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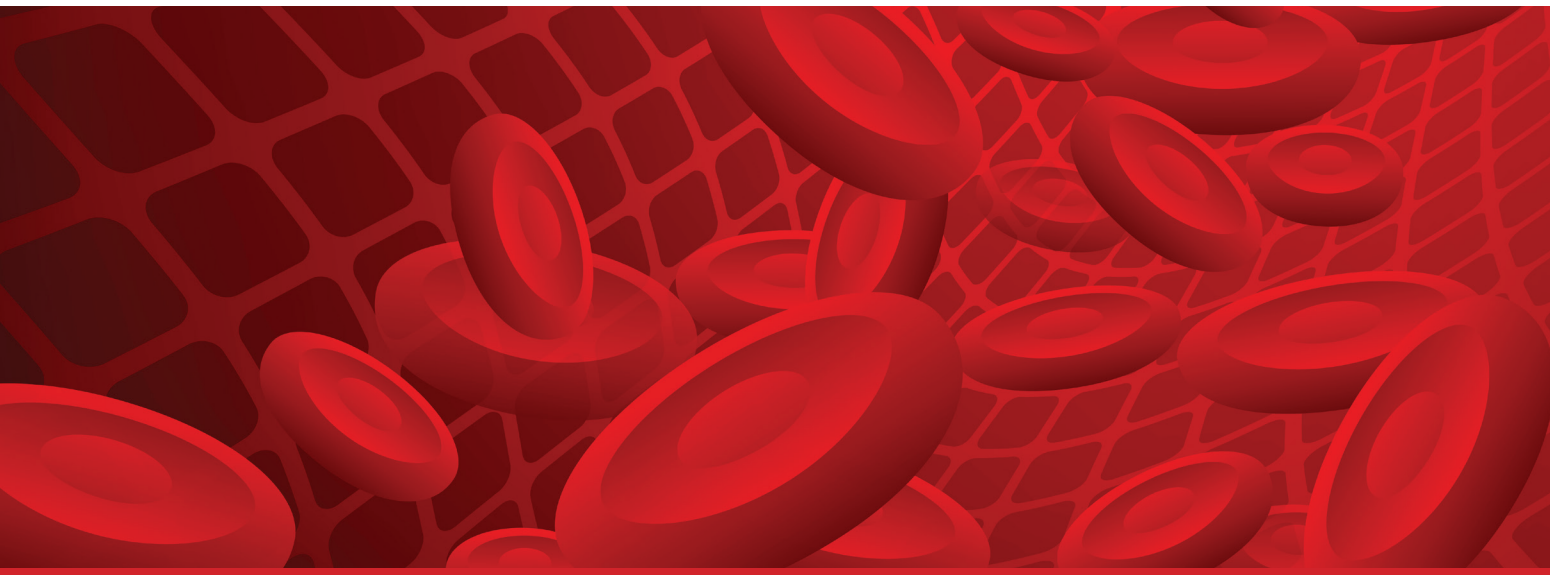
PTH:T IHiT



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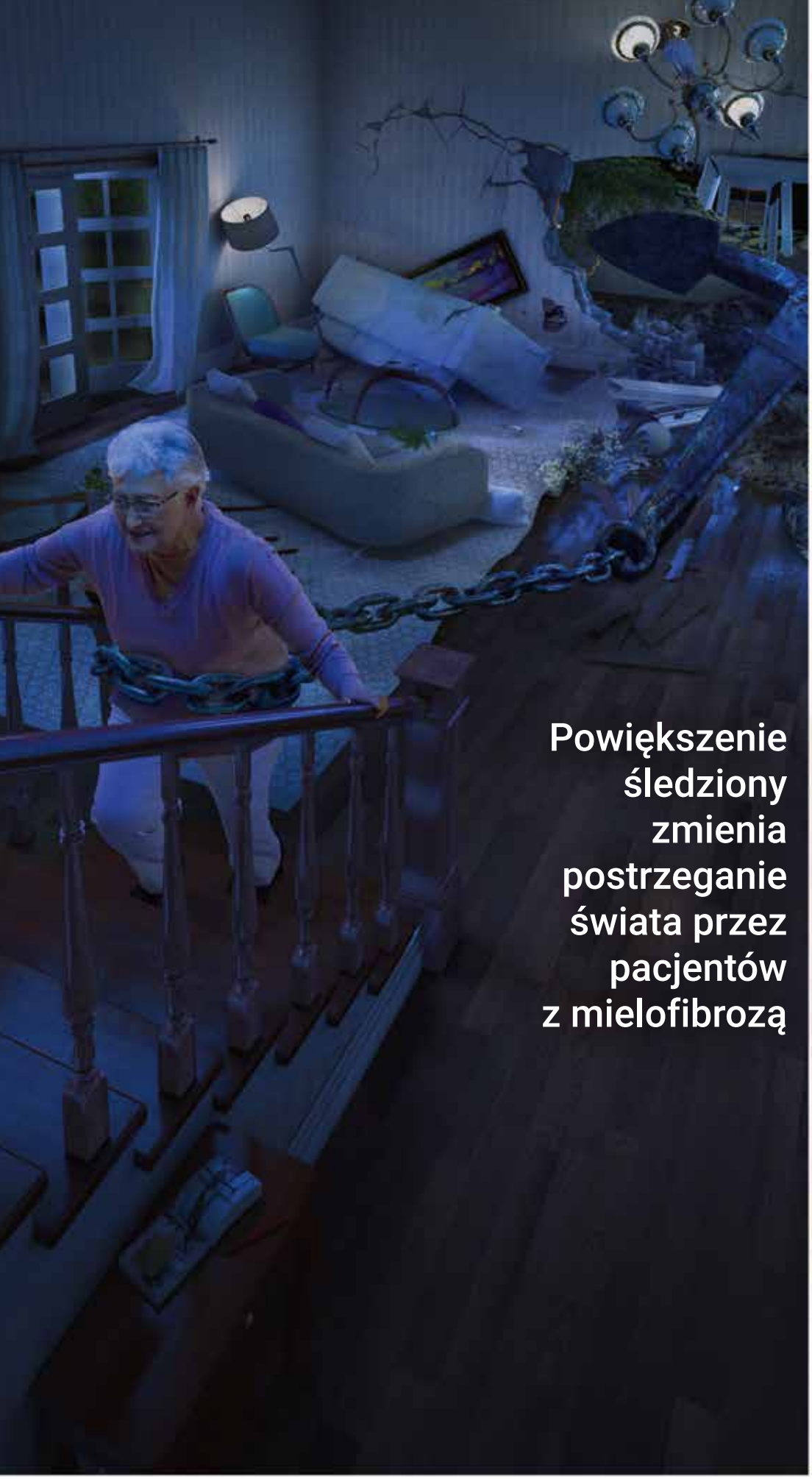
1. Charakterystyka produktu leczniczego Rydapt 02/2021.
2. Zgodnie z aktualnym Obwieszczeniem Ministra Zdrowia w sprawie wykazu refundowanych leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych. Program lekowy: • Załącznik B.114. Leczenie chorych na ostrą białaczkę szpikową (ICD-10 C92.0) • Załącznik B.115. Leczenie agresywnej mastocytozy układowej, mastocytozy układowej z współistniejącym nowotworem układu krwiotwórczego oraz białaczki mastocytarnej (ICD-10: C96.2, C94.3, D47.9).

NOVARTIS



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1. Charakterystyka produktu leczniczego Inrebic.

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INFORMACJA O LEKU (październik 2021)

Nazwa produktu leczniczego: Inrebic (fedratynib) 100 mg kapsułki twarde.

Skład: Każda kapsułka twarda zawiera fedratynibu dwuchlorowodorek jednowodny, co odpowiada 100 mg fedratynibu. **Postać farmaceutyczna:** kapsułka twarda. **Wskazania do stosowania**: Produkt leczniczy Inrebic jest wskazany w leczeniu powiększenia śledziony związanego z chorobą lub objawów występujących u dorosłych pacjentów z pierwotnym włóknieniem szpiku (znanym także jako przewlekłe idiopatyczne włóknienie szpiku), włóknieniem szpiku poprzedzonym czerwienicą prawdziwą lub włóknieniem szpiku poprzedzonym nadpłytkowością samoistną u pacjentów, którzy nie byli wcześniej leczeni inhibitorem kinazy janusowej (ang. *Janus Associated Kinase*), JAK lub byli leczeni ruksolitynibem. **Dawkowanie i sposób podawania**: Leczenie produktem Inrebic powinno być rozpoczęte, a następnie monitorowane przez lekarzy doświadczonych w stosowaniu przeciwnowotworowych produktów leczniczych. Dawkowanie: Przed rozpoczęciem leczenia produktem Inrebic, u pacjentów leczonych dotychczas ruksolitynibem, należy stopniowo zmniejszyć dawkę ruksolitynibu, a następnie zakończyć podawanie ruksolitynibu, zgodnie z charakterystyką produktu leczniczego ruksolitynibu. Przed rozpoczęciem leczenia produktem Inrebic należy oznaczyć początkowe stężenie tiaminy (witamina B1), wykonać morfologię krwi, badania czynności wątroby, oznaczyć stężenie amylazy i lipazy, azot mocznika (ang. *blood urea nitrogen*, BUN) i stężenie kreatyniny we krwi. Następnie badania należy powtarzać okresowo podczas leczenia oraz w uzasadnionych klinicznie sytuacjach. Nie należy rozpoczynać leczenia produktem Inrebic u pacjentów z niewyrównanym niedoborem tiaminy. Nie zaleca się rozpoczynania leczenia produktem Inrebic u pacjentów, u których początkowa liczba płytek krwi jest mniejsza niż 50 x 10^9/L oraz bezwzględna liczba neutrofili (ang. *absolute neutrophil count*, ANC) jest mniejsza niż 1.0 x 10^9/l. Zaleca się profilaktyczne stosowanie leków przeciwwymiotnych zgodnie z lokalną praktyką przez pierwsze 8 tygodni leczenia i kontynuowanie ich stosowania zgodnie ze wskazaniami klinicznymi. Przyjmowanie produktu Inrebic z posiłkiem o wysokiej zawartości tłuszczu może zmniejszyć częstość występowania nudności i wymiotów. Zalecana dawka produktu Inrebic wynosi 400 mg raz na dobę. Leczenie może być kontynuowane tak długo, jak długo pacjenci odnoszą korzyści kliniczne. W przypadku wystąpienia objawów toksyczności hematologicznej lub niehematologicznej należy rozważyć zmianę dawkowania (Tabela 1). Leczenie produktem Inrebic należy zakończyć, jeśli pacjent nie toleruje dawki 200 mg na dobę. W przypadku pominięcia dawki, następna zaplanowana dawka powinna zostać przyjęta następnego dnia. Nie należy przyjmować dodatkowych kapsułek w celu uzupełnienia pominiętej dawki. *Zmiany dawkowania:* W Tabeli 1 przedstawiono schemat zmiany dawkowania w przypadku wystąpienia objawów toksyczności hematologicznej, niehematologicznej i w przypadku leczenia encefalopatii Wernickego. *Zwiększanie stężenia tiaminy*: Przed rozpoczęciem oraz w trakcie leczenia należy wyrównać niedobór tiaminy, jeżeli jej stężenie jest zbyt małe. Podczas leczenia należy okresowo oznaczać stężenie tiaminy (np. co miesiąc przez pierwsze 3 miesiące, a następnie co 3 miesiące) i zgodnie ze wskazaniami klinicznymi. *Zmiany dawkowania podczas jednoczesnego stosowania silnych inhibitorów CYP3A4*: Jeżeli nie można uniknąć jednoczesnego stosowania silnych inhibitorów CYP3A4, należy zmniejszyć dawkę produktu Inrebic do 200 mg. Należy uważnie monitorować bezpieczeństwo pacjentów (np. co najmniej raz w tygodniu). W przypadku przerwania jednoczesnego podawania silnego inhibitora CYP3A4, dawkę produktu Inrebic należy zwiększyć do 300 mg raz na dobę w ciągu pierwszych dwóch tygodni po przerwaniu leczenia inhibitorem CYP3A4, a następnie do 400 mg raz na dobę, w zależności od tolerancji. W razie potrzeby należy dokonać dodatkowych zmian dawkowania w oparciu o wyniki monitorowania bezpieczeństwa stosowania i skuteczności produktu Inrebic. *Ponowne zwiększanie dawki*: Jeżeli działanie niepożądane spowodowane przez produkt Inrebic, które było powodem zmniejszenia dawki, jest skutecznie kontrolowane i objawy toksyczności ustąpią na co najmniej 28 dni, dawka może zostać ponownie zwiększona o jeden poziom dawkowania na miesiąc, do osiągnięcia dawki początkowej. Ponowne zwiększanie dawki nie jest zalecane, jeżeli zmniejszenie dawki było spowodowane objawami toksyczności niehematologicznej stopnia 4., zwiększeniem aktywności aminotransferazy alaninowej (AlAT), aminotransferazy asparaginianowej (AspAT) lub stężenia bilirubiny całkowitej stopnia ≥ 3. albo nawrotem objawów toksyczności hematologicznej stopnia 4.

Tabela 1: Zmniejszenie dawki w przypadku toksyczności hematologicznej, niehematologicznej i leczenia encefalopatii Wernickego

Toksyczność hematologiczna	**Zmniejszenie dawki**
Małopłytkowość stopnia 3. z aktywnym krwawieniem (liczba płytek krwi < 50 x 10^9/l) lub małopłytkowość stopnia 4. (liczba płytek krwi < 25 x 10^9/l)	Przerwać stosowanie produktu Inrebic do czasu ustąpienia objawów do stopnia ≤ 2. (liczba płytek krwi < 75 x 10^9/l) lub uzyskania wartości początkowych. Wznowić stosowanie w dawce dobowej mniejszej o 100 mg od ostatniej stosowanej dawki.
Neutropenia stopnia 4. (bezwzględna liczba neutrofili [ANC] < 0,5 x 10^9/l)	Przerywać stosowanie produktu Inrebic do czasu ustąpienia objawów do stopnia ≤ 2. (ANC < 1,5 x 10^9/l) lub uzyskania wartości początkowych. Wznowić w dawce dobowej mniejszej o 100 mg od ostatniej stosowanej dawki. Zgodnie z decyzją lekarza można zastosować czynniki wzrostu kolonii granulocytów.
Niedokrwistość stopnia 3. i wyższego, wskazana transfuzja (stężenie hemoglobiny < 8,0 g/dl)	Przerwać stosowanie produktu Inrebic, do czasu ustąpienia objawów do stopnia ≤ 2. (stężenie hemoglobiny ≤ 10,0 g/dl) lub uzyskania wartości początkowych. Wznowić stosowanie w dawce dobowej mniejszej o 100 mg od ostatniej stosowanej dawki.
Nawrót objawów toksyczności hematologicznej stopnia 4.	Zakończyć stosowanie produktu Inrebic zgodnie z decyzją lekarza.
Toksyczność niehematologiczna	**Zmniejszenie dawki**
Nudności, wymioty lub biegunka stopnia ≥ 3., nieodpowiadająca na leczenie wspomagające w ciągu 48 godzin	Przerwać stosowanie produktu Inrebic, do czasu ustąpienia objawów do stopnia ≤ 1. lub uzyskania wartości początkowych. Wznowić stosowanie w dawce dobowej mniejszej o 100 mg od ostatniej stosowanej dawki.
Objawy toksyczności stopnia ≥ 3. związane z aktywnością AlAT/ AspAT (> 5,0 do 20,0 x górna granica normy [GGN] lub stężeniem bilirubiny (> 3,0 do 10,0 GGN).	Przerwać stosowanie produktu Inrebic do czasu ustąpienia objawów do ≤ stopnia 1. (AspAT/AlAT [> GGN - 3,0 x GGN] lub bilirubina [> GGN - 1,5 x GGN]) lub do czasu uzyskania wartości początkowej. Wznowić stosowanie w dawce dobowej mniejszej o 100 mg od ostatniej stosowanej dawki. Monitorować aktywność AlAT, AspAT i stężenie bilirubiny (całkowitą i bezpośrednią) co 2 tygodnie przez co najmniej 3 miesiące po zmniejszeniu dawki. W przypadku ponownego wystąpienia objawów toksyczności stopnia 3. lub wyższego, zakończyć leczenie produktem Inrebic.
Aktywność amylazy i (lub) lipazy stopnia ≥ 3. (> 2,0 do 5,0 x GGN)	Przerwać stosowanie produktu Inrebic do czasu ustąpienia objawów do stopnia 1. (> GGN - 1.5 x GGN) lub do czasu uzyskania wartości początkowych. Wznowić stosowanie produktu leczniczego w dawce dobowej mniejszej o 100 mg od ostatniej stosowanej dawki. Monitorować aktywność amylazy i (lub) lipazy co 2 tygodnie przez co najmniej 3 miesiące po zmniejszeniu dawki. W przypadku ponownego wystąpienia objawów toksyczności stopnia 3. lub wyższego, zakończyć leczenie produktem Inrebic.
Stopień ≥ 3. innych objawów toksyczności niehematologicznych	Przerwać stosowanie produktu Inrebic do czasu ustąpienia objawów do stopnia ≤ 1. lub uzyskania wartości jak w punkcie wyjściowym. Ponownie rozpocząć stosowanie produktu leczniczego w dawce dobowej o 100 mg mniejszej od ostatniej stosowanej dawki.
Wyrównywanie niedoboru tiaminy i leczenie encefalopatii Wernickego	**Zmniejszenie dawki**
Dla stężeń tiaminy < zakres normalny (74 do 222 nmol/l), ale ≥ 30 nmol/l bez objawów przedmiotowych lub podmiotowych encefalopatii Wernickego	Przerwać stosowanie produktu Inrebic. Przyjmować doustnie 100 mg tiaminy na dobę do momentu wyrównania niedoboru*. Rozważyć wznowienie leczenia produktem Inrebic, gdy stężenie tiaminy będzie w granicach normy*.
Dla poziomów tiaminy < 30 nmol/l bez objawów przedmiotowych lub podmiotowych encefalopatii Wernickego	Przerwać stosowanie produktu Inrebic. Rozpocząć leczenie roztworem tiaminy do podania parenteralnego w dawkach terapeutycznych aż do przywrócenia stężenia tiaminy do normy*. Rozważyć wznowienie leczenia produktem Inrebic, gdy stężenie tiaminy będzie w granicach normy*.
W przypadku objawów podmiotowych lub przedmiotowych encefalopatii Wernickego, niezależnie od stężenia tiaminy	Przerwać stosowanie produktu Inrebic i natychmiast podać tiaminę parenteralnie w dawkach terapeutycznych.

* zakres normalny stężeń tiaminy może różnić się w zależności od metody oznaczenia stosowanej przez dane laboratorium

Szczególne grupy pacjentów: Zaburzenia czynności nerek: U pacjentów z ciężkimi zaburzeniami czynności nerek (klirens kreatyniny [CLcr] 15 ml/min do 29 ml/min według Cockcrofta-Gaulta [C-G]), dawka powinna zostać zmniejszona do 200 mg. Nie zaleca się modyfikacji dawki początkowej u pacjentów z łagodnymi lub umiarkowanymi zaburzeniami czynności nerek (CLcr 30 ml/min do 89 ml/min według C-G). Ze względu na potencjalny wzrost ekspozycji, pacjenci z wcześniej występującym umiarkowanym zaburzeniem czynności nerek, mogą wymagać co najmniej cotygodniowego monitorowania bezpieczeństwa i w razie konieczności zmian dawkowania w oparciu o działania niepożądane. *Zaburzenia czynności wątroby:* Nie badano farmakokinetyki produktu Inrebic u pacjentów z ciężkimi zaburzeniami czynności wątroby. Należy unikać stosowania produktu Inrebic u pacjentów z ciężkimi zaburzeniami czynności wątroby (klasa C w skali Child-Pugh lub stężenie bilirubiny całkowitej > 3 razy GGN i każde zwiększenie aktywności AspAT). Zmiana dawki początkowej nie jest konieczna u pacjentów z łagodnymi lub umiarkowanymi zaburzeniami czynności wątroby. *Pacjenci w podeszłym wieku:* U pacjentów w podeszłym wieku (> 65 lat) nie jest wymagane dostosowywanie dawki. *Dzieci i młodzież:* Nie określono dotychczas bezpieczeństwa stosowania ani skuteczności produktu leczniczego Inrebic u dzieci i młodzieży w wieku do 18 lat. Dane nie są dostępne. Sposób podawania: Podanie doustne. Nie należy otwierać, łamać ani żuć kapsułek. Kapsułki należy połykać w całości, najlepiej z wodą. Można je przyjmować z posiłkiem lub bez. Przyjmowanie z posiłkiem o dużej zawartości tłuszczu może zmniejszyć częstość występowania nudności i wymiotów, dlatego zaleca się przyjmowanie z posiłkiem. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą. Ciąża. **Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania**: Encefalopatia, w tym encefalopatia Wernickego: Zgłaszano przypadki ciężkich i śmiertelnych encefalopatii, w tym encefalopatii Wernickego, u pacjentów przyjmujących produkt Inrebic. Encefalopatia Wernickego jest nagłym stanem neurologicznym spowodowanym niedoborem tiaminy (witamina B1). Objawy przedmiotowe i podmiotowe encefalopatii Wernickego mogą obejmować ataksję, zmiany stanu psychicznego i oftalmoplegię (np. oczopląs, podwójne widzenie). Wszelkie zmiany stanu psychicznego, dezorientacja lub upośledzenie pamięci powinny budzić obawy dotyczące potencjalnej encefalopatii, w tym encefalopatii Wernickego i wskazywać konieczność szybkiego przeprowadzenia pełnej oceny, w tym przeprowadzenia badania neurologicznego, oceny stężenia tiaminy i obrazowania. Stężenia tiaminy i stan odżywienia pacjentów należy oceniać przed rozpoczęciem leczenia produktem Inrebic, okresowo podczas leczenia (np. co miesiąc przez pierwsze 3 miesiące, a następnie co 3 miesiące) i zgodnie ze wskazaniami klinicznymi. Nie należy rozpoczynać leczenia produktem Inrebic u pacjentów z niedoborem tiaminy. Przed rozpoczęciem leczenia i w trakcie leczenia należy uzupełniać niedobór tiaminy. W przypadku podejrzenia encefalopatii, należy natychmiast przerwać leczenie produktem Inrebic i rozpocząć podanie parenteralne tiaminy podczas oceny pod kątem wszystkich możliwych przyczyn. Należy monitorować pacjenta do momentu ustąpienia lub poprawy objawów i uzupełnienia niedoboru tiaminy. Niedokrwistość, małopłytkowość i neutropenia: Leczenie produktem Inrebic może powodować niedokrwistość, małopłytkowość i neutropenię. Morfologię krwi należy wykonywać w punkcie początkowym, okresowo podczas leczenia i zgodnie z zaleceniami klinicznymi. Nie badano działania produktu Inrebic u pacjentów z początkową liczbą płytek krwi < 50 x 10^9/l oraz ANC < 1,0 x 10^9/L. *Niedokrwistość:* Niedokrwistość zazwyczaj występuje w ciągu pierwszych 3 miesięcy leczenia. U pacjentów ze stężeniem hemoglobiny poniżej 10,0 g/dl na początku leczenia prawdopodobieństwo wystąpienia niedokrwistości stopnia 3. lub wyższego podczas leczenia jest większe i powinno być uważnie monitorowane (np. raz w tygodniu przez pierwszy miesiąc, do czasu zwiększenia stężenia hemoglobiny). U pacjentów, u których wystąpi niedokrwistość, może być konieczna transfuzja krwi. Należy rozważyć zmniejszenie dawki u pacjentów, u których wystąpi niedokrwistość, szczególnie w przypadku osób, które będą wymagać transfuzji krwinek czerwonych. *Małopłytkowość:* Małopłytkowość zazwyczaj występuje w ciągu pierwszych 3 miesięcy leczenia. U pacjentów z małą liczbą płytek krwi (< 100 x 10^9/l) na początku leczenia bardziej prawdopodobne jest wystąpienie małopłytkowości stopnia 3. lub wyższego w trakcie leczenia i należy ich uważnie monitorować (np. raz w tygodniu przez pierwszy miesiąc, do czasu zwiększenia liczby płytek krwi). Małopłytkowość jest zazwyczaj odwracalna i można ją wyrównać poprzez leczenie wspomagające, takie jak przerwy w dawkowaniu, zmniejszenie dawki i (lub) transfuzje płytek krwi w razie potrzeby. Należy poinformować pacjentów o zwiększonym ryzyku wystąpienia krwawienia związanego z małopłytkowością. *Neutropenia*: Neutropenia była zazwyczaj odwracalna i była wyrównywana przez tymczasowe przerwanie stosowania produktu Inrebic. Zdarzenia ze strony układu pokarmowego: Nudności, wymioty i biegunka są najczęstszymi działaniami niepożądanymi u pacjentów przyjmujących produkt Inrebic. Większość działań niepożądanych była zdarzeniami stopnia 1. lub 2. i zazwyczaj występowały w ciągu pierwszych 2 tygodni leczenia. Podczas stosowania produktu Inrebic należy rozważyć odpowiednie profilaktyczne leczenie przeciwwymiotne (np. antagoniści receptora 5-HT3). Należy niezwłocznie włączyć leczenie biegunki lekami przeciwbiegunkowymi w momencie wystąpienia pierwszych objawów. W przypadku nudności, wymiotów i biegunki stopnia 3. lub wyższego, które nie reagują na leczenie wspomagające w ciągu 48 godzin, stosowanie produktu Inrebic należy przerwać do ustąpienia do stopnia 1. lub poziomu niższego lub wyjściowego. Ponownie rozpocząć stosowanie produktu leczniczego w dawce dobowej o 100 mg niższej od ostatniej stosowanej dawki. Stężenie tiaminy należy monitorować i uzupełniać zgodnie z potrzebami. Hepatotoksyczność: Podczas stosowania produktu Inrebic zgłaszano przypadki zwiększenia aktywności AlAT i AspAT oraz zgłoszono jeden przypadek niewydolności wątroby. Czynność wątroby powinna być monitorowana u pacjentów w punkcie początkowym, co najmniej raz w miesiącu przez pierwsze 3 miesiące, okresowo podczas leczenia i zgodnie ze wskazaniami klinicznymi. Po zaobserwowaniu objawów toksyczności pacjenci powinni być monitorowani co najmniej co 2 tygodnie aż do ustąpienia objawów. Wzrost aktywności AlAT i AspAT był zasadniczo odwracalny po wprowadzeniu zmian dawkowania lub zakończeniu leczenia. Zwiększona aktywność amylazy i (lub) lipazy: Odnotowano zwiększenie aktywności amylazy i (lub) lipazy podczas stosowania produktu Inrebic i zgłoszono jeden przypadek zapalenia trzustki. Aktywność amylazy i lipazy powinna być monitorowa u pacjentów w punkcie początkowym, co najmniej raz w miesiącu przez pierwsze 3 miesiące, okresowo podczas leczenia i zgodnie ze wskazaniami klinicznymi. Po zaobserwowaniu objawów toksyczności pacjenci powinni być monitorowani co najmniej

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co 2 tygodnie aż do ustąpienia objawów. W przypadku zwiększenia aktywności amylazy i (lub) lipazy stopnia 3. lub wyższego zaleca się wprowadzenie zmiany dawki. Podwyższone stężenie kreatyniny: Odnotowano zwiększenie stężenia kreatyniny podczas stosowania produktu Inrebic. Stężenie kreatyniny powinno być monitorowane u pacjentów w punkcie początkowym, co najmniej raz w miesiącu przez pierwsze 3 miesiące, okresowo podczas leczenia i zgodnie ze wskazaniami klinicznymi. W przypadku ciężkich zaburzeń czynności nerek (CLcr 15 ml/min do 29 ml/min według C-G) zaleca się zmianę dawki. Interakcje: Jednoczesne stosowanie produktu Inrebic z silnymi inhibitorami CYP3A4 zwiększa ekspozycję na produkt Inrebic. Zwiększona ekspozycja na produkt Inrebic może zwiększyć ryzyko wystąpienia działań niepożądanych. W przypadku silnych inhibitorów CYP3A4 należy rozważyć alternatywne metody leczenia, które nie wywołują silnego działania hamującego aktywności CYP3A4. Jeżeli nie można zastosować zamienników silnych inhibitorów CYP3A4, dawkę produktu Inrebic należy zmniejszyć podczas stosowania silnych inhibitorów CYP3A4 (np. ketokonazol, rytonawir). Należy uważnie monitorować pacjentów (np. co najmniej raz w tygodniu) pod kątem bezpieczeństwa stosowania. Długotrwałe stosowanie umiarkowanych inhibitorów CYP3A4 może wymagać ścisłego monitorowania bezpieczeństwa pacjentów, oraz, w razie konieczności, zmiany dawkowania w oparciu o występujące działania niepożądane. Leki hamujące jednocześnie CYP3A4 i CYP2C19 (np. flukonazol, fluwoksamina) lub połączenia leków hamujących CYP3A4 i CYP2C19 mogą zwiększyć ekspozycję na produkt Inrebic i należy unikać ich stosowania u pacjentów przyjmujących Inrebic. Leki umiarkowanie lub silnie indukujące CYP3A4 (np. fenytoina, ryfampicyna, efawirenz) mogą zmniejszyć ekspozycję na produkt Inrebic i należy unikać ich stosowania u pacjentów przyjmujących produkt Inrebic. Jeśli produkt Inrebic ma być stosowany razem z substratem CYP3A4 (np. midazolam, symwastatyna), CYP2C19 (np. omeprazol, S-mefenytoina) lub CYP2D6 (np. metoprolol, dekstrometorfan), należy w razie potrzeby modyfikować dawki leków stosowanych w skojarzeniu oraz ściśle monitorować bezpieczeństwo stosowania i skuteczność. Jeśli produkt Inrebic ma być stosowany razem z lekami, które są wydalane przy udziale transportera kationów organicznych (ang. *organic cation transporter*, OCT) OCT2 oraz transportera wielolekowego i wypływu toksyn (ang. *multidrug and toxin extrusion*, MATE) (MATE)1/2 K (np. metformina), należy zachować ostrożność i w razie potrzeby zmodyfikować dawkę. Nie badano jednoczesnego stosowania krwiotwórczych czynników wzrostu oraz produktu Inrebic. Bezpieczeństwo stosowania i skuteczność takiego połączenia nie są znane. Szczególne grupy pacjentów: *Pacjenci w podeszłym wieku:* Doświadczenie w stosowaniu u pacjentów w wieku 75 lat i powyżej jest ograniczone. W badaniach klinicznych, 13,8% (28/203) pacjentów leczonych produktem Inrebic było w wieku 75 lat i powyżej, w tej grupie ciężkie działania niepożądane i działania niepożądane prowadzące do przerwania leczenia występowały częściej. Substancje pomocnicze: Kapsułki produktu Inrebic zawierają mniej niż 1 mmol (23 mg) sodu na dawkę, to znaczy lek uznaje się za „wolny od sodu". **Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: Ogólne informacje dotyczące bezpieczeństwa stosowania produktu Inrebic zebrano od 608 pacjentów, którzy otrzymywali stałe dawki Inrebic w badaniach klinicznych fazy 1, 2 i 3. Pierwotne lub wtórne włóknienie szpiku (JAKARTA, JAKARTA2, ARD11936): W badaniach klinicznych z udziałem pacjentów z pierwotnym włóknieniem szpiku (ang. *myelofibrosis*, MF), włóknieniem szpiku poprzedzonym czerwienicą prawdziwą (ang. *post polycythaemia vera myelofibrosis*, post-PV MF) lub włóknieniem szpiku poprzedzonym nadpłytkowością samoistną (ang. *post essential thrombocythemia myelofibrosis*, post-ET MF), przyjmujących produkt Inrebic w dawce 400 mg (N=203), w tym pacjentów po wcześniejszej ekspozycji na ruksolitynib (N=97; JAKARTA2) mediana ekspozycji wynosiła 35,6 tygodnia (przedział od 0,7 do 114,6 tygodni) a mediana liczby rozpoczętych cykli (1 cykl = 28 dni) wynosiła 9. Sześćdziesiąt trzy procent z 203 pacjentów było leczonych przez 6 miesięcy lub dłużej, a 38% przez 12 miesięcy lub dłużej. Wśród 203 pacjentów z MF leczonych dawką 400 mg produktu Inrebic w badaniach klinicznych, najczęstszymi niehematologicznymi działaniami niepożądanym były: biegunka (67,5%), nudności (61,6%) i wymioty (44,8%). Najczęstszymi hematologicznymi działaniami niepożądanymi były: niedokrwistość (99,0%) i małopłytkowość (68,5%), na podstawie badań laboratoryjnych (Tabela 2). Najczęstszymi ciężkimi działaniami niepożądanymi u pacjentów z MF leczonych dawką 400 mg były: niedokrwistość (2,5% na podstawie zgłaszanych działań niepożądanych, a nie badań laboratoryjnych) i biegunka (1,5%). Zakończenie udziału w badaniu ze względu na działania niepożądane, niezależnie od przyczyny, dotyczyło 24% pacjentów przyjmujących dawkę 400 mg produktu Inrebic. Tabelaryczne zestawienie działań niepożądanych: Działania niepożądane obserwowane w badaniach klinicznych przez cały czas trwania leczenia (Tabela 2) wymieniono według klasyfikacji układów i narządów MedDRA. W obrębie każdej klasy układów i narządów, działania niepożądane są wymienione według częstości występowania, zaczynając od działań obserwowanych najczęściej. Częstość występowania zdefiniowano w następujący sposób: bardzo często (≥ 1/10); często (≥1/100 do <1/10); niezbyt często (≥1/1000 do <1/100); rzadko (≥1/10 000 do <1/1000); bardzo rzadko (<1/10 000) i nieznana (częstość nie może być określona na podstawie dostępnych danych).

Tabela 2: Wszystkie działania niepożądane produktu leczniczego według klasyfikacji układów i narządów oraz preferowanej terminologii

Klasyfikacja układów i narządów	Działanie niepożądane	Wszystkie stopnie Częstość
Zakażenia i zarażenia pasożytnicze	Zakażenie dróg moczowych	Bardzo często
Zaburzenia krwi i układu chłonnego	Niedokrwistość[a]	Bardzo często
	Małopłytkowość[a]	Bardzo często
	Neutropenia[a]	Bardzo często
	Krwawienie[b]	Bardzo często
Zaburzenia metabolizmu i odżywiania	Podwyższona aktywność lipazy[a]	Bardzo często
	Podwyższona aktywność amylazy[a]	Bardzo często
Zaburzenia układu nerwowego	Ból głowy	Bardzo często
	Encefalopatia Wernickego	Często
	Zawroty głowy	Często
Zaburzenia naczyniowe	Nadciśnienie	Często
Zaburzenia żołądka i jelit	Biegunka	Bardzo często
	Wymioty	Bardzo często
	Nudności	Bardzo często
	Zaparcia	Bardzo często
	Niestrawność	Często
Zaburzenia wątroby i dróg żółciowych	Zwiększona aktywność aminotransferazy alaninowej[a]	Bardzo często
	Zwiększona aktywność aminotransferazy asparaginianowej[a]	Bardzo często
Zaburzenia mięśniowo-szkieletowe i tkanki łącznej	Ból kości	Często
	Kurcze mięśni	Bardzo często
	Ból kończyn	Często
Zaburzenia nerek i dróg moczowych	Wzrost stężenia kreatyniny we krwi[a]	Bardzo często
	Dyzuria	Często
Zaburzenia ogólne i stany w miejscu podania	Zmęczenie/astenia	Bardzo często
Badania diagnostyczne	Zwiększenie masy ciała	Często

MedDRA (Medical dictionary of regulatory activities) = Słownik terminów medycznych dla czynności regulacyjnych
SMQ (Standardized MedDRA Quer) = standaryzowany wpise MedDRA (grupowanie kilku preferowanych terminów MedDRA w celu ujęcia koncepcji medycznej).
[a] Częstość opiera się na badaniach laboratoryjnych.
[b] Krwawienie obejmuje wszelkie rodzaje związane z małopłytkowością wymagającą interwencji klinicznej. Krwawienie ocenia się przy użyciu terminów związanych z krwotokami MedDRA SMQ (szeroki zakres).

Opis wybranych działań niepożądanych: *Encefalopatia, w tym encefalopatia Wernickego*: Ciężkie przypadki encefalopatii, w tym 1 potwierdzony przypadek encefalopatii Wernickego zgłoszono u 1,3% (8/608) pacjentów przyjmujących produkt Inrebic w badaniach klinicznych; 7 pacjentów przyjmowało produkt Inrebic w dawce 500 mg na dobę przed wystąpieniem objawów neurologicznych i występowały u nich czynniki predysponujące, takie jak niedożywienie, działania niepożądane ze strony żołądka i jelit oraz inne czynniki ryzyka, które mogą doprowadzić do niedoboru tiaminy. U jednego pacjenta leczonego produktem Inrebic w dawce 400 mg stwierdzono encefalopatię wątrobową. Większość zdarzeń ustąpiła z pewnymi pozostającymi objawami neurologicznymi, w tym utratą pamięci, zaburzeniami poznawczymi i zawrotami głowy, z wyjątkiem jednego przypadku śmiertelnego (1/608; 0,16%). Był to pacjent z rakiem głowy i szyi, przerzutami do mózgu, trudnościami z jedzeniem i utratą masy ciała, który otrzymywał fedratynib w dawce 500 mg w ramach badania w innym wskazaniu. *Toksyczny wpływ na układ pokarmowy:* Nudności, wymioty i biegunka są najczęstszymi działaniami niepożądanymi u pacjentów przyjmujących produkt Inrebic. U pacjentów z MF przyjmujących produkt Inrebic w dawce 400 mg, biegunka wystąpiła u 68% pacjentów, nudności u 62% pacjentów a wymioty u 45% pacjentów. Biegunka, nudności i wymioty stopnia 3. wystąpiły odpowiednio u 5%, 0,5% i 2% pacjentów. Mediana czasu do wystąpienia nudności, wymiotów i biegunki dowolnego stopnia wynosiła 2 dni, przy czym w 75% przypadków wystąpiły one w ciągu 3 tygodni od rozpoczęcia leczenia. Przerwy w przyjmowaniu i zmniejszenie dawki z powodu objawów toksyczności ze strony układu pokarmowego zgłoszono odpowiednio u 11% i 9% pacjentów. Stosowanie produktu Inrebic w dawce 400 mg zakończono z powodu wystąpienia objawów toksyczności ze strony układu pokarmowego u 4% pacjentów. *Niedokrwistość:* U 52% pacjentów z pierwotnym lub wtórnym włóknieniem szpiku leczonych produktem Inrebic w dawce 400 mg, wystąpiła niedokrwistość stopnia 3. Mediana czasu do pierwszego wystąpienia niedokrwistości stopnia 3. wynosiła około 60 dni, przy czym w 75% przypadków wystąpiła ona w ciągu 4 miesięcy od rozpoczęcia leczenia. 58% pacjentów leczonych produktem Inrebic w dawce 400 mg otrzymywało transfuzje krwinek czerwonych, a stosowanie produktu Inrebic w dawce 400 mg z powodu niedokrwistości zakończono u 1,5% pacjentów. *Małopłytkowość:* U pacjentów z pierwotnym lub wtórnym zwłóknieniem szpiku leczonych produktem Inrebic w dawce 400 mg, odpowiednio u 14% i 9% pacjentów wystąpiła trombocytopenia stopnia 3. i 4. Mediana czasu do pierwszego wystąpienia niedokrwistości stopnia 3. lub niedokrwistości stopnia 4. wynosiła około 70 dni, przy czym w 75% przypadków wystąpiła ona w ciągu 7 miesięcy od rozpoczęcia leczenia. 9% pacjentów leczonych produktem Inrebic w dawce 400 mg otrzymywało transfuzje płytek krwi. Krwawienie (związane z małopłytkowością), które wymagało interwencji klinicznej wystąpiło u 11% pacjentów. U 3% pacjentów zakończono leczenie z powodu małopłytkowości. *Neutropenia* Neutropenia stopnia 4. wystąpiła u 3,5% pacjentów, a u 0,5% pacjentów przerwano stosowania leku z powodu neutropenii. *Hepatotoksyczność*: Zwiększenie aktywności AlAT i AspAT (wszystkie stopnie) wystąpiły odpowiednio u 52% i 59% pacjentów, w tym stopnia 3. i 4. u odpowiednio 3% i 2% pacjentów przyjmujących produkt Inrebic w dawce 400 mg. Mediana czasu do wystąpienia zwiększenia aktywności transaminazy dowolnego stopnia wynosiła około 1 miesiąca, przy czym w 75% przypadków wystąpiło ono w ciągu 3 miesięcy od rozpoczęcia leczenia. *Zwiększenie aktywności amylazy i (lub) lipazy:* Zwiększenie aktywności amylazy i (lub) lipazy (wszystkie stopnie) wystąpiło odpowiednio u 24% i 40% pacjentów z MF. Większość tych zdarzeń była stopnia 1. lub 2., a odpowiednio u 2,5% i 12% pacjentów była stopnia 3. lub 4. Mediana czasu do pierwszego wystąpienia zwiększenia aktywności amylazy lub lipazy dowolnego stopnia 16 dni, przy czym w 75% przypadków, wystąpiła ono w ciągu 3 miesięcy od rozpoczęcia leczenia. Zakończenie leczenia z powodu zwiększenia aktywności amylazy i (lub) lipazy wystąpiło u 1,0% pacjentów przyjmujących produkt Inrebic w dawce 400 mg. *Zwiększone stężenie kreatyniny*: Zwiększone stężenie kreatyniny (wszystkie stopnie) wystąpiło u 74% pacjentów z MF przyjmujących produkt Inrebic w dawce 400 mg. Zwiększenie stężenia było zazwyczaj bezobjawowymi zdarzeniami stopnia 1. lub 2., przy czym zwiększenie stopnia 3. zaobserwowano u 3% pacjentów. Mediana czasu do pierwszego wystąpienia zwiększenia stężenia kreatyniny dowolnego stopnia wynosiła 27 dni, przy czym w 75% przypadków wystąpiła ona w ciągu 3 miesięcy od rozpoczęcia leczenia. Przerwanie i zmniejszenie dawkowania z powodu zwiększonego stężenia kreatyniny zgłoszono odpowiednio u 1% i 0,5% pacjentów. U 1,5% pacjentów przyjmujących produkt Inrebic w dawce 400 mg zakończono leczenie z powodu zwiększenia stężenia kreatyniny.

▼Niniejszy produkt leczniczy będzie dodatkowo monitorowany. Umożliwi to szybkie zidentyfikowanie nowych informacji o bezpieczeństwie. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane.
Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem:
Departament Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, 02-222 Warszawa, Tel.: + 48 22 49 21 301, Faks: + 48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl
Podmiot Odpowiedzialny: Bristol Myers Squibb Pharma EEIG, Plaza 254, Blanchardstown Corporate Park 2, Dublin 15, D15 T867, Irlandia .
Numer pozwolenia na dopuszczenie do obrotu wydanych przez Komisję Europejską: EU/1/20/1514/001.
Kategoria dostępności: Produkt leczniczy wydawany na receptę do zastrzeżonego stosowania.
Przed zastosowaniem leku należy zapoznać się z Charakterystyką Produktu Leczniczego.
Przedstawiciel podmiotu odpowiedzialnego: Celgene Sp. z o.o.,
Al. Armii Ludowej 26, 00-609 Warszawa. Tel. (22) 260 64 00.
Data przygotowania: październik 2021 r. (na podstawie ChPL z dnia 2021-10-19).

Rydapt® *Midostauryna*

▼Niniejszy produkt leczniczy będzie dodatkowo monitorowany. Umożliwi to szybkie zidentyfikowanie nowych informacji o bezpieczeństwie. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane. Aby dowiedzieć się, jak zgłaszać działania niepożądane – patrz punkt „Działania niepożądane".

