of AD patients [54]. In addition, we postulate that sTREM2 could be one of the molecular indicators of activated neuropathological mechanisms in Alzheimer's disease.

We evaluated the relationships between proteins associated with inflammation and classical biomarkers. Among all tested inflammatory biomarkers only sTREM2 showed correlation with tau and pTau in AD patients. Interestingly, this correlation between sTREM2 and tau proteins was observed even in at the early stages of the disease, specifically in the MCI group. Our findings suggest that an increase in the concentration of anti-inflammatory proteins may be linked to the activation of the immune response to the intrathecal synthesis of the misfolded proteins, acting as a protective mechanism. It is important to note that this correlation is stronger in the earlier stages of the disease but also maintains in patients with fully developed AD. Higher levels of sTREM2 suggest microglia activation and might be confirming undergoing neurodegeneration. The results are in agreement with other authors [55,56]. A study on a hTau (human MAPT expressed but not endogenous mouse Mapt) mouse model revealed that TREM2 deficiency exacerbates tau phosphorylation and aggregation throughout the early stages of the disease. Additionally, a recent study described that microglia-derived sTREM2 selectively binds to transeglin 2 and inhibits the RhoA/ROCK/GSK3β signaling pathway thereby reducing tau hyperphosphorylation [57].

Furthermore, we assesed the diagnostic usefulness of the tested proteins based on the AUC results. The best discriminatory capability was demonstrated for sTREM1 and NGAL in the MCI group, better than other tested proteins, including established Alzheimer's biomarkers. While discriminating between the AD and CTRL, results were also high, especially for NGAL although the AUC values did not surpass those of the classical biomarkers. This might suggest that these proteins might be most effective in the early stages of neurodegeneration.

Understanding inflammatory pathomechanism may allow to restrict AD pathology and may open avenues for novel diagnostics tools, as well as therapeutic targets. Findings from our study suggest that harmful action of some proinflammatory molecules microglia and astrocytic activation-mediated may participate in the progression of the disease at the early stages. However, we acknowledge that our study has limitations in terms of the study cohort population. Nevertheless, in the later stages of the disease anti-inflammatory proteins, including sTREM2, appear to play a crucial role as mechanisms against the development of hyperphosphorylated tau pathology.

4. Materials and Methods

4.1. Material

The study population consisted of 80 subjects (n = 55 women, n = 25 men; median age: 74 (63.3–80)) recruited at the Department of Neurology, Jagiellonian University Hospital, Krakow, Poland, and included 42 AD patients (n = 33 women, n = 9 men; age: 75.5 (64–80)), 18 subjects with MCI (n = 11 women, n = 7 men; age: 75.5 (70.3–78)), and 20 non-demented controls (n = 12 women, n = 8 men; age: 68 (63.3–76.8)). The Bialystok University study (No. R-I-002/103/2019) was approved by the Ethics Committee, and the research was carried out in accordance with the Declaration of Helsinki in the Department of Neurodegeneration Diagnostics at the Medical University of Bialystok. Prior to any procedures, each patient signed an informed consent form. The clinical diagnosis of the research groups

involved the use of standard medical, physical, and neurological examinations, laboratory screening tests, a neurocognitive assessment, and brain computed tomography or magnetic resonance imaging. Cases of Alzheimer's disease with sporadic occurrences formed the AD group. Throughout their medical interview, none of the study's patients disclosed a family history of Alzheimer's. The diagnosis of AD has been determined using the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria [58,59]. To provide the most accurate clinical diagnosis of AD, neurochemical data (levels of Aβ1-42, tau, and pTau181 as well as values of the Aβ1-42/Aβ1-40 ratio) were combined with neuroimaging and neuropsychological tests. The MMSE score (range 0–30) was used to determine dementia severity (AD patients (MMSE: 22 (19–24)), MCI patients (MMSE: 27.5 (26–29)), and controls (MMSE: 28.1 (27–30))). Patients from whole study group (AD, MCI and CTRL) with raised albumin quotients (QAlb), a sign of dysfunction in the blood–CSF barrier, abnormalities in CT or MRI scans, and suspected cerebrovascular disorders (such as cerebral hemorrhage, aortic aneurysm, intracranial aneurysm, stroke, or arteriovenous malformation) and also with visible signs of blood in CSF were excluded from the study.

