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Znaczenie kliniczne polimorfizmu haptoglobiny u pacjentów ze spondyloartropatią

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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Spis treści

Wykaz stosowanych skrótów

ASAS-EULAR – Assessment of SpondyloArthritis International Society-European Alliance of Associations for Rheumatology

ASDAS – Ankylosing Spondylitis Disease Activity Score

BASDAI – Bath Ankylosing Spondylitis Disease Activity Index

bLMPCh (bDMARDs) – biologiczne leki modyfikujące przebieg choroby (*biologic disease-modifying anti-rheumatic drugs*)

csLMPCh (tsDMARDs) – celowane syntetyczne leki modyfikujące przebieg choroby (*targeted synthetic disease-modifying antirheumatic drugs*)

ELISA – test immunoenzymatyczny (*enzyme-linked immunosorbent assay*)

Hp – haptoglobina (*haptoglobin)*

NLPZ (NSAIDs) – niesteroidowe leki przeciwzapalne (*non-steroidal anti-inflammatory drugs*)

MRI – rezonans magnetyczny (*magnetic resonance imaging)*

TNF – czynnik martwicy nowotworów (*tumor necrosis factor)*

VAS – wizualna skala analogowa *(visual analogue scale)*

Streszczenie w języku polskim

Spondyloartropatie to grupa zapalnych chorób stawów kręgosłupa i stawów obwodowych o złożonej patogenezie, obejmującej uwarunkowania genetyczne, środowiskowe oraz mechanizmy immunologiczne. Na przestrzeni ostatnich lat silnie podkreślany jest związek spondyloartropatii z osią jelito-stawy.

Zgodnie z dostępną literaturą polimorfizm haptoglobiny, będący jednym z białek ostrej fazy, którego główną funkcją jest wiązanie wolnej hemoglobiny we krwi, może mieć związek z przebiegiem i skutecznością leczenia chorób autoimmunologicznych i zapalnych. W mojej pracy przedstawiłam hipotezę, że różne fenotypy haptoglobiny uwarunkowane jej polimorfizmem mogą determinować odmienny przebieg spondyloartropatii i wpływać na efekty terapii. Aby kompleksowo scharakteryzować wpływ polimorfizmu haptoglobiny w tej chorobie w swoich analizach uwzględniłam również cząsteczkę opisywaną w literaturze jako prekursor Hp2- zonulinę, która ma związek ze zwiększoną przepuszczalnością jelit.

Spondyloartropatie dzieli się na postacie osiowe lub obwodowe w zależności od dominujących objawów. Leczenie spondyloartropatii osiowej, na której skupiłam się w mojej pracy obejmuje dwa etapy: w pierwszej kolejności stosowane są niesteroidowe leki przeciwzapalne (NLPZ), następnie w przypadku ich nieskuteczności wdraża się terapię biologicznymi lekami modyfikującymi przebieg choroby (bLMPCh) (najczęściej) lub celowanymi syntetycznymi lekami modyfikującymi przebieg choroby (csLMPCh). Skuteczność terapii jest ograniczona, a przyczyny tych ograniczeń nie zostały jeszcze w pełni wyjaśnione.

Pierwszy artykuł to praca poglądowa, w której opisałam istniejące dane literaturowe na temat rozkładu fenotypów haptoglobiny w spondyloartropatiach oraz dokonałam analizy znanych szlaków zapalnych związanych z patogenezą spondyloartropatii, w których polimorfizm haptoglobiny i aktywność zonuliny mogą mieć znaczenie. W artykule tym przedstawiłam zasadność wyboru polimorfizmu haptoglobiny jako czynnika, który może wpływać na przebieg spondyloartropatii. Dodatkowo, omówiłam znaczenie zonuliny w dezintegracji bariery jelitowej w kontekście rozwoju choroby oraz zaproponowałam potencjalną ścieżkę terapeutyczną, wykorzystującą inhibitor zonuliny.

W drugim artykule zaprezentowałam wyniki oryginalnego badania, w którym udało mi się zidentyfikować predyktory złej odpowiedzi na leczenie niesteroidowymi lekami

przeciwzapalnymi w osiowej spondyloartropatii. Przede wszystkim sprawdziłam czy pośród wykrytych predyktorów znajduje się haptoglobina, jej polimorfizm lub zonulina. Wyniki przeprowadzonych analiz wykazały, że pacjenci z wysokim poziomem zonuliny mają istotnie wyższe ryzyko złej odpowiedzi na leczenie standardowe. Podobnego związku nie zaobserwowałam dla haptoglobiny i jej polimorfizmu. Sam polimorfizm haptoglobiny nie był związany z żadnym z parametrów aktywności choroby, ani nie był powiązany z zonuliną.

Co ważne, w moim badaniu po raz pierwszy udało się scharakteryzować pacjentów z osiową spondyloartropatią, którzy mają wysokie ryzyko nieskuteczności kontynuacji terapii NLPZ. Są to chorzy z zesztywniającym zapaleniem stawów kręgosłupa, o długim czasie trwania choroby, z zajęciem stawów krzyżowo-biodrowych w badaniu RTG, z aktywnym zapaleniem stawów krzyżowo-biodrowych w badaniu MRI, z wysokim wskaźnikiem BASDAI, wysokimi wartościami subiektywnej oceny nasilenia bólu kręgosłupa w skali VAS oraz wspomnianym wcześniej, wysokim poziomem zonuliny w surowicy.

Trzeci artykuł wchodzący w skład niniejszego cyklu zawiera wyniki badania nad predyktorami złej odpowiedzi na leczenie biologiczne w osiowej spondyloartropatii po 12 tygodniach terapii. Przeprowadzone przeze mnie analizy dowiodły, że również w tym przypadku wyższe stężenia zonuliny są powiązane z niepowodzeniem terapii i jest to niezależne od wszystkich pozostałych badanych zmiennych. Natomiast wyższe stężenia haptoglobiny występowały u chorych, którzy dobrze reagowali na leczenie biologiczne. Stężenie haptoglobiny było istotnie związane z polimorfizmem haptoglobiny, ale sam polimorfizm nie był powiązany z odpowiedzią na leczenie biologiczne ani ze wskaźnikami aktywności spondyloartropatii. Podobnie jak w poprzednim badaniu, zonulina nie wykazywała istotnego związku z określonym fenotypem haptoglobiny i występowała również u chorych nieposiadających genu Hp2.

Warto odnotować, że wśród predyktorów nieskuteczności leczenia biologicznego zidentyfikowałam inne, oprócz zonuliny, czynniki powiązane z zaburzonym funkcjonowaniem jelit i dysbiozą takie jak starszy wiek, nieswoiste zapalenie jelit w wywiadzie oraz częste stosowanie antybiotyków z powodu infekcji.

Podsumowując, wyniki przeprowadzonych przeze mnie badań w odniesieniu do spondyloartropatii osiowej wskazują na to, że polimorfizm haptoglobiny nie ma znaczenia klinicznego w przebiegu i leczeniu tej choroby. Moja praca natomiast pozwoliła na

zidentyfikowanie predyktorów nieskuteczności terapii zarówno standardowej, jak i biologicznej w tym schorzeniu. Wykorzystanie tych wyników może pomóc w przyszłości w prowadzeniu leczenia bardziej dostosowanego do pacjenta i unikaniu przedłużonej terapii standardowej u chorych, u których taka kontynuacja cechuje się dużym ryzykiem niepowodzenia. Identyfikacja zonuliny jako czynnika związanego z odpowiedzią na leczenie w spondyloartropatii wskazuje na nowe kierunki i możliwości leczenia z wykorzystaniem inhibitora zonuliny, niezależnie od posiadanego fenotypu haptoglobiny. Wyniki przeprowadzonych przeze mnie analiz uzasadniają prowadzenie w przyszłości badań nad rolą osi jelitowo-stawowej w leczeniu spondyloartropatii.

Streszczenie w języku angielskim

Spondyloarthritis is a group of inflammatory joint diseases of the spine and peripheral joints with a complex pathogenesis involving genetic conditions, environmental factors and immunological mechanisms. Over recent years, the association of spondyloarthritis with the gut-joint axis has been strongly emphasised.

According to the available literature, the polymorphism of haptoglobin, which is one of the acute phase proteins whose main function is to bind free haemoglobin in the blood, may be associated with the course and treatment efficacy of autoimmune and inflammatory diseases. In my dissertation, I presented the hypothesis that different phenotypes of haptoglobin determined by its polymorphism may result in the different course of spondyloarthritis and affect the outcome of therapy. In order to comprehensively characterise the impact of haptoglobin polymorphism in this disease, in my analyses I also included a molecule described in the literature as a precursor of Hp2- zonulin, which is related to increased intestinal permeability.

Spondyloarthritis may be axial or peripheral depending on the predominant symptoms. The treatment of axial spondyloarthritis, which is the focus of my work, involves two stages: first, non-steroidal anti-inflammatory drugs (NSAIDs) are used, then, if these are ineffective, biological disease-modifying antirheumatic drugs (bDMARDs) (most commonly) or targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) therapy is implemented. The effectiveness of the therapy is limited and the reasons for these limitations have not yet been fully elucidated.

The first article is a review paper in which I described existing literature data on the distribution of haptoglobin phenotypes in spondyloarthritis and analysed inflammatory pathways involved in the pathogenesis of spondyloarthritis in which haptoglobin polymorphism and zonulin activity may be relevant. In this article, I presented the rationale for selecting haptoglobin polymorphism as a factor that may influence the course of spondyloarthritis. Additionally, I discussed the importance of zonulin on intestinal barrier disintegration in the context of disease progression and proposed a potential therapeutic pathway using a zonulin inhibitor.

In the second article, I described the results of an original study in which I was able to identify predictors of poor response to treatment with non-steroidal anti-inflammatory drugs in axial spondyloarthritis. First of all, I checked whether haptoglobin, its polymorphism or zonulin were

among detected predictors. The results of the analyses indicate that patients with high levels of zonulin have a significantly higher risk of poor response to standard treatment. No similar association was detected for haptoglobin and its polymorphism. Haptoglobin polymorphism alone was not associated with any of the disease activity parameters, nor was it linked to zonulin.

Importantly, my study has for the first time characterised patients with axial spondyloarthritis who have a high risk of failing to continue NSAIDs therapy. These are patients with ankylosing spondylitis, long duration of disease, sacroiliac joint involvement on X-ray, active sacroiliitis on MRI, high BASDAI, high values of subjective assessment of severity of the back pain on the VAS scale and the aforementioned high serum zonulin levels.

The third article in this series reports the results of a study on predictors of poor response to biological therapy in axial spondyloarthritis after 12 weeks of therapy. The analyses found that, again, higher zonulin concentrations were associated with treatment failure, independently of all other examined variables. In contrast, higher haptoglobin concentrations were found in patients who responded well to biological treatment. Haptoglobin concentration was significantly associated with the haptoglobin polymorphism, but the polymorphism itself was not associated with response to biological treatment or with indicators of spondyloarthritis activity. As in the previous study, zonulin was not significantly related to a specific haptoglobin phenotype and was also present in patients not carrying the Hp2 gene.

It is noteworthy that among the predictors of ineffectiveness of biological treatment, other factors besides zonulin have been identified as associated with impaired intestinal function and dysbiosis, such as older age, history of inflammatory bowel disease and frequent use of antibiotics for infections.

In conclusion, my findings in relation to axial spondyloarthritis show that the haptoglobin polymorphism is not clinically relevant in the course and treatment of this disease. My work, on the other hand, has identified predictors of failure of both standard and biological therapy in this disorder. This will help to guide more patient-specific treatment in the future and avoid prolonged standard therapy in patients where such continuation has a high risk of failure. The identification of zonulin as a factor associated with treatment response in spondyloarthritis points to new directions and treatment options using a zonulin inhibitor, regardless of the present haptoglobin phenotype. Based on my analyses, future research into the role of the gut-joint axis in the treatment of this disease is warranted.

Wstęp uzasadniający połączenie wskazanych publikacji w jeden cykl, jak i komentujący osiągnięcia naukowe kandydata na tle dotychczasowego stanu wiedzy.

Przedstawiony cykl publikacji, których jestem pierwszym autorem dotyczy znaczenia klinicznego haptoglobiny, jej polimorfizmu i zonuliny w przebiegu spondyloartopatii oraz możliwości wykorzystania wyżej wymienionych czynników w przewidywania skuteczności leczenia tej choroby.

Spondyloartropatie to określenie grupy chorób reumatycznych o podobnej patogenezie i objawach klinicznych, gdzie dochodzi do zapalenia stawów kręgosłupa i stawów obwodowych. Spondyloartropatie są zaliczane do chorób zapalnych o podłożu immunologicznym (*immune-mediated inflammatory diseases*), ponieważ w ich patogenezie uczestniczą zarówno czynniki autozapalne jak i autoimmunologiczne [1].

Częstość występowania spondyloartropatii mieści się w przedziale 0.3-1.9%, co czyni ją jedną z najczęściej występujących chorób reumatycznych [2]. Główne podtypy choroby to zesztywniające zapalenie stawów kręgosłupa, łuszczycowe zapalenie stawów , reaktywne zapalenie stawów, zapalenie stawów w przebiegu nieswoistych zapaleń jelit i spondyloartropatia niezróżnicowana. Z uwagi na manifestację kliniczną możemy wyróżnić spondyloartopatie osiowe (dominuje zapalny ból kręgosłupa ze sztywnością poranną) i obwodowe (głównym objawem jest zapalenie stawów obwodowych i przyczepów ścięgnistych). Postać osiowa występuje częściej i na niej skupiłam się w mojej pracy. Warto zaznaczyć, że istniejące kryteria klasyfikacyjne spondyloartropatii, które różnicują formę osiową i obwodową nie stanowią sztywnego podziału na dwie odrębne choroby o różnej etiologii. W moim badaniu znacząca liczba pacjentów wykazywała także objawy związane ze stawami obwodowymi oraz przyczepami ścięgnistymi.

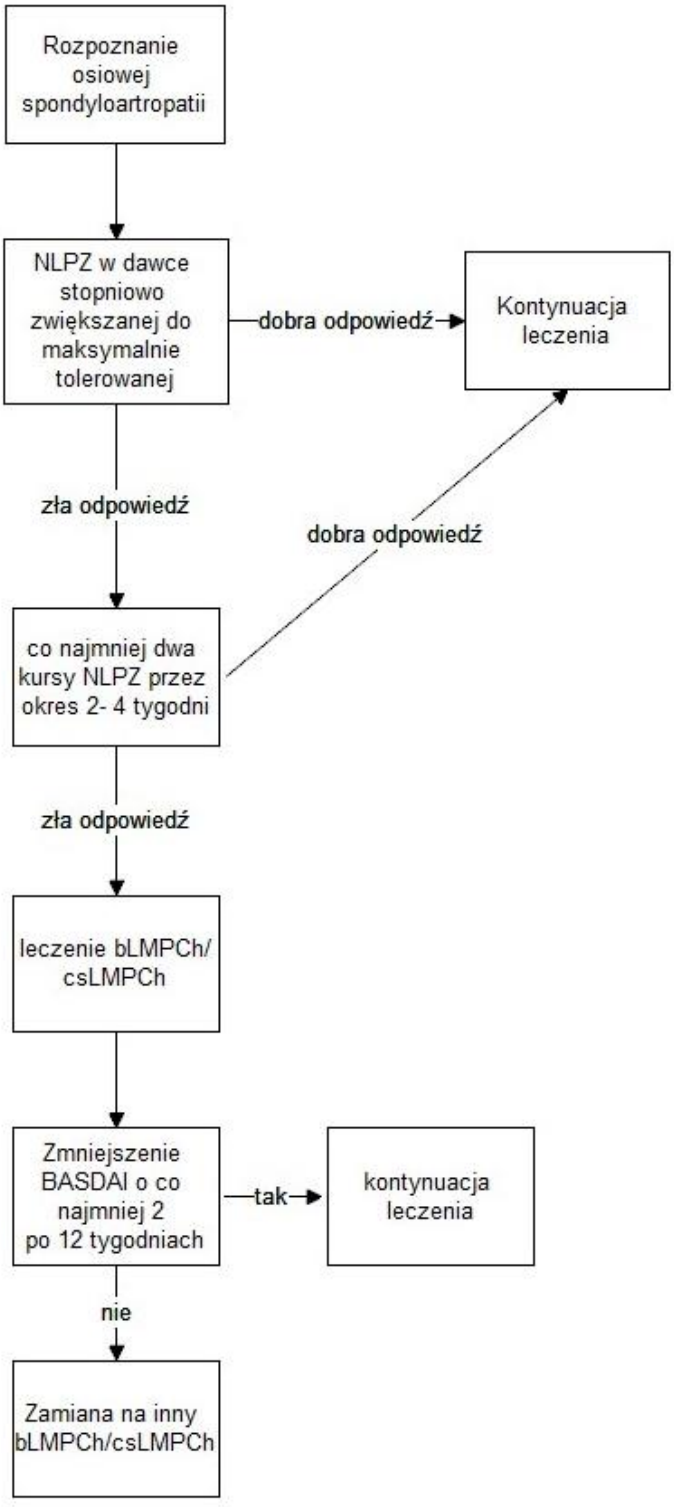
Zajęcie stawów krzyżowo-biodrowych i stawów kręgosłupa często prowadzi do trwałych uszkodzeń w ich obrębie, co manifestuje się poprzez powstawanie nadżerek, nowotworzenie kości (syndesmofity) oraz całkowite zesztywnienie stawów (ankyloza). Wiąże się to ze znacznym ograniczeniem ruchomości kręgosłupa i kalectwem. Jest to tym bardziej dotkliwe społecznie, że na spondyloartropatie chorują głównie osoby młode, przed 45 rokiem życia.

Pierwsza linia leczenia osiowej postaci spondyloartropatii obejmuje grupę niesteroidowych leków przeciwzapalnych. Zgodnie z zaleceniami ASAS-EULAR przed rozważeniem drugiej linii leczenia, należy wypróbować co najmniej dwa leki z tej grupy w maksymalnej dawce przez okres co najmniej jednego miesiąca [3]. W przypadku utrzymywania się wysokiej aktywności choroby mierzonej za pomocą wskaźników ASDAS lub BASDAI (w badaniu użyto wskaźnika BASDAI) oraz pozytywnej opinii reumatologa, pacjent jest kwalifikowany do terapii lekami biologicznymi lub syntetycznymi celowanymi lekami modyfikującymi przebieg choroby (Rycina 1). Pomimo zastosowania drugiej linii leczenia spora część pacjentów nie uzyskuje remisji choroby (po 6 miesiącach terapii najczęściej stosowanymi inhibitorami TNF alfa odsetek remisji wynosi 11-48%) [4].

W przypadku leczenia niesteroidowymi lekami przeciwzapalnymi istnieją pojedyncze prace poświęcone ocenie ich skuteczności w osiągnięciu remisji w osiowej spondyloartropatii oraz nie istniały żadne dane na temat predyktorów nieskuteczności terapii tymi lekami [5-7]. Opisywane natomiast w literaturze predyktory złej odpowiedzi na leczenie biologiczne były poszukiwane zazwyczaj wśród standardowych markerów aktywności choroby i znanych czynników związanych z chorobą [4,8].