Postać, skład: Kapsułka, miękka. Każda kapsułka miękka zawiera 25 mg midostauryny. Substancje pomocnicze o znanym działaniu: Każda kapsułka miękka zawiera około 83 mg bezwodnego etanolu i 415 mg hydroksystearynianu makrogloglicerolu. **Wskazania:** Lek Rydapt jest wskazany: • w skojarzeniu ze standardową chemioterapią indukcyjną daunarubicyną i cytarabiną oraz konsolidacyjną dużymi dawkami cytarabiny, oraz u pacjentów z całkowitą odpowiedzią, a następnie jako monoterapia podtrzymująca lekiem Rydapt u dorosłych pacjentów z noworozpoznaną ostrą białaczką szpikową (ang. *acute myeloid leukaemia* – AML) z mutacją genu FLT3 (patrz punkt 4.2 ChPL); • w monoterapii w leczeniu dorosłych pacjentów z agresywną mastocytozą układową (ASM), mastocytozą układową z nowotworem układu krwiotwórczego (SM-AHN) lub białaczką mastocytarną (MCL). **Dawkowanie:** Leczenie lekiem Rydapt powinno być rozpoczynane przez lekarza posiadającego doświadczenie w stosowaniu leków przeciwnowotworowych. Przed przyjęciem midostauryny, u pacjentów z AML należy potwierdzić występowanie mutacji FLT3 (wewnątrztandemowej duplikacji [ITD] lub mutacji w obrębie domeny kinazy tyrozynowej [TKD]) przy pomocy zwalidowanego testu. Dawkowanie: Lek Rydapt należy przyjmować doustnie dwa razy na dobę w odstępach około 12-godzinnych. Kapsułki należy przyjmować z pokarmem. Leki zapobiegające wymiotom należy podawać zgodnie z lokalnie obowiązującą praktyką medyczną, w zależności od tolerancji pacjenta. *AML:* Zalecana dawka leku Rydapt wynosi 50 mg doustnie dwa razy na dobę. Lek Rydapt jest podawany od 8 do 21 dnia cyklu chemioterapii indukcyjnej i konsolidacyjnej, a następnie u pacjentów z całkowitą odpowiedzią codziennie jako monoterapia podtrzymująca do nawrotu choroby przez maksymalnie 12 cykli po 28 dni każdy (patrz punkt 4.1). U pacjentów będących biorcami przeszczepu krwiotwórczych komórek macierzystych (ang. *stem cell transplant* – SCT) leczenie lekiem Rydapt należy przerwać 48 godzin przed kondycjonującym schematem leczenia poprzedzającym SCT. *Modyfikacje dawkowania w AML:* W Tabeli 1 przedstawiono zalecenia dotyczące modyfikacji dawkowania u pacjentów z AML.

Tabela 1. Zalecenia dotyczące przerwania, zmniejszenia dawki i zakończenia podawania leku Rydapt u pacjentów z AML

Faza	**Kryteria**	**Dawkowanie leku Rydapt**
Indukcji, konsolidacji i leczenia podtrzymującego	Nacieki w płucach stopnia 3/4	Przerwać podawanie leku Rydapt przez pozostałą część cyklu. Wznowić podawanie leku Rydapt w takiej samej dawce, gdy naciek zmniejszy się do stopnia ≤1.
	Inne niehematologiczne działania toksyczne stopnia 3/4	Przerwać podawanie leku Rydapt do czasu złagodzenia do stopnia ≤2 działań toksycznych uznawanych za posiadające przynajmniej możliwy związek z lekiem Rydapt, a następnie wznowić podawanie leku Rydapt.
	Odstęp QTc >470 ms i ≤500 ms	Zmniejszyć dawkę leku Rydapt do 50 mg raz na dobę przez pozostałą część cyklu. Wznowić podawanie leku Rydapt w dawce początkowej w następnym cyklu, jeśli odstęp QTc zmniejszy się do ≤470 ms na początku tego cyklu. W przeciwnym razie kontynuować podawanie leku Rydapt w dawce 50 mg raz na dobę.
	Odstęp QTc >500 ms	Wstrzymać lub przerwać podawanie leku Rydapt przez pozostałą część cyklu. Jeśli odstęp QTc zmniejszy się do ≤470 ms tuż przed rozpoczęciem kolejnego cyklu, wznowić podawanie leku Rydapt w dawce początkowej. Jeśli odstęp QTc nie zmniejszy się do czasu rozpoczęcia kolejnego cyklu, nie podawać leku Rydapt podczas tego cyklu. Podawanie leku Rydapt można wstrzymać na tyle cykli, ile to konieczne do czasu poprawy wartości QTc.
Tylko leczenie podtrzymujące	Neutropenia stopnia 4 (ANC <0,5 x 10^9/l)	Przerwać podawanie leku Rydapt do czasu, gdy ANC ≥1,0 x 10^9/l, następnie wznowić podawanie w dawce 50 mg dwa razy na dobę. Jeśli neutropenia (ANC <1,0 x 10^9/l) utrzymuje się przez >2 tygodnie i istnieją podejrzenia, że ma ona związek z lekiem Rydapt, podawanie leku Rydapt należy zakończyć.
	Utrzymujące się działania toksyczne stopnia 1/2	Utrzymujące się działania toksyczne o 1. lub 2. stopniu nasilenia, które pacjenci uznają za niemożliwe do zaakceptowania mogą spowodować przerwanie leczenia nawet na 28 dni.
ANC (ang. *Absolute Neutrophil Count*): bezwzględna liczba neutrofilów		

ASM, SM-AHN i MCL: Zalecana dawka początkowa leku Rydapt to 100 mg doustnie dwa razy na dobę. Leczenie należy kontynuować tak długo, jak długo obserwuje się korzyści kliniczne lub do czasu wystąpienia niemożliwych do zaakceptowania działań toksycznych. *Modyfikacje dawkowania w ASM, SM-AHN i MCL:* W Tabeli 2 przedstawiono modyfikacje dotyczące dawkowania leku Rydapt u pacjentów z ASM, SM-AHN i MCL.

Tabela 2. Zalecenia dotyczące przerwania, zmniejszenia dawki i zakończenia podawania leku Rydapt u pacjentów z ASM, SM-AHN lub MCL

Kryteria	**Dawkowanie leku Rydapt**
ANC <1,0 x 10^9/l przypisywane produktowi Rydapt u pacjentów bez MCL lub ANC poniżej 0,5 x 10^9/l przypisywane produktowi Rydapt u pacjentów z wyjściową wartością ANC wynoszącą 0,5-1,5 x 10^9/l	Przerwać podawanie leku Rydapt do czasu, gdy ANC wyniesie ≥1,0 x 10^9/l, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli mała liczba ANC utrzymuje się przez >21 dni i istnieją podejrzenia, że ma to związek z lekiem Rydapt.
Liczba płytek krwi mniejsza niż 50 x 10^9/l przypisywana produktowi Rydapt u pacjentów bez MCL lub liczba płytek krwi mniejsza niż 25 x 10^9/l przypisywana produktowi Rydapt u pacjentów z wyjściową liczbą płytek krwi wynoszącą 25-75 x 10^9/l	Przerwać podawanie leku Rydapt do czasu, gdy liczba płytek krwi wyniesie 50 x 10^9/l lub więcej, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli mała liczba płytek krwi utrzymuje się przez >21 dni i istnieją podejrzenia, że ma to związek z lekiem Rydapt.
Stężenie hemoglobiny poniżej 8 g/dl przypisywane produktowi Rydapt u pacjentów bez MCL lub zagrażająca życiu niedokrwistość przypisywana produktowi Rydapt u pacjentów z wyjściowym stężeniem hemoglobiny wynoszącym 8-10 g/dl	Przerwać podawanie leku Rydapt do czasu, gdy stężenie hemoglobiny wyniesie 8 g/dl lub więcej, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli małe stężenie hemoglobiny utrzymuje się przez >21 dni i istnieją podejrzenia, że ma to związek z lekiem Rydapt.
Nudności i (lub) wymioty stopnia 3/4 pomimo stosowania optymalnej terapii przeciwwymiotnej	Przerwać podawanie leku Rydapt na 3 dni (6 dawek), następnie wznowić podawanie w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, stopniowo zwiększać dawkę do 100 mg dwa razy na dobę.
Inne niehematologiczne działania toksyczne stopnia 3/4	Przerwać podawanie leku Rydapt do czasu, gdy nasilenie zdarzenia zmniejszy się do stopnia ≤2, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli działanie toksyczne nie zmniejszy się do stopnia ≤2 w ciągu 21 dni lub jeśli dojdzie do nawrotu ciężkiego działania toksycznego przy stosowaniu zmniejszonej dawki leku Rydapt.
ANC: bezwzględna liczba neutrofilów. Stopień nasilenia wg CTCAE: stopień 1 = objawy łagodne; 2 = objawy umiarkowane; 3 = objawy ciężkie; 4 = objawy zagrażające życiu.	

Pominięcie dawki: W przypadku pominięcia dawki należy przyjąć kolejną dawkę o wyznaczonej porze. W przypadku wystąpienia wymiotów nie należy przyjmować dodatkowej dawki leku Rydapt, ale przyjąć kolejną dawkę o wyznaczonej porze. *Szczególne populacje pacjentów: Osoby w podeszłym wieku (≥65 lat):* Nie ma konieczności dostosowania schematu dawkowania u pacjentów w wieku powyżej 65 lat (patrz punkt 5.2 ChPL). Istnieje ograniczone doświadczenie ze stosowaniem midostauryny u pacjentów z AML w wieku 60-70 lat oraz brak jest doświadczenia ze stosowaniem u pacjentów z AML w wieku powyżej 70 lat. U pacjentów w wieku ≥60 lat, lek Rydapt powinien być stosowany wyłącznie u pacjentów kwalifikujących się do intensywnej chemioterapii indukcyjnej, o odpowiednim stanie sprawności i bez istotnych chorób współistniejących. *Zaburzenia czynności nerek:* Nie ma konieczności dostosowania dawki u pacjentów z łagodnymi lub umiarkowanymi zaburzeniami czynności nerek. Doświadczenie kliniczne u pacjentów z ciężkimi zaburzeniami czynności nerek jest ograniczone i brak jest dostępnych danych od pacjentów ze schyłkową niewydolnością nerek (patrz punkt 4.4 i 5.2 ChPL). *Zaburzenia czynności wątroby:* Nie ma konieczności dostosowania dawki u pacjentów z łagodnymi lub umiarkowanymi (stopnia A lub B w skali Child-Pugh) zaburzeniami czynności wątroby (patrz punkt 5.2 ChPL). Nie ukończono żadnego badania z udziałem pacjentów z ciężkimi (stopnia C wg Child-Pugh C) zaburzeniami czynności wątroby (patrz punkt 4.4 ChPL). *Ostra białaczka promielocytowa:* Lek Rydapt nie był badany u pacjentów z ostrą białaczką promielocytową i dlatego jego stosowanie nie jest zalecane w tej populacji pacjentów. *Dzieci i młodzież:* Produkt leczniczy Rydapt nie powinien być stosowany w skojarzeniu ze schematami intensywnej chemioterapii skojarzonej przeznaczonymi dla dzieci i młodzieży z AML, obejmującymi antracykliny, fludarabinę i cytarabinę z uwagi na ryzyko przedłużającej się normalizacji parametrów hematologicznych (na przykład przedłużającą się ciężką neutropenię i małopłytkowość) (patrz punkty 4.4 i 5.1). Sposób podawania: Lek Rydapt jest przeznaczony do podawania doustnego. Kapsułki należy połykać w całości, popijając szklanką wody. Nie należy ich otwierać, rozgryzać ani żuć, co zapewni dostarczenie odpowiedniej dawki leku i pozwoli uniknąć nieprzyjemnego smaku zawartości kapsułki. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą wymienioną w punkcie 6.1. Jednoczesne podawanie silnych induktorów CYP3A4, np. ryfampicyny, ziela dziurawca (*Hypericum perforatum*), karbamazepiny, enzalutamidu, fenytoiny (patrz punkt 4.5 ChPL). **Środki ostrożności/Ostrzeżenia:** Neutropenia i zakażenia: U pacjentów otrzymujących lek Rydapt w monoterapii i w skojarzeniu z chemioterapią występowała neutropenia (patrz punkt 4.8 ChPL). Ciężka neutropenia (ANC <0,5 x 10^9/l) była na ogół odwracalna po wstrzymaniu podawania leku Rydapt aż do powrotu liczby neutrofilów do wartości początkowych i odstawieniu leku w badaniach z ASM, SM-AHN i MCL. Należy regularnie kontrolować liczbę białych krwinek, zwłaszcza na początku leczenia. U pacjentów, u których wystąpi ciężka neutropenia o niewyjaśnionej etiologii, leczenie lekiem Rydapt należy przerwać do czasu, gdy ANC wyniesie ≥1,0 x 10^9/l, zgodnie z zaleceniami przedstawionymi w Tabelach 1 i 2. Lek Rydapt należy odstawić u pacjentów, u których wystąpi nawracająca lub przedłużająca się ciężka neutropenia, podejrzewana o związek z lekiem Rydapt (patrz punkt 4.2 ChPL). Przed rozpoczęciem podawania leku Rydapt w monoterapii należy opanować wszelkie czynne, ciężkie zakażenia. Należy monitorować pacjentów pod kątem przedmiotowych i podmiotowych objawów zakażenia, w tym wszelkich zakażeń związanych ze stosowaniem aparatury medycznej, a w przypadku rozpoznania zakażenia, należy szybko wdrożyć odpowiednie leczenie, w tym w razie konieczności, odstawić lek Rydapt. Zaburzenia czynności serca: Pacjenci z objawową zastoinową niewydolnością serca byli wykluczeni z badań klinicznych. W badaniach z ASM, SM-AHN i MCL występowały zaburzenia czynności serca, takie jak zastoinowa niewydolność serca (ang. *congestive heart failure* – CHF) (w tym przypadki śmiertelne) i przejściowe obniżenie frakcji wyrzutowej lewej komory (ang. *left ventricular ejection fraction* – LVEF). W randomizowanym badaniu z AML nie obserwowano różnic dotyczących CHF pomiędzy grupą otrzymującą Rydapt + chemioterapię a grupą otrzymującą placebo + chemioterapię. U pacjentów podlegających ryzyku Rydapt należy stosować z zachowaniem ostrożności, a pacjenci wymagają ścisłego monitorowania poprzez ocenę LVEF, jeśli wystąpią wskazania kliniczne (na początku leczenia i w czasie jego trwania). U pacjentów leczonych midostauryną odnotowano zwiększoną częstość wydłużenia odstępu QTc (patrz punkt 4.8 ChPL), jednak nie znaleziono mechanistycznego wyjaśnienia dla tej obserwacji. Należy zachować ostrożność u pacjentów z ryzykiem wydłużenia QTc (np. spowodowanym jednoczesnym stosowaniem produktów leczniczych i (lub) zaburzeniami równowagi elektrolitowej). Należy rozważyć ocenę odstępu QT w badaniu EKG, jeśli Rydapt jest przyjmowany jednocześnie z produktami leczniczymi, które mogą wydłużać odstęp QT.

Toksyczność płucna: U pacjentów leczonych lekiem Rydapt w monoterapii lub w skojarzeniu z chemioterapią występowała choroba śródmiąższowa płuc i zapalenie płuc, w niektórych przypadkach zakończone zgonem. Należy monitorować pacjentów pod kątem objawów płucnych wskazujących na chorobę śródmiąższową płuc lub zapalenie płuc oraz odstawić Rydapt u pacjentów z objawami płucnymi wskazującymi na chorobę śródmiąższową płuc lub zapalenie płuc, o nasileniu ≥stopnia 3 (wg NCI CTCAE). Toksyczne działanie na zarodek i płód oraz karmienie piersią: Należy poinformować kobiety w ciąży o potencjalnym ryzyku dla płodu; należy doradzić kobietom w wieku rozrodczym wykonanie testu ciążowego w okresie 7 dni przed rozpoczęciem leczenia produktem leczniczym Rydapt oraz stosowanie skutecznej antykoncepcji podczas leczenia produktem leczniczym Rydapt i przez co najmniej 4 miesiące po jego zakończeniu. Kobiety stosujące antykoncepcję hormonalną powinny dodatkowo stosować barierową metodę antykoncepcyjną. Z uwagi na możliwość wystąpienia ciężkich działań niepożądanych produktu leczniczego Rydapt u dzieci karmionych piersią, kobiety powinny zaprzestać karmienia piersią podczas leczenia produktem leczniczy Rydapt i przez co najmniej 4 miesiące po zakończeniu leczenia (patrz punkt 4.6 ChPL). Dzieci i młodzież: Produkt leczniczy Rydapt nie powinien być stosowany w skojarzeniu ze schematami intensywnej chemioterapii skojarzonej przeznaczonymi dla dzieci i młodzieży z AML, obejmującymi antracykliny, fludarabinę i cytarabinę z uwagi na ryzyko przedłużającej się normalizacji parametrów hematologicznych (na przykład przedłużającą się ciężką neutropenię i małopłytkowość) (patrz punkty 4.2 i 5.1 ChPL). Ciężkie zaburzenia czynności wątroby: Należy zachować ostrożność rozważając podanie midostauryny pacjentom z ciężkimi zaburzeniami czynności wątroby i starannie ich monitorować w kierunku działań toksycznych (patrz punkt 5.2 ChPL). Ciężkie zaburzenia czynności nerek: Należy zachować ostrożność rozważając podanie midostauryny pacjentom z ciężkimi zaburzeniami czynności nerek lub schyłkową niewydolnością nerek i należy uważnie monitorować pacjentów pod kątem działań toksycznych (patrz punkt 5.2 ChPL). Interakcje: Wymaga się zachowania ostrożności, gdy midostauryna jest przepisywana jednocześnie z produktami leczniczymi będącymi silnymi inhibitorami CYP3A4, takimi jak m. in. leki przeciwgrzybicze (np. ketokonazol), pewne leki antywirusowe (np. rytonawir), antybiotyki makrolidowe (np. klarytromycyna) i nefazodon, ponieważ mogą one zwiększać stężenie midostauryny w osoczu, zwłaszcza w przypadku (ponownego) rozpoczynania leczenia midostauryną (patrz punkt 4.5 ChPL). Należy rozważyć zastosowanie alternatywnych produktów leczniczych nieposiadających silnego działania hamującego na CYP3A4. W sytuacji braku zadowalającej alternatywy terapeutycznej należy uważnie monitorować pacjentów pod kątem działań toksycznych związanych z midostauryną. Substancje pomocnicze: Lek Rydapt zawiera hydroksystearynian makrogologlicerolu, który może powodować dyskomfort żołądkowy i biegunkę. Ten lek zawiera 666 mg alkoholu (etanolu) w każdej 200 mg dawce (maksymalnej dawce dobowej), co jest równoważne 14 % obj. bezwodnego etanolu. Ilość alkoholu w 200 mg dawce tego leku jest równoważna 16,9 ml piwa lub 7,0 ml wina. Mała ilość alkoholu w tym leku nie będzie powodowała zauważalnych skutków. Alkohol może być szkodliwy u pacjentów z problemami związanymi z alkoholem, padaczką lub chorobami wątroby bądź podczas ciąży lub karmienia piersią. Wpływ na płodność, ciążę i laktację: Kobiety w wieku rozrodczym: Kobiety w wieku rozrodczym należy poinformować, że badania na zwierzętach wykazują szkodliwy wpływ midostauryny na rozwijający się płód. Aktywnym seksualnie kobietom w wieku rozrodczym należy doradzić wykonanie testu ciążowego w ciągu 7 dni przed rozpoczęciem leczenia lekiem Rydapt oraz stosowanie skutecznej antykoncepcji (metod ze wskaźnikiem ciąż wynoszącym mniej niż 1%) podczas przyjmowania leku Rydapt i przez co najmniej 4 miesiące po zakończeniu leczenia lekiem Rydapt. Obecnie nie wiadomo, czy midostauryna może zmniejszać skuteczność hormonalnych środków antykoncepcyjnych i dlatego kobiety stosujące antykoncepcję hormonalną powinny dodatkowo używać barierowych metod antykoncepcyjnych. Ciąża: Midostauryna może powodować uszkodzenie płodu, gdy jest podawana kobietom w ciąży. Brak jest odpowiednich, dobrze kontrolowanych badań z udziałem kobiet ciężarnych. Badania wpływu na reprodukcję prowadzone na szczurach i królikach wykazały, że midostauryna wywoływała działanie toksyczne na płód (patrz punkt 5.3 ChPL). Lek Rydapt nie jest zalecany do stosowania w okresie ciąży lub u kobiet w wieku rozrodczym niestosujących skutecznej metody antykoncepcji. Należy poinformować kobiety w ciąży o potencjalnym ryzyku dla płodu. Karmienie piersią: Nie wiadomo, czy midostauryna lub jej czynne metabolity przenikają do mleka kobiecego. Dostępne dane pochodzące z badań na zwierzętach wykazały, że midostauryna i jej czynne metabolity przenikają do mleka karmiących szczurów. Karmienie piersią należy przerwać podczas leczenia lekiem Rydapt i przez co najmniej 4 miesiące po zakończeniu leczenia. Płodność: Brak jest danych dotyczących wpływu leku Rydapt na płodność ludzi. Badania na zwierzętach, którym podawano midostaurynę wykazały zaburzenia płodności (patrz punkt 5.3 ChPL). **Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: *AML:* Ocena bezpieczeństwa stosowania leku Rydapt (podawanego w dawce 50 mg dwa razy na dobę) u pacjentów z noworozpoznaną AML z mutacją FLT3 opiera się na wynikach randomizowanego, podwójnie zaślepionego badania III fazy kontrolowanego placebo z udziałem 717 pacjentów. Ogólna mediana czasu trwania ekspozycji wyniosła 42 dni (zakres od 2 do 576 dni) u pacjentów z grupy otrzymującej Rydapt w skojarzeniu ze standardową chemioterapią w porównaniu z 34 dniami (zakres od 1 do 465 dni) u pacjentów z grupy otrzymującej placebo w skojarzeniu ze standardową chemioterapią. Mediana czasu trwania ekspozycji na lek w fazie leczenia podtrzymującego wyniosła 11 miesięcy w obu grupach badania (16 do 520 dni u pacjentów otrzymujących Rydapt oraz 22 do 381 dni u pacjentów z grupy placebo) u 205 pacjentów (120 z grupy otrzymującej Rydapt i 85 z grupy otrzymującej placebo), którzy weszli do fazy leczenia podtrzymującego. Najczęstszymi działaniami niepożądanymi (ang. *adverse drug reaction* – ADR) w grupie otrzymującej Rydapt były: gorączka neutropeniczna (83,4%), nudności (83,4%), złuszczające zapalenie skóry (61,6%), wymioty (60,7%), ból głowy (45,9%), wybroczyny (35,8%) i gorączka (34,5%). Najczęstszymi ADR stopnia 3/4 były gorączką neutropeniczna (83,5%), limfopenia (20,0%), zakażenia związane z zastosowaniem aparatury medycznej (15,7%), złuszczające zapalenie skóry (13,6%), hiperglikemia (7,0%) i nudności (5,8%). Najczęstszymi odchyleniami w wynikach badań laboratoryjnych było zmniejszenie stężenia hemoglobiny (97,3%), zmniejszenie ANC (86,7%), zwiększenie aktywności AIAT (84,2%), zwiększenie aktywności AspAT (73,9%) i hipokaliemia (61,7%). Najczęstszymi odchyleniami w wynikach badań laboratoryjnych w 3/4 stopniu nasilenia było zmniejszenie ANC (85,8%), zmniejszenie stężenia hemoglobiny (78,5%), zwiększenie aktywności AIAT (19,4%) i hipokaliemia (13,9%). Poważne ADR wystąpiły z podobną częstością u pacjentów leczonych lekiem Rydapt, jak u pacjentów z grupy otrzymującej placebo. Najczęstszym poważnym ADR w obu grupach była gorączka neutropeniczna (16%). Zakończenie leczenia z powodu jakiegokolwiek działania niepożądanego miało miejsce u 3,1% pacjentów z grupy otrzymującej Rydapt w porównaniu z 1,3% pacjentów z grupy otrzymującej placebo. Najczęstszym działaniem niepożądanym stopnia 3/4 prowadzącym do zakończenia leczenia w grupie otrzymującej Rydapt było złuszczające zapalenie skóry (1,2%). *Profil bezpieczeństwa w fazie leczenia podtrzymującego:* W Tabeli 3 przedstawiono częstość występowania ADR w całym okresie badania, jednak po dokonaniu odrębnej oceny dla fazy leczenia podtrzymującego (monoterapia lekiem Rydapt lub placebo) stwierdzono różnicę dotyczącą rodzaju i nasilenia ADR. Całkowita częstość występowania ADR w fazie leczenia podtrzymującego była na ogół mniejsza niż w fazie leczenia indukcyjnego i konsolidacyjnego. Częstość występowania działań niepożądanych była jednak większa w grupie otrzymującej Rydapt niż w grupie placebo w fazie leczenia podtrzymującego. Do ADR występujących częściej w grupie midostauryny w porównaniu z grupą placebo w fazie leczenia podtrzymującego należały: nudności (46,4% w porównaniu z 17,9%), hiperglikemia (20,2% w porównaniu z 12,5%), wymioty (19% w porównaniu z 5,4%) i wydłużenie odstępu QT (11,9% w porównaniu z 5,4%). Większość zgłaszanych nieprawidłowości hematologicznych występowało w fazie indukcji i konsolidacji, gdy pacjenci otrzymywali Rydapt lub placebo w skojarzeniu z chemioterapią. Najczęstszymi zaburzeniami hematologicznymi stopnia 3/4 zgłaszanymi u pacjentów w fazie leczenia podtrzymującego lekiem Rydapt było zmniejszenie ANC (20,8% w por. z 18,8%) i leukopenia (7,5% w por. z 5,9%). ADR zgłaszane w fazie leczenia podtrzymującego były przyczyną przerwania leczenia u 1,2% pacjentów w grupie otrzymującej Rydapt i u żadnego pacjenta z grupy placebo. *ASM, SM-AHN i MCL:* Bezpieczeństwo stosowania leku Rydapt (100 mg dwa razy na dobę) podawanego w monoterapii pacjentom z ASM, SM-AHN i MCL było oceniane u 142 pacjentów w dwóch otwartych, wieloośrodkowych badaniach z jedną grupą leczenia. Mediana czasu trwania ekspozycji na lek Rydapt wyniosła 11,4 miesiąca (zakres: 0 do 81 miesięcy). Najczęstszymi ADR były nudności (82%), wymioty (68%), biegunka (51%), obrzęki obwodowe (35%) i uczucie zmęczenia (31%). Najczęstszymi ADR stopnia 3/4 były uczucie zmęczenia (8,5%), posocznica (7,7%), zapalenie płuc (7%), gorączka neutropeniczna (7%) i biegunka (6,3%). Najczęstszymi niehematologicznymi odchyleniami w wynikach badań laboratoryjnych były: hiperglikemia (93,7%), wzrost stężenia bilirubiny całkowitej (40,1%), wzrost aktywności lipazy (39,4%), wzrost aktywności aminotransferazy asparaginianowej (AST) (33,8%) i wzrost aktywności aminotransferazy alaninowej (ALT) (33,1%), natomiast do najczęstszych hematologicznych odchyleń w wynikach badań laboratoryjnych należało zmniejszenie bezwzględnej liczby limfocytów (73,2%) i zmniejszenie ANC (58,5%). Najczęstszymi odchyleniami w wynikach badań laboratoryjnych stopnia 3/4 było zmniejszenie bezwzględnej liczby limfocytów (45,8%), zmniejszenie ANC (26,8%), hiperglikemia (19%) i wzrost aktywności lipazy (17,6%). Modyfikacje dawkowania (przerwanie podawania leku lub dostosowanie dawki) z powodu ADR miały miejsce u 31% pacjentów. Najczęstszymi ADR powodującymi konieczność modyfikacji dawki (częstość występowania ≥5%) były nudności i wymioty. ADR, które doprowadziły do zakończenia leczenia wystąpiły u 9,2% pacjentów. Najczęstszymi (częstość występowania ≥1%) były: gorączka neutropeniczna, nudności, wymioty i wysięk opłucnowy. Wykaz działań niepożądanych: ADR wymieniono według klasyfikacji układów i narządów MedDRA. W każdej klasie układów i narządów ADR zostały przedstawione według częstości występowania, poczynając od najczęstszych według następującej konwencji (CIOMS III): bardzo często (≥1/10); często (≥1/100 do <1/10); niezbyt często (≥1/1000 do <1/100); rzadko (≥1/10 000 do <1/1000); bardzo rzadko (<1/10 000); nieznana (częstość nie może być określona na podstawie dostępnych danych). W obrębie każdej grupy działania niepożądane przedstawiono zgodnie ze zmniejszającym się nasileniem. *AML:* Działania niepożądane obserwowane w badaniu klinicznym III fazy u pacjentów z noworozpoznaną AML z mutacją FLT3. ***Bardzo często:*** zakażenie związane z zastosowaniem aparatury medycznej, gorączka neutropeniczna, wybroczyny, limfopenia, nadwrażliwość, bezsenność, ból głowy, hipotensja, krwawienie z nosa, ból krtani, duszność, nudności, wymioty, zapalenie jamy ustnej, ból w górnej części brzucha, guzki krwawnicze, złuszczające zapalenie skóry, nadmierne pocenie się, ból pleców, ból stawów, gorączka, zmniejszenie stężenia hemoglobiny*, zmniejszenie ANC*, zwiększenie aktywności AIAT*, zwiększenie aktywności AspAT*, hipokaliemia*, hiperglikemia, hipernatremia*, wydłużenie czasu kaolinowo-kefalinowego. ***Często:*** zakażenie górnych dróg oddechowych, hiperurykemia, omdlenie, drżenie, obrzęk powiek, częstoskurcz zatokowy, nadciśnienie, wysięk osierdziowy, wysięk opłucnowy, zapalenie nosogardła, zespół ostrej niewydolności oddechowej, dyskomfort w obrębie odbytnicy i odbytu, dyskomfort w jamie brzusznej, suchość skóry, zapalenie rogówki, ból kości, ból kończyny, ból szyi, zakrzepica związana z obecnością cewnika, hiperkalcemia*, zwiększenie masy ciała. ***Niezbyt często:*** posocznica neutropeniczna. *ASM, SM-AHN i MCL:* Działania niepożądane obserwowane w badaniach klinicznych z ASM, SM-AHN i MCL. ***Bardzo często:*** zakażenie układu moczowego, zakażenie górnych dróg oddechowych, ból głowy, zawroty głowy, duszność, kaszel, wysięk opłucnowy, krwawienie z nosa, nudności, wymioty, biegunka, zaparcie, obrzęk obwodowy, uczucie zmęczenia, gorączka, hiperglikemia (nie na czczo)*, zmniejszenie bezwzględnej liczby limfocytów*, zmniejszenie ANC*, zwiększenie stężenia bilirubiny całkowitej*, zwiększenie aktywności lipazy*, zwiększenie aktywności AspAT*, zwiększenie aktywności AIAT*, zwiększenie aktywności amylazy*. ***Często:*** zapalenie płuc, posocznica, zapalenie oskrzeli, opryszczka jamy ustnej, zapalenie pęcherza moczowego, zapalenie zatok, róża, półpasiec, gorączka neutropeniczna, nadwrażliwość, zaburzenia uwagi, drżenie, układowe zawroty głowy, hipotensja, krwiak, ból części ustnej gardła, niestrawność, krwotok żołądkowo-jelitowy, osłabienie, dreszcze, obrzęk, zwiększenie masy ciała, stłuczenia, upadki. 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* Częstość podana w oparciu o wyniki badań laboratoryjnych.

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Jeśli u pacjenta wystąpi ciężka reakcja związana z infuzją, anafilaksja, ciężka reakcja alergiczna lub zespół uwalniania cytokin związany z infuzją, należy przerwać dalsze podawanie produktu SYLVANT. Należy rozważyć odstawienie produktu leczniczego, jeśli wystąpi opóźnienie większe niż 2 dawki z powodu toksyczności związanej z leczeniem w ciągu pierwszych 48 tygodni. **6. Przeciwwskazania:** Ciężka nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą. **7. Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania:** Jednocześnie występujące ciężkie zakażenia: Zakażenia, w tym miejscowe, należy wyleczyć przed zastosowaniem produktu SYLVANT. W trakcie badań klinicznych stwierdzano ciężkie zakażenia, w tym zapalenie płuc i posocznicę. W badaniu klinicznym stwierdzano hipoglobulinemię u 4 do 11,3% pacjentów. U 4 do 11% pacjentów w badaniu MCD (Badanie 1) stwierdzano zmniejszenie poniżej normy stężeń IgG, IgA lub IgM. Ze wszystkich badań klinicznych produktu SYLVANT wykluczano pacjentów z istotnymi klinicznie zakażeniami, w tym z dodatnim wynikiem testu na antygen powierzchniowy WZW B. U pacjentów ze szpiczakiem mnogim zgłoszono dwa przypadki reaktywacji WZW B podczas podawania produktu SYLVANT jednocześnie z dużą dawką deksametazonu oraz bortezomibem, melfalanem i prednizonem. Produkt SYLVANT może maskować objawy przedmiotowe i podmiotowe ostrego stanu zapalnego, w tym hamowanie gorączki i białek ostrej fazy, takich jak białko C-reaktywne (CRP). Dlatego w celu wykrycia ciężkich zakażeń należy dokładnie obserwować pacjentów otrzymujących leczenie. Szczepienia: Nie należy podawać żywych, atenuowanych szczepionek jednocześnie lub w okresie 4 tygodni przed rozpoczęciem podawania produktu SYLVANT, gdyż bezpieczeństwo kliniczne nie zostało ustalone. Parametry lipidowe: U pacjentów leczonych produktem SYLVANT stwierdzano zwiększenie stężeń (parametrów lipidowych) trójglicerydów i cholesterolu. Z pacjentami należy postępować zgodnie z aktualnymi wytycznymi klinicznymi dotyczącymi hiperlipidemii. Reakcje związane z infuzją i nadwrażliwość: Podczas dożylnej infuzji produktu SYLVANT łagodne i umiarkowane reakcje związane z infuzją można złagodzić, zmniejszając szybkość wlewu lub go zatrzymując. Po ustąpieniu reakcji można rozważyć wznowienie infuzji z mniejszą prędkością oraz podawanie leków przeciwhistaminowych, paracetamolu i kortykosteroidów. U pacjentów, którzy nie tolerują infuzji mimo podjętych ww. działań, produkt SYLVANT należy odstawić. Podczas lub po infuzji leczenie należy zakończyć u pacjentów, którzy mają ciężkie reakcje nadwrażliwości podczas infuzji (np. anafilaksja). Postępowanie z ciężkimi reakcjami związanymi z infuzją powinno być zależne od objawów podmiotowych i przedmiotowych reakcji. Odpowiedni personel i produkty lecznicze muszą być dostępne w razie wystąpienia reakcji anafilaktycznej. Nowotwory złośliwe: Produkty lecznicze wpływające na układ immunologiczny mogą zwiększać ryzyko wystąpienia nowotworów złośliwych. Na podstawie ograniczonych doświadczeń z siltuksymabem aktualne dane nie wskazują na zwiększone ryzyko wystąpienia nowotworów złośliwych. Perforacja przewodu pokarmowego: W badaniach klinicznych siltuksymabu stwierdzano perforację przewodu pokarmowego, jednak nie w badaniach MCD. Należy stosować z ostrożnością u pacjentów z ryzykiem perforacji przewodu pokarmowego. Natychmiast badać pacjentów wykazujących objawy, które mogą być związane lub wskazywać na perforację przewodu pokarmowego. Zaburzenia czynności wątroby: Po leczeniu produktem SYLVANT w badaniach klinicznych zgłaszano przemijające lub okresowe, łagodne do umiarkowanego zwiększenie aktywności aminotransferaz wątrobowych lub innych parametrów badań czynnościowych wątroby takich jak stężenie bilirubiny. Należy obserwować pacjentów z zaburzeniami czynności wątroby oraz ze zwiększoną aktywnością aminotransferaz i stężeniem bilirubiny podczas leczenia produktem SYLVANT. **8. Działania niepożądane:** Najczęściej zgłaszanymi działaniami niepożądanymi w badaniach klinicznych dotyczących choroby Castlemana (CD) występującymi u pacjentów otrzymujących siltuksymab były: bardzo często (≥ 1/10): zakażenia (w tym infekcje górnych dróg oddechowych), świąd, wysypka, ból stawów, zawroty głowy, ból głowy, nadciśnienie, nudności, ból brzucha, wymioty, zaparcia, biegunka, refluks żołądkowo-przełykowy, owrzodzenie jamy ustnej, neutropenia, trombocytopenia, hipertrójglicerydemia, hiperurykemia, zaburzenia czynności nerek, obrzęk zlokalizowany, zwiększenie masy ciała; często (≥ 1/100 do < 1/10): reakcja anafilaktyczna, hipercholesterolemia. Reakcje związane z infuzją i nadwrażliwość: W badaniach klinicznych siltuksymabu reakcja związana z infuzją lub nadwrażliwość występowała u 5,1% (ciężka reakcja u 0,8%) pacjentów leczonych siltuksymabem w monoterapii. W długoterminowym leczeniu pacjentów z MCD siltuksymabem w zalecanej dawce 11 mg/kg co 3 tygodnie reakcje związane z infuzją i nadwrażliwość występowały z częstością 6,3% (1,3% w przypadku ciężkich reakcji). Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, 02-222 Warszawa, tel.: +48 22 49 21 301, faks: +48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl. **9. Podmiot odpowiedzialny posiadający pozwolenie na dopuszczenie do obrotu:** EUSA Pharma (Netherlands) B.V., Beechavenue 54, 1119 PW Schiphol-Rijk, Holandia. **10. Numery pozwolenia na dopuszczenie do obrotu:** EU/1/14/928/001, EU/1/14/928/002. **Kategoria dostępności leku SYLVANT:** Lek wydawany jest z przepisu lekarza – Rp.

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PERSPECTIVE

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"Acta Haematologica Polonica" awarded 100 points by Ministry of Education and Science!

Jan Styczyński*, Agata Marjańska

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Ministry of Education and Science (MEiN, *Ministerstwo Edukacji i Nauki*) awarded our journal with 100 points for parametrization [1]. This has ranked "Acta Haematologica Polonica" among the most prestigious Polish journals.

"Acta Haematologica Polonica" was created in 1970, being the journal of Polish Society of Haematologists and Transfusiologists and Insitute of Haematology and Transfusion Medicine [2]. The journal being 52 years old is the most important and influential journal in the field of Polish hematology and transfusion medicine [3]. The journal covers all aspects of hematology, presented by Polish and international scientists. From two years all papers in the journal are published exclusively in English. From current year the journal is published bimonthly [4]. A number of papers prepared by international groups were published in "Acta Haematologica Polonica" [5, 6]. Authors from Western Europe and America as well as all over the world have coauthored papers published in the journal. The rejection rate in 2021 reached 30%. Still, the chances for PubMed and impact factor depends on citation papers published in "Acta Haematologica Polonica" in recent two calendar years in international journals. It can be done only by entire society!

Conflict of interest

Nothing to disclose.

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Authors' contributions

Both authors contributed equally to the paper.

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EDITORIAL

VIA MEDICA

Therapeutic drug monitoring: a key point in optimal treatment of invasive fungal disease

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Invasive fungal disease (IFD) remains a complication with high mortality, particularly in patients with acute leukemia, and in the setting of hematopoietic cell transplantation. In the strategy of management of IFD, azoles are one of the mainstay drugs. In order to maintain the correct therapeutic range of azoles which is both efficient and safe for the patient, therapeutic drug monitoring (TDM) is recommended [1]. In alignment with this, Polish scientific societies have recently recommended the use of posaconazole and voriconazole in various clinical conditions and strategies, with the support of TDM [2–5].

In this issue of "Acta Haematologica Polonica", Liszka et al. [6] in a pilot study present, for the first time in Poland, the use of TDM in IFD. The authors show that therapeutic drug monitoring of posaconazole is an effective approach to therapy of invasive fungal infections in pediatric patients. To determine drug concentration, they used the high-performance liquid chromatography with fluorescence detector (HPLC-FLD) method, which is currently regarded as the optimal standard for TDM for azoles [1, 7].

We must always bear in mind that the likelihood of toxicity associated with supratherapeutic azole serum concentrations can be as high as quadruple that of therapeutic concentrations [7], and that adequate TDM can prevent many adverse events. Our previous experience with IFD and its management [8, 9] indicate the need for more efficacious antifungal treatment.

Nevertheless, the current Polish guidelines [2, 5] are in line with increasing safety and efficacy in the treatment of infectious complications, something particularly necessary due to the development of new targeted anticancer therapy [10]. Therapeutic drug monitoring for antifungal azoles is still an unmet medical need, although we have taken a significant step forward.

Authors' contributions

JS — sole author.

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Nothing to disclose.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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VIA MEDICA

Antimicrobial prophylaxis in adults and children undergoing hematopoietic cell transplantation: 2021 Polish recommendations

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Abstract

Infections are still one of the most common causes of death after hematopoietic cell transplantation (HCT). Antimicrobial prophylaxis plays a crucial role in decreasing non-relapse mortality after HCT.

The objective of this guideline paper was the presentation of current recommendations of antimicrobial prophylaxis for children and adults after hematopoietic cell transplantation, prepared in cooperation with Polish scientific hematological societies. Recommendations were prepared by the working group and finally approved by all 23 Polish transplant centers for children and adults. Existing (European Conference on Infections in Leukemia (ECIL) and European Society of Blood and Marrow Transplantation (EBMT) guidelines, as well as the results of a survey performed among all Polish transplant centers, were the background material for the working group. Recommendations are presented in sections dedicated to antibacterial prophylaxis, antifungal prophylaxis, antiviral prophylaxis, as well as prophylaxis of toxoplasmosis and infections with *Pneumocystis jiroveci*. Recommendations on the principles of vaccination against COVID-19 are provided based on the state of knowledge in September 2021. A section on guidelines of environmental prophylaxis is also presented.

Key words: hematopoietic cell transplantation, children, adults, incidence, outcome, bacterial infections, viral infections, invasive fungal disease

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Introduction

Despite progress in the diagnostic field and the pharmacotherapy of infections, they are still one of the most common causes of death after hematopoietic cell transplantation (HCT). According to the latest analysis published by the European Society for Blood and Marrow Transplantation (EBMT), infections were responsible for about 22% of deaths, being the second commonest cause of death after autologous HCT (auto-HCT), and the third commonest cause after allogeneic HCT (allo-HCT) [1].

The post-HCT prophylaxis is adjusted to the risk of specific infections, which in turn depend mainly on the time elapsed after HCT. Traditionally, four phases with different frequencies of infectious categories are distinguished: the very early or pre-engraftment phase (up to 30 days post-HCT); the early post-engraftment phase (between days 30–100); the late post-engraftment phase (>100 days and up to one year); and the very late post-transplant phase (>1 year post-HCT). In the allo-HCT setting, prophylaxis also covers the risk of immunosuppression and graft-versus-host disease (GvHD).

Most infectious complications are reported in the very early and early periods post-HCT. However, in some patients, delayed immune reconstitution leads to a long-lasting vulnerability to certain infections. That is why appropriate antimicrobial prophylaxis remains one of the most critical aspects of post-transplantation care. In Europe, the major trends in this area are designed by the European Conference on Infections in Leukemia (ECIL), in nine editions 2005–2021 covering bacterial, fungal, viral and parasitic infections [2–16].

The objective of this guideline paper was to present current recommendations of antimicrobial prophylaxis in children and adults after hematopoietic cell transplantation, prepared in cooperation with Polish scientific hematological societies.

Material and methods

Experts in the field of infectious complications after HCT were invited to prepare recommendations on antimicrobial prophylaxis, both for children and adults, after HCT.

Table I. Grading system of recommendations

Strength of recommendation (SoR)	Definition
Grade A	Strong support of recommendation for use
Grade B	Moderate support of recommendation for use
Grade C	Marginal support of recommendation for use
Grade D	Support for recommendation against use
Quality of evidence (QoE)	**Definition**
Level I	Evidence from at least one properly designed randomized, controlled trial (orientated on primary endpoint of trial)
Level II	Evidence from at least one well-designed clinical trial (including secondary endpoints), without randomization; from cohort or case-controlled analytic studies (preferably from >1 center; from multiple time series; or from dramatic results of uncontrolled experiments
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

Recommendations were analyzed and approved by representatives of all transplant centers which had previously participated in a survey on practices of antimicrobial prophylaxis in Polish transplant centers [17, 18].