The control group comprised individuals who were not experiencing subjective memory impairments and did not meet the MCI criteria but who might experience recurring headaches. None of the patients in this group displayed any meaningful changes in the levels of the recognized biomarkers for AD (Aβ1-42, tau, and pTau181), which allowed the exclusion of the symptoms' organic background. An Erlangen Score of 0 points across all 18 of the participants in this group supported these findings.

4.2. Biochemical Measurements

CSF samples were taken using lumbar punctures in the L3/L4 or L4/L5 interspace and transferred into polypropylene tubes. All CSF samples were frozen at −80 °C, aliquoted, and centrifuged before analysis. The concentrations of analyzed proteins (NGAL, CXCL11, sTREM1, sTREM2, Aβ1-42, Aβ1-40, tau, and pTau181) in the CSF were measured in the Department of Neurodegeneration Diagnostics, Medical University of Bialystok, Poland.

Neurochemical dementia diagnostics (NDD) biomarker concentrations were measured using IBL kits (RE59661, RE59651, Hamburg, Germany) for Aβ1-42 and Aβ1-40 and Fujirebio kits (81572, 81574, Gent, Belgium) for tau and pTau181 proteins.

R&D Systems, Abingdon, UK, supplied the ELISA kits that were used for the NGAL analysis. Luminex Human Discovery assay plates from R&D Systems, Abingdon, UK, and a Luminex 200 analyzer (multiplexing, multiparametric, fluorescence laser reading system on microspheres for the simultaneous determination of multiple parameters) were used for the analysis of CXCL11, sTREM1, and sTREM2. For every standard, control, and sample duplicate measurements were evaluated in accordance with the manufacturer's protocols.

4.3. Statistical Analysis

The PMCMRplus package in the statistical software RStudio (Version 1.4.1106, Boston, MA, USA) and Statistica 13.3 (StatSoft Polska, Krakow, Poland) were used to perform nonparametric tests. The Shapiro–Wilk test demonstrated that the protein concentrations were not distributed normally. The comparisons between the AD, MCI, and control groups were performed using the Kruskal–Wallis test. The post hoc Dwass–Steele–Critchlow–Fligner test was then used to assess significant differences between the levels of the tested groups to determine which groups had statistically significant differences. The results are presented as medians and interquartile ranges. Statistical significance was set at $p < 0.05$. In addition, the receiver operating characteristic (ROC) curve and area under curve (AUC) analysis was used to determine the diagnostic usefulness of the tested proteins as potential neuroinflammation-related biomarkers for AD.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms25147543/s1.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Bialystok Ethics Committee (No. R-I-002/103/2019).

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

Data Availability Statement: The original data presented in the study are openly available this publication.

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Conflicts of Interest: A.K.-P. has received a consultation and/or lecture honoraria from Roche company. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). B.M. has received consultation and/or lecture honoraria from Abbott, Wiener, Roche, Cormay, Biameditek and TK Biotech.

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Uchwała nr: R-I-002/459/2018

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Ocena przydatności oznaczeń wybranych biomarkerów w diagnostyce chorób zwyrodnieniowych układu nerwowego" przez prof. dr hab. Barbarę Mroczko wraz z zespołem badawczym z UMB.