W celu zrozumienia przyczyn braku skuteczności leczenia w spondyloartropatiach, w tym identyfikacji nowych predyktorów odpowiedzi na istniejące terapie, postanowiłam zbadać znaczenie innych czynników, które dotychczas nie były brane pod uwagę w tego typu analizach, lecz są powiązane z patogenezą chorób autoimmunologicznych i zapalnych.

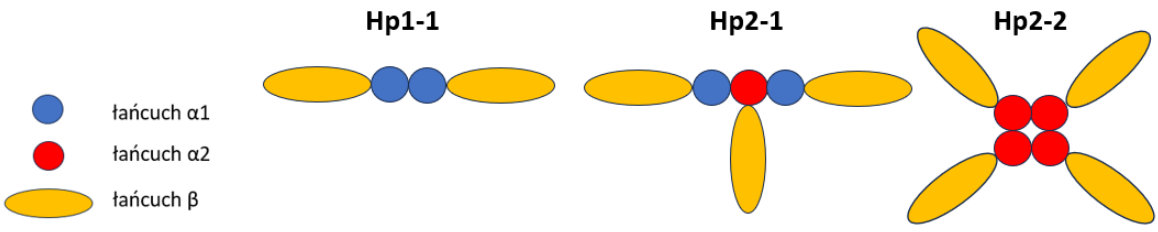
Haptoglobina wydała mi się szczególnie interesującym białkiem ze względu na swoje unikalne właściwości biofizyczne i immunomodulacyjne, co czyni ją obiektem badań jako potencjalnego biomarkera w wielu chorobach autoimmunologicznych, infekcyjnych i nowotworowych [9-12]. Oprócz wiązania wolnej hemoglobiny podczas hemolizy, haptoglobina pełni również funkcję białka ostrej fazy i odgrywa wiele istotnych ról w procesie zapalnym (takich jak hamowanie produkcji wolnych rodników i TNF alfa przez komórki zapalne, blokowanie produkcji prostaglandyn oraz modulacja odpowiedzi limfocytów T) [13]. Dodatkowo, stężenie haptoglobiny wzrasta lokalnie w przypadku zapalenia stawów [14].



Rycina 1- Schemat leczenia osiowej spondyloartropatii.
Opracowano na podstawie rekomendacji ASAS/EULAR z 2022 r.

Cechy strukturalne, fizyczne i biologiczne cząsteczek haptoglobiny są zależne od jej polimorfizmu. Istnieją trzy główne fenotypy haptoglobiny Hp1-1, Hp2-1 oraz Hp2-2 (Rycina 2). Cząsteczki Hp2-2 mają największą masę, słabo przenikają do przestrzeni pozanaczyniowej, a stężenie haptoglobiny u osoby z takim fenotypem jest niższe niż w pozostałych dwóch przypadkach. Ponadto Hp2-2 jest białkiem o najsłabszych właściwościach przeciwzapalnych, antyoksydacyjnych oraz najsłabiej hamuje syntezę prostaglandyn – jest to ważny mechanizm patogenetyczny w spondyloartropatii, na który ukierunkowane jest leczenie niesteroidowymi lekami przeciwzapalnymi [15]. Fenotyp Hp2-2 jest opisywany w literaturze jako związany z gorszym rokowaniem w niektórych chorobach autoimmunologicznych, zakaźnych, nowotworowych oraz w chorobach układu sercowo-naczyniowego [16-18]. Co więcej, prekursorem Hp2-2 jest cząsteczka pre-Hp2 nazywana zonuliną, która wpływa na zwiększoną przepuszczalność jelit [19]. Istnieją dane na temat zwiększonej ekspresji zonuliny w jelitach u pacjentów z zesztywniającym zapaleniem stawów kręgosłupa [20].

Wiązanie hemoglobiny	silne	umiarkowane	słabe
Aktywność antyoksydacyjna	silna	umiarkowana	słaba
Hamowanie syntezy prostaglandyn	silne	umiarkowane	słabe



Rycina 2- Różnice w budowie i właściwościach haptoglobiny w zależności od fenotypu.
Opracowano na podstawie danych literaturowych [9].

Pierwszy artykuł cyklu przedstawia, w których szlakach immunologicznych i zapalnych związanych z patogenezą spondyloartropatii polimorfizm haptoglobiny może mieć znaczenie i wpływać na odmienny przebieg choroby [21]. Ponadto w pracy tej opisuję, jak subkliniczne zapalenie jelit, obserwowane u około 60 % pacjentów ze spondyloartropatią, może być związane z dysbiozą i zaburzoną integralnością jelit, na którą ma wpływ zonulina. Jest to poniekąd nawiązanie do istniejącej zależności pomiędzy zapaleniem jelit i stawów, czyli osi

jelito-stawy [22]. Jest to pierwsza opublikowana praca poglądowa na temat możliwej roli haptoglobiny, jej polimorfizmu oraz zonuliny w różnych mechanizmach patofizjologicznych spondyloartropatii.

Drugi artykuł stanowi rezultat moich badań, które umożliwiły identyfikację predyktorów złej odpowiedzi na leczenie niesteroidowymi lekami przeciwzapalnymi w osiowej spondyloartropatii ze szczególnym uwzględnieniem haptoglobiny, jej polimorfizmu i zonuliny [23]. Przedstawione przeze mnie wyniki pokazują, że zwiększone stężenie zonuliny jest związane ze złą odpowiedzią na leczenie i może być użytecznym wskaźnikiem niepowodzenia terapii standardowej w tej chorobie. Wpływ zonuliny nie był zależny od polimorfizmu haptoglobiny i nie był z nią skorelowany. Jest to pierwsza praca, która pomaga wyszczególnić pacjentów z osiową postacią spondyloartropatii, u których kontynuacja terapii pierwszej linii wiąże się z dużym ryzykiem niepowodzenia i u który należy wcześniej rozważyć inne metody leczenia, być może szybszą kwalifikację do terapii drugiej linii. Wśród negatywnych predyktorów , oprócz wysokiego stężenia zonuliny, wymienione zostały: postać zesztywniającego zapalenia stawów kręgosłupa, długi czas trwania objawów, wysoka aktywność choroby (mierzona wskaźnikami BASDAI i VAS), aktywne zapalenie stawów krzyżowo-biodrowych w badaniu rezonansu magnetycznego, zmiany radiologiczne w stawach krzyżowo-biodrowych. Początkowe stężenie haptoglobiny we krwi i polimorfizm haptoglobiny okazały się nie mieć istotnego związku z odpowiedzią na leczenie niesteroidowymi lekami przeciwzapalnymi.

Badanie, którego wyniki zostały zaprezentowane w trzecim artykule wchodzącym w skład cyklu, pozwoliło znaleźć potencjalne przyczyny nieskuteczności leczenia biologicznego w osiowej spondyloartropatii [24]. Predyktorami złej odpowiedzi na leczenie biologiczne okazały się być czynniki związane z zaburzeniami mikroflory i zwiększoną przepuszczalnością jelit (starszy wiek, częste infekcje leczone antybiotykami, zapalne choroby jelit w wywiadzie, wyższe stężenia zonuliny). W artykule postawiona została hipoteza, że przyczyną nieskuteczności terapii biologicznej są zaburzenia prawidłowego funkcjonowania jelit. Wniosek jest taki, że lecząc tylko jedno ze składowych osi jelito-stawy możemy mieć problem z uzyskaniem remisji u pacjenta. Jest to spójne z dotychczasową wiedzą na temat wpływu zaburzeń mikrobiomu jelitowego oraz chorób zapalnych jelit na przebieg spondyloartropatii [25-27]. Dodatkowo wyniki analiz zmian w składzie flory

jelitowej podczas leczenia biologicznego w spondyloartropatii wskazują na użyteczność tego parametru do monitorowania efektów terapii [28-30].

Co interesujące, haptoglobina w stężeniach wysokich występowała wyłącznie u pacjentów dobrze odpowiadających na leczenie, niezależnie od jej fenotypu.

W obydwu badaniach polimorfizm haptoglobiny nie był związany z parametrami aktywności spondyloartropatii.

Podsumowując, prace wchodzące w skład cyklu publikacji w sposób spójny poruszają tematykę związaną ze znaczeniem klinicznym polimorfizmu haptoglobiny w przebiegu i leczeniu najczęstszej postaci spondyloartropatii. Sam polimorfizm haptoglobiny nie okazał się być czynnikiem determinującym odpowiedź na leczenie osiowej spondyloartropatii, natomiast haptoglobina i zonulina stanowią obiecujące wskaźniki użyteczne w przewidywaniu skuteczności terapii w tej chorobie. Fenotyp Hp2-2 nie wykazywał związku z wyższą aktywnością choroby ani nawet z wyższym poziomem zonuliny. Występowanie zonuliny również u osób nieposiadających allelu Hp2 oznacza, że zonulina badana za pomocą dostępnych komercyjnie testów ELISA musi być zawsze rozpatrywana jako tzw. peptydy z rodziny zonuliny (*zonulin family peptides*), a nie jako wyłącznie cząsteczka pre-Hp2. Podobne obserwacje zgłaszali również inni autorzy prac [31-33].

Cały cykl badań stanowi preludium do dalszych badań, gdyż po raz pierwszy rzuca nowe światło na potencjalne metody leczenia spondyloartropatii przy użyciu inhibitora zonuliny (octanu larazotydu). Aktualnie octan larazotydu wydaje się obiecującą opcją w terapii celiaklii [34]. Jest to nowe podejście do leczenia tej choroby, skupiające się na regulowaniu właściwej funkcji jelit, co stanowi kolejny element osi zapalenia jelitowo-stawowego. Dopiero przyszłe badania, których celem będzie opracowanie skutecznych metod przywracania homeostazy w jelitach, mogą określić, czy może to zwiększyć częstość uzyskiwania remisji w spondyloartropatii. Wyniki mojej pracy oraz istniejące pierwsze obserwacje z badań nad przeszczepianiem flory jelitowej, stosowaniem probiotyków i zmian w składzie diety u pacjentów ze spondyloartropatią są nadzieją na znalezienie nowej drogi terapeutycznej w tej chorobie [35-38].

Założenia i cel pracy

W spondyloartropatii nadal istnieje znaczny odsetek pacjentów, którzy nie reagują na leczenie. W dobie medycyny spersonalizowanej poszukiwanie wskaźników oceniających efektywność terapii pozwala zwiększyć szansę na dobranie skutecznego leku dla chorego. Mając to na uwadze, uznałam za celowe ustalenie znaczenia nowych czynników w przewidywaniu skuteczności leczenia w spondyloartropatii osiowej, które mogą być powiązane z patogenezą choroby i tym samym przyczynić się do lepszego zrozumienia problemów w osiąganiu remisji.

Celem mojej pracy było zbadanie związku poszczególnych fenotypów haptoglobiny z odpowiedzią na standardowe i biologiczne leczenie w najczęściej występującej osiowej postaci spondyloartropatii.

W mojej dysertacji chciałam zweryfikować hipotezę, opartą na istniejących danych literaturowych, która zakładała, że pacjenci ze spondyloartropatią i fenotypem Hp2-2 mają gorszy przebieg choroby objawiający się słabszą odpowiedzią na istniejące leczenie oraz wyższą aktywnością choroby.

Szczegółowe cele pracy są następujące:

1. Analiza istniejących badań na temat polimorfizmu haptoglobiny w kontekście chorób autoimmunologicznych, zapalnych i w spondyloartropatii.
2. Analiza mechanizmów patogenetycznych w spondyloartropatii powiązanych z haptoglobiną, jej polimorfizmem i zonuliną.
3. Wskazanie nowych kierunków badań na temat przebiegu spondyloartropatii związanych z polimorfizmem haptoglobiny i zonuliną.
4. Zbadanie związku haptoglobiny, polimorfizmu haptoglobiny i zonuliny z odpowiedzią na leczenie niesteroidowymi lekami przeciwzapalnymi oraz leczenie biologiczne (odpowiedź wczesna po 12 tygodniach) w spondyloartropatii osiowej.
5. Zbadanie związku polimorfizmu haptoglobiny z zonuliną.
6. Analiza związku polimorfizmu haptoglobiny, stężenia haptoglobiny i zonuliny ze wskaźnikami aktywności choroby w spondyloartropatii osiowej.
7. Analiza znaczenia zonuliny w spondyloartropatii.

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Review

Haptoglobin and Its Related Protein, Zonulin—What Is Their Role in Spondyloarthropathy?

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Abstract: Haptoglobin (Hp) is an acute phase protein which supports the immune response and protects tissues from free radicals. Its concentration correlates with disease activity in spondyloarthropathies (SpAs). The Hp polymorphism determines the functional differences between Hp1 and Hp2 protein products. The role of the Hp polymorphism has been demonstrated in many diseases. In particular, the Hp 2-2 phenotype has been associated with the unfavorable course of some inflammatory and autoimmune disorders. Its potential role in modulating the immune system in SpA is still unknown. This article contains pathophysiological considerations on the potential relationship between Hp, its polymorphism and SpA.

Keywords: haptoglobin polymorphism; inflammation; pathogenesis; spondyloarthropathy; zonulin

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1. Introduction

Spondyloarthropathy is one of the most common rheumatic diseases whose prevalence varies between 0.4 and 1.9% in different countries [1]. The heterogeneity of SpA is the result of numerous overlapping environmental and genetic factors which make the overall pathogenesis of the disease still elusive.

The dominant role of the innate immune system in the pathophysiology of SpA indicates its autoinflammatory character rather than an autoimmune one, although it is ultimately classified as an immune-mediated disease [2]. Several cytokine pathways are involved in the inflammatory process in SpA but it is the increased production of free oxygen radicals that directly corresponds to the destruction of tissues. Increased oxidative stress and its link to disease activity was demonstrated in the prototype form of SpA-ankylosing spondylitis (AS) [3].

Hp is a molecule that regulates the immune response and reduces oxidative stress; thus, its role may be crucial in the inflammatory pathways implicated in SpA [4]. Increased local expression of Hp was described in arthritis [5]. Hp is described as an inhibitor of collagen degradation and an important factor in cell migration [6]. Both of these processes characterize arthritis.

Human Hp is α^2-sialoglycoprotein that belongs to acute phase proteins. Its synthesis, mainly in hepatocytes, is stimulated by proinflammatory cytokines IL-1, IL-6 and tumor necrosis factor α (TNFα). There are three major Hp phenotypes: Hp 1-1, Hp 2-1 and Hp 2-2, which arise from diversity in α chain compositions. Hp phenotypes differ in molecular size and structure, which determine their biological properties. The main role of Hp is connected with the binding capacity of hemoglobin (Hb). It forms a soluble complex with Hb, which is not filtered in the kidneys but is broken down in the liver, thus preventing kidneys from damage. Moreover, Hp dampens the inflammation by inhibiting the synthesis of prostaglandins, leukotrienes and cathepsin B. It reduces oxidative stress of iron-derived

reactive oxygen connected with free Hb release. Hp 2-2 is the least effective in terms of antioxidative and anti-inflammatory activities due to its polymeric form weakly moving to the tissues [4,7,8].

Elevated serum Hp concentrations and a positive correlation between Hp levels and disease activity parameters have been observed in SpAs. Hp is an inflammatory marker used in the evaluation of treatment efficacy in many clinical trials [9–11]. An interesting issue is how Hp shapes the anti-inflammatory response in SpA and whether there are significant differences between Hp phenotypes in this respect. It also seems important for Hp2 gene carriers as the Hp2 precursor (pre-Hp2) molecule, zonulin, was upregulated in a recently conducted study in AS patients [12].

This article will focus on pathophysiological mechanisms in SpA in which Hp and its polymorphism may be crucial. We propose that Hp and its related protein, zonulin, may have important functions in the pathogenesis of SpA. Uncovering what role Hp and its polymorphism play in SpA would be advantageous in the future.

2. Distribution of Haptoglobin Phenotypes in Spondyloarthropathies

The distribution of general Hp alleles presents the frequencies of 0.4 for Hp1 and 0.6 for Hp2 in Europe [4]. The most widespread theory of evolutionary and structural biology of Hp states that the gene Hp2 occurred approximately 2 million years ago in India and is evolutionarily younger than the Hp1 gene [13]. Its spread beyond the Asian continent indicates the existence of certain advantages over the Hp1 allele. The hypothesis says that the appearance and increasing prevalence of the Hp2 allele is related to parasitic infections, especially malaria [14]. Nevertheless, in light of recent studies, this theory seems to be controversial, because it is also likely that the Hp2 gene is much older than previously assumed [15].

The anti-inflammatory properties of Hp depend on the phenotype and are the weakest for the Hp 2-2 phenotype. Hp 2-2 is associated with a higher predisposition to autoimmune and inflammatory diseases and worse outcomes of many of them [14,16–18]. The lower concentrations of Hp in serum and tissues in patients with inflammatory bowel diseases and Hp 2-2 phenotype may be associated with higher concentrations of proinflammatory cytokines compared to other patients [19].

The first published study on the distribution of Hp phenotypes in patients with rheumatoid spondylitis dates back to 1962. [20]. No statistical significance was found between the study group (45 Caucasian males) and the healthy volunteers, but it was noted that the differences in the distribution of the Hp1-1 phenotype between these two groups were at the borderline of statistical significance. The authors suggested conducting the study on a larger group of patients.

No significant difference in Hp frequency in AS (48 Caucasian individuals) and no correlation between Hp phenotype and serum C-reactive protein (CRP) were found by Sitton and Dixon [21]. The authors observed disturbed proportions of Hp 2-1 and Hp 2-2 phenotypes compared to patients with rheumatoid arthritis in favor of Hp 2-1. The relatively small size study is its limitation, as listed by the authors. Nothing is known about other factors that could affect the CRP value either. No data are given on, e.g., medicines taken by patients.

Baeten et al. investigated the expression of CD 163 (a scavenger receptor for Hb–Hp complexes) in patients with SpAs (130 Belgian residents) and showed no difference in the distribution of Hp phenotypes compared to both: the normal distribution in the Belgian population and between the subgroups of SpA [22]. The Hp 1-1 phenotype was weakly correlated with some of the disease activity parameters (CRP and erythrocyte sedimentation rate (ESR)) but not with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) or with the Bath Ankylosing Spondylitis Metrology Index (BASMI).

The cross-sectional character of the study is its limitation. It is worth noting that the examined patients with SpAs were on various treatments modifying the course of

the disease and some of them were untreated. Therefore, conclusions on the relationship between disease activity and Hp polymorphism are difficult to draw.

Surprisingly, different results from above were published by Soliev et al. from the Medical Institute in Tashkent [23]. The authors observed the significantly more frequent occurrence of the Hp 2-2 phenotype in ankylosing spondylitis, Hp 1-1 in psoriatic arthritis and Hp 2-1 in reactive arthritis. A total of 100 patients with SpAs, residents of Uzbekistan, were examined. The control group in this study had a similar distribution of Hp phenotypes to the European population. In the context of this research, it is worth noting the finding of Ciccia et al. who demonstrated upregulation of zonulin in the intestines of AS patients [12]. Zonulin is a relatively newly discovered molecule, having been identified in 2000 by Fasano and colleagues [24]. In 2009, zonulin was identified as pre-Hp2, an uncleaved form of mature Hp2 [25]. Importantly, the function of zonulin as a modulator of intestinal barrier tightness has been described. Until this discovery, pre-Hp2 was thought to be inactive. There are two identified zonulin triggers so far: gliadin and bacteria [26]. Of additional interest are the results of the proteomic analysis in AS performed by Liu et al. [27]. This study showed significantly increased expression of Hp precursor (pre-Hp) in AS patients compared to the control group. Moreover, it was shown that pre-Hp epitopes bind with high affinity to an allele HLA B*2705—the subtype which is the SpA risk factor for Caucasians. The authors found that the acute anterior uveitis is connected with an allele, HLA A*0201, especially when it coexists with HLA B*2705. HLA A*0201 possess properties that are particularly easy to combine with pre-Hp, similar to HLA B*2705.