The presented guidelines are based on ECIL recommendations, adapted to Polish conditions, and supplemented by the results of the survey.

Recommendations are graded based on the modified system of recent ECIL meetings, taking into account the strength of the recommendation and the quality of evidence (Table I) [2, 3].

Prophylaxis of bacterial infections

Prophylaxis of bacterial infections in adults

The risk of infections in the pre-engraftment period should be individually assessed for each transplant patient, evaluating patient-, disease-, and treatment-related factors [19]. The level and duration of neutropenia, as well as mucosal damage, are the crucial risk factors of bacterial infections in the pre-engraftment phase. In the late post-transplant period, patients are at risk of infections caused by encapsulated bacteria (e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae*) due to hypogammaglobulinemia and functional hyposplenism, resulting from GvHD and its treatment [20, 21].

Prophylaxis of bacterial infections in pre-engraftment period

Fluoroquinolone prophylaxis is recommended in transplant patients who are at high risk of bacterial infections due to expected profound and protracted neutropenia (defined as <100 neutrophils/mL for ≥7 days) (Table II) [19, 22, 23]. This refers mainly to allo-HCT with myeloablative conditioning (MAC). In patients treated with allo-HCT with non-myeloablative (NMA) protocols, or with auto-HCT, the risk of prolonged and profound neutropenia and, consequently, the risk of bacterial infections, is lower. In these settings, the possible benefits of prevention should be weighed against its potential harm related to the negative influence of fluoroquinolones on the intestinal microbiome, increased risk of *Clostridium difficile* infection, and colonization/infection with fluoroquinolone-resistant or multidrug-resistant strains [22, 24]. Emerging data reporting an increasing frequency of fluoroquinolone-resistant gram-negative bacilli strongly indicates that the antibiotic policy of the transplant center should be adjusted in light of the local epidemiological data [24]. The implementation of an antimicrobial stewardship program is highly recommended in every center to promote optimal management of antibiotics [20, 22, 24].

Prophylaxis of bacterial infections in late post-transplant period (>100 days post-HCT)

Oral prophylaxis with penicillin is recommended during the immunosuppressive treatment for GvHD or hypogammaglobulinemia in areas with low pneumococcal resistance [20, 25]. Interchangeably, other agents, according to local antibiotic resistance patterns, might be used [20, 25]. An appropriate macrolide may be substituted depending on local practice [20, 25]. An applicable post-transplant vaccination schedule with Pneumococcal and *Haemophilus influenzae* vaccines plays a **crucial role** and should be performed in all HCT recipients [20, 25]. Supplementation with intravenous immunoglobulins (IVIG) is useful in patients with severe hypogammaglobulinemia (serum IgG level <4 g/L) with accompanying infections [20].

Prophylaxis of bacterial infections in children

Bacterial infections remain a significant challenge in HCT recipients, being an important cause of morbidity in these patients [26]. The incidence of these infections depends on various factors including underlying disease, the phase of treatment, MAC chemotherapy, immunosuppression induced by conditioning, and immunosuppressive effect of the prevention and/or treatment of GvHD [27]. Increasing

Table II. Recommendations for antibacterial prophylaxis in adults

Population	Recommendation	SoR/QoE
Pre-engraftment period (up to day 30 and beyond)		
Allo-HCT (MAC)	Fluoroquinolone prophylaxis	AII
Allo-HCT (NMA)	Fluoroquinolone prophylaxis	BIII
Auto-HCT	Fluoroquinolone prophylaxis	CII
Late post-transplant period (>100 days post-HCT)		
Allo-HCT (MAC, NMA)	Penicillin prophylaxis	BIII
	Macrolide prophylaxis	CIII
	Intravenous immunoglobulins (IVIG)	CIII
	Vaccination:	
	• pneumococcal vaccine	BI
	• *Haemophilus influenzae* vaccine	BII
Auto-HCT	Penicillin prophylaxis if TBI used in conditioning	CIII
	Vaccination:	
	• pneumococcal vaccine	BI
	• *Haemophilus influenzae* vaccine	BII

allo-HCT — allogeneic hematopoietic cell transplantation; MAC — myeloablative conditioning; NMA — non-myeloablative conditioning; auto-HCT — autologous hematopoietic cell transplantation; TBI — total body irradiation

antimicrobial resistance negatively affects prognosis and can influence eligibility for HCT. The high rate of bacterial infection in the peri-transplant period and the emergence of resistance to key antibiotic groups have led to widespread broad-spectrum antibiotic use [3]. Systemic antibacterial prophylaxis is one approach that can reduce the risk of bacterial infections, but carries the risk of drug toxicity or the emergence of antibiotic resistance.

We suggest that systemic antibacterial prophylaxis **should not be used routinely** for pediatric patients undergoing allo- or auto-HCT during pre-engraftment neutropenia or for those receiving systemic immunosuppression for the treatment of GvHD (DIII) (Table III). The evidence base that underlies this recommendation shows that antibacterial prophylaxis does not reduce mortality of HCT recipients, while it may have a certain impact on resistance in bacteriemia isolates [28]. Besides the risk of increasing microbial resistance, antibiotics usually given in antibacterial prophylaxis are associated with negative consequences, i.e. musculoskeletal problems or central nervous system-related adverse events after fluoroquinolones and allergic reactions or gastrointestinal disorders after penicillin V [29]. Furthermore, as HCT recipients are routinely managed in a hospital during the high-risk period, there is an opportunity for very early empiric antibiotic administration and supportive care to reduce complications of bacteremia and severe sepsis [30].

Table III. Recommendations for antibacterial prophylaxis in children

Prophylaxis of bacterial infection in children	SoR/QoE
Recommendation: Routine antibacterial prophylaxis for pediatric patients undergoing HCT during pre-engraftment neutropenia is not recommended	DIII

As the recommendation is not supported by a randomized, controlled trial, a careful risk-benefit evaluation might favor antibacterial prophylaxis in particular patients.

It is important to emphasize that although routine antibacterial prophylaxis for HCT pediatric patients is not recommended, the implementation must always consider individual patient- and treatment-specific risk factors in deciding who merits antibacterial prophylaxis.

Prophylaxis of fungal infections

Prophylaxis of fungal infections in adults

In 2018, the European Conference on Infections in Leukemia (ECIL), the EBMT, the European Organization for Research and Treatment of Cancer, the Immunocompromised Host Society, and the European LeukemiaNet (ELN) published joint recommendations for antifungal prophylaxis [16].

These recommendations were adopted by national centers [31]. The choice of appropriate agent should be phase-specific, and adjusted to the risk of invasive fungal disease (IFD) and the local epidemiology of fungal infections, including construction works. For patients undergoing auto-HCT, routine prophylaxis is not recommended, although fluconazole 400 mg/day as prevention of *Candida* mucositis during the very early neutropenic phase may be considered (BIII) [32]. For allo-HCT, identified risk factors for mold IFD in the pre-engraftment/engraftment phases include active leukemia, cord blood transplantation (CBT), prior IFD, an alternative donor (AD) HCT recipient with iron overload or early/recurrent cytomegalovirus (CMV) infection or acute GvHD or delayed engraftment (≥3-week neutropenia) or high-dose steroids for more than one week (≥2 mg/kg) [33]. The risk factors for the post-engraftment phase include acute GvHD grade III-IV, grade II after AD-HCT, acute GvHD non-responsive to steroids, acute GvHD receiving steroids, and age >40 years, subsequent acute and chronic GvHD, secondary neutropenia or recurrent CMV infection after AD-HCT [33, 34].

Antifungal prophylaxis in adults undergoing HCT: neutropenic phase (pre-engraftment)

In centers with mold IFD incidence <5%, fluconazole is recommended (AI) when combined with mold-directed screening with biomarkers (galactomannan, GM) and/or high resolution computed tomography (HRCT), while an alternative approach should be used in centers with higher incidence (AIII). According to the latest update, mold-active

Table IV. Recommendations for primary antifungal prophylaxis in adults

Anti-fungal agent	Pre-engraftment		Post-engraftment
	Low risk of mold IFD	High risk of mold IFD	High-risk GvHD
Fluconazole p.o./i.v.	AI	DIII	DIII
Posaconazole (TDM) p.o.	BII	BII	AI
Voriconazole (TDM) p.o./i.v.	BI	BI	BI
Micafungin* i.v.	BI	CI	CII
Liposomal amphotericin B i.v.	CII	CII	CII
Aerosolized liposomal amphotericin B (plus fluconazole)	CIII	BII	No data

*No data for other echinocandins: anidulafungin and caspofungin; p.o. — per os; i.v. — intravenous; TDM — therapeutic drug monitoring (recommended)

azoles voriconazole 2 × 200 mg (BI) or posaconazole 3 × 200 mg (oral solution) or 1 × 300 mg (tablets) after a one-day loading dose of 2 × 300 mg (BII) should be used. Alternative options include micafungin 50 mg intravenous (i.v.) in low-risk (BI) and high-risk centers (CI) and liposomal amphotericin B IV (CII) [35]. Aerosolized liposomal amphotericin B in a dose of 10 mg twice weekly added to fluconazole 400 mg remains the option for high-risk centers (BII) [16].

Antifungal prophylaxis in adults undergoing HCT: post-engraftment phase

The experts contraindicate fluconazole in patients with GvHD and high-risk factors (AIII), while oral posaconazole continues to be the drug of choice (AI) [16, 36]. A low level of recommendations (CII) for micafungin and liposomal amphotericin B is based on limited data from randomized trials [16, 35]. The summarized recommendations are presented in Table IV.

The use of azoles poses the risk of drug-to-drug interactions in the HCT recipients, and Polish transplant centers avoid their use during the conditioning [17]. Centers should apply therapeutic drug monitoring (TDM) in the case of voriconazole (plasma target 1–6 mg/L) and posaconazole (plasma target >0.7 mg/L) [16]. Itraconazole, available in Poland in the form of capsules, has no recommendations for antifungal prophylaxis. Preliminary data supports isavuconazole's utility, but more trials are needed to determine its primary prevention role due to reported breakthrough IFD [37, 38].

Prophylaxis of fungal infections in children

Primary antifungal prophylaxis in children is strongly recommended irrespective of primary diagnosis during the neutropenic phase and until immune reconstitution, and in children with GvHD according to updated guidelines by ECIL-8 [2], and Polish recommendations, including dosing of antifungals [31]. Monitoring of *Aspergillus* using serum galactomannan (GM) levels is feasible, yet the negative predictive value is relatively high and other molds remain undetected. According to ECIL-8 guidelines, GM monitoring is valuable in children not receiving mold-active prophylaxis, but experts discourage its use in those receiving mold-active prophylaxis due to false-negative results [2].

Antifungal prophylaxis in children undergoing allo-HCT: neutropenic phase (pre-engraftment)

Primary antifungal prophylaxis is recommended in children undergoing allo-HCT in the neutropenic phase until engraftment (BII). Therapeutic options include fluconazole (effective only against selected yeasts), micafungin, posaconazole, or voriconazole. Due to possible drug-drug interactions, voriconazole should not be used during high-dose chemotherapy.

Antifungal prophylaxis in children undergoing allo-HCT: post-engraftment phase

In the absence of GvHD, antifungal prophylaxis should be continued after engraftment until immune recovery. In the presence of GvHD treated with augmented immunosuppressive therapy, prophylaxis against mold and yeast infections is recommended (AII). The available options include posaconazole (BI) and voriconazole (BI). Posaconazole oral suspension remains the treatment of choice (BI). Its twice-daily body-weight-based dosing algorithm has been proposed by Welzen et al. (Table V) [39]. Alternatively, for pediatric patients, from 1 month to 12 years of age, a starting dose of posaconazole 6 mg/kg three times daily may be used [40]. Parallel administration of proton pump inhibitors should be avoided during posaconazole prophylaxis. Posaconazole i.v. may be used alternatively for children with acute GVHD weighing >40 kg (300 mg daily i.v.). Posaconazole TDM will hopefully be shortly developed in Poland and offered to all transplant centers. A pilot study has been started in cooperation between Wrocław center and MonitLab in Poznań.

Antifungal prophylaxis in children undergoing auto-HCT

Fluconazole as primary prophylaxis against *Candida albicans* should be considered. Micafungin or caspofungin may be administered in patients with *C. glabrata/C. krusei* colonization.

Table V. Posaconazole dosing algorithm in children

Body weight [kg]	Dosing in	
	[mg]	[mL]
10–14	2 × 120	2 × 3
15–19	2 × 160	2 × 4
20–24	2 × 200	2 × 5
25–29	2 × 220	2 × 5.5
30–34	2 × 260	2 × 6.5
35–39	2 × 280	2 × 7
>40	2 × 300	2 × 7.5

Antifungal prophylaxis in children with low risk for IFD development

Antifungal prophylaxis is recommended in selected patients with additional risk factors.

Secondary antifungal prophylaxis

Secondary antifungal chemoprophylaxis is recommended, targeted against the previous fungal pathogen, for as long as the patient is neutropenic or under immunosuppression (AII). Either posaconazole or voriconazole may be considered (Table VI). Off-label isavuconazole may be used in very high-risk patients with previous fungal infections treated successfully with this agent.

Prophylaxis of viral infections

Prevention of CMV infection and disease

CMV infection has been associated with increased non-relapse mortality (NRM) in allo-HCT recipients [41]. Antiviral prophylaxis aims to prevent CMV replication in seropositive patients.

Universal prophylaxis leads to toxicities, detrimental for patients, including clinically significant myelosuppression associated with prolonged use of gancyclovir or valgancyclovir, which may also increase NRM. Moreover, high doses of acyclovir or valacyclovir reduced the risk of CMV infection, but not CMV disease, in randomized studies [42, 43].

Letermovir, a CMV-terminase inhibitor, significantly reduced CMV infection or reactivation and all-cause mortality at 24 weeks in CMV seropositive allo-HCT recipients, with no significant side effects [44]. Letermovir is active only against CMV, and therefore, acyclovir or valacyclovir is necessary to cover herpes simplex virus (HSV)/varicella zoster virus (VZV) prophylaxis. Patients who receive prophylaxis with letermovir should have CMV viremia monitored after drug discontinuation.

Currently, letermovir is the drug of choice for a universal CMV prophylaxis in CMV-seropositive allo-HCT recipients (Table VII). CMV infection and disease risk remains a concern among high-risk patients, including haploidentical HCT (haplo-HCT), CBT, and T-cell-depleted (TCD) graft recipients. In this context, letermovir may be preferentially considered in this patient population.

Table VI. Recommendations for antifungal prophylaxis in children undergoing hematopoietic cell transplantation (HCT)

Drug	Allo-HCT, neutropenic phase	Allo--HCT, GvHD	Auto--HCT	Secondary prophylaxis
Posaconazole	+	++		+
Micafungin	+		+	
Fluconazole	+		+	
Voriconazole	+	+		+
Isavuconazole				(+)

allo-HCT — allogeneic hematopoietic cell transplantation; GvHD — graft-versus-host disease

Table VII. Recommendations for cytomegalovirus infection prophylaxis in allogeneic hematopoietic cell transplantation (allo-HCT) recipients [4]

Therapeutic agent	Adult patients	Pediatric patients
Letermovir 480 mg/day (240 mg/ /day, if cyclosporin is co-administered), starts on day of transplantation or up to 28 days afterwards, for 100 days after HCT	AI	–
Valacyclovir	BI	BI
Gancyclovir (2 × 5 mg/kg/day)	CI	CI
Valgancyclovir	CII	CII
Acyclovir	CI	CI
Foscarnet (180 mg/kg/day in 2–3 doses)	DII	CI
Intravenous immunoglobulin	DI	DI

Prevention of primary CMV infection

CMV-seronegative recipients should receive blood products from seronegative donors or leucocyte-depleted blood products, and the quality standard of less than 1×10^6 residual leukocytes per unit should be warranted (AI) [45, 46]. A CMV-seronegative donor should be chosen, when possible, for a CMV-seronegative recipient (AI), for haplo-HCT (AIII) [4]. In a MAC unrelated allo-HCT setting, for the CMV-seropositive recipient, a CMV-seropositive recipient should be chosen (BII). In haplo-HCT with post-transplant cyclophosphamide, a CMV-seropositive or seronegative donor is suitable (BII) [4].

Prevention of CMV infection and disease in children

In high risk pediatric patients including haplo-HCT, CBT and TCD graft recipients, several agents are recommended for CMV prophylaxis: valacyclovir (BI) [43, 47], gancyclovir (CI)

Table VIII. Recommendations for prevention of Epstein-Bárr virus (EBV)-post-transplant lymphoproliferative disorder (PTLD) and other EBV-related diseases [6]

Recommendation	Grading
Allo-HCT recipients and donors should be tested for EBV antibodies	AII
For EBV-seronegative recipients, an EBV-seronegative donor should be chosen	BII
For EBV-seropositive recipients, an EBV-seropositive donor might be preferred because of presence of EBV-CTL	CIII
Allo-HCT recipients at high risk for EBV-PTLD should be closely monitored for signs and symptoms related to PTLD	AII
After high-risk allo-HCT, prospective EBV-DNA-emia monitoring should be introduced	AII
For HLA-fully matched related, non-T-cell depleted graft recipients without GvHD, routine EBV-DNA-emia screening is not recommended	DII
For auto-HCT patients, routine monitoring for EBV-DNA-emia before and after HCT is not recommended	DIII

allo-HCT — allogeneic hematopoietic cell transplantation; CTL — cytotoxic T lymphocyte; HLA — human leukocyte antigen; GvHD — graft-versus-host disease

[48, 49], acyclovir (CI) [42], foscarnet (CI) [50] and valganciclovir (CII). Data on letermovir use in children [51, 52] is insufficient to provide any recommendations for its use in a pediatric population. Due to a minor effect on prevention of CMV infection and disease, CMV-specific immunoglobulins and polyvalent IVIG are currently not recommended (DI) [53].

Prophylaxis of EBV-post-transplant lymphoproliferative disorder (PTLD) in adults and children

Active prevention is based on optimization of donor choice, conditioning regimen, and GvHD prevention (Table VIII). Data on Epstein-Bárr virus (EBV) prophylaxis in transplant settings is limited. Passive prophylaxis approaches, including a low dose of rituximab, unselected donor lymphocyte infusion (DLI), or EBV-specific cytotoxic T lymphocyte (EBV--CTL) infusion, can decrease the incidence of EBV-DNAemia. It should be noted, however, that the use of DLI for EBV-DNAemia or EBV-post-transplant lymphoproliferative disorder (PTLD) is not reported nowadays by transplant centers. Rituximab-based preemptive therapy can prevent the development of EBV-PTLD, benefiting recipients with higher loads of EBV-DNA. To date, there is no consensus as to when to initiate prophylactic or preemptive treatment. Current preemptive strategies for EBV-PTLD include reduction (RI) or withdrawal of immunosuppression therapy when feasible (AII) [54], rituximab (AII) [55, 56], and EBV-CTL (CIII) [57, 58]. Antiviral drugs are currently not recommended (DIII) [59] in the prophylaxis of EBV-reactivation (Table IX). RI is defined as a sustained decrease of at least 20% of the daily dose of immunosuppressive drugs [6].

Table IX. Preemptive treatment strategy for Epstein-Bárr virus (EBV)-post-transplant lymphoproliferative disorder (PTLD)

Therapeutic agent/strategy	Grading
Rituximab (B-cell depletion)	AII
Reduction or withdrawal of immunosuppressive therapy (RI)	AII
Adoptive transfer of EBV-CTL	CII
DLI	CIII
Antiviral agents	DII
IVIg, interferon	DIII

CTL — cytotoxic T lymphocyte; DLI — donor lymphocyte infusion; IVIg — intravenous immunoglobulin

Table X. Recommendations for herpes simplex virus (HSV) disease prophylaxis in HSV-seropositive adult patients [13, 60]

Therapeutic agent	Grading
Acyclovir from 3 × 200 mg to 2 × 800 mg/day p.o.	AI
Acyclovir 2 × 250 mg/m^2 or 2 × 5 mg/kg i.v.	AI
Valacyclovir 2 × 500 mg/day p.o.	AII
Famcyclovir 2 × 500 mg/day	BIII

p.o. — per os; i.v. — intravenous

Prophylaxis of HSV disease

Prophylaxis with an antiviral drug in herpes simplex virus (HSV)-seronegative allo-HCT recipients is not recommended (DIII) because primary HSV infections in this population are unusual. Due to the high rate of HSV reactivation in HSV-seropositive patients, prophylactic oral or IV acyclovir has been administered routinely in allo-HCT recipients, lasting 3–5 weeks after HCT (Table X). Allo-HCT recipients who develop GVHD or receive immunosuppressive treatment require prolonged HSV prophylaxis (BII). Intravenous acyclovir should be considered for patients with poor drug absorption or due to severe mucositis (CIII). Valacyclovir is an alternative prophylactic agent with good bioavailability, and famcyclovir may be considered as an alternative treatment [13, 60]. Famcyclovir is not yet available in Poland.

There are no specific recommendations for HSV prevention in a pediatric setting. In addition, there is no data on famcyclovir's safety and efficacy in children younger than 12 years. That is why a recommendation for this drug in a pediatric population cannot be given. However, according to the medical product characteristics, acyclovir dosing in children >2 years old is the same as for adults, while a half dose is recommended for children <2 years old.

Table XI. Recommendations for voricella zoster virus (VZV) disease prophylaxis for allogeneic hematopoietic cell transplantation (allo-HCT) recipients [13, 60]

Prophylaxis of VZV after allo-HCT	Grading
Acyclovir 2 × 800 mg/d p.o. (for children: 2 × 20 mg/kg) or valacyclovir once or twice daily: • for one year after allo-HCT or • in the presence of GvHD and immunosuppressive therapy beyond one year	 AII BII
Prophylaxis after VZV exposure in VZV-seronegative patients	
Passive immunization with specific VZIG i.v. or i.m. (0.2–1 mL/kg) or normal IVIG (300–500 mg/kg) should be given immediately after exposure (<96 h), for patients who have chronic GvHD, or who are on immunosuppressive treatment, or who have undergone HCT within the last two years	AII
Acyclovir 4 × 800 mg/daily (4 × 600 mg/m^2 for children) or valacyclovir 3 × 1,000 mg/daily, (3 × 500 mg if body weight <40 kg), or famcyclovir 3 × 500 mg/daily; therapy should be administered from days 3–21 after exposure	AIII
If a second exposure occurs >21 days after a passive immunization or after the prophylaxis administration, a prophylaxis should be reintroduced	CIII
Seronegative HCT recipients should receive prophylaxis in case of exposure to a VZV vaccine having a varicella-like rash	BIII
Prophylaxis after VZV exposure for VZV-seropositive patients is optional	CIII

p.o. — per os; GvHD — graft-versus-host disease; VZIG — varicella-zoster immune globulin; i.v. — intravenous; i.m. — intramuscular; IVIg — intravenous immunoglobulin

Prophylaxis of VZV disease

The determination of VZV serostatus before transplantation should be done in all allo-HCT recipients (AIII). Acyclovir and valacyclovir prophylaxis were proven effective in several trials, and they are a primary preventive approach in the allo-HCT setting (Tables XI, XII) Prophylaxis in auto-HCT recipients is controversial [13]. Recommendations for adults and children are the same.

VZV-seronegative HCT recipients should not have contact with people with chickenpox or zoster (AII) and vaccine recipients experiencing a rash after varicella vaccine (BIII). Vaccination of VZV-seronegative individuals who may be in contact with the patients during transplantation is a worthwhile preventive strategy, but should be performed at least four weeks before the start of conditioning (BIII). Zoster immune globulin (ZIG) and varicella-zoster immune globulin (VZIG) are passive antibody prophylaxis in seronegative recipients after exposure to varicella. If passive immunization is not available, acyclovir, valacyclovir, or famcyclovir as post-exposure prophylaxis are recommended.

Prophylaxis for BK Polyomavirus-associated hemorrhagic cystitis (BKV-HC)

No randomized controlled trial has yet been published to give a specific recommendation for the prevention of BKV-HC in adults and children. Anti-BKV prophylaxis is not available. Hyperhydration plus forced diuresis during conditioning regimen (BII) and/or bladder irrigation through a triple lumen Foley catheter are of limited value in a prophylactic setting (CII) [7, 61]. The risk of fluid overload for hyperhydration and the invasiveness of catheter positioning must be taken into consideration.

Fluoroquinolones (ciprofloxacin) are not effective for prophylaxis, and in fact increase the risk of antibiotic resistance (DII). Two studies showed a reduction of BKV replication in ciprofloxacin recipients, but without any impact on BKV-HC incidence [62, 63].

Table XII. Administration of acyclovir in children

Indication	Dose
Prophylaxis HSV	2 × 5 mg/kg p.o.
Treatment of HSV mucositis	3 × 5 mg/kg i.v.; 10 days
Treatment of HSV visceral	3 × 10 mg/kg; 14–21 days
Prophylaxis VZV in IgG--seropositives	2 × 20 mg/kg (max. 2 × 800 mg)
Post-exposure prophylaxis VZV in IgG-seronegatives	2 × 20 mg/kg to 4 × 600 mg/m^2; 21 days

p.o. — per os; HSV — herpes simplex virus; VZV — voricella zoster virus; IgG — immunoglobulin G

Prevention for HHV-6B infection (encephalitis)

Patients after CBT are at significant risk for both HHV-6 reactivation and HHV-6 encephalitis [64]. Routine testing of blood for HHV-6B in allo-HCT and testing donors or recipients for inherited chromosomally integrated HHV6 (CI-HHV-6) is not recommended due to a lack of recognized preemptive treatment thresholds, and the uncertain efficacy of preemptive therapy (DII). HHV-6 PCR results must be interpreted with caution due to the possibility of CI-HHV-6 in the recipient or allografted cells. In patients with CI-HHV-6, latent HHV-6 DNA will be detected by PCR test in all clinical samples. However, this may not reveal viral replication, and antivirals will not reduce the viral load (in whole blood samples, HHV-6 viral loads are typically >10^6 copies/mL). Encephalitis is the only well recognized HHV-6 disease in allo-HCT recipients; other clinical manifestations are less

Table XIII. Recommendations for adenovirus disease prophylaxis for allogeneic hematopoietic cell transplantation recipients [14]

Recommendation	Grading
Prophylaxis with an antiviral drug is not recommended	BIII
In patients with viremia and at least one risk factor, preemptive therapy with cidofovir (3–5 mg/kg/week for 2–3 weeks, followed by 3–5 mg/kg every other week or 1 mg/kg, three times a week)	BII

established. There is insufficient evidence to recommend the routine use of antiviral prophylaxis or preemptive therapy for HHV-6 infection in adults and children (DII) [5].

Prophylaxis of adenovirus (ADV) infection

ADV infection is diagnosed mainly in the 2–3 months after allo-HCT. Although some patients are asymptomatic and eliminate the virus spontaneously, a quick increase of viral load to multi-organ disease with organ insufficiency is possible. Nevertheless, prophylaxis with any antiviral drug is not recommended for adults and children (BIII) (Table XIII). Cidofovir is recommended for preemptive antiviral treatment in allo-HCT patients with viremia and at least one risk factor both for children (allo-HCT with *in vivo* or *ex vivo* T-cell depletion; allo-HCT with unrelated donor graft; allo-HCT with unrelated cord blood graft; grade III–IV graft-versus-host disease; and severe lymphopenia with <200 cells/μL PB) and for adults (allo-HCT with haploidentical donor or unrelated cord blood graft; grade III–IV GvHD; severe lymphopenia with <200 cells/μL PB; and treatment with alemtuzumab) (BII) [14].

Prevention of community-acquired respiratory virus infection (CARV) in adults and children

Good personal hygiene (i.e. frequent hand washing, covering mouth when coughing and sneezing, safe disposal of nasal and oral secretions) is strongly recommended for allo-HCT patients and their contact persons (AII) (Table XIV). Patients undergoing HCT should avoid people with respiratory tract infections in the hospital and the community (AII). Young children should not be allowed to visit patients and wards because of the greater risk of CARV transmission with prolonged shedding (BII). Entering HCT wards and seeing HCT patients should be restricted for healthcare workers and visitors with respiratory tract infections (AII). In HCT patients with respiratory tract infection inside care facilities, infection control measures should be incorporated, including isolation rooms and protective equipment (coat, gloves, masks, eye protection) for staff and visitors (AII). Outpatients with respiratory tract infections should be placed in rooms separated from other HCT patients

Table XIV. Recommendations on prevention of community-acquired respiratory virus (CARV) for allogeneic hematopoietic cell transplantation (allo-HCT) recipients [12]

Recommendation	Grading
Good personal hygiene (frequent hand washing, covering mouth when coughing and sneezing, safe disposal of nasal and oral secretions) is strongly recommended for allo-HCT patients and contact persons	AII
HCT patients should avoid contact with people with respiratory tract infections in the hospital and the community	AII
Young children should not be allowed to visit patients and wards because of the greater risk of CARV exposure, prolonged shedding, and transmission	BII
Healthcare workers and visitors with respiratory tract infections should be prevented from visiting HCT patients and wards	AII
In HCT patients with respiratory tract infection inside care facilities, infection control measures should be used, including isolation rooms and protective equipment to support staff and visitors (coat, gloves, masks, eye protection)	AII
Outpatients with respiratory tract infections should be admitted and treated in rooms separated from other HCT patients and the HCT ward	AII
In pediatric HCT recipients aged <2 years, palivizumab may be considered during RSV outbreaks in the community, indicating an increased exposure	CIII
In HCT recipients with hypogammaglobulinemia (<4 g/L), IVIg may reduce the risk of morbidity and mortality secondary to respiratory tract infections caused by CARV	CIII

IVIg — intravenous immunoglobulin

and an HCT ward (AII). In pediatric HCT recipients aged <2 years, palivizumab, an intravenous monoclonal antibody specific for respiratory syncytial virus (RSV), may be considered during RSV outbreaks in the community (CIII). In HCT recipients with hypogammaglobulinemia (<4 g/L), IVIg may reduce the risk of morbidity and mortality secondary to respiratory tract infections caused by CARV (CIII) [12].

Prophylaxis of influenza in adults and children

To avoid transmission of influenza, patients and healthcare staff should apply the same general precautions for preventing CARV infection.

Vaccination with seasonal inactivated influenza vaccine is recommended yearly in all HCT recipients older than 6 months (AII) (Table XV). The vaccine is given at least three months after HCT, and preferably prior to the flu season (BIII). Due to the risk of suboptimal immunity in case

Table XV. Recommendations on prevention of influenza for hematopoietic cell transplantation (HCT) recipients [15]

Prevention of influenza with vaccination	Grading
Vaccination with seasonal inactivated influenza vaccine is recommended in all HCT recipients older than 6 months	AII
The vaccine should be given at least three months after HCT, preferably prior to the influenza season	BIII
Post-exposure antiviral prophylaxis	
Oseltamivir for at least 10 days	CIII

of vaccination performed <6 months post-HCT, a second dose of vaccine can be considered [60]. After exposure to a confirmed or probable case of flu, antiviral prophylaxis with oseltamivir for at least 10 days is recommended in HCT recipients who are less than 12 months after HCT, or later if immunocompromised (CIII) [15].

Prophylaxis of SARS-CoV-2/COVID-19 in adults and children

A new coronavirus — severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) — appeared at the end of 2019, and caused a disease called COVID-19. The WHO classified COVID-19 as a pandemic on 11 March 2020 [65]. Mortality among HCT patients caused by this disease was over 22% in adults and 7% in children [66].

There is no specific antiviral agent active against SARS-CoV-2. Thus, non-specific preventive measures typical for CARV infections are mandatory. A number of new-generation vaccines are available nowadays. We recommend following EBMT guidelines: HCT patients should be vaccinated with whatever vaccine is available but not live-attenuated vaccine or vaccine containing replicating viral vectors [67]. Vaccination against COVID-19 should take priority over the regular vaccinations program; the vaccine should routinely be administered alone. The vaccination program should be initiated at least three months after HCT; although if the transmission rate is low, it is advised to wait six months after HCT to initiate vaccination. Controlled GvHD is not a contraindication for vaccination [67]. It will be necessary to administer additional or periodic shots. As of September 2021, children over 12 years should be vaccinated. As of December 2021, children over 5 years should be vaccinated (note added at proof).

Prophylaxis for toxoplasmosis

Toxoplasmosis is a life-threatening condition with an incidence depending on seroprevalence in the population. Toxoplasmosis is seen mainly after allo-HCT, but cases after auto-HCT have been published. The disease occurs mainly as reactivation in previously infected individuals, however primary infection or re-infection after transplant can be also observed. The main risk factors for its development include patient seropositivity, allo-HCT and GVHD.

Recommendations on prevention of toxoplasmosis [68]:

1. In all patients and their donors, serostatus should be determined prior to transplant.
2. Avoiding eating uncooked meat of any type or drinking contaminated water should be recommended to all patients before and after transplant.
3. Primary chemoprophylaxis should be offered to seronegative recipients transplanted from a seropositive donor.
4. Secondary chemoprophylaxis is recommended to all seropositive recipients, regardless of donor serostatus.
5. Chemoprophylaxis is based on TMP/SMX taken at least three times per week for ≥6 months:
   a) 80/400 mg daily,
   b) 160/800 mg, three days per week.
6. Alternative chemoprophylaxis includes:
   a) Pyrimethamine and sulfadoxine (fancidar): 2–3 tablets per week,
   b) Atovaquone: 1,500 mg daily,
   c) Dapsone: 100 mg daily.
7. Weekly qPCR monitoring is advocated when prophylaxis is not being used, is used for less than six months, or when the dose or type of prophylactic medicines are not adequate (i.e. pentamidine).

Prophylaxis for *Pneumocystis jiroveci*

Pneumocystis jiroveci (PjP, previously known as PcP) causes life-threatening pneumonia in immunocompromised patients. According to ECIL guidance, primary prophylaxis should be offered to all recipients after allo-HCT.

The drug of choice is TMP/SMX (trimethoprim/sulfamethoxazole) in a dose of 80/400 mg daily or 160/800 mg, three times weekly from engraftment for at least six months or longer where immunosuppression is ongoing. Second-line alternatives include: pentamidine aerosolized (300 mg per month) or IV, or atovaquone (1,500 mg per day), or dapsone (2 × 50 mg, daily) [8].

Environmental prophylaxis

Global recommendations on protective environment in the HCT setting have been prepared internationally (Table XVI) [69] but adopted locally [18, 70]. Additionally, general precautions for patients and healthcare staff to prevent CARV infection should always be implemented in everyday clinical practice.

Discussion

Antimicrobial prophylaxis plays a crucial role in lessening NRM after HCT [71]. These joint recommendations summarize the most important aspects based on available data to

Table XVI. Global Recommendations on Protective Environment

Global Recommendations on Protective Environment (GRPE)	Grading
1. Ventilation: ≥12 air changes per hour	AIII
2. Central or point-of-use HEPA filters with 99.97% efficiency for removing particles ≤0.3 μm in diameter	AIII
3. Filters should be replaced regularly based on manufacturers' recommendations, and, when there is ongoing construction, filtration efficiency should be monitored frequently to best determine appropriate time for replacement	AIII
4. Directed airflow so that air intake occurs at one side of room and air exhaust occurs at opposite side	BIII
5. Consistent positive air pressure differential between patient's room and hallway ≥2.5 Pa	BIII
6. Well-sealed rooms (e.g. filling gaps between walls and windows, outlets, floor, and ceiling) should always be used for HCT patients to prevent infiltration of air from outside room that could allow entry of spores and hinder maintenance of proper pressure differential	BIII
7. Continuous pressure monitoring, especially while rooms are occupied	BIII
8. Self-closing doors to maintain constant pressure differentials	BIII
9. Monitoring systems that will set off an alarm when pressure differential between any protective environment room and adjacent hallway or anteroom falls <2.5 Pa, to alert staff to possible engineering failures	CIII
10. To enable nursing staff to observe HCT recipient even when doors are closed, windows can be installed in either door or wall of HCT recipient's room	CIII

help transplant physicians in their everyday practice. We have tried to adjust these recommendations to a local market. However, we also included new antimicrobial agents like letermovir and isavuconazole.

Although limited refunding options severely hinder access to them in Poland, we believe that this situation will change in the near future. The market of azoles differs from that of other European countries. That is why itraconazole in oral suspension was not mentioned, while we listed posaconazole in tablets, as this form may soon become available in Poland. Although, according to the current ECIL or EBMT guidelines, isavuconazole is nowadays not listed in recommendations for prophylactic treatment, we provided this possibility both for children and adults. This is due to the increasing number of new publications regarding its off-label use in antifungal prophylaxis in a population of patients with hematological malignancies and patients undergoing allogeneic transplantation. There are several advantages of this drug compared to other antifungal azoles: less drug interactions, less hepatic toxicity, and less renal impairment.

Antibacterial prophylaxis remains an unsolved issue due to the side effects of fluoroquinolones, unfavorable influence on gut microbiota, and increased bacterial resistance to this group of antibiotics. Transplant centers follow different strategies: continue this type of prophylaxis, or abandon pharmacological prevention with increased attention to neutropenic fever, or replace fluoroquinolones with another agent. Randomized prospective studies should be designed to determine which attitude is the optimal solution for HCT patients.

Moreover, there is an urgent need to implement and standardize TDM of voriconazole and posaconazole in our laboratories. This would increase the safety and efficacy of antifungal management.

Regarding CARV, we have given general recommendations with no stress on the current SARS-CoV-2 pandemic since most preventive precautions are common while the therapeutic approach is very dynamic and systematically updated by the EBMT.

Due to constant development in the field of HCT and new evidence regarding antimicrobial prophylaxis and treatment being published constantly, regular updates of these recommendations will be required.

Authors' contributions

Design of study: JS, LG. Analysis of recommendations and final approval: all authors. The following authors wrote primary versions of respective sections: AP (Introduction, Antifungal prophylaxis in adults, Discussion); KK (Antifungal prophylaxis in children); AF (Antibacterial prophylaxis in adults); OZS (Antibacterial prophylaxis in children); BPJ (Antiviral prophylaxis in adults); KC (Antiviral prophylaxis in children); LG (Prophylaxis of toxoplasmosis and infections with PjP); JS (Introduction, Methods, COVID-19 prophylaxis, Environmental prophylaxis, Discussion).

Conflicts of interest

JS — lecture fees or participant in meetings supported by Pfizer, MSD, Gilead, TEVA, and Astellas. KC — lecture fees or participant in meetings supported by MSD and Gilead. KK — lecture fees or participant in meetings supported by Pfizer, MSD, and Gilead. BB — lecture fees or participant in meetings supported by Amgen, and Celgene/BMS. All other authors declared no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical

Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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Impact of clonal hematopoiesis on outcomes in patients with aplastic anemia

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Abstract

Over the years, not only have the T-cell mediated immune mechanisms of aplastic anemia (AA) involved in AA development started to become better understood, but there is now also a better understanding of the roles played by somatic mutations, cytogenetic abnormalities and defective telomerase functions and other genetically-related factors.

Somatic gene mutations suggestive of clonal hematopoiesis are detected in approximately one third of patients with AA. Recent studies have suggested that some of these may predict a better response to immunosuppressive therapy, whereas others indicate poorer outcomes with higher risks of clonal evolution to myelodysplastic syndrome or acute myeloid leukemia, and that therefore better results may be obtained based on allogeneic stem cell transplantation. Furthermore, recent advances in molecular techniques may be useful in differentiating aplastic anemia from hypocellular myelodysplastic syndrome and other clonal hematopoiesises of indeterminate potential. All of these are summarized in this review which includes further insights into treatment personalization based on the molecular pathogenesis of AA.

Key words: aplastic anemia, clonal hematopoiesis, outcomes, allogeneic stem cell transplantation

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Introduction

Aplastic anemia (AA) is a rare form of bone marrow failure caused by autoimmune destruction of hematopoietic progenitor stem cells with a clinical picture dominated by pancytopenia [1, 2].

For many years, it was thought to be based solely on the response of T-cell mediated immune mechanisms to toxic agents, including cytotoxic drugs, some medications, irradiation, toxins or infections such as viruses [3, 4]. In the majority of cases, some genetic abnormalities are also relevant. In all cases, an extensive differential diagnostic work-up should be performed (Table I) to exclude other pancytopenia causes (Table II) and thus to establish the diagnosis of AA. The appropriate decisions and choices of therapy, along with an assessment of risk stratification, are based on the Camitta classification of AA (Table III) [5–7].

The incidence of AA is, on average, 2 cases per million in Europe. The incidence is roughly three times higher in Asia, which may indicate some genetic or environmental factors [8–11]. Several hypotheses have been proposed to explain why the incidence of AA is higher in Asia than in Europe and North America, but the most probable seems to be host genetics such as HLA types and nucleotide polymorphisms in some cytokine genes [12]. There is no difference in the incidence of AA between men and women, but as most cases are observed before the age of 40, a genetic predisposition to AA has been suggested. Although clonal evolution of AA to paroxysmal nocturnal hemoglobinuria

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Table I. Proposed diagnostic procedures for aplastic anemia (AA)

Category	Tests
Peripheral blood testing	CBC, differential, reticulocyte count
	Flow cytometry for PNH
Bone marrow examination	Bone marrow smear
	Flow cytometry
	Cytogenetics
	Trephine biopsy
Rheumatoid disease screening	Antinuclear antibodies
	Rheumatoid factor
Liver function tests	ALT, AST, bilirubin serum levels
Viral infection testing	HBV, HCV, EBV, CMV, HHV-6, HIV, parvovirus B19
Visual imaging	CT, PET-CT, MRI, US for searching solid tumors and lymphoproliferative neoplasms

CBC — complete blood count; PNH — paroxysmal nocturnal hemoglobinuria; ALT — alanine aminotransferase; AST — aspartate aminotransferase; HBV — hepatitis B virus; HCV — hepatitis C virus; EBV — Epstein-Bárr virus; CMV — cytomegalovirus; HHV-6 — human herpesvirus 6; HIV — human immunodeficiency virus; CT — computed tomography; PET-CT — positron emission tomography-computed tomography; MRI — magnetic resonance imaging; US — ultrasonography

Table II. Differential diagnosis of aplastic anemia

Infectious diseases	Cancers	Other
HBV, HCV	MDS	Megaloblastic anemia
EBV, CMV	AML	PNH
HHV-6	Myelofibrosis	HLH
HIV	ALL	
Parvovirus B19	NHL	
Mycobacterial infections	HCL	
	Solid tumor metastases	

HBV — hepatitis B virus; HCV — hepatitis C virus, EBV — Epstein-Bárr virus; CMV — cytomegalovirus; HHV-6 — human herpesvirus 6; HIV — human immunodeficiency virus; MDS — myelodysplastic syndrome; AML — acute myeloid leukemia; ALL — acute lymphoblastic leukemia; NHL — non-Hodgkin lymphoma; HCL — hairy cell leukemia; PNH — paroxysmal nocturnal hemoglobinuria; HLH — hemophagocytic lymphohistiocytosis

Table III. Camitta criteria for aplastic anemia stratification

Stage	Criteria
Severe aplastic anemia (SAA)	Bone marrow cellularity <25% (or 25–50% with <30% residual hematopoietic cells), plus at least two of the following peripheral blood findings: • neutrophils $<0.5 \times 10^9$ • platelets $<20 \times 10^9$/L • reticulocytes $<20 \times 10^9$/L
Very severe aplastic anemia (VSAA)	As SAA, but neutrophils less than 0.2×10^9/L
Non-severe aplastic anemia (NSAA)	Criteria for SAA or VSAA not fulfilled and decreased bone marrow cellularity, plus at least two of the following peripheral blood findings: • neutrophils $<1.5 \times 10^9$ • platelets $<100 \times 10^9$/L • hemoglobin <10 g/dL

(PNH), hypocellular myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) is often observed [13], co-existing somatic mutations may predispose to this process.