Z-ca Przewodniczącej Komisji Bioetycznej UMB

dr n. farm. Krzysztof Chrzanowski

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1. *Doroszkiewicz J, Mroczko P, Kulczyńska-Przybik A. Inflammation in the CNS: Understanding Various Aspects of the Pathogenesis of Alzheimer's Disease. Curr Alzheimer Res. 2022;19(1):16-31. doi:10.2174/1567205018666211202143935*

2. *Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Borawska R, Zajkowska M, Słowik A, Mroczko B. Potential Utility of Cerebrospinal Fluid Glycoprotein Nonmetastatic Melanoma Protein B as a Neuroinflammatory Diagnostic Biomarker in Mild Cognitive Impairment and Alzheimer's Disease. J Clin Med. 2023;12(14):4689. Published 2023 Jul 14. doi:10.3390/jcm12144689*

3. *Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

wchodzących w skład mojej rozprawy doktorskiej polegał na ustaleniu koncepcji, metodologii i planu badań, wykonaniu badań, interpretacji wyników, analizie statystycznej, przeglądzie elektronicznych baz publikacji naukowych i zgromadzeniu piśmiennictwa oraz przygotowaniu manuskryptu.

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wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na ocenie merytorycznej pracy oraz edycji manuskryptu.

2. *Julia Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, **Mroczko B**. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na ocenie merytorycznej pracy oraz edycji manuskryptu..

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Julię Doroszkiewicz jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

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1. *Doroszkiewicz J, Mroczko P,* ***Kulczyńska-Przybik A.*** *Inflammation in the CNS: Understanding Various Aspects of the Pathogenesis of Alzheimer's Disease. Curr Alzheimer Res. 2022;19(1):16-31. doi:10.2174/1567205018666211202143935*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na ustaleniu koncepcji pracy, ocenie merytorycznej pracy, edycji manuskryptu.

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Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Julię Doroszkiewicz jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

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Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Julię Doroszkiewicz jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

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Podpis (czytelny)

Miejscowość, data

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Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Borawska R, Zajkowska M,* ***Słowik A****, Mroczko B. Potential Utility of Cerebrospinal Fluid Glycoprotein Nonmetastatic Melanoma Protein B as a Neuroinflammatory Diagnostic Biomarker in Mild Cognitive Impairment and Alzheimer's Disease. J Clin Med. 2023;12(14):4689. Published 2023 Jul 14. doi:10.3390/jcm12144689*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na kwalifikacji pacjentów oraz zbieraniu materiału biologicznego do badań.

2. *Julia Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R,* ***Słowik A****, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na kwalifikacji pacjentów oraz zbieraniu materiału biologicznego do badań.

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.....

Podpis (czytelny)

Agnieszka Słowik

Miejscowość, data

Mölndal, 04.12.24 r.

Dr Maciej Dulewicz
Imiona i nazwisko współautora

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Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Doroszkiewicz J, Kulczyńska-Przybik A,* ***Dulewicz M,*** *Borawska R, Zajkowska M, Słowik A, Mroczko B. Potential Utility of Cerebrospinal Fluid Glycoprotein Nonmetastatic Melanoma Protein B as a Neuroinflammatory Diagnostic Biomarker in Mild Cognitive Impairment and Alzheimer's Disease. J Clin Med. 2023;12(14):4689. Published 2023 Jul 14. doi:10.3390/jcm12144689*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na opracowaniu statystycznym wyników i przygotowaniu rycin.

2. *Julia Doroszkiewicz J, Kulczyńska-Przybik A,* ***Dulewicz M,*** *Mroczko J, Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

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Maciej Dulewicz
.....
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place, date
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Statement

I declare that my participation in the publication:

1. *Julia Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R, Słowik A, Zetterberg H, **Hanrieder J**, Blennow K, Mroczko B. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

constituting part of a special scientific achievement, doctoral dissertation of Ms Julia Doroszkiewicz, M.Sc., consisted in substantive checking the content of the publication.

At the same time, I consent to the use of the publication by Ms Julia Doroszkiewicz, M.Sc. in the procedure for conferring the doctoral degree.

.....
Signature

Mölndal, 04.12.2024
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SciLifeLab, University of Gothenburg, Gothenburg, Sweden

Statement

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Signature
04.12.2024

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Mölndal, 04.12.24.

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Department of Psychiatry and Neurochemistry,
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Statement

I declare that my participation in the publication:

1. *Julia Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R, Słowik A, Zetterberg H, Hanrieder J,* ***Blennow K****, Mroczko B. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

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At the same time, I consent to the use of the publication by Ms Julia Doroszkiewicz, M.Sc. in the procedure for conferring the doctoral degree.