Taken together, there has been no clear evidence of abnormal distribution of Hp phenotypes among people with SpA and there is no good data of the relationship between the Hp polymorphism and disease activity in SpA. However, the distribution of the Hp genes themselves seems to be relevant in the context of the functions that their precursors perform, especially the pre-Hp2- zonulin molecule, already well described in the literature.

3. The Role of Zonulin and Haptoglobin in Chronic Gut Inflammation

The idea that the gut joint axis is an important pathophysiological component of SpA is growing. Interestingly, studies have shown that inflammation of the intestines is correlated with the disease activity. Subclinical intestinal inflammation can be found in up to 68% of patients with SpAs [28]. Microscopic gut inflammation in axial SpA was described as related to younger age, progressive disease, male sex and higher disease activity [29]. In another study, the degree of bone marrow oedema in sacroilliac joints of patients with axial SpAs was linked to gut inflammation and male sex [30]. Gut inflammation seems to be a predictor of SpA progression to AS [28]. Inflammatory changes in the gut appear to be driven by an altered microbiome which causes an increased response from the immune cells especially through IL-23 cytokine release [31–33]. The pathogenic responsiveness to bacteria antigens and perturbation in the gut microenvironment may be associated with HLA B27 function [34–36]. Impaired intestinal barrier which leads to increased intestinal permeability has been demonstrated in SpA [12,36,37]. It is likely that the translocation of bacterial products plays an important role in the initiation of inflammation in the joints and in the uvea [38–40]. Marquez et al. revealed a protective function of Hp in the intestines [19]. Hp deficient mice developed severe inflammatory colitis with a particularly high production of IL 17. In this study, a higher frequency of Hp2 gene in the group with inflammatory bowel diseases than in the control group was presented.

Fasano et al. showed that zonulin regulates the tight junctions in the intestines and that increased gut permeability is associated with zonulin expression [41]. Ciccia et al. had similar observations [12]. The authors studied gut vascular and epithelial barrier impairment in AS patients and found that downregulation of endothelial and epithelial tight junction proteins is associated with zonulin. It was shown that high serum levels of lipopolysaccharide, lipopolysaccharide (LPS)-binding protein and intestinal fatty acid-BP together with the zonulin modified the activity of peripheral blood monocytes. This study demonstrated that zonulin, due to its affinity for the CD 163 receptor, leads to expansion of

CD 163+ c-MAF + monocytes compatible with M2 macrophages. M2 macrophages were shown to be expanded in the peripheral blood, the gut and the synovium in patients with SpAs [42]. In another study, the number of macrophages CD 163+ (M2) in the synovium of patients with SpAs correlated with the disease activity and decreased during anti-TNF therapy [43].

The role of the microbiome in the development of SpA was shown in a study on transgenic HLA B27 rats [44]. The lack of commensal bacteria effectively protected them from the development of arthritis. Ciccia et al. demonstrated that ileal inflammation and perturbation in epithelial tight junctions in HLA B27 positive rats could be reversed by antibiotic treatments [12]. In another study, mice deprived of the normal Toll-like receptor 4(TLR4)—the lipopolysaccharide (LPS) sensor—were less likely to develop arthritis [45]. Additionally, the levels of proinflammatory factors in their synovial tissue were lower. TLR 4 was necessary to induce LPS-dependent arthritis.

LPSs increase the expression of the IL-23p19 gene in dendritic cells as well as activate innate lymphoid cells type 3 (ILC3) [46]. Polarization of innate lymphoid cells towards ILC3 may also result from the direct action of IL-23 [47]. Increased amounts of ILC3 were detected in the inflamed gut of patients with AS and correlated with disease activity [48]. ILC3 expresses integrin $\alpha 4\beta 7$ and thus provides circulation between the intestine and the active inflammatory sites such as bone marrow and joints rich in $\alpha 4\beta 7$ ligand [33,48].

On the other hand, innate immune cells, such as macrophages, NK cells, and neutrophils that are involved in intestinal inflammation, have receptors for Hp-Mac-1 leucocyte integrin b2 (CD11b/CD18) [49]. CD11b/18 together with other receptors is involved in regulating gene expression in response to LPSs. Further, Ling Zeng showed that macrophages in AS patients produce more IL-23 and TNF α in response to LPSs than in the control group. For some reason, these cells are particularly easily activated by LPS [50]. Hp was shown to dampen the LPS driven immune response mainly by inhibiting the monocyte and macrophage functions. This effect was selective and was associated with a decrease in production of TNF α, IL-10 and IL-12p70 [51].

An interesting issue in the context of differences in the course of SpA between men and women is the gender-specific anti-inflammatory properties of Hp in response to bacterial LPS, as was shown in the in vitro study. Raju et al. investigated changes in Hp levels in relation to the presence of LPS, TNF alpha and sex hormones [52]. The study showed that Hp was responsible for the endotoxin tolerance (ET) state, caused by a fall in TNF α levels, and was reversible when Hp was blocked. Hp suppressed the proinflammatory cytokines, released in response to bacterial LPSs, more strongly in the presence of estrogen. The opposite effect was observed by adding testosterone to the test blood, which caused an increase in TNF α. The authors conclude that this finding is consistent with observations of worse prognoses in the case of bacterial sepsis in males. On the other hand, higher levels of estrogen in females have long been suggested to correlate with a higher incidence of autoimmune diseases. The question is, can this be related to more frequent occurrence of AS among men and faster progression of the disease in their case?

To summarize, microbiomes, increased intestinal permeability and inflammation play important roles in the pathogenesis of SpA, as indicated by numerous reports from the literature. The regulation of response to LPS involves Hp and zonulin. There are some differences in the pattern of this reaction between the Hp phenotypes and it seems that the response is gender-specific. Zonulin in AS patients was shown to be linked with an impaired gut barrier, which may indicate a worse course of the disease in carriers of the Hp2 gene.

4. The Role of Haptoglobin in Inflammatory Pathways

The IL23/IL17 axis is an important cytokine pathway in SpA and a crucial part of antibacterial immunity [53]. One of the main sources of IL-23 in SpAs are macrophages with receptor CD 163 (M2) [50]. Ciccia et al. showed that polarization of macrophages towards M2 may happen upon exposure to zonulin; thus, it may enhance the IL23/IL17

axis [12]. Prostaglandins create the next most important inflammatory pathway in SpA, which affects this axis. A number of studies have reported that prostaglandin E2 (PGE2) leads to an increase in IL-23 production by bone marrow-derived dendritic cells and an increase in IL-23 receptor expression on Th17 cells [46,54,55]. Therefore, PGE2 shifts the immune response towards Th17. Additionally, IL-23 was shown to stimulate Th17 cells to produce PGE2 [56]. The recent studies have demonstrated that overexpression of prostaglandin E2 receptor 4 (EP4) on Th17 lymphocytes and monocytes is associated with disease activity and progression in AS [57,58]. Moreover, prostaglandins play an important role in new bone formation by EP2 and EP4 receptors [59]. Cyclooxygenase (COX) inhibition by nonsteroidal anti-inflammatory drugs has proven to be effective in SpA treatment [60,61].

Shim has shown that prostaglandins stimulate Hp synthesis [62]. On the other hand, Hp blocks COX. This process is phenotype-dependent and is the least pronounced for Hp 2-2 [4]. However, there are no data showing that inhibition of prostaglandin synthesis by Hp and that phenotype strength of this process have any clinical implications in SpA. This is a particularly interesting issue in terms of new bone formation.

Hp itself was demonstrated to modulate the response of lymphocytes, Th 17 [19]. It is unknown whether this process is phenotype-dependent. Moreover, Arredouani et al. showed a direct effect of Hp on T lymphocytes by significantly stronger suppression of Th2 cytokines (IL-4, IL-5, IL-10, IL-13) than Th1 cytokines (IFN gamma and IL-2) [63]. This outcome was observed for both Hp 1-1 and Hp 2-1 phenotypes. Unfortunately, the Hp 2-2 phenotype has not been studied for this purpose.

To conclude, Hp interacts with the receptors of immune cells and clearly takes part in the cytokine pathways important for SpA pathogenesis. A particularly interesting issue is the Hp phenotypic relationship of the prostaglandin synthesis blockade.

5. Haptoglobin and Oxidative Stress

Oxidative stress is based on increased production of reactive oxygen species (ROS) and the insufficiency of the system's antioxidant potential to balance them. Inflammation can easily cause oxidative stress, but, on the other hand, ROS activate the genes involved in inflammation [64]. Thus, these two processes constitute a rather inseparable pathophysiological aspect.

One of the main sources of ROS are inflammatory cells. This way of defense against pathogens is particularly important for innate immune cells. Neutrophils and macrophage activation may lead to respiratory burst. Excessive generation of ROS may lead to cellular damage and death [65]. Interestingly, a study conducted on macrophages obtained from HLA B27-transgenic rats showed that stimulation of macrophages through IFN gamma and LPS leads to an increase in the production of ROS [66]. ROS reduction with antioxidant N-acetylcysteine significantly reduces transcriptions of proinflammatory cytokines.

In general, increased numbers of oxidative stress biomarkers were observed in patients with AS and PsA [67]. There are numerous reports from the literature showing that oxidative stress has an important function in AS pathogenesis [3,68]. There are hypotheses stating that high ROS toxicity may be responsible for articular cartilage damage and bone loss in AS [69]. Pathological bone formation may also be associated with Wnt/Beta-catenin and BMP/Smad pathways activation triggered by ROS [70]. The last meta-analysis of 2020 showed that some oxidative stress markers correlate with disease activity in AS [71]. In one study, it was observed that oxidative stress can be reduced by infliximab therapy [72].

The antioxidant properties of Hp are mainly related to its ability to form complexes with Hb. The smallest antioxidative capacity has the phenotype Hp 2-2 [4]. However, it seems that Hp is also a strong antioxidant, regardless of its ability to bind Hb. Additionally, Hp increases the resistance of the cell to oxidative stress and this property also seems phenotypically dependent [73]. Additionally, Hp can directly bind to neutrophils, inhibiting production of ROS and influencing their responses to other agonists [74]. It is interesting that TNF α, by stimulating p 55 receptors on neutrophils, leads to release of Hp [75].

Moreover, TNF α is one of the main factors responsible for the production of free oxygen radicals in AS [76].

Summarizing, Hp is produced during pro-oxidative conditions such as inflammation and has important antioxidant functions. Oxidative stress is another pathway in SpA, which may be influenced by Hp.

6. Conclusions and Research Directions

Despite the scientific basis on which Hp and its precursors are linked to immune response, there is little research on their influence in the pathogenesis of SpA. Considering that Hp and zonulin are associated with the immunological pathways distinctive for SpA, such studies could prove very worthwhile. It seems highly possible that interplay between genetic susceptibility to SpA and environmental factors may be prevented by intercellular tight junctions regulated by zonulin.

In this context, research using larazotide acetate seems interesting. Larazotide acetate (also known as AT-1001) is an oral synthetic peptide that blocks the action of zonulin by increasing the integrity of the intestinal barrier and reducing the immunoreactivity associated with its impairment [77]. Studies on AT 1001 in patients with coeliac disease showed reductions in gastrointestinal symptoms compared to those on a gluten-free diets alone [78] and during gluten challenge [79]. Would AT 1001 be effective in the therapy of SpA in Hp2 gene carriers?

Figure 1 depicts the immunological pathways in SpA regarding the mechanism of actions of Hp and pre-Hp2. These are the sites where Hp polymorphism may be relevant.

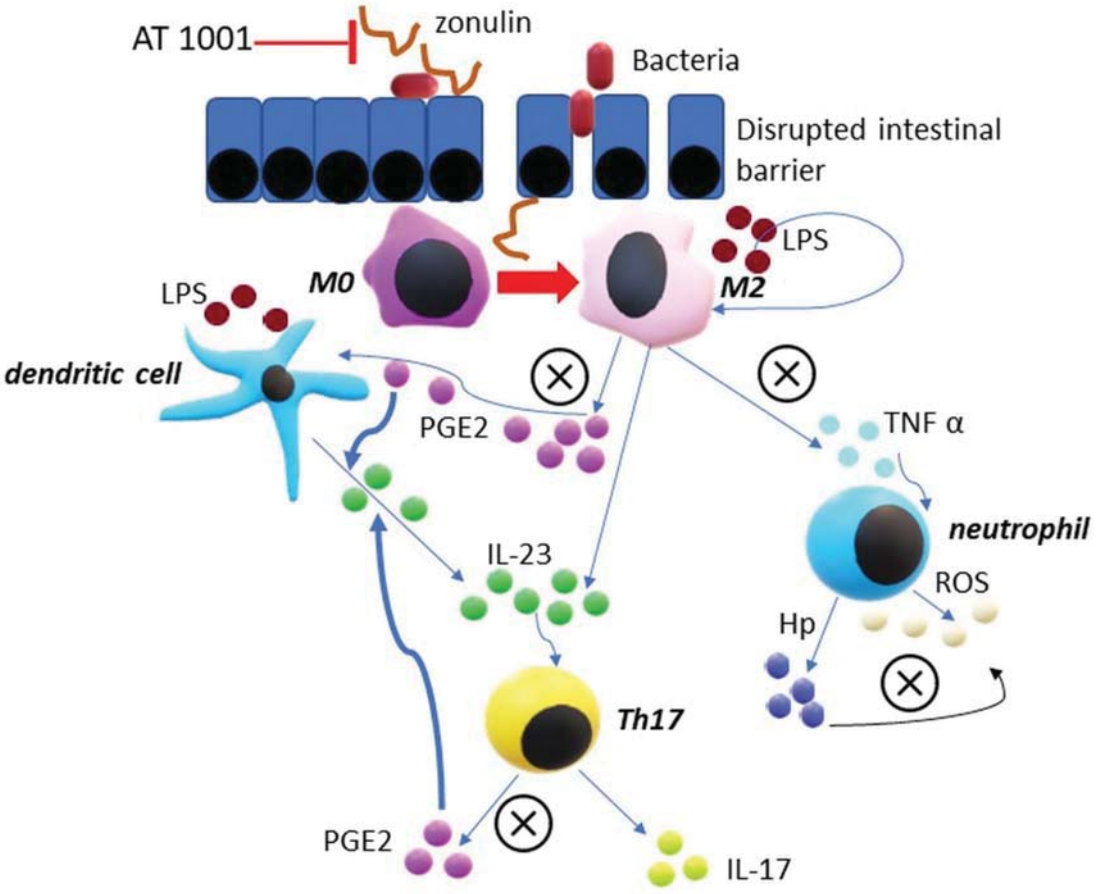

Figure 1. Proposed model of haptoglobin and zonulin impact on pathophysiology of spondyloarthropathies. Enteric bacteria stimulate zonulin secretion. Zonulin increases intestinal permeability and causes bacteria and lipopolysaccharides

(LPSs) to penetrate the intestinal wall. LPSs lead to the polarization of macrophages towards M2, activate dendritic cells and boost production of proinflammatory cytokines by them. IL-23 strongly stimulates lymphocytes' Th17 to secrete cytokine IL-17 and PGE2. PGE2 in turn strengthens the response of Th17 cells to IL-23 and increases production of IL-23 by dendritic cells. TNF is a powerful oxidative stress trigger but on the other hand leads to the release of Hp from neutrophils. Hp has antioxidant properties and reduces macrophage M2 response to LPSs by decreasing TNF alpha secretion. Hp also inhibits the production of prostaglandins in other signal pathways between cells. AT 1001 inhibits zonulin functions and may represent a novel therapeutic option in SpA. ⊗ = sites of haptoglobin inhibitory action.

Returning to the topic of our work—haptoglobin and its related protein, zonulin—what is their role in spondyloarthropathy?—currently, we are not able to answer this question unequivocally on the basis of the available literature. There is very little research on the subject. However, we want to draw attention to the issue of potential immunomodulatory functions of Hp and zonulin in SpA. Research on this subject, especially with regard to the polymorphisms of Hp and pre-Hp2, could help us understand the difficult pathogenesis of this disease and to develop better and more effective methods of treatment.

Whether the enhancement of the natural barrier by the use of a zonulin blocker could inhibit the development of SpA and alleviate the course of the disease is questionable, although it is worth answering this question one day.

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Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin

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Abstract
According to the Assessment of SpondyloArthritis International Society-European Alliance of Associations for Rheumatology (ASAS-EULAR) recommendations for the management of axial spondyloarthritis (axSpA), patients should undergo at least two courses of non-steroidal anti-inflammatory drugs (NSAIDs) therapy. In our study, we enrolled axSpA patients both at onset and in a flare who had already been treated with NSAIDs ineffectively. Subsequently, according to the recommendations, they received modified NSAID treatment as another attempt to the first-line drug therapy and were monitored from there. We aimed to identify risk factors for treatment failure after 4 weeks (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score ≥ 4) especially amongst zonulin and haptoglobin concentrations, and haptoglobin polymorphism. Treatment failure was observed in 71% of patients, and the following variables were contributed for occurrence of this state: higher zonulin levels, ankylosing spondylitis, X-ray sacroiliitis, magnetic resonance imaging sacroiliitis, long duration of symptoms, high BASDAI, and high value of spinal pain intensity on visual analogue scale. In addition, the following positive correlations were found: haptoglobin concentration with C-reactive protein ($r=0.56$; $p=0.0004$), and erythrocyte sedimentation rate ($r=0.62$; $p<0.0001$), as well as between zonulin levels and white blood count ($r=0.5$; $p=0.0003$). The results of the study presented the identified factors related to the standard treatment failure in axSpA, amongst them zonulin levels. They might be applied to point out the patients for whom the search for a more appropriate method of treatment should be considered.

Keywords Anti-inflammatory agents · Ankylosing spondylitis · Haptoglobins · Non-steroidal · Sacroiliitis · Zonulin

Introduction

Spondyloarthritis (SpA) is a chronic rheumatic disease affecting the spine, sacroiliac and peripheral joints with numerous extra-articular manifestations, such as uveitis and subclinical gut inflammation. The pathogenesis of SpA is complex and includes features of both autoimmune and auto-inflammatory diseases [1].

SpA can be divided into peripheral and axial forms depending on the predominant symptoms, although features of both can overlap. AxSpA is defined as a form with involvement of the spine and sacroiliac joints, where the predominant symptom is back pain. Amongst axSpA, we distinguish a non-radiographic form (nr-SpA) with no changes in the sacroiliac joints described on radiographs and a radiographic form called AS. AS is considered the more advanced form, which may be one of the later stages of

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nr-SpA [2, 3]. However, this hypothesis has not been definitively confirmed.