Irrespective of the identification of the cause of pancytopenia in the course of AA, the responses to immunosuppressive treatment confirm the thesis of autoimmune injury to hematopoietic stem cells and stem cell progenitors [14–16]. The primary role of T-cell cytotoxic lymphocytes along with the additional effect of interferon gamma and tumor necrosis factor (TNF) on the inhibition of hematopoietic stem cell (HSC) production together with an increasing FAS receptor expression (the first sign of apoptosis) all contribute to immune-mediated destruction of HSCs [17–22]. The human leukocyte antigen (HLA) genes play key roles in mediating the immune response, especially HLA class II alleles. A Chinese study identified HLA-DRB1, DQB1 and DPB1 alleles predisposing to AA development [23]. The dysfunction of T regulatory cells is increased NK cells and autoantibodies, which are also involved in HSC immune destruction in AA [24–28].

Inherited bone marrow failure syndromes

Several genetic disorders including Schwachman-Diamond syndrome (which leads to a reduction in hematopoietic stem cells' ability to repair DNA because of genetic lesions), congenital amegakaryocytic thrombocytopenia (*MPL* gene), Diamond Blackfan anemia (*SBDF* gene), Fanconi anemia, some GATA2 spectrum disorders, congenital keratosis, SRP72, and congenital pure red cell aplasia have all been identified as familiar cases of AA [29–34]. Careful history-taking and physical examinations may be helpful in the identification of germ-like genetic bone marrow failure disorders associated with AA and included in differential diagnostics in children, adolescents and young adults (Table IV) [6, 35]. Next-generation sequencing technologies have facilitated the discovery of mutations that cause pancytopenia and lead to aplastic anemia. All of them carry a high risk of MDS/AML, and some of them are associated with an especially high risk of a range of solid tumors. Thus a tailored stem cell transplantation regimen, such as reduced intensity conditioning, may be the optimal treatment. This is especially true for Fanconi anemia, dyskeratosis congenita, Diamond Blackfan anemia, and Shwachman-Diamond syndrome, not only because of the high risk of clonal evolution, but also due to the high risk of morbidity and mortality [36–38].

Table IV. Selected anomalies in physical examination indicative of inherited aplastic anemia

Anomaly	Disease or mutation
Short stature	FA, DKC, DBA, SDS, SAMD9
Microcephaly	FA, DKC
Café-au-lait skin lesions	FA
Abnormal skin pigmentation, dystrophic nail and oral leucoplakia	DC
Skeletal anomalies	SDS
Erythema nodosum, warts and molluscum	GATA2
Absent radii	TARS
Abnormal thumbs	FA, DBA
Hypertelorism, epicanthal folds	DBA
Cerebellar ataxia	SAMD9L

FA — Fanconi anemia; DBA — Diamond Blackfan anemia; SDS — Shwachman-Diamond syndrome; DC — dyskeratosis congenita; TARS — thrombocytopenia-absentradii syndrome

Somatic mutations in AA

Recurrent mutations and variants have been detected in up to 50% of patients with AA using targeted next generation sequencing hematopoiesis [39–42]. Although some of these mutations are limited to AA, such as *PIGA* [43] and *BCOR/BCORL1* mutations, others are frequently found in myeloid malignancies, including *ASXL1* and *DNMT3A*. Moreover, *DNMT3A*-mutated and *ASXL*1-mutated clones tend to increase in size over time, whereas *BCOR*- and *BCORL1*-mutated and *PIGA*-mutated clones decrease or remain stable [44].

Impact of somatic mutations on outcomes

Several reports have evaluated the clinical significance of somatic mutations in AA. Firstly, it has been shown that the response to immunosuppressive therapy is better in patients with *PIGA*, *BCOR* and *BCORL1* mutations [45]. In the study by Hosokawa et al. [45], the presence of increased glycosylphosphatidylinositol-anchored protein--deficient cells correlated with a positive response to immunosuppressive therapy and prognosis, and thus was found helpful in choosing the optimal treatment for trisomy +8 patients with AA or low-risk MD. Although the natural history of AA patients with PNH clones has been studied, no impact on progression to symptomatic PNH or transformation to AML/MDS has been observed [46]. Furthermore, higher rates of overall and progression-free survival have been found in these subgroups of mutations [44]. However, other somatic mutations such as DNMT3A and ASXL1 are associated with worse outcomes. Recently, a study into mutation status and the differences between severe and non-severe AA by Patel et al. [47] detected at least one mutation in 19% of patients with AA at the time of diagnosis, independent of the severity of the AA. However, patients with severe AA had a higher mutation rate compared to moderate AA (56% vs. 19%), which corresponds to the unstable hematopoietic clones and higher risk of clonal evolution [47].

Finally, the effect of somatic mutations on a higher risk of progression to MDS/AML was revealed by Kulasekararaj et al. [42]. Furthermore, other specific mutations are likely predictors of secondary MDS [48]. The effect of the therapy applied also influences the mutational status, and *BCOR/ /BCORL1* mutations may expand during the course of IST [48]. Negoro et al. demonstrated that, in serial samples of AA without evolution to MDS, clones with *GATA2*, *PHF6*, *RUNX1*, *SMC3*, *TET2* and *BCORL1* mutations decreased in size during the course of AA, whereas *ASXL1*, *CALR*, *CUX1*, *ETV6*, *EZH2*, *G3BP1*, *RIT1*, *U2AF1*, and *ZRSR2* expanded. In contrast, *DNMT3A*, *BCOR*, and *CEBPA* clones showed individually variable behavior with regard to clonal dynamics [48]. Lastly, Negoro et al. [48] also demonstrated the clinical impact of MDS-driver mutations found in AA at presentation, which transformed to MDS and had a shorter median progression-free survival and overall survival compared to cases without such somatic alterations. Other researchers have postulated that clonal dynamics might be highly variable and may not predict response to therapy in individual patients.

Telomerases abnormalities

Telomere shortening is found in up to 35% of patients with AA [49, 50]. It is known that this can result in chromosomal instability and may lead to evolution to MDS/AML [51]. To resist the attrition, germ-like cells utilize telomerase reverse transcriptase (TERT), telomerase RNA component (TERC) telomerase genes, and the stabilizing protein dyskerin (DKC1) to assemble the telomerase complex and maintain telomere length [52]. It has been found that several mutations in *TERT, TERC-DKC1* (stabilizing protein dyskerin) and *RTEL1* (regulator of telomere elongation helicase 1) are associated with telomere shortening in AA patients [53, 54].

Shortened telomere length at diagnosis in patients with AA has been shown to correlate with poorer outcomes [55–57], particularly due to an inadequate response to immunosuppressive therapy. Moreover, some mutations like *TERT* or *TERC* mutations [54, 58] are associated with transformation to MDS/AML [51, 55, 59, 60]. Sex hormones or other pharmacological agents have been shown to be effective in up-regulating telomere length and reducing the risk of clonal evolution to AML [61]. A frequency of up to 38% of clonal patterns of X-chromosome inactivation in female patients with AA has been observed [62].

Cytogenetic abnormalities

The most common cytogenetic abnormality is monosomy 7 (–7), occurring in up to 13% of AA cases. Overall, this is associated with a poorer prognosis and a high risk of progression to MDS or AML [63, 64]. Evaluation of the karyotypes in patients with MDS secondary to AA revealed the presence of chromosomes 6, 7 and 8 abnormalities [64] which suggests that these cytogenetic abnormalities, at the initial diagnosis or developed later in patients with AA, can promote progression to MDS/AML. Some cytogenetic abnormalities such as trisomy 8 or del(13q) are associated with a favorable response to immunosuppressive therapy [65–67]. Although they are commonly found in other myeloid malignancies, they are related to a low risk of transformation to MDS or AML [57, 63, 68]. There are many cytogenetic abnormalities whose clinical impact on outcomes remains to be established [69].

Circulating exosomal microRNAs

MicroRNAs (miRNAs) can regulate T cell differentiation and plasticity by targeting their corresponding message RNAs (mRNAs), which play important roles in many autoimmune diseases and also AA [70–73].

Among several specific miRNAs which regulate RNA silencing and post-transcriptional regulation of gene expression to have been studied in AA and MDS, Guidice et al. [74] identified 25 exosomal microRNAs uniquely or frequently present in AA and/or MDS. One of these, mir-126-5p, with its higher expression at diagnosis in patients with AA, was associated with a shorter progression-free survival and a poorer response to therapy. In another study by Hosokawa, two miRNAs were identified: miR-150-5p which regulated the induction of T-cell differentiation, and miR-146b-5p which was involved in innate immune response. Both of these increased in AA patients, whereas miR-1 was decreased in AA [75]. Moreover, the elevated expression of miR-150-5p was significantly reduced after successful immunosuppressive therapy but did not change in non-responders, indicating the clinical utility of miR-150-5p for disease monitoring [75].

Management of patients with aplastic anemia

Prior to initiating treatment for AA, other causes of pancytopenia should be excluded, particularly inherited bone marrow failure syndrome (IBMFS), hypoplastic MDS and some others transient causes of pancytopenia including drugs or infections. As AA may be associated with PNH, detection of the PNH clone is more indicative for AA than any other cause of pancytopenia and bone marrow failure. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered to be the only curative procedure for patients with severe aplastic anemia (SAA), it is recommended that younger patients, particularly children, undergo careful evaluation of concomitant illnesses and performance status to determine unfit or frail patients before intensive therapies, including allo-HSCT or immunosuppressive therapy (IST) [antithymocyte globulin (ATG) or cyclosporine A (CsA)], due to treatment-related mortality and morbidity [76–78]. Figure 1 shows a practical therapeutic algorithm in SAA [European Group for Blood and Marrow Transplantation (EBMT) algorithm for SAA in 2019, modified] [5]. In cases of the detection of clonal hematopoiesis, especially monosomy 7 (–7) or other abnormalities related to high-risk MDS or insufficient response to IST in patients with SAA below the age of 60, if these patients are assessed as eligible for transplant but have no identical sibling donor, an alternative donor should be sought.

Clonal hematopoiesis and supportive therapy

All patients with AA require ongoing supportive care to alleviate symptoms and reduce the adverse effects related to pancytopenia. Most studies have reported that infections were the predominant cause of death; therefore recommendations for infection prevention are included in several guidelines, independent of the intensity of AA treatment, both for transplant- or IST-eligible patients and for less fit patients on ongoing supportive care [6, 76, 79–81].

Granulocyte colony-stimulating factor

Hematopoietic growth factor, granulocyte colony-stimulating factor (G-CSF) stimulates granulocyte progenitors as well as stem cells for proliferation and differentiation. A randomized prospective trial on patients with newly diagnosed severe AA (n =192), receiving ATG and cyclosporine, with and without G-CSF, did not demonstrate any impact of G-CSF on the outcome of severe AA, independent of cytogenetic abnormalities. Overall survival and progression-free survival was comparable in both groups, as well as the risk of clonal abnormalities and myeloid neoplasm development [82]. Moreover, the results of a metanalysis of four studies confirm that the usage of G-CSF in IST is not associated with a higher occurrence of clonal evolution into malignant neoplasm and PNH in SAA patients [83]. On the other hand, a rapid granulocyte recovery in patients treated with IST with G-CSF addiction may identify early non-responders, and perhaps indicate the need for urgent transplantation [84, 85].

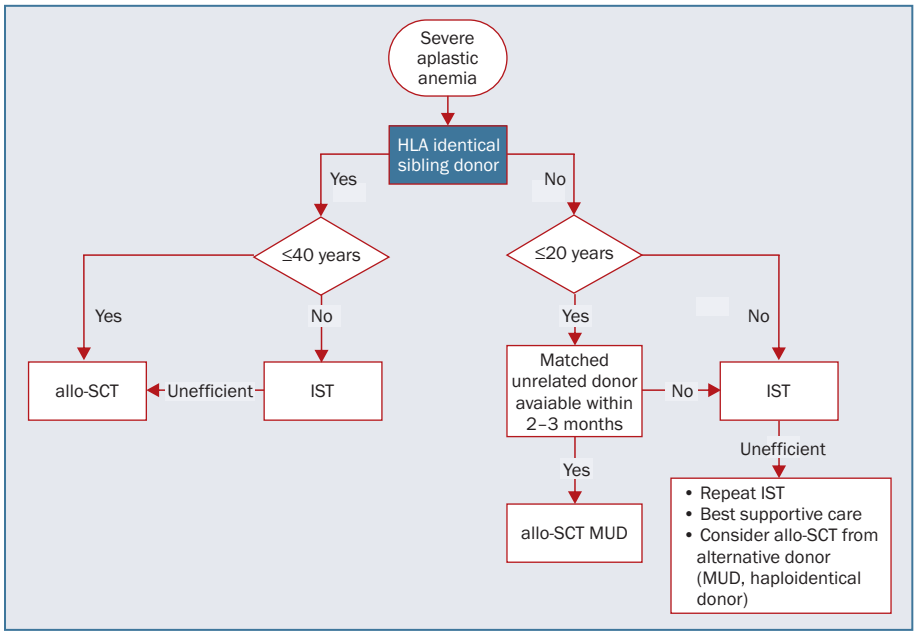

Figure 1. Therapeutic algorithm in severe aplastic anemia; HLA — human leukocyte antigen; allo-SCT — allogeneic stem cell transplantation; IST — mmunosuppressive therapy; MUD — matched unrelated donor

Eltrombopag

Eltrombopag (EPAG), an oral thrombopoietin (TPO) receptor agonist used in immune thrombocytopenia treatment, is a new therapeutic option in transplant-ineligible SAA patients. The role of TPO in hematopoiesis is not limited only to thrombopoiesis: a TPO receptor c-Mpl is present on hematopoietic stem and progenitor cells (HSPCs), and its lack in murine models leads to HSPC deficiency [86]. EPAG is efficient at SAA refractory to IST and in some patients it restores trilineage hematopoiesis with a sustained response even after discontinuation of the treatment [87–89]. Nevertheless, a risk of clonal evolution during this treatment remains an area of concern. Two prospective studies of EPAG usage in treatment naïve and second in refractory/relapsed SAA have not shown a higher risk of clonal evolution or myeloid neoplasm development compared to historical data [87, 88]. On the other hand, in phase 1/2 EPAG in R/R SAA (18%) have developed new cytogenetic abnormalities, most of these (87%) within six months of beginning treatment. However, some were unstable and disappeared after EPAG withdrawal. Chromosome 7 abnormalities were observed in 8% (7/83) of patients, and four of them had persistent aberration in control cytogenetic testing one month after drug discontinuation. Nevertheless, none of them progressed to MDS/AML [88].

The impact of EPAG on the overall risk of cytogenetic progression, clonal evolution, and/or clinical progression to MDS/AML in patients with SAA requires further investigation. Due to an insufficient response to IST, patients who are platelet transfusion-dependent may receive EPAG as secondary SAA therapy, but its high costs limit the widespread application of this treatment option in many countries [79, 90].

Survival after hematopoietic stem cell transplantation

A recent study demonstrated that in some situations, despite the identification of certain genetic abnormalities of germline monoallelic deleterious variants in the Fanconi anemia gene in patients with idiopathic AA (21 variants in 730 patients), the abnormalities do not influence the outcome of hematopoietic cell transplantation [91].

Generally, although allogeneic HSCT has shown an improvement in survival rates, particularly for HLA-matched unrelated donor transplants, haploidentical transplantation has been proposed as the effective treatment for severe aplastic anemia and it is increasingly being used [15]. The optimal choice of haploidentical donor has also been the subject of research [92]. Furthermore, a recent meta-analysis of 5,336 patients comparing front-line treatments for AA showed significantly longer survival among AA patients undergoing first-line allo-HSCT compared to IST. On the other hand, one of the most important complications after allo-HSCT is graft-versus-host disease, and this needs to be carefully balanced against the concerns of IST [93].

It has to be emphasized that the choice of initial treatment for patients with newly diagnosed AA still requires a comprehensive evaluation of donor availability, patient age, expected quality of life, and the risk of disease relapse or clonal evolution after IST [94].

Conclusions

There are difficulties in differentiating between AA and MDS due to the high prevalence of clonal hematopoiesis in AA with genetic abnormalities overlapping with MDS. Furthermore, a better understanding of the pathogenesis of AA with respect to somatic mutations, cytogenetic abnormalities and defective telomerase functions, and their impacts on the response to IST, along with a balancing of the risk of clonal progression to MDS/AML, may in future allow for treatment personalization with precise indications for upfront allo-HSCT.

Author's contributions

The authors participated equally in writing the article.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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ORIGINAL RESEARCH ARTICLE

Prevalence and demographic characteristics of Hodgkin lymphoma in Colombia, according to Ministry of Health data

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Abstract

Introduction: Hodgkin lymphoma accounts for approximately 10% of lymphoma cases. The epidemiology of Hodgkin lymphoma has always been a source of fascination to researchers due to its heterogeneous characteristics. Since 1993 in Colombia, the coverage of health services has been extended, and there is now an extensive registry of healthcare processes through the Social Protection Comprehensive Information System. The aim of our study was to calculate the prevalence, and describe the demographic characteristics, of Hodgkin lymphoma in Colombia.

Material and methods: This is a descriptive cross-sectional study with data from the Comprehensive Social Protection Information System of the Ministry of Health of Colombia between 2015 and 2019.

Results: 4,396 cases were identified, giving a prevalence of 8.9 per 100,000 inhabitants; of these, 55% were men, with a male:female ratio of 1.2:1, with a higher prevalence in the 75–79 years age group. The departments with the highest prevalence were Risaralda (13.33), Bogotá D.C. (13.30), Boyacá (11.9), Quindío (11.49) and Santander (11.44).

Conclusions: Our prevalence is higher than that reported based on population-based cancer registries in Colombia 5.4 per 100,000 inhabitants between 2016 and 2020. We suggest that the differences found are due to the lack of population-based cancer registries in Colombian cities in which there is a higher prevalence of this entity and greater aging of the population.

Key words: Hodgkin lymphoma, epidemiology, Colombia, prevalence

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Introduction

Hodgkin lymphoma is a hematopoietic neoplasm characterized by the presence of Reed-Sternberg cells in an inflammatory infiltrate. Hodgkin lymphoma accounts for approximately 10% of lymphoma cases [1]. Its etiology is not clear, although it has been documented that immunosuppression and Epstein-Bárr virus (EBV) infection increase the risk of developing it [2–4].

This lymphoid malignancy involves peripheral lymph nodes and can also affect organs such as the liver, lung, and bone marrow [5]. It is subdivided according to its morphology and immunohistochemistry into classical (90%) and nodular lymphocyte predominant (10%) [6].

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Epidemiological studies allow for estimation of the population affected by the disease and reflect the sociodemographic characteristics of those who suffer from it. Since Law 100 of 1993 was established in Colombia, the coverage of health services has been extended, and there is now an extensive registry of healthcare processes through the Social Protection Comprehensive Information System (known as SISPRO by its Spanish acronym) [6]. The purpose of this study was to establish the prevalence of Hodgkin lymphoma in Colombia and to describe the demographic characteristics of patients based on the official records of the Colombian Ministry of Health.

Material and methods

This was a descriptive cross-sectional study based on information from the Integrated Social Protection Information System (SISPRO) of the Colombian Ministry of Health. The Colombian healthcare system has one of the largest coverages in Latin America, reaching 95.97% of the 49 million inhabitants, according to the most recent measurement made in May 2020 [7]. The Ministry of Health and Social Protection of Colombia has developed a tool for collecting and storing information, called SISPRO. This tool stores and processes the basic data that the General Social Security System in Health requires for management, regulation and control processes; the data is taken from the Individual Health Services Delivery Registry (known as RIPS by its Spanish acronym), which medical staff are obliged to fill out after each episode of outpatient or inpatient medical care. All healthcare providers (hospitals and health centers) are required by law to send such information to the SISPRO, using the corresponding International Classification of Diseases, 10th revision (ICD-10) code for the primary diagnosis. Therefore, the said registry offers consolidated data of the entire population that demands services within the social security system in Colombia.

The SISPRO receives data from different sources, which are carefully checked by the Ministry of Health and Social Protection. The data sent is subject to continuous quality control tests. The data is checked against other sources of information (such as the population census, national health surveys, or other administrative records), before being entered into the SISPRO. If inconsistencies are detected, the data is sent back to the reporting institutions for review and correction, with the goal of improving the quality of data collected. The information contained in the SISPRO is accessible to the public via the online dynamic tables of the Ministry of Health of Colombia. For the present study, information was obtained from the care performed between 1 January, 2015 and 31 December, 2019, identifying the primary diagnosis for the care in accordance with the International Classification of Diseases, Tenth Revision (ICD-10) codes.

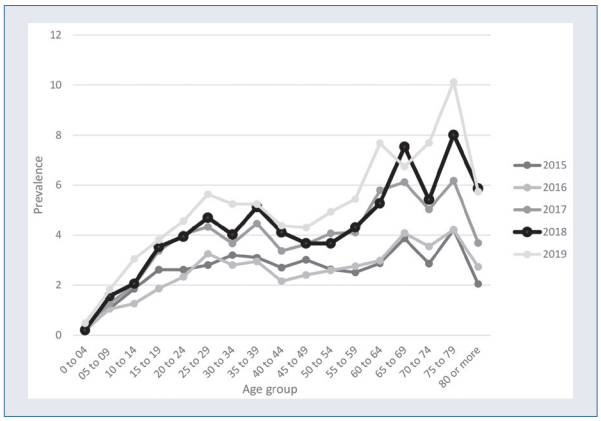

Figure 1. Age-specific unadjusted prevalence of patients with Hodgkin lymphoma 2015–2019. Prevalence is calculated per 100,000 inhabitants

The Individual Health Services Delivery Registry (RIPS) demographic information was obtained for patients diagnosed with Hodgkin lymphoma (ICD-10 codes: C81.0, C81.1, C81.2, C81.3, C81.4, C81.7, and C81.9).

Information regarding population size, geographic distribution by department, sex and age (classified by five-year groups), was obtained from the official projections of the National Administrative Department of Statistics (DANE), based on the projections of the 2005 national census [8]. DANE is the organization that processes official population statistics in Colombia. Hodgkin lymphoma prevalence rates standardized by age per 100,000 population were calculated from 2015 to 2019 (Figure 1). The numerator was the number of Hodgkin lymphoma cases in patients of any age (divided by five-year groups) which were reported to the SISPRO. The denominator was the number of inhabitants reported by DANE in the population projections for the entire country, and for each geographical area (Figure 2).

Results

Between 1 January, 2015 and 31 December, 2019, 4,396 cases of Hodgkin lymphoma were reported, giving a global prevalence of 8.9 per 100,000 inhabitants. Men accounted for 55% of cases, giving a male:female ratio of 1.2:1.

When analyzing the prevalence by five-year age groups, a gradual increase in people over 55 years of age was identified, with a higher prevalence in the 75–79 years age group (22.2 per 100,000). Due to the characteristics of the registry, it was impossible to determine the incidence or the time of evolution of the disease.

The distribution of classical Hodgkin lymphoma by histopathological subtypes according to the ICD-10 code provided was nodular sclerosis (67.7%), mixed cellularity (16.6%), lymphocyte predominant (13.1%), and lymphocyte depletion (2.6%).

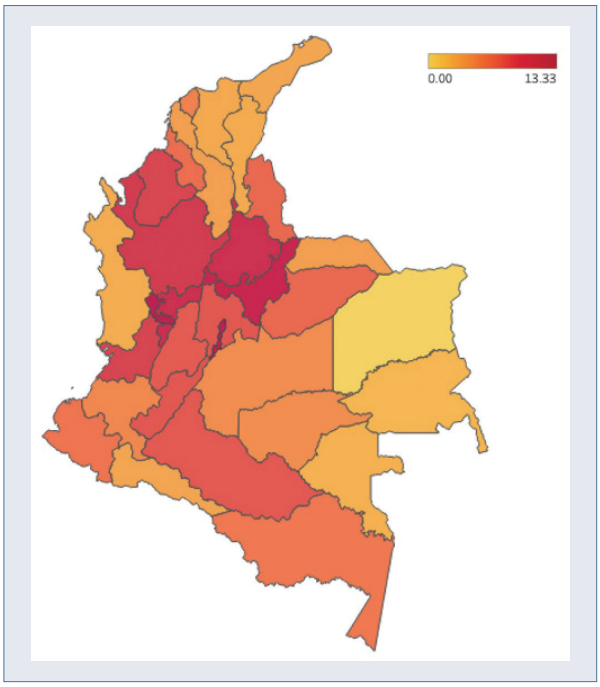

Figure 2. Geographical distribution of prevalence of Hodgkin lymphoma by department, adjusted to Colombian population. Prevalence is calculated per 100,000 inhabitants

An analysis of the distribution by department showed that the highest prevalences were registered in the most industrialized departments: Risaralda (13.33), Bogotá D.C. (13.30), Boyacá (11.9), Quindío (11.49) and Santander (11.44). Departments such as Archipelago of San Andrés, Providencia and Santa Catalina, Guainía, Vaupés, Cesar and Magdalena had the lowest prevalence.

Discussion

Hodgkin lymphoma accounts for 10% of lymphomas and 0.6% of cancers diagnosed each year worldwide [9]. Its prevalence has been described in various parts of the world through population-based cancer registries, but there have been few studies on its prevalence in each of the different countries. Latin America is undergoing an accelerated process of demographic transition, with greater aging of the population, which leads to an increase in the prevalence of low mortality entities such as Hodgkin lymphoma [10].

A study conducted in the United States based on health services information systems (Medicare databases), reported a prevalence of 46.9 cases per 100,000 inhabitants between 2013 and 2014 [11]. The United Kingdom's population-based Hematological Malignancy Research Network found a 5-year prevalence of 12.9 cases per 100,000 inhabitants [12]. Our study based on SISPRO data reports a prevalence of 8.9 cases per 100,000.

Hodgkin lymphoma is classified as either classical or nodular lymphocyte predominant. The four subtypes of classical Hodgkin lymphoma differ in their presentation, epidemiology and association with the EBV [13]. In general, patients with lymphocyte depletion and mixed cellularity subtype have a worse prognosis [14]. In a study of 454 patients in Turkey, the following description of subtypes was made: nodular sclerosis (52.7%), mixed cellularity (32.5%), lymphocyte-rich (8.5%), and lymphocyte depletion (4.2%) [15]. In our study, the most frequent subtype was nodular sclerosis (67.7%), followed by mixed cellularity (16.6%), lymphocyte-rich (13.1%) and lymphocyte depletion (2.6%); the frequency of presentation of the subtypes varies from that of the published studies because the assignment of the subtype depends upon the attending physician during the consultation.

Hodgkin lymphoma occurs more often in men, with the exception of the nodular sclerosis subtype, which mostly affects young women, and its presentation by age in industrialized countries shows a bimodal curve, with the most significant peak in young adults (15 to 34 years) and the second most significant in those over 50 [16]. Our study showed that the greatest number of cases occurred in adults older than 75 years, which is probably related to the aging of our country's population, and to the fact that the population includes surviving patients, because it was impossible to determine whether it was a new case or a previously treated patient.

The study by Glaser et al. [17] reported how the place of birth influences the incidence of Hodgkin lymphoma, and found a higher incidence in Hispanics and children of Hispanics in the second bimodal peak. Previous studies have shown that the incidence rate is higher than the mortality rate, considering that a high percentage of Hodgkin lymphoma patients live long enough to die from other causes, such as in the series of the Memorial Sloan Kettering Cancer Center, in which a 22-year follow-up found a 30% mortality, 47% of which was attributable to other causes [18].

It has been reported that 1,700 cases occur every year in the United Kingdom, with a slight predominance in men (1.2:1), and a greater number of cases during the fourth and fifth decades of life [19]. It was estimated that in 2017, 8,260 new cases of Hodgkin lymphoma were diagnosed in the United States [20].

The prevalence for the population reported in the Global Cancer Observatory GLOBOCAN between 2016 and 2020, by the International Agency for Research on Cancer (IARC) in Colombia, is 5.4 per 100,000 inhabitants. The following is the prevalence per 100,000 inhabitants found in other countries: Brazil (5.2), Argentina (8.4), Portugal (8.4) Spain (11.1), France (12.4), United Kingdom (13.9), and Italy (13.9) [21].

The United States National Cancer Institute (NCI), through the Surveillance, Epidemiology, and End Results

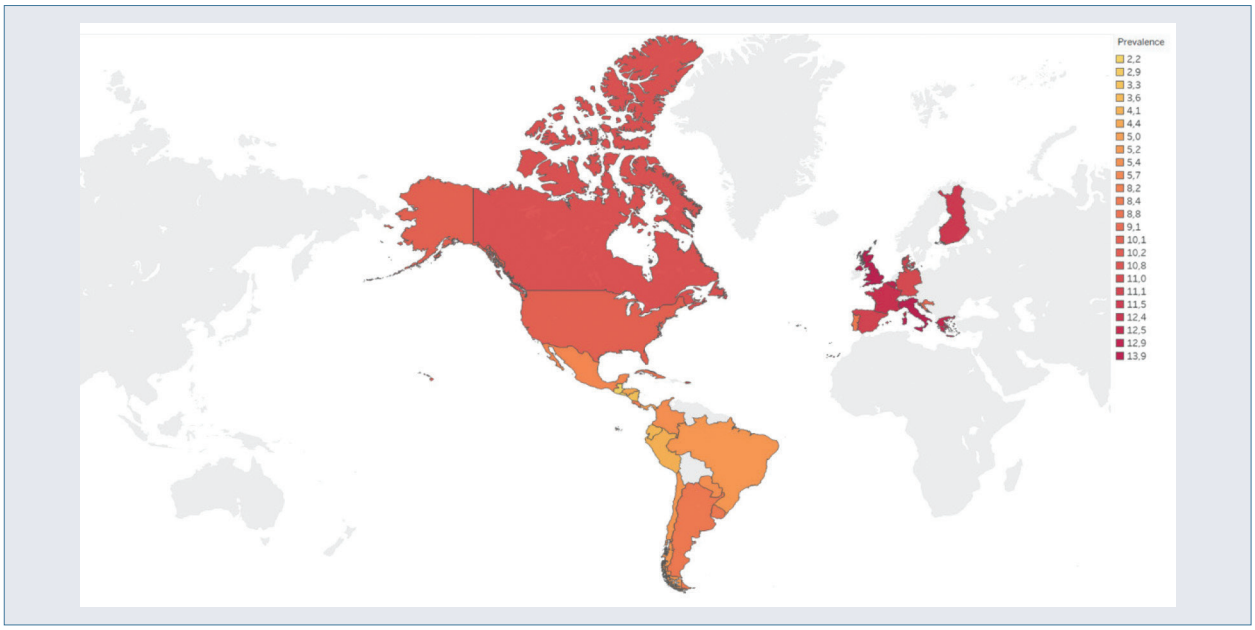

Figure 3. Prevalence of Hodgkin lymphoma in several countries. Prevalence is calculated per 100,000 inhabitants (source: the Global Cancer Observatory)

(SEER) Program, reported that by 2017 there were 215,531 people living in this country with Hodgkin lymphoma [22].

Population-based cancer registries from the cities of Cali, Pasto, Manizales and Bucaramanga have been the only sources of information on cancer in Colombia in recent years [23]. However, there is no detailed information on Hodgkin lymphoma from other major cities with particular sociodemographic characteristics, including Bogotá and Medellín, the two largest cities in Colombia. The inclusion of these two cities, and other cities in general, could explain the higher rate of Hodgkin lymphoma found in our study compared to the results previously presented by the IARC in Colombia through the Global Cancer Observatory (GLOBOCAN). This study carried out in Colombia allows us to determine the prevalence in all departments of the country, to describe the demographic characteristics of the patients, and to compare them to the statistics of population-based cancer registries.

The last report of the National Cancer Institute of Colombia, for the 2007–2011 five-year period, was carried out using the IARC methods to estimate survival in countries with a high human development index. These methods assume that cancer survival for Colombia by location is the same as that obtained from the weighted average of the absolute survival for each location recorded in the cities of Hong Kong, Qidong, Shanghai and Tianjin, in China; Barshi, Bhopal, Chennai, Karunagappally and Mumbai, in India; Chiang Mai, Khon Kaen, Lampang and Songkhla, in Thailand, and the city of Kampala, in Uganda. For the estimation of prevalence, it is assumed that the annual incidence is constant during the period. During this five-year period, a total of 1,086 cases were reported, 66% of which were in men; the departments with the highest number of cases were Risaralda, Bogotá D.C., Boyacá, Quindío and Santander; the lowest number of cases was registered in the Archipelago of San Andrés, Providencia and Santa Catalina, Guainía, Vaupés, Cesar and Magdalena [24].

Unlike other studies carried out, our study evaluated all medical care performed in the country in which Hodgkin lymphoma has been considered as the main diagnosis. Taking into account the high coverage of the healthcare system, in order to calculate the prevalence it is not necessary to make estimates through population registries. The difference in prevalence in our study may be related to the greater availability of data from cities with a greater number of cases. However, a limitation of our study is the possible incorrect reporting by doctors at the time of entering the ICD-10 code in the medical records, as well as the fact that they do not specify how the diagnosis was performed. The nature of the available information does not allow us to calculate the time of evolution of the disease or to estimate its incidence and severity.

The present study shows the prevalence of Hodgkin lymphoma through the report and registry available in the SISPRO of the Ministry of Health of Colombia, and constitutes one of the different ways of calculating the prevalence and epidemiologically assessing this disease; in addition, it provides complementary information to other methodological approaches, to establish the frequency of this disease in our population.

Colombia is a middle-income country with limited resources affecting research. The starting point of this work was to make a register with more reliable data about the cancer situation nationwide, with a specific interest in lymphoma. It is extremely important to perform a population analysis in middle-income countries such as this report, since we have too little information to allow analysis of the disease impact and any possible actions in terms of public health geared towards improving treatment for hematological neoplasm patients. Colombia also requires more robust cancer registries to provide more reliable information about what is happening in the country. As we have a registry seeded by all the health professionals around the country, we invite other colleagues to perform a more precise registration to improve and take advantage of the information provided.

Conclusions

The present study provides demographic and epidemiological information regarding Hodgkin lymphoma in Colombia. The figures are higher than those reported in the literature. Our study shows information taken from the database of the Ministry of Health of Colombia, with which demographic and epidemiological analyses of the population, and projections for the care of patients in our country, are made.

Authors' contributions

DF — study design and administrative support; MA — important clinical data; LM — developed first draft of manuscript; DF and MA — assisted in writing and editing of manuscript. All authors — data analysis and interpretation, data checkup, critical revision and final approval.

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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ORIGINAL RESEARCH ARTICLE

Clinical spectrum of neutropenia in children — analysis of 109 cases

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Abstract

Introduction: The aim of this study was to retrospectively analyze the course of neutropenia in children hospitalized at a single pediatric hematology and oncology center, with particular emphasis on the assessment of risk factors for severe infectious complications.

Material and methods: The study included 109 children diagnosed with neutropenia unrelated to malignancy. The etiology, laboratory and genetic test results, and clinical data and course were analyzed.

Results: More than half (53.2%) of the patients were ultimately diagnosed with benign childhood neutropenia. 74.5% of the children presented a chronic course of neutropenia, with a mean duration of 22 months. The duration of neutropenia had a significant impact on its clinical course: none of the patients with acute neutropenia had severe infections or required treatment. Among the patients with chronic neutropenia, a positive family history (p <0.002), comorbidities (p <0.005), severe infectious complications (p <0.001) and the need for specific treatment (p <0.004) were observed statistically more often in children with the congenital form of the disease.

Conclusions: Neutropenia in children usually has a benign course, but the prognosis largely depends on duration and etiology. History, clinical course, and ancillary test results should be carefully interpreted to identify patients with congenital neutropenia, due to the higher risk of complications and the need to treat patients in this group.

Key words: children, neutropenia unrelated to malignancy

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Introduction

Neutropenia is relatively common in children [1]. It is usually a symptom rather than a disease. Based on the absolute number of granulocytes (ANC), we classify neutropenia as being severe, moderate or mild. The duration of neutropenia can also be taken into account as a division criterion, allowing us to distinguish acute and chronic neutropenia [2]. Due to the pathomechanism of granulocyte count decrease, we can also distinguish primary from secondary neutropenia. In the case of primary neutropenia, a correct diagnosis can be challenging, but recently, thanks to the dynamic development of diagnostic techniques, clinicians have become more aware of the genetic background of the disease [3]. Depending on the type of neutropenia, different clinical variants are possible: from a frequently asymptomatic course in benign chronic childhood neutropenia (autoimmune or idiopathic background), to the

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occurrence of frequent, serious and life-threatening infections in congenital neutropenia.

The following analysis aims to determine the characteristics of pediatric patients hospitalized for neutropenia, including specific groups separated by its etiology. This is a retrospective analysis of children diagnosed with neutropenia unrelated to disease and oncological treatment, hospitalized at a single pediatric hematology and oncology center between January 2005 and April 2020. Based on clinical data, laboratory and genetic test results, we wanted to analyze the clinical course, the risk of severe infectious complications, and the need for granulocyte colony-stimulating factor (G-CSF) treatment or antibiotic prophylaxis in patients from each group. We hope that our experience will bolster understanding about this heterogeneous group of patients and help to establish principles for the diagnosis and treatment of children with neutropenia.

Material and methods

Patients

The study included patients aged from 1 month to 18 years hospitalized due to neutropenia in the Department of Pediatrics, Hematology and Oncology of the Medical University Hospital No. 1 in Bydgoszcz, Poland. Neutropenia associated with neoplastic disease, myelodysplastic or myeloproliferative syndromes and their treatment was excluded from the study group.

Clinical analysis

The following data, obtained from medical records, was evaluated:

- basic patient characteristics such as gender, age at diagnosis of neutropenia, and duration of neutropenia;
- family history, with special attention paid to neutropenia and other hematological diseases, autoimmune diseases, and immune disorders;
- clinical data on course of neutropenia (i.e. frequency of infections, including severe infections such as sepsis; the presence of comorbidities);
- laboratory test results: blood count values at diagnosis, blood microscopic picture, serum levels of immunoglobulin major classes;
- other laboratory tests performed as necessary to further diagnosis, such as myelogram, presence of antigranulocytic antibodies, vitamin B_{12} and folic acid levels, chromosome fragility test with mitomycin;
- microbiological and serological tests for diagnosis of infection as a complication of neutropenia;
- any need for treatment with G-CSF or antibiotic prophylaxis.

Genetic analysis

Genetic testing was performed based on next-generation sequencing (NGS) techniques and molecular karyotyping. Detected point mutations or microdeletions were verified by testing the child's parents. Point mutations were additionally confirmed by Sanger sequencing.

Definitions

- **Duration**: up to 3 months: acute neutropenia, >3 months: chronic neutropenia.
- **ANC:** agranulocytosis (<100/μL); severe neutropenia (100–500/μL); moderate neutropenia (500––1,000/μL); mild neutropenia <lower age limit but >1,000/μL.
- **Etiology:**
  - congenital neutropenia (positive family history, diagnosis of genetically determined disease) with neutropenia as the only clinical manifestation and neutropenia as part of the disease syndrome;
  - secondary neutropenia: autoimmune; secondary to infection, idiopathic.

Classification

A classification considering the etiology of neutropenia with definitions is shown in Table I.

Principles of therapy

Decisions to initiate treatment in children were made after analyzing the severity of neutropenia and the risk of complications. Treatment with G-CSF was initiated in patients with severe neutropenia or agranulocytosis who were at high risk for severe infections. In accordance with the recommendations of the Neutropenia Committee of the Marrow Failure Syndrome Group of the Associazione Italiana Emato-Oncologia Pediatrica (AIEOP), in children with severe congenital neutropenia the starting dose of G-CSF was 5 μg/kg/day [8]. In patients with secondary neutropenia, lower doses of G-CSF (0.5–3 μg/kg/day) were used [1]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was used in children with congenital neutropenia who were unresponsive to G-CSF treatment with severe clinical course [8]. Antibiotic prophylaxis was used in children in whom infectious complications were observed, with a characteristic microorganism of known susceptibility to antibiotics used for prophylaxis.

Statistical analysis

Data analysis was performed using Statistica software for Windows version 10. Categorical variables were compared by chi-square test and non-categorical variables by Mann-Whitney U test. A value of p <0.05 was considered statistically significant.

Table I. Classification of neutropenia in children by etiology (acc. to [4–7])

Diagnosis	Definition
Acute infection-induced neutropenia	Neutropenia secondary to infection, lasting up to three months. Decrease in granulocyte counts in neutropenia secondary to infection can occur by a variety of mechanisms, including formation of antigranulocytic antibodies or direct bone marrow suppression by toxins produced by pathogens
Post-infection persistent neutropenia	Neutropenia secondary to infection, lasting more than three months. Mechanism of onset as in acute infection-induced neutropenia
Benign chronic childhood neutropenia	Autoimmune or idiopathic neutropenia lasting 3+ months and characterized by benign clinical course. Autoimmune neutropenia is diagnosed when serum autoantibodies directed against granulocytes are detected. Idiopathic neutropenia is diagnosed when extensive diagnostic work-up fails to identify cause
Severe congenital neutropenia	Severe neutropenia genetically determined, observed from birth, progressing with serious infectious complications already in neonatal period and infancy. This is a group of diseases inherited in an autosomal recessive (Kostmann syndrome, *HAX1* gene mutation) manner and an autosomal dominant (*ELANE* gene mutation) manner
Specific genetic syndrome associated with neutropenia	Genetically determined diseases in which neutropenia occurs as part of complex syndromes involving multiple organ dysfunction (e.g. Shwachman-Diamond syndrome, WHIM, Fanconi anemia, *etc.*)

WHIM — warts, hypogammaglobulinemia, immunodeficiency, myelocathexis

Results

Demographic analysis

During the analysis period, neutropenia was diagnosed in 109 patients, comprising 65 (59.6%) boys and 44 girls (40.4%). The age at which the disease was diagnosed ranged from 1 month to 17 years, but most (71.6%) of the patients were younger than 5 years. The mean age of the children at diagnosis was 49 months, with a median of 18 months. Agranulocytosis was observed in 23 patients (21.1%), severe neutropenia in 46 (42.2%), moderate neutropenia in 29 (26.6%), and mild neutropenia in 11 (10.1%).

Clinical data

Mild infections were the most common reason for diagnosis and blood counts on which neutropenia was diagnosed in 59 children (54.1%). In 19 patients (17.4%), neutropenia was found incidentally in an examination performed for another reason. In this group, as many as 12 children were diagnosed with idiopathic neutropenia. Severe infection, as the first manifestation and reason for neutropenia diagnosis, occurred in 10 children (9.2%), of which congenital neutropenia was finally diagnosed in four.

The family history was eventful in 27 patients (24.8%), of which neutropenia was also observed in 13 members of the immediate family (parents, siblings), and in the remaining 14 we observed autoimmune diseases, oncological diseases and other hematological disorders. Coexisting chronic diseases were found in 25 children (22.9%).

Diagnoses

More than half of the children (n =58, 53.2%) were eventually diagnosed with benign chronic childhood neutropenia in the form of autoimmune (n =15, 13.8%) or idiopathic neutropenia (n =43, 39.4%). 18 (16.5%) were diagnosed with acute infectious neutropenia, and another 14 (12.8%) with persistent postinfectious neutropenia. In patients with neutropenia secondary to infection, the etiologic agent was identified in seven cases (one adenovirus infection, two parvovirus B_{19} infections, two cytomegalovirus infections, one streptococcal angina, and one co-infection with adenovirus and parvovirus B_{19}); urinary tract infection was diagnosed in another two patients, and acute otitis media in one. In the remaining children, respiratory tract infection was diagnosed without identification of the causative microorganism. Severe congenital neutropenia (SCN) was diagnosed in one patient. In another 14 children (12.8%), certain genetic syndromes associated with neutropenia were diagnosed; this subgroup included such entities as Schwachmann-Diamond syndrome (n =3), severe combined immunodeficiency (SCID) (n =3), autoimmune lymphoproliferative syndrome (ALPS) (n =2), Fanconi anemia (n =1), Pelger-Huet anomaly (n =1), transaldolase deficiency (n =1), WHIM (warts, hypogammaglobulinemia, immunodeficiency, myelocathexis) syndrome (n =1), Noonan syndrome (n =1), and Blackfan-Diamond syndrome (n =1) (see Table II).