Signature K. Blennow

Białystok, 04.12.2024
Miejscowość, data

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Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Julia Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M,* ***Mroczko J,*** *Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. Associations between Microglia and Astrocytic Proteins and Tau Biomarkers across the Continuum of Alzheimer's Disease. Int. J. Mol. Sci. 2024, 25, 7543. https://doi.org/10.3390/ijms25147543*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na udziale w przygotowaniu manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Julię Doroszkiewicz jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Jan Mroczko
Podpis (czytelny)

Białystok, 04.12.2024
Miejscowość, data

Dr n. pr. Piotr Mroczko
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Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Doroszkiewicz J, **Mroczko P**, Kulczyńska-Przybik A. Inflammation in the CNS: Understanding Various Aspects of the Pathogenesis of Alzheimer's Disease. Curr Alzheimer Res. 2022;19(1):16-31. doi:10.2174/1567205018666211202143935*

wchodzącej w skład rozprawy doktorskiej Pani mgr Julii Doroszkiewicz polegał na opisaniu aspektów prawnych.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Julię Doroszkiewicz jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Piotr Mroczko
.....
Podpis (czytelny)

16. Dorobek naukowy

Łączna wartość Impact Factor całego dorobku naukowego: **49.008**

Łączna ilość punktów MNiSW całego dorobku naukowego: **1500**

Wskaźnik Hirscha według Scopus: **5**

Cytowania według Scopus: **160**

16.1 Wykaz publikacji stanowiących podstawę rozprawy doktorskiej

1. Doroszkiewicz J, Mroczko P, Kulczyńska-Przybik A. Inflammation in the CNS: Understanding Various Aspects of the Pathogenesis of Alzheimer's Disease. Curr Alzheimer Res. 2022;19(1):16-31. doi:10.2174/1567205018666211202143935

IF: **2,1** MNiSW: **100**

2. Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Borawska R, Zajkowska M, Słowik A, Mroczko B. Potential Utility of Cerebrospinal Fluid Glycoprotein Nonmetastatic Melanoma Protein B as a Neuroinflammatory Diagnostic Biomarker in Mild Cognitive Impairment and Alzheimer's Disease. *J Clin Med*. 2023;12(14):4689. Published 2023 Jul 14. doi:10.3390/jcm12144689

IF: **3,0** MNiSW: **140**

3. Doroszkiewicz J, Kulczyńska-Przybik A, Dulewicz M, Mroczko J, Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. Associations between microglia and astrocytic proteins and tau biomarkers across the continuum of Alzheimer’s disease . Int J Mol Sci. 2024;25(14):7543. Published 2024 Jul 9. doi:10.3390/ijms25147543

IF: **4,9** MNiSW: **140**

16.2 Wykaz innych publikacji naukowych

1. **Doroszkiewicz J**, Groblewska M, Mroczko B. The Role of Gut Microbiota and Gut-Brain Interplay in Selected Diseases of the Central Nervous System. Int J Mol Sci. 2021;22(18):10028. Published 2021 Sep 17. doi:10.3390/ijms221810028

IF: **6,208** MNiSW: **140**

2. **Doroszkiewicz J**, Groblewska M, Mroczko B. Molecular Biomarkers and Their Implications for the Early Diagnosis of Selected Neurodegenerative Diseases. Int J Mol Sci. 2022;23(9):4610. Published 2022 Apr 21. doi:10.3390/ijms23094610

IF: **5,6** MNiSW: **140**

3. Kulczyńska-Przybik A, Dulewicz M, **Doroszkiewicz J**, Borawska R, Litman-Zawadzka A, Arslan D, Kułakowska A, Kochanowicz J, Mroczko B. Comparative Analysis of Neurodegeneration and Axonal Dysfunction Biomarkers in the Cerebrospinal Fluid of Patients with Multiple Sclerosis. J Clin Med. 2022;11(14):4122. Published 2022 Jul 15. doi:10.3390/jcm11144122