The ongoing spinal inflammation may lead to pathological bone remodelling and syndesmophytes formation. A key role in this process is played by prostaglandin PGE2 produced by enzymes called cyclooxygenases (COX) [4]. COX are in turn blocked by NSAIDs. This mechanism of action is probably responsible for the inhibition of radiographic spinal progression in axSpA [5, 6].

According to ASAS-EULAR recommendations for the management of axSpA, the first-line drugs are NSAIDs. After failure of ≥ 2 NSAIDs over 4 weeks at the maximum or maximum tolerated dose, biological treatment should be considered [7].

Despite the proven efficacy of NSAIDs in axSpA, there are not many studies reporting how many patients, after the recommended duration of therapy, still have high disease activity and remain candidates for biological therapy. In view of the existing "window of opportunity", which ensures good and long-lasting treatment effects in the case of early implementation, it should be indicated for which patients first-line NSAIDs therapy is no longer sufficient [8]. The current analysis identified such risk factors to support clinicians in making therapeutic decisions for patients with axSpA. We focussed particular attention on haptoglobin (Hp), its polymorphisms and zonulin as potential contributors for reasons described below.

Hp is an immune response modulating molecule with antioxidant properties. It occurs in three forms: Hp 1–1, Hp 2–1 and Hp 2–2. Each phenotype has a various structure and mass, which determines its different physiochemical properties [9]. The main role of Hp in the body is to bind free haemoglobin which prevents kidney damage. The Hp 2–2 phenotype is considered the most immunogenic in comparison to the others and is associated with a higher predisposition to autoimmune diseases and worse outcomes. This may result from its weaker anti-inflammatory activity [10, 11]. A particularly interesting property of Hp is, similarly to NSAIDs, the blocking of COX. The inhibitory effect on prostaglandin synthesis is phenotypically dependent. As Hp polymorphism signifies the presence of a structurally and functionally different Hp, we decided to test whether this has a bearing on clinical status in axSpA and on the response to NSAID treatment.

The presence of the Hp2 gene is also associated with the production of zonulin, a protein that affects increased intestinal permeability [12]. Zonulin is a group of structurally and functionally related proteins—the zonulin family peptides (ZFPs), where the first identified protein of this group was the pre-Hp2 molecule [13].

A disturbed intestinal barrier and increased expression of zonulin has been shown in patients with AS [14]. The intestinal bacterial flora and the gut inflammation reveal an important role in the pathogenesis of SpA [15]. This is supported by the results of studies that demonstrated an association between subclinical gut inflammation with high disease activity and earlier age of onset. Gut inflammation was also related to disease progression [16, 17].

The potential role of Hp and zonulin in SpA was expounded by Chmielinska et al. [18]. In a presented model of SpA pathogenesis, the authors describe how zonulin-induced increased intestinal permeability leads to the activation of immune pathways crucial to the disease. Hp, as an immunomodulatory molecule, controls the potency of these responses by regulating the reactions of immune cells to lipopolysaccharides, which may be crucial for suppressing inflammation very early.

The purpose of our study was to investigate whether the factors that are associated with inflammation in the gut (zonulin) and are involved in the inflammatory response (Hp) may be related to the poor response to NSAIDs therapy in axSpA. Given the differences in structure, anti-inflammatory potency and other properties of Hp molecules reported in the literature, depending on phenotype, we decided to assay and study Hp polymorphism in this regard. Since there are no data on the predictors of treatment failure to NSAIDs, we expanded our analysis to other factors.

Materials and methods

Study populations

50 of consecutive sampling adult patients hospitalised between November 2020 and October 2022 at the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw were enrolled in this prospective observational cohort study.

The exact scheme of the study has been illustrated in the flowchart (Fig. 1).

There was one criterion of inclusion:inflammatory back pain [19] being the cause of hospital admission.

Exclusion criteria included:

- Not meeting the classification criteria for axSpA according to ASAS 2010 [20]
- Biological treatment ever
- Not taking NSAIDs before hospitalisation
- No modification of NSAIDs treatment during hospitalisation
- Contraindications to NSAIDs
- Diseases and conditions affecting haptoglobin levels: (1) active infection, (2) haemolytic anaemia, (3) active malignancy, (4) concomitant other inflammatory connective tissue disease, (5) pregnancy [21]

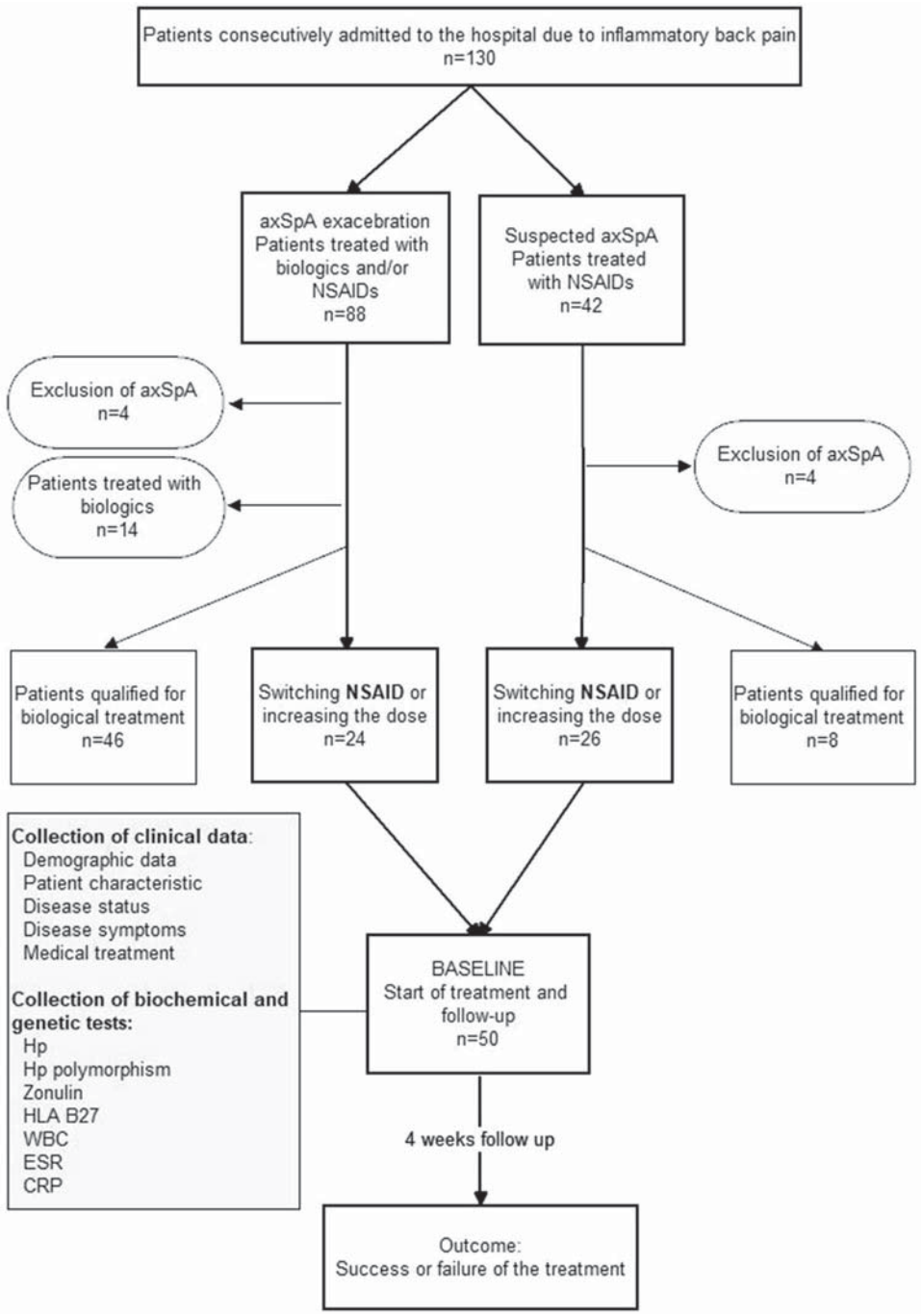

Fig. 1 Study design. *axSpA* axial spondyloarthritis, *BASDAI* Bath Ankylosing Spondylitis Diseas Activity Index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *Hp* haptoglobin, *NSAID* non-steroidal anti-inflammatory drug, *WBC* white blood count

– Reactive arthritis due to the often self-limiting course of the disease

Amongst participants were patients without a previous diagnosis of axSpA ($n=26$) and in exacerbation ($n=24$). Before inclusion in the study, the diagnosis of axSpA has been re-evaluated.

Clinical data, biochemical and genetic tests of patients were collected at baseline (Table 1). Each patient was examined by a rheumatologist, who assessed the number of swollen joints, tendons and the presence of extra-articular manifestations. Arthritis and tendinitis were confirmed by ultrasound.

Gastrointestinal symptoms were considered to be present if they were chronic and included symptoms, such as bloating, abdominal pain, diarrhoea and constipation. Frequent infections meant recurrent infections, without the criterion of antibiotics.

Table 1 Baseline characteristics for all patients ($n = 50$)

	Data missing (n)	n (%) or median (IQR)
Female, n (%)	0	23 (46.0)
Age, median (IQR), years	0	37.2 (25.9–45.1)
axPsA, n (%)	0	6 (12.0)
AS, n (%)	0	24 (48.0)
nr-axSpA, n (%)	0	20 (40.0)
HLA B27 positivity, n (%)	1	38 (77.6)
Hp 1–1 phenotype, n (%)	6	15 (34.1)
Hp 2–1 phenotype, n (%)	6	18 (40.9)
Hp 2–2 phenotype, n (%)	6	11 (25.0)
Symptom duration, median (IQR), years	0	6.5 (2.3–11.0)
Years since diagnosis, median (IQR), years	0	0.0 (0.0–2.3)
Family history of SpA, n (%)	0	6 (12.0)
History of frequent infections, n (%)	0	8 (16.0)
Active or past smokers, n (%)	0	10 (20.0)
IBD, n (%)	0	8 (16.0)
Gastrointestinal symptoms, n (%)	0	20 (40.0)
Concomitant diseases, n (%)	1	40 (81.6)
BASDAI, median (IQR)	0	5.7 (4.1–7.3)
VAS, median (IQR), mm	1	63.0 (39.0–76.0)
MRI sacroiliitis, n (%)	16	26 (76.5)
Syndesmophytes, n (%)	1	2 (4.1)
No changes, n (%)	0	11 (22.0)
X-ray sacroiliitis of ≥ 1 joint in grade ≥ 1, n (%) (0–1)	0	3 (6.0)
≥ 2, n (%) (1–2)	0	19 (38.0)
≥ 3, n (%) (2–3)	0	14 (28.0)
≥ 4, n (%) (3–4)	0	3 (6.0)
Active arthritis, n (%)	0	11 (22.0)
Active tenditis, n (%)	0	2 (4.0)
Uveitis, n (%)	0	0 (0.0)
Uveitis ever, n (%)	0	10 (20.0)
Buttock pain, n (%)	0	19 (38.0)
ESR, median (IQR), mm/h	0	7.5 (5.0–20.0)
CRP, median (IQR), mg/l	0	5.0 (4.0–13.0)
WBC, median (IQR), 10^9/L	0	7.2 (5.4–9.0)
Haptoglobin, median (IQR), mg/dl	15	391.1 (238.3–736.6)
Zonulin, median (IQR), ng/ml	1	35.6 (28.9–50.9)
NSAIDs, n (%)	0	50 (100.0)
DMARDs, n (%)	0	24 (48.0)
GCS, n (%)	0	6 (12.0)

AS ankylosing spondylitis, *axPsA* axial psoriatic arthritis, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *CRP* C-reactive protein, *DMARDs* classic disease-modifying anti-rheumatic drugs, *ESR* erythrocyte sedimentation rate, *GCS* gluco-corticosteroids, *Hp* haptoglobin, *IBD* inflammatory bowel disease, *IQR* inter-quartile range, *MRI* magnetic resonance imaging, *nr-axSpA* non-radiographic axial spondyloarthritis, *NSAIDs* non-steroidal anti-inflammatory drugs, *SpA* spondyloarthritis general, *WBC* white blood count, *VAS* value of spinal pain intensity on visual analogue scale

Presence of magnetic resonance imaging (MRI) sacroiliitis was defined according to the definition of a positive MRI for axSpA classification [22].

Sacroiliitis grading on X-ray was used according to the modified New York criteria [23].

The study was approved by the Bioethics Committee at the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw (Date: 23 October 2020; No KBT-5/1/2020). All patients signed an informed consent to participate in the study.

Study size

To determine the sample size, we used assumptions on the presence of X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 and assumptions about the percentages of failures in each group: X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 (+) and (–).

It was deduced, based on clinical experience, that the percentages of X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 in group (+) and group (–) amongst patients admitted to the hospital for suspected or exacerbated axSpA would be 2:1, respectively.

The risk of treatment failure as measured by the odds ratio will be 5.0 times higher in the group (+) compared to group (–).

Assuming that the tests examining the association of this factor (X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2) with the risk of treatment failure will be a two-tailed test, the alpha error will not exceed 0.05, and the power of the test will be at least 80%, then the number of patients to show such a risk as significant will be 60 patients.

Assessment of disease activity and response to treatment

Each patient had their disease activity assessed using the BASDAI index [24] at inclusion in the study and four weeks after dose escalation or starting a new NSAID on a specially designed website. An outcome was defined as treatment failure (BASDAI score ≥ 4) or success (BASDAI < 4) after four weeks. In case of doubt about the accuracy of the completed BASDAI questionnaire and apparent high disease activity after four weeks, a phone rheumatological consultation was performed.

In addition, the following parameters associated with disease activity were assessed at baseline: value of spinal pain intensity assessed by patient on the visual analogue scale (VAS) (0–100 mm), C-reactive protein (CRP), erythrocyte sedimentation (ESR), Hp, zonulin, WBC, degree of sacroiliac joint involvement on X-ray (X-ray sacroiliitis), MRI sacroiliitis, number of swollen and tender joints and tendons, uveitis involvement, gluteal pain.

Blood samples and method for the determination of haptoglobin, zonulin and haptoglobin polymorphism

Zonulin

For quantitative determination of zonulin serum levels, samples from 50 patients were separated from peripheral venous blood at room temperature and stored at – 86 °C until analysis. The levels of circulating zonulin in serum were determined using commercially available ELISA kits (Immunodiagnostik AG, Bensheim, Germany), according to the manufacturer's instructions. The minimum level of detection for zonulin was 0.183 ng/ml. The developed colour reaction was measured at OD450 units on an ELISA reader (El × 800, BIO-TEK Instruments).

Haptoglobin

Serum samples from 50 patients were separated from peripheral venous blood at room temperature and frozen at – 86 °C until analysis. HPT levels (ng/ml) in serum were measured with enzyme-linked immunosorbent assay kits (ELISA: Aviva Systems Biology, San Diego, CA, USA)) according to the manufacturer's instructions. The limit of detection of human HPT was determined to be 0.8196 ng/ml. Each sample was assayed in duplicate and the intra-assay coefficient of variation was 4.571%. Plates were read at an absorbance of 450 nm on LT-4000MS reader (Labtech International Ltd, Great Britain). Concentration was determined following a linear standard curve fit as per instruction's recommendation.

Haptoglobin polymorphism

Whole blood samples from 50 patients were collected in EDTA tubes. Genomic DNA was obtained using a Blood DNA Mini kit (A&A Biotechnology, Poland). Hp-1–Hp2 polymorphism genotyping was done by allele-specific PCR. For the Hp-1- and Hp2-specific sequences amplification, we used the primers A (5′-GAGGGGAGCTTG CCTTTCCATTG-3′) and B (5′-GAGATTTTTGAGCCC TGGCTGGT-3′′), whereas for the Hp2-specific sequence amplify, we used the primers C (5′-CCTGCCTCGTATTAA CTGCACCAT-3′) and D (5′-CCGAGTGCTCCACATAGC CATGT-3′). 50 ng of DNA was amplified in 10 µl of the reaction mixture (Taq PCR Master Mix, EURx, Gdanska, Poland, and primers form Genomed, Warsaw, Poland). The temperature profile for primer AB reaction was at 95 °C for 1 min, followed by 35 cycles of 95 °C for 1 min, 66 °C for 1 min, 72 °C for 3 min and extension at 72 °C for 7 min. PCR products underwent electrophoresis in a 1.8% agarose

gel containing ethidium bromide, and the Hp genotypes were determined by observing the DNA fragments under UV light.

Statistics

Baseline characteristics were presented by means of medians (IQR) and percentages, and they were used for quantitative and qualitative variables, respectively. Relationships between qualitative variables are tested using Fisher's exact test, whilst relations between qualitative and quantitative variables are tested using the Mann–Whitney test. The relationships between quantitative variables are tested using Spearman correlation analysis. The P values are used to assess significance, and the Spearman correlation coefficient is used to estimate the strength of the relationship. In a crucial part of the analysis, to identify significant factors contributing to poor response, logistic regression was used. Odds ratios (OR) and the probability of treatment failure were applied to estimate effect sizes for both quantitative and qualitative variables. For the former ones, the curve reflecting the relation between the baseline and the probability of poor response was estimated and presented as a graph where the numbers on the vertical axis refer to the probability of treatment failure. Also for the qualitative variable, the probability of treatment failure is presented in the table for each level of the variable examined. For each qualitative factor tested, the minus signs always refer to the reference levels (control groups, OR = 1.00) of the factor being tested as a potential predictor of treatment failure, whilst for quantitative variables, the odds ratio is estimated per unit of the variable tested. Collection of data and all analyses were performed with the aid of the SAS System (SAS/STAT® User's Guide. Cary, NC. 2023).

Results

Patient characteristics

Table 1 shows data on demographic and clinical features of patients with laboratory analyses at the index day including Hp, Hp phenotypes and zonulin determinations.

All patients enrolled to the study had predominant axial symptoms and met the classification criteria for axSpA according to ASAS 2010. The types of axSpA in our study presented as follows: ankylosing spondylitis (AS) 48%, non-radiographic axial spondyloarthr (nr-axSpA) 40% and axial psoriatic arthritis (axPsA) 12%. All patients were already using NSAIDs at the time of hospitalisation. Nevertheless, almost half of them (48%) received classic disease-modifying anti-rheumatic (DMARDs) and 12% glucocorticosteroids (GCS) because of a history of concomitant arthritis or uveitis. At inclusion to the study, no patient had active uveitis, 22% had active arthritis and only 4% active tendinitis. All patients reported inflammatory back pain. The median BASDAI was 5.7 (4.1–7.3), the median duration of symptoms 6.5 (2.3–11.0) years. The median Hp and the zonulin levels were 391.1 (238.3–736.6) mg/dl and 35.6 (28.9–50.9) ng/ml, respectively. The percentage distribution of individual Hp phenotypes was as follows: Hp 1–1: 34%, Hp 2–1: 41%, Hp 2–2: 25%.

Predictors of poor response to NSAID treatment

We obtained data from 48 previously eligible patients who completed the BASDAI questionnaire on the website after four weeks of treatment, and the following analysis was conducted on this group.

Table 2 illustrates the factors associated with poor response to standard treatment 4 weeks after changing NSAIDs or escalation to the maximum dose.