Two children were found to have vitamin B12 deficiency, and in another two the cause of neutropenia was undetermined. The characteristics of the patients in each group are set out in Table III.

The duration of neutropenia ranged from 1 week to 98 months, with a mean of 22 months and a median of 10 months. In the congenital neutropenia group, the mean duration was 41 months, and was statistically significantly higher compared to the other patients (p <0.02).

Acute neutropenia was present in 27 patients (24.8%). In this group, the majority were children with acute neutropenia with infection (Figure 1). None of the children in this group required G-CSF treatment or the inclusion of

Table II. Characteristics of patients with congenital neutropenia

Diagnosis*	N	Diagnosis based on	Age**	Therapy
Schwachmann-Diamond syndrome	3	NGS – *SBDS* mutation	2, 48; 120	G-CSF
SCID	3	NGS – *RAG1* mutation (n =2); ADA	8; 3; 4	G-CSF, allo-HSCT
ALPS	2	Clinical picture	24; 84	G-CSF, sirolimus
Fanconi anemia	1	Chromosome fragility test	192	–
Pelger-Huet anomaly	1	No data available	66	–
Transaldolase deficiency	1	Confirmed by genetic testing at another center	2	Antibiotic prophylaxis
WHIM syndrome	1	NGS – *CXCR4* mutation	5	–
SCN	1	NGS – *ELANE* mutation	1	G-CSF, allo-HSCT
Noonan syndrome	1	No data available	60	–
Blackfan-Diamond syndrome	1	*RPL35* deletion	1	–

**Final diagnosis based on genetic testing, clinical symptoms or additional tests; *age at diagnosis (in months); NGS – next-generation sequencing; G-CSF – granulocyte colony-stimulating factor; SCID – severe combined immunodeficiency; allo-HSCT – allogeneic hematopoietic stem cell transplantation; ALPS – autoimmune lymphoproliferative syndrome; WHIM – warts, hypogammaglobulinemia, immunodeficiency, myelokathexis; SCN – severe congenital neutropenia

Table III. Characteristics of study group according to diagnosed cause of neutropenia

Parameter	Congenital	Chronic benign	Acute infection-induced	Post-infection prolonged	Other
Number of patients	15/109	58/109	18/109	14/109	4/109
	(13.8%)	(53.2%)	(16.5%)	(12.8%)	(3.7%)
Age at diagnosis in months (mean; min–max value)	41	34	91	63	65
	(1–192)	(1–192)	(7–199)	(1–183)	(2–204)
Male	9/15	35/58	12/18	7/14	2/4
	(60.0%)	(60.3%)	(66.7%)	(50.0%)	(50.0%)
Female	6/15	23/58	6/18	7/14	2/4
	(40.0%)	(39.7%)	(33.3%)	(50.0%)	(50.0%)
ANC <500/μL	11/15	43/58	6/18	5/14	3/4
	(73.3%)	(74.1%)	(33.3%)	(35.7%)	(75.0%)
Duration in months (mean)	41.3	26.2	1.3	14.2	3.4
Transient nature	8/15	53/58	18/18	14/14	4/4
	(53.3%)	(91.4%)	(100%)	(100%)	(100%)
Eventful family history	9/15	11/58	2/18	4/14	1/4
	(60.0%)	(19.9%)	(11.1%)	(28.6%)	(25%)
Infections	13/15	41/58	4/18	1/14	0/4
	(86.7%)	(70.7%)	(22.2%)	(7.1%)	(0%)
Severe infections	9/15	5/58	0/18	1/14	0/4
	(60.0%)	(8.6%)	(0.0%)	(7.1%)	(0%)
Coexisting immunodeficiency	14/15	15/58	1/18	3/14	1/4
	(93.3%)	(25.9%)	(5.6%)	(21.4%)	(25.0%)
Coexisting chronic diseases	9/15	12/58	4/18	1/14	0/4
	(60.0%)	(20.7%)	(22.2%)	(7.1%)	(0%)
Treatment	8/15	15/58	0/18	1/14	0/4
	(53.3%)	(25.9%)	(0%)	(7.1%)	(0%)

ANC – absolute neutrophil count

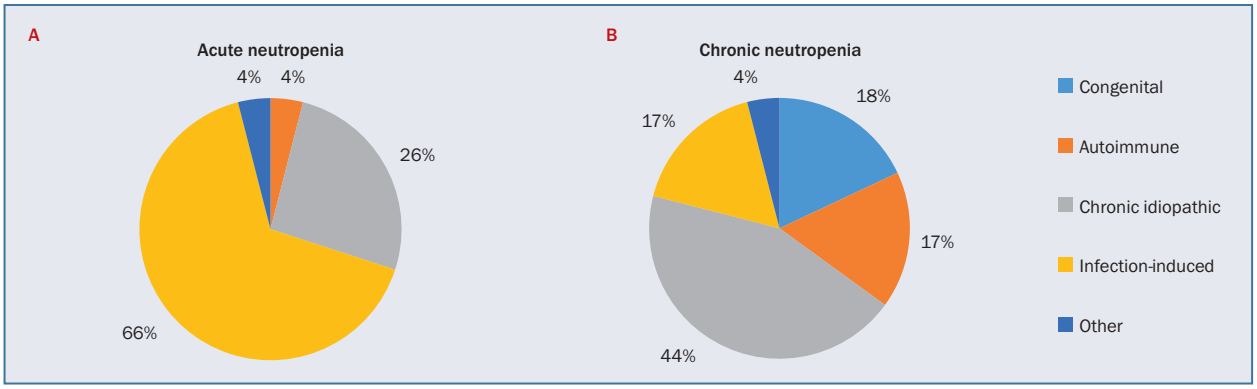

Figure 1A, B. Causes of acute and chronic neutropenia

antibiotic prophylaxis. Severe infections requiring hospitalization did not occur in any child during the follow-up period.

Chronic neutropenia was found in the remaining 82 patients, representing 74.5% of the children. Benign chronic neutropenia in children was the most common finding (Figure 1). Twenty-four children (29.3% of patients with chronic neutropenia) required treatment. Of these, four with congenital neutropenia underwent an allo-HSCT procedure. Among the patients with chronic neutropenia, its transient nature was observed in 70 children (85.4%).

Clinical course

During the observation period, infections occurred in 76 children (69.7%) in the whole study group; these were mostly mild infections typical for childhood. Serious infections were found in 23 children (21.1%), of whom four with congenital neutropenia had multiple, recurrent, and complicated life-threatening infections. Serious infections were statistically significantly more common in children with congenital neutropenia (p <0.001); these patients also had an almost ten-fold increased risk of sepsis during the follow-up period compared to other patients (Table IV).

Patients with benign chronic childhood neutropenia had a similar clinical course, regardless of the etiology of neutropenia (autoimmune vs idiopathic). In the comparative analysis of the two groups there were no statistically significant differences in parameters such as age of child at diagnosis (p <0.75), duration of neutropenia (p <0.79), percentage of children with severe neutropenia and agranulocytosis at diagnosis (p <0.44 for agranulocytosis and p <0.65 for severe neutropenia, respectively), or occurrence of severe infections (p <0.65). The only statistically significant difference between the two groups was the percentage of children in whom the decision to initiate G-CSF treatment was made (53.3% of patients with autoimmune neutropenia and 16.3% with idiopathic neutropenia, respectively, p <0.005).

Treatment

In 23 patients (21.1%), the decision was made to initiate treatment in the form of antibiotic prophylaxis or G-CSF treatment. In three of them, antibiotic prophylaxis alone was used, in 19 G-CSF therapy alone, and in one patient both forms of treatment were implemented.

In three patients, amoxicillin was used for antibiotic prophylaxis, in one case in combination with cotrimoxazole administered twice a week, and in one boy trimethoprim was used.

G-CSF treatment was used in children at high risk of severe infectious complications (patients with chronic severe neutropenia or agranulocytosis, with pre-existing severe infections). For benign chronic childhood neutropenia, both autoimmune and idiopathic, lower doses of G-CSF (0.5–3 µg/kg/day) were used and were usually sufficient to achieve an increase in granulocyte count >1,000/µL that was sustained after treatment. Children with congenital neutropenia were more than five times more likely to require treatment than other patients (Table IV), and treatment with G-CSF was usually not satisfactory, despite daily administration and higher doses (5 µg/kg initially, multiple higher doses in case of no response). In four children with congenital neutropenia (three with severe combined immunodeficiency (SCID) and one with severe congenital neutropenia associated with *ELANE* gene mutation), an allogeneic blood stem cell transplantation procedure was performed, because this was the only therapeutic option leading to a cure. All patients achieved disease remission after transplantation, but patients with SCID required intravenous immunoglobulin (IVIG) supplementation due to persistent hypogammaglobulinemia after transplantation. A boy with neutropenia due to *ELANE* gene mutation despite normalization of blood morphology parameters after allogeneic hematopoietic stem cell transplantation (allo-HSCT) died due to post-transplant complications.

Table IV. Odds ratio of diagnosis of congenital neutropenia and other neutropenia for selected data of family history, duration of neutropenia, clinical course of neutropenia

Parameter to be assessed	OR	Confidence of OR (–95%; 95%)	*P* value
Male sex	1.018	0.335; 3.095	0.9751
Transient nature of neutropenia	**0.078**	**0.021; 0.288**	**0.0001**
Eventful family history	**6.333**	**1.998; 20.077**	**0.0017**
Infections	3.683	0.784; 17.303	0.0986
Fever	1.761	0.589; 5.266	0.3115
UTI	4.450	0.942; 21.031	0.0596
Acute otitis media	1.453	0.282; 7.488	0.6551
Sepsis	**8.900**	**2.192; 36.142**	**0.0022**
Coexisting chronic diseases	**5.176**	**1.652; 16.223**	**0.0048**
Need for treatment	**5.571**	**1.767; 17.564**	**0.0034**
Antibiotic prophylaxis	7.077	0.916; 54.656	0.0606
G-CSF treatment	1.915	0.538 6.824	0.3162

Values in **bold** are statistically significant; OR — odds ratio; UTI — urinary tract infection; G-CSF — granulocyte colony-stimulating factor

Discussion

Neutropenia is relatively common in children, but is usually secondary in nature, with remission occurring during the follow-up period. Hospitalization in children with neutropenia is necessary if infectious complications develop or if the diagnosis is broadened, especially when congenital neutropenia is suspected.

To the best of our knowledge, this study is the largest single-center analysis of pediatric patients with neutropenia in Poland. In order to identify at-risk patients, a variety of methods was used in the diagnostic process, including modern genetic diagnostic techniques such as NGS, which also enabled the diagnosis of rare and ultra-rare diseases developing alongside neutropenia.

On the basis of the analysis of the presented data, it can be concluded that in most cases neutropenia was characterized by a mild course, and in more than half of the patients spontaneous remission was observed within the first year of follow-up. The group of patients with congenital neutropenia was the most vulnerable to infectious complications. They were characterized by a statistically significantly higher risk of severe infections and the need for treatment. Children from this group were more frequently affected by coexisting chronic diseases and a positive family history, and more often the first manifestation of neutropenia was severe infection.

The presented observations are consistent with reports from other centers [2]. The duration of neutropenia had a significant impact on its clinical course, regardless of the severity of neutropenia at the time of diagnosis. In children with acute neutropenia, severe infections were not observed, nor did they require treatment.

The largest group consisted of children with benign chronic childhood neutropenia. This group included both children with autoimmune and idiopathic neutropenia. Because of the similar clinical course, some authors have suggested that idiopathic neutropenia may in fact have an autoimmune basis [3]. This is supported by the fact that only in 60–74% of patients with autoimmune neutropenia are antigranulocytic antibodies detected [9, 10]. This may be caused by too low titers of antibodies, not exceeding the detection threshold of the method, or by too long an interval between the appearance of antibodies and the test, when a decrease in ANC resulting from autoimmunity is observed, but autoantibodies are not detected [11].

The only statistically significant difference between our two groups of patients was the percentage of children who received G-CSF treatment. Apart from that, no statistically significant differences were observed in terms of clinical course or main parameters describing neutropenia.

The smallest group consisted of children with congenital neutropenia. It is worth underscoring that congenital neutropenia is an extremely rare disorder, which means that the diagnosis is often made late. Another factor complicating the diagnosis is the fact that the group of diseases classified as congenital neutropenia is very heterogeneous and may manifest with the onset of neutropenia at an older age. In our analysis, we also observed great heterogeneity in the group of patients with congenital neutropenia, ranging from severe congenital neutropenia based on *ELANE* gene mutation, through neutropenia coexisting with primary immunodeficiency (SCID), children with diseases manifesting in infancy (Swachmann-Diamond syndrome, Fanconi anemia), as well as children with congenital neutropenia of mild course (Pelger-Huet anomaly).

One patient in this group was diagnosed with warts, hypogammaglobulinemia, immunodeficiency, myelocathexis (WHIM) syndrome, an extremely rare genetically determined disease with neutropenia that had only been described in 105 patients worldwide up to 2019 [12]. This syndrome is caused by a mutation of the CXCR4 receptor, inherited in an autosomal dominant manner. Neutropenia in WHIM syndrome is due to myelocatexia, a phenomenon involving retention of mature granulocytes in the bone marrow [12, 13]. It is noteworthy that in the described patient, the clinical course was uncharacteristic (absence of viral warts), and only thanks to NGS were we able to establish the diagnosis. This is an excellent illustration of the fact that in rare and ultra-rare diseases, it is often only thanks to modern genetic tests that a diagnosis can be made [14].

Children with neutropenia secondary to infection, both acute and persistent, had the mildest clinical course. In this group, only one patient developed severe infections requiring hospitalization during the follow-up period. This confirms previous observations that neutropenia secondary to infection is characterized by an uncomplicated course due to, among other things, its usually episodic nature, the older age of children, and the rare coexistence of chronic diseases (only in 15.6% of children with neutropenia secondary to infection) [5, 15–17].

Interestingly, drug-induced neutropenia was not observed in any of the hospitalized patients, which contradicts data from other centers [2, 18]. This is probably due to the fact that children under immunological care for drug-induced neutropenia usually do not require hospitalization because the cause of the decrease in neutrophil count is known, and the treatment of choice is drug switching, dose reduction or withdrawal [19].

In the study group, treatment was initiated in children with a history of severe infections or in patients with neutropenia associated with a high risk of serious infectious complications.

In children with severe congenital neutropenia, the starting dose of G-CSF was 5 μg/kg/day, with doses increased as needed [8], but despite high doses of G-CSF satisfactory ANC levels were not always achieved. When treatment was responded to, the increase in granulocyte counts did not necessarily translate into clinical improvement in this group, probably due to pre-existing severe infections and their complications.

Benign chronic childhood neutropenia, whether autoimmune or idiopathic, has traditionally been treated with lower doses of G-CSF (0.5–3 μg/kg/day) and this has usually been sufficient to achieve an increase in granulocyte count >1,000/μL. In our group of patients, an initial dose of 5 μg/kg body weight three times per week was used and then the lowest effective dose to be used two or three times per week was sought. This treatment was usually initiated in children with previous serious infections, was usually well tolerated, and led to a satisfactory response and a decrease in infectious complications. As mentioned above, decisions to start G-CSF treatment were the only statistically significant difference between children with autoimmune and idiopathic neutropenia, with one in two children with autoimmune neutropenia, and one in six with idiopathic neutropenia, choosing to do so. This is probably due to concerns that the administration of G-CSF will disturb the clinical picture and the results of laboratory tests where there is a need to broaden or repeat the diagnostics in patients diagnosed with idiopathic neutropenia.

In the present group, blood stem cell transplantation was performed in three patients with SCID and one with SCN. All of these children had severe, life-threatening infections requiring several weeks of hospitalization in early childhood. Among these patients, the three with SCID achieved remission of the underlying disease and withdrawal of neutropenia, whereas the boy with SCN died due to post-transplant complications.

Antibiotic prophylaxis was used in individual patients, always after careful consideration of the pros and cons of such an approach. It was implemented in children with recurrent infections with a specific pathogen, and was maintained in cases of good clinical response.

Conclusions

In conclusion, based on the data analyzed, the occurrence of severe infections was more likely in children with neutropenia lasting more than three months, and children with congenital neutropenia were most at risk. An eventful family history, the coexistence of other chronic diseases, and the persistent nature of neutropenia, as well as the occurrence of severe infectious complications, also as the first manifestation of neutropenia: these features characterized patients who were eventually diagnosed with congenital neutropenia.

The mildest course was observed in children with neutropenia secondary to infection, which was also characterized by rapid, spontaneous remission.

Based on the above analysis, it is clearly vital in the diagnosis of children with neutropenia to take a thorough history and to evaluate laboratory findings in conjunction with the child's clinical assessment. Neutropenia itself is only an abnormality in laboratory tests, and doctors' experience and knowledge are crucial in assessing the risk of complications associated with it, and in deciding on both in-depth diagnostics and treatment.

Authors' contributions

JK — data collection and interpretation, statistical analysis, literature collection and description of results. NB, MR-P, EC, EW, AD, AU, EG — data collection and interpretation. JS, MW — critical review for important intellectual

content. SK — thesis draft, critical review for important intellectual content, acceptance of final version for publication.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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ORIGINAL RESEARCH ARTICLE

Ferritin and transferrin saturation cannot be used to diagnose iron-deficiency anemia in critically ill patients

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Abstract

Introduction: Iron-deficiency anemia (IDA) is the most common anemia globally. The frequency of IDA among critically ill patients is not known. The aim of our study was to analyse performance of standard iron metabolism parameters for diagnosis of IDA in the critically ill.

Material and methods: We performed a retrospective analysis of consecutive anemic patients admitted to the intensive care unit. We based on various cut-off values for ferritin and/or transferrin saturation (TfS), determined the incidence of IDA.

Results: The population consisted of 27 (53%) men and 24 (47%) women. The median hemoglobin concentration was 96 [interquartile range (IQR) 87–109] g/L. The studied population had markedly increased concentrations of C-reactive protein [119 (IQR 44–196) mg/L], and ferritin [686 (385–1,114) µg/L], whereas iron concentration and TfS were below reference values. Depending on the cut-off value chosen IDA could be diagnosed in between 7.8% (ferritin <100 µg/L +TfS <20%) and 56.9% (TfS <20%) of patients.

Conclusions: Ferritin and transferrin saturation cannot be used for a precise diagnosis of IDA caused by absolute or functional iron deficiency in the critically ill.

Key words: ferritin, intensive care unit, iron-deficiency anemia, transferrin saturation

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Introduction

Anemia constitutes a global healthcare challenge [1]. The incidence of anemia among patients admitted to the intensive care unit (ICU) reaches up to 66% [2], in a prospective cohort study by Thomas et al. even 98% [3]. As a result of disease processes and iatrogenic blood loss due to laboratory testing [4, 5], many patients develop anemia during ICU hospitalization. Anemia has several negative consequences: tissue hypoxia, myocardial infarction, ischemic stroke, infection, and increased mortality [6]. Therefore, timely diagnosis of anemia is a fundamental component of patient blood management (PBM) — the concept of conservation of a patient's own blood through multiple interventions [7]. The cause of anemia should be established and appropriate treatment introduced. IDA is the most common anemia globally [1, 8]. The frequency of IDA among critically ill patients is not known. Although algorithms for differential diagnosis of preoperative anemia have been proposed [9, 10], there are no algorithms for

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diagnosis of IDA in critically ill patients. Ferritin concentration and transferrin saturation are standard tests used for diagnosis of ID in most guidelines according to a recent systematic review [11]. However, these proteins are acute phase reactants and their concentration may fluctuate when systemic inflammation is present. In the acute phase ferritin concentration rises, whereas transferrin saturation in both directions. Systemic inflammatory response is frequently encountered in ICU patients [12]. As ferritin and transferrin are produced in the liver, liver dysfunction may also have impact on their concentration. Apart from true IDA, many critically ill patients may have functional ID [13, 14]. Functional ID is defined as inappropriately low iron stores in the setting of inflammation [15]. Functional ID is caused by inflammatory cytokines and is a hallmark of anemia of inflammation (AI), previously known as anemia of chronic disease [16]. AI may accompany acute and chronic inflammatory states such as critical illness, obesity or advanced cancer [17]. All these factors make iron metabolism diagnostics complicated in critically ill patients.

Using concurrent determination of ferritin and transferrin saturation, attempts have been made to distinguish between absolute and functional ID [9, 15]. The aim of this study was to assess the usefulness of ferritin and transferrin saturation in diagnosis of IDA in a population of critically ill patients.

Material and methods

We performed a retrospective analysis of consecutive anemic patients admitted to the intensive care unit of a university-affiliated medical center between January and July 2020. Anemia was defined as hemoglobin (Hb) concentration below 130 g/L in both sexes, as it was postulated in the perioperative setting [9]. Exclusion criteria were as follows: blood loss within 120 days (n =11), RBC transfusion within 120 days (n =15), iron supplementation within 120 days (n =0), and history of a hematological disorder other than anemia (n =0). Basic demographic and laboratory data were retrieved: sex, age, primary diagnosis, iron metabolism parameters (iron, ferritin, transferrin), complete blood count, inflammatory marker [C-reactive protein (CRP)], liver function tests (LFT) [alanine aminotransferase (ALT); aspartate aminotransferase (AST); bilirubin), kidney function tests [creatinine; and blood urea nitrogen (BUN)]. Transferrin saturation (TfS) was calculated according to the formula: (iron [μg/dL]//transferrin [mg/dL]) ×71.6 [%]. All laboratory parameters were determined at the ICU admission. Reference values for all laboratory parameters were as per a local laboratory performing determinations (Tables I, II).

Based on cut-off values for ferritin (<100 μg/L), transferrin saturation (<20%) and combined ferritin and transferrin saturation (ferritin <100 μg/L and TfS <20% or ferritin <300 μg/L and TfS <20%) reported in literature [18–20], we determined the incidence of IDA in our study group. Analysis of associations between ferritin/transferrin and parameters of inflammation and liver function was performed in order to determine the possible impact of systemic inflammation and liver dysfunction on the results obtained.

Table I. Selected laboratory parameters in the study population

Parameter	Me (IQR)	Reference value/range [9]
Hb [g/L]	96 (87–109)	<130
MCV [fL]	92 (88–96)	84–98
MCH [pg]	29 (28–32)	27–31
MCHC [g/dL]	33 (31–34)	32–36
RDW-CV [%]	15 (14–18)	11–16
RDW-SD [fL]	52 (44–57)	39–50
CRP [mg/L]	119 (44–196)	<5
ALT [U/L]	27 (17–68)	<34
AST [U/L]	49 (28–96)	<31
Bilirubin [mg/dL]	0.6 (0.5–0.9)	0.3–1.2
Creatinine [mg/dL]	0.95 (0.79–1.89)	0.84–1.25
BUN [mg/dL]	29 (21–56)	7.9–20

Me — median value; IQR — interquartile range; Hb — hemoglobin; MCV — mean corpuscular volume; MCH — mean corpuscular hemoglobin; MCHC — mean corpuscular hemoglobin concentration; RDW-CV — red blood cell distribution width coefficient of variation; RDW-SD — red blood cell distribution standard deviation; CRP — C-reactive protein; ALT — alanine aminotransferase; AST — aspartate aminotransferase; BUN — blood urea nitrogen

Statistical analysis was performed using licensed statistical software (MedCalc v.18.2.1, MedCalc Software, Ostend, Belgium). Quantitative variables with normal distribution were presented as means and standard deviations, and with non-normal distribution as medians and interquartile ranges (IQR). The distribution of variables was verified by the d'Agostino-Pearson test. Correlations were assessed based on the Pearson correlation coefficient (normal distribution) or Spearman's rank (non-normal distribution) correlation coefficient. P <0.05 was considered statistically significant.

Due to the observational retrospective character of this study there was no requirement for bioethics committee approval. Patient data have been anonymised.

Results

The study population consisted of 51 patients, 27 (53%) men and 24 (47%) women, median age was 64 (IQR 57–72) years. Primary diagnoses in the study population are presented in Table III. The four most frequent primary diagnoses were subarachnoidal hemorrhage, pneumonia, sudden cardiac arrest, and heart failure (n =29). Selected laboratory parameters in the study population are presented in Table II. The median Hb concentration in the study

Table II. Iron metabolism parameters in the study population

Parameter	Me (IQR) all patients	Me (IQR) women	Me (IQR) men	*P* value for sex	Reference range
Ferritin [µg/L]	686 (385–1,114)	567 (342–826)	773 (521–2,407)	0.2	4.6–204
Iron [µg/dL]	37 (18–78)	37 (19–74)	36 (19–81)	0.9	60–180
Transferrin [mg/dL]	159 (118–195)	162 (112–188)	159 (119–203)	0.6	200–360
TfS [%]	16 (9–36)	17 (9–38)	16 (9–35)	0.9	20–45

Me — median value; IQR — interquartile range; TfS — transferrin saturation

Table III. Primary diagnoses in the study population

Primary diagnosis	N
Neurological:	
• subarachnoid hemorrhage	8
• meningitis	2
• intracranial hypertension	2
• brain tumor	1
• craniocephalic trauma	1
• ischemic stroke	1
• encephalitis	1
• epilepsy	1
Heart failure	6
Post sudden cardiac arrest	7
Gastrointestinal:	
• acute pancreatitis	2
• bowel perforation	1
• peritonitis	1
• bowel obstruction	1
Pneumonia	8
Acute respiratory failure	3
Septic shock	3
Others	2

Table IV. Incidence of iron-deficiency anemia in the study population

Parameter	% of population
Ferritin <100 µg/L	11.8
Ferritin <100 µg/L + TfS <20%	7.8
Ferritin <300 µg/L + TfS <20%	15.7
TfS <20%	56.9

TfS — transferrin saturation

Table V. Associations between parameters of iron metabolism and other parameters

Association	Correlation coefficient	*P*-value
Ferritin–CRP	0.22	0.13
Transferrin–CRP	**–0.28**	**0.04**
Ferritin–bilirubin	0.14	0.30
Ferritin–ALT	0.27	0.05
Ferritin–AST	**0.31**	**0.03**
Transferrin–bilirubin	–0.21	0.14
Transferrin–ALT	0.02	0.88
Transferrin–AST	–0.09	0.53
Ferritin–MCV	0.25	0.08
Ferritin–MCH	0.26	0.07
Ferritin–MCHC	0.05	0.71
Ferritin–RDW-CV	0.19	0.19
Ferritin–RDW-SD	**0.44**	**0.001**
Transferrin–MCV	–0.01	0.92
Transferrin–MCH	0.02	0.90
Transferrin–MCHC	–0.02	0.87
Transferrin–RDW-CV	**–0.36**	**0.01**
Transferrin–RDW-SD	**–0.40**	**0.004**

CRP — C-reactive protein; AST — aspartate aminotransferase; ALT — alanine aminotransferase; MCV — mean corpuscular volume; MCH — mean corpuscular hemoglobin; MCHC — mean corpuscular hemoglobin concentration; RDW-CV — red blood cell distribution width coefficient of variation; RDW-SD — red blood cell distribution standard deviation; **in bold: statistically significant associations**

population was 96 (IQR 87–109) g/L, well below the cut-off value for anemia. The studied population had a markedly increased concentration of CRP, with a median value of 119 (44–196) mg/L. Liver and kidney function tests were within reference ranges. As far as iron metabolism parameters are concerned, ferritin concentration in our patients was more than 3-fold upper limit of normal iron and transferrin saturation were below the reference values. Iron metabolism parameters in the study population are presented in Table II. The number of study subjects diagnosed with IDA based on different reported cut-off values of iron metabolism parameters is presented in Table IV. Depending on the cut-off value chosen, IDA could be diagnosed in between 7.8% (ferritin <100 µg/L +TfS <20%) and 56.9% (TfS <20%) of patients.

We found weak correlations between iron metabolism parameters and parameters of inflammation and LFT (AST).

There were also some weak correlations between standard iron metabolism and erythrocyte (red blood cell distribution width coefficient of variation/standard deviation) parameters (Table V).

Discussion

In our study, we used standard iron metabolism parameters to diagnose IDA in critically ill patients hospitalized in the ICU. In order to do this, we used different reported in the literature cut-off values for ferritin (<100, <300 µg/L) or TfS (<20%) alone [18–20].We also combined different cut-off values for both these parameters together. The more than 7-fold disparity in the percentage of patients diagnosed with IDA using the different criteria (i.e. from7.8% to 56.9%) shows that we were unable to precisely diagnose IDA (absolute or functional ID) in critically ill patients using these standard iron metabolism parameters. The incidence of IDA among patients admitted to the ICU reaches up to 66% [2]. The reason for this disparity lies in the population studied. Systemic inflammatory response is frequently present in patients hospitalized in the ICU and is associated with the following conditions: trauma, surgery, acute pancreatitis, burns, and sepsis. Ferritin is an acute phase reactant and its concentration rises in systemic inflammatory response. Transferrin concentration in an acute phase may change in both directions. Median ferritin concentration in our study was more than double the upper limit of normal (686, IQR 385–1,114 µg/L), thus lowering the number of patients diagnosed with IDA. The high ferritin concentration in our study was due to systemic inflammatory response present in the patients, which was heralded by median CRP concentration well above the reference value (119, IQR 44–196 mg/L). However we did not find any correlation between ferritin and CRP concentration (correlation coefficient 0.22, p =0.13). On the other hand, median concentration of iron (37, IQR 18–78 µg/dL) was well below the reference range (60–180 µg/dL) and transferrin concentration was slightly below the reference range (median 159, IQR 118–195, reference 200–360 mg/dL), lowering the calculated TfS, and hence increasing the number of patients diagnosed with IDA.

In order to precisely diagnose IDA in critically ill patients, it is of the utmost importance to accurately assess the amount of iron stored (absolute deficiency) and iron available for erythropoiesis (functional deficiency). Therefore other parameters have been proposed [21]. One of these parameters is the percentage of hypochromic red cells (%HRC), a figure which reflects recent iron reduction [15, 22]. Another is reticulocyte hemoglobin equivalent (Ret–Hb) which reflects functional iron reserve available for immature erythrocytes in the previous 3–4 days [23]. Ret–Hb quickly normalizes with iron supplementation and can be used in treatment monitoring [24]. Another parameter is hepcidin. Hepcidin is a peptide hormone produced in the liver which along with its receptor (ferroportin) regulates iron transport and availability in the body [25, 26]. This is gradually used more frequently in diagnosis of functional IDA [27, 28].

Our study has some limitations. The first might be the number of study subjects. We analysed 51 subjects. However this was a pragmatic study aimed at finding the incidence of IDA in a heterogenous group of our critically ill patients using standard parameters of iron metabolism. We did not diagnose other types of anemia that could be present in our ICU patients (e.g. folate, vitamin B_{12}, vitamin A deficiency, immune hemolytic anemia). Our only goal was to analyze the incidence of IDA in our ICU population based on standard parameters of iron metabolism. Another limitation is that we used only one laboratory parameter of inflammation (CRP), however this has been used previously in the context of anemia and inflammation [9]. Although our study was single-center, wide variability in iron metabolism results obtained from different laboratories was shown, so identical laboratory equipment must be used in multi-center studies. The variability in results obtained in different laboratories can reach as high as 50% [19]. Standard parameters of iron metabolism have diurnal variability. ICU patients are admitted at different times of the day, and this could impact on the results obtained. However some new parameters also have diurnal variability (hepcidin).

Conclusions

Ferritin and transferrin saturation cannot be used for a precise diagnosis of anemia caused by absolute or functional iron deficiency in critically ill patients, due to the systemic inflammatory response frequently encountered in these patients.

Authors' contributions

PFC — design, data collection, final manuscript, MPP — statistical analysis, final manuscript, ŁJK — critical review, final manuscript.

Conflict of interests

None.

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None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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From classical Langerhans cell histiocytosis to Erdheim--Chester disease: different sides of the same coin?

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Abstract

Introduction: We analysed the clinical course of classical histiocytosis (LCH) in children, and the clinical differences, diagnostic difficulties and different therapeutic strategy in a child with a rare variant of LCH in the form of Erdheim-Chester disease (ECD).

Material and methods: We conducted a retrospective single-center analysis of the clinical course of classic LCH in 54 children who were diagnosed with the disease over the last 40 years, and the differences shown in a patient with diagnosed ECD. The clinical response was assessed according to the LCH programs valid at the time for the classic form of LCH and in the child with ECD.

Results: The multi-system form of LCH was diagnosed more often than the single-system form. The skull bones were the most common localization of LCH in both forms of the disease. Recurrence of the disease occurred in about 5% of patients, and death in one (1.9%) patient. The course of the child with ECD was more turbulent, with rapid progression, the involvement of critical organs, and no response to standard chemotherapy according to the LCH 2009 protocol. After a molecular diagnosis was specified, therapy with vemurafenib, a *BRAF-V600E* kinase inhibitor, followed by allogeneic hematopoietic stem cells transplantation (allo-HSCT) was applied. The basic disease has been in remission for the last 12 months.

Conclusions: The lack of an expected response to LCH therapy should indicate the possibility of rare forms of histiocytic hyperplasia. Molecular tests are an important element in the diagnosis of histiocytosis, and allow the precise selection of the most appropriate, targeted therapy. BRAF-V600E kinase inhibitors are highly safe and effective in the treatment of LCH and ECD with a confirmed *BRAF-V600E* mutation, although allo-HSCT should be considered in selected cases.

Key words: Erdheim-Chester disease, BRAF-V600E mutation, vemurafenib, children

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Introduction

Langerhans cell histiocytosis (LCH) is a very rare disease, with a frequency of 0.1–1 new case per 100,000 children per year. Most often it is diagnosed up to the age of 6 [1]. LCH is a heterogeneous group of diseases characterized by uncontrolled growth, proliferation and differentiation of cells of the mononuclear-phagocytic system. It belongs to the family of clonal proliferative diseases, and lies on the border with neoplastic diseases. At the root of LCH are disturbances in the mitogen-activated kinase (MAPK) pathway. Their occurrence is associated with a more serious course

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and a higher probability of progression and recurrence. One of the rare forms of histiocytic hyperplasia in children is Erdheim-Chester disease (ECD). ECD's pathogenesis is related to the *BRAF-V600* mutation [1, 2].

Histiocytosis can affect any organ or system, with the occurrence of permanent complications in 20–30% of patients, including death. The skeletal system (80%), skin (33%) and then the pituitary gland (25%) are most often involved. The disease is also localized in parenchymal organs: liver, spleen, hematopoietic system and lungs (15% each), lymph nodes (5–10%) and the central nervous system excluding the pituitary gland (2–4%). The clinical course varies from self-limited to rapidly progressive, leading to death.

Treatment varies according to the severity of the disease. The response to first-line treatment is important prognostic information, and helps in the selection of a further therapeutic strategy, often based on genetic tests, allowing for the selection of the most effective therapy [3–6]. The number of randomized clinical trials is limited in the literature, and many aspects of management of this condition remain controversial.

The current diagnostic procedure in pediatric LCH has been developed by the Histiocytic Society and also based on evidence-based-medicine papers, published between 2009 and 2020 [4, 7, 8]. In the diagnosis of histiocytosis, a histopathological examination with immunophenotyping is decisive and differentiating. The current guidelines suggest mandatory genetic testing to detect mutations in the MAPK pathway, especially the mutation identifying *BRAF*. Clinical and laboratory data allows the patient to be classified as either single system or multi system, as well as to determine the involvement of critical organs. Basic tests include blood tests (hematological, biochemical, liver and kidney function, coagulation parameters and lipid profile), urine tests, and imaging tests (lung X-ray, abdominal ultrasound). Scintigraphy of the skeletal system, as well as positron emission tomography with computed tomography (PET-CT) in patients with indications for systemic treatment, is very important. Such meticulous diagnostics allows all affected organs to be identified and facilitates monitoring of the response to treatment. The study designs in LCH patients have been presented many times and are consistent in different countries, according to different working groups [4–6].

LCH in the past, the mysterious histiocytosis X, still eludes exhaustive classification. The most recent classification divides disorders in the histiocytosis group into five groups (Tables I and II): LCH, ECD, indeterminate cell histiocytosis (ICH), extra-cutaneous JXG, as well as mixed forms of LCH and ECD belonging to the group L histiocytosis [3, 4, 9, 10].

Erdheim-Chester disease is a rare non-Langerhans cell histiocytosis (non-LCH), first described as a "lipid granulomatosis" by Jakob Erdheim and William Chester in 1930 [11]. So far, about 1,500 cases of ECD have been reported worldwide, but only 11 in children [12]. The age at onset of the disease is 43–55 years, and it is more common in men [13–16]. Average survival time in adults is c. 32 months [17]. ECD is a multi-system clonal hematopoietic disease which comprises the infiltration of tissues by histiocytes taking on the characteristic appearance of foam cells [18–20]. Somatic genetic mutations are responsible for clonal growth of the bone marrow, as a result of which the MAPK pathways are activated in the inflammatory environment [7, 19]. The most common mutation in the MAPK pathway in ECD is the *BRAF-V600E* mutation (60–100% of patients) [13, 16, 20–22]. Other mutations are *PIK3CA* in 11% and *NRAS* in 4% of patients [23]. A strong systemic inflammatory reaction is associated with the activation of the Th1-dependent response, resulting in the production

Table I. Classification of histiocytoses (source [9])

Histiocytosis group	Diseases
L	LCH
	Indeterminate-cell histiocytosis (ICH)
	Erdheim-Chester disease (ECD)
	Mixed LCH/ECD
C	Cutaneous non-LCH
	Xanthomatous granuloma (XG) family: JXG, AXG, SRH, BCH, GEH, PNH
	Non-XG family: cutaneous RDD, NXG, other
	Cutaneous non-LCH with a major systemic component
	XG family: XD
	Non-XG family: MRH
R	Familial RDD
	Sporadic RDD
	Classical RDD
	Extranodal RDD
	RDD with neoplasia or immune disease
	Unclassified
M	Primary malignant histiocytoses
	Secondary malignant histiocytoses
H	Primary HLH: monogenic inherited conditions leading to HLH
	Secondary HLH (non-Mendelian HLH)
	HLH of unknown or uncertain origin

LCH — Langerhans cell histiocytosis; JXG — juvenile xanthogranuloma; AXG — adult xanthogranuloma; SRH — solitary reticulohistiocytoma; BCH — benign cephalic histiocytosis; GEH — generalized eruptive histiocytosis; PNH — progressive nodular histiocytosis; RDD — Rosai-Dorfman disease; NXG — necrobiotic xanthogranuloma; XD — xanthoma disseminatum; MRH — multicentric reticulohistiocytosis

Table II. Proposed new classification of histiocytosis group L (acc. to [10])

1. Langerhans cell histiocytosis (LCH)
LCH SS
LCH lung+
LCH MS-RO+
LCH MS-RO
Associated with other myelo-/lymphoproliferative diseases
2. Intermediate cell histiocytosis (ICH)
3. Erdheim-Chester disease (ECD)
ECD — classical type
ECD without bone involvement
Associated with other myelo-/lymphoproliferative diseases
4. Extracutaneous and disseminated juvenile xanthogranulomatosis (JXG) with activating MAP kinase mutation or ALK translocation
5. Mixed form ECD and LCH

SS — single organ; MS — mixed systems; RO — risk organ

of the cytokines interferon (IFN) alpha, interleukin (IL) 1/ /IL-1 receptor antagonist IL-1-RA, IL-6, IL-12 and monocyte chemoattractant protein 1 (MCP-1) [7, 19].

In a typical histopathological presentation, in addition to foam histiocytes, giant Tauton cells and stromal fibrosis are common. In immunohistochemistry, histiocytes show positive reactions for CD68, CD163 and factor XIIIa, negative for CD1a and langerin. Most commonly, the S100 protein is not detected. Unlike ECD, histiocytic cells in LCH have the phenotype of CD1a+, S100+ and Langerin+ [15, 19, 20]. Histopathological and immunohistochemical images in ECD and in juvenile xanthogranuloma (JXG) are almost the same, and therefore it is believed that ECD is a variant of JXG but without dominant skin involvement [20]. In the course of ECD, any organ may be involved, most often the long bones (in 80–90% of patients), the retroperitoneal space within the kidneys (60%), the cardiovascular system (50%+), the central nervous system (40–50%), the lungs (15–33%), and less commonly the skin, lymph nodes, liver, and spleen [1, 5, 12, 23–28]. The multi-organ form of ECD consists of skeletal failure, with the most common symmetrical bilateral osteosclerotic changes in the long bones around the knee joints.

Bone pain occurs in 39% of patients, and is localized in the distal sections of the lower limbs [28]. In children, the involvement of the skull, mandible, ribs and spine bones is more common [2]. In the multi-system form, the next affected organ is the kidneys, giving the typical image of infiltrates in computed tomography, the so-called 'hairy kidney'. Cardiovascular symptoms in ECD may include a pseudo-tumor in the right atrium, changes in the coronary arteries, and pericarditis. Lung involvement is frequent, with cellular infiltration and thickening of the alveoli with pleural effusion. Swelling of the optic nerve and the presence of retrobulbar masses can cause exophthalmos and impaired motor function, as well as fibrotic changes along the optic nerve, extending up to the hypothalamus and pituitary gland, with the possibility of diabetes insipidus. Yellow tufts (xanthelasma), as well as yellow or red-brown foci, may appear on the skin [11, 16, 20, 25, 27, 29].

The first ECD therapies were based on chemotherapy (vinblastine, cyclophosphamide, anthracyclines), radiotherapy, steroid therapy, interferon alpha, and anti-cytokine drugs (anakinra, infliximab and tocilizumab), and showed improvement in only 50% of patients [11, 12, 16]. The discovery and frequent occurrence of the *BRAF-V600E* mutation in ECD revolutionized the understanding of the pathogenesis of the disease and led to research focused on appropriate therapies showing high efficacy [15, 30]. In 2012, the BRAF and extracellular-signal regulated kinase (MEK) inhibitors, vemurafenib and cobimetinib, respectively, were introduced to the treatment of ECD, and in 2017 the US Food and Drug Administration (FDA) approved vemurafenib for the treatment of BRAF+ ECD [11]. Anti-BRAF therapy (vemurafenib) is currently recommended at diagnosis in all BRAF+ patients, except for asymptomatic patients [7, 19, 20] (Figure 1). BRAF and MEK inhibitors are believed to be effective and safe drugs in life-threatening forms of ECD [3, 4, 5, 9]. The prognosis for ECD is generally good, but there are cases where second-line treatment and hematopoietic cell transplantation are required.

Due to the rarity of LCH, each initiation of therapy requires verification not only of the current literature but also of the available pharmacotherapy, in both registration and off-label modes. In the absence of clearly defined management protocols, it is also necessary to perform a retrospective evaluation of the effectiveness and safety of the applied therapy in previously treated patients.

The aim of our study was to analyze the clinical course and treatment effects of classic LCH, diagnosed in children over the last 40 years at our Clinic, and to analyze the course and treatment of ECD in a 3-year-old girl with a critical analysis of the similarities and differences in both forms of histiocytic hyperplasia.