IF: **3,9** MNiSW: **140**

4. **Doroszkiewicz J**, Mroczko B. New Possibilities in the Therapeutic Approach to Alzheimer's Disease. Int J Mol Sci. 2022;23(16):8902. Published 2022 Aug 10. doi:10.3390/ijms23168902

IF: **5,6** MNiSW: **140**

5. **Doroszkiewicz J**, Mroczko J, Rutkowski P, Mroczko B. Molecular Aspects of a Diet as a New Pathway in the Prevention and Treatment of Alzheimer's Disease. Int J Mol Sci. 2023;24(13):10751. Published 2023 Jun 28. doi:10.3390/ijms241310751

IF: **4,9** MNiSW: **140**

6. Kulczyńska-Przybik A, Dulewicz M, **Doroszkiewicz J**, Borawska R, Słowik A, Zetterberg H, Hanrieder J, Blennow K, Mroczko B. The Relationships between Cerebrospinal Fluid Glial (CXCL12, CX3CL, YKL-40) and Synaptic Biomarkers (Ng, NPTXR) in Early Alzheimer's Disease. Int J Mol Sci. 2023;24(17):13166. Published 2023 Aug 24. doi:10.3390/ijms241713166

IF: **4,9** MNiSW: **140**

7. **Doroszkiewicz J**, Farhan JA, Mroczko J, Winkel I, Perkowski M, Mroczko B. Common and Trace Metals in Alzheimer's and Parkinson's Diseases. Int J Mol Sci. 2023;24(21):15721. Published 2023 Oct 29. doi:10.3390/ijms242115721

IF: **4,9** MNiSW: **140**

8. **Doroszkiewicz J**, Mroczko J, Winkel I, Mroczko B. Metabolic and Immune System Dysregulation: Unraveling the Connections between Alzheimer's Disease, Diabetes, Inflammatory Bowel Diseases, and Rheumatoid Arthritis. J Clin Med. 2024;13(17):5057. Published 2024 Aug 26. doi:10.3390/jcm13175057

IF: **3** MNiSW: **140**

16.3 Komunikaty zjazdowe z konferencji polskich i zagranicznych

1. **Doroszkiewicz Julia**, Kulczyńska-Przybik Agnieszka, Kaczyńska Aleksandra, Muszyński Paweł, Słowik Agnieszka, Borawska Renata, Dulewicz Maciej, Litman-Zawadzka Ala, Mroczko Barbara. Fraktalkina (CX3CL1) jako nowy potencjalny biomarker choroby Alzheimera. II Ogólnopolska Konferencja Naukowa "Choroby neurodegeneracyjne - objawy, diagnostyka, leczenie", 17 listopada 2020, prezentacja ustna.

2. Kaczyńska Aleksandra, Kulczyńska-Przybik Agnieszka, **Doroszkiewicz Julia**, Muszyński Paweł, Słowik Agnieszka, Borawska Renata, Dulewicz Maciej, Litman-Zawadzka Ala, Mroczko Barbara. Zależność pomiędzy markerem stanu zapalnego mikrogleju - białkiem YKL-40 a dysfunkcją bariery krew-płyn mózgowo-rdzeniowy u pacjentów z demencją. II

Ogólnopolska Konferencja Naukowa "Choroby neurodegeneracyjne - objawy, diagnostyka, leczenie", 17 listopada 2020, prezentacja ustna.

3. Kaczyńska Aleksandra, Krawiec Anita, Kulczyńska-Przybik Agnieszka, **Doroszkiewicz Julia**, Borawska Renata, Litman-Zawadzka Ala, Słowik Agnieszka, Mroczko Barbara. Rola chemokiny CXCL12 w chorobie Alzheimera. VII Ogólnopolska Konferencja Studentów Medycyny Laboratoryjnej i Młodych Diagnostów "Wschodząca Diagnostyka". 5 czerwca 2021, prezentacja ustna.

4. Mroczko Barbara, Kulczyńska-Przybik Agnieszka, Borawski Bartłomiej, Dulewicz Maciej, **Doroszkiewicz Julia**, Borawska Renata, Litman-Zawadzka Ala, Rutkowski Robert, Mariak Zenon. The evaluation of atypical chemokine receptor (ACKR) levels in serum of patients with tumors of central nerves system (CNS). 24th IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine, Munich, Nov 28-Dec 02, 2021, poster.

5. **Doroszkiewicz Julia**, Kulczyńska-Przybik Agnieszka, Dulewicz Maciej, Borawska Renata, Krawiec Anita, Słowik Agnieszka, Mroczko Barbara. The cerebrospinal fluid Interleukin 8 (IL-8) concentration in Alzheimer's Disease (AD). Alzheimer's Association International Conference 2021, Denver (on-line), July 26-30, 2021, poster.

6. Kulczyńska-Przybik Agnieszka, Dulewicz Maciej, Borawska Renata, **Doroszkiewicz Julia**, Kaczyńska Aleksandra, Litman-Zawadzka Ala, Słowik Agnieszka, Mroczko Barbara. Upregulation of microRNA-451a in the blood of the patients with Alzheimer's disease (AD). Alzheimer's Association International Conference 2021, Denver (on-line), July 26-30, 2021, poster.

7. Mroczko Barbara, Kulczyńska-Przybik Agnieszka, Borawska Renata, Dulewicz Maciej, **Doroszkiewicz Julia**, Słowik Agnieszka. The diagnostic significance of the chemokine CXCL12 in Alzheimer's disease. Alzheimer's Association International Conference 2021, Denver (on-line), July 26-30, 2021, poster.

8. **Doroszkiewicz Julia**, Kulczyńska-Przybik Agnieszka, Dulewicz Maciej, Borawska Renata, Słowik Agnieszka, Mroczko Barbara. The cerebrospinal fluid Glycoprotein Nonmetastatic Melanoma Protein B (GPNMB) concentration in Alzheimer's Disease (AD). Alzheimer's Association International Conference, San Diego, California, July 31 - August 3, 2022, poster.

9. Mroczko Barbara, Kulczyńska-Przybik Agnieszka, Borawska Renata, Dulewicz Maciej, **Doroszkiewicz Julia**, Karpiuk Magdalena, Słowik Agnieszka. The significance of the

Calbindin-D in Alzheimer's disease. Alzheimer's Association International Conference, San Diego, California, July 31 - August 3, 2022, poster.

10. Kulczyńska-Przybik Agnieszka, Dulewicz Maciej, Mroczko Piotr, Borawska Renata, **Doroszkiewicz Julia**, Litman-Zawadzka Ala, Arslan Daria, Słowik Agnieszka. The assessment of ubiquitin C-terminal hydrolase-1 (UCH-L1) in patients with Alzheimer's disease. Alzheimer's Association International Conference, San Diego, California, July 31 - August 3, 2022, poster.

11. **Doroszkiewicz Julia**, Kulczyńska-Przybik Agnieszka, Dulewicz Maciej, Borawska Renata, Słowik Agnieszka, Mroczko Barbara. The cerebrospinal fluid soluble TREM-1 concentration in Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI). Alzheimer's Association International Conference, Amsterdam, Netherlands and Online, July 16 - July 20, 2023, poster.

16.4 Dodatkowe aktywności

2022-2024 - Stypendium Naukowe Rektora Uniwersytetu Medycznego w Białymstoku za uzyskanie wyróżniających wyników w nauce oraz osiągnięcia naukowe.

2022,2023 - Współorganizator zajęć z dziećmi i młodzieżą na Podlaskim Festiwalu Nauki i Sztuki, Uniwersytetu Medycznego w Białymstoku. Temat zajęć: Choroba Alzheimera – epidemia XXI wieku.

2020 – obecnie - Członkostwo w Krajowej Izbie Diagnostów Laboratoryjnych.

2015 – obecnie - Aktywne uczestnictwo w Chórze Uniwersytetu Medycznego w Białymstoku.