70.8% of patients failed to respond to continuation of standard treatment. The risk of poor response was significantly higher in patients who were switched from one NSAID to another than in the group where dose was escalated to maximum (OR = 14.5, 95% CI 3.24–64.9, $p = 0.0001$).

The rates of poor responses were: 91% in patients with AS (OR = 10.0, 95% CI 1.83–54.60, $p = 0.008$), 85% in the presence of X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 (OR = 10.44, 95% CI 2.45−44.40, $p = 0.002$), 79% in patients with active MRI sacroiliitis (OR = 6.33, 95% CI 1.11–36.00, $p = 0.037$).

More than 75% of patients who failed to respond to treatment: had a duration of symptoms of more than 5 years (per 5 years OR = 2.16, 95% CI 1.19–5.13, $p = 0.036$), BASDAI > 4.5 (OR = 2.20, 95% CI 1.36–3.58, $p = 0.001$), VAS > 45 mm (per 10 mm OR = 1.51, 95% CI 1.14–2.02, $p = 0.005$), zonulin level above 40 ng/ml (per 10 ng/ml OR = 1.62, 95% CI 1.00–2.62, $p = 0.049$) (Fig. 2 panels A, B, C, D respectively).

Approximately 15% of patients (7/48) discontinued NSAIDs (5/7) or reduced the dose of the drug (2/7) during follow-up. The reason for this was minor pain according to the patient, which could be tolerated.

Associations of Hp, its polymorphism and zonulin with indices of disease activity in SpA

The relationship between Hp, Hp polymorphism, zonulin and indicators of disease activity in axSpA was calculated (Table 3). For comparison, we performed analyses between the same disease activity measures and the common laboratory parameters (CRP, ESR and WBC), which are listed in Table 4.

Table 2 Prediction of failure of NSAIDs therapy

Factor	% of failure	OR	95% CI	*p*
Onset	69.2	1.00		
Flare	72.7	1.19	0.34–4.16	0.79
Female	68.2	0.79	0.23–2.75	0.71
Male	73.1	1.00		
Age (per 10 years)	[a]	1.55	0.92–2.75	0.11
Symptom duration (per 5 years)	Figure 2	2.16	1.19–5.13	0.036
Symptom duration (per 10 years)		4.67	1.42–26.4	0.036
Family history of SpA				
+	66.7	0.80	0.13–4.96	0.81
−	71.4	1.00		
History of frequent infections				
+	62.5	0.63	0.13–3.10	0.57
−	72.5	1.00		
Current or past smoker				
+	80.0	1.85	0.34–10.04	0.49
−	68.4	1.00		
Concomitant diseases				
+	74.4	1.74	0.35–8.63	0.50
−	62.5	1.00		
Treatment with DMARDs or GCS				
+	66.7	0.67	0.19–2.34	0.53
−	75.0	1.00		
NSAIDs change	87.9	14.5	3.24–64.9	0.0001
NSAIDs dose escalation	33.3	1.00		
SpA type				
nr-axSpA	50.0	1.0		
AS	90.9	10.0	1.83–54.60	0.008
axPsA	66.7	2.0	0.30–13.51	0.48
Arthritis				
+	70.0	0.95	0.21–4.36	0.95
−	71.1	1.00		
History of Uveitis				
+	70.0	0.95	0.21–4.36	0.95
−	71.0	1.00		
Buttock pain				
+	68.4	0.83	0.23–2.92	0.77
−	72.4	1.00		
IBD				
+	71.4	1.03	0.18–6.09	0.97
−	70.7	1.00		
Gastrointestinal symptoms				
+	68.4	0.83	0.23–2.92	0.77
−	72.4	1.00		
X-ray sacroiliitis				
+	81.1	1.00		
−	36.4	0.13	0.03–0.59	0.008
X-ray sacroiliitis of ≥ 1 SI joint in grade ≥ 2				
+	85.3	10.44	2.45–44.40	0.002
−	35.7	1.00		
HLAB27				

Table 2 (continued)

Factor	% of failure	OR	95% CI	*p*
+	71.1	1.23	0.26–5.80	0.80
−	66.7	1.00		
MRI sacroiliitis				
+	79.2	6.33	1.11–36.00	0.037
−	37.5	1.00		
WBC (10^9/L)	[a]	1.29	0.94–1.78	0.11
ESR (mm/h)	[a]	1.02	0.98–1.07	0.29
CRP (mg/l)	[a]	1.03	0.98–1.09	0.30
BASDAI (1 score)	Figure 2	2.20	1.36–3.58	0.001
VAS (mm)	Figure 2	1.04	1.01–1.07	0.005
VAS (per 10 mm)		1.51	1.14–2.02	0.005
Haptoglobin (mg/dl)	[a]	1.00	1.00–1.00	0.21
Haptoglobin (per 200 mg/dl)		1.36	0.84–2.21	0.21
Zonulin (ng/ml)	Figure 2	1.05	1.00–1.11	0.049
Zonulin (per 10 ng/ml)		1.62	1.00–2.62	0.049
Haptoglobin phenotype				
Hp 1–1	73.3	1.00		
Hp 2–1	64.7	0.67	0.15–3.04	0.60
Hp 2–2	72.7	0.97	0.17–5.59	0.97

AS ankylosing spondylitis, *axPsA* axial psoriatic arthritis *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *CRP* C-reactive protein, *DMARDs* classic disease-modifying antirheumatic drugs, *ESR* erythrocyte sedimentation rate, *GCS* glucocorticosteroids, *Hp* haptoglobin, *IBD* inflammatory bowel disease, *IQR* inter-quartile range, *nr-axSpA* non-radiographic axial spondyloarthritis, *MRI* magnetic resonance imaging, *NSAIDs* non-steroidal anti-inflammatory drugs, *SpA* spondyloarthritis general, *WBC* white blood count, *VAS* value of spinal pain intensity on visual analogue scale

[a]Quantitative variables not statistically significant are not shown in the graphs

Hp correlated well with inflammatory markers: CRP ($r = 0.56$, $p = 0.0004$), ESR ($r = 0.62$, $p \leq 0.0001$), on the borderline of significance with WBC ($r = 0.33$, $p = 0.056$) and zonulin ($r = 0.32$, $p = 0.063$). The median Hp concentrations for the different Hp phenotypes in mg/dl were as follows: Hp 2–1: 400.4 (238.3–754.3); Hp 1–1: 397.6 (325.4–621.3); Hp 2–2: 171.6 (104.3–297.8), $p = 0.048$.

Interestingly, zonulin correlated strongly with WBC ($r = 0.50$, $p = 0.0003$), stronger than CRP ($r = 0.34$, $p = 0.015$). WBC was on the borderline of significance correlated with MRI sacroiliitis ($p = 0.070$). CRP was the only factor correlated significantly with arthritis ($p = 0.014$). On the borderline of statistical significance was the correlation of zonulin with CRP ($r = 0.26$, $p = 0.076$). The median zonulin concentrations in the different Hp phenotypes in ng/ml were as follows: Hp 1–1: 46.4 (28.9–57.2); Hp 2–1: 42.4 (29.0–50.7); Hp 2–2: 32.6 (26.4–53.3). Surprisingly, the highest zonulin levels were recorded in patients with the Hp 1–1 phenotype, although the differences were not statistically significant, $p < 0.65$.

Hp, Hp polymorphism, zonulin nor any inflammatory indicator was correlated with BASDAI or MRI sacroiliitis.

Discussion

As far as we know, this is the first study focussed on investigating predictors of poor response to conventional therapy in axSpA and examining the impact of zonulin, haptoglobin and its polymorphism in this issue. It is also the first time that the association of zonulin with disease activity parameters in SpA has been evaluated.

An interesting finding from our study is that approximately 71% of patients had BASDA ≥ 4 after 4 weeks of NSAID treatment—which means that for the vast majority of patients, this chosen treatment strategy was unsuccessful. This is certainly surprising in the context of the widely recognised efficacy of NSAIDs [6, 25–27], but not so much unusual if we relate the results to other studies showing that remission with NSAID treatment is not common [28, 29].

In cross-sectional study of 246 patients with AS, high disease activity defined as BASDAI ≥ 4 was diagnosed in 64% of patients who had previously used NSAIDs [30]. The authors noted the magnitude of poor disease activity control amongst outpatients with AS. As the data were collected from email questionnaires, it is reasonable to assume

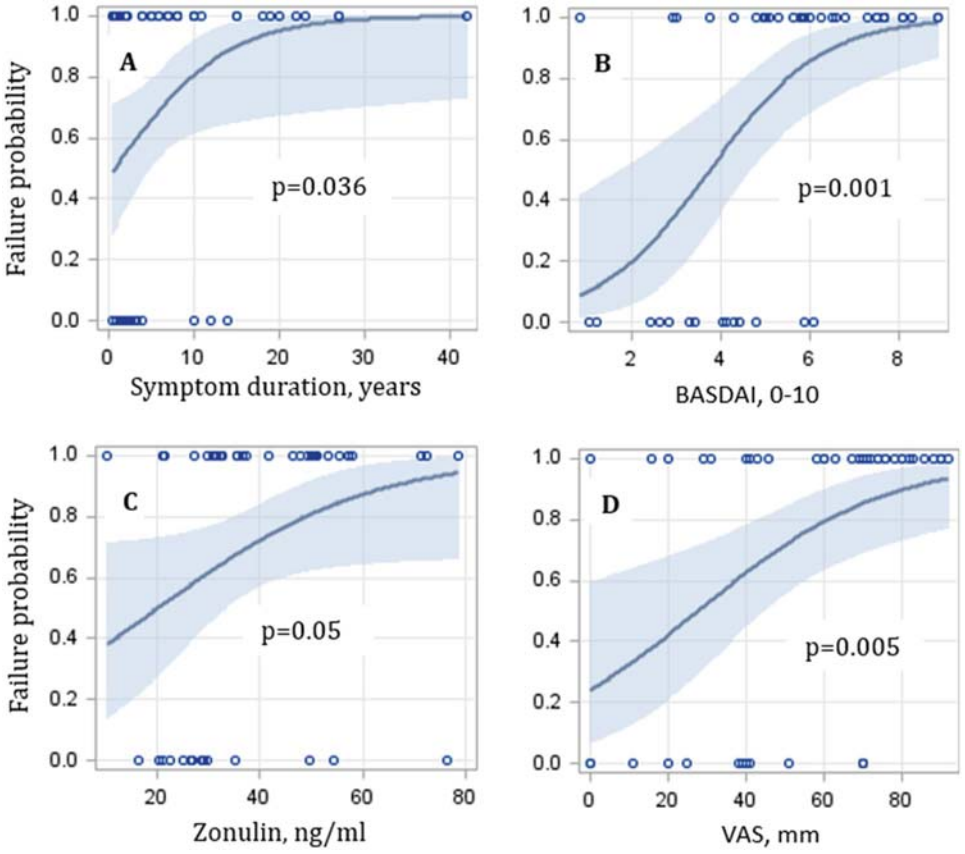

Fig. 2 Prediction of failure of NSAIDs therapy for quantitative variables

Table 3 Association between Hp, Hp polymorphism, zonulin and disease activity markers

	Hp		Hp phenotype	Zonulin
Hp 1–1	397.6 (325.4–621.3)[a]	$p=0.048$	–	$p=0.65$
Hp 2–1	400.4 (238.3–754.3)[a]			
Hp 2–2	171.6 (104.3–297.8)[a]			
Zonulin	$r=0.32$ $p=0.063$		$p=0.65$	–
CRP	$r=0.56$ $p=0.0004$		$p=0.91$	$r=0.26$ $p=0.076$
ESR	$r=0.62$ $p<0.0001$		$p=0.27$	$r=0.09$ $p=0.52$
WBC	$r=0.33$ $p=0.056$		$p=0.98$	$r=0.50$ $p=0.0003$
BASDAI	$r=0.24$ $p=0.17$		$p=0.48$	$r=0.17$ $p=0.23$
VAS	$r=0.16$ $p=0.35$		$p=0.75$	$r=0.13$ $p=0.36$
MRI sacroiliitis	$p<1.0$		$p<1.0$	$p=0.15$
Arthritis	$p=0.29$		$p=0.51$	$p=0.82$

BASDAI Bath Ankylosing Spondylitis Disease Activity Index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *Hp* haptoglobin, *MRI* magnetic resonance imaging, *VAS* value of spinal pain intensity on visual analogue scale, *WBC* white blood count

[a]Hp level, median (IQR), mg/dl

Table 4 Association between indices of disease activity

	BASDAI	MRI sacroiliitis	Arthritis
WBC	$r=0.02$ $p=0.91$	(+) 7.6 (5.6–8.7)[a] (−) 5.5 (5.0–5.9)[a] $p<0.080$	(+) 9.3 (6.0–10.7)[b] (−) 6.3 (5.4–8.4)[b] $p=0.070$
CRP	$r=0.21$ $p=0.14$	$p<0.45$	(+) 20 (6–42)[b] (−) 5 (2–11)[b] $p=0.014$
ESR	$r=0.25$ $p=0.082$	$p<0.56$	$p=0.16$

BASDAI Bath Ankylosing Spondylitis Disease Activity Index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *MRI* magnetic resonance imaging, *WBC* white blood count

[a]WBC level, median (IQR), 10^9/L in MRI positive (+) and negative (−) groups

[b]WBC level, median (IQR), 10^9/L and CRP level, median (IQR), mg/l in arthritis positive (+) and negative (−) groups

that patients did not visit doctors because of an exacerbation of their symptoms. Similarly, in our study, patients who reported high disease activity on the online questionnaire 4 weeks after the change in treatment were informed by phone that they should have consulted rheumatology again. What surprised us, apart from the proportion of the poor treatment response, was that the majority of patients with complaints did not intend to see a doctor. The most common reason was unawareness of the possibility of other treatment methods and that pain can and should be managed. This may indicate an underestimation of the problem of high activity and low efficacy of long-term NSAID use amongst patients with axSpA. It also shows the urgency of improving communication between a patient and a doctor.

Barakliakos et al. showed that therapy with full-dose NSAIDs in patients who have not previously used maximum doses was effective in most cases after four weeks of treatment [27]. However, 44% of patients sustained BASDAI ≥ 4 and remained candidates for biological treatment. ASDAS40 reached a total of 35% and only 16% achieved partial remission. The research had a different design to ours, as participants had their NSAIDs changed after one week of treatment in case of ineffectiveness. In our study, patients were taking NSAIDs continuously. Moreover, most patients had a history of NSAIDs failure and this was a strong predictor of non-response to standard treatment (OR = 14.5, 95% CI 3.24 – 64.9, $p=0.0001$).

Interestingly, the authors reported a low proportion of patients who remained on the maximum dose of the drug (13%). This is in contrast to our study where the percentage was as high as 85%. Perhaps this means that our study group had more active disease.

It must therefore be suspected that the poorer efficacy of NSAIDs in our study was mainly due to the high proportion of subjects in whom this therapy was previously ineffective. This is similar to the authors' description of higher frequency of non-response after 4 weeks in the group of patients who had their NSAIDs changed after just one week due to treatment failure. The authors did not find amongst the following factors: duration of symptoms, MRI sacroiliitis, gender, CRP positive predictors of poor response to NSAIDs after 4 weeks of treatment (data was not shown in the article).

In contrast, we have identified patients with axSpA who are unlikely to benefit from continued NSAIDs treatment. These are patients with: long duration of symptoms, AS form of axSpA, high disease activity as measured by BASDAI (especially above 4.5) and VAS (especially above 45 mm), active MRI sacroiliitis, radiological changes in SI joints (X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 is sufficient) and high zonulin levels (especially if levels exceed 40 ng/ml).

The discovery of zonulin as a predictor of poor response is consistent with our assumption that increased intestinal permeability sustains inflammation and interferes with achieving remission despite anti-inflammatory treatment [18]. Zonulin was not associated with Hp polymorphism, but was correlated with Hp at the limit of significance. This shows, as in other studies and was finally confirmed by the discoverer of zonulin, that zonulin is in fact a group of proteins associated with increased intestinal permeability and it is not just a precursor of Hp2 [13, 31]. Its correlation with WBC may indicate a link to inflammation at the cellular level. As zonulin has been known to interact with immune cells in a direct manner [14], how zonulin shapes the immune response and how it interacts with immune cells is an interesting question that requires further research.

The results of our study are all the more interesting as a recently published paper demonstrated the efficacy of a zonulin antagonist in increasing intestinal tightness and preventing the development of rheumatoid arthritis [32]. The authors suggested the intestinal barrier is where autoimmunity can progress to arthritis. This hypothesis is commonly proposed also by other authors [33, 34]. Gracey et al. concluded that the high rate of treatment failure in SpA may have its origin in persistent gut inflammation [15].

The association of Hp with disease activity measures in AS has been reported by some studies [35–38]. However, our research on axSpA patients failed to show a correlation of Hp with either BASDAI or MRI sacroiliitis. In contrast, Hp was strongly associated with CRP and ESR. The explanation for these linkage is arguably that Hp production is stimulated by pro-inflammatory cytokines and represents an acute phase protein. On the other hand, no inflammatory parameter showed association with BASDAI or MRI sacroiliitis. CRP was the only variable which correlated with arthritis. We demonstrate that Hp does not appear to be a better biomarker of disease activity than CRP, although it

would be appropriate to investigate the relationship of Hp also with the Ankylosing Spondylitis Disease Activity Score (ASDAS). Correlations of Hp with ASDAS were observed in a study of AS patients treated with golimumab [37].

Individual Hp phenotypes were reflected in significant differences in Hp concentrations, but were not associated with any activity disease parameters. This is opposite to that reported by the authors Beaten et al. where the Hp-1–1 phenotype was associated with higher inflammatory CRP and ESR indices [39].

It must be considered that we also enrolled people with axPsA, whereas most studies do not include individuals with such a defined disease in axSpA. It should also be noted that the participants were patients who had been admitted to hospital for persistent complaints of SpA, whilst some of them had already been diagnosed with the disease earlier and the others were newly diagnosed during hospitalisation. However, this was not linked to the risk of a poor response.

Additionally, not all of the subjects had a baseline BASDAI ≥ 4. However, all patients required a change in treatment due to difficulties in daily functioning. This management is in line with ASAS-EULAR recommendations, which states that the main goal of treatment is to provide the best possible quality of life for patients and the ability to participate in social life completely [7].

In our study, we did not use the NSAID equivalent scoring system recommended by ASAS, but this is not a limitation of our study as the purpose was not to investigate the effectiveness of NSAIDs [40].

The strength of our study is its observational nature, which reflects real-life situation. An important advantage of our research is that it included patients who were re-evaluated for a correctly made diagnosis before inclusion in the study (concerns patients with exacerbation). The differential diagnosis of spinal pain was also carried out.

Our study has some limitations. First, the treatment failure was defined using BASDAI index instead of ASDAS, which is more appropriate disease activity assessment tool [7]. However, it requires CRP/ESR measurement, which for logistical reasons were not possible for our study after patient was discharged from the hospital.

Second, the inclusion of only the hospitalised group raises concerns about selection bias. However, the specificity of healthcare for patients with rheumatic diseases in Poland takes place mainly in a hospital setting due to the limited possibilities and availability of outpatient care. The hospital in this case often takes on the role of an outpatient clinic. Consequently, our group should not be regarded as representing a particularly severe course of axSpA.