Material and methods

Our study consisted of two parts. Firstly, we looked at the epidemiology and treatment outcomes of LCH in children in a 40-year (1980–2020) single center study. Medical records concerning hospitalization and outpatient treatment of patients were assessed. Due to the limited access to historical documentation, it was possible to obtain only some clinical data. Secondly, we assessed the diagnostic process in a patient with atypical symptoms and initial lack of response to treatment according to the classic LCH

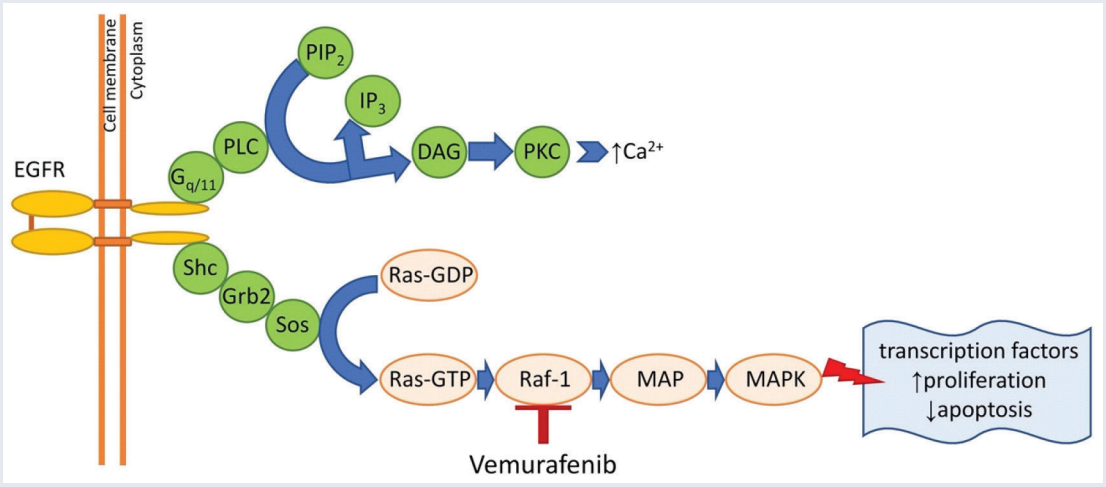

Figure 1. Schematic illustration of vemurafenib intervention in proliferation and apoptosis process; Ca^{2+} — calcium ions; DAG — diacylglycerol; EGFR — endothelial growth factor receptor; GDP — guanosine diphosphate; GTP — guanosine triphosphate; GRB2 — growth factor receptor-bound protein 2; $G_{q/11}$ — G protein type $G_{q/11}$; IP_3 — inositol 1,4,5-trisphosphate; MAP — mitogen-activated protein; MAPK — mitogen-activated protein kinase; PIP_2 — phosphatidylinositol 4,5-bisphosphate; PLC — phospholipase type C; PKC — protein kinase type C; RAF-1 — serine/threonine-specific protein kinases; RAS — small GTPase; Shc — signaling adaptor protein; Sos — guanosine nucleotide exchange factor

treatment protocol, the importance of molecular tests for correct diagnosis, and the effects of targeted treatment with the subsequent option of hematopoietic cell transplantation.

Results

Epidemiology, clinical manifestation and results of LCH treatment in children 1980–2020

Between 1980 and 2020, 1,785 children were diagnosed and treated at the Department of Pediatrics, Hematology and Oncology, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland. The number of patients with hematological malignancies (acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and LCH was 1,069 (59.9%), including 54 with LCH, which accounts for 3.0% of all malignant neoplasms and 5.1% of all hematological tumors.

In the studied group of children, the multi-system form was observed more often (in 54%, *see* Table III). The dominant clinical manifestations of LCH in any form were bone lesions located in the bones of the skull in several patients with intracranial penetration and infiltration of the dura mater. Bone lesions also occurred in the eye socket, in the mastoid process, in the ribs, in the mandible, in the long bones, and in one patient in the vertebrae of the thoracic spine and the pubic bone. The next most frequently affected organ was the skin. In the multi-system form, changes in the skeletal system, lungs, liver, spleen, peripheral lymph nodes and pancreas were simultaneously observed. Recurrence of the disease occurred in a total of three patients (5.5%). One patient died (1.85%).

The treatment was applied in accordance with the recommendations in force at the time. In recent years, the treatment was implemented according to the Langerhans Cell Histiocytosis 2009 protocol, Histiocyte Society Evaluation and Treatment Guidelines MS-LCH.

In 1/54 patients with primary diagnosed multiorgan LCH, the course was atypical, with no involvement of the skeletal system, but with involvement of the critical organs: liver, spleen and bone marrow. No improvement was achieved after the treatment according to LCH 2009. Due to the disease progression, the diagnostics was extended and a molecular test was performed. The obtained results made it possible to verify the diagnosis at ECD and to implement targeted and effective therapy.

Erdheim-Chester disease: analysis of diagnostic and therapeutic process

A 3-years-old girl with an unburdened perinatal history, and with a family history of sarcoidosis in the father and inhaled allergy in the mother, was admitted to our department. By the age of 2.5 years, she had been diagnosed with recurrent abdominal pain and diarrhea. Primary malabsorption syndrome and inflammatory disease of the gastrointestinal tract were excluded, and allergy to cow's milk protein and rice confirmed. After introducing the diet, the child's condition improved moderately. At the age of 2.7 years, the child developed a high fever

Table III. Characteristics of patients with Langerhans cell histiocytosis (LCH)

Variable	Value
Number of patients	54 (100%)
Age (years): median (range)	6.25 (0.5–17.8)
Sex: female vs. male	26 (48.2%)/28 (51.8%)
Organ involvement:	
• bone	44 (81.5%)
• skin	16 (30%)
• hematopoietic	2(3.7%)
• spleen	2 (3.7%)
• liver	2 (3.7%)
• lungs	2 (3.7%)
• eye	1 (1.85%)
• ear	3 (5.5 %)
• diabetes insipidus	5 (9.3%)
• central nervous system	5 (9.3%)
• lymph nodes	3 (5.5%)
Form of disease:	
• single-system LCH	25 (46%)
• multisystem LCH with or without involvement of risk organs	29 (54%)

with generalized lymphadenopathy, hepatosplenomegaly and bilinear cytopenia. Bone marrow biopsy revealed hypoplasia and suspected aplastic anemia. Skin purpura, hepatosplenomegaly, and a large, bloated abdomen were observed. The clinical presentation was dominated by severe abdominal pain and diarrhea. Laboratory tests revealed a decreased activity of amylases in blood serum, and in the CT examination, a manifestation of the pancreas typical for organ inflammation. Acute pancreatitis was treated, but there was no improvement. As part of the differential diagnosis, bacterial, viral, fungal and parasitic infections, Gaucher disease, Niemann-Pick disease, sarcoidosis, autoimmune lymphoproliferative syndrome, and phagocytosis disorders were excluded. Due to persistent bone marrow insufficiency and exocrine pancreatic insufficiency, Shwachman-Diamond syndrome was suspected, which was excluded by molecular examination. Myelodysplastic and hemophagocytic syndromes were also excluded. The presence of histiocytes with a foam-like cytoplasm, CD68+, part XIII+, CD1a-, Langerin -, in a few S-100+ cells was found. The bedding showed the loss of reticulin fibers. The image corresponded to a diagnosis of disseminated juvenile xanthogranuloma.

High-resolution computed tomography of the chest (HRCT) revealed inflammatory changes and areas of 'frosted glass', as well as nodules in the lungs that may correspond to infiltrates in the course of histiocytosis. No pathological changes were found in the scintigraphy of the skeletal system. The PET-CT examination showed no signs of an active malignant proliferative process. There were also no deviations in cardiological and neurological examination. Treatment according to the LCH-2009 protocol (prednisone, vinblastine) was started, after which the progression of clinical symptoms and increasing signs of bone marrow failure were observed. Another bone marrow biopsy test revealed the presence of CD68+, CD1a–, S-100–, CD14+, CD163+ protein, moreover the *BRAF-V600E* mutation was found in bone marrow cells.

Eventually, ECD was diagnosed. After obtaining the approval of the Therapeutic Committee for off-label therapy, anti-BRAF therapy with the drug vemurafenib at a dose of 10 mg/kg bw/day was initiated, achieving a spectacular clinical improvement. The symptoms of the gastrointestinal tract subsided, and normalization of blood cell count and biochemical parameters was observed. The parenchymal organs of the abdominal cavity decreased significantly. Normal CT image of the pancreas was observed. Anti-BRAF therapy was used for three months without any side effects. Subsequently, an allogeneic hematopoietic stem cell (allo-HSCT) transplantation was performed from a matched unrelated donor. The post-transplant course was uneventful. In the control tests performed two months after allo-HSCT, no *BRAF-V600E* mutation was found. The bone marrow image was rich in cells with reconstruction of all lines of the hematopoietic system. Currently, the child is in the 11th month of follow-up after allo-HSCT, in clinical and molecular ECD remission.

Discussion

Histiocytic hyperplasia is a rare form of cancer in children. In our Clinic, they were diagnosed in 3% of pediatric patients diagnosed with any neoplastic disease, and in c.5% of pediatric patients diagnosed with hematological neoplasms. The classic form of LCH is a disease with a good prognosis: 5-year survival in children is 90%. However, relapses are observed in as many as one third of patients [9]. In our presented study, recurrence of LCH was reported in 5.5% of children.

The course of the rare forms of histiocytic hyperplasia, including ECD, is different.

The age at onset was between 1 year and 14 years [12, 14]. In all children, the skeletal system was affected (long bones, bones of the cranial vault, jaw, mandible, pelvic bones, ribs and spine). Infiltrations in the lungs and retroperitoneal space were noted in two patients, and in the central nervous system in three. One patient had hepatosplenomegaly [2, 14]. The most common involvement, of the bones, skin, cardiovascular system and central nervous system, was not confirmed in the presented child. Her clinical picture was dominated by bone marrow failure, severe abdominal pain and diarrhea with hypoalbuminemia associated with infiltrates in the pancreas and its exocrine insufficiency. The consequence of bone marrow aplasia was the necessity to use numerous blood transfusions and

the treatment of life-threatening infections. The presented patient had the clinical manifestation with predominant myelo-pancreatic-pulmonary disease of Erdheim-Chester.

Among the children with ECD described so far, none of them had bone marrow failure [2, 14], which was the dominant symptom in our patient. The literature lacks data on the frequency of malignant bone marrow transformation in children with ECD. However, it is known from the literature that ECD is associated with an increased risk of hyperplasia of the hematopoietic system, mainly the myeloid lineage [25]. Papo et al. [26] reported cases of chronic myelomonocytic leukemia, myelodysplastic and myeloproliferative syndromes, and *polycythemia vera* in 10% of adult ECD patients. In a study by Cohen-Aubart et al. [19], 15.8% of adult ECD patients developed myeloid neoplasm with present genetic abnormalities in the *TET2, ASXL1, DNMT3A*, and *NRAS* genes. These patients were significantly more often elderly, and the BRAF-V600E mutation was found significantly more often [19].

In the case reports of childhood ECD, delays in diagnosis of the disease, ranging from six months to six years, are frequently underscored [2]. It cannot be ruled out that the symptoms of the disease had appeared in our patient much earlier, as the girl had had abdominal pain and recurrent diarrhea from age 9 months. The causes of the ailments were not established in the extensive diagnostics, and dietary treatment did not bring about any significant improvement. For a year before the correct diagnosis, the girl was treated with pancreatin, with quite good results. This would suggest some initial changes in the pancreas. In the diagnosis of histiocytosis, a biopsy of the affected organ should be carried out together with the assessment of the MAPK pathway mutation. Due to the similarities between ECD, JXG and LCH, it is sometimes necessary to repeat the histopathological examination, especially when the patient does not respond to the treatment according to the LCH protocol [14]. This was the case in the described patient, who initially received treatment with vinblastine and steroids, but, due to disease progression, another bone marrow trephine biopsy was performed with the assessment of the *BRAF-V600E* mutation.

Confirmation of the *BRAF-V600E* mutation firstly differentiates ECD from JXG, and secondly allows for an effective targeted therapy. Among 11 reported children with ECD, only two were tested for the *BRAF-V600E* mutation, which was confirmed in one. MAPK inhibitors were not used in the therapy [14]. The literature also lacks data on hematopoietic cell transplantation in patients with ECD. The presented patient was the first child to develop bone marrow failure in the course of ECD. Taking into account the lack of data on anti-BRAF therapy in children worldwide (duration of therapy, side effects, effectiveness) and the documented risk of bone marrow malignant transformation, it was decided to transplant the patient with allogeneic hematopoietic cells with preceding anti-BRAF therapy. This treatment led to a complete recovery.

Conclusions

Erdheim-Chester disease is extremely rare in children, but it should be considered in all cases of histiocytosis, especially in multi-system forms, with poor response to standard therapy. Molecular diagnostics should be sought. The described case of a child is also an example of the diagnostic difficulties in bone marrow aplasia. The clinical presentation of the patient and the cytomorphological manifestation of the bone marrow initially corresponded to aplastic anemia, but the decisive factor was the histopathological and immunohistochemical results of bone marrow trephine biopsy and confirmation of *BRAF-V600E* mutation in histiocytes. The heterogeneity and diversity of the clinical symptoms, and the atypical age of the patient were the reasons for the long drawn-out differential diagnosis, but with therapeutic success through the use of anti-BRAF therapy followed by the procedure of hematopoietic cells transplantation.

Authors' contributions

EG — study design, data collection and analysis, interpretation of results, writing manuscript; JS — study design, critical revision of manuscript; all other Authors contributed to provision of important clinical data, interpretation of results, and final approval.

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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ORIGINAL RESEARCH ARTICLE

Therapeutic drug monitoring of posaconazole for effective prophylaxis of invasive fungal infections in pediatric patients: a pilot study

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Abstract

Introduction: Posaconazole, a second-generation triazole agent, is a drug with wide interindividual variation in bioavailability and variability of pharmacokinetics. The European Conference on Infections in Leukemia recommends the implementation of posaconazole therapeutic drug monitoring (TDM) in children to provide certainty regarding the efficacy of treatment and to increase its safety. A concentration of posaconazole in plasma in prophylactic regimens of above 0.7 mg/L is recommended.

The aim of our pilot study was to discover the most effective method of posaconazole plasma concentration measurement, and to analyze posaconazole pharmacokinetics profiles in pediatric patients on posaconazole prophylaxis.

Material and methods: Children receiving oncological treatment or hematopoietic stem cell transplantation in the Department of Pediatric Bone Marrow Transplantation, Oncology, and Hematology in Wroclaw Medical University Hospital were included in the trial. Posaconazole concentration was determined at the MonitLab™ laboratory using a newly validated high-performance liquid chromatography with fluorescence detector method.

Results: Initial analysis shows that the major problem in patients on antifungal prophylaxis is inadequately low plasma drug concentration.

Conclusions: Clinical trials on TDM of posaconazole should be developed.

Key words: posaconazole, therapeutic drug monitoring (TDM), antifungal prophylaxis

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Introduction

The appropriate medication dosage often depends on a patient's individual metabolism patterns, and therefore closer cooperation between physicians and pharmacologists seems sensible. One solution that enables therapy to be customized to the individual patient's needs is therapeutic drug monitoring (TDM).

TDM provides certainty as to the efficacy of treatment by confirming that the plasma concentration of medicine is fixed within the desired level. It increases the safety and efficacy of the implementation of the drugs within a narrow therapeutic range (NTR).

Posaconazole is a second-generation triazole agent with a broad spectrum of activity against a range of important fungal pathogens including: *Aspergillus spp.*,

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Candida spp., Mucormycetes spp., and *Fusarium spp.* As a result, it is widely used in pediatric onco-hematological wards, because fungal infections are one of the most frequent complications that occur in immunocompromised patients. Appropriate prophylaxis and targeted treatment decrease mortality rates significantly. However, it should be remembered that the therapeutic efficacy of posaconazole is conditional upon achieving a plasma drug concentration target at a steady state, which should be considered as an equivalent to the minimum inhibitory concentration (MIC_{90}) for a pathogen causing an infection.

Posaconazole is a drug with wide interindividual variation in bioavailability and well-recognized variability of pharmacokinetics. For that reason, the European Conference on Infections in Leukemia (ECIL) recommends the implementation of posaconazole TDM in children as routine practice to ensure an adequate exposure, and to optimize the clinical response [1].

Medicines involved in drug-to-drug interactions (DDIs) can be divided into two groups: victims — i.e. drugs that are directly affected by DDIs, and perpetrators — i.e. drugs that cause DDIs. Posaconazole can act in both ways. It can be affected as a victim drug by interactions with medicines that interact with uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes or P-glycoprotein. Nearly 20% of posaconazole is glucuronidated by UGT1A4. Both inducers (e.g. rifampicin, some anticonvulsants) and inhibitors of the enzyme (e.g. verapamil, cyclosporin A, macrolides) can alter posaconazole's plasma concentration.

On the other hand, posaconazole is also an inhibitor of CYP3A4, so it affects the metabolism of drugs that are substrates for that enzyme, such as cyclosporin A [2].

Although posaconazole is characterized by better tolerance and fewer interactions than other azoles, its pharmacokinetics is affected also by other factors such as: the clinical condition of the patient, the patient's age, the route of drug administration, and the means of the patient's feeding, all of which can be associated with altered medicine absorption. It is recommended to ingest this medication with a meal that is rich in lipids, or with soda drinks that increase the acidity. Proton pump inhibitors, as well as metoclopramide or gastrointestinal tract disorders such as mucositis or diarrhea, are known to decrease the bioavailability of posaconazole [2]. Because of this, these patients require especially precise dose adjustments according to posaconazole's plasma concentration in order to provide effective antifungal treatment or prophylaxis.

In Poland, two posaconazole formulations are available:

- suspension (this has possible bioavailability issues, is reimbursed for antimold prophylaxis in patients who underwent hematopoietic stem cell transplantation (HSCT) and who receive high dose immunosuppressive drugs due to graft-versus-host disease (GvHD) — children with myelodysplastic syndrome (MDS) prepared for HSCT, secondary antifungal prophylaxis after HSCT, chronic granulomatous disease with frequent fungal organ infections, children with acute myeloblastic leukemia (AML), myelodysplastic syndrome, acute lymphoblastic leukemia (ALL), recurrence of ALL or AML, malignant lymphomas or solid tumors receiving chemotherapy that can lead to prolonged neutropenia with high risk of fungal infections, and secondary antimold prophylaxis until chemotherapy or immunosuppressive therapy is completed;
- intravenous formulation (this is expensive, and is used in rare cases of patients who are unable to take the suspension).

Until now, slow-release tablets have not been available in Poland, although they offer much better bioavailability and stability [2].

According to ECIL-8 recommendations, the suggested concentration of posaconazole in plasma in prophylactic regimens is a concentration of above 0.7 mg/L [1]. It has been demonstrated that a concentration above 3 mg/L can be associated with the occurrence of side effects. If we derive our data from voriconazole, then a concentration of above 6 mg/L would be considered as associated with toxicity (predominantly neurotoxicity) [3].

TDM in clinical practice is based on measuring plasma drug concentration at designated intervals, when the steady state in patients' blood is obtained. For posaconazole, this is reached after 7–10 days of therapy. The main purpose of the implementation of posaconazole TDM in everyday practice is to improve the safety of posaconazole therapy in a way that will enable us to achieve the optimal medical outcome and limit side effects. To implement TDM of posaconazole in standard routine practice, we need a method of serum concentration assessment that is simple, quick, and cost effective.

The aim of our pilot study was to find the most effective method of posaconazole pharmacokinetics (PK) measurement, and to analyze PK profiles in pediatric patients on posaconazole prophylaxis.

Material and methods

Our subjects were selected from patients treated in the Department of Pediatric Bone Marrow Transplantation, Oncology, and Hematology at Wroclaw Medical University, Wroclaw, Poland. The protocol for this study was approved by the Wroclaw Medical University Ethics Committee before the study began. All the patients were administered posaconazole according to the Welzen's et al. [4] dosing recommendations. In the first phase of our clinical trial, we assessed a group of patients on posaconazole prophylaxis. We measured plasma drug concentration and we tried to review the impact of additional factors on posaconazole pharmacokinetics, factors that when compared to clinical data could help us to reach conclusions as to adequate and safe dosage modification in the future.

Table I. Clinical trial results

No.	Diagnosis	Age in years	Weight in kg	Treatment stage	Day on POSA	C 0H	C 3H	C 5H	C 8H	Median. Con.	Drugs with possible interaction with POSA
1	CD40 ligand def.	2	11	Day +14 after allo-HSCT	25	0.74	1.02	1.05	1.21	1.04	Acyclovir, colistin, meropenem, teicoplanin, *cyclosporin*
2	ALL	4	14.6	Day +49 after allo-HSCT	60	0.87	1.18	0.92	0.63	0.90	Sulfamethoxazole/ /trimethoprim, ursodeoxycholic acid
3	ALL	7	30.5	Day 6 HC1 IntReALL 2010	10	0.67	0.72	0.66	0.56	0.67	Sulfamethoxazole/ trimethoprim, acyclovir, ciprofloxacin, **omeprazole**
4	AML	3	11.5	Day –8 before allo-HSCT	90	0.44	0.63	0.56	0.50	0.53	Acyclovir, meropenem, oxybutynin
5	ALL	10	30	Year after allo-HSCT, GvHD	90	2.46	2.77	2.71	2.39	2.59	Sulfamethoxazole/ /trimethoprim, acyclovir, **omeprazole**, mycofenolate mofetil, methylprednisolone, levothyroxine, ursodeoxycholic acid
6	ALL	16	61	Day +33 after allo-HSCT, GvHD	8	0.26	0.32	0.30	0.33	0.31	Acyclovir, **omeprazole**, methylprednisolone, *cyclosporin*, teicoplanin, ursodeoxycholic acid
7	Lymphoma	16	55.5	Day +27 after allo-HSCT	8	1.42	1.02	1.56	1.23	1.33	Acyclovir, *cyclosporin*, rifaximin, ursodeoxycholic acid
8	WAS	5	18	Day +50 after allo-HSCT, GvHD	18	0.96	1.06	1.02	0.77	0.99	Amlodipine, atenolol, mycophenolate mofetil, colistin, *cyclosporin*, etanercept, foscarnet sodium, methylprednisolone, letermovir, rifaximin
9	Lymphoma	16	59	Day +3 after allo-HSCT	15	0.37	0.45	0.39	0.27	0.38	Sulfamethoxazole/trimethoprim, hydrocortisone, colistin, acyclovir, ursodeoxycholic acid, cyclosporin
10	ALL	11	33	Day +10 after CAR-T	400	0.69	0.86	0.84	0.76	0.80	Sulfamethoxazole/ /trimethoprim, ciprofloxacin, acyclovir, levetiracetam, tocilizumab
11	ALL	6	30	17 days after Protocol II AIEOP-BFM ALL 2017	7	3.06	3.65	2.93	2.75	3.00	Sulfamethoxazole/trimethoprim, nystatin
12	AML	4	18	Day +18 after HSCT	28	0.7	0.62	0.63	0.55	0.63	Sulfamethoxazole/ /trimethoprim, ciprofloxacin, acyclovir, cefepime, *cyclosporin*, teicoplanin, ursodeoxycholic acid

→

Table I (cont.). Clinical trial results

No.	Diagnosis	Age in years	Weight in kg	Treatment stage	Day on POSA	C 0H	C 3H	C 5H	C 8H	Median. Con.	Drugs with possible interaction with POSA
13	ALL	2	11.5	One day after Ara--C, Cons. A AIEOP-BFM ALL 2017	7	3.63	4.46	4.3	6.07	4.38	Sulfamethoxazole/ /trimethoprim, colistin, acyclovir
14	Rhabdoid tumor	1	8.6	Day –1 before auto-HSCT	7	0.44	0.55	0.59	0.72	0.57	Sulfamethoxazole/trimethoprim, acyclovir
15	AML	1	8.8	Day +7 after allo-HSCT	19	0.18	0.32	0.23	0.18	0.21	Sulfamethoxazole/ /trimethoprim, colistin, acyclovir, cyclosporin

Drugs in **bold** are those known to take part in drug-to-drug interactions with posaconazole (POSA) suspension, leading to **decreases** in its bioavailbility and serum concentration. Drugs in *italics* are those known to take part in drug-to-drug interactions with posaconazole suspension, leading to an *increase* in its serum concentration; C 0H, C3H, C5H, C8H — time points of taking samples from the patients, C 0H — just before the drug administration and then 3, 5, 8 hours after; allo-HSCT — allogeneic hematopoietic stem cell transplantation; ALL — acute lymphoblastic leukemia; GvHD — graft-versus-host disease; AML — acute myeloblastic leukemia; CAR-T — chimeric antigen receptor T cells; WAS — Wiskott-Aldrich syndrome; Ara-C — cytosine arabinoside

Blood samples were obtained from 15 patients during their hospitalization. The samples were taken after seven days of treatment with posaconazole, after reaching the drug steady state at four time points: 0 — just before the drug administration, and then three, five and eight hours after. The sample volume needed for analysis was 0.5 mL. All blood samples were centrifuged (3,500 g at 20°C for 5 min) and plasma was stored at –20°C until analysis. For each plasma sample, posaconazole concentration was determined at the MonitLab™ laboratory in Poznan, Poland using a newly validated high-performance liquid chromatography with fluorescence detector (HPLC-FLD) method. Chromatographic separation was achieved with the mobile phase consisting of acetonitrile/water with a flow rate of 1.3 mL/min. Each sample injection volume was 2 µL. 50 µL of patient plasma was used for the analysis. Protein precipitation procedure was applied for the extraction of posaconazole from plasma samples. The validation of the analytical method was carried out according to the guidelines on bioanalytical method validation of the European Medicines Agency (EMA).

Results

Demographics and TDM

The ages of our preliminary group of patients ranged from one to 16 years. 10 out of 15 patients included in the trial were diagnosed with leukemia, two patients suffered from lymphoma, and three had immunodeficiency syndromes such as CD40 ligand deficiency and Wiskott-Aldrich syndrome (WAS). 6/15 patients (40%) included in the trial presented with the desired plasma concentration of posaconazole in the intervals between doses. In 7/15 patients (47%), the plasma concentrations of posaconazole were lower, and were considered to be insufficient. All of these patients remained in the HSCT procedure, either before (n =2) or after HSCT (n =5). Two out of three patients who received parallel omeprazole had low posaconazole plasma concentrations.

In two patients (13%), plasma posaconazole concentration reached an alarming level, bordering toxicity. Both these patients showed no known toxicity symptoms. Both were patients with ALL. The first patient was after the II protocol and the second one was during the Ib protocol (AIEOP-BFM ALL 2017) (Table I). This led us to hypothesize that the reason for the elevated plasma concentration of posaconazole was related to metabolic drug interaction.

Interactions and side effects

Our initial study results revealed that insufficient plasma drug concentration is the major problem in patients on antifungal prophylaxis. Inadequately low concentrations appear more frequently than do toxic levels. We cannot assess the correlation between the length of prophylaxis implementation in a patient and the low serum concentration of posaconazole so far. The problem affects both children on long-term treatment as well as patients who have just started oral antifungal prophylaxis. On the other hand, alarmingly high plasma serum concentrations of the drug occurred among patients who had just started their prophylactic regimen – both of the patients were tested on day 7 following the start of their oral posaconazole prophylactic regimen. Nevertheless, posaconazole treatment-related severe toxicity, such as hepatotoxicity or cardiotoxicity, occurs very rarely, i.e. <1% [2]. Frequent adverse effects include gastrointestinal disorders, hypokalemia and pyrexia.

Discussion

The main purpose of the implementation of posaconazole TDM into everyday practice is to improve the safety and

efficacy of posaconazole therapy in pediatric patients in a way that will enable the achievement of optimal prophylaxis efficacy and minimize any possible side effects.

To perform TDM of posaconazole in clinical practice, accurate, validated analytical methods have to be available. Up to now, a dozen methods for the estimation of posaconazole in biological samples have been published. High-performance liquid chromatography with UV [5–8] or FLD [9–11] detection and liquid chromatography-tandem mass spectrometry [12–16] remain the most advantageous in terms of therapeutic monitoring of drugs, because they enable reliable results to be obtained in a relatively short time.

Liquid chromatography with mass spectrometry and tandem mass spectrometry are preferable in sensitivity and specificity to ultraviolet or fluorescence detections. However, such instruments are expensive to purchase and are not affordable in many laboratories. The assay costs often exceed those of the other techniques, which are based on fluorescence (FLD) or ultraviolet (UV) detection. An elaborated procedure involving FLD detection is likely to be a more suitable alternative to routine TDM measurements of posaconazole.

Another point to consider is the sample pretreatment procedure. It is very important to simplify the analytical procedure so that it can be successfully used in therapeutic drug monitoring. Previously published HPLC-UV and FLD methods of posaconazole level measurement still use liquid-liquid extraction (LLE) [6–8, 11] or solid-phase extraction (SPE) [17] to separate the drug from the biological matrix. This is expensive and time-consuming.

Our method is much faster and more easily performed. It is based on one step plasma protein precipitation which reduces the potential errors resulting from changes in extraction efficiency. An extremely important facet of TDM practice is the aliquot of the matrix that is required for analysis, especially in pediatric patients. When compared to previously published HPLC-UV [6–8] or HPLC-FLD [9–11] methods in which the sample volume ranges from 100 to 500 µL, the current method allows the determination of posaconazole in plasma with a smaller serum sample (50 µL) and a smaller injection volume (2 µL). Finally, the total chromatographic run time of the method used is less than 7 min, which is very important in TDM practice when results must be obtained as quickly as possible.

There are some limitations to our study. This was a single-center retrospective study with a small number of patients. The correlation between dosage, the plasma concentration of the drug, and the outcome of treatment was not assessed. No dose adjustment or repeat TDM measurements were performed.

We still have insufficient knowledge on which to build final conclusions, but undoubtedly clinical trials on TDM of posaconazole should be developed. It seems to be especially useful and important to secure reasonable treatment with drugs of unpredictable pharmacokinetics such as posaconazole. Despite the implementation of the drug according to the international dosage recommendations, we failed to obtain the desired plasma concentration in a significant proportion of children.

One possible conclusion after our trial could be that we are unable to assess which factors really influence posaconazole serum concentration. Hence, we will not be able to avoid or modify them. Due to that, therapeutic drug monitoring applied in routine practice should be implemented in Poland.

ECIL-4 and ECIL-8, the Polish Society of Pediatric Oncology and Hematology guidelines on antifungal prophylaxis, clearly recommend the use of TDM in children below a weight of 40 kg [1, 18, 19].

We will aim to assess an extended group of patients with the future roll-out of our research.

Authors' contributions

KL, MR, PZ, KK — study design, MR, PZ — development of a method of posaconazole plasma concentration measurement, KL, KK — pharmacokinetic profiles and other data analysis, KL, MR, PZ, KK — manuscript preparation.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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ORIGINAL RESEARCH ARTICLE

VIA MEDICA

Comparison of various diagnostic methods in assessing platelet count in patients with immune thrombocytopenia

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Abstract

Introduction: Accurate platelet count (PLTC) in immune thrombocytopenia (ITP) is important in order to make therapeutic decisions. The basic method of assessing PLTC is peripheral blood morphology with EDTA or with citrate. The older way of assessing PLTC is measurement under the microscope (FONIO), and the newer way is the fluorescent method. The purpose of this study was to compare PLTC methods, and find the most reliable.

Material and methods: PLTC was assessed using five methods (EDTA, citrate, FONIO, fluorescent, and immunofluorescent methods) in adult patients with previously untreated ITP.

Results: 66 patients were enrolled in the study. The median age was 56 years and 56% were men. Median PLTC in EDTA was 69 G/L, in citrate 69 G/L, in fluorescence 69 G/L, in FONIO 90 G/L, and in immunofluorescence 83 G/L. A significant difference in PLTC was observed in comparing EDTA to immunofluorescence (53% ±123%), followed by FONIO (51% ±91%), PLTC from immunofluorescence differed from the fluorescent method by 40% ±78%.

Conclusions: The most valuable method for obtaining PLTC is the immunofluorescent method. These findings are especially important in helping to make therapeutic decisions during a challenging time for accessing medical care such as a pandemic.

Key words: flow cytometry, immunofluorescence, immune thrombocytopenia, platelet count

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Introduction

Immune thrombocytopenia (ITP) is an acquired disease characterized by isolated low platelet count (PLTC) and an increased risk of bleeding [1]. The pathophysiology underlying this disease is still not completely understood. So far, several disease mechanisms have been proposed, but the most plausible includes increased platelet destruction due to sensitization of platelets by autoantibodies. It has been observed that these autoantibodies are mainly IgG, usually connected with IgM, directed against glycoprotein (GP) complexes IIb/IIIa, GPIb/IX and GPIa/IIa [2].

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ITP can be classified as 'primary' when occurring without any underlying disease, or 'secondary' when associated with another disease such as connective tissue disorder, a viral infection, or certain drugs. According to the American Society of Hematology (ASH) guidelines, ITP is diagnosed by identifying PLTC of less than 100 G/L in the absence of other causes or disorders [3].

Despite evidence that the PLTC by itself has limitations as an effective marker of the need for treatment, it is still the most commonly applied parameter in ITP patients. There is clinical value in differences in PLTC, especially when the amount is below 20–30 G/L, because of the influence on therapeutic decisions like starting immunosuppressive treatment [4].

The basic method of assessing PLTC is peripheral blood morphology anticoagulated with ethylene diamine tetracetic acid (EDTA) or with citrate. The older version involves counting platelets under the microscope, while the newer is the fluorescent method. There is a need to identify better laboratory tests of bleeding risk, other than provided by PLTC, which fails to provide information on platelets aggregation and their function. The answer may be flow cytometry (FCM) with the immunofluorescent method, which until now has only been available in highly specialized centralized laboratories, but in future could be an inpatient testing method that might provide additional guidance [5].

To date, there has been no publication comparing all five different PLTC methods.

In our study, we evaluated and compared for the first time the five different methods of evaluating PLTC in ITP patients: peripheral blood morphology with EDTA and with citrate, counting platelets under the microscope, fluorescent and immunofluorescent methods. We believe this to be an important study, especially in the extremely challenging conditions of a pandemic.

Material and methods

Our study involved a group of 66 patients with an ITP diagnosis according to the ASH 2019 guidelines [3]. The inclusion criteria were: age 18+, previously untreated, and primary disease. All the included patients had not received any medical treatment for at least one month before sampling. The group was composed of 27 women and 39 men, mean age 56 years [standard deviation (SD) 19]. In all patients, we evaluated PLTC using five different methods: EDTA and citrate in peripheral blood, manually under the microscope, and fluorescent and immunofluorescent methods.

About 5 mL of blood was drawn from a vein in the patient's inner elbow region. The freshly collected whole blood samples were divided into tubes with tripotassium salt of EDTA and trisodium citrate and counted for PLTC by an automatic hematology analyzer, Sysmex XN100 (Sysmex Corporation, Kobe, Japan). Precautions were taken to ensure there were no time lapses. After blood collection, it was analyzed within four hours. The calibration status of the Sysmex analyzer was initially checked by the manufacturer. Quality control samples and maintenance procedures were performed daily according to the manufacturer's instructions.

Microscope method

PLTC was also evaluated by manual platelet counting under the microscope (FONIO) [8]. A direct smear was made by placing a drop of blood onto a microscope slide and spreading it into a thin layer. Slides were stained with Giemsa stain, and reviewed for PLTC and platelet aggregates or clumping.

Fluorescent method

We also used the new fluorescent method (FFC) for counting platelets on the Sysmex XN1000 analyzer. In platelet counting by the FFC, platelets are stained with a fluorescent oxazine dye that is specifically bound with nucleic acid-rich platelet organelles, for example ribosomes or mitochondria. These are irradiated with a semiconductor laser beam, and then the forward scattered light and side fluorescence intensities of each platelet are plotted on a 2D scattergram to differentiate and count the platelets. FFC helps in specific differentiation of platelets from other blood cells and from interfering particles such as red blood cell fragments. Moreover, the analyzed sample volume of the FFC channel is about five times larger than those of standard methods. This provides highly precise data even in situations when the PLTC is extremely low.

Immunofluorescent method

Coagulation sodium citrate samples were used to evaluate the platelet count by the immunofluorescent method. The staining and gating protocol followed that proposed by the London Laboratory Service Group [7, 8] (Figure 1). Whole blood was incubated with FITC-labeled monoclonal antibody CD41a and CD61 (Becton Dickinson, San Jose, CA, USA). FCM measurements were carried out on FASC Lyric (Becton-Dickinson).

Statistical evaluation

For the statistical analysis of data obtained, the range of the measured variable, mean, median and SD were calculated, using statistical software (STATISTICA v.7.0, Tulsa, OK, USA). Data was presented as a median or mean SD values. The differences between values were evaluated with non-parametric Mann-Whitney test. For assessment of correlations, Spearman's rank correlation coefficient or Chi-squared test were performed where necessary. *P* values of less than 0.05 were considered statistically significant.

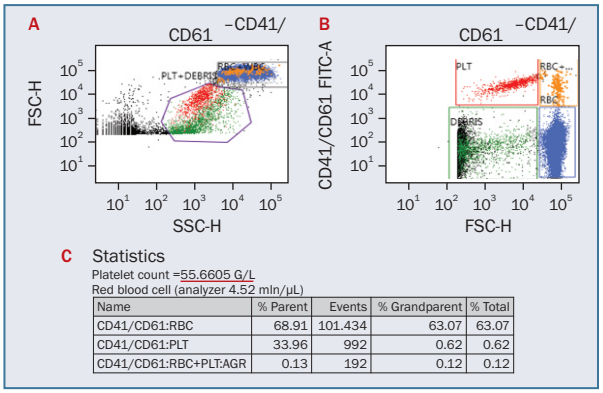

Figure 1. Gates used for analysis of platelets identified as CD61+ population (**A, B**). Statistical formula for platelet count in immunofluorescent method with flow cytometer (**C**); RBC — red blood cells; PLT — platelets; AGR — aggregates

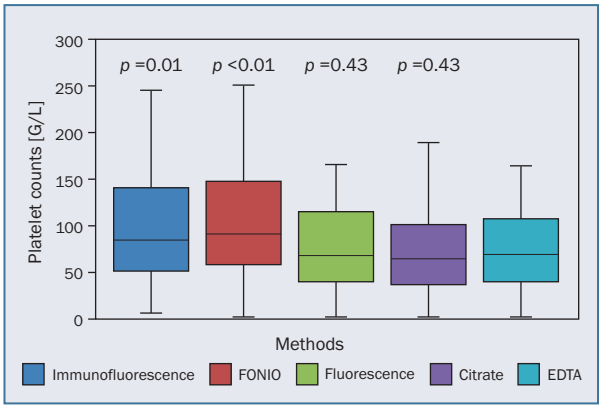

Figure 2. Platelet counts [G/L] determined using immunofluorescence (dark blue), FONIO (red), fluorescence (green), citrate (violet), and standard analyzer ethylene diamine tetracetic acid (EDTA; light blue) methods

Ethics committee

This study was approved by the local ethics committee, and all patients provided written informed consent to participate.

Results

We enrolled 66 patients with previously untreated ITP. The majority of patients were male (n =37, 56%), with median age at diagnosis of 56 years (± 19). The median PLTC in the EDTA method was 69 G/L (1–164), with citrate 69 G/L (1–205), with fluorescence 69 G/L (2–164), with FONIO 90 G/L (1–250), and with immunofluorescence 83 G/L (5–283 SD ±65) (Figure 2). The characteristics of the patients are set out in Table I.

The standard method (PLTC in peripheral blood count with EDTA) was compared to the other available diagnostic methods including the FONIO, citrate morphology, fluorescent, and immunofluorescent methods. The mean and median of the measurements from the immunofluorescent method (p =0.01) and the FONIO method (p <0.01) were significantly higher than from the analyzer. The biggest difference in PLTC was observed in the results comparing the immunofluorescence (53% ±123%), method followed by the FONIO method (51% ±91%) (Figure 3). The mean and median of the measurements with FFC and citrate did not differ significantly from the measurement from the analyzer. No difference was observed in PLTC when comparing the standard method to the FFC (7% ±43%) and the morphology with citrate (7% ±58%). The PLTC results obtained with immunofluorescence differed from fluorescence by 40% ±78%.

Because of the possibility of platelet aggregation, the obtained results were correlated with standard morphological platelet parameters [platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR)], with the number of white and red blood cells, with the fraction of immature platelets, and with the mean fluorescence intensity (MFI). There were no statistical differences observed in the measurements of the above-mentioned parameters.

Table I. Characteristics of study group

Parameter	Value
Number of patients	n =66; male 56%
Age	56 ±19 years
Mean platelet level	
EDTA analyzer	69 (1–164) G/L
Citrate analyzer	69 (1–205) G/L
Fluorescent method	69 (2–164) G/L
Microscope (FONIO)	90 (1–250) G/L
Immunofluorescent method	83 (5–283) G/L
Platelet parameters	
Platelet distribution width (PDW) [fL]	16 (9–25)
Mean platelet volume (MPV) [fL]	12 (9–15)
Platelet large cell ratio (P-LCR) [%]	41 (10–59)
Immature platelet fraction (IPF) [%]	18 (3–42)
Red blood cell (RBC) [million/µL]	5 (3–6)
White blood cell (WBC) [thousand/µL]	7 (3–29)
Mean fluorescence intensity (MFI)	25.504 (10.559–59.667)

EDTA — ethylene diamine tetracetic acid

Discussion

Determining the exact PLTC is crucial in ITP patients because of the decision to start treatment. To date, the most commonly used method to assess and observe PLTC in ITP diagnosis has been morphology for EDTA. This is a quick and

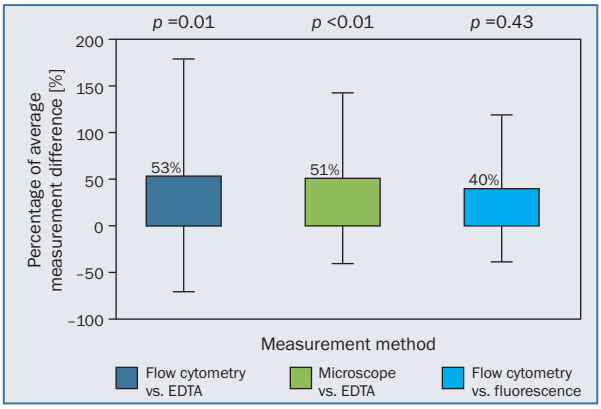

Figure 3. Comparison of mean differences in platelet count measurements depending on method; EDTA — ethylene diamine tetracetic acid

cheap method, but has unfortunately important drawbacks. One of them is EDTA-dependent pseudo-thrombocytopenia, a commonly known laboratory phenomenon. Its prevalence has been observed to vary between 0.1–2% among hospitalized patients and 15–17% in patients with an ITP diagnosis [9, 10]. A possible reason for this phenomenon may be agglutinating antibodies that recognize cytoadhesive receptors on platelet GPIIb/IIIa, which as a result cause platelet clumping [11].

We should be very careful in interpreting PLTC in ITP patients obtained with conventional EDTA methods which have limitations in platelet measurements such as poor accuracy and precision in the low PLTC and interference by nonplatelet particles.

The perfect method for ITP patients is still being sought. The best method of counting platelets in samples from ITP patients is still a matter of debate. Due to its high imprecision and laboriousness, the manual method has fallen out of favor and been replaced by immunological platelet counting using FCM. In the study by Gatt et al. [8], the PLTC was assessed by standard EDTA measurement and the immunofluorescent method. The results were similar to those observed in our study. The PLTC assessed by FCM was higher than that measured by the standard EDTA method (mean difference 4 G/L, $p = -0.0011$). There was an excellent correlation between the counts determined by the EDTA and immunofluorescence ($r = 0.89$, $p < 0.0001$). In the work by Bowles et al. [12] in ITP patients, measurement of the platelet count by the standard EDTA method frequently underestimated the PLTC as defined by the immunofluorescent method. In a group of 35 enrolled patients, the mean PLTC by standard EDTA method was 44 G/L and by immunofluorescence was 56 G/L ($p < 0.001$). Similar results were observed by Harrison et al. [13].

Both these studies support our finding that the consistent common discrepancy in the EDTA method of evaluating ITP patients underestimates PLTC.

The platelet is an interesting but difficult cell to study. The immunofluorescent method provides a rapid, accurate and reproducible test for PLTC, and might be adopted by laboratories with appropriate FCM experience. One of the reasons why immunofluorescence may be more accurate and extremely useful in ITP patients is detecting the immature (reticulated) platelets [14, 15]. These are not routinely assessed by hematology analyzers, but are crucial in the evaluation of the bone marrow response to thrombocytopenia.

A great hope for the future is the newly developed XN-Series automated hematology analyzer, equipped with a novel dedicated channel for platelet analysis, which is based on the FFC method. Tanaka et al. [16] observed that FFC gave a higher correlation with the immunofluorescent method compared to the standard EDTA method for samples with a PLTC ≤50 G/L. The same results were noted by Sun et al. [17]. PLTC counted using FFC channel was much more accurate than other diagnostic methods including standard EDTA, especially in thrombocytopenic patients.

FFC may be a more precise and accurate method, even in ITP patients. Unfortunately, this observation was not confirmed in our study.

It has been observed that ITP patients differ in their tendency to bleed despite similarly low platelet counts. Moreover, it has been appreciated that, as with the inherited platelet disorders, hemostasis depends not only on platelet count, but also on platelet function.

On the other hand, there are study results addressing the question of whether tests of platelet function may predict bleeding in ITP patients, as most tests of platelet function are affected by low PLTC [18, 19]. Nowadays, immunofluorescence has many applications in the diagnostic work-up of not only PLTC but also platelet function testing [20, 21]. Platelet analysis by FCM has been applied to the detection of platelet antigens, platelet surface-bound proteins, platelet activation, measurement of reticulated platelets, intracellular calcium studies, and the measurement of platelet microparticles *in vivo* and *in vitro* [22–27]. It has been used to evaluate platelet function in research studies, and therefore these assays require clinical validation before they can be used as the standard diagnostic tool in ITP patients.

There were some limitations of our study that should be acknowledged. It was a small group of ITP patients who were enrolled. This was supposed to be an exploratory study in order to gather the information necessary to estimate for a larger follow-up study. We believe that it would be very important to perform an outcome study to prospectively evaluate the PLTC methods, as well as bleeding and thrombosis examination in a further group of patients. Moreover, there should be cost and benefit analyses made before the possible introduction of newer ways of assessing PLTC in ITP patients.

The second drawback of our study was that we did not investigate the correlation with clinical outcomes, although there were no life-threatening bleeding episodes in any patient in our study.

In our study, similar to previous studies, the immunofluorescent method seems to be the most valuable PLTC method in ITP patients. Clinical applications of FCM analysis have been pursued in individual specialized medical centers so far. This technique has not found widespread adoption in clinical laboratories, mostly due to the difficult standardization process of the method and the inherent biovariability in comparing normal and abnormal platelets.

Despite these hurdles, it seems certain that immunofluorescence in ITP patients will continue to evolve into more practical and robust procedures that may eventually become the standard hematological method, and not only a specialized research tool.

Conclusions

Accurate PLTC is very important in ITP patients. According to our study, the most reflective test for PLTC is the immunofluorescent method. These findings are especially important during a difficult time in accessing medical care in order to make therapeutic decisions such as a pandemic.

FCM has emerged as an important technology for the study of platelets. Unfortunately, this method is still expensive and labor-intensive, and so should be reserved for selected patients and situations. This also highlights the need to identify novel ways of assessing PLTC in various types of thrombocytopenia.

Authors' contributions

Michał Witkowski, Piotr Smolewski — study design; Magdalena Witkowska, Michał Witkowski, Agata Majchrzak, Marzena Tybura-Sawicka — data collection; Michał Witkowski, Marzena Tybura-Sawicka — statistical analysis; Michał Witkowski, Piotr Smolewski — data interpretation; Michał Witkowski, Magdalena Witkowska, Tadeusz Robak — manuscript preparation; Magdalena Witkowska, Michał Witkowski — literature search.

Conflicts of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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ORIGINAL RESEARCH ARTICLE

Length of hospital stay in treatment of venous thromboembolism: do outcomes vary according to preference of anticoagulant? A retrospective analysis

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Abstract

Introduction: Regarding the choice of novel or traditional oral anticoagulants for the treatment of different entities of venous thromboembolism (VTE), there is contrasting, little, or no evidence to put forward. We here assess the impact of various anticoagulants in reducing the length of stay (LOS) in patients with acute VTE. Our objectives were: 1) to compare LOS among novel and traditional anticoagulants groups on discharge, and 2) to determine the clinical risk factors responsible for lengthier hospital stay in patients having acute VTE.

Material and methods: We conducted a retrospective data analysis of 161 consecutively admitted patients in the Life line Hospital, Karachi, Pakistan with a recent diagnosis of VTE. Lengths of stay with various anticoagulants on discharge were compared. Bleeding complications and readmission outcomes were compared, along with determination of independent predictors by multivariate analysis for LOS among groups.

Results: Patients discharged on a vitamin K antagonist (warfarin) had significantly longer hospital stays compared to patients on rivaroxaban (7.65 days vs. 5.21 days, p <0.001). Patients discharged exclusively on enoxaparin (hospital stay duration of 3.30 days) or on a combination of enoxaparin and warfarin (hospital stay duration of 4.26 days,) when compared for LOS for rivaroxaban, showed statistical significance (p <0.0001).

Conclusions: Warfarin has significantly longer LOS compared to rivaroxaban. Bleeding outcomes and readmissions compared among anticoagulant discharged groups were found to be statistically insignificant. Novel anticoagulants have an observable impact on the length of hospital stay in patients with acute venous thromboembolism.

Key words: venous thromboembolism, pulmonary embolism, rivaroxaban, enoxaparin, warfarin

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Introduction

Acute venous thromboembolism has long been a grave medical challenge worldwide, and is evidently associated with multiple medical disorders and situations. Deep vein thrombosis (DVT), and pulmonary embolism (PE), either or both, are the typical manifestations of acute venous thromboembolism (VTE), where venous thromboembolism is a frequently encountered ailment, having a yearly incidence of 1 to 2 per 1,000 people [1, 2]. The symptomatology ranges from swelling of an extremity, shortness of breath, to shock or death. Its management depends on the position along with the degree of the VTE, wherein anticoagulation therapy, systemic thrombolysis or thrombolysis via catheterization, and surgical embolectomy, are the most widely used methods. The gold standard in any form of venous thromboembolism is the institution of anticoagulation. The initiation of therapy is with any parenteral agent along with the later introduction of other more novel agents e.g. oral anticoagulants dabigatran, rivaroxaban, or apixaban in a controlled manner under vigilant observation. These agents are far more convenient to use as the need for spanning or tapering has almost been abolished with their introduction to the anticoagulant profile. Although anticoagulants have added positively to the venous thromboembolism armamentarium, there are health and financial challenges. In the USA alone, around 600,000 cases of VTE are admitted yearly and despite appropriate treatment, mortality remains high [3, 4].

Where the early therapy involves parenteral anticoagulation, the goal is to prevent extension and recurrence of any sort of thrombosis [5]. Deep venous thrombosis, although acute, is treated mostly at home rather than in hospital, while pulmonary embolism is an acute medical emergency which is essentially treated in hospital keeping in mind its higher short-term mortality [5]. Conventionally, subcutaneous or intravenous heparin, either low-molecular-weight heparin or unfractionated heparin, is instituted initially combined with a vitamin K antagonist (VKA), often warfarin, given alongside or immediately after the heparin has been initiated [5]. There are many restrictions on the use of VKAs, such as slow-acting warfarin having a narrow therapeutic range and volatile anticoagulation because of various interactions and inconstant metabolism [6]. So, in order to acquire the desired anticoagulation effects, constant monitoring and accurate dose adjustment are mandatory.

As far as the domain of anticoagulants is concerned, there are some more novel and promising agents being offered in the USA, such as dabigatran (a direct thrombin inhibitor), rivaroxaban, edoxaban, and apixaban (factor Xa inhibitors). When compared to the traditional anticoagulants, the newer agents demonstrate swifter inception with few adverse effects and interactions, and a hassle-free follow up investigative routine [6].

As far as management and monitoring in acute venous thromboembolic states such as DVT and PE are concerned, both the EINSTEIN-DVT and EINSTEIN-PE trials have found rivaroxaban to be equally as effective, if not more so, as enoxaparin and warfarin [7–9]. The same EINSTEIN trials, using the inpatient records of admissions retrospectively, revealed a substantially reduced hospital stay in those patients who received anticoagulation through rivaroxaban compared to those who received enoxaparin/VKA [10]. Another Canadian/USA analysis of EINSTEIN-DVT and EINSTEIN-PE patients also established a mean reduction of about 1.6 days in hospital stay for rivaroxaban-treated patients compared to others treated with enoxaparin/VKA [11].

The shorter length of stay, and reduced readmissions, for treatment with rivaroxaban as compared to warfarin and other contemporary agents also has the benefit of being more cost effective. This has been validated by a case-control study over a period of six months [12].

The available data, apart from clinical studies, is scanty to serve the purpose of validating rivaroxaban as a superior anticoagulant. Having seen the evidence of earlier studies from other parts of the world, in an effort to validate the results, we carried out this study aiming to demonstrate the same superiority of rivaroxaban over other anticoagulants in shortening LOS in hospital, improving hassle-free management and monitoring of anticoagulation, as well as reducing the number of readmissions.

Materials and methods

This was a retrospective data analysis of patients consecutively admitted to the Life line Hospital in Karachi, Pakistan with a recent diagnosis of VTE between January and December 2019. All the patients, who had a confirmed diagnosis of VTE, including DVT and PE, were retrieved from hospital records. 161 patients in total having VTE were enrolled for the study duration. Patients aged over 18 years who had a confirmed diagnosis of VTE were included. Patients with evidence of VTE within 24 hours of admission, nosocomial infections, iatrogenic overinfusion, pregnancy, or who had any contraindication to anticoagulation were excluded. Patients with a high risk of PE as evidenced by surgical embolectomy or catheter-delivered systemic thrombolytic or thrombolytic agents were also excluded from the study.

Deep vein thrombosis

The criteria from the American College of Radiology were applied in order to confirm the diagnosis through sonology in accordance with the criteria entailing venous non-compressibility of the involved vein with thrombus echogenicity from within the venous lumen, venous distension of vein, complete Doppler or spectral signal loss from within venous lumen, absent flow phaticity, and muted Valsalva or augmentation response [13].

Pulmonary embolism

Patients suspected for pulmonary embolism were stratified by using a modified Wells Score [14]: >4 for a likely diagnosis, <4 for unlikely, along with D dimer level. Patients with a modified Wells Score >4 were further confirmed objectively via a computerized tomography pulmonary angiogram (CTPA). Pulmonary embolism was confirmed by the following criteria [15, 16] on CTPA:

- A large filling defect due to arterial occlusion and enlarged size with respect to adjacent vessels;
- A 'polo mint' sign (a partial filling defect enclosed by contrast material) evidenced on images attained vertical to the long axis of a vessel and a 'railway track' sign (demonstrated on vertical images of the vessel);
- Acute angles formed with the arterial wall due to a peripheral intraluminal filling defect.

Primary and secondary outcomes

The primary outcome of our study was length of stay in hospital. Bleeding complications, either during index admission or on subsequent readmission, were considered as the secondary outcome.

Bleeding complications

Bleeding complications were defined and classified in accordance with a previously published study [17] where patients had central nervous system bleed, a fall of hemoglobin level to >2 g/dL, and the prerequisite transfusion of two or more packed cell units. Patients who did not meet the major bleeding criteria were labeled as having minor bleeding complications.

Statistical analysis

Demographic features and clinical physiognomies of the patients on different anticoagulants were shown with their frequency, means ±standard deviation or median (inter-quartile range). For comparison among the groups, one-way analysis of variance (ANOVA), Kruskal-Wallis test and exact Pearson's Chi-square test were applied as required. Statistical association between anticoagulants and length of stay in hospital among the patients having VTE were determined by Kruskal-Wallis test. For multiple comparison of length of hospital stay within four types of anticoagulant discharge medication in patients having VTE, Bonferroni's adjustment was applied. A Wilcoxon test was used for variables (categorical) having two categories. For multiple categories of categorical variables among groups, we used a Kruskal-Wallis test.

Results

In our study, multiple demographic and clinical risk factors were taken into account and statistically analyzed for prolonged hospital stay or outcome with treatment of VTE with various anticoagulation agents.

Demographic parameters

Among demographic profiles, the mean ages of patients on rivaroxaban, on warfarin, on both enoxaparin and warfarin, and on enoxaparin alone, were 60.02 ±10.4, 55.50 ±14.68, 59.69 ±11.54, and 56.37 ±13.46 years respectively. Of the 161 patients, there were 71 (44.09%) males and 90 (55.90%) females in all groups. Gender distribution and ages among various groups are set out in Table I.

Clinical risk factors

Nearly half of the patients, 69/161 (42.8%), were discharged on warfarin, where patients with DVT and DM were in the majority. 49/161 (30.43%) patients were discharged on rivaroxaban as an anticoagulant, where the majority of patients had DVT, DM and malignancies. 30 patients (18.6%) were discharged on combined enoxaparin and warfarin, with the majority having DM and DVT. Various clinical risk factors such as gender, age, hyperthrombophilic state, malignancies, hormones, immobilization, chronic liver disease (CLD), congestive cardiac failure (CCF), acute coronary syndrome (ACS), chronic renal failure (CRF), chronic obstructive pulmonary disease (COPD), deep vein thrombosis (DVT), and PE alone were compared among all four groups of anticoagulation discharge with reference to the length of hospital stay, as shown in Table I. Hyperthrombophilic state (p <0.001), deep vein thrombosis (p <0.012), COPD (p <0.001), and CKD (p <0.028) were found to be statistically significant for prolonging the length of stay in hospital among the groups discharged on anticoagulants, as shown in Table I.

Discharged anticoagulants and length of hospital stay

A significant overall statistical association (p <0.001) was found between duration of stay in hospital and various anticoagulants on discharge such as rivaroxaban, warfarin sodium, enoxaparin with warfarin, and enoxaparin alone. Patients who were discharged on anticoagulation with warfarin had significantly longer LOS compared to those sent home on rivaroxaban (7.65 vs. 5.21 days, p <0.001; Table II). Patients who were discharged exclusively on enoxaparin (3.30 days), or enoxaparin and warfarin (4.26 days), when compared for length of stay with rivaroxaban, also showed statistical significance (p <0.0001).

Outcome measures

Bleeding complications

Bleeding complications were assessed and compared as an outcome through univariate analysis among the discharged anticoagulant patients. Major bleeding complications appeared in five (10.9%) patients on rivaroxaban, seven (10.1%) with warfarin, two (6.7%) on enoxaparin plus warfarin therapy, and in 0 (0%) enoxaparin-taking patients. Mild

Table I. Length of Stay among patients discharged on various anticoagulants (clinical risk factors)

Groups	Warfarin (69)		Enoxaparin and warfarin (30)		Rivaroxaban (46)		Enoxaparin (16)		*P*-value
Age (years)	55.50 ±14.68		59.69 ±11.54		60.02 ±10.49		56.37 ±13.46		
Male	27	23	50.0%	39.1%	13	43.3%	8	50.0%	0.665
Hormones	1	1	2.2%	1.4%	3	10.0%	1	6.2%	0.181
Immobilization	3	5	10.9%	4.3%	6	20.0%	2	12.5%	0.113
Hyper thrombophilia	4	6	13.0%	5.8%	9	30.0%	11	68.8%	<0.001
Malignancy	8	10	21.7%	11.6%	9	30.0%	4	25.0%	0.148
Surgery	5	3	6.5%	7.2%	2	6.7%	2	12.5%	0.878
Post traumatic	1	4	8.7%	1.4%	1	3.3%	2	12.5%	0.154
Surgery	5	3	6.5%	7.2%	2	6.7%	2	12.5%	0.878
Congestive heart failure	4	3	6.5%	5.8%	6	20.0%	1	6.2%	0.114
Diabetes mellitus	22	18	39.1%	31.9%	15	50.0%	8	50.0%	0.284
Chronic kidney disease	7	6	13.0%	10.1%	10	33.3%	2	12.5%	0.028
Chronic obstructive pulmonary disease	4	3	6.5%	5.8%	1	3.3%	7	43.8%	<0.001
Chronic liver disease	1	2	4.3%	1.4%	1	3.3%	1	6.2%	0.707
Pulmonary embolism alone	1	2	4.3%	1.4%	2	6.7%	2	12.5%	0.227
Deep vein thrombosis	64	44	95.7%	92.8%	30	100.0%	12	75.0%	0.012
Unknown	31	20	43.5%	44.9%	13	43.3%	4	25.0%	0.533

Table II. Correlation of outcomes with various anticoagulants on discharge

Groups	Warfarin sodium		Warfarin with enoxaparin		Rivaroxaban		Enoxaparin alone		*P*-value
Length of Stay (days)	7.65 ±1.29		4.26 ±1.01		5.21 ±1.20		3.30 ±1.40		<0.001
Median (inter-quartile range)	7.73 (1.55)		4.35 (1.74)		5.55 (1.98)		3.79 (2.55)		
Readmission	3	4.3%	2	6.7%	3	6.5%	1	6.2%	0.949
Bleeding manifestations									0.802
Major	7	10.1%	2	6.7%	5	10.9%	0	0.0%	
Mild	3	4.3%	1	3.3%	2	4.3%	0	0.0%	
None	59	85.5%	27	90.0%	39	84.8%	16	100.0%	

bleeding was observed in two (4.3%) rivaroxaban patients, three (4.3%) on warfarin, one (3.3%) on enoxaparin plus warfarin, and in none of the enoxaparin-taking patients. Bleeding outcome when compared among anticoagulant discharged groups was found to be statistically insignificant (p <0.802) (see Table II).

Readmissions

Readmissions, as another outcome parameter among these groups, were observed to be three (6.5%) on rivaroxaban, three (4.3%) on warfarin, two (6.7%) on enoxaparin plus warfarin, and one (6.2%) on enoxaparin. When compared, it was statistically insignificant, with a p value of 0.949 (see Table II).

Duration of hospital stay (independent predictors)

Various independent predictors such as age, gender, and conditions such as COPD, CCF, and CKD were assessed by multivariate analysis for Length of Stay among groups of patients discharged on various anticoagulants. Anticoagulants like enoxaparin, enoxaparin + warfarin, and warfarin alone with reference to rivaroxaban were also assessed as independent predictors for Length of Stay among groups of patients. Among all independent predictors for Length of Stay, only the discharged anticoagulants enoxaparin, enoxaparin + warfarin, and warfarin were found to be significant (p <0.001, <0.03 and <0.001) (see Table III).

Table III. Independent predictors of longer hospital stay among patients with different anticoagulants

Predictors	Estimate of regression	Standard error of regression	t	*P*-value	95% confidence interval for B	
					Lower bound	Upper bound
Demography						
Age	–0.00064	0.008	–0.082	0.934	–0.016	0.015
Gender	–0.013	0.202	–0.064	0.949	–0.413	0.387
Anticoagulants						
Warfarin	2.358	0.243	9.723	<0.001	1.879	2.838
Enoxaparin and warfarin	–0.913	0.306	–2.983	0.003	–1.518	–0.308
Enoxaparin	–1.809	0.419	–4.312	<0.001	–2.638	0.980
Clinical risk factors						
Chronic kidney disease	–0.399	0.292	–1.367	0.174	–0.975	0.178
Chronic obstructive pulmonary airway disease	–0.175	0.391	–0.447	0.656	–0.948	0.598
Congestive cardiac failure	–0.221	0.357	–0.619	0.537	–0.927	0.485
Causes of VTE						
Hyper-thrombophilia	0.054	0.305	0.177	0.860	–0.549	0.657
Hormonal	0.180	0.547	0.330	0.742	–0.900	1.261
Malignancy	–0.452	0.261	–1.732	0.085	–0.967	0.064
Immobilization	0.482	0.342	1.411	0.160	–0.193	1.158
Surgery	–0.544	0.392	–1.388	0.167	–1.319	0.231

Discussion

Our study compared the effect of various anticoagulation drugs on patients with VTE. The duration of hospital stay, bleeding, and readmissions were assessed as outcome parameters. Age and gender distribution were compared among anticoagulation groups (warfarin, rivaroxaban, warfarin and enoxaparin, and enoxaparin alone) and found to be statistically insignificant. However, an earlier study [17] has shown age to be statistically significant among anticoagulation groups. The majority of patients in our study were on warfarin, followed by rivaroxaban, enoxaparin + warfarin, and enoxaparin alone, and this is in accordance with an earlier study [17]. Warfarin is the most cost-effective drug among all groups, which explains its being the most used anticoagulant group. Among various clinical risk factors such as thrombophilia, CCF, COPD, and CKD, only hyper-thrombophilic state, deep vein thrombosis, COPD and CKD were statistically significant for prolonging the length of stay in hospital among the discharged groups of anticoagulants.

Ruggles et al. [18] have shown heart failure, CKD and coronary artery disease to be significant clinical risk factors for prolonging hospital stay among patients with thromboembolism on various anticoagulants. A previous similar study [17] has also shown CKD, malignancy and hypercoagulable state as significant risk factors prolonging hospitalization in patients with thromboembolism on anticoagulants. An earlier study [18] among intensive care unit (ICU) patients on anticoagulants had shown length of stay for warfarin to be 3.0 days [95% confidence interval (CI) 1.9–3.9; p <0.001] more than for patients on dabigatran, and 2.4 days longer (95% CI 0.9–3.7; p =0.003) than for patients on rivaroxaban. However, that study showed no difference in hospital stay between rivaroxaban and dabigatran, although it differed from the current study as they also compared novel anticoagulants like dabigatran and rivaroxaban.

Saint et al. [19] compared duration of hospital stay among patients managed through novel oral anticoagulants (rivaroxaban) with warfarin and warfarin plus a parenteral agent for venous thromboembolism. They found a shorter duration of hospital stay (2.63 vs. 5.33 days; p <0.05) for rivaroxaban. Our current study showed slightly more major bleeding in patients discharged on warfarin, although overall comparison of bleeding among various groups of anticoagulants showed no significant difference. When bleeding as an outcome parameter for length of hospital stay was compared among groups, length of stay was shortest with

rivaroxaban. Ruggles et al. [18] also assessed length of stay among patients having bleeding due to various anticoagulant groups, and showed shortest length of stay with dabigatran and rivaroxaban as compared to warfarin. An earlier study [17] reported similar results to our study. Readmission as an outcome parameter in our study did not differ among various groups of anticoagulants, which is similar to the earlier study [17]. Readmission in our study was lower than that reported in the EINSTEIN trial [7]. Rivaroxaban, warfarin, enoxaparin plus warfarin, and enoxaparin alone were found to be significant independent predictors of length of stay in our study. The earlier study found similar results [17].

Conclusions

In our study, various demographic features and clinical characteristics of patients with VTE have been analyzed for consecutive durations of hospital stay, while comparing the impact to the array of contemporary versus novel anticoagulant drugs.

It is noteworthy that patients who were discharged on anticoagulation with warfarin had significantly longer LOS compared to those sent home on rivaroxaban (p <0.001). Bleeding outcome and readmissions when compared among anticoagulant discharged groups were found to be statistically insignificant: p <0.802, and p <0.949 respectively. Among all independent predictors of length of stay, only discharged anticoagulants enoxaparin, enoxaparin +warfarin, and warfarin, were found to be significant (p <0.001, p <0.03 and p <0.001, respectively).

Therefore, our study concludes that the preference of novel anticoagulants has an observable impact on the length of hospital stay in patients with acute venous thromboembolism.

Authors' contributions

IHN and AT: design of study; SNZR: statistical analysis; MR, RZG and SKF: manuscript writing, data collection; AT: final approval

Conflicts of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to biomedical journals.

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Long-term follow-up of pediatric patients with EBV-related post-transplant lymphoproliferative disorder

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Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a rare life-threatening complication developing after transplantation, which is caused by suppression of T-cell function [1–4]. PTLD after allogeneic hematopoietic stem cell transplantation (allo-HCT) is usually caused by Epstein-Bárr virus (EBV). Given recent progress in diagnostics and therapy [5, 6], survival from EBV-PTLD has improved from 15% [1] to almost 70% [7]. Nonetheless, little is still known regarding the long-term follow-up of patients treated for EBV-PTLD in the early period after allo-HCT.

We previously reported early outcomes of PTLD patients transplanted in our center between 2007 and 2011 [8]. We hypothesized that after the completion of treatment for PTLD, it has no further impact on overall long-term survival. To establish the truth of this, we performed a long-term follow-up analysis of a group of patients with EBV-PTLD in a single-center pediatric transplant center experience.

Material and methods

Design of study

The following inclusion criteria were implemented for the study: a biopsy-proven or probable EBV-PTLD diagnosis and the use of rituximab in treatment either as single therapy or administered together with other therapeutic approaches as combination therapy. We retrospectively analyzed 12 patients (Table I) with EBV-PTLD after allo-HCT, who were transplanted between 2007 and 2011, whose early outcomes of therapy for PTLD have been presented previously [8]. Our study was approved by the Bioethical Committee of *Collegium Medicum*, Nicolaus Copernicus University, Bydgoszcz, Poland.

Treatment

First line tretament for EBV-PTLD was rituximab, administered at a weekly dose of 375 mg/m^2 intravenously. Reduction of immunosuppression was done whenever possible. Second line therapy was scheduled as chemotherapy in cases of a partial response, stable or progressive disease. Other therapies such as surgery or antiviral agents (mainly cidofovir) were not used in our patients, as recommended by the ECIL [5, 6].

Definitions

Commonly used definitions, specified under Sixth European Conference on Infections in Leukemia (ECIL-6), were used [6]. Proven PTLD was diagnosed when a biopsy or other invasive procedure was performed and EBV was detected in

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Table I. Clinical data of patients

Patient	Age (y)	Diagnosis	Donor, HLA match	Relapse	Survival	Cause of death
1	15.7	AML	UD; 8/10	No	No (0.1y)	PTLD, multiorgan failure
2	19.6	HL	UD; 10/10	No	No (1.1y)	Pneumonia, GvHD
3	19.3	AML	UD; 9/10	No	No (0.3y)	GvHD, CMV, PTLD
4	7.8	ALL	UD; 10/10	No	Yes (11.5y)	
5	6.2	AML	UD; 10/10	No	Yes (11.4y)	
6	4.6	ALL	UD; 9/10	Yes (2.9y)	Yes (10.6y)	
7	16.7	SAA	MSD; 10/10	No	Yes (10.5y)	
8	19.1	ES	UD; 10/10	Yes (0.4y)	No (0.5y)	Relapse, progression
9	18.2	ALL	UD; 9/10	No	No (0.6y)	Septic shock, poor graft function
10	2.4	ALL	UD; 9/10	Yes (0.1y)	No (0.3y)	Relapse, progression
11	11.4	ALL	UD; 10/10	No	No (0.1y)	PTLD, multiorgan failure
12	5.3	AML	UD; 10/10	Yes (1.6y)	No (2.7y)	Relapse, progression

HLA — human leukocyte antigen; AML — acute myeloid leukemia; UD — unrelated donor; PTLD — post-transplant lymphoproliferative disorder; HL — Hodgkin lymphoma; GvHD — graft-versus-host disease; CMV — cytomegalovirus; ALL — acute lymphoblastic leukemia; SAA — severe aplastic anemia; MSD — matched sibling donor; y — years; ES — Ewing sarcoma

a specimen obtained with a test with appropriate sensitivity and specificity, together with symptoms and signs from the affected organ [6]. Probable PTLD was defined when a biopsy was not performed, and when clinically presenting as significant lymphoadenopathy or other endorgan disease accompanied by a high EBV-DNA blood load, and in the absence of other etiological factors or established diseases [6]. Response to treatment was assessed at clinical level, classified as complete remission, partial response, stable, or progressive disease, according to the standard definitions [9].

Statistical analysis

The probabilities of relapse incidence, relapse-free survival, and overall survival were determined using the Kaplan-Meier method. The time from the date of PTLD diagnosis to the date of death due to PTLD, or to the date of death due to other causes, or to the date of the last follow-up, was considered.

Results

Demographics

Patients were transplanted due to acute leukemia (n =9), relapsed/refractory Hodgkin lymphoma, Ewing sarcoma (ES), or severe aplastic anemia. The median age at transplant was 11.4 years (range: 2.4–19). PTLD developed at a median of two months after HCT (range: 0.7–3.5). PTLD was proven by biopsy in four cases; the remaining eight cases were considered to be probable PTLD. In 11/12 cases, transplant was performed from an unrelated donor (six matched, five mismatched). In 11/12 cases, peripheral blood was the source of cells. All patients had lymph node presentation, and seven also had extranodal involvement.

Long-term survival after PTLD

After treatment with a median of four doses (range 2–9) of rituximab, 9/12 patients reached complete remission, while 3/12 died due to progression of PTLD (rapid progression in two patients, slow progression with concomitant chronic GvHD and refractory CMV infection in one). The 100-day survival from PTLD was 0.75, as determined using the Kaplan-Meier method.

In 4/9 patients with remission of PTLD, a relapse of primary disease occurred [two acute lymphoblastic leukemia (ALL), one acute myeloid leukemia (AML), and one ES], after a median of 1.1 years (range: 0.2–2.9). The 3-year relapse-free survival for patients surviving PTLD was 0.56, and the relapse incidence was 0.44.

In three cases, relapse/progression was the cause of death after 0.2–1.0 years. In the patient with AML, a second allo-HCT was performed, followed by a second EBV--PTLD, treated successfully with rituximab. However, another AML relapse occurred and the patient eventually died. Two other patients died due to other complications: one pneumonia with GvHD, and one septic shock with myelosuppression. Finally, 4/12 patients are still alive at 10.5–11.5 years (median 11.0) after allo-HCT, with overall survival of 0.25. The long-term overall survival of patients cured of PTLD was 0.44.

Long-term complications

Of the four long-term survivors, all four suffered from prolonged hypogammaglobulinemia: in three cases this was diagnosed as secondary disease with immunoglobulin supplementation for 2–4 years. Primary hypogammaglobulinemia was diagnosed in one child, who has been on replacement therapy for more than 10 years.

Two of the four surviving patients have long-term endocrinological complications: a 15-year-old boy with hypothyreoidism, and a 17-year-old girl with hypogonadotropic hypogonadism. Autoimmune background of hypothyreoidism was excluded. Both patients are on replacement therapy, with L-thyroxin and with estradiol with lutein, respectively. One patient developed hypertension.

Discussion

In this study, we analyzed the long-term follow-up of a group of patients who were treated for EBV-PTLD after allo-HCT in a single pediatric experience. The short-term survival from PTLD was very good, reaching 75% after completion of rituximab-based therapy. Nevertheless, the main finding of our study was a relatively high relapse incidence and decreased long-term overall survival.

There are several explanations for these results. Firstly, theoretically the treatment of EBV-PTLD could contribute to a relapse of primary disease. We are not in favor of this explanation, which has been outlined in the literature [10]. Kinch et al. [10], in a large cohort study, showed that overall survival of patients with clinically significant EBV-DNA-emia at 5 years was 52% for rituximab-treated patients, which was not inferior to all other patients post-transplant. They concluded that rituximab-based treatment for patients with EBV-DNA-emia did not negatively affect their long-term survival. This finding additionally supports the strategy of monitoring EBV and the use of preemptive therapy in high-risk patients against the development of PTLD. Secondly, we are dealing with aggressive forms of primary disease, both in patients with acute leukemia, and with refractory ES, who had already poorly responded to previous autologous hematopoietic stem cell transplantation (auto-HCT). The third explanation is a development of this concept, i.e. that it may be related to a high predisposition of some patients to malignant transformation. Since PTLD, by definition, should be regarded as a malignancy developing after HCT, this explanation might be the most plausible one.

Another finding of our study is long-term hypogammaglobulinemia, prolonged for a period of at least two years in every patient. This complication may be a clear consequence of therapy with rituximab, which depleted CD20-positive lymphocytes B, thus compromising the production of immunoglobulins. Nevertheless, in one patient we diagnosed primary hypogammaglobulinemia, as there was no spontaneous recovery in B-cell function in this patient during 10 years of follow-up.

In two survivors, we found hormonal dysfunction of thyroid function or sex hormones. Both deficits should, however, be regarded as late complications of high-dose chemotherapy and transplantation, rather than of rituximab-based treatment of PTLD [11].

Apart from PTLD, Epstein-Bárr virus contributes to several diseases after HCT including hemophagocytic lymphohistiocytosis and chronic fatigue, and it increases the risk of several malignancies and the development of graft-versus-host disease [6, 12].

The limitation of our study is the clearly small group of patients. We wanted, however, to point out the complicated correlations between various factors contributing to the final outcomes of transplant therapy and treatment of PTLD.

Summary

We found a relatively high-rate of relapse of primary disease in pediatric patients treated for EBV-related post-transplant lymphoproliferative disorder after allogeneic hematopoietic cell transplantation. This finding is probably more related to the aggressiveness of malignancies in this selected group of patients, rather than to rituximab-based therapy applied for PTLD.

Authors' contributions

Design of the study: JS. Provision of clinical data: PG, MS, KJ, KC, RD, MRP, JK. Interpretation of data: JS, PG, MS, DR. Methdological analysis: JS, DR. Genetic analysis: TG. Writing manuscript: PG, JS, MS. Final approval: all authors.

Conflict of interest

None.

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None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to biomedical journals.

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CLINICAL VIGNETTE

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Diagnostic and treatment dilemmas in severe course of multicentric Castleman disease

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Introduction

Castleman disease (CD) is a rare benign lymphoproliferative disorder occurring in two forms: unicentric (UCD), described by Castleman in 1956, and multicentric (MCD), described in 1978 by Gaba [1, 2]. In UCD, 50% of patients remain asymptomatic, while MCD can manifest as systemic inflammation resulting from excessive production of proinflammatory cytokines, especially interleukin-6 (IL-6) [3]. Subtypes of MCD include idiopathic MCD (iMCD) with TAFRO (thrombocytopenia, anasarca/ascites, reticulin bone marrow fibrosis, renal failure, organomegaly) and non-TAFRO clinicopathological variants. Both subtypes may have overlapping clinical features, making their distinction very difficult. We herein present such an interesting case.

Case description

In September 2020, a 50-year-old woman was admitted with a 3-week history of malaise, fever, stabbing pain in the right hypochondrium, itching of forearms, loss of appetite, and general swelling. She had had cardiac infarction at the age of 47, 15 pack-years of smoking, and a family history of systemic lupus erythematosus (SLE). Initial laboratory testing showed elevated C-reactive protein [CRP; 196.3 mg/L; normal range (N) <5], thrombocytosis (714 G/L) and leukocytosis (20.75 G/L) with normal hemoglobin (12.9 g/dL). Computed tomography (CT) revealed pleural effusion, hepatosplenomegaly, and mediastinal lymphadenopathy up to 26 mm (Figure 1A, B).

Despite sequential empiric antibiotics, the patient's general condition deteriorated. She developed dyspnea, progressive anasarca, ascites, and worsening renal function (creatinine 3.15 mg/dL), requiring continuous renal replacement therapy.

Differential diagnoses included infections, autoimmune disorders, and neoplasms. The work-up for multiple viruses [human herpesvirus-8 (HHV-8), human immunodeficiency virus (HIV), Epstein-Bárr virus (EBV), cytomegalovirus (CMV)] was negative. Serum amyloid A was 1,440 mg/L (N <6.4 mg/L). Autoantibody profile revealed the presence of nonspecific anti-nuclear antibodies (ANA)-Hep2 (1:2,560), anti-Sjögren syndrome antibodies (SSA)-Ro52 antibodies (+++), positive lupus anticoagulant with negative anticardiolipin, and anti-beta$_2$-glicoprotein antibodies, but she did not meet the criteria of any autoimmune disease.

A bone marrow biopsy was not diagnostic. Histological evaluation of lymph node biopsy revealed regressed germinal centers, overgrowth of the parafollicular zone with multiple vessels, and plasmacytic infiltration consistent with the hyaline-vascular type of CD (Figure 1C–E). Additional tests revealed hypoalbuminemia 18 g/L (N 35–50 g/L), increased alkaline phosphatase 202 U/L (N 39–100 U/L), and lactate dehydrogenase 254 U/L (N 125–220 U/L), with unaltered aminotransferases. Serum protein electrophoresis was normal. IgG remained within the normal range (10.82 g/L). Serum IL-6 was elevated 152 pg/mL (N <5.9 pg/mL). Hemoglobin decreased to 8.9 g/dL. Given the patient's clinical presentation,

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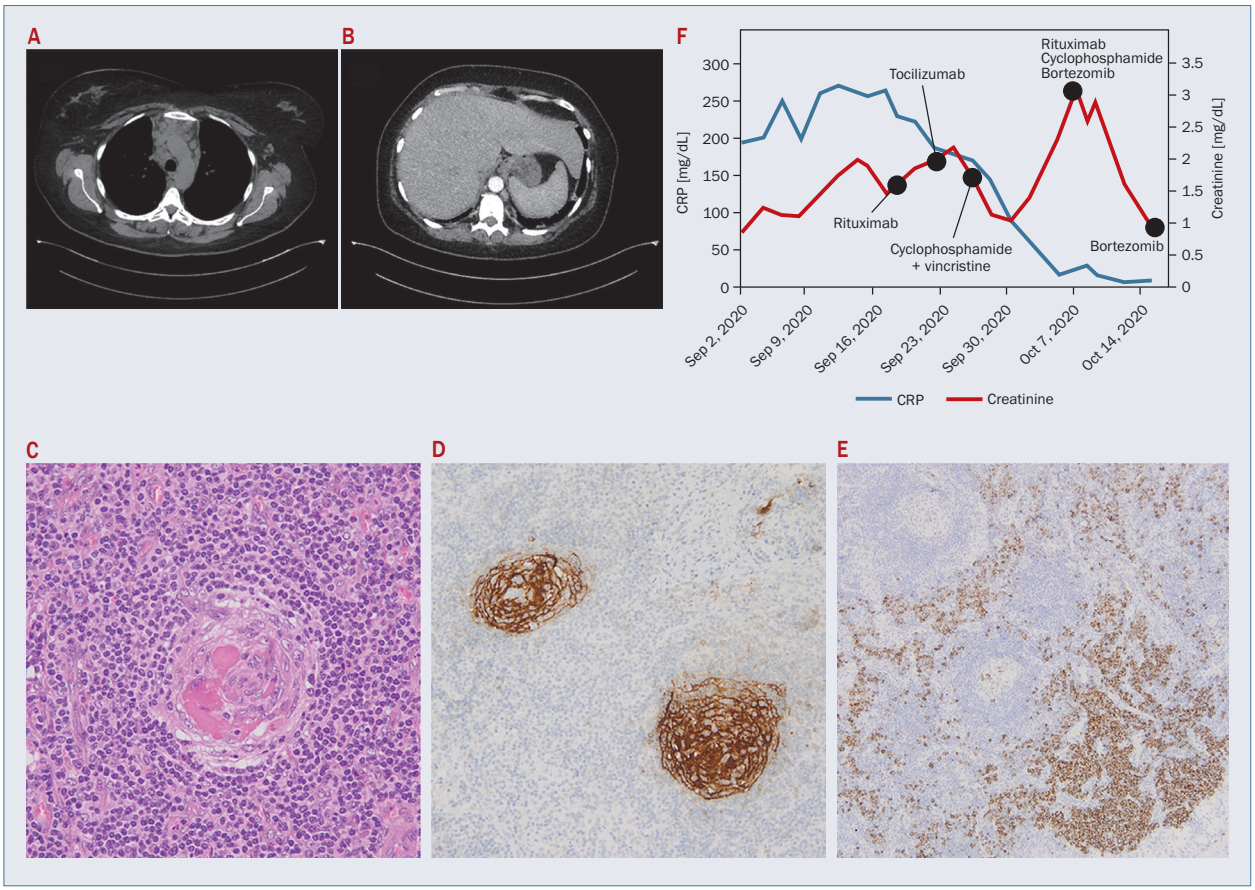

Figure 1. Chest computed tomography (CT) (**A**) and abdomen CT (**B**) performed at admission. Histological view of lymph nodes; **C.** Regressed germinal centers penetrated by hyalinized blood vessels surrounded by hyperplastic mantle zone. Proliferation of endothelial venules in interfollicular areas was observed; **D.** CD23 staining demonstrates atrophic germinal centers with prominent meshwork of follicular dendritic cells; **E.** Focal aggregates of CD138-positive polytypic plasma cells in interfollicular areas; **F.** Graph showing impact of targeted treatment administration (marked with dots) on C-reactive protein (CRP) and creatinine fluctuations during hospitalization

including non-infectious lymphadenopathy, hepatosplenomegaly, anasarca, renal failure, and histology, the findings indicated a severe course of iMCD or iMCD with TAFRO syndrome.

Treatment included initially a high dose of methylprednisolone and rituximab (375 mg/m^2), followed by tocilizumab (8 mg/kg), without regression of the clinical symptoms. Therefore, cyclophosphamide and vincristine were administered, leading to a brief improvement followed by deterioration. She received a second dose of rituximab, cyclophosphamide and, to target infiltrating plasmacytes and due to immunomodulatory proprieties, we added bortezomib (1.3 mg/m^2). After this combined therapy, a remarkable improvement occurred in the patient's general condition (Figure 1F). She continued the maintenance therapy with cyclophosphamide 500 mg, bortezomib 1.3 mg/m^2 and dexamethasone 20 mg once a week for six months at the Daily Clinic, and then treatment was discontinued. Up to now (9 months), she remains in complete remission confirmed in CT.

Discussion

The exact cause of iMCD is unknown. The overlapping clinical and pathological symptoms with autoimmune disorders such as SLE, Sjögren syndrome, and rheumatoid arthritis (RA), suggest that immune dysregulation and cytokines overproduction may contribute to iMCD [4]. The three most likely mechanisms responsible for hypercytokinemia are: 1) autoimmune driven by autoantibodies (the systemic inflammatory disease hypothesis); 2) ectopic cytokine secretion by malignant or benign cells within lymph nodes (the paraneoplastic hypothesis); and 3) viral signaling by a non-HHV-8 virus [5].

In our case, neither viral infections nor neoplasm was identified. This makes the systemic inflammatory disease hypothesis the most probable explanation of her symptoms. Specifically, the identified ANA-Hep2 and anti-SSA antibodies could induce hypercytokinemia. However, multisystemic involvement can be seen in many autoimmune disorders, and almost all lymph nodes of patients with

RA, and 15–30% with SLE, present lesions similar to hyaline-vascular or mixed type CD [6, 7]. In-depth diagnostics led two rheumatologists to agree on the diagnosis of iMCD.

The diagnostic criteria for iMCD require the fulfillment of both major criteria and at least 2/11 minor criteria [8]. Our case met both major criteria and fulfilled minor criteria, both laboratory (CRP, anemia, hypoalbuminemia, renal failure) and clinical (fever, fatigue, hepatosplenomegaly, anasarca, ascites). In TAFRO, lymphadenopathy is mild (<1.5 cm) with a smaller extent of plasmacytosis and myelofibrosis present in the bone marrow. Clinically, patients present with polyserositis and renal dysfunction [9, 10]. Constitutional symptoms, hepatosplenomegaly, and renal failure are present in both TAFRO and non-TAFRO iMCD. Platelet count helps to differentiate non-TAFRO iMCD (thrombocytosis) from TAFRO (thrombocytopenia), while hypergammaglobulinemia and plasmacytic infiltration of the lymph nodes are more typical for non-TAFRO iMCD.

In our case, the presence of severe anasarca, fever, renal failure, and organomegaly suggested TAFRO-iMCD, while lymph nodes >1.5 cm and thrombocytosis suggested non-TAFRO. Unfortunately, we lack data from the bone marrow. Non-TAFRO iMCD is corroborated by anemia, thrombocytosis, renal failure, and plasmacytic infiltration in lymph nodes, but not by hypergammaglobulinemia.

In summary, the whole clinical picture is fairly consistent with severe non-TAFRO iMCD. Our third line treatment combined the standard chemotherapy with bortezomib, listed as an option in the literature, which turned out to be effective. However, we cannot exclude the overlapping postponed effect of the immunotherapy targeting the IL-6 receptor.

Authors' contributions

MT — clinical analysis, writing manuscript; AP — clinical analysis, writing manuscript; MK — histopathological revision, microscopic images, critical revision; MD — clinical analysis, critical revision; E.Z. – clinical analysis, critical revision; WB — histopathological revision, microscopic images, critical revision; MB — clinical analysis, critical revision; JMZ — clinical analysis, writing manuscript.

Conflicts of interest

None.

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None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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YESCARTA®▼

(aksykabtagen cyloleucel)

▼Niniejszy produkt leczniczy będzie dodatkowo monitorowany. Umożliwi to szybkie zidentyfikowanie nowych informacji o bezpieczeństwie. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane. Aby dowiedzieć się, jak zgłaszać działania niepożądane – patrz punkt 4.8.

1. NAZWA PRODUKTU LECZNICZEGO: Yescarta, 0,4-2 × 10^8 komórek, dyspersja do infuzji. **2. SKŁAD JAKOŚCIOWY I ILOŚCIOWY: 2.1 Opis ogólny:** Yescarta (aksykabtagen cyloleucel) to genetycznie zmodyfikowane autologiczne limfocyty T skierowane przeciw CD19 stosowane w immunoterapii. Aby przygotować produkt Yescarta, od pacjenta pobiera się limfocyty T, które są następnie genetycznie modyfikowane w warunkach *ex vivo* metodą transdukcji retrowirusowej w celu uzyskania ekspresji chimerowego receptora antygenowego (ang. *chimeric antigen receptor*, CAR) zawierającego mysi jednołańcuchowy fragment Fv anty-CD19 połączony z domeną kostymulującą CD-28 i domeną sygnalizacyjną CD3-zeta. Żywotne limfocyty CAR-T anty-CD19 są namnażane i z powrotem wprowadzane za pomocą infuzji do organizmu pacjenta, gdzie mogą rozpoznawać i eliminować komórki docelowe prezentujące CD19. **2.2 Skład jakościowy i ilościowy:** Każdy worek do jednorazowej infuzji ze swoistym dla danego pacjenta produktem Yescarta zawiera około 68 ml dyspersji limfocytów CAR-T anty-CD19, co umożliwia otrzymanie docelowej dawki wynoszącej 2 × 10^6 żywotnych limfocytów CAR-T anty-CD19/kg masy ciała (zakres od 1 × 10^6 do 2 × 10^6 limfocytów/kg) z maksymalną liczbą limfocytów CAR-T anty-CD19 wynoszącą 2 × 10^8. Substancje pomocnicze o znanym działaniu: Każdy worek z produktem Yescarta zawiera 300 mg sodu. Pełny wykaz substancji pomocniczych, patrz punkt 6.1 ChPL. **3. POSTAĆ FARMACEUTYCZNA:** Dyspersja do infuzji. Klarowna do opalizującej dyspersja w kolorze od białego do czerwonego. **4. SZCZEGÓŁOWE DANE KLINICZNE: 4.1 Wskazania do stosowania:** Produkt Yescarta jest wskazany w leczeniu nawrotowego lub opornego na leczenie chłoniaka rozlanego z dużych komórek B (ang. *diffuse large B-cell lymphoma*, DLBCL) i pierwotnego chłoniaka śródpiersia z dużych komórek B (ang. *primary mediastinal large B-cell lymphoma*, PMBCL) u dorosłych pacjentów, którzy uprzednio otrzymali co najmniej dwie linie leczenia systemowego. **4.2 Dawkowanie i sposób podawania:** Produkt Yescarta musi być podawany w kwalifikowanym ośrodku leczniczym przez lekarza mającego doświadczenie w leczeniu złośliwych nowotworów krwi oraz przeszkolonego w zakresie podawania i leczenia pacjentów produktem Yescarta. Na wypadek wystąpienia zespołu uwalniania cytokin (ang. *cytokine release syndrome*, CRS) przed infuzją musi być dostępna przynajmniej 1 dawka tocilizumabu i sprzęt ratunkowy. Ośrodek, w którym odbywa się leczenie musi mieć zapewniony dostęp do dodatkowej dawki tocilizumabu w ciągu 8 godzin od uprzedniego podania każdej dawki. Dawkowanie: Yescarta to produkt przeznaczony wyłącznie do stosowania autologicznego (patrz punkt 4.4). Pojedyncza dawka produktu Yescarta zawiera 2 × 10^6 żywotnych limfocytów CAR-T na kilogram masy ciała (lub maksymalnie 2 × 10^8 żywotnych limfocytów CAR-T dla pacjentów o masie ciała równej 100 kg i większej) w około 68 ml dyspersji w worku do infuzji. Należy potwierdzić dostępność produktu Yescarta przed rozpoczęciem limfodeplecji. *Leczenie wstępne (chemioterapia limfodeplecyjna):* W 5., 4. i 3. dniu przed infuzją produktu Yescarta należy zastosować chemioterapię limfodeplecyjną, składającą się z cyklofosfamidu w dawce 500 mg/m^2 podawanego dożylnie i fludarabiny w dawce 30 mg/m^2 podawanej dożylnie. *Premedykacja:* Zaleca się podanie paracetamolu w dawce 500-1000 mg doustnie i difenhydraminy w dawce 12,5 do 25 mg dożylnie lub doustnie (lub równoważnej) na około 1 godzinę przed infuzją produktu Yescarta. Nie zaleca się podawania ogólnoustrojowych kortykosteroidów w ramach profilaktyki, ponieważ mogą mieć wpływ na działanie produktu Yescarta. *Monitorowanie:* Po infuzji pacjentów należy monitorować codziennie przez pierwsze 10 dni po infuzji pod kątem objawów przedmiotowych i podmiotowych potencjalnego CRS, zdarzeń neurologicznych i innych toksyczności. Lekarze powinni rozważyć hospitalizację pacjenta przez pierwsze 10 dni po infuzji lub w momencie pojawienia się pierwszych objawów przedmiotowych lub podmiotowych CRS i (lub) zdarzeń neurologicznych. Po pierwszych 10 dniach od infuzji o monitorowaniu pacjenta zdecyduje lekarz. Pacjentów należy poinformować o konieczności pozostania w pobliżu kwalifikowanej placówki klinicznej przez co najmniej 4 tygodnie po infuzji. Specjalne grupy pacjentów: *Pacjenci z zakażeniem ludzkim wirusem niedoboru odporności (ang. human immunodeficiency virus, HIV), wirusowym zapaleniem wątroby typu B (ang. hepatitis B virus, HBV) oraz wirusowym zapaleniem wątroby typu C (ang. hepatitis C virus, HCV):* Brak doświadczenia klinicznego ze stosowaniem u pacjentów z aktywnym zakażeniem HIV, HBV lub HCV. *Dzieci i młodzież:* Nie określono jeszcze bezpieczeństwa stosowania ani skuteczności produktu leczniczego Yescarta u dzieci i młodzieży w wieku poniżej 18 lat. Dane nie są dostępne. *Osoby w podeszłym wieku:* Nie jest wymagane dostosowywanie dawki u pacjentów w wieku 65 lat i starszych. Skuteczność leczenia była zgodna ze skutecznością leczenia w całej leczonej populacji pacjentów. Sposób podawania: Produkt Yescarta jest podawany drogą infuzji dożylnej. Produktu Yescarta nie wolno naświetlać. NIE używać filtra do deplecji leukocytów. *Środki ostrożności, które należy podjąć przed użyciem lub podaniem produktu leczniczego:* Ten produkt leczniczy zawiera genetycznie zmodyfikowane ludzkie komórki krwi. Fachowy personel medyczny przygotowujący produkt Yescarta powinien stosować odpowiednie środki ostrożności (nosić rękawice i okulary), aby uniknąć potencjalnego przeniesienia chorób zakaźnych. *Przygotowanie do infuzji:* Zweryfikować zgodność tożsamości pacjenta (ang. *identity,* ID) z oznaczeniami na kasecie z produktem Yescarta. Nie wolno wyjmować worka z produktem Yescarta z metalowej kasety, gdy brak zgodności informacji na etykiecie dotyczącej danego pacjenta z danymi pacjenta, dla którego przeznaczony jest ten produkt. Po potwierdzeniu ID pacjenta wyjąć worek z produktem Yescarta z metalowej kasety. Sprawdzić, czy dane pacjenta na etykiecie metalowej kasety są zgodne z danymi na etykiecie worka. Przed rozmrożeniem sprawdzić, czy worek z produktem nie ma jakichkolwiek uszkodzeń. W razie uszkodzenia worka postępować zgodnie z lokalnymi wytycznymi dotyczącymi postępowania z odpadami materiałów pochodzenia ludzkiego (lub niezwłocznie przekazać informację do firmy Kite). Umieścić worek do infuzji w drugim worku. Rozmrozić produkt Yescarta w temperaturze wynoszącej około 37°C w kąpieli wodnej lub metodą suchego rozmrażania do momentu, gdy w worku infuzyjnym nie będzie widocznego lodu. Delikatnie wymieszać zawartość worka, aby rozprowadzić grudki materiału komórkowego. Jeśli nadal widoczne są grudki materiału komórkowego, kontynuować delikatne mieszanie zawartości worka. Powinno to doprowadzić do rozproszenia małych grudek materiału komórkowego. Produktu Yescarta nie należy myć, wirować ani powtórnie odtwarzać zawiesiny na nowych nośnikach przed infuzją. Rozmrażanie powinno trwać około 3 do 5 minut. Po rozmrożeniu produkt Yescarta zachowuje trwałość w temperaturze pokojowej (od 20°C do 25°C) do 3 godzin. Infuzję produktu Yescarta należy jednak rozpocząć w ciągu 30 minut od rozmrożenia. *Podawanie:* Tylko do stosowania autologicznego. Tocilizumab oraz sprzęt ratunkowy powinny być dostępne przed infuzją i podczas monitorowania. Nie wolno używać filtra do deplecji leukocytów. Zaleca się założenie centralnego dostępu żylnego w celu podania produktu Yescarta. Ponownie zweryfikować ID pacjenta w celu potwierdzenia zgodności z oznaczeniami pacjenta na worku z produktem Yescarta. Przed infuzją wypełnić zestaw do infuzji roztworem chlorku sodu (0,154 mmol sodu/ml). Cała zawartość worka z produktem Yescarta powinna zostać podana w infuzji w ciągu 30 minut metodą grawitacyjną lub z użyciem objętościowej pompy infuzyjnej. Delikatnie masować worek podczas infuzji produktu Yescarta, aby zapobiec tworzeniu się grudek materiału komórkowego. Po podaniu w infuzji całej zawartości worka, przepłukać zestaw do infuzji roztworem chlorku sodu (0,154 mmol sodu/ml) z tą samą prędkością infuzji, aby upewnić się, że produkt Yescarta został podany w całości. Instrukcja dotycząca postępowania, przypadkowego narażenia oraz usuwania tego produktu, patrz punkt 6.6 ChPL. **4.3 Przeciwwskazania:** Nadwrażliwość na substancję czynną lub którąkolwiek substancję pomocniczą wymienioną w punkcie 6.1 ChPL. Należy uwzględnić przeciwwskazania dotyczące chemioterapii limfodeplecyjnej. **4.4 Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania:** Identyfikowalność: Należy bezwzględnie przestrzegać wymogów identyfikowalności produktów leczniczych stosowanych w zaawansowanych terapiach komórkowych. Aby zapewnić identyfikowalność nazwę produktu leczniczego, numer serii oraz imię i nazwisko leczonego pacjenta należy przechowywać przez okres 30 lat po upłynięciu terminu ważności produktu. Ogólne: Produkt Yescarta jest przeznaczony wyłącznie do stosowania autologicznego i nie wolno podawać go innym pacjentom. Przed infuzją: tożsamość pacjenta musi być zgodna z danymi identyfikacyjnymi na worku infuzyjnym i kasecie z produktem Yescarta. Nie należy podawać infuzji produktu Yescarta, gdy informacja na etykiecie dotyczącej danego pacjenta nie jest zgodna z danymi pacjenta, dla którego przeznaczony jest produkt. Pacjentów należy monitorować codziennie przez pierwsze 10 dni po infuzji w celu wykrycia przedmiotowych i podmiotowych objawów potencjalnego CRS, zdarzeń neurologicznych i innych toksyczności. Lekarze powinni rozważyć hospitalizację pacjenta przez pierwsze 10 dni po infuzji lub w momencie wystąpienia pierwszych objawów przedmiotowych/podmiotowych CRS i (lub) zdarzeń neurologicznych. Po pierwszych 10 dniach po infuzji o monitorowaniu pacjenta zdecyduje lekarz. Należy poinformować pacjentów o konieczności pozostawania w pobliżu kwalifikowanego ośrodka leczniczego przez co najmniej 4 tygodnie po infuzji oraz o konieczności uzyskania natychmiastowej pomocy medycznej w razie wystąpienia przedmiotowych lub podmiotowych objawów CRS lub neurologicznych działań niepożądanych. W zależności od stopnia nasilenia danego działania niepożądanego należy rozważyć monitorowanie parametrów życiowych i czynności narządów. Powody odroczenia leczenia: Ze względu na zagrożenia związane z leczeniem produktem Yescarta podanie infuzji należy wstrzymać, jeśli u pacjenta występuje którykolwiek z następujących stanów: utrzymujące się ciężkie działania niepożądane (w szczególności działania dotyczące płuc, serca lub niedociśnienie), w tym związane z wcześniejszymi chemioterapiami; czynne, niekontrolowane zakażenie; czynna choroba przeszczep przeciwko gospodarzowi (ang. *graft-versus-host disease*, GVHD). Badania serologiczne: Przed pobraniem komórek do wytworzenia produktu Yescarta, należy wykonać badania przesiewowe na obecność HBV, HCV i HIV (patrz punkt 4.2). Oddawanie krwi, narządów, tkanek i komórek: Pacjenci leczeni produktem Yescarta nie powinni być dawcami krwi, narządów, tkanek ani komórek do przeszczepienia. Choroba współistniejąca: Pacjenci z czynnym zaburzeniem OUN lub z zaburzeniami czynności nerek, wątroby, płuc lub serca mogą być bardziej podatni na skutki działań niepożądanych opisanych poniżej i wymagają szczególnej uwagi. Pierwotny chłoniak ośrodkowego układu nerwowego (OUN): Brak doświadczenia związanego ze stosowaniem produktu Yescarta u pacjentów z pierwotnym chłoniakiem OUN. W związku z tym nie określono stosunku ryzyka do korzyści leczenia produktem Yescarta dla tej populacji. Zespół uwalniania cytokin: U prawie wszystkich pacjentów wystąpił CRS któregoś stopnia. W przypadku stosowania produktu Yescarta bardzo często stwierdzano ciężkie przypadki CRS, w tym reakcje zagrażające życiu i prowadzące do zgonu, a czas do ich wystąpienia wynosił od 1 do 12 dni (patrz punkt 4.8). O leczeniu CRS decyduje lekarz na podstawie występujących u pacjenta objawów klinicznych oraz zgodnie z algorytmem postępowania w CRS przedstawionym w tabeli 1. W leczeniu CRS o nasileniu umiarkowanym lub ciężkim, związanego z podaniem produktu Yescarta, stosowano terapię opartą na inhibitorze receptora interleukiny 6 (IL-6), takim jak tocilizumab. Ośrodek, w którym odbywa się infuzja produktu Yescarta, musi przystępując do niej dysponować co najmniej 1 dostępną do podania dawką tocilizumabu dla każdego pacjenta. Ośrodek, w którym odbywa się leczenie, musi mieć dostęp do dodatkowej dawki tocilizumabu w ciągu 8 godzin od podania każdej z uprzednich dawek. Pacjentów należy codziennie monitorować w kwalifikowanej placówce klinicznej przez co najmniej 10 dni po infuzji pod kątem objawów przedmiotowych i podmiotowych CRS. Po upływie pierwszych 10 dni od infuzji o monitorowaniu pacjentów zdecyduje lekarz. Należy poinformować pacjentów o konieczności pozostawania w pobliżu kwalifikowanej placówki klinicznej przez co najmniej 4 tygodnie po infuzji oraz o konieczności uzyskania natychmiastowej pomocy medycznej w razie wystąpienia objawów przedmiotowych lub podmiotowych CRS. Opracowano algorytmy postępowania mające na celu łagodzenie niektórych objawów CRS występujących u pacjentów przyjmujących produkt Yescarta. Obejmują one stosowanie tocilizumabu lub tocilizumabu z kortykosteroidami w przypadkach CRS o nasileniu umiarkowanym, ciężkim lub zagrażającym życiu, jak przedstawiono w tabeli 1. Pacjentów, u których wystąpi CRS w stopniu 2. lub wyższym (np. niedociśnienie, brak odpowiedzi po podaniu płynów lub hipoksja wymagająca podania dodatkowego tlenu), należy monitorować z wykorzystaniem ciągłej telemetrii kardiologicznej oraz pulsoksymetrii. U pacjentów z ciężkim CRS należy rozważyć wykonanie echokardiogramu w celu oceny czynności serca. W przypadku ciężkiego lub zagrażającego życiu CRS należy rozważyć wdrożenie intensywnej terapii w ramach leczenia wspomagającego. Produktu Yescarta nie należy podawać pacjentom, u których występuje czynne zakażenie lub choroba zapalna, dopóki stany te nie ustąpią. Wiadomo, że CRS jest związany z niewydolnością narządów docelowych (np. wątroby, nerek, serca i płuc). Ponadto w przebiegu CRS może dojść do nasilenia występujących zaburzeń tych narządów. U pacjentów z istotnymi klinicznie zaburzeniami kardiologicznymi należy stosować standardy opieki w stanach krytycznych oraz rozważyć takie środki, jak echokardiografia. W celu rozpoznania CRS należy wykluczyć inne przyczyny ogólnoustrojowej odpowiedzi zapalnej, w tym zakażenie. W razie wystąpienia gorączki neutropenicznej należy dokonać oceny pod kątem zakażenia i zastosować antybiotyki o szerokim spektrum działania, płyny oraz leczenie podtrzymujące zgodnie z istniejącymi wskazaniami medycznymi. W przypadku pacjentów z ciężkim lub nieodpowiadającym na leczenie CRS należy rozważyć możliwość wystąpienia limfohistiocytozy hemofagocytarnej/zespołu aktywacji makrofagów (ang. *haemophagocytic lymphohistiocytosis/macrophage activation syndrome*, HLH/MAS). Produkt Yescarta nadal rozprzestrzenia się i utrzymuje w organizmie po podaniu tocilizumabu oraz kortykosteroidów. Nie zaleca się stosowania antagonistów czynnika martwicy nowotworu (ang. *tumour necrosis factor*, TNF) w leczeniu CRS związanego z zastosowaniem produktu Yescarta. **Tabela 1: Algorytm postępowania w zespole uwalniania cytokin (CRS).** Nasilenie CRS[a]: Stopień 1.: Objawy wymagają zastosowania tylko leczenia objawowego (np. gorączka, nudności, zmęczenie, ból głowy, ból mięśni, złe samopoczucie). *Tocilizumab:* W razie braku poprawy po 24 godzinach postępować jak w stopniu 2. *Kortykosteroidy:* N/A. Stopień 2.: Objawy wymagają umiarkowanej interwencji, po której ustępują. Zapotrzebowanie na tlen wynoszące mniej niż 40% FiO_2 lub hipotensja odpowiadająca na podanie płynów lub małej dawki jednego leku wazopresyjnego, lub toksyczność narządowa 2. stopnia[b]. *Tocilizumab:* Podać tocilizumab[c] w dawce 8 mg/kg dożylnie przez 1 godzinę (nie przekraczać dawki 800 mg). W razie potrzeby podawać tocilizumab co 8 godzin, gdy brak odpowiedzi na dożylne podanie płynów lub rośnie zapotrzebowanie na tlenoterapię. Nie podawać więcej niż 3 dawki w ciągu 24 godzin; maksymalna całkowita liczba dawek to 4 dawki, jeśli brak poprawy klinicznej dotyczącej objawów przedmiotowych i podmiotowych CRS lub jeśli brak odpowiedzi na drugą lub kolejną dawkę tocilizumabu, należy rozważyć zastosowanie alternatywnych sposobów leczenia CRS. *Kortykosteroidy:* Leczenie jak w stopniu 3., gdy brak poprawy w ciągu 24 godzin od podania tocilizumabu. Stopień 3.: Objawy wymagają poważnej interwencji, po której ustępują. Zapotrzebowanie na tlen przekraczające lub równe 40% FiO_2 lub hipotensja wymagająca podania dużej dawki leku wazopresyjnego albo zastosowania wielu leków wazopresyjnych, lub toksyczność narządowa 3. stopnia, albo hipertransaminazemia 4. stopnia. *Tocilizumab:* Leczenie jak w stopniu 2. *Kortykosteroidy:* Podać metyloprednizolon dożylnie w dawce 1 mg/kg dwa razy na dobę lub równoważną dawkę deksametazonu (np. 10 mg dożylnie co 6 godzin). Kontynuować podawanie kortykosteroidów do momentu, gdy zdarzenie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a następnie stopniowo zmniejszać dawkę. Gdy brak poprawy, zastosować leczenie jak w stopniu 4. (poniżej). Stopień 4.: Objawy zagrażające życiu. Pacjent wymaga zastosowania respiratora lub ciągłej hemodializy żylno-żylnej, lub toksyczność narządowa 4. stopnia (z wyjątkiem hipertransaminazemii). *Tocilizumab:* Leczenie jak w stopniu 2. *Kortykosteroidy:* Podawać metyloprednizolon dożylnie w dawce dobowej 1000 mg przez 3 dni, gdy jest poprawa – stosować leczenie jak powyżej. Rozważyć podanie innych leków immunosupresyjnych, gdy brak poprawy lub pogorszenie stanu. N/A = brak/nie dotyczy. *(a) Lee et al 2014. (b) Leczenie neurologicznych działań niepożądanych – patrz Tabela 2. (c) Szczegółowe informacje można znaleźć w charakterystyce produktu leczniczego tocilizumabu.* Neurologiczne działania niepożądane: U pacjentów leczonych produktem Yescarta bardzo często obserwowano ciężkie neurologiczne działania niepożądane, które mogą zagrażać życiu lub prowadzić do zgonu (patrz punkt 4.8). Pacjenci z zaburzeniami OUN w wywiadzie, takimi jak drgawki lub niedokrwienie naczyniowo-mózgowe, mogą być narażeni na większe ryzyko. Zgłaszano przypadki zakończonego zgonem i ciężkiego obrzęku mózgu u pacjentów leczonych produktem Yescarta. Pacjentów należy monitorować pod kątem objawów przedmiotowych i podmiotowych neurologicznych działań niepożądanych (Tabela 2). Po infuzji pacjentów należy monitorować w kwalifikowanej placówce opieki zdrowotnej co najmniej codziennie przez pierwsze 10 dni pod kątem objawów przedmiotowych i podmiotowych toksyczności neurologicznej. Po tych pierwszych 10 dniach o monitorowaniu pacjenta zdecyduje lekarz. Pacjentów należy poinformować o konieczności pozostania w pobliżu kwalifikowanej placówki klinicznej przynajmniej przez 4 tygodnie po infuzji i natychmiastowego zwrócenia się po pomoc medyczną, jeśli wystąpią objawy przedmiotowe lub podmiotowe toksyczności neurologicznej. W zależności od stopnia nasilenia danego działania niepożądanego należy rozważyć monitorowanie parametrów życiowych i wydolności narządów. Pacjentów, u których wystąpi toksyczność neurologiczna stopnia 2. lub wyższego, należy monitorować z wykorzystaniem ciągłej telemetrii kardiologicznej oraz pulsoksymetrii. W razie ciężkiej lub zagrażającej życiu toksyczności neurologicznej należy wdrożyć intensywną terapię wspomagającą. Należy rozważyć podanie leków przeciwdrgawkowych niemających działania uspokajającego w ramach profilaktyki przeciwdrgawkowej zgodnie ze wskazaniami klinicznymi dla działań niepożądanych 2. lub wyższego stopnia. Opracowano algorytmy postępowania mające na celu złagodzenie neurologicznych działań niepożądanych występujących u pacjentów przyjmujących produkt Yescarta. Obejmują one stosowanie tocilizumabu (w razie współistniejącego CRS) i (lub) kortykosteroidów w przypadku umiarkowanych, ciężkich lub zagrażających życiu neurologicznych działań niepożądanych, jak przedstawiono w tabeli 2. **Tabela 2: Algorytmy postępowania dotyczące objawów neurologicznych działań niepożądanych.** Stopień 2.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Gdy brak poprawy w ciągu 24 godzin po podaniu tocilizumabu: podać deksametazon dożylnie w dawce 10 mg co 6 godzin, jeśli pacjent nie przyjmuje już innych kortykosteroidów. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. *Bez współistniejącego CRS:* Podawać deksametazon dożylnie w dawce 10 mg co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. Stopień 3.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Dodatkowo z pierwszą dawką tocilizumabu podać deksametazon dożylnie w dawce 10 mg, powtarzając co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. *Bez współistniejącego CRS:* Podawać deksametazon dożylnie w dawce 10 mg co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. Stopień 4.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Wraz z pierwszą dawką tocilizumabu podać metyloprednizolon dożylnie w dobowej dawce 1000 mg oraz kontynuować podawanie metyloprednizolonu dożylnie w dobowej dawce 1000 mg przez 2 kolejne dni; w przypadku poprawy stosować leczenie jak powyżej. Gdy brak poprawy, rozważyć podanie metyloprednizolonu 1000 mg dożylnie 3 razy na dobę lub alternatywne leczenie[a]. *Bez współistniejącego CRS:* Podawać metyloprednizolon dożylnie w dawce dobowej 1000 mg przez 3 dni, gdy poprawa – stosować leczenie jak powyżej. Gdy brak poprawy, rozważyć podanie metyloprednizolonu 1000 mg dożylnie 3 razy na dobę lub alternatywne leczenie[a]. *(a) Alternatywne leczenie obejmuje (między innymi): anakinrę, siltuksymab, ruksolitynib, cyklofosfamid, immunoglobuliny podawane dożylnie* (*ang. intravenous immunoglobulin,* IVIG) *oraz globulinę anty-tymocytarną* (*ang. anti-thymocyte globulin,* ATG). Zakażenia i gorączka neutropeniczna: Podczas stosowania produktu Yescarta bardzo często obserwowano ciężkie zakażenia (patrz punkt 4.8). Pacjentów należy monitorować pod kątem objawów przedmiotowych i podmiotowych zakażenia przed, w trakcie i po podaniu produktu Yescarta w infuzji oraz zastosować odpowiednie leczenie. Należy zastosować profilaktykę przeciwbakteryjną zgodnie z obowiązującymi standardowymi wytycznymi. Po infuzji produktu Yescarta u pacjentów stwierdzano gorączkę neutropeniczną (patrz punkt 4.8) i może ona występować jednocześnie z CRS. W przypadku wystąpienia gorączki neutropenicznej należy ocenić, czy nie jest to zakażenie, i zastosować antybiotyki o szerokim spektrum działania, płyny oraz leczenie objawowe zgodnie ze wskazaniami medycznymi. Reaktywacja HBV: U pacjentów leczonych lekami skierowanymi przeciwko limfocytom B może dojść do reaktywacji HBV, w niektórych przypadkach prowadzącej do piorunującego zapalenia wątroby, niewydolności wątroby i zgonu. Przed pobraniem komórek do wytworzenia produktu Yescarta należy przeprowadzić badanie przesiewowe w kierunku HBV, HCV i HIV. Przedłużająca się cytopenia: Po chemioterapii limfodeplecyjnej i infuzji produktu Yescarta u pacjentów przez kilka tygodni może występować cytopenia. Odnotowano bardzo częste występowanie przedłużającej się cytopenii stopnia 3. lub wyższego, w tym małopłytkowość, neutropenię i niedokrwistość, po infuzji produktu Yescarta. Należy monitorować wyniki badań morfologii krwi po infuzji produktu Yescarta. Hipogammaglobulinemia: U pacjentów leczonych produktem Yescarta może wystąpić aplazja limfocytów B, prowadząca do hipogammaglobulinemii. Hipogammaglobulinemię obserwowano bardzo często u pacjentów leczonych produktem Yescarta. Należy monitorować stężenie immunoglobulin po leczeniu produktem Yescarta oraz stosować leczenie obejmujące profilaktykę zakażeń, profilaktykę antybiotykową oraz immunoglobulinową terapię zastępczą. Reakcje nadwrażliwości: Po infuzji produktu Yescarta mogą wystąpić reakcje alergiczne. Ciężkie reakcje nadwrażliwości, w tym reakcja anafilaktyczna, mogą być spowodowane obecnością dimetylosulfotlenku (ang. *dimethylsulfoxide*, DMSO) lub resztkowej gentamycyny w produkcie Yescarta. Wtórne nowotwory złośliwe: U pacjentów leczonych produktem Yescarta mogą rozwinąć się wtórne nowotwory złośliwe. Pacjentów należy monitorować przez całe życie pod kątem wtórnych nowotworów złośliwych. W razie pojawienia się wtórnego nowotworu złośliwego należy skontaktować się z firmą w celu uzyskania instrukcji dotyczących pobrania od pacjenta próbek do badań. Zespół rozpadu guza (ang. *tumour lysis syndrome*, TLS): Rzadko obserwowano występowanie TLS, który może mieć ciężką postać. W celu zminimalizowania ryzyka wystąpienia TLS pacjenci ze zwiększonym stężeniem kwasu moczowego lub dużą łączną masą guza powinni otrzymywać allopurynol lub inne leczenie profilaktyczne przed infuzją produktu Yescarta. Pacjentów należy monitorować pod kątem przedmiotowych i podmiotowych objawów TLS, a objawy należy leczyć zgodnie ze standardowymi wytycznymi. Wcześniejsze leczenie terapią anty-CD19: Doświadczenie związane ze stosowaniem produktu Yescarta u pacjentów, którzy wcześniej otrzymali leczenie celowane przeciwko CD19, jest ograniczone. Nie zaleca się stosowania produktu Yescarta, jeśli u pacjenta nastąpił nawrót choroby bez ekspresji CD19 po uprzedniej terapii anty-CD19. Substancje pomocnicze: Ten produkt leczniczy zawiera 300 mg sodu na worek infuzyjny, co odpowiada 15% maksymalnego dziennego dopuszczalnego spożycia sodu, wynoszącego 2 g dla osoby dorosłej, zgodnie z zaleceniami WHO. Oczekuje się, że pacjenci zostaną wpisani do rejestru pacjentów, na podstawie którego prowadzona będzie obserwacja w celu lepszego poznania długoterminowego bezpieczeństwa stosowania i skuteczności produktu Yescarta. **4.8 Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: Dane dotyczące bezpieczeństwa opisane w tym punkcie odzwierciedlają skutki narażenia na produkt Yescarta w badaniu fazy I/II ZUMA-1, w którym 108 pacjentów z nawrotowym/opornym chłoniakiem nieziarniczym (NHL) z limfocytów B otrzymywało limfocyty CAR-T w zalecanej dawce w zależności od masy ciała. Mediana okresu obserwacji wyniosła 27,4 miesiąca. Do najbardziej poważnych i najczęstszych działań niepożądanych należały CRS (93%), encefalopatia (58%) oraz zakażenia (39%). Ciężkie działania niepożądane wystąpiły u 56% pacjentów. Do najczęstszych działań niepożądanych należały: encefalopatia (22%), zakażenia nieswoistymi patogenami (16%), zakażenia bakteryjne (6%), gorączka neutropeniczna (6%), zakażenia wirusowe (5%) i gorączka (5%). Najczęściej występujące działania niepożądane stopnia 3. lub wyższego obejmowały encefalopatię (31%), zakażenia nieswoistymi patogenami (19%), CRS (11%), zakażenie bakteryjne (9%), afazję (7%), zakażenie wirusowe (6%), stan majaczeniowy (6%), hipotensję (6%) i nadciśnienie (6%). Tabelaryczna lista działań niepożądanych: Działania niepożądane zgłaszane w badaniach klinicznych oraz po wprowadzeniu produktu do obrotu przedstawiono poniżej. Działania te są wymienione według klasyfikacji układów i narządów oraz częstości występowania. Częstości występowania określone są w następujący sposób: bardzo często (≥ 1/10), często (≥ 1/100 do < 1/10), niezbyt często (≥ 1/1000 do < 1/100), rzadko (≥ 1/10 000 do < 1/1000) i bardzo rzadko (< 1/10 000). W obrębie każdej grupy o określonej częstości występowania działania niepożądane wymieniono zgodnie ze zmniejszającym się nasileniem. **Tabela 3: Działania niepożądane związane ze stosowaniem produktu Yescarta.** *Zakażenia i zakażenia pasożytnicze:* Bardzo często: zakażenia nieswoistymi patogenami, zakażenie wirusowe, zakażenie bakteryjne. Często: zakażenie grzybicze. *Zaburzenia krwi i układu chłonnego:* Bardzo często: leukopenia, neutropenia, niedokrwistość, małopłytkowość. Często: koagulopatia. *Zaburzenia układu immunologicznego:* Bardzo często: zespół uwalniania cytokin, hipogammaglobulinemia. Często: nadwrażliwość, histiocytoza hemofagocytarna. *Zaburzenia metabolizmu i odżywiania:* Bardzo często: odwodnienie, zmniejszone łaknienie, hipofosfatemia, hiponatremia, zmniejszenie masy ciała. Często: hipokalcemia, hipoalbuminemia. *Zaburzenia psychiczne:* Bardzo często: majaczenia, lęk. Często: bezsenność. *Zaburzenia układu nerwowego:* Bardzo często: encefalopatia, ból głowy, drżenie, zawroty głowy, afazja. Często: ataksja, neuropatia, drgawki, dyskalkulia, mioklonie. Niezbyt często: obrzęk rdzenia kręgowego, zapalenie rdzenia kręgowego, porażenie czterokończynowe. *Zaburzenia serca:* Bardzo często: tachykardia, arytmia. Często: zatrzymanie akcji serca, niewydolność serca. *Zaburzenia naczyniowe:* Bardzo często: niedociśnienie, nadciśnienie. Często: zakrzepica, zespół przesiąkania włośniczek. *Zaburzenia układu oddechowego, klatki piersiowej i śródpiersia:* Bardzo często: kaszel, duszność, hipoksja, wysięk opłucnowy. Często: obrzęk płuc. *Zaburzenia żołądka i jelit:* Bardzo często: biegunka, nudności, wymioty, zaparcia, ból brzucha, suchość w jamie ustnej. Często: dysfagia*. *Zaburzenia skóry i tkanki podskórnej:* Często: wysypka. *Zaburzenia układu mięśniowo-szkieletowego i tkanki łącznej:* Bardzo często: zaburzenia ruchowe, ból kończyn, ból pleców, ból stawów, ból mięśni. *Zaburzenia nerek i dróg moczowych:* Często: niewydolność nerek. *Zaburzenia ogólne i stany w miejscu podania:* Bardzo często: zmęczenie, gorączka, obrzęk, dreszcze. *Badania diagnostyczne:* Bardzo często: zwiększone stężenie aminotransferazy alaninowej, zwiększone stężenie aminotransferazy asparaginowej. Często: zwiększone stężenie bilirubiny. W tabeli 3 ujęto wyłącznie przypadki cytopenii, które spowodowały (I) wystąpienie nowych lub zaostrzenie istniejących następstw klinicznych, (II) wymagały leczenia lub (III) modyfikacji aktualnego leczenia. * Dysfagię zgłaszano w ramach toksycznego działania na układ nerwowy i encefalopatii. Opis wybranych działań niepożądanych: *Zespół uwalniania cytokin:* CRS wystąpił u 93% pacjentów. U jedenastu procent (11%) pacjentów wystąpił CRS stopnia 3. lub wyższego (ciężki, zagrażający życiu oraz prowadzący do zgonu). Mediana czasu do wystąpienia CRS wynosiła 2 dni (zakres od 1 do 12 dni), a mediana czasu trwania 7 dni (zakres od 2 do 29 dni). U dziewięćdziesięciu ośmiu procent pacjentów (98%) doszło do ustąpienia CRS. Najczęściej występujące objawy przedmiotowe i podmiotowe związane z CRS obejmowały gorączkę (83%), niedociśnienie (44%), tachykardię (24%), hipoksję (23%) i dreszcze (20%). Ciężkie działania niepożądane, które mogą być związane z CRS, obejmowały: ostre uszkodzenie nerek, migotanie przedsionków, tachykardię komorową, zatrzymanie akcji serca, niewydolność serca, zespół przesiąkania włośniczek, niedociśnienie, hipoksję i HLH/MAS. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Neurologiczne działania niepożądane:* Neurologiczne działania niepożądane wystąpiły u 67% pacjentów. U trzydziestu dwóch procent (32%) pacjentów wystąpiły działania niepożądane stopnia 3. lub wyższego (ciężkie lub zagrażające życiu). Mediana czasu do wystąpienia działań niepożądanych wynosiła 5 dni (zakres od 1 do 17 dni). Mediana czasu trwania wynosiła 13 dni (zakres: od 1 do 191 dni). U większości pacjentów neurologiczne działania niepożądane ustąpiły, z wyjątkiem 4 pacjentów, u których niepożądane działania neurologiczne utrzymywały się do momentu zgonu; zgony te były spowodowane innymi przyczynami. Najczęstszymi objawami przedmiotowymi i podmiotowymi związanymi z neurologicznymi działaniami niepożądanymi były: encefalopatia (58%), ból głowy (40%), drżenie (31%), zawroty głowy (21%), afazja (18%) i stan majaczeniowy (17%). Zgłaszano ciężkie działania niepożądane, w tym encefalopatię (22%), afazję (4%), stan majaczeniowy (4%) i drgawki (1%), u pacjentów, którym podawano produkt Yescarta. Inne neurologiczne działania niepożądane były zgłaszane rzadziej w badaniach klinicznych i obejmowały dysfagię (5%), zapalenie rdzenia kręgowego (0,2%) i porażenie czterokończynowe (0,2%). W okresie po wprowadzeniu produktu do obrotu zgłoszono wystąpienie obrzęku rdzenia kręgowego, w kontekście neurotoksyczności. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Gorączka neutropeniczna i zakażenia:* Gorączkę neutropeniczną zaobserwowano u 36% pacjentów po infuzji produktu Yescarta. Zakażenia wystąpiły u 39% pacjentów w badaniu ZUMA-1. Zakażenia stopnia 3. lub wyższego (ciężkie, zagrażające życiu lub prowadzące do zgonu) wystąpiły u 26% pacjentów. Zakażenia nieswoistymi patogenami oraz zakażenia bakteryjne i wirusowe stopnia 3. lub wyższego wystąpiły u, odpowiednio, 19%, 9% i 6% pacjentów. Najczęstszym miejscem zakażenia były drogi oddechowe. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Przedłużająca się cytopenia:* Neutropenia (w tym gorączka neutropeniczna), niedokrwistość i małopłytkowość stopnia 3. lub wyższego wystąpiły u, odpowiednio, 80%, 45% i 40% pacjentów. Przedłużająca się (utrzymująca się w dniu 30. lub pojawiająca się w dniu 30. lub później) neutropenia, małopłytkowość i niedokrwistość stopnia 3. lub wyższego wystąpiła u, odpowiednio, 26%, 24% i 10% pacjentów. Utrzymująca się neutropenia, małopłytkowość i niedokrwistość stopnia 3. lub wyższego po dniu 93. wystąpiła u, odpowiednio, 11%, 7% i 3% pacjentów. Wskazówki dotyczące leczenia – patrz punkt 4.4. *Hipogammaglobulinemia:* W badaniu ZUMA-1 hipogammaglobulinemia wystąpiła u 16% pacjentów. Łącznie 33 (31%) ze 108 pacjentów otrzymywało dożylne leczenie immunoglobulinami w momencie przeprowadzania analizy po 24 miesiącach. Wskazówki dotyczące leczenia – patrz punkt 4.4. Immunogenność: Immunogenność produktu Yescarta oceniano za pomocą testu immunoenzymatycznego (ELISA) wykrywającego przeciwciała wiążące FMC63, z których wywodzą się przeciwciała anty-CD19 CAR. U trzech pacjentów przed leczeniem produktem Yescarta badanie przeciwciał anty-FMC63 dało wynik dodatni. Wpływ tych przeciwciał na skuteczność lub bezpieczeństwo stosowania nie był dostrzegalny. Specjalne grupy pacjentów: Doświadczenie dotyczące stosowania produktu Yescarta u pacjentów w wieku 75 lat i starszych jest ograniczone. Na ogół bezpieczeństwo stosowania oraz skuteczność były podobne jak w przypadku pacjentów w wieku ≥ 65 lat i < 65 lat leczonych produktem Yescarta. Wyniki były zgodne z wynikami uzyskanymi w grupach pacjentów z oceną w skali Eastern Cooperative Oncology Group (ECOG) 0 i 1 oraz według płci. Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem *Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, PL-02 222 Warszawa, tel.: +48 22 49 21 301, faks: +48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl.* **7. PODMIOT ODPOWIEDZIALNY POSIADAJĄCY POZWOLENIE NA DOPUSZCZENIE DO OBROTU:** Kite Pharma EU B.V., Tufsteen 1, 2132 NT Hoofddorp, Holandia. **8. NUMER POZWOLENIA NA DOPUSZCZENIE DO OBROTU:** EU/1/18/1299/001. **DATA WYDANIA PIERWSZEGO POZWOLENIA NA DOPUSZCZENIE DO OBROTU I DATA PRZEDŁUŻENIA POZWOLENIA:** Data wydania pierwszego pozwolenia na dopuszczenie do obrotu: 23 sierpnia 2018. **DATA ZATWIERDZENIA LUB CZĘŚCIOWEJ ZMIANY TEKSTU CHARAKTERYSTYKI PRODUKTU LECZNICZEGO:** 04/2021. Szczegółowe informacje o tym produkcie leczniczym są dostępne na stronie internetowej Europejskiej Agencji Leków http://www.ema.europa.eu.

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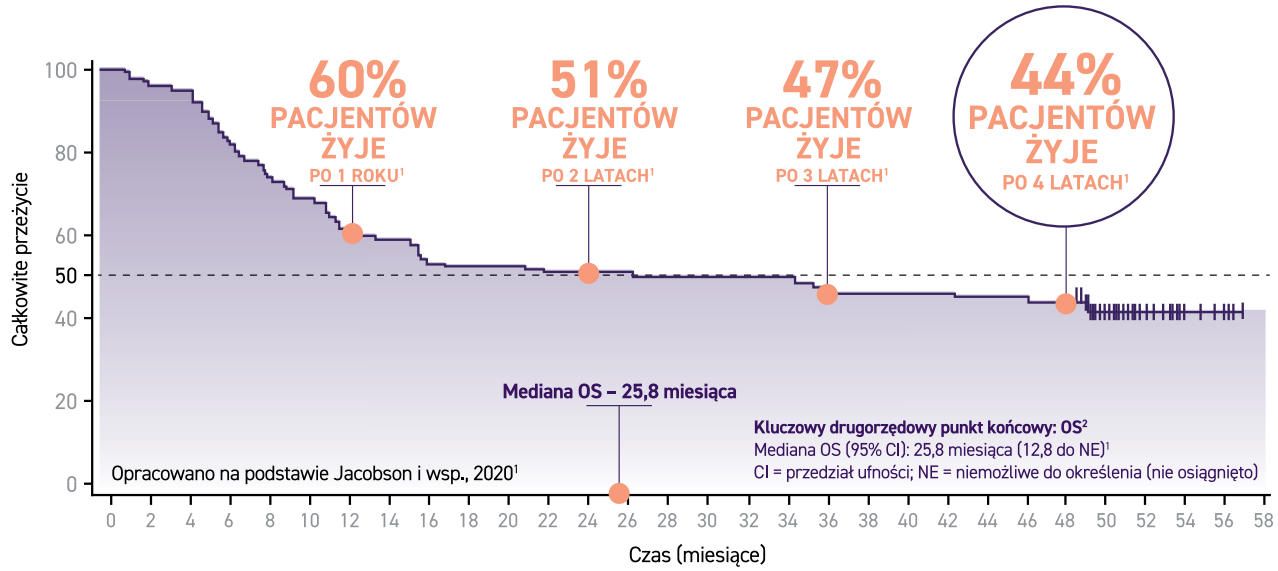

Pacjenci narażeni	101	97	93	80	74	69	61	60	54	53	53	51	51	50	50	50	50	50	47	47	47	46	46	45	44	28	16	6	1	0
(Pacjenci odcięci)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(15)	(27)	(37)	(42)	(43)

* Wartości szacunkowe wg Kaplana-Meiera dla 3-letniego i 4-letniego wskaźnika OS wyniosły, odpowiednio, 47% i 44%.

Piśmiennictwo: 1. Jacobson C et al. Poster presented at: American Society for Hematology Annual Meeting (Virtual); December 5-8, 2020. 2. Locke FL et al. Lancet Oncol 2019; 20(1): 31-42.

PL-YES-0007. Data przygotowania: 06.2021

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