Since we were unable to collect the required number of patients (the estimated sample size was 60), this may have been the reason why not all risk factors were found. In addition, the sample size itself was calculated based on presumptive data corresponding to only one factor: X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2. Only for this factor, based on our experience and the literature, we were able to assess the preliminary risk of treatment failure in the groups: X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 (+) and X-ray sacroiliitis of ≥ 1 joint in grade: ≥ 2 (–) (the exact procedure for calculating the sample size is placed in the Methods section). Such reasoning was not carried out for the other factors, because analogous data, even heavily speculative for them, were virtually unobtainable. Perhaps if the power of the study had been greater, other factors with current significance levels greater than 0.05 might have become significant.

However, this study is the first to provide data on the prevalence of qualitative factors or values of quantitative factors and the incidence of treatment failure in axSpA patients previously unsuccessfully treated with NSAIDs.

In addition, we found that on a fewer than the calculated number of patient, it was possible to find significant risk factors for the outcome.

Another limitation is that the study was not powered by subgroups analysis due to the small sample size. Given the heterogeneity of the group, such analyses would be advisable. For the same reason, a multivariate logistic regression was not performed.

The results of our analysis cannot be applied to the entire group of patients with axSpA who are treated with NSAIDs, because we investigated patients who were already using NSAIDs ineffectively. There is also a need for studies in the NSAID-naïve group, whereas our study group, without very narrow inclusion criteria, reflects the patients that we most commonly encounter in daily practice.

Conclusions

Consideration should be given to revising the recommendations for continuation of NSAIDs treatment in axSpA patients with identified risk factors. The presented results may enhance decision of earlier initiation of biological treatment.

Although zonulin correlated strongly with leukocytes, it proved to be a predictor for poor response and leukocytes did not, nor did CRP and ESR. Thus, zonulin is independent of inflammatory measures candidate as a useful marker of treatment failure to NSAIDs.

We report zonulin for further study as a potential promising new biomarker to assess prediction of treatment failure in SpA.

Need for further research focussed on the role of zonulin in SpA and response to therapies including biologic treatment.

Haptoglobin, despite being considered in previous reports as a reliable biomarker in SpA, did not show a correlation with BASDAI or MRI sacroiliitis in our study.

The Hp polymorphism does not seem to have any clinical relevance in axSpA.

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Data availability The data that support the findings of this study are available to investigators upon request to the corresponding author.

Declarations

Conflict of interest Magdalena Chmielińska, Marzena Olesińska, Anna Felis-Giemza, Agnieszka Paradowska-Gorycka, Karolina Palej, Julita Rejmer-Szcześniak and Dariusz Szukiewicz declare no competing interests.

Ethical approval The study was approved by the Bioethics Committee at the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw (Date: 23 October 2020; No KBT-5/1/2020). All patients who participated in the study signed an informed consent.

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The failure of biological treatment in axial spondyloarthritis is linked to the factors related to increased intestinal permeability and dysbiosis: prospective observational cohort study

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Abstract

Background A significant number of patients with axial spondyloarthritis (axSpA) do not respond to biological therapy. Therefore, we decided to investigate the specificity of this group of patients and, in particular, whether haptoglobin (Hp), its polymorphism and zonulin, in addition to other clinical features, are predictors of poor response to biological treatment.

Methods 48 patients with axSpA who were unsuccessfully treated with standard drugs were converted to biological treatment, and from this time on, a 12-week follow-up was started to assess the failure of biological treatment (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) decrease < 2 points). Predictors of treatment failure were identified using logistic regression analysis.

Results 21% of subjects had biological treatment failure. Patients who had a higher zonulin level, a history of frequent infections, were older, had inflammatory bowel disease (IBD), had a lower Hp level at the time of inclusion in biological therapy showed an increased risk of treatment failure.

Conclusions The results of the study support the hypothesis that the effectiveness of biological treatment of axSpA is limited by changed microbiota and intestinal epithelial barrier dysfunction, as an increased risk of biological treatment failure was observed in patients who were older, had higher zonulin level, IBD and repeated courses of antibiotics due to frequent infections. Therefore, starting biological treatment should be followed by reducing intestinal permeability and regulating the disturbed gut microbiome.

Keywords Biological therapy · Haptoglobins · Intestinal barrier function · Spondylitis, Ankylosing · Treatment failure · Zonulin

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Introduction

Currently, the therapeutic management for axial spondyloarthropathy follows the guidelines of Assessment of SpondyloArthritis International Society-European Alliance of Associations for Rheumatology (ASAS-EULAR), which recommends the inclusion of treatment with biological or targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs) after treatment failure with non-steroidal anti-inflammatory drugs (NSAIDs) and a positive rheumatologist's opinion on b/tsDMARD treatment [1].

According to available research data, approximately 40–65% of patients do not respond to biological treatment, depending on the defined outcome measures, the drug used and the treatment duration [2–5]. The remission rate is even lower, although it is rarely used as an endpoint in trials, probably because it is much more difficult to achieve [5]. In addition, the initial successful treatment often becomes ineffective after some time, forcing a change of therapy [6].

There is a number of studies on predictors of a good response to biological treatment [7–9]. The ASAS-EULAR recommendations list C-reactive protein (CRP) and MRI sacroiliitis as factors that increase the likelihood of response to tumour necrosis factor inhibitors (TNFi) [1]. Thus, the rheumatologists better identify the group of patients for whom this treatment modality has the highest chance of success. For patients without these predictive factors, the choice of biological treatment is more difficult but, in the absence of an NSAIDs effect, probably the only option, along with tsDMARDs.

In our study, we changed the previous way of approaching the problem of treatment choice after failed NSAID therapy. We focused on the potential factors related to the resistance to bDMARDs.

Based on existing data on the pathogenesis of axSpA, we decided to expand the search for predictors of response to biological treatment to include variables that were not previously considered as potential predictors: the variable associated with inflammation (Hp), related to increased intestinal permeability (zonulin, IBD) and disturbed intestinal flora (repeated courses of antibiotics due to frequent infections) [10–13]. We necessarily included the Hp polymorphism because the structure of the Hp molecule and its functional properties are polymorphism-dependent [14].

Especially since many data indicate that Hp 2–2 is a phenotype associated with a worse course of certain diseases, including autoimmune disorders [15–18].

Given that a high proportion of patients have normal CRP and erythrocyte sedimentation rate (ESR), we considered that another inflammatory marker might prove to be a better predictor of response to treatment.

Hp is not only an acute-phase protein, but is also involved in modulating the response of immune cells to various cytokine signals associated with inflammation and the lipopolysaccharide response [19]. On the other hand, zonulin itself is a precursor of Hp2—as the first eukaryotic member of the zonulin family peptides (ZFP) [20]. Existing enzyme-linked immunosorbent assays (ELISAs) for zonulin, however, also detect other proteins from ZFP [21]. Zonulin appears to be associated with the gut-joint axis and in our last report we demonstrated its association with poor response to NSAIDs [22].

The aim of this study was to investigate which of the selected clinical features of axSpA extended by Hp concentration, its polymorphism and zonulin concentration predict biological treatment failure.

Materials and methods

Study design

The prospective observational cohort study included patients with axSpA converted to biological treatment after failure of NSAIDs therapy according to ASAS-EULAR recommendations [1]. The consecutive sampling patients hospitalized between November 2020 and October 2022 at the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw were recruited to the study. The study design and enrollment process is presented on the flowchart (Fig. 1). The diagram is a part of the flowchart from our previous study and reflects its section on biological treatment [22]. All participants signed an informed consent. The study received positive approval from the Bioethics Committee at the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw (Date: 23 October 2020; No KBT- 5/1/2020).

Sample size

The sample size was calculated based on the following assumptions:

1. The ratio of patients with an elevated CRP (+) and a normal CRP (−) who will be admitted to the hospital for spinal inflammatory back pain in the course of axSpA will be 2:1.
2. The treatment failure rate in the CRP (+) and CRP (−) groups will be 0.3 and 0.7, respectively.

Assuming an alpha error will not exceed 0.05 and a test power will be at least 80%, the calculated number of patients was 66.

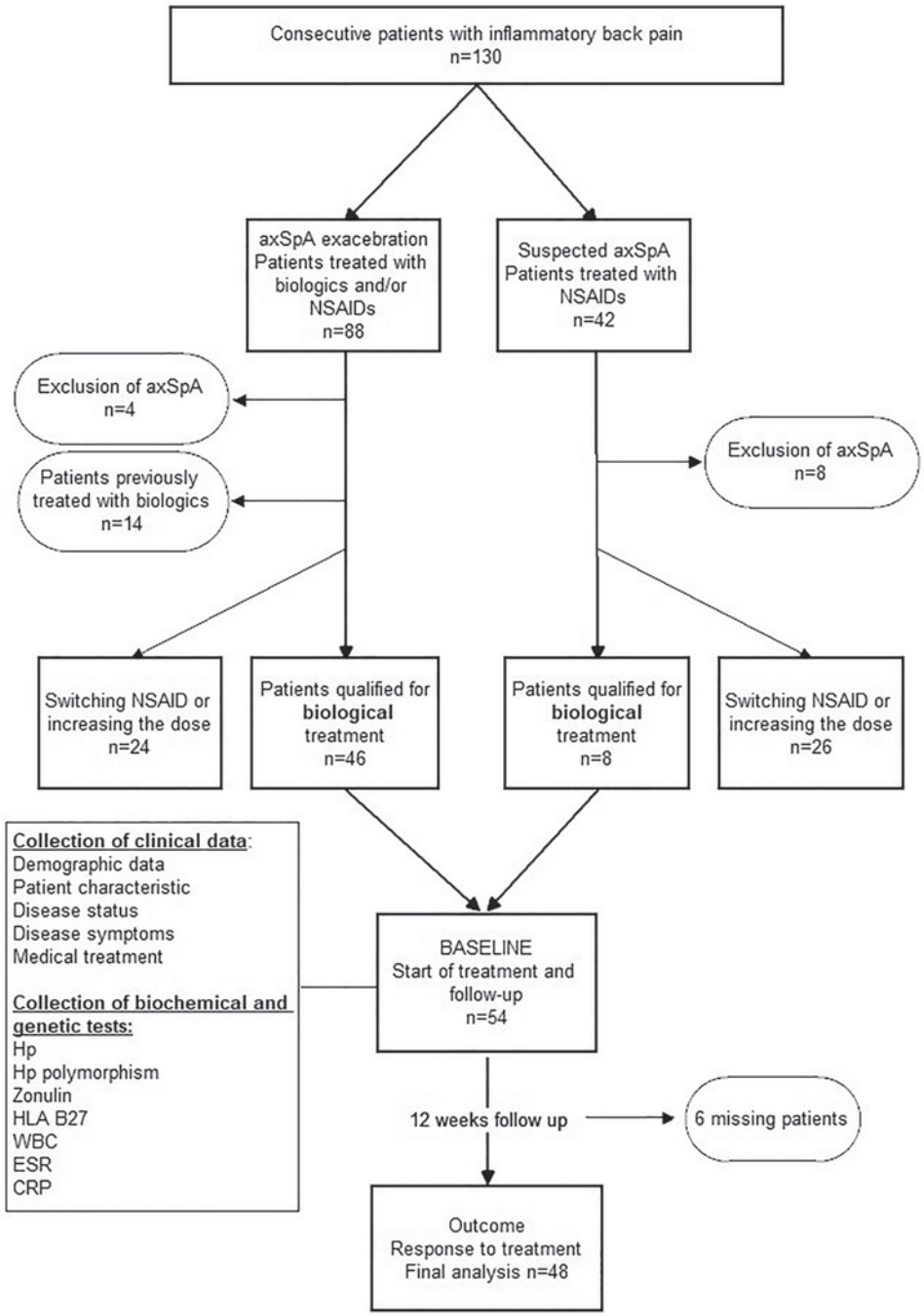

Fig. 1 Flowcharts of study design and patients enrolment process. *axPsA* axial psoriatic arthritis, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *CRP-C* reactive protein, *ESR* erythrocyte sedimentation rate, *HLA-B27* human leukocyte antigen B27, *Hp* haptoglobin, *NSAIDs* non-steroidal anti-inflammatory drugs, *WBC* white blood count

Patients

54 patients with non-radiographic axSpA (nr-axSpA), axial psoriatic arthritis (axPsA), ankylosing spondylitis (AS) who met the axSpA classification criteria according to ASAS 2010 and who were qualified for biological treatment according to the ASAS-EULAR recommendations were included in the study [1, 23]. The inclusion criterion was the presence of inflammatory spinal pain as a reason for hospitalisation [24]. Exclusion criteria included conditions affecting Hp levels: active infection, haemolytic anaemia, active malignancy, concomitant other inflammatory connective tissue disease, pregnancy [25]. Additional exclusion criteria consisted of: back pain unrelated to axSpA, qualification for therapy other than bDMARDs, previous use of bDMARDs and eligibility for biological treatment for reasons other than treatment failure with NSAIDs.

Data collection and study measures

At baseline, we collected clinical data on demographics (sex, age), patient characteristics (body mass index, SpA subtype, disease duration, family history of SpA, smoking status, history of frequent infection), disease status (disease activity measured by BASDAI, severity of back pain according to visual analogue scale (VAS) by the patient, degree of sacroiliac joint involvement on X-ray, presence of sacroiliitis on magnetic resonance imaging (MRI), presence of syndesmophytes), symptoms (arthritis, tendinitis, present and past history of uveitis, buttock pain), medical treatment (biological and conventional DMARDs, glucocorticosteroids (GCS)) and comorbidities. The selected biochemical and genetic parameters were determinated: Hp level, Hp polymorphism, zonulin level, CRP, ESR, white blood count (WBC), human leukocyte antigen B27 (HLA-B27). The data collected is detailed in Table 1. Frequent infections were defined as recurrent and requiring antibiotics.

Disease activity was assessed with BASDAI which is an instrument routinely used in clinical practice based on patient self-scoring on the severity of various ailments such as spinal and peripheral joint pain, discomfort in pressure-sensitive areas, morning stiffness and fatigue. Each question is scored on a scale of 0–10, where 10 represents the most severe complaints. BASDAI is calculated using a formula and scores $>=4$ are considered high disease activity [26]. Some patients had a sacroiliac joint MRI ordered if there was doubt about the nature of their back pain. The majority of patients, however, had this examination already performed previously but at different times and sometimes outside of the research site, therefore the results of these examinations were not included in our analysis. MRI sacroiliitis was graded as positive or negative according to the ASAS classification [27]. All patients had their sacroiliac joint structural damage assessed by X-ray. Sacroiliitis x-ray grading was according to the New York criteria [28]. Additional differential diagnosis of spinal pain was also performed.

Biological treatment

Dosing of individual bDMARDs was according to the summary of product characteristics: adalimumab 40 mg every 2 weeks; etanercept 50 mg every week; golimumab 50 mg every month; certolizumab: first 3 doses 400 mg every 2 weeks, then 200 mg every 2 weeks; ixekizumab: first dose 160 mg, then 80 mg every 4 weeks; secukinumab: first 5 doses 150 mg per week, then 150 mg per month.

In our study, IBD should be regarded as a concomitant disease not a reason for implementing biological treatment. For IBD requiring biological treatment, patients are managed by gastroenterology departments. None of our patients required biological treatment for IBD. No patient required an increase in the dose of secukinumab due to severe psoriasis.

Outcome

After 12 weeks of biological treatment, each patient had the disease activity assessed using BASDAI scale by completing a questionnaire on a website specially prepared for this study.

Biological treatment failure was defined as a decrease in BASDAI of less than 2 points according to the ASAS-EULAR criteria [1].

Serum analysis for haptoglobin, haptoglobin polymorphism and zonulin

Haptoglobin

Serum Hp concentrations (ng/ml) in patients were measured by ELISA (Aviva Systems Biology, San Diego, CA, USA)) according to the manufacturer's instructions. The detection limit of human Hp was 0.8196 ng/ml. Each sample was tested twice, and the intra-assay coefficient of variation was 4.571%. The plates were read at 450 nm absorbance on an LT-4000MS reader (Labtech International Ltd, UK). The concentration was determined after fitting a linear standard curve as recommended in the manual.

Haptoglobin polymorphism

Genomic DNA was extracted from 200 µL of whole blood samples from 48 patients while using a Blood DNA Mini kit (A&A Biotechnology, Poland) following the manufacturer's instructions. Hp-1–Hp2 polymorphism was detected by the allele-specific PCR. For the Hp-1- and Hp2-specific sequences amplification, primer A sequence was 5′-GAG GGGAGCTTGCCTTTCCATTG-3′ and primer B sequence was 5′-GAGATTTTTGAGCCCTGGCTGGT-3′′. For the Hp2-specific sequence amplify, we used the primers: C 5′-CCTGCCTCGTATTAACTGCACCAT-3′ and D 5′-CCG AGTGCTCCACATAGCCATGT-3′. Reaction mixture contained: 50 ng of genomic DNA, 10 pmol of each primers, and Taq PCR Master Mix (EURx, Gdanska, Poland). Reaction condition for primer AB was as follows: 95 °C for 1 min, 35 cycles of 95 °C for 1 min, 66 °C for 1 min, 72 °C for 3 min and a final extension at 72 °C for 7 min. PCR products were separated on a 1.8% agarose gel containing ethidium bromide, and the Hp genotypes were determined by observing the DNA fragments under UV light.

Table 1 Baseline characteristics of the patients, $n = 48$

	Data missing (*n*)	*n* (%) or median (IQR)
Patients demographics		
Female, *n* (%)	0	27 (56,3)
Age, median (IQR), years	0	36.7 (30.8–44.4)
Patient characteristic		
BMI, kg/m^2	1	24.3 (17.3–33.5)
axPsA, *n* (%)	0	14 (29.2)
AS, *n* (%)	0	27 (56.3)
nr-axSpA, *n* (%)	0	7 (14.6)
HLA B27 positivity, *n* (%)	0	38 (79.2)
Hp 1–1 phenotype, *n* (%)	3	10 (22.2)
Hp 1–2 phenotype, *n* (%)	3	20 (44.4)
Hp 2–2 phenotype, *n* (%)	3	15 (33.3)
Symptom duration, median (IQR), years	0	8 (4–15.5)
Years since diagnosis, median (IQR), years	0	1.2 (0.2–3.5)
Family history of SpA, *n* (%)	0	8 (16.7)
Active or past smokers, *n* (%)	0	6 (12.5)
History of frequent infections, *n* (%)	0	12 (25)
Disease status		
BASDAI, median (IQR)	0	6.9 (5.6–8.0)
VAS, median (IQR), mm	1	71 (60–82)
MRI sacroiliitis, *n* (%)	30	12 (66.7)
Syndesmophytes, *n* (%)	0	7 (14.6)
x-ray sacroiliitis		
No changes, *n* (%)	0	5 (10.4)
x-ray sacroiliitis of ≥ 1 joint in grade:	0	1 (2.1)
≥ 1, *n* (%) (0–1)		
≥ 2, *n* (%) (1–2)	0	22 (45.8)
≥ 3, *n* (%) (2–3)	0	15 (31.3)
≥ 4, *n* (%) (3–4)	0	4 (8.3)
Disease symptoms		
Arthritis, *n* (%)	0	15 (31.3)
Tendinitis, *n* (%)	0	10 (20.8)
Uveitis, *n* (%)	0	2 (4.2)
Uveitis ever, *n* (%)	0	12 (25)
Buttock pain, *n* (%)	1	26 (55.3)
Laboratory analyses		
ESR, median (IQR), mm/h	0	13 (7–29)
ESR, ranges, mm/h	0	2.0–87.0
CRP, median (IQR), mg/l	0	9 (5–18)
CRP, ranges, mg/l	0	1–163
WBC, median (IQR), 10 9 /L	0	7 (5.5–8.5)
WBC, ranges, 10 9 /L	0	3.4–11.1
Haptoglobin, median (IQR), mg/dl	17	381.6 (233.7–512.2)
Haptoglobin, ranges, mg/dl	17	132.5–980.8
Zonulin, median (IQR), ng/ml	0	40.5 (25.0–55.2)
Zonulin, ranges, ng/ml	0	11.8–105.4
Biological treatment		
Anty-TNF overall, *n* (%)	0	38 (79.2)
Adalimumab, *n* (%)	0	23 (47.9)
Etanercept, *n* (%)	0	3 (6.3)
Certolizumab, *n* (%)	0	9 (18.8)

Table 1 (continued)

	Data missing (*n*)	*n* (%) or median (IQR)
Golimumab, *n* (%)	0	3 (6.3)
Secukinumab, *n* (%)	0	8 (16.7)
Ixekizumab, *n* (%)	0	2 (4.2)
Other medication		
NSAIDs, *n* (%)	0	48 (100)
cDMARDS, n (%)	0	21 (43.8)
GCS, n (%)	0	5 (10.4)
Concomitant disease		
Overall, *n* (%)	0	40 (83.3)
IBD, *n* (%)	0	10 (20.8)
Gastrointestinal symptoms, *n* (%)	0	18 (37.5)

Anti-TNF therapy anti tumor necrosing factor therapy (adalimumab, certolizumab, etanercept, golimumab), *AS* ankylosing spondylitis, *axPsA* axial psoriatic arthritis, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BMI* body mass index, *bDMARDs* biological disease-modifying antirheumatic drugs, *cDMARDs* classic disease-modifying antirheumatic drugs, *CRP C*-reactive protein, *ESR* erythrocyte sedimentation rate, *GCS* glucocorticosteroids, *Hp* haptoglobin, *IBD* inflammatory bowel disease, *IQR* interquartile range, *nr-axSpA* non-radiographic axial spondyloarthritis, *MRI* magnetic resonance imaging, *NSAIDs* non-steroidal anti-inflammatory drugs, *SpA* spondyloarthritis general, *WBC* white blood count, *VAS* value of spinal pain intensity on visual analogue scale

Zonulin

Enzyme-linked immunosorbent assay kits (ELISA) for zonulin was performed with commercially available ELISA kits (Immunodiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. Samples from patients were separated from peripheral venous blood at room temperature and stored at − 86 °C until analysis. The minimum detection level of 0.183 ng/ml was used. Serum zonulin concentrations were detected at a wavelength of 450 nm using the microplate reader (El × 800, BIO-TEK Instruments).

Statistics

Descriptive data are presented using means of medians (IQR) and percentages when referring to quantitative and qualitative variables, respectively. The Spearman correlation analysis was used to assess relationships between quantitative variables, Fisher's exact test to analyse associations between qualitative variables and the Mann–Whitney test to find relations between qualitative and quantitative variables. To assess significance the P values were used, and to estimate the strength of the associations the Spearman correlation coefficient was applied. A p-value of less than 0.05 was considered significant for all tests. To identify significant factors contributing to poor response to bDMARDs, univariate logistic regression was performed. In addition, the series of two-factor analyses with baseline variables were conducted to investigate the zonulin factor as an independent predictor of treatment failure. To estimate effect sizes odds ratios (OR) and the probability of treatment failure were used. For quantitative variables, the risk of treatment failure is presented as a curve in the graph, where the vertical axis shows the probability of a poor response. For qualitative variables, the risk of treatment failure is presented in the table, where the minus sign refers to the reference group for the given factor (control group, OR = 1.00). Odds ratios for quantitative variables are calculated per unit for each variable. Statistical analysis and data collection were performed with the SAS System (SAS/STAT® User's Guide. Cary, NC. 2023).

Results

Of 48 participants sufficient data were available. Patient characteristics are listed in Table 1. The majority of patients were AS patients (56.3%) and the most used bDMARDs were iTNF (79.2%) and adalimumab (47.9%). At baseline all patients were taking NSAIDs and 43.8% ($n = 21$) of them used additionally conventional disease-modifying drugs (cDMARDs) because of concomitant peripheral arthritis, tendinitis or uveitis. The doses of taken cDMARDs were as follows: methotrexate 10 mg–25 mg per week, sulfasalazine 2 g–3 g per day, one person was taking leflunomide 20 mg per day. 10.4% ($n = 5$) of subjects were using GCS at baseline due to active arthritis at doses of 4–16 mg per day prescribed to be gradually reduced and discontinued within a few weeks. The doses of cDMARDs did not differ between responders and non-responders. The use of cDMARDs or GCS at baseline increased the risk of poor response to

biological treatment, but the result was not statistically significant (OR = 3.58, 95% CI 0.80 – 16.05, $p = 0.096$).

Few patients had an MRI of the sacroiliac joints (37.5%) and, of these, a positive was described in 66.7%. The majority of patients had an elevated CRP (71%). None of our patients had exacerbated psoriatic lesions or symptoms of IBD exacerbation.

The median Hp concentration was 381.6 (233.7–512.2) mg/dl and zonulin was 40.5 (25.0–55.2) ng/ml. The distribution of the Hp polymorphism was as follows: Hp1-1: 22%, Hp2-1: 44.4%, 2–2: 33.3%. Hp concentrations varied according to Hp phenotype and were significantly highest in subjects with the Hp1-1 phenotype (median Hp level,mg/dl: Hp1-1: 497.9 (387.8–650.9); Hp2-1: 436.7 (355.4–526.0); Hp2-2: 190.8 (162.1–305.1); $p = 0.0025$). Hp polymorphism was not associated with parameters of inflammatory activity, disease activity and zonulin level (Table 1S).

As in the previous study [21], we recorded the highest zonulin levels in individuals with the Hp1-1 phenotype, but the differences were not statistically significant $p = 0.93$ (median zonulin concentrations, ng/ml: Hp1-1: 42.6 (25.3–56.4); Hp2-1: 41.8 (28.7–55.8); Hp2-2: 41.2 (26.5–55.2)). The presence of zonulin in those with the Hp1-1 phenotype demonstrates that commercially available ELISAs detect other ZFP family proteins than just pre-Hp2. Zonulin was not correlated with Hp ($r = -0.004$, $p = 0.98$) and ESR ($r = 0.11$, $p = 0.46$) but it was significantly correlated with CRP ($r = 0.3$; $p = 0.045$). Zonulin levels were not significantly different in patients with IBD or current gastrointestinal symptoms.

Approximately 21% of subjects had treatment failure (baseline BASDAI decline < 2 scores). Predictors increasing the risk of biological treatment failure were previous history of frequent infections (OR = 4.43, 95% CI 1.00–19.58, $p = 0.049$) and higher zonulin levels (per 10 ng/ml OR = 1.39, 95% CI 1.02–2.00, $p = 0.048$), which remained significant after adjusting for the majority of potential confounders (Table 2) and (Table 3). Figure 2 shows the response to biological treatment depending on zonulin and Hp levels. Good response to bDMARDs was greater in those who had higher levels of Hp (per 200 mg/dl OR = 0.19, 95% CI 0.02–0.76, $p = 0.053$). All subjects who had treatment failure to bDMARDs had Hp levels below 400 mg/dl. Hp was not associated with either ESR ($r = 0.17$, $p = 0.36$) or CRP ($r = 0.27$, $p = 0.14$).

Furthermore, in two-factor analyses with zonulin, older age (OR = 1.09, 95% CI 1.00 – 1.18, $p = 0.047$) and IBD (OR = 7.56, 95% CI 1.06 – 54.06, $p = 0.044$) have also proved to be predictors of poor response to biological treatment independently of zonulin (Table 3).

We did not report any disease activity markers (WBC, ESR, CRP, arthritis, tenditis, BASDAI, VAS) to be significantly associated with our endpoint. Although it all reduced the risk of poor response. Furthermore, all patients with active MRI sacroiliitis responded well to bDMARDs and therefore logistic regression calculations with this factor could not be performed.

Data on the incidence of treatment failure according to the different factors are included in supplement Table 2S.

Discussion

Failure to biological treatment is an important issue and challenge for today's rheumatology. The current literature focuses on identifying predictors of good response to bDMARDs, while there is a great need to find the causes of the resistance. In addition, only known and routinely assessed markers of disease activity are usually considered for research [29–31].

In our study, we went deeper into the pathogenesis of axSpA and decided to include factors that have not been considered so far in the context of biological treatment failure. The idea to explore factors related to the microbiome and increased intestinal permeability came from the constantly arising number of reports on the importance of the gut-joints axis [32–35]. Disruption of the intestinal barrier function has been shown to predict onset of arthritis and zonulin was the main agent associated with this process [34, 36].

In addition, zonulin was shown to be up-regulated in ileal samples of patients with AS. The authors demonstrated that zonulin is able to stimulate the expansion of macrophages with the M2 phenotype, which are involved in SpA gut inflammation and synovitis [13].

Interestingly, as in our previous study, higher levels of zonulin were associated with poor response to treatment in axSpA [22]. It appears that persistently increased intestinal permeability 'interferes' with therapy. This may be due to the continuous stimulation of the immune system by intestinal antigens, probably related to dysbiosis. It is possible that the condition of intestinal damage and disturbance of the microbiome itself is linked to NSAID use [37].

This is a very exciting result, especially in the context of the study showing that the use of the zonulin antagonist larazotide acetate can inhibit arthritis [36].

The lack of association of zonulin with gastrointestinal symptoms and IBD shows difficulty in selecting individuals with increased intestinal permeability based on the medical history and clinical symptoms. Similar results have already been reported in other studies [38, 39]. It is likely that the relationship of IBD and gastrointestinal with biological treatment failure did not depend only on a damaged intestinal barrier. In our study, these two factors were zonulin-independent predictors (OR = 7.56, 95% CI 1.06 – 54.06, $p = 0.044$; OR = 4.63, 95% CI 0.91 – 23.48 $p = 0.064$, respectively). It seems that healing the gastrointestinal tract,

Table 2 Baseline predictors of treatment failure to bDMARDs

Variable	Univariate analyses, OR (95% CI)
Gender (female vs male)	0.73 (0.18 – 2.94)
Age (per 10 years)	1.88 (0.90 – 3.96)
BMI	1.14 (0.96 – 1.35)
Symptom duration (per 5 years)	1.44 (0.94–2.22)
Family history of SpA (ref negative)	1.33 (0.23 – 7.89)
History of frequent infections (ref negative)	4.43 (1 – 19.58)
Concomitant diseases (ref negative)	2.03 (0.22 – 18.77)
Treatment with cDMARDs or GCS (ref negative)	3.58 (0.80 – 16.05)
Biological treatment (anti-TNF therapy vs other bDMARDs)	0.54 (0.11 – 2.65)
Gastrointestinal symptoms (ref negative)	3.25 (0.77 – 13.69)
AS	0.73 (0.18 – 2.94)
nr-axSpA	*
axPsA	3.22 (0.76 – 13.71)
History of uveitis (ref negative)	0.7 (0.13 – 3.87)
Buttock pain (ref negative)	1.28 (0.31 – 5.28)
IBD (ref negative)	3.56 (0.77 – 16.53)
x-ray sacroiliitis	*
x-ray sacroiliitis of ≥ 1 SI joint in grade ≥ 3	1.02 (0.25 – 4.24)
Uveitis (ref negative)	4.11 (0.23 – 72.21)
Arthritis (ref negative)	0.93 (0.20 – 4.23)
Tenditis (ref negative)	0.94 (0.17 – 5.31)
HLAB27 (ref negative)	0.28 (0.06 – 1.31)
MRI sacroiliitis	*
WBC (10 9 /L)	0.66 (0.41–1.04)
ESR (mm/h)	0.95 (0.89 – 1.02)
CRP (mg/l)	0.98 (0.93 – 1.04)
CRP > 5 (mg/l)	0.54 (0.13 – 2.30)
BASDAI (1 score)	0.82 (0.50 – 1.32)
VAS (mm)	0.99 (0.95 – 1.04)
Haptoglobin (mg/dl)	0.99 (0.98 – 0.99)
Haptoglobin (per 200 mg/dl)	0.19 (0.02 –0.76)
Zonulin (ng/ml)	1.03 (1.00 – 1.07)
Zonulin (per 10 ng/ml)	1.39 (1.02 – 2.0)
Haptoglobin phenotype (Hp 2–1 vs Hp 1–1)	0.41 (0.07–2.56)
Haptoglobin phenotype (Hp 2–2 vs Hp 1–1)	0.85 (0.14 – 5.0)

Anti-TNF therapy anti tumor necrosing factor therapy (adalimumab, certolizumab, etanercept, golimumab), *AS* ankylosing spondylitis, *axPsA* axial psoriatic arthritis, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BMI* body mass index, *bDMARDs* biological disease-modifying antirheumatic drugs, *cDMARDs* classic disease-modifying antirheumatic drugs, *CRP C*-reactive protein, *CRP > 5 mg/l* is deemed to be increased, *ESR* erythrocyte sedimentation rate, *GCS* glucocorticosteroids, *Hp*-haptoglobin, *IBD* inflammatory bowel disease, *nr-axSpA* non-radiographic axial spondyloarthritis, *MRI* magnetic resonance imaging, *NSAIDs* non-steroidal anti-inflammatory drugs, *SpA* spondyloarthritis general, *WBC* white blood count, *VAS* value of spinal pain intensity on visual analogue scale

*Variables where logistic regression calculations could not be performed. See also Table 2S

whatever the cause, may increase the possibility of therapeutic success in axSpA.

Our hypothesis related to the influence of the microbiome on response to treatment seems to be supported by two other factors: age and the history of frequent infections, which independently of zonulin were negative predictors (OR = 1.09, $p = 0.047$; 95% CI 1.00 – 1.18, OR = 8.63, 95% CI 1.43 – 52.21, $p = 0.019$, respectively). According to the results of another study, the composition of the gut microbiota at baseline may have a better predictive value for response to TNFi than indicators of disease activity including CRP.

Table 3 Predictors of treatment failure to bDMARDs in series of two-factor analyses with zonulin and other baseline variables

Zonulin OR (95% CI)		Variable OR (95% CI)		
1.04	(1.00 – 1.07)	Female	1.18	(0.25 – 5.70)
1.04	(1.01 – 1.08)	Age	1.09	(1.00 – 1.18)
1.04	(1.00 – 1.07)	BMI	1.14	(1.00 – 1.21)
1.04	(1.01 – 1.08)	Symptom duration	1.10	(1.00 – 1.21)
1.03	(1.00 – 1.07)	Family History of SpA	0.98	(0.14 – 6.83)
1.05	(1.01 – 1.09)	History of frequent infections	8.63	(1.43 – 52.21)
1.04	(1.00 – 1.07)	Concomitant diseases	2.29	(0.21 – 25.09)
1.04	(1.00 – 1.07)	Treatment with DMARDs or GCS	3.77	(0.77 – 18.41)
1.04	(1.00 – 1.07)	Treatment with anti-TNF	0.50	(0.09 – 2.74)
1.03	(1.00 – 1.07)	Treatment with other bDMARDs	1.70	(0.38 – 7.62)
1.04	(1.00 – 1.08)	Gastrointestinal symptoms	4.63	(0.91 – 23.48)
1.04	(1.00 – 1.07)	AS	0.49	(0.10 – 2.32)
1.03	(1.00 – 1.07)	nr-axSpA	*	*
1.05	(1.00 – 1.10)	axPsA	5.91	(0.99 – 35.1)
1.04	(1.00 – 1.07)	History of uveitis	0.50	(0.08 – 3.22)
1.04	(1.00 – 1.08)	Buttock pain	2.09	(0.40 – 10.77)
1.05	(1.01 – 1.09)	IBD	7.56	(1.06 – 54.06)
1.03	(1.00 – 1.06)	No x-ray sacroiliitis	*	*
1.04	(1.00 – 1.07)	x-ray sacroiliitis of ≥ 1 SI joint in grade ≥ 3	0.75	(0.16 – 3.53)
1.04	(1.00 – 1.07)	Uveitis	6.99	(0.30 – 161.5)
1.04	(1.00 – 1.07)	Arthritis	0.57	(0.10 – 3.25)
1.03	(1.00 – 1.07)	Tenditis	0.87	(0.15 – 5.14)
1.04	(1.00 – 1.07)	HLA B27 (+)	0.27	(0.05 – 1.37)
1.07	(0.95 – 1.20)	MRI sacroiliitis (+)	*	*
1.03	(1.00 – 1.07)	WBC (10 9 /L)	0.69	(0.44 – 1.10)
1.04	(1.00 – 1.07)	ESR (mm/h)	0.95	(0.88 – 1.02)
1.04	(1.00 – 1.07)	CRP (mg/l)	0.96	(0.90 – 1.04)
1.04	(1.00 – 1.07)	BASDAI (1 score)	0.78	(0.47 – 1.32)
1.03	(1.00 – 1.07)	VAS (mm)	0.99	(0.95 – 1.04)
1.04	(0.98 – 1.10)	Haptoglobin (mg/dl)	0.99	(0.98 – 1.00)
1.03	(1.00 – 1.07)	Haptoglobin phenotype:		
		Hp 1–1	1.00	
		Hp 2–1	0.45	(0.07 – 3.00)
		Hp 2–2	0.90	(0.14 – 5.86)

Anti-TNF therapy anti tumor necrosing factor therapy (adalimumab, certolizumab, etanercept, golimumab), *AS*-ankylosing spondylitis, *axPsA* axial psoriatic arthritis, *BMI* body mass index, *BASDAI*-Bath Ankylosing Spondylitis Disease Activity Index, *bDMARDs* biological disease-modifying antirheumatic drugs, *cDMARDs*-classic disease-modifying antirheumatic drugs, *CRP-C*-reactive protein, *GCS* glucocorticosteroids, *Hp*-haptoglobin, *IBD* inflammatory bowel disease, *nr-axSpA* non-radiographic axial spondyloarthritis, *MRI* sacroiliitis-sacroiliitis visible on magnetic resonance imaging, *NSAIDs* non-steroidal anti-inflammatory drugs, other *bDMARDs* other biological disease-modifying antirheumatic drugs (iksekizumab, secukinumab), *other SpA* spondyloarthritis general, *WBC*-white blood count, *VAS*-value of spinal pain intensity on visual analogue scale

*Variables where logistic regression calculations could not be performed. See also Table 2S

A history of frequent infections increased the risk of treatment failure of bDMARDs by more than eightfold! This probably may stem from the disruption of the gut microflora caused by frequent antibiotic use.

Age, on the other hand, is linked to dysbiosis [40]. Previous reports have already shown a decrease in treatment effectiveness with age in axSpA, but this was usually associated with the presence of advanced degenerative changes in the spine or a higher degree of x-ray sacroiliitis [41–43]. In our study, x-ray sacroiliitis was not associated with response to treatment, whereas age was. Age was also not correlated with the degree of x-ray sacroiliitis.

Vallier et al. demonstrated that the composition of the gut microbiota at baseline in axSpA patients presented better

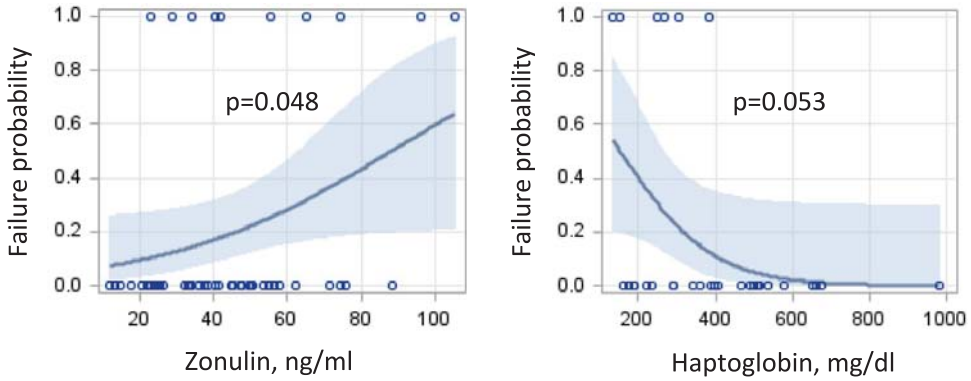

Fig. 2 Prediction of biological treatment failure by zonulin and haptoglobin concentrations at admission to the hospital

predictive value for response to TNFi than indicators of disease activity including CRP [44].

Also, psoriasis treatment studies have noted differences in response to bDMARDs depending on the composition of the microbiome [45, 46].

Similarly, in another study, concomitant diseases were associated with worse treatment effects with TNFi in axSpA [47]. In our study concomitant diseases did not significantly increase this risk (OR = 2.03, 95% CI 0.22 – 18.77, $p = 0.53$).

As in our previous report, zonulin was detected in all patients, not only in Hp2 antigen carriers, and, as before, was highest in those with the Hp 1–1 phenotype ($p = 0.91$), demonstrating that the ELISA detected more than just the pre-Hp2 molecule [21, 22].

Zonulin was significantly correlated with CRP, although CRP alone was not associated with treatment response. This is a different result from most studies, which have shown the superiority of increased CRP in predicting good response to bDMARDs, especially TNFi [29, 30, 47, 48]. However, higher values of the inflammatory indices (WBC, CRP, ESR) reduced the risk of poor response, but not significantly. A substantially better predictive value demonstrated Hp level.

High levels of Hp were present in patients responding well to biological therapy. In fact, Hp concentrations above 400 mg/dl were the cut-off point for responders. The result was on the borderline of significance ($p = 0.056$), which may result from the small size of the group. It is possible that, like CRP, Hp level reflects inflammation and define those who may benefit from biological treatment.

Although there were significant differences in Hp levels between phenotypes none of the phenotypes proved to be a predictor of treatment failure to bDMARDs.

MRI sacroiliitis only occurred in patients who responded well to treatment, which is in line with other study results indicating MRI sacroiliitis as a predictor of good response to standard and biological therapy [22, 30, 49, 50]. In our analysis we had a lot of missing data, the result was not statistically significant. Also, all patients without radiographic changes in the sacroiliac joints responded well, reflecting the good efficacy of biological treatment at an early stage of the disease.

A strong part of this study is its observational character, which reflects 'real-life' clinical situations with consecutive sampling patients.

In addition, we analysed risk factors for a poor response to bDMARDs that had never been considered until now. We shed new light on certain aspects in the approach to treating patients with axSpA and identified factors that can be modified and increase the chance of therapeutic success.

Our study had also some limitations. Due to the COVID-19 pandemic, we were unable to collect more patients and the final sample size is smaller than intended. For this reason, we did not perform separate predictors analyses for different groups of bDMARDs. Although we were not able to do multivariate analysis due to small sample size, we performed a univariate analysis with zonulin referring to the factor of most interest.

In the assessment of axSpA disease activity, we did not use the Ankylosing Spondylitis Disease Activity Score (ASDAS), which is more appropriate for this purpose. Determination of inflammatory indices after 12 weeks of biological treatment was difficult due to pandemic.

It may seem that the inclusion of only hospitalised patients in the study raises the risk of a sample selection bias. However, in Poland, diagnosis of spondyloarthritis is most often made in the hospital setting due to limitations in the operation of outpatient clinics, whereas qualification for biological treatment can only take place in hospital. Instead, hospitalisation made it possible to quickly rule out possible other causes of the back pain.

Conclusions

In our study, we identified factors associated with intestinal dysfunction (zonulin, older age, IBD, frequent use of antibiotics) that are valuable for the prediction biological treatment failure in axSpA. We presume that by modifying the

gut microbiota and/or using a zonulin inhibitor, treatment outcomes may be improved.

This is a new approach to the therapy of this disease, as it focuses on the second element of the gut-joint axis, the regulation of normal intestinal function. Whether this will help increase the effectiveness of therapy and achieve remission in SpA may only present future studies aimed at restoring homeostasis in the gut.

Furthermore, Hp appears to be, regardless of its polymorphism, a potential predictive marker of response to bDMARDs, which needs to be confirmed in further research. According to our analysis, it may prove to be a better predictor than other indices of inflammation.

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Data availability The data analysed during this study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest Magdalena Chmielińska, Anna Felis-Giemza, Marzena Olesińska, Agnieszka Paradowska-Gorycka and Dariusz Szukiewicz have no conflict of interest to declare.

Ethics approval The study was approved by the Bioethics Committee at the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw (Date: 23 October 2020; No KBT-5/1/2020). All patients who participated in the study signed an informed consent. This study was performed in line with the principles of the Declaration of Helsinki.

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Podsumowanie i wnioski

W niniejszej pracy zostały zebrane i opracowane dane na temat klinicznego znaczenia polimorfizmu haptoglobiny w przebiegu spondyloartropatii. W pierwszym artykule omówiłam dostępne dane literaturowe i wyniki badań dotyczące polimorfizmu haptoglobiny i jej pochodnej cząsteczki – zonuliny w spondyloartropatii i chorobach o podobnej etiologii [21]. Stanowiło to podstawę do postawienia przeze mnie hipotezy o gorszym przebiegu choroby u osób z fenotypem Hp2-2, ponieważ wytwarzana przez nich haptoglobina ma słabsze właściwości przeciwzapalne i niższe stężenie w przestrzeni pozanaczyniowej, np. w stawach [9,14]. Dodatkowo zonulina, cząsteczka opisywana jako prekursor Hp2, jest związana ze zwiększoną przepuszczalnością jelit i rozregulowaniem osi jelito-stawy [19]. Mogłoby to oznaczać, że nosiciele genu Hp2 są genetycznie obciążeni predyspozycją do subklinicznego zapalenia jelit i w związku z tym podtrzymywania stanu zapalnego poprzez oś jelitowo-stawową. W konsekwencji skuteczność dostępnych terapii powinna być wówczas gorsza. Rozregulowanie osi jelito-stawy jako ważny element w oporności na terapie potwierdzałyby również inne zidentyfikowane predyktory nieskuteczności leczenia bLMPCh w moim badaniu, czyli starszy wiek, częste kuracje antybiotykami z powodu infekcji oraz wywiad w kierunku nieswoistych zapaleń jelit [24]. Wszystkie te czynniki są związane z nieprawidłowym funkcjonowaniem jelit i dysbiozą [40-42].

W wyniku przeprowadzonych badań wykazałam, że rzeczywiście zwiększony poziom zonuliny predysponuje do gorszej odpowiedzi na zarówno standardowe, jak i biologiczne leczenie w spondyloartropatii osiowej [23-24]. Natomiast zonulina wykrywana przez dostępne testy ELISA nie jest wyłącznie prekursorem Hp2, ponieważ występuje również u pacjentów z fenotypem Hp1-1. Jest to spójne z innymi opublikowanymi pracami, w których uzyskano podobne wyniki i dowiedziono, że komercyjne testy ELISA (pomimo opisu zawartego w informacji o produkcie) wykrywają nie tylko cząsteczkę pre-Hp2, ale również inne tzw. peptydy z rodziny zonuliny (*zonulin family peptides*) o podobnej budowie strukturalnej i funkcji, jak zostało to zresztą opisane przez samego odkrywcę tej cząsteczki [43].

Dodatkowo Hp2-2 i pozostałe fenotypy nie były związane z odpowiedzią na leczenie niesteroidowymi lekami przeciwzapalnymi i biologicznymi w moich badaniach. Polimorfizm haptoglobiny nie był również skorelowany z żadnym ze wskaźników aktywności choroby zarówno w pierwszej jak i drugiej pracy.

Co interesujące, wysokie stężenie haptoglobiny okazało się pozytywnym predyktorem w odpowiedzi na leczenie biologiczne, natomiast takiej zależności nie znaleziono w stosunku do wymienianych w rekomendacjach ASAS/EULAR predyktorów: CRP i aktywnego zapalenia stawów krzyżowo-biodrowych. Może to wynikać z tego, że siła predykcji tych wskaźników opiera się głównie na badaniach z inhibitorami TNF alfa, a w moim badaniu pacjenci stosowali również inne leki biologiczne. Stężenia haptoglobiny powyżej 400 mg/dl występowały tylko u osób dobrze odpowiadających na bLMPCh. Haptoglobina była silnie skorelowana ze wskaźnikami stanu zapalnego CRP ($r=0.56$, $p=0.0004$) i OB ($r=0.62$, $p<0.0001$), ale tylko w grupie pacjentów zakwalifikowanych do dalszego leczenia NLPZ [23]. Haptoglobina może okazać się lepszym predyktorem niż standardowo oznaczane wskaźniki zapalne w odpowiedzi na leczenie bLMPCh. Wymaga to jednak dalszych badań. Uzyskany wynik mieścił się na granicy istotności statystycznej, co prawdopodobnie jest spowodowane małą grupą badaną ($p=0.053$).

Podsumowując:

1. Polimorfizm haptoglobiny nie jest związany z odpowiedzią na leczenie ani z aktywnością choroby u pacjentów ze spondyloartropatią osiową, co wskazuje na brak jego znaczenia klinicznego w tej chorobie.

2. Zonulina jest predyktorem odpowiedzi na leczenie w osiowej spondyloartropatii zarówno w leczeniu standardowym jak i biologicznym, niezależenie od polimorfizmu haptoglobiny. Oznacza to, że zwiększona przepuszczalność jelit może być kluczowym czynnikiem w oporności na stosowane terapie w osiowej spondyloartropatii, uczestniczyć w mechanizmach podtrzymywania stanu zapalnego w tej chorobie i stanowić jego źródło. Jest to nowatorskie odkrycie, które może stworzyć nowe perspektywy leczenia tej choroby za pomocą np. inhibitora zonuliny.

3. Wyniki moich badań mogą pomóc w opracowaniu nowych rekomendacji leczenia osiowej postaci spondyloartropatii, które będą uwzględniać czynniki ryzyka niepowodzenia istniejących terapii. Dodatkowo wskazują na zasadność przeprowadzenia podobnych analiz w różnych podtypach spondyloartropatii.

Piśmiennictwo

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Warszawa, 23.10.2020 r.

Decyzja Komisji Bioetycznej
przy Narodowym Instytucie Geriatrii, Reumatologii i Rehabilitacji w Warszawie
nr KBT-5/1/2020

Komisja Bioetyczna przy Narodowym Instytucie Geriatrii, Reumatologii i Rehabilitacji w Warszawie, ul. Spartańska 1, działająca zgodnie z zasadami GCP, zapoznała się z następującymi dokumentami dotyczącymi projektu badawczego pt. **„Znaczenie kliniczne polimorfizmu haptoglobiny w przebiegu i leczeniu spondyloartropatii"**:

1. **Wniosek kierownika projektu do Komisji Bioetycznej ze zgodą Kierownika Kliniki i Polikliniki Układowych Chorób Tkanki Łącznej NIGRiR na przeprowadzenie badania;**
2. **Podanie kierownika projektu do Dyrektora Narodowego Instytutu Geriatrii, Reumatologii i Rehabilitacji ze zgodą na przeprowadzenie badania;**
3. **Podania kierownika projektu do kierowników Klinik: Reumatologii oraz Wczesnego Zapalenia Stawów NIGRiR ze zgodą na przeprowadzenie badania;**
4. **Opis programu badania;**
5. **Wzór Karty Obserwacji Klinicznej – Case Report Form (CRF – version 1.0 – 01.07.20);**
6. **Informacja dla pacjenta;**
7. **Formularz świadomej zgody na udział w badaniu;**
8. **Kopia trójstronnej umowy o współpracy dotyczącej badania naukowego pomiędzy Warszawskim Uniwersytetem Medycznym, Narodowym Instytutem Geriatrii, Reumatologii i Rehabilitacji oraz Badaczem – Magdaleną Chmielińską.**

Badanie naukowe będzie realizowane w Klinice i Poliklinice Układowych Chorób Tkanki Łącznej NIGRiR we współpracy z Zakładem Biologii Molekularnej, Kliniką Reumatologii, Kliniką Wczesnego Zapalenia Stawów Instytutu oraz Warszawskim Uniwersytetem Medycznym w ramach Katedry i Zakładu Patologii Ogólnej i Doświadczalnej, a także Zakładu Epidemiologii i Biostatystyki. W projekcie wezmą udział pacjenci z rozpoznaną spondyloartropatią hospitalizowani lub leczeni ambulatoryjnie w Narodowym Instytucie Geriatrii, Reumatologii i Rehabilitacji. Kierownikiem projektu jest lek. Magdalena Chmielińska z zespołu Kliniki i Polikliniki Układowych Chorób Tkanki Łącznej NIGRiR. Wyniki badań zostaną wykorzystane w pracy doktorskiej dr Magdaleny Chmielińskiej prowadzonej pod kierunkiem prof. dr hab. n. med. Dariusza Szukiewicza z Katedry i Zakładu Patologii Ogólnej i Doświadczalnej WUM.

Komisja Bioetyczna przy NIGRiR w głosowaniu tajnym nad akceptacją zgłoszonego projektu wyraziła zgodę na rozpoczęcie badań zgodnie z przedstawionym protokołem.

PRZEWODNICZĄCY
KOMISJI BIOETYCZNEJ
przy Narodowym Instytucie Geriatrii,
Reumatologii i Rehabilitacji w Warszawie
prof. dr hab. n. med. Piotr Głuszko

Warszawa
(miejscowość, data)
07/05/2024

Marzena Olesińska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Haptoglobin and Its Related Protein, Zonulin-What Is Their Role in Spondyloarthropathy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:Przegląd dostępnej literatury , analiza danych

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 85 %,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji pracy, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu, wizualizacja

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

M. Olesińska
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 07.05.2014
.....
(miejscowość, data)

Katarzyna Romanowska-Próchnicka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „ Haptoglobin and Its Related Protein, Zonulin-What Is Their Role in Spondyloarthropathy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Przegląd dostępnej literatury , uwagi merytoryczne, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 85 %,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji pracy, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu, wizualizacja
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 2024.05.02.

.....
(miejscowość, data)

Dariusz Szukiewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Haptoglobin and Its Related Protein, Zonulin-What Is Their Role in Spondyloarthropathy" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Analiza danych, edycja manuskryptu,

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 85 %,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji pracy, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu, wizualizacja

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej

(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa
(miejscowość, data)
07/05/2024

Marzena Olesińska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

nadzorowanie prac badawczych, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 77 %,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

M. Olesińska
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 30.04.14
(miejscowość, data)

Anna Felis-Giemza
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

nadzorowanie prac badawczych, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 77 %,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej

(imię i nazwisko kandydata do stopnia)

Anna Felis-Giemza
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 06/05/24
(miejscowość, data)

Agnieszka Paradowska-Gorycka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Nadzór nad prowadzeniem badań związanych z oznaczeniem polimorfizmu haptoglobiny, zonuliny i haptoglobiny, pisanie manuskryptu , analiza danych

Mój udział procentowy w przygotowaniu publikacji określam jako 10 %.
Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 77 %,
(imię i nazwisko kandydata do stopnia)
obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

Warszawa 30.09.19
(miejscowość, data)

Karolina Palej
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

nadzorowanie prac badawczych, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 77 %,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej

(imię i nazwisko kandydata do stopnia)

Karolina Palej
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 07.05.2024 r.
(miejscowość, data)

Julita Rejmer-Szcześniak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

nadzorowanie prac badawczych, wizualizacja wyników, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 77 %,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

Julita Rejmer-Szcześniak
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 2024.05.02.
.....
(miejscowość, data)

Dariusz Szukiewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of treatment failure of non-steroidal anti-inflammatory drugs in patients with axial spondyloarthritis with focus on haptoglobin, haptoglobin polymorphism and zonulin" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Analiza danych, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 77 %,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej

(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 30.04.24
(miejscowość, data)

Anna Felis-Giemza
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „The failure of biological treatment in axial spondyloarthritis is linked to the factors related to increased intestinal permeability and dysbiosis: prospective observational cohort study" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

pisanie manuskryptu, wizualizacja wyników, wskazówki dotyczące metodyki, analiza danych

Mój udział procentowy w przygotowaniu publikacji określam jako 2,5 %.
Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 80 %,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji, opracowanie metodyki, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej

(imię i nazwisko kandydata do stopnia)

Anna Felis-Giemza
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa
(miejscowość, data)

07/05/2024

Marzena Olesińska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „The failure of biological treatment in axial spondyloarthritis is linked to the factors related to increased intestinal permeability and dysbiosis: prospective observational cohort study" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Nadzorowanie prac badawczych, analiza danych, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 2,5 %.
Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 80 %,
(imię i nazwisko kandydata do stopnia)
obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

M. Olesińska
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 06/05/24
(miejscowość, data)

Agnieszka Paradowska-Gorycka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „The failure of biological treatment in axial spondyloarthritis is linked to the factors related to increased intestinal permeability and dysbiosis: prospective observational cohort study" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Nadzór nad pracami związanymi z oznaczaniem polimorfizmu Hp, zonuliny i haptoglobiny, pisanie manuskryptu , opracowanie metodyki, edycja manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 10 %.
Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 80 %,
(imię i nazwisko kandydata do stopnia)
obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 2024.05.02
.....
(miejscowość, data)

Dariusz Szukiewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „The failure of biological treatment in axial spondyloarthritis is linked to the factors related to increased intestinal permeability and dysbiosis: prospective observational cohort study" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

Analiza danych, edycja manuskryptu, wskazówki dotyczące metodologii

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.
Wkład Magdaleny Chmielińskiej w powstawanie publikacji określam jako 80 %,
(imię i nazwisko kandydata do stopnia)
obejmował on: opracowanie koncepcji badania, metodyki, zbieranie danych, wizualizacja wyników, własny wkład finansowy w zakup części odczynników do badania, przegląd literatury, analiza danych, napisanie manuskryptu, edycja manuskryptu,
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Chmielińskiej
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników