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DLA PACJENTÓW Z WYSOKIM RYZYKIEM CYTOGENETYCZNYM W SZPICZAKU PLAZMOCYTOWYM[1]

PODARUJ CZAS, KTÓREGO PRAGNĄ

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Ninlaro (iksazomib) w połączeniu z RD znosi ryzyko cytogenetyczne u chorych na szpiczaka plazmocytowego. Wykazano, że schemat leczenia Ninlaro + RD poprawił PFS u pacjentów z wysokim ryzykiem cytogenetycznym prawie o rok (21,4 miesiąca dla Ninlaro + RD vs. 9,7 miesiąca dla schematu placebo + RD[1]).

Jednocześnie to pierwszy i jedyny doustny inhibitor proteasomu, który w schemacie z RD można przyjmować w domu[1].

RD - lenalidomid i deksametazon
PFS – mediana przeżycia bez progresji choroby

1. Avet-Loiseau i wsp., Blood, 2017, 130, 2610-2618

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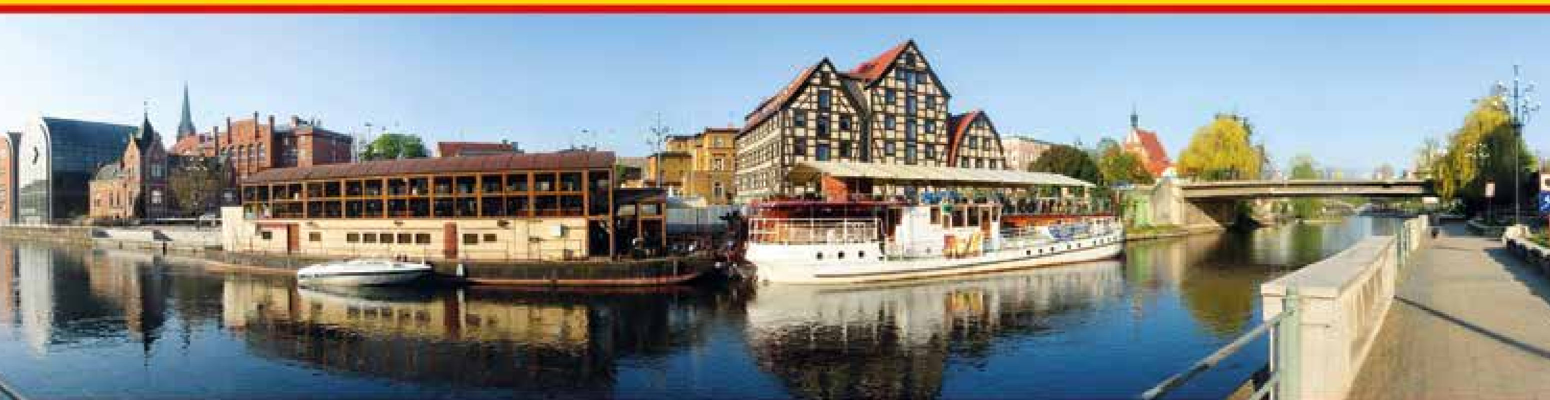

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Schemat dawkowania: Iksazomib w skojarzeniu z lenalidomidem i deksametazonem

	28-dniowy cykl (4-tygodniowy cykl)							
	Tydzień 1		**Tydzień 2**		**Tydzień 3**		**Tydzień 4**	
	Dzień 1	Dni 2-7	Dzień 8	Dni 9-14	Dzień 15	Dni 16-21	Dzień 22	Dni 23-28
Iksazomib	✔		✔		✔			
Lenalidomid	✔	✔ Raz na dobę	✔	✔ Raz na dobę	✔	✔ Raz na dobę		
Deksametazon	✔		✔		✔		✔	

✔ = podanie produktu leczniczego

Aby uzyskać dodatkowe informacje dotyczące lenalidomidu i deksametazonu należy zapoznać się z Charakterystykami Produktów Leczniczych (ChPL) tych leków. Przed rozpoczęciem nowego cyklu leczenia: bezwzględna liczba neutrofili powinna wynosić ≥ 1000/mm³; liczba płytek krwi powinna wynosić ≥ 75 000/mm³; według oceny lekarza, objawy toksyczności niehematologicznej powinny powrócić do stanu początkowego lub zmniejszyć się co najmniej do stopnia ≤ 1. Leczenie należy kontynuować do stwierdzenia progresji choroby lub wystąpienia nieakceptowanej toksyczności. Podstawą leczenia iksazomibem w skojarzeniu z lenalidomidem i deksametazonem dłuższego niż 24 cykle powinna być indywidualna ocena stosunku korzyści do ryzyka, ponieważ dane dotyczące tolerancji i toksyczności leku po upływie 24 cykli leczenia są ograniczone. *Opóźnione podanie lub pominięcie dawki:* W przypadku opóźnionego podania lub pominięcia dawki iksazomibu pacjent może zażyć tę dawkę, jeżeli do kolejnej planowanej dawki pozostają co najmniej 72 godziny. Nie należy uzupełniać pominiętej dawki, jeżeli do kolejnej planowanej dawki pozostaje mniej niż 72 godziny. Nie należy zażywać podwójnej dawki w celu uzupełnienia pominiętej dawki. Jeżeli po zażyciu dawki u pacjenta wystąpią wymioty, nie należy powtarzać dawki, lecz wznowić dawkowanie w terminie kolejnej planowanej dawki. *Dostosowanie dawki:* Schemat zmniejszania dawki iksazomibu przedstawiono w Tabeli 1, zaś wytyczne dotyczące dostosowania dawki tego produktu leczniczego opisano w Tabeli 2.

Tabela 1: Schemat zmniejszania dawki iksazomibu

Zalecana dawka początkowa*	Pierwsze zmniejszenie dawki do	Drugie zmniejszenie dawki do	Zakończenie leczenia
4 mg	3 mg	2,3 mg	

* W przypadku występowania umiarkowanych lub ciężkich zaburzeń czynności wątroby, ciężkich zaburzeń czynności nerek lub schyłkowej niewydolności nerek wymagającej dializy zalecana zmniejszona dawka wynosi 3 mg.

W przypadku współwystępujących objawów toksyczności w postaci trombocytopenii, neutropenii i wysypki zaleca się stosowanie schematu naprzemiennego dostosowywania dawek iksazomibu i lenalidomidu. W przypadku wystąpienia wymienionych wyżej objawów toksyczności pierwszym krokiem do zmiany dawki jest ograniczenie lub przerwanie stosowania lenalidomidu. Schemat zmniejszania dawki lenalidomidu w przypadku wystąpienia wymienionych wyżej objawów toksyczności - patrz punkt 4.2 ChPL lenalidomidu.

Tabela 2: Zalecane dostosowanie dawki iksazomibu stosowanego w skojarzeniu z lenalidomidem i deksametazonem

Objawy toksyczności hematologicznej	**Zalecane postępowanie**
Trombocytopenia (liczba płytek krwi)	
Liczba płytek krwi < 30 000/mm³	• Należy wstrzymać leczenie iksazomibem i lenalidomidem dopóki liczba płytek krwi nie powróci do ≥30 000/mm³. • W przypadku uzyskania poprawy należy wznowić leczenie lenalidomidem w kolejnej mniejszej dawce podanej w ChPL tego produktu oraz wznowić leczenie iksazomibem w dawce ostatnio stosowanej. • W przypadku ponownego zmniejszenia liczby płytek krwi < 30 000/mm³ należy wstrzymać leczenie iksazomibem i lenalidomidem dopóki liczba płytek krwi nie powróci do ≥ 30 000/mm³. • W przypadku uzyskania poprawy należy wznowić leczenie iksazomibem w kolejnej mniejszej dawce oraz wznowić leczenie lenalidomidem w dawce ostatnio stosowanej.*

Objawy toksyczności hematologicznej	Zalecane postępowanie
Neutropenia (bezwzględna liczba neutrofili)	
Bezwzględna liczba neutrofili < 500/mm^3	• Należy wstrzymać leczenie iksazomibem i lenalidomidem dopóki bezwzględna liczba neutrofili nie powróci do poziomu ≥ 500/mm^3. Należy rozważyć uzupełnienie leczenia o czynniki stymulujące tworzenie kolonii granulocytów (G-CSF) zgodnie z wytycznymi klinicznymi. • W przypadku uzyskania poprawy należy wznowić leczenie lenalidomidem w kolejnej mniejszej dawce zgodnie z informacjami dotyczącymi stosowania tego produktu i wznowić leczenie iksazomibem w dawce ostatnio stosowanej. • W przypadku ponownego spadku bezwzględnej liczby neutrofili do poziomu < 500/mm^3 należy wstrzymać leczenie iksazomibem i lenalidomidem dopóki bezwzględna liczba neutrofili nie powróci do poziomu ≥ 500/mm^3. • W przypadku uzyskania poprawy należy wznowić leczenie iksazomibem w kolejnej mniejszej dawce oraz wznowić leczenie lenalidomidem w dawce ostatnio stosowanej.*
Objawy toksyczności niehematologicznej	**Zalecane postępowanie**
Wysypka	
Stopień† 2. lub 3.	• Należy wstrzymać leczenie lenalidomidem dopóki nasilenie wysypki nie zmniejszy się co najmniej do stopnia 1. • Po uzyskaniu poprawy należy wznowić leczenie lenalidomidem w kolejnej mniejszej dawce zgodnie z ChPL tego produktu. • W przypadku powtórnego wystąpienia wysypki w stopniu 2. lub 3. należy wstrzymać leczenie iksazomibem i lenalidomidem dopóki nasilenie wysypki nie zmniejszy się co najmniej do stopnia 1. • W przypadku uzyskania poprawy należy wznowić leczenie iksazomibem w kolejnej mniejszej dawce oraz wznowić leczenie lenalidomidem w dawce ostatnio stosowanej.*
Stopień† 4.	Należy przerwać leczenie.
Neuropatia obwodowa	
Neuropatia obwodowa stopnia 1. ze współwystępującym bólem lub neuropatia obwodowa stopnia 2.	• Należy wstrzymać leczenie iksazomibem dopóki nasilenie neuropatii obwodowej nie zmniejszy się co najmniej do stopnia 1. bez współwystępującego bólu lub stan pacjenta nie powróci do stanu początkowego. • W przypadku uzyskania poprawy należy wznowić leczenie iksazomibem w dawce ostatnio stosowanej.
Neuropatia obwodowa stopnia 2. ze współwystępującym bólem lub neuropatia obwodowa stopnia 3.	• Należy wstrzymać leczenie iksazomibem. Należy wznowić leczenie iksazomibem dopiero wtedy, gdy nasilenie objawów toksyczności zmniejszy się co najmniej do stopnia 1. lub stan pacjenta powróci do stanu początkowego, według oceny lekarza. • W przypadku uzyskania poprawy należy wznowić leczenie iksazomibem w kolejnej mniejszej dawce.
Neuropatia obwodowa stopnia 4.	Należy przerwać leczenie.
Inne objawy toksyczności niehematologicznej	
Inne objawy toksyczności niehematologicznej stopnia 3. lub 4.	• Należy wstrzymać leczenie iksazomibem. Należy wznowić leczenie iksazomibem dopiero wtedy, gdy nasilenie objawów toksyczności zmniejszy się co najmniej do stopnia 1. lub stan pacjenta powróci do stanu początkowego, według uznania lekarza. • Jeżeli objawy mają związek z iksazomibem, w przypadku oceny poprawy należy wznowić leczenie tym produktem w kolejnej mniejszej dawce.

* W przypadku wystąpienia dodatkowych objawów należy zastosować schemat naprzemiennego dostosowania dawki lenalidomidu i iksazomibu.

† Nasilenie objawów oceniane na podstawie wspólnych kryteriów terminologii dla zdarzeń niepożądanych instytutu National Cancer Institute (ang. Common Terminology Criteria for Adverse Events, CTCAE) w wersji 4.03.

Jednocześnie stosowane produkty lecznicze: U pacjentów leczonych iksazomibem należy rozważyć zastosowanie profilaktyki przeciwwirusowej w celu zmniejszenia ryzyka reaktywacji wirusa wywołującego półpaśca. U pacjentów włączonych do badań klinicznych iksazomibem, u których zastosowano profilaktykę przeciwwirusową, częstość występowania zakażeń wirusem półpaśca była niższa w porównaniu z pacjentami, u których nie zastosowano leczenia profilaktycznego. U pacjentów leczonych iksazomibem w skojarzeniu z lenalidomidem i deksametazonem zaleca się stosowanie profilaktyki przeciwzakrzepowej, a decyzję w tej kwestii należy podjąć na podstawie oceny czynników ryzyka występujących u pacjenta i jego stanu klinicznego. W razie konieczności jednoczesnego podawania innych produktów leczniczych, należy zapoznać się z informacjami podanymi w aktualnych wersjach ChPL lenalidomidu i deksametazonu. Szczególne grupy pacjentów: *Pacjenci w podeszłym wieku:* U pacjentów w wieku powyżej 65 lat nie jest konieczne dostosowywanie dawki iksazomibu. W grupie pacjentów w wieku powyżej 75 lat przerwanie leczenia zgłoszono u 13 pacjentów (28%) otrzymujących iksazomib i u 10 pacjentów (16%) otrzymujących placebo. W grupie pacjentów w wieku powyżej 75 lat zaburzenia rytmu serca zaobserwowano u 10 pacjentów (21%) otrzymujących iksazomib i u 9 pacjentów (15%) otrzymujących placebo. *Zaburzenia czynności wątroby:* U pacjentów z łagodnymi zaburzeniami czynności wątroby (bilirubina całkowita ≤ górna granica normy [GGN] i aminotransferaza asparaginowa [AspAT] > GGN lub bilirubina całkowita > 1-1,5 x GGN i dowolna wartość AspAT) nie jest konieczne dostosowanie dawki iksazomibu. U pacjentów z umiarkowanymi (bilirubina całkowita > 1,53 x GGN) lub ciężkimi (bilirubina całkowita > 3 x GGN) zaburzeniami czynności wątroby zaleca się stosowanie zmniejszonej dawki wynoszącej 3 mg. *Zaburzenia czynności nerek:* U pacjentów z łagodnymi lub

umiarkowanymi zaburzeniami czynności nerek (klirens kreatyniny ≥ 30 ml/min) nie jest konieczne dostosowanie dawki iksazomibu. U pacjentów z ciężkimi zaburzeniami czynności nerek (klirens kreatyniny < 30 ml/min) lub schyłkową niewydolnością nerek wymagającą dializy zaleca się stosowanie zmniejszonej dawki wynoszącej 3 mg. Iksazomib nie jest usuwany w procesie dializy, dlatego może być stosowany niezależnie od harmonogramu dializ. Zalecenia dotyczące dawkowania lenalidomidu u pacjentów z zaburzeniami czynności nerek opisano w ChPL lenalidomidu. *Dzieci i młodzież:* Nie określono bezpieczeństwa stosowania ani skuteczności iksazomibu u dzieci w wieku poniżej 18 lat. Dane nie są dostępne. Sposób podawania: Iksazomib jest przeznaczony do podawania doustnego. Iksazomib należy zażywać mniej więcej o tej samej porze w 1., 8. i 15. dniu każdego cyklu leczenia, co najmniej na 1 godzinę przed posiłkiem lub co najmniej 2 godziny po posiłku. Kapsułkę należy połknąć w całości, popijając wodą. Nie należy jej łamać, rozgryzać ani otwierać. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą. Ponieważ iksazomib jest stosowany w skojarzeniu z lenalidomidem i deksametazonem, należy zapoznać się z dodatkowymi przeciwwskazaniami wymienionymi w ChPL tych produktów leczniczych. **Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania:** Ponieważ iksazomib jest stosowany w skojarzeniu z lenalidomidem i deksametazonem, należy zapoznać się z dodatkowymi specjalnymi ostrzeżeniami i środkami ostrożności dotyczącym stosowania wymienionymi w ChPL tych produktów leczniczych. Małopłytkowość: U pacjentów leczonych iksazomibem zgłaszano małopłytkowość (patrz punkt 4.8), przy czym liczba płytek krwi była zwykle najmniejsza między 14. a 21. dniem każdego 28-dniowego cyklu leczenia, a przed rozpoczęciem kolejnego cyklu zwiększała się ponownie do wartości początkowej. W trakcie leczenia iksazomibem należy monitorować liczbę płytek krwi co najmniej raz w miesiącu. Częstsze monitorowanie liczby płytek krwi należy rozważyć w pierwszych trzech cyklach zgodnie z ChPL lenalidomidu. Małopłytkowość można kontrolować przez dostosowywanie dawkowania i przetoczenia płytek krwi zgodnie ze standardowymi wskazaniami medycznymi. Objawy toksyczności ze strony układu pokarmowego: U pacjentów leczonych iksazomibem obserwowano biegunkę, zaparcie, nudności i wymioty, które sporadycznie wymagały zastosowania leków przeciwwymiotnych i przeciwbiegunkowych oraz leczenia podtrzymującego. W przypadku występowania ciężkich objawów toksyczności (stopnia 3. lub 4.) należy odpowiednio dostosować dawkę. W przypadku wystąpienia ciężkich zaburzeń żołądkowo-jelitowych zaleca się monitorowanie stężenia potasu w surowicy krwi. Neuropatia obwodowa: U pacjentów leczonych iksazomibem obserwowano objawy neuropatii obwodowej. Należy monitorować stan pacjenta w celu wykrycia objawów neuropatii obwodowej. U pacjentów, u których doszło do wystąpienia lub nasilenia objawów neuropatii obwodowej, może być konieczna modyfikacja dawki. Obrzęk obwodowy: U pacjentów leczonych iksazomibem obserwowano objawy obrzęku obwodowego. Należy monitorować stan pacjenta w celu wykrycia przyczyn i, w razie konieczności, zastosować leczenie objawowe. Należy dostosować dawkę deksametazonu zgodnie z informacjami dotyczącymi stosowania lub według dawki iksazomibu zalecanej w przypadku występowania objawów stopnia 3. lub 4. Reakcje skórne: U pacjentów leczonych iksazomibem obserwowano występowanie wysypki. Należy wówczas zastosować leczenie objawowe lub zmodyfikować dawkę w przypadku wystąpienia objawów stopnia 2. lub wyższego. Mikroangiopatia zakrzepowa Zgłaszano przypadki mikroangiopatii zakrzepowej (ang. thrombotic microangiopathy, TMA), w tym zakrzepowej plamicy małopłytkowej (ang. thrombotic thrombocytopenic purpura, TTP) u pacjentów otrzymujących iksazomib. Niektóre z tych przypadków zakończyły się zgonem. Należy monitorować pacjenta w kierunku przedmiotowych i podmiotowych objawów TMA. W razie podejrzenia takiego rozpoznania należy wstrzymać podawanie iksazomibu i ocenić pacjenta w kierunku TMA. W razie wykluczenia rozpoznania TMA można wznowić leczenie iksazomibem. Nie określono bezpieczeństwa wznowienia leczenia iksazomibem u pacjentów z przebytą TMA. Hepatotoksyczność: U pacjentów leczonych iksazomibem rzadko obserwowano uszkodzenie wątroby wywołane lekami, uszkodzenie komórek wątroby, stłuszczenie wątroby, cholestatyczne zapalenie wątroby i hepatotoksyczność. Należy regularnie monitorować enzymy wątrobowe i dostosować dawkę w przypadku wystąpienia objawów stopnia 3. lub 4. Ciąża: Należy unikać zajścia w ciążę podczas leczenia iksazomibem. Jeżeli iksazomib stosowany jest u kobiety w ciąży lub jeżeli podczas jego stosowania pacjentka zajdzie w ciążę, należy poinformować ją o możliwym zagrożeniu dla płodu. Kobiety w wieku rozrodczym muszą stosować wysoce skuteczną metodę antykoncepcji w trakcie leczenia iksazomibem i w okresie 90 dni po zakończeniu leczenia. W przypadku stosowania hormonalnych środków antykoncepcyjnych należy dodatkowo stosować mechaniczne metody antykoncepcji. Zespół tylnej odwracalnej encefalopatii: U pacjentów otrzymujących iksazomib wystąpiły przypadki zespołu tylnej odwracalnej encefalopatii (ang. *posterior reversible encephalopathy syndrome*, PRES). Zespół PRES jest rzadkim, odwracalnym zaburzeniem neurologicznym, który może objawiać się napadami drgawkowymi, nadciśnieniem, bólami głowy, zaburzeniami świadomości i zaburzeniami widzenia. Rozpoznanie zespołu PRES należy potwierdzić metodami obrazowania mózgu, najlepiej techniki obrazowania metodą rezonansu magnetycznego. U pacjentów, u których doszło do wystąpienia PRES, należy przerwać stosowanie iksazomibu. Silne induktory CYP3A: Silne induktory mogą zmniejszać skuteczność iksazomibu, dlatego należy unikać jednoczesnego stosowania produktu z silnymi induktorami CYP3A, takimi jak karbamazepina, fenytoina, ryfampicyna i ziele dziurawca (*Hypericum perforatum*). Jeżeli nie można uniknąć jednoczesnego stosowania iksazomibu w skojarzeniu z silnym induktorem CYP3A, należy ściśle monitorować stan pacjenta w celu kontrolowania objawów choroby. **Działania niepożądane:** Ponieważ iksazomib jest stosowany w skojarzeniu z lenalidomidem i deksametazonem, należy zapoznać się z dodatkowymi informacjami o działaniach niepożądanych wymienionymi w ChPL tych produktów. Podsumowanie profilu bezpieczeństwa: Poniższe informacje stanowią zestawione dane dotyczące bezpieczeństwa uzyskane w ramach globalnego, zasadniczego badania klinicznego fazy III (C16010) (n=720) i Chińskiego Badania Kontynuacyjnego prowadzonego metodą podwójnie ślepej próby z grupą kontrolną otrzymującą placebo (C16010) (n=115). W grupie 417 pacjentów leczonych iksazomibem i 418 pacjentów otrzymujących placebo najczęściej (≥ 20%) zgłaszano następujące działania niepożądane: biegunka (odpowiednio 39% i 32%), małopłytkowość (odpowiednio 33% i 21%), neutropenia (odpowiednio 33% i 30%), zaparcia (odpowiednio 30% i 22%), neuropatia obwodowa (odpowiednio 25% i 20%), nudności (23% i 18%), obrzęki obwodowe (odpowiednio 23% i 17%), wymioty (odpowiednio 20% i 10%) oraz zakażenie górnych dróg oddechowych (odpowiednio 21% i 16%). Ciężkie działania niepożądane zaobserwowane u co najmniej 2% pacjentów obejmują małopłytkowość (2%) i biegunkę (2%). Tabelaryczne zestawienie działań niepożądanych: Działania niepożądane zostały wymienione poniżej według następującej klasyfikacji częstości występowania: bardzo często (≥ 1/10), często (≥ 1/100 do < 1/10), niezbyt często (≥ 1/1 000 do < 1/100), rzadko (≥ 1/10 000 do < 1/1 000), bardzo rzadko (< 1/10 000), częstość nieznana (nie może być określona na podstawie dostępnych danych). W ramach poszczególnych grup klasyfikacji układów i narządów działania niepożądane uszeregowano zgodnie z częstością ich występowania, począwszy od tych najczęściej występujących. W obrębie każdej grupy o określonej częstości występowania, działania niepożądane wymienione są zgodnie ze zmniejszającym się nasileniem.

Tabela 3: Działania niepożądane zgłoszone u pacjentów leczonych iksazomibem w skojarzeniu z lenalidomidem i deksametazonem (wszystkie stopnie nasilenia, stopień 3. i stopień 4.)

Klasyfikacja układów i narządów / Działanie niepożądane	Działania niepożądane (wszystkie stopnie nasilenia)	Działania niepożądane stopnia 3.	Działania niepożądane stopnia 4.
Zakażenia i zarażenia pasożytnicze			
Zakażenie górnych dróg oddechowych	Bardzo często	Niezbyt często	–
Półpasiec	Często	Często	–
Zaburzenia krwi i układu chłonnego			
Małopłytkowość*	Bardzo często	Bardzo często	Często
Neutropenia*	Bardzo często	Bardzo często	Często
Mikroangiopatia zakrzepowa	Rzadko	–	Rzadko

Klasyfikacja układów i narządów / Działanie niepożądane	Działania niepożądane (wszystkie stopnie nasilenia)	Działania niepożądane stopnia 3.	Działania niepożądane stopnia 4.
Zaburzenia krwi i układu chłonnego			
Zakrzepowa plamica małopłytkowa†	Rzadko	Rzadko	Rzadko
Zaburzenia układu nerwowego			
Neuropatie obwodowe*	Bardzo często	Często	–
Zaburzenia żołądka i jelit			
Biegunka	Bardzo często	Często	–
Nudności	Bardzo często	Często	–
Wymioty	Bardzo często	Niezbyt często	–
Zaparcie	Bardzo często	Niezbyt często	–
Zaburzenia skóry i tkanki podskórnej			
Wysypka*	Bardzo często	Często	–
Zaburzenia mięśniowo-szkieletowe i tkanki łącznej			
Ból pleców	Bardzo często	Niezbyt często	–
Zaburzenia ogólne i stany w miejscu podania			
Obrzęki obwodowe	Bardzo często	Często	–

Uwaga: Uwzględniono nazwy działań niepożądanych zgodne z preferowaną terminologią MedDRA w wersji 16.0.
*Termin łączy w sobie preferowaną terminologię. †Zgłaszana poza badaniami III fazy

Opis wybranych działań niepożądanych: *Przerwanie leczenia:* Przerwanie leczenia co najmniej jednym z trzech produktów leczniczych nastąpiło dla każdego działania niepożądanego u ≤ 1% pacjentów otrzymujących iksazomib. *Małopłytkowość:* W trakcie leczenia liczba płytek krwi nie przekraczała 10 000/mm^3 u 3% pacjentów otrzymujących iksazomib i u 1% pacjentów otrzymujących placebo. W trakcie leczenia u mniej niż 1% pacjentów w obu schematach leczenia liczba płytek krwi nie przekraczała 5000/mm^3. Małopłytkowość była przyczyną przerwania leczenia co najmniej jednym z trzech produktów leczniczych u mniej niż 1% pacjentów otrzymujących iksazomib i u 1% pacjentów otrzymujących placebo. Małopłytkowość nie spowodowała zwiększenia częstości występowania zdarzeń krwotocznych ani przetoczeń płytek krwi. *Objawy toksyczności ze strony układu pokarmowego:* Biegunka była przyczyną przerwania leczenia co najmniej jednym z trzech produktów leczniczych u 1% pacjentów otrzymujących iksazomib i u mniej niż 1% pacjentów otrzymujących placebo. *Wysypka:* Wysypkę zaobserwowano u 18% pacjentów leczonych iksazomibem i u 10% pacjentów otrzymujących placebo. W obu grupach najczęściej zgłaszano wysypkę plamisto-grudkową i plamistą. Wysypkę 3. stopnia obserwowano u 2% pacjentów leczonych iksazomibem i u 1% pacjentów otrzymujących placebo. Wysypka była przyczyną przerwania leczenia co najmniej jednym z trzech produktów leczniczych u mniej niż 1% pacjentów w obu grupach. *Neuropatia obwodowa:* Neuropatię obwodową obserwowano u 25% pacjentów leczonych iksazomibem i u 20% pacjentów otrzymujących placebo. Działania niepożądane 3. stopnia związane z neuropatią obwodową obserwowano u 2% w obu schematach. Najczęściej zgłaszanym działaniem niepożądanym była czuciowa neuropatia obwodowa (występowała u 16% pacjentów stosujących iksazomib i u 12% pacjentów otrzymujących placebo). W obu grupach pacjentów ruchowa neuropatia obwodowa była zgłaszana niezbyt często (< 1%). Neuropatia obwodowa była przyczyną przerwania leczenia co najmniej jednym z trzech produktów leczniczych u 1% pacjentów leczonych iksazomibem i u 1% pacjentów otrzymujących placebo. Zaburzenia oka: Mimo, że zaburzenia oka zgłaszano z użyciem różnorodnej, preferowanej terminologii, ogółem częstość ich występowania u pacjentów leczonych iksazomibem i u pacjentów otrzymujących placebo wyniosła odpowiednio 24% i 15%. Najczęściej obserwowanymi działaniami niepożądanymi były: nieostre widzenie (występujące u 5% pacjentów przyjmujących iksazomib i 4% pacjentów otrzymujących placebo), suche oko (występujące u 4% pacjentów przyjmujących iksazomib i 1% pacjentów otrzymujących placebo), zapalenie spojówek (występujące u 5% pacjentów przyjmujących iksazomib i 1% pacjentów otrzymujących placebo) i zaćma (występująca u 4% pacjentów przyjmujących iksazomib i u 5% pacjentów otrzymujących placebo). Działania niepożądane stopnia 3. zgłaszano u 2% pacjentów w obu schematach. *Inne działania niepożądane:* Poza badaniami III fazy niezbyt często zgłaszano następujące poważne działania niepożądane: ostra gorączkowa dermatoza neutrofilowa (zespół Sweeta), zespół Stevensa-Johnsona, poprzeczne zapalenie rdzenia, zespół odwracalnej tylnej encefalopatii i zespół rozpadu guza. W zestawieniu danych uzyskanych w ramach globalnego, zasadniczego badania klinicznego fazy III (C16060) (n=720) i Chińskiego Badania Kontynuacyjnego prowadzonego metodą podwójnie ślepej próby z grupą kontrolną otrzymującą placebo (C16010) (n=115), następujące działania niepożądane wystąpiły z podobną częstością u pacjentów leczonych iksazomibem i u pacjentów otrzymujących placebo: zmęczenie (odpowiednio 26% i 24%), utrata apetytu (odpowiednio 12% i 9%), hipotensja (4% w obu grupach), niewydolność serca† (3% w obu grupach), zaburzenia rytmu serca† (odpowiednio 12% i 11%) oraz zaburzenia czynności wątroby obejmujące zmiany aktywności enzymów† (odpowiednio 8% i 6%). Częstość występowania ciężkich przypadków hipokaliemii (stopnia 3. lub 4.) była wyższa u pacjentów leczonych iksazomibem (5%) niż u pacjentów otrzymujących placebo (< 1%). U pacjentów leczonych iksazomibem w skojarzeniu z lenalidomidem i deksametazonem niezbyt często zgłaszano przypadki grzybiczego i wirusowego zapalenia płuc zakończonego zgonem. † Grupowanie kilku preferowanych terminów słownika MedDRA w celu opisania zagadnienia medycznego (ang. *Standarized MedDRA Query*, SQM). 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. 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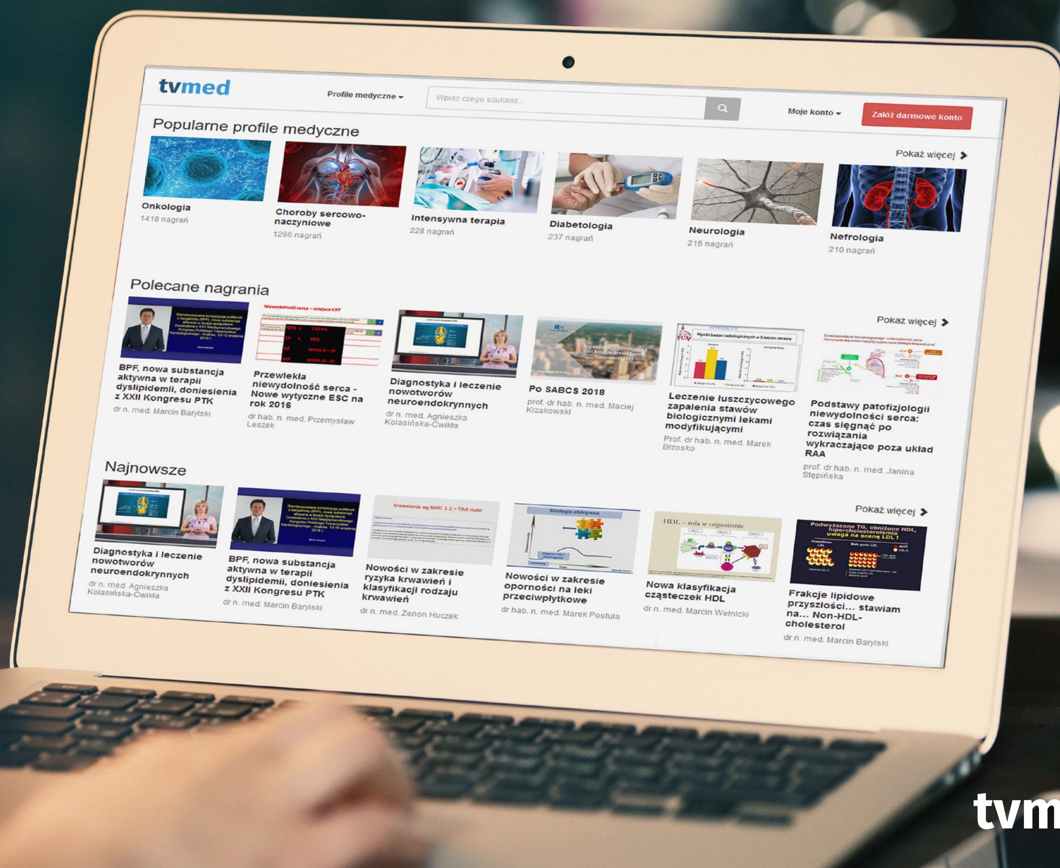

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On behalf of the Editorial Board, I would like to thank all Reviewers for accepting the invitation and the time devoted to reviewing the manuscripts. Thank you for contribution to the development and quality of the journal and sharing your expertise with the readers.

Prof. dr hab. n. med. Jan Styczyński
— Editor-in-Chief

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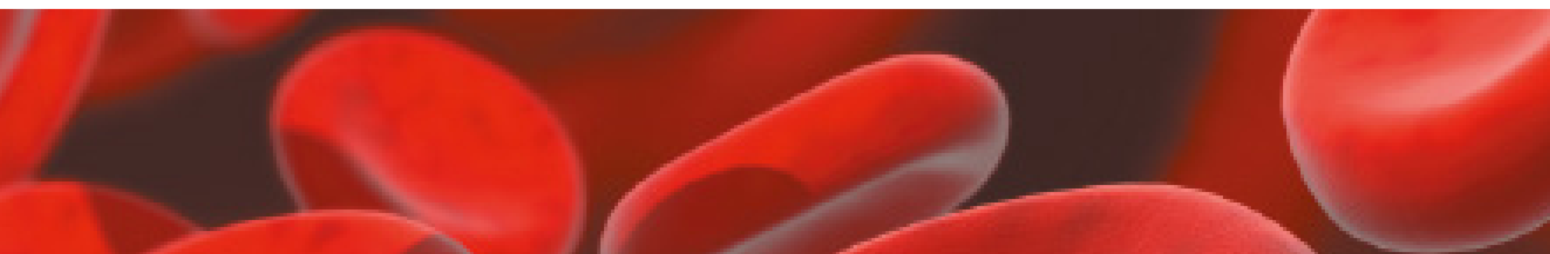

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"Acta Haematologica Polonica": on its new road

Jan Styczyński

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"Acta Haematologica Polonica" was launched in 1970 as the journal of the Polish Society of Haematology, later the Polish Society of Haematology and Transfusion Medicine together with the Institute of Haematology and Transfusion Medicine [1]. Until 2020, it was published as a quarterly journal. It has now become a bimonthly journal, with a primary electronic edition. This shift will enable faster publication.

2020 will be remembered by the entire world for the never-ending struggle with the SARS-CoV-2/COVID-19 (severe acute respiratory syndrome coronavirus 2/coronavirus disease 19) pandemic that severely affected our lives, our careers, and our patients. 2021 began with the roll-out of vaccinations against the virus. Bearing in mind that vaccinations are one of the greatest achievements in the history of humankind and the history of medicine [1–3], we all hope that this signals the beginning of the end of the pandemic.

A new year calls for new directions, but our fresh perspective is based on the substantial achievements of our Society, and the position of respect that we have earned [4–7].

I call on all members of the haematology and transfusion medicine profession to engage with Acta Haematologica Polonica. Publish your achievements and cite papers published in our journal. We are all readers, authors, and reviewers of „Acta Haematologica Polonica"!

Authors' contributions

JS — sole author.

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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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EDITORIAL

VIA MEDICA

Polish haematology: looking to the future

Wiesław Wiktor Jędrzejczak

Department of Haematology, Transplantation and Internal Medicine, Medical University of Warsaw, Warsaw, Poland

In this issue, Giebel et al. [1] report on the status of haemato-oncology in Poland. They summarise achievements and describe its current situation. Polish haematology is most certainly at a crossroads. There has been a changing of the guard. Within just a few years, the 'baby boomer' generation (those born shortly after World War II) has transferred practically all chair positions to a younger generation of doctors born in the late 1960s and the 1970s. This is the generation whose careers began after the change of Poland's political and economic system. This generation will be responsible for developments in the field over the next 20–30 years.

Since the start of the 21st century, the number of Polish haematologists has more than doubled, reaching now almost 500 fully trained specialists, with more than 100 in training. There are haematology centres in all regions (previously almost half of Poland's 16 voivodships had none) but the greatest challenge is the rapidly growing number of patients. This is due to the ageing of society and even more so to the increased effectiveness of therapy. Progress in haematology which now is almost instantaneously incorporated into practice is generating more and more people surviving with chronic disease who need services.

An interesting analysis of the survival of patients with various cancers was published by the Polish National Institute of Public Health. The overall effects of cancer treatment in Poland are inferior when compared to the countries of what we used to call 'Western Europe'. However, there is one exception: leukemia. Poland, while not leading, is not far behind these latter countries [2]. Poland is one of the few countries in which bone marrow transplantation technology was independently developed, rather than being imported from abroad [3–6]. While this was largely due to necessity (development took place in the early 1980s when Poland was under martial law and foreign travel was not permitted), this is a clear demonstration of the innovative potential of Polish haematology.

Today, Poland has nearly closed the gap in the number of haematopoietic cell transplantations (1,848 transplantations in 2019) between it and more developed countries, and has become a 'superpower' in terms of the number of registered potential donors of haematopoietic cells (almost 1.8 million registered and typed people). This is a figure exceeded only by the US, Germany, and Brazil. Moreover, Poland has become the second of only two countries (the first being Germany) to provide more transplants to foreign patients than to its own (1,294 vs. 265 in 2019) [7, 8].

While Polish haematology centres participate actively in global clinical trials, there is still a limited number of trials initiated and designed in Poland. The Polish pharmaceutical industry is concentrated on the production of generic drugs, and the next step should include also some innovative molecules. However, these have to be invented or discovered by the chemists!

The successful introduction of Polish-made cladribine has shown that Polish haematology is capable of designing and performing trials with the aim of introducing new drugs to clinical practice, when gaining early access to new molecules [9, 10]. Polish haematology is well integrated into European haematology, with Polish centres being very active in the European Leukemia Net, the European Haematology Association, and the European Society for Blood and Marrow Transplantation.

The future depends both on encouraging further research in Poland, and actively participating in international collaboration.

Authors' contributions

WWJ — sole author.

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REVIEW ARTICLE

VIA MEDICA

Current status and achievements of Polish haemato-oncology

Sebastian Giebel[1], Grzegorz Basak[2], Maria Bieniaszewska[3], Tomasz Czerw[1], Anna Czyż[4], Joanna Drozd-Sokołowska[2], Dominik Dytfeld[5], Krzysztof Giannopoulos[6], Lidia Gil[5], Grzegorz Helbig[7], Jerzy Hołowiecki[1], Iwona Hus[8], Krzysztof Jamroziak[8, 9], Wiesław W. Jędrzejczak[2], Wojciech Jurczak[10], Artur Jurczyszyn[11], Ewa Lech-Marańda[8], Krzysztof Lewandowski[5], Krzysztof Mądry[2], Monika Prochorec-Sobieszek[12], Tadeusz Robak[13], Tomasz Sacha[11], Małgorzata Sokołowska-Wojdyło[14], Michał Szymczyk[9], Jan Walewski[9], Agnieszka Wierzbowska[13], Dariusz Wołowiec[4], Tomasz Wróbel[4], Jan Maciej Zaucha[3], Jan Styczyński[15, *]

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Abstract

The number of newly diagnosed haematological malignancies in Polish adults and children is about 9,000 a year, which constitutes about 5.5% of all malignancies in the country. Adult patients with haematological malignancies are diagnosed and treated in 42 institutions in Poland. The scientific and educational support for this activity is provided under the umbrella of the Polish Society of Haematologists and Transfusiologists (PTHiT, *Polskie Towarzystwo Hematologów i Transfuzjologów*), the Polish Adult Leukemia Group (PALG), the Polish Lymphoma Research Group (PLRG), the Polish Myeloma Study Group (PMSG), the Polish Myeloma Consortium (PMC), and consultants in haematology.

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The aim of this position paper is to present the current status and progress in therapy of haematological malignancies in Polish haematology adult centres, focusing on the activity of PALG, PLRG, and PMSG. The achievements of Polish haemato-oncology at the beginning of the third decade of the 21st century are set out in this paper. Polish haemato-oncology today has an important international position based on contributions to the development of knowledge, international cooperation, and a high quality of patient care. In many instances, clinical trials run by Polish collaborative groups have influenced international standards. Polish haematologists have been the authors of treatment recommendations, and their research has indicated areas for further research.

Key words: Polish Society of Haematologists and Transfusiologists, Polish Adult Leukemia Group, Polish Lymphoma Research Group, Polish Myeloma Study Group

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Introduction — haematology in Poland

Adult patients with haematological malignancies are diagnosed and treated in 42 institutions in Poland. The scientific and educational support for this activity is provided under the umbrella of the Polish Society of Haematologists and Transfusiologists (PTHiT, *Polskie Towarzystwo Hematologów i Transfuzjologów*), the Polish Adult Leukemia Group (PALG), the Polish Lymphoma Research Group (PLRG), the Polish Myeloma Study Group (PMSG), and consultants in haematology.

The Polish Society of Haematology (PTH, *Polskie Towarzystwo Hematologiczne*) was created in 1949 by Prof. Tadeusz Tempka. From the first meeting of the Society in 1950 in Kraków, Prof. Tempka was its President up to 1972. From 1972 to 1976, Prof. Hugon Kowarzyk was the President. He changed the name of the society to the PTHiT in 1975. The next Presidents were: Prof. Józef Japa (1976–1987), Prof. Janusz Hansz (1987–1995), Prof. Wiesław W. Jędrzejczak (1995–2003), Prof. Andrzej Hellmann (2003–2011), Prof. Tadeusz Robak (2011–2019) and Prof. Iwona Hus (since 2019). The Society has biannual meetings. PTHiT meetings were held in Białystok 2001; Gdańsk 2003; Katowice/Wisła 2005; Warsaw 2007; Wrocław 2009; Lublin 2011; Poznań 2013; Szczecin 2015, Warsaw 2017, and Łódź in 2019. The 29th Meeting will be held in Bydgoszcz in 2021.

The position of national consultant in haematology was held consecutively by Prof. Lech Konopka (1997–2001), Prof. Jerzy Hołowiecki (2001–2002), Prof. Wiesław Jędrzejczak (2002–2014), Prof. Dariusz Wołowiec (2014–2016), again Prof. Jędrzejczak (2016–2018), and Prof. Ewa Lech-Marańda (since 2018). The current team of regional consultants comprises: Lidia Usnarska-Zubkiewicz (woj. dolnośląskie), Małgorzata Całbecka (woj. kujawsko-pomorskie), Marek Hus (woj. lubelskie), Katarzyna Brzeźniakiewicz-Janus (woj. lubuskie), Piotr Smolewski (woj. łódzkie), Tomasz Sacha (woj. małopolskie), Bożena Katarzyna Budziszewska (woj. mazowieckie), Dariusz Woszczyk (woj. opolskie), Mirosław Markiewicz (woj. podkarpackie), Jarosław Andrzej Piszcz (woj. podlaskie), Wojciech Homenda (woj. pomorskie), Małgorzata Wojciechowska (woj. warmińsko-mazurskie), Małgorzata Krawczyk-Kuliś (woj. śląskie), Marcin Pasiarski (woj. świętokrzyskie), Lidia Gil (woj. wielkopolskie), and Barbara Zdziarska (woj. zachodniopomorskie).

The Polish Adult Leukemia Group was created in 1975 and chaired by Prof. Jerzy Hołowiecki up to 2014, followed by Prof. Sebastian Giebel. The Polish Lymphoma Research Group was created in 2008 and chaired by Dr Janusz Meder followed by Prof. Sebastian Giebel. PALG and PLRG include a total of 36 haematology adult centres actively participating in scientific activity.

The aim of this position paper is to present the current status, achievements and progress in the therapy of haematological malignancies in Polish haematology adult centres, focusing on the activity of PALG, PLRG, and PMSG.

Epidemiology of haematological malignancies in Poland

Morbidity

According to the National Cancer Registry, the number of newly diagnosed malignancies in the Polish population in 2017 was 164,875 including 8,988 (5.45%) haematological malignancies (Table I) [1]. However, it is worth noting that the National Cancer Registry does not include newly diagnosed patients with myelodysplastic syndromes (D46) or myeloproliferative neoplasms such as polycythemia vera (D45), primary myelofibrosis (D47.1), and essential thrombocythemia (D75.2). Data from the National Health Fund (NFZ, *Narodowy Fundusz Zdrowia*) from 2014 indicates there were 1,444 new patients with myelodysplastic syndromes and 3,382 patients with myeloproliferative neoplasms [2].

The National Cancer Registry provides the following general information regarding the epidemiology of the most frequent haematological malignancies [1].

Table I. Newly diagnosed haematological malignancies and morbidity rates in Poland in 2017 (no age limits) [1]

ICD-10	Malignancy	Newly diagnosed (2017)	Morbidity rate	Standardised morbidity ratio
C00-D09	All malignancies	164,875 (100%)	429.11	323.50
C81	Hodgkin's lymphoma	753 (0.46%)	1.96	1.90
C82	Follicular lymphoma	442 (0.27%)	1.15	0.93
C83	Non-follicular lymphoma	1,535 (0.93%)	4.00	3.11
C84	Other specified types of T/NK-cell lymphoma	244 (0.15%)	0.64	0.51
C85	Other and unspecified types of non-Hodgkin's lymphoma	848 (0.51%)	2.21	1.67
C88	Malignant immunoproliferative diseases	67 (0.04%)	0.17	0.13
C90	Multiple myeloma and malignant plasma cell neoplasms	1,600 (0.97%)	4.16	3.02
C91	Lymphoid leukemia	1,801 (1.09%)	4.69	3.78
C92	Myeloid leukemia	1,119 (0.68%)	2.91	2.28
C93	Monocytic leukemia	71 (0.04%)	0.18	0.13
C94	Other leukemias of specified cell type	375 (0.23%)	0.98	0.72
C95	Leukemia of unspecified cell type	57 (0.03%)	0.15	0.11
C96	Other and unspecified malignant neoplasms of lymphoid, haematopoietic and related tissue	76 (0.05%)	0.20	0.16
	All haematological malignancies (C81–C96)	8,988 (5.45%)	23.40	18.45

The morbidity rate is the frequency or proportion with which a disease appears in a population. Standardised morbidity ratio is the ratio between the observed number of new diagnoses in a study population and the number of new diagnoses that would be expected based on the age- and sex-specific rates in a standard population and the population size of the study population by the same age/sex groups (European population is assumed as standard population); ICD-10 — International Statistical Classification of Diseases and Health-Related Problems; NK — natural killers

Leukemia

Patients with leukemia comprise 2% of malignancies. The number of newly diagnosed patients with leukemia in 2017 was 3,423. There is an age-dependent increasing trend in the number of newly diagnosed patients. The risk of leukemia in children, adolescents and young adults (AYA) is $4/10^5$, and this risk steadily increases after the age of 50.

Myeloid leukemia

Myeloid leukemia (acute and chronic, as reported in ICD-10) comprises 0.7% of malignancies. The number of newly diagnosed patients with myeloid leukemia in 2017 was 1,119, with 55% of diagnoses at age 55–79. Patients diagnosed with myeloid leukemia between 2003 and 2005 had a 1-year survival rate of 45.5% in males and 46.6% in females.

Lymphoid leukemia

Lymphoid leukemia (acute and chronic, as reported in ICD-10) comprises 1.1% of malignancies. The number of newly diagnosed patients with lymphoid leukemia in 2017 was 1,801. There are two peak incidence periods: childhood and old age. Patients diagnosed with myeloid leukemia between 2003 and 2005 had a 1-year survival rate of 74.7% in males and 77.3% in females.

Multiple myeloma (MM)

This comprises 1% of malignancies. The number of newly diagnosed patients with MM in 2017 was 1,600, with 75% of diagnoses in those aged over 60. The peak incidence is for people in their 70s: $20/10^5$ in males and $15/10^5$ in females. Patients diagnosed with MM between 2003 and 2005 had a 1-year survival rate of 62.9% in males and 66.0% in females.

Hodgkin's lymphoma (HL)

This comprises 0.5% of malignancies. The number of newly diagnosed patients with HL in 2017 was 753. More than 50% of new diagnoses in males, and 65% in females, are made between the ages of 15 and 40. The peak incidences occur between 25–30 and after the age of 70. Patients diagnosed with HL between 2003 and 2005 had a 1-year survival rate of 89.2% in males and 91.5% in females.

Non-Hodgkin's lymphoma (NHL)

This comprises 2% of malignancies. The number of newly diagnosed patients with NHL in 2017 was 3,069. More than 70% of new NHL diagnoses are made after the age of 50. NHL comprises 7.5% in children, and 5% in age 20–44. Patients diagnosed with NHL between 2003 and 2005 had a 1-year survival rate of 68.5% in males and 70.2% in females.

Acute myeloid leukemia

Treatment of newly diagnosed AML

Therapeutic management in AML depends on prognostic factors, particularly the patient's age and cytogenetic as

well as molecular features [3]. Achievement of complete remission (CR) after induction treatment is a prerequisite for successful therapy and prolongation of overall survival (OS). In PALG studies, attempts have been made to modify the standard DA-60 induction protocol (daunorubicin [DNR] 60 mg/m^2 for three consecutive days in combination with cytosine arabinoside [Ara-C] at a dose of 100–200 mg//m^2/day for seven days) in order to improve the efficacy of induction treatment in AML patients eligible for intensive chemotherapy.

Two multicentre randomised trials conducted by PALG demonstrated that the addition of cladribine at a dose of 5 mg/m^2/day in a two-hour infusion for five consecutive days adhering to the DA induction (DAC protocol) had a beneficial impact in newly diagnosed AML patients ≤60 years of age [3, 4]. In the DAC arm, CR rate was significantly higher (67.5%) and the occurrence of primary resistance to chemotherapy rarer (21%) compared to standard DA-60 therapy (56%; p =0.01 and 34%; p =0.004, respectively). The addition of cladribine also exerted a significant effect on OS improvement (3-year OS, 45% vs. 33%), particularly in patients >50 years, with high leukocytosis (>50 G/L), and in the group with unfavourable karyotype [4]. A retrospective analysis showed that the DAC treatment was associated with improved CR and OS rates in the subgroup of patients with FLT3-ITD mutation [5]. In AML patients older than 60 years, DAC appeared to be superior in the subgroup aged 60–65 (CR rate: DAC 51% vs. DA 29%; p =0.02). What's more, patients with good and intermediate karyotypes benefited from the addition of cladribine also in terms of OS (p =0.02) [6]. No differences in haematological and non-haematological toxicity between the DA and DAC regimens in all trials were observed. In a prospective study, outcomes in 509 elderly patients with AML with different treatment approaches depending on Eastern Cooperative Oncology Group (ECOG) performance status and Charlson Comorbidity Index (CCI) were estimated [7].

Treatment of refractory and relapsed AML

A significant limitation of AML treatment is the impossibility of achieving CR with standard induction chemotherapy in 20–30% of patients. In more than half of the patients with CR, leukemia relapse can occur within three years of the initial diagnosis.

The results of the multicentre PALG phase II clinical trials have indicated that cladribine and high-dose Ara-C combined with G-CSF (CLAG) and mitoxantrone (CLAG-M) is a highly efficient treatment for refractory and relapsed AML [8, 9]. The results of the subsequent PALG phase II study showed that the CLAG-M protocol has high antileukemic activity and moderate toxicity in poor prognosis patients with primary drug-resistant AML or in those demonstrating early relapse (1CR <6 months) or relapse after stem cell transplantation [10]. Based on these promising results, in the next phase II trial CLAM (cladribine + Ara-C + mitoxantrone ± G-CSF) was used as an early second induction on day 16 based on bone marrow blasts on day 14 in AML patients aged under 60 years who received DAC as first induction. The study results showed that CLAM used as early second induction might improve CR/CRi rates for younger AML patients with poor early response to DAC induction, but this approach may be associated with higher mortality [11].

Treatment of acute promyelocytic leukemia (APL)

In Poland, patients with acute promyelocytic leukemia (APL) are treated in cooperation with the PETHEMA (*Programa Español para el Tratamiento de las Hemopatías Malignas*) group and according to current PETHEMA protocols. A retrospective analysis of a real-life Polish population showed that early death (ED) remains a major problem in APL, and that shortening the time between the initial contact with a healthcare professional and all-trans retinoic acid administration, as well as the use of appropriate supportive care, could improve the outcomes of an unselected APL population [12].

Currently ongoing clinical trials

The PALG-AML1/2016 study aims to compare the safety and efficacy of two commonly used induction (DAC vs. DA-90) and salvage (CLAG-M vs. FLAG-IDA) regimens in AML. This trial is also the first international randomised trial regarding AML induction to prospectively evaluate the impact of measurable residual disease (MRD) on overall survival, using multi-modality testing (flow-cytometry, next-generation sequencing, and PCR) of serial samples. (ClinicalTrials.gov Identifier: NCT03257241). In the PALG-AML-1/2018 phase I/Ib trial, the safety and efficacy of a combination of CPX-351 with cladribine in elderly patients with relapsed/refractory acute myeloid leukemia is being analysed (EudraCT: 2020-002535-29).

Chronic myeloid leukemia

In monitoring the treatment results of chronic myeloid leukemia (CML) with the use of tyrosine kinase inhibitors (TKIs), special attention is paid to appropriate follow-up with molecular methods, which should be performed in certified laboratories able to issue the results of real-time quantitative PCR (RQ-PCR) using the international scale (IS). Polish Molecular Laboratories have been organised in the National Network and have joined the European Leukemia Net and EUTOS projects dedicated to the standardisation of quantitative *BCR-ABL1* analysis in patients with CML [13]. The National Molecular Reference Laboratory has been established in Kraków, and this has successfully conducted standardisation and certification procedure in

16 Polish laboratories currently issuing results using the IS [14]. Poland was the first European country with registered imatinib generics in 2014. PALG has established a webpage-based registry to evaluate prospectively the efficacy and tolerability of imatinib generics in a large cohort of adult patients. The report after one year of follow-up of 726 patients (99 previously untreated and 627 patients switched from branded to generic imatinib) showed equal efficacy and tolerability of imatinib generics compared to the branded drug [15].

Myeloproliferative neoplasms Ph-negative

The discovery of mutations that finally confirmed the clonality of Ph-negative myeloproliferative neoplasms resulted in the development of molecular studies also in Polish research centres. This allowed for the characterisation of the Polish population in terms of the presence of new mutations [16], as well as the detection of new mutations in atypical exons of the *JAK* gene and the *MPL* gene. Also the subject of research interest was the relationship between the occurrence of complications and the diversified course of diseases depending on the changes in the genome.

In 2018, the results of studies on the evolution of the clinical picture of myeloproliferative neoplasms were published, showing no effect of allele burden on the occurrence of polycythemic transformation [17]. As a result of the cooperation of the centres of the Myeloproliferative Neoplasms Working Group, analysis of the incidence of secondary von Willebrand syndrome in myeloproliferative neoplasms has been published, where it has been shown that this haemostatic pathology is not limited to essential thrombocythemia [18]. In 2019, the preliminary results of a multicentre study of Polish patients treated with ruxolitinib due to myelofibrosis were presented.

Myelodysplastic syndromes (MDS)

In Poland, the standard of care of MDS patients adheres to the current ELN recommendations. In 2008, the MDS Working Group within PALG included 960 MDS patients into a retrospective Polish MDS Registry. Since 2009, 2,513 MDS, AML <30% BM blasts and MDS/MPN patients have been registered prospectively. Cytogenetic results availability improved between 2009–2010 and 2018–2019 from 45% to 77%. Serum ferritin (SF) had a significant impact on outcomes. Patients with higher than 1,000 ng/mL SF versus patients with SF <1,000 ng/mL had a median survival of 320 days versus 568 days (p =0.014) [19]. Assuming that azacitidine (AZA) treated patients are at higher risk of serious infection, especially within the first three AZA cycles, between 2009 and 2016 296 patients were retrospectively analysed. It was found that red blood cell transfusion dependency [odds ratio (OR) =2.38], neutropenia <0.8 $\times 10^9$/L (OR =3.03), platelet count <50 $\times 10^9$/L (OR =2.63), albumin level <35 g/L (OR =2.04) and ECOG performance status ≥2 (OR =2.19) all had a significant impact on infectious risk. A subset of patients was selected with high risk of infection rate, 73% versus 25%, and worse clinical outcome, 8 versus 29 months survival [20].

Hypereosinophilic syndrome

Hypereosinophilic syndrome (HES) is a group of rare disorders with a unique clinical picture and challenging treatment. Over the last 20 years, we have witnessed an eruption of molecular findings leading to improved understanding of HES pathogenesis. A small proportion of HES patients may have an abnormal T-cell population responsible for the overproduction of eosinophilopoietic cytokines. These patients are defined to have lymphocytic variant HES. Peripheral blood samples from 42 HES patients were studied for the presence of T-cell receptor rearrangement by PCR and aberrant T lymphocytes by flow cytometry. Clonal T-cell rearrangements were detected in 18 individuals (42.8%) whereas an abnormal T-cell population was revealed only in three patients, with the conclusion that T-cell abnormalities are frequently found in HES [21]. Approximately 10% of HES patients present an interstitial deletion in chromosome 4q12 leading to the expression of *FIP1L1-PDGFR*α (F/P) — an imatinib-sensitive gene fusion.

Within the Polish Hypereosinophilic Syndrome Study Group, 32 patients were identified as meeting HES criteria and expressing F/P. Male gender greatly predominated (94%) and splenomegaly and pulmonary involvements were most frequently observed. Treatment with imatinib mesylate (IM) resulted in haematological and molecular CR in 100% of the studied patients. The response was rapid and durable. Imatinib at 100 mg weekly was sufficient to maintain CR in long-term follow-up. The updated results after a median of 12 years on IM have confirmed its excellent efficacy and safety. None of the patients exhibited IM resistance or transformed into acute leukemia. Seven patients stopped IM after achieving long-term remission, and two of them remained in CR for more than seven years after IM discontinuation. IM re-initiation leads to second CR in nearly all patients [22, 23].

Acute lymphoblastic leukemia (ALL)

The treatment of ALL in adults has been based on BFM-like protocols in Poland for more than two decades. Since 1996, adult patients in Poland have been treated with a uniform PALG protocol, with treatment outcomes recorded in prospective studies. A randomised trial demonstrated that the use of granulocyte-colony stimulating factor enabled better adherence to chemotherapy and

improved overall survival of ALL patients [24]. In a phase II study, the safety and efficacy of an originally-developed FLAM regimen (fludarabine, cytarabine, and mitoxantrone) was assessed for patients with relapsed/refractory ALL [25]. The PALG 4-2002 study showed that MRD level ≥0.1% of bone marrow cells after induction assessed by flow cytometry should be considered an independent risk factor for treatment decisions in adult ALL [26]. The combination of MRD status with conventional risk stratification system identified a subgroup of patients allocated to the SR group with MRD ≥0.1% after induction who had a risk of relapse of 71% at three years, versus 9% in the remaining subjects (p =0.001) [26]. The prognostic value of MRD may be further increased when cytogenetic features are included in the model [27]. Consequently, flow cytometric MRD positive status, i.e. ≥0.1% after induction and ≥0.01% after consolidation, is considered an independent risk factor in the current ALL7 PALG protocol which was introduced in October 2018. Since L-asparaginase is one the core drugs in PALG protocols, recommendations were published on the use of L-asparaginase in ALL [28]. PALG is currently initiating a randomised, multicentre trial to compare obinutuzumab versus rituximab in newly diagnosed CD20-positive B-cell ALL. A Polish group led an international collaboration to establish recommendations regarding the role of allo-HCT in Ph-negative ALL [29].

Chronic lymphocytic leukemia (CLL)

Biology, prognostic factors and the treatment of CLL have been important targets of research for Polish haematologists in recent decades. Basic and pre-clinical studies performed in several Polish academic haematology departments have contributed to the current understanding of the molecular background and immunology of CLL [30]. However, the greatest achievement was the creation of a CLL clinical research programme within the PALG by Prof. Robak that was aimed at the active development of new therapies for CLL, mainly purine nucleoside analogue cladribine (2-CdA)-based combinations [31, 32]. Besides a number of early phase studies, this successful cooperation within PALG led to the setting up of four large prospective, multi-centre randomised phase III clinical trials (PALG CLL1-4) that included a total of 1,288 untreated CLL patients between 1995 and 2011. As a result, several active regimens such as CC (cladribine and cyclophosphamide) or RCC (rituximab, cladribine, and cyclophosphamide) have been recommended as up-front therapy of CLL by national guidelines and implemented into routine care in Poland. Furthermore, rare CLL complications have constituted another area of study where Polish observations have enabled better characterisation of epidemiology and clinical course of autoimmune conditions, and secondary neoplasms including Richter's syndrome [33].

Follicular lymphoma

The treatment of follicular lymphoma (FL) in Poland has been under the supervision of the PLRG since 2008. Two randomised clinical trials (RCT) were conducted, PLRG1 and PLRG4. In the pre-rituximab era, treatment-naïve patients with indolent lymphomas, including 28% of patients with follicular lymphoma, were enrolled into phase III PLRG1 RCT, comparing the efficacy of three protocols: cladribine monotherapy, CC (cladribine and cyclophosphamide), and CVP (cyclophosphamide, vincristine, and prednisone). Protocols containing cladribine yielded significantly better overall responses and complete remission rates and progression-free survival (PFS) times, but not OS [34]. PLRG4, phase III RCT, conducted already in the rituximab era, compared two induction chemoimmunotherapy protocols, R-CVP and R-CHOP, followed by rituximab maintenance, for indolent lymphomas [35]. Patients with FL constituted 42%, while patients with marginal zone lymphoma comprised 38%, among 250 enrolled patients. The frequency of response did not differ between the study arms and the time-to-event endpoints i.e. event-free survival (EFS), PFS and OS, were similar. The only indolent lymphoma with a significantly worse outcome was small lymphocytic lymphoma. Five-year EFS for the whole group reached 61% and 56% in the R-CHOP and the R-CVP arms, respectively. The occurrence of early progression of FL (POD24) was similarly frequent in both arms (13.7 vs. 16.7%, p >0.05). Grade III/IV adverse events occurred more frequently in the R-CHOP arm compared to the R-CVP arm (55.1 vs. 18.2%). Based on the results of this trial, the R-CVP regimen is preferentially used in PLRG centres for follicular lymphoma patients requiring therapy. In earlier years, PLRG centres participated also in studies involving patients with relapsed/refractory indolent lymphomas, including FL, treated either with four weekly doses of rituximab [36] or radioimmunotherapy [37].

Hodgkin's lymphoma (HL)

A new era in the treatment of Hodgkin's lymphoma (HL) in Poland started in the past decade with the use of positron emission tomography (PET/CT) for staging [38], interim and final response assessment. Centres allied to the PLRG took part in the validation study of the Deauville Scale, conducted a prospective trial assessing the role of very early interim PET (after one cycle) [39], and developed a new chemotherapy regimen composed of bendamustine, gemcitabine and dexamethasone (BGD) [40] for relapsed/refractory HL. BGD is now being assessed in a prospective study (BURGUND, EudraCT: 2017-001966-97) in patients with progressive disease after first line treatment. The PLRG, together with international partners, has proposed a personalised PET-adapted treatment of early non-bulky HL (eHL) using an innovative risk and response-adapted

strategy. The RAFTING study (EudraCT: 2020-002382-33) aims to assess the efficacy of standard treatment followed by nivolumab in high risk eHL patients defined by positive interim PET (iPET) and/or by high (>84 cm^3) initial metabolic tumour volume (MTV) and chemotherapy alone in low risk eHL (defined by both a low MTV and a negative iPET) and the rate of HL relapses in low-risk patients that could be salvaged with delayed radiotherapy and nivolumab maintenance.

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive B cell lymphoma subtype, and prognosis depends on the efficacy of first line therapy. Polish Lymphoma Research Group (PLRG) centres have participated in the most important multicentre studies establishing R-CHOP as the present standard of care (Prof. Jan Walewski), and protocols investigating the potential role of Bruton tyrosine kinase inhibitors and immunomodulating agents (Prof. Wojciech Jurczak). PLRG, in a national multicentre study, confirmed the role of R-CHOP in high risk DLBCL patients [41]. Cardiovascular toxicity of doxorubicin was addressed in several studies, confirming premature cardiovascular mortality [42], investigating the role of arterial hypertension [43], and the feasibility of cardioprotection [44]. Pre-existing diabetes was identified as an independent risk factor of adverse prognosis [45]. In a multicentre approach, PLRG investigated the efficacy of the PREBEN regimen (pixantrone, rituximab, etoposide, and bendamustine) in relapsing refractory cases [46].

Hairy cell leukemia

Hairy cell leukemia (HCL), a chronic lymphoproliferative disorder, responds well to treatment, and one course of cladribine (2-chlorodeoxyadenosine, 2-CdA) usually induces a durable CR. However, there are several administration schedules of this drug and no superiority has been shown of one schedule over the others. Robak et al. [47] demonstrated CR obtained in 75% of patients after 5-day intravenous infusions of 2-CdA, and in 76% after 7-day courses. Intermittent 2-hour infusions and continuous 24-hour infusions yielded CR in 82.6% and 66.7% of patients respectively. In another study, Robak et al. [48] compared the efficacy and toxicity of a standard 5-day 2-CdA protocol with a schedule of six weekly 2-CdA infusions. Neither the efficacy nor the toxicity profile was significantly different between the groups. In particular, CR was obtained in 76% of patients in the group of daily 2-CdA administration and in 72% in the weekly administration group. Both PFS and OS were similar in both groups. 2-CdA at 0.12 mg/kg in 2-hour i.v. infusion for five days; or alternatively 2-CdA at 0.12 mg/kg in 2-hour intravenous infusion once a week for six weeks are currently considered in Poland as the standard first line treatment for classical HCL.

Peripheral T cell lymphomas

Peripheral T-cell lymphomas (PTCL) are a heterogenous group of rare diseases that are challenging to treat. Patients with PTCL are treated with the CHOP or CHOP-like regimens used as an induction chemotherapy followed by autologous haematopoietic stem cell transplantation (auto-HCT) as a consolidation of first response. To expand the published experience, Czyż et al. [49] conducted a multicentre, retrospective review of 65 patients with PTCL who underwent auto-HCT as a consolidation of first response achieved with either initial induction chemotherapy or salvage chemotherapy. With the median follow-up of 53 months (range 7–157 months), the 5-year OS and PFS for all patients were 61.5% and 59.4%, respectively. Bone marrow involvement at diagnosis and less than partial remission after induction chemotherapy were factors independently predictive for OS and PFS. Maciejka-Kłembowska et al. published a data report from the Polish Paediatric Leukemia/Lymphoma Study Group on clinical features and treatment outcomes of PTCL in 10 children [50]. Different regimens, including CHOP and protocols for lymphoblastic lymphoma, were used. The 5-year OS and event-free survival rates were 63.9% and 81%, respectively. Three children underwent allogeneic HCT, and all of them remain alive and in complete remission.

Mantle cell lymphoma

The mantle cell lymphoma (MCL) treatment paradigm has evolved over recent years in Poland. The National Research Institute of Oncology joined the European Mantle Cell Lymphoma Network (EMCLN) and co-performed two landmark academic clinical trials for untreated MCL patients. In the 'younger' trial, a comparison of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) and autologous haematopoietic cell transplantation (auto-HCT) consolidation versus alternating cycles of R-CHOP and R-DHAP (rituximab, cisplatin, cytarabine, and dexamethasone) with auto-HCT showed improved time-to-treatment failure [median 3.9 vs. 9.1 years and 40% vs. 65% at 5 year (p =0.038)] for the treatment containing cytarabine [51].

The 'elderly' trial demonstrated that the recommended treatment in elderly patients is R-CHOP and rituximab maintenance [52, 53]. Median overall survival (OS) after R-CHOP was 6.4 versus 3.9 years after R-FC (rituximab, fludarabine, and cyclophosphamide) (p =0.0054). Patients responding to R-CHOP had median progression-free survival (PFS) and OS of 5.4 and 9.8 years when randomised to rituximab versus 1.9 (p <0.0001) and 7.1 years (p =0.0026) when randomised to interferon maintenance. Based on the

results of this study, rituximab maintenance after immunochemotherapy treatment became the standard procedure in elderly patients. The Polish Lymphoma Research Group (PLRG), as the EMCLN sponsor's delegate in Poland, is currently conducting two subsequent first line trials: TRIANGLE for younger and MCL-R2 for elderly patients.

Burkitt's lymphoma

A major improvement in the outcomes of adult Burkitt's lymphoma patients was achieved in Poland after implementing intensive chemotherapy CODOX-M/IVAC within the UKLG LY06 study [54]. Long-term survival increased from the previous less than 20% to close to 70%. Toxicity of treatment was substantial, with the rate of treatment-related death approaching 10%. Efficacy was further improved with the introduction of the GMALL-B-ALL/NHL-2002 protocol of short intensive chemotherapy combined with rituximab including high-dose methotrexate, cytarabine, and triple intrathecal therapy [55]. This largest prospective multicentre trial for adult patients with Burkitt's lymphoma/leukemia involved 363 patients aged 16 to 85 from 98 European centres including the Oncology Institute in Warsaw. The rate of complete remission was 88%, 5-year survival was 80%, and 5-year progression-free survival was 71%. There was no lethal toxicity. Given the high cure rates across a range of prognostic groups including age, IPI, and feasibility of immunochemotherapy in elderly patients, the GMALL-B-ALL/NHL-2002 protocol is now preferentially used in Poland.

Cutaneous lymphomas

The diagnosis and treatment of cutaneous lymphomas remains a major challenge [56]. Epigenetic dysregulation seems to play an important role in the development and progression of Sézary syndrome (SS). The *TMEM244* gene is ectopically expressed in SS patients, SS-derived cell lines, and, to a lesser extent, in MF. *TMEM244* expression is negatively correlated with the methylation level of its promoter. *TMEM244* expression can be activated *in vitro* by the CRISPR-dCas9-induced specific demethylation of *TMEM244* promoter region. Since both *TMEM244* expression and its promoter demethylation can be potentially used as markers in SS and some other T-cell lymphomas [57], *STAT5* but also *STAT6* and to a lesser extent *STAT3* seems to be constitutively activated in Cutaneous T-cell lymphomas (CTCL). Downregulation of STAT5b protein in advanced-stage CTCL appears to contribute to its pathogenesis. STATs seem to be a promising target for new effective therapeutic agents in CTCL [58]. WP1220 is a synthetic compound that potently inhibits p-STAT3 and the growth of CTCL cell lines. Topical treatment of index skin lesions in stage I–III MF have revealed safety and some efficacy in MF in the Phase 1b study performed at the Medical University of Gdańsk [59].

Multiple myeloma

The Polish Myeloma Study Group (PMSG) was established by Prof. Anna Dmoszyńska in cooperation with Prof. Maria Kraj in 2005. One of the original clinical protocols was the evaluation of the efficacy and safety of multiple myeloma (MM) patient therapy with the CTD regimen (cyclophosphamide, thalidomide, and dexamethasone) based on a low dose of thalidomide (100 mg/d) [60]. Other clinical and multicentre studies of the PMSG were focused on therapy with lenalidomide, pomalidomide and bortezomib as well as the identification of novel cytogenetic prognostic factors [61–66].

The most significant change in the field of multiple myeloma in Poland in recent years was the launch of several prospective clinical trials initiated by Polish investigators. On the one hand this provided the opportunity to play a significant role in the development of new treatment strategies worldwide, and on the other hand it provided access to non-reimbursed drugs to Polish patients. PMC006 (ATLAS), a study that the Polish Myeloma Consortium launched in 2017, was the first non-commercial investigator-initiated clinical study in Poland; it completed the recruitment of its planned 160 patients in September 2020. This study assessed two different methods of maintenance after auto-PBSCT: R versus KRD.

There are three other important studies ongoing: the PMC007 (OBI1), which assesses the efficacy of obinutuzumab in patients with refractory or recurrent Waldenstroem macroglobulinemia; the PMC008 (PREDATOR), where daratumumab is used to treat patients with biochemical relapse of myeloma; and the PMC010 (COBRA), which assesses the effectiveness of KRD versus RVD in patients with newly diagnosed multiple myeloma. The Polish Myeloma Study Group and the Polish Myeloma Consortium are also active in the development of the MM Registry. Therefore, the prospective observational study PMC009 (POMOST) was launched. In this trial, the treatment conditions and epidemiology of myeloma in Poland are prospectively assessed on a population of 1,500 patients. PMG and PMC also took part, together with the Łazarski University and the National Health Fund, in a pilot project to analyse the epidemiology and therapy of myeloma based on the sources of the NFZ [67]. PMSG regularly publishes recommendations for the diagnosis and treatment of MM. Several retrospective nationwide or international studies have been performed under the auspices of PMSG, including MM patients with CNS involvement [68], primary and secondary plasma cell leukemia [69, 70], primary refractory disease [71], t(14;16) [72], and biclonal MM [73]. As a result, it has been shown that the outcomes of advanced stage MM

patients aged 21–40 are comparable to those of patients aged 41–60 [74].

Autologous haematopoietic cell transplantation

In 2019, 1,162 auto-HCTs (306 per 10 million inhabitants) were performed in Poland. Throughout the past decade, work has been underway aimed at the introduction and optimisation of a haematopoietic stem cell chemo-mobilisation protocol with the use of intermediate-dose cytarabine (ID-Ara-C) which is currently commonly used in Polish centres. ID-Ara-C refers to: cytarabine as a i.v. infusion at a dose of 0.4 g/m^2 twice daily on days +1 and +2 (total dose, 1.6 g/m^2), with 10 μg/kg filgrastim started on day +5. In a pilot report, its efficacy was shown when given as a second-line salvage mobilisation regimen. In a subsequent retrospective analysis, the greater benefit of ID-Ara-C compared to 4 g/m^2 cyclophosphamide as first-line mobilisation in patients with myeloma and lymphoma was demonstrated [75]. In a multicentre analysis from the PLRG, the superior efficacy of ID-Ara-C over DHAP plus G-CSF regimen in lymphoma patients was noted [76]. Finally, in a randomised trial, ID-Ara-C was shown to be superior to G-CSF alone for myeloma patients in terms of a greater proportion of patients achieving a $CD34^+$ cell yield sufficient for tandem auto-HCT (98% vs. 70%), a higher median number of collected $CD34^+$ cells (20.2 vs. 5.9 ×10^6 cells/kg), and faster haematopoietic recovery after auto-HCT [77].

Allogeneic haematopoietic cell transplantation

Six hundred and eighty-six allogeneic hematopoietic stem cell transplantations (allo-HCT) were performed in Poland in 2019 in 17 centres (12 adult and five paediatric). They included 185 transplantations from family members, 423 from unrelated people, and 78 from haploidentical donors. Since 2013, local Polish donors have predominated among unrelated donors (63% in 2019). The Polish unrelated donor registry is one of the biggest in the world, accounting for more than 1.7 million volunteers. The most frequent indication for allo-HCT was acute myeloid leukemia (32% of procedures from family, 36% from unrelated, and 49% from haploidentical), followed by acute lymphoblastic leukemia (21% from family and 20% from unrelated). Poland has been a very active member of the European Society for Blood and Marrow Transplantation (EBMT). In particular, two Polish investigators have been elected chairs of working parties and members of the EBMT's Scientific Council: Prof. Jan Styczyński (Infectious Diseases Working Party, 2016–2020) and Prof. Grzegorz Basak (Transplant Complications Working Party, 2017–2021). In addition, Prof. Sebastian Giebel since 2006 has been ALL subcommittee chair and secretary of the Acute Leukemia Working Party. Polish authors have substantially contributed to understanding the role of NK cell alloreactivity after allo-HCT [78], genetic polymorphisms contributing to GVHD [79], the role of pharmaco-economics [80], and the management of post-transplant lymphoproliferative disease (PTLD) [81], as well as having developed a number of international recommendations including treatment and outcome of viral infections following allo-HCT [82] and GVHD [83].

Infectious complications

Infectious complications constitute the major cause of morbidity and mortality in haematology and transplant patients. Cooperation between the Infection Disease Study Group of PALG with the Polish paediatric group PSPOH and the Infectious Diseases Working Party of EBMT has resulted in a number of publications. Polish epidemiological analysis on stem cell transplant recipients performed by PALG and PTHIT together with the Polish Society of Paediatric Oncology and Haematology (PTOHD, PSPOH) in a nationwide study revealed a high incidence of bacterial infections in children compared to adults (36% vs. 27.6%; p <0.0001), although with a substantial rate of Gram-negative bacteria in adults. Fungal infections were seen also more often in children (25.3% vs. 6.3%; p <0.0001), as well as viral infections (88.0% vs. 74.9%; p <0.0001). Infection-related mortality was lower in children than in adults (7.8% vs. 18.4%; p <0.0001) [84]. Sub-analysis of MM patients confirmed the importance of multidrug bacterial infections (37.5% of Gram-negative; 54% of Gram-positive) during neutropenia after HCT, influencing mortality [85]. Important data regarding mucormycosis in haematology and transplant settings was published recently, based on 10-year observations. Analysis revealed high mortality (82%) in patients undergoing allo-HCT, despite targeted therapy [86]. The treatment of infectious complications in haematology/transplant patients in Poland is based on systematically updated national recommendations. Recent guidance concerns vaccination in adult patients with haematological malignancies and after stem cell transplantation [87–89], the management of invasive fungal infections [90], the management of CMV infections after allo-HCT [91], and the management of infections after CAR T therapy [92].

Haematopathology

The National Histopathological Lymphoma Registry project (NHLR) was implemented in Poland in 2014 by haematopathologists from 24 pathology departments in accordance with the 2008 WHO Classification of Tumours of Haematopoietic and Lymphatic Tissues. DLBCL (32.9%; 2,587), CLL/SLL (31.84%; 2,504), HL (13.37; 1,567), PCM (13.32%, 1,561) and MCL (9.04%; 711) were the most frequent

in a group of 11,718 tested patients. Major differences between NHLR and European and American data on lymphoma subtypes included a higher incidence of DLBCL, and lower incidences of FL and MALT [93]. The next study, based on 2000–2014 data from the Polish National Cancer Registry, confirmed a lower FL incidence rate in Poland compared to other European countries. FL was ranked fourth in incidence (CR $0.72/10^5$, SR $0.87/10^5$) among all reported mature B-cell non-Hodgkin lymphomas, after CLL//SLL (CR $3.62/10^5$, SR $4.99/10^5$), PCM (CR $3.78/10^5$, SR $4.97/10^5$) and DLBCL, NOS (CR $2.13/10^5$, SR $2.65/10^5$) [94]. A scheme of comprehensive haematopathological diagnostics of aggressive B-cell lymphomas based on morphology, immune profile and evaluation of *MYC*, *BCL2*, and *BCL6* gene statuses was proposed, and this was introduced in Poland after the publication of revised WHO classifications in 2017 [95].

Molecular diagnostics

During the last ten years, progress has been made in the molecular diagnostics of haematological malignancies in Poland in terms of the spectrum of techniques used, as well as its standardisation. In 2010, the standardisation of *BCR-ABL1* measurement by RQ-PCR in CML patients was carried out in eight laboratories in cooperation with the ELN [14]. The results of the *BCR-ABL1* KD mutation analysis in imatinib-resistant patients were also published [96]. The RQ-PCR technique was improved by modification of the e13a2 and e14a2 transcript measurement in 2019 [97]. Similar progress has been made in the Ph-negative MPN. In 2015, the first results of the molecular characteristics of Polish patients were published [16]. The quality of *JAK2 V617F* quantification was assessed in an international study [98]. A comparison of qPCR and ddPCR sensitivity in the quantification of the *JAK2 V617F* mutation allele burden was performed in 2019 [99]. Further progress was made thanks to the introduction of GEP and NGS to myeloid malignancies diagnostics and MRD assessment [100–102]. A development has also been made in the CLL thanks to the cooperation with ERIC (European Research Initiative on CLL) and the creation of a reference laboratories network in Poland.

Summary

Polish haemato-oncology in the 2020s has an important international position based on many contributions to the development of knowledge, cooperation, and a high quality of patient care. In many instances, clinical trials run by Polish collaborative groups have influenced international standards. Polish haematologists have been the authors of many treatment recommendations, and their research has suggested areas for further research.

Authors' contributions

SG, JS, LG — design of study. All authors — collection of data and manuscript writing, critical revision and approval.

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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REVIEW ARTICLE

VIA MEDICA

Clinical implications of cytogenetic and molecular aberrations in multiple myeloma

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Abstract

Multiple myeloma (MM) is an incurable haematological malignancy affecting approximately 7:100,000 people. Monoclonal gammopathy of undetermined significance (MGUS) and 'smouldering' MM precede symptomatic MM. Cytogenetics in MM is the most powerful prognostication tool incorporated into different classifications, including the Revised International Staging System (R-ISS) and the Mayo Clinic Risk Stratification for Multiple Myeloma (mSMART). Methods commonly used to test for cytogenetic aberrations include conventional karyotyping and fluorescence *in situ* hybridisation (FISH), although the difficulty of obtaining metaphases in plasma cells results in low yields.

Therefore, new genomic tools are essential to explore the complex landscape of genetic alterations in MM. These include next generation sequencing, a highly sensitive method to monitor minimal residual disease. The serial evolution of MGUS to MM is accompanied by a range of heterogenous genetic abnormalities, divided into primary (involving mostly chromosome 14 translocations and trisomies) and secondary genetic aberration events (involving mostly 17p, 1p, 13q deletions, 1q gain, or MYC translocations). Based on the primary genetic aberration results, strong prognostic features of MM have been identified with distinct clinical characteristics. High risk aberrations include 17p deletion, t(4;14), t(14;16), t(14;20) and chromosome 1 abnormalities. The incorporation of novel drugs and maintenance strategies in conjunction with autologous stem cell transplantation partially overcome the adverse effect of some of these genetic aberrations. Nonetheless, survival remains worse in this group compared to standard risk patients. Clinical decisions regarding treatment should be based on the cytogenetic results. The establishment of individualised and mutation-targeted therapies are of the greatest importance in future studies.

Key words: myeloma, cytogenetics, prognosis, high risk, genomics, aberration

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Introduction

Multiple myeloma (MM) is a neoplastic plasma cell disorder which affects approximately seven in every 100,000 people, giving it the second highest incidence among all haematological malignancies in the Western world [1–3]. MM is preceded by a pre-malignant stage called monoclonal gammopathy of undetermined significance (MGUS), which is present in over 3% of the population >50-years-old, and the incidence increases with age [4]. The risk of progression of MGUS to MM is approximately 1% per year depending on prognostic features (e.g. paraprotein concentration, immunoglobulin isotype and free light chain ratio) [5]. While MGUS is a 'benign precursor state' which is devoid of any myeloma defining events (MDE), MM can cause severe symptoms and end-stage organ damage including renal insufficiency (present in 20% of newly diagnosed patients), anaemia (haemoglobin <12 g/dL present in 73% of patients), skeletal lesions (present in approximately 65–75% of patients) or hypercalcemia (calcium level >11 mg/dL present in 13% of patients) [6]. According to the updated International Myeloma Working

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Table I. For diagnosis of multiple myeloma, the criterium of clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma should be met, and any one or more of the following myeloma-defining events (SLiM CRAB) should be present

Calcium	Hypercalcemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than upper limit of normal or >2.75 mmol/L (>11 mg/dL)
Renal Insufficiency	Renal insufficiency: creatinine clearance <40 mL per minute or serum creatinine >177 μmol/L (>2 mg/dL)
Anaemia	Anaemia: haemoglobin value of >2 g/dL below lower limit of normal, or haemoglobin value <10 g/dL
Bones	Bone lesions: one or more osteolytic lesions on skeletal radiography, computed tomography (CT), or positron emission tomography-CT (PET-CT)
Sixty	Clonal bone marrow plasma cell percentage ≥60%
Light chains	Involved: uninvolved serum free light chain (FLC) ratio ≥100 (involved free light chain level must be ≥100 mg/L)
Magnetic resonance	>1 focal lesion on magnetic resonance imaging (MRI) studies (at least 5 mm in size)

Group (IMWG) criteria, the diagnosis requires ≥10% clonal bone marrow plasma cells or a biopsy-proven plasmacytoma plus evidence of one or more MDE included in the SLiM-CRAB acronym (Table I) [7]. However, unlike other haematological malignancies, no specific cytogenetic pathognomonic criterium for MM exists.

The disease is considered incurable, and survival may vary from a few months to over 15 years; this diversity is most strongly driven by the genetic abnormalities present in the plasma cells [8].

In the era of modern drugs including proteasome inhibitors (PIs) (bortezomib/carfilzomib/ixazomib), immunomodulatory drugs (IMIDs) (thalidomide/lenalidomide/pomalidomide), monoclonal antibodies (daratumumab/isatuximab//elotuzumab), and antibody drug conjugates (belantamab mafodotin), median overall survival reaches 6+ years [9].

Several features affect survival: host characteristics, tumour burden and biology (cytogenetics) as well as the response to treatment. Laboratory risk factors for a poorer prognosis have been identified, including serum beta$_2$-microglobulin (β2M), low albumin and/or elevated lactate dehydrogenase. However, genetic changes seem to play the largest role in prognosis in MM [10].

Studying myeloma genetics has been always limited due to low proliferation potential of the plasma cells for standard karyotype analysis. Nonetheless, in recent years the development of genetic tools including microarrays and next-generation sequencing (NGS) has led to substantial progress in understanding the genetics of MM [11].

MM remains a highly heterogenous and complex disease with varied genetic aberrations resulting in multiple subclones. A major clone dominates throughout the disease course, although minor clones may evolve and be responsible for chemoresistance or serve as a reservoir for relapses or progressions [11, 12]. Interestingly, in a recent study by Merz et al., the presence of subclones was prognostic for smouldering MM to transform into MM; however, the risk of progression was not constant and changed due to clonal evolution [13]. Nonetheless, the mechanism and driving factors resulting in the development of how these subclones emerge, how they are selected and what is their prognostic implication, need to be investigated in future studies.

In general, the pathogenesis of MM can be visualised by two steps: the establishment of MGUS and progression from MGUS to MM (Figure 1) [14]. This process is accompanied by different cytogenetic changes (Table II). The evolution of MGUS is thought to be a result of abnormal antigen stimulation and is caused by primary cytogenetic aberration events, so-called 'disease-initiating' aberrations. The primary cytogenetic abnormalities help to classify MGUS and MM into several subtypes and consist mostly of trisomies and translocations involving chromosome 14 [5]. Additionally, primary cytogenetic abnormalities are mostly non-overlapping [15]. The second step occurs rather due to random cytogenetic mutations (called secondary cytogenetic aberration events) resulting in the evolution to symptomatic MM and further MM progression [16]. Secondary cytogenetic aberrations might overlap, and are typically subclonal (Figure 1) [5].

This review provides an overview of the different cytogenetic approaches used to stratify MM patients. We will discuss cytogenetic and molecular aberrations, their frequency, and clinical and prognostic implications.

Laboratory testing strategy

Conventional cytogenetics (G-band karyotyping)

Conventional cytogenetics is a well-established and universally available test, and it also provides a whole genome analysis at once. On the other hand, the detection of chromosomal abnormalities using conventional cytogenetics is limited due to low proliferative activity of malignant plasma cells and low number of plasma cells in an often haemodiluted bone marrow aspirate. Chromosomal abnormalities are detected in only 30% of patients without CD138$^+$ cell enrichment [17]. In plasma cell leukemia, cytogenetic aberrations are more common (up to 68% of cases) [18]. Despite this limitation, karyotyping provides essential

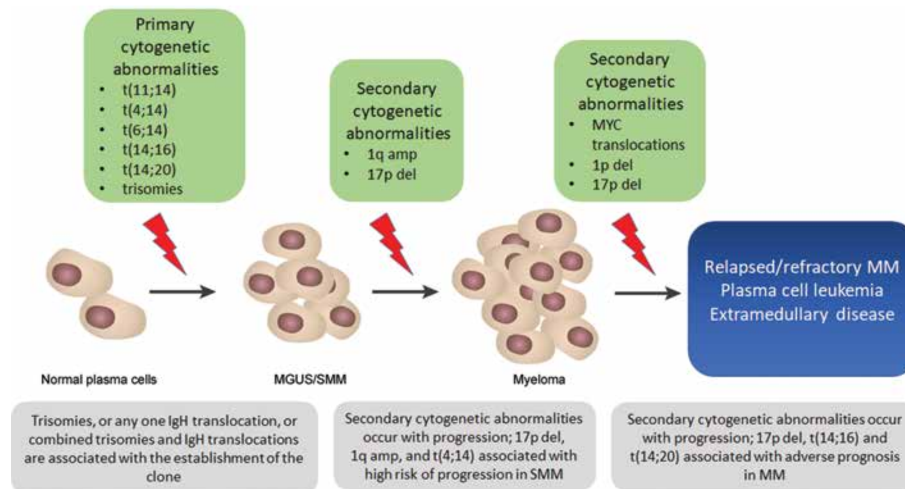

Figure 1. Cytogenetic abnormalities in multiple myeloma (MM) (modified from [14]); amp — amplification; del — deletion; IgH — immunoglobulin heavy chain; MGUS — monoclonal gammopathy of undetermined significance; SMM — smoldering multiple myeloma; t — translocation

Table II. Genetic abnormalities in multiple myeloma, their frequency and clinical impact

Cytogenetic abnormalities		Frequency [%]	Clinical impact, additional characteristics
Primary	t(4;14)(p16;q32)	10–15	Adverse, good response to proteasome inhibitors, unfavourable for any immunomodulatory drug
	t(6;14)(p21;q32)	2	Neutral
	t(11;14)(q13;q32)	15–20	Neutral, sensitive to venetoclax
	t(14;16)(q32;q23)	2–3	Adverse
	t(14;20)(q32;q12)	1	Adverse
	Hypodiploidy	13–20	Adverse
	Hyperdiploidy	50	Favourable, good response to lenalidomide in patients with trisomies
Secondary	13q deletion	45–50	Adverse
	MYC translocation	15–20	Neutral or adverse
	1q21 gain	35–40	Adverse, might be implicated in bortezomib resistance
	1p32 deletion	30	Adverse
	17p deletion	At diagnosis <10, at relapses >30%	Adverse, possible central nervous system involvement, pomalidomide seems to be beneficial

information on numerical and structural chromosomal changes. Numerical aberrations include hyperdiploidy or non-hyperdiploidy, whereas structural abnormalities include translocations or copy number aberrations (i.e. deletions or gains). Furthermore, conventional karyotyping is an independent prognostic factor even in the era of modern testing modalities [e.g. fluorescence *in situ* hybridisation (FISH), NGS, and gene-expression profiling (GEP)], and should be routinely performed as an initial diagnostic workup especially in situations where a FISH test is unavailable [19, 20].

FISH analysis

FISH testing is based on the use of fluorescent probes that bind to highly complementary nucleic acid sequence. Using a variety of FISH probes, numerous genetic alterations might be detected, including numerical amplifications or deletions, structural rearrangements and translocations [21]. On conventional cytogenetics, in patients with complex karyotype, some aberrations (e.g. translocations) are cryptic, and FISH enables their detection [22]. Furthermore, a FISH test does not depend on the proliferation rate, and the probes can be applied on nondividing cells, mainly on interphase cells [21]. However, the detection sensitivity is limited by the number of plasma cells in the whole bone marrow, which is especially low in patients after treatment or early in the diagnosis or relapse (when plasma cells range from 1–20%) [23]. Consequently, FISH is run mainly on CD138^{+} enriched cells, and different techniques are used to target and enrich the monoclonal plasma cells, e.g. immunostaining (cytoplasmic immunoglobulin FISH, cIg-FISH),

Table III. Staging strategies in multiple myeloma according to different working groups

Staging system	Stage	Criteria
International Staging System (ISS)	Stage I	β2M <3.5 mg/dL and albumin ≥3.5 g/dL
	Stage II	Neither I nor III
	Stage III	β2M ≥5.5 mg/dL
Revised International Staging System (R-ISS)	Stage I	ISS I, standard risk by FISH (no high-risk chromosomal abnormalities) and normal LDH
	Stage II	Neither R-ISS I nor III
	Stage III	ISS III, either high risk by FISH [del(17p) and/or t(4;14) and/or t(14;16)] or high LDH (> upper limit of normal)
Mayo Clinic Risk Stratification for Multiple Myeloma (mSMART)	Standard risk	Trisomies
		t(11;14)
		t(6;14)
	High risk	t(4;14)
		t(14:16)
		t(14;20)
		17p deletion
		1q gain
		R-ISS stage III
		High plasma cell S-phase (cut-off varies)
		GEP: high risk signature
		Double-hit: any two high-risk factors
		Triple-hit: any three or more high-risk factors
Gene-expression-based signatures		Presence of alterations detected by: UAMS, Skyline 92–HOVON, IFM

β2M — $beta_2$ microglobulin; FISH — fluorescence *in situ* hybridisation; GEP — gene expression profiling; LDH — lactate dehydrogenase

selection by magnetic cell sorting (MACS), fluorescence-activated cell sorting (FACS), as well as targeted manual scoring or customised automated image analysis [24].

The European Myeloma Network (EMN) guidelines recommend that at least 100 cells should be scored when analysing FISH [23]. The cut-off value for positivity remains a controversial issue and varies from laboratory to laboratory; there is no uniform criterion. It is related to the testing strategy and the various ways to establish cut-off for different FISH patterns in different laboratories [25, 26]. It is recommended by the EMN to use conservative cut-off levels: 10% for fusion or break-apart probes and 20% for numerical abnormalities [23], although in clinical practice the threshold for a positive test is very often much lower.

Molecular methods

Although not used in daily clinical practice outside clinical trials, advanced oncogenomic analyses are very important tools to understand the complex genetic landscape, and hence clinical outcomes, of MM patients. Different genomic analysis methods exist to research into the various processes of translating the genomic information. The most comprehensive test, NGS, is a process that allows the determination of the sequence of nucleotides in a section of DNA. This provides a full, integrated spectrum of gene mutations, aneuploidies, segmental copy-number changes and translocations. Utilising NGS has identified several recurrent gene mutations in newly diagnosed MM patients, including the most common mitogen activated protein kinase (MAPK) pathway, NF-κB pathway and TP53 pathway genes [27]. Although NGS is not yet universally available, in the future it will be an important tool in the diagnosis and monitoring of the minimal residual disease (MRD) due to its high sensitivity [28]. Of note, large sequencing studies in MM are being conducted to determine the relevance of different mutations, and preliminary results are promising (CoMMpass, The Myeloma Genome Project) [29, 30]. The GEP analyses the RNA expression of different genes pertinent to different functions [31]. It is an important marker to detect high risk patients (Table III) [32], and it also helps to establish classifiers for prognostication in addition to the International Staging System (ISS) or FISH known high risk factors [33]. Several genes lists have been found to have strong prognostic information, including commercially available GEP70 and SKY92 profiles [32, 34]. A single-nucleotide polymorphism (SNP)-microarray is a technique of the hybridisation of fragmented single-stranded DNA to arrays containing hundreds of thousands of unique nucleotide probe sequences. SNP is the smallest genetic variation that can occur within a DNA sequence. SNP microarray can detect small copy number changes such as gene deletion, chromothripsis, complex copy number changes, and amplification, as well as copy neutral loss of heterozygosity (CN-LOH), which is an important oncogenic event [25]. It also detects aberrations in small populations (subclones) due to clonal evolution [25], of which the most common are complex MYC 8q24 rearrangements or amplifications, 1q gain, 1p and 17p deletions [35, 36].

Using the genomic tools, several molecular classifications have been established in MM. They have been also incorporated into well-known staging systems to more precisely estimate an individual patient's disease risk and clinical outcome. Incorporating the novel molecular methods into clinical use and establishing personalised therapies based on the molecular findings will be of the utmost urgency.

Cytogenetic abnormalities

Primary cytogenetic abnormalities

Hyperdiploidy

Hyperdiploidy, commonly involving gain of odd-numbered chromosomes (trisomies), is detected in approximately 50% of patients with MM [5, 37]. Trisomies 3 and 5 are known to improve overall survival, but trisomy 21 impairs overall survival [38]. Patients with trisomies have particularly good response to IMIDs [39]. In approximately 10% of patients, trisomies and IgH translocations coexist [37]. The prognostic impact of this coexistence is a matter of controversy. Kumar et al. reported the presence of trisomies in patients with high risk translocations, namely t(4;14), t(14;16), t(14;20), or TP53 deletion, treated with modern drugs (PI and IMiD- based regimens). The adverse impact associated with these prognostic markers was partially ameliorated [37]. In contrast, Pawlyn et al. showed that in high risk cytogenetic patients coexistent hyperdiploidy did not abrogate a poor prognosis, although the patients were treated with older conventional drugs (cyclophosphamide with thalidomide and dexamethasone, or with vincristine) [40]. Hyperdiploidy is often accompanied by other structural chromosome changes (e.g. translocations, deletions or duplications) and a hyperdiploid karyotype with ≥2 of those aberrations should be considered as an independent high risk factor [41].

Non-hyperdiploidy

Non-hyperdiploidy is defined as hypodiploid, pseudodiploid, and near-tetraploid karyotypes. Hypodiploidy is defined as having ≤44 chromosomes in a cell, pseudodiploidy as having 45–46 chromosomes in a cell, and near-tetraploid karyotype originates from doubling of the hypodiploid and pseudodiploid karyotypes (>75 chromosomes) [42]. Hypodiploidy was an important independent factor for worse overall survival in multivariate analysis that included Durie and Salmon stage, treatment or bone marrow plasmocytosis [43]. In the non-hyperdiploid population, hypodiploid cases are associated with a higher prevalence of genetic alterations and a worse prognosis [42].

IGH translocations

IGH translocations, with a breakpoint on chromosome 14 band q32, are often present in tumours of the lymphoid lineage due to the common physiological DNA rearrangements at the immunoglobulin heavy chain (IGH) *locus* that might have been displayed incorrectly [44]. The aberration in IGH in MM occurs mostly during class switching, and the juxtaposition of an oncogene next to the IGH *locus* results in overexpression of the affected oncogene [45]. In MM, the IGH translocations occur in up to 60% of patients [15, 46]. There are five main translocation partner chromosomes including the t(4;14)(p16;q32) — multiple myeloma set domain (MMSET), t(6;14)(p21;q32) — cyclin D3 gene, t(11;14)(q13;q32) — cyclin D1 gene, t(14;16)(q32;q23) — musculoaponeurotic fibrosarcoma (C-MAF), and t(14;20)(q32;q12) — musculoaponeurotic fibrosarcoma oncology family, protein B (MAFB) [45]. Moreover, the recurrent IGH translocations (mostly with partner chromosomes 11, 4, and 16) are highly associated with nonhyperdiploid karyotype [47].

t(4;14)(p16;q32)

Translocation t(4;14), present in 10–15% of newly diagnosed cases, is not detectable by conventional karyotyping [25, 48, 49]. It is associated with poor prognosis and is considered to be a high-risk prognostic factor [48, 49]. Translocation t(4;14) is associated with immature morphology, higher tumour mass, and more frequent chromosome 13 abnormalities [50]. It is also reported to be less frequently associated with bone lesions, which consequently might be associated with delayed diagnosis [51]. Patients with t(4;14) seem to benefit from bortezomib-based therapy and bortezomib maintenance, and lenalidomide maintenance also might provide better survival in this group of patients [52, 53, 54]. It is also recommended to implement autologous stem cell transplantation (ASCT) as soon as possible in this cytogenetic subtype, and tandem ASCT should be considered [5]. Moreover, data from the MM-003 trial shows that pomalidomide plus low-dose dexamethasone is an effective strategy in relapsed/refractory settings in patients with t(4;14) [55].

t(11;14)(q13;q32)

The most common IGH translocation, accounting for 15–20% of cases, is t(11;14) [48, 49, 56]. This translocation results in upregulation and overexpression of the cyclin D1 that might be detected by immunohistochemistry [57]. Translocation t(11;14) is more prevalent in plasma cell leukemia patients as well as in light chain amyloidosis patients than in MM patients [58, 59, 60]. Although the prognostic impact is considered to be standard, some studies suggest that the overall survival and response rates are inferior to other standard risk patients [61, 62]. Translocation t(11;14) is more frequent among the African-American population and exerts negative influence on the survival in African-Americans compared to non-African-Americans [63, 64]. It is associated with lymphoplasmacytic morphology and frequently with low serum monoclonal protein or nonsecretory MM, and it is also less likely to coexist with hyperdiploid karyotype [50, 57]. In t(11;14) the expression of CD20 is often present [65]. The Mayo Clinic experts recommend the use of combined bortezomib and lenalidomide regimens followed by early ASCT and lenalidomide maintenance in t(11;14) patients [5]. The t(11;14) is associated with high expression of BCL2 protein and several studies have suggested the efficacy of the use of venetoclax, the BCL2 inhibitor, in t(11;14) positive patients.

t(14;16)(q32;q23)

The more common MAF translocation, t(14;16), present in 2–5% of newly diagnosed cases, is hardly detectable by conventional karyotyping [25, 49, 56]. Translocation t(14;16) is associated with adverse outcomes, even in the era of novel drugs, something recently confirmed by a large international sample study conducted by our group [49, 66, 67]. Renal failure as a MDE is more frequently observed in patients with t(14;16) than in other cytogenetic subtypes. It is associated with high levels of free light chain in serum, which might partially explain the poorer prognosis in t(14;16) positive group of patients [51, 68]. It has been observed that t(14;16) is associated with negativity in CD56 expression and high proliferative activity, which might predispose toward an adverse outcome [69]. According to the Mayo Clinic experts, the treatment approach should be similar as in t(4;14), and in patients with renal failure it is recommended to combine bortezomib with cyclophosphamide, thalidomide or adriamycin over lenalidomide [5]. Our study suggests that at least a three-drug induction regimen (including IMID and PI) should be used, ASCT should be performed wherever possible [67, 68], and tandem ASCT should be considered [5]. Maintenance should be based on bortezomib [5].

t(14;20)(q32;q12)

The second MAF translocation, t(14;20), has a low prevalence, 1%, and also exhibits adverse impact on outcomes, comparable to t(14;16) [56, 70, 71]. Translocation t(14;20) is also associated with higher frequency of renal failure due to high levels of free light chains in serum [51, 68]. Treatment based on PI should be used in an induction therapy [68]. Double ASCT should be considered as an upfront treatment strategy in this group of patients [70], and maintenance should include bortezomib [5].

Secondary cytogenetic abnormalities

MYC translocation

MYC translocations are secondary events and occur at late stages of tumour progression. They are present in 15–20% of patients [48, 72]. Most MYC translocations involve IGH *locus*, however other genes might be also involved (*IGL, IGK, FAM46C, FOXO3* or *BMP6*) [56]. As a result of the juxtaposition, the expression of c-MYC is increased [73]. The impact of MYC translocation on survival is believed to be negative, although it is still a matter of debate [73, 74]. It has been suggested that the negative impact on outcome of MYC is restricted to hyperdiploid MM and is caused by an interaction between t(MYC) and gain of 1q21 [72].

1q gain

1q gain is one of the most common aberrations in MM, present in approximately 30% of patients [75]. It is associated with end-organ damage and a higher tumour burden [75]. It has been shown that 1q gain is associated with the evolution from MGUS to MM, and the copy number of the 1q gain increases with disease progression [76]. Although the prognostic impact is a contentious issue, recent studies suggest that 1q gain has a strong, independent negative impact on survival, even in the era of modern drugs and ASCT [75, 77]. Although neither IMID- and PI-based treatment regimens nor ASCT seem to prolong survival in patients with 1q gain, a recent Mayo Clinic study suggested that PI-based strategies might show a slightly better effect on outcomes [75]. Nonetheless, another study showed that with increasing number of copies of 1q gain, bortezomib resistance also increases [78].

1p deletion

1p deletion is present in approximately 20% of patients and has an adverse impact on outcomes [79, 80]. These patients might need more intensive treatment, similar to that for other high-risk MM subtypes [5, 80].

13q deletion

13q deletion is present in 45–50% of patients with MM [48]. The 13q deletion often coexists with other cytogenetic abnormalities, and the presence and time of occurrence of 13q deletion depends on the presence of specific concurrent abnormalities [81]. 13q deletion detected by conventional karyotyping appears to predict poor outcomes (present in 10–15% of newly diagnosed patients) [82]. It has been shown that bortezomib might overcome the adverse impact of 13q deletion [83].

17p deletion

17p deletion (associated with loss of the *TP53* gene) is present in 10% of newly diagnosed patients, and the incidence increases with disease progression [48, 56]. It is present in the majority of cases of plasma cell leukemia [84]. 17p deletion is considered to be the most detrimental prognostic factor of high-risk disease and a poor outcome [66, 71]. The size of clone carrying the abnormality seems to be significant for the prognosis, although a recent study found contradictory results [85]. The bi-allelic inactivation of TP53 has an additional negative impact on survival [86]. TP53 deletions are associated with more aggressive disease course and complications including plasmocytomas and hypercalcemia [49]. The studies on the influence of different treatment strategies are inconsistent. BMT CTN 0702 trial results showed no survival benefit of either tandem ASCT or intensive treatment that included ASCT with a subsequent four cycles of bortezomib and lenalidomide followed by lenalidomide maintenance in patients with 17p deletion [87]. Moreover, Lakshman et al. showed that a deletion 17p positive patient did not benefit from IMID plus PI-based induction as well as early ASCT [85]. On the other hand, according

to the recent study based on the EMN02/HO95 trial, patients with 17p deletion were benefiting from intensive treatment including tandem ASCT and bortezomib-based induction therapy [88].

Despite all these differences, recommendations suggest using induction therapy based on IMIDs and PI (bortezomib or carfilzomib) and subsequently considering tandem ASCT and prolonged bortezomib maintenance [71, 89]. Of note, pomalidomide seems to be particularly active in 17p deletion [90].

Specific mutations

In recent years, the genomic landscape of MM has been thoroughly studied, and several recurrent mutations identified, most of them involving genes implicated in the translocation of chromosome 14 or MAPK pathway, critical in cell growth and survival [91]. Some of them are related to the cytogenetic MM subtype, e.g. *FGFR3* or *PRKD2* genes mutations occur mainly in t(4;14) positive patients; *CCND1, KRAS* and *IRF4* genes mutations are detected in t(11;14) positive patients or *TP53* gene mutation is present in 17p deletion positive patients [5]. The *MAPK* genes mutations are represented by *KRAS* and *NRAS*, present in 40% of patients as well as *BRAF* genes mutations present in approximately 4% of patients [92]. Patients with mutations in *NRAS* or *KRAS* have worse survival than those with wild-type *RAS* genes [93]. It has been shown that patients with *NRAS* mutation exhibit reduced sensitivity to bortezomib [94]. For the MAP kinase pathway, several mutation-specific drugs are tested, e.g. vemurafenib in BRAF positive tumours or trametinib in AKT mutation [5].

Double/triple hit MM

High risk cytogenetic aberrations, especially secondary genetic aberration events, often overlap. For example, in a study by Boyd et al. in a group of patients with adverse IGH translocation [t(4;14), t(14;16) or t(14;20)], almost 72% had additionally 1q gain, and 12.4% had 17p deletion [95]. To address the prognosis of patients with multiple adverse cytogenetic aberrations, the mSMART classification includes the concepts of 'double hit' (when any two high risk factors are present) and 'triple hit' (when any three high risk factors are present) as a high risk stage with poor prognosis (Table III) [7]. A recent study showed the predictive value of double- and triple-hit MM, with double-hit MM having OS of 6 months vs. 32 months for patients with one high risk factor, and 57 months for patients with no high risk factor [96].

Staging approaches

The most universally accepted staging systems are set out in Table III. The Mayo Clinic recommends both conventional cytogenetic and FISH tests, and FISH is preferred if both are not available. All patients should be stratified and classified into standard- or high-risk groups using the mSMART criteria (Table III) and the FISH set should include detecting at least t(11;14), t(4;14), t(14;16), t(6;14), t(14;20), trisomies, and 17p deletion [71, 97]. The EMN recommends performing FISH after CD138$^+$ plasma cell enrichment, and the analysis should include at least t(4;14) and 17p deletion; analysis of t(14;16), 1q gain and 1p deletion [98]. The National Comprehensive Cancer Network (NCCN) recommends FISH panel on bone marrow which includes 13 deletion, 17p deletion, t(4;14), t(11;14), t(14;16), t(14:20), 1q amplification and 1p deletion as an initial work-up [99]. According to the IMWG, the essential testing should include either cIg-FISH or FISH carried out on the nuclei from purified plasma cells. The minimum panel required for prognostic estimation should include t(4;14), t(14;16) and 17p deletions. A more comprehensive panel should include testing for t(11;14), 13 deletion, ploidy category and chromosome 1 abnormalities [100]. The Polish guidelines for the basic evaluation of cytogenetic prognosis suggest stepwise FISH testing: the first step includes *TP53* gene and *IGH* gene. If the IGH rearrangement is present, gene *FGFR3*/t(4;14) should be verified. If there is no *FGFR3* fusion, further analysis should include *MAF*/t(14;16) gene status. Extended testing should include additionally t(14;16), t(14;20), chromosome 1 status, t(11;14), *MYC* rearrangement, 13 deletion and the 5, 9 and 15 chromosome aberrations. Karyotyping is an optional study according to the Polish experts' recommendations [101].

It should be highlighted that the risk factors described for newly diagnosed patients might be applicable to the relapse or refractory setting. However, in that case, other factors seem to be equally crucial. For example, the resistance to primary treatment (primary refractory patients), or a short response after first line treatment with ASCT have poor prognosis, even if other high risk factors were not detected [102, 103]. Another powerful predictor of outcome in MM is MRD status after induction treatment, and its negativity predicts better survival [104]. Although not routinely monitored, MRD has a growing significance in monitoring the disease in clinical trials. MRD can be monitored by multiparameter flow cytometry or using molecular techniques like polymerase chain reaction (PCR) or NGS on a bone marrow sample. Studies evaluating NGS in MRD have shown that its negativity surpasses the traditional complete remission criterium in predicting better outcomes in MM patients [105, 106], and its sensitivity reaches 10^{-6} [107]. Limitations in utilising NGS in MRD monitoring include lack of standardisation, low availability, and frequent haemodilution of the bone marrow sample [98].

Conclusions

MM is a highly heterogenous, gnomically evolving and ever-changing disease, with no disease-identifying unique

molecular aberration. The coexisting numerous subclones and potential lack of functionality of some mutations make interpretation even more difficult. Nonetheless, based on the cytogenetic and molecular landscape, several different subtypes of MM have been identified with different clinical characteristics and prognosis, and clinical decisions regarding treatment should be made based on the cytogenetic results. The establishment of individualised and cytogenetic subtype- or mutation-targeted treatment strategies are of the utmost importance in future studies.

Authors' contributions

SGM — wrote manuscript; DHV, AJ — critically revised manuscript.

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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REVIEW ARTICLE

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Idiopathic multicentric Castleman disease: pathogenesis, clinical presentation and recommendations for treatment based on the Castleman Disease Collaborative Network (CDCN)

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Abstract

Castleman disease is a very rare, lymphoproliferative disease, driven by dysregulation of the cytokine interleukin 6 (IL-6) and other proinflammatory cytokines with the development of symptoms of systemic inflammation, reactive proliferation of lymphocytes, and damage to numerous organs. HHV-8 infection plays a major role in the pathogenesis of human immunodeficiency virus (HIV+) multicentric Castleman disease (MCD). The aetiology of the idiopathic form of MCD (HIV/ /HHV-8 negative) is unknown.

In 2017, a panel of Castleman Disease Collaborative Network (CDCN) experts developed unified diagnostic criteria necessary for the diagnosis of idiopathic MCD. The disease has a diverse course, with a broad spectrum from mild to severe. The drug of choice according to the CDCN recommendation of 2018 for patients requiring treatment is anti--IL-6 monoclonal antibody, siltuximab. Immunomodulatory and immunosuppressive drugs are an alternative first-line treatment when anti-IL-6/IL-6R drugs are unavailable, and in subsequent lines of treatment after the failure of anti-IL-6 antibodies. Chemotherapy is reserved for the treatment of severe forms of the disease. The prognosis in most patients is moderately good. Unfortunately, the availability of the new therapies remains limited.

Key words: multicentric Castleman disease, interleukin 6, siltuximab

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Introduction

Castleman disease (CD) is a very rare, non-neoplastic lymph node hyperplasia of unknown aetiology. Excessive release of interleukin 6 (IL-6) and other pro-inflammatory cytokines results in systemic inflammation, reactive lymphocyte proliferation, and organ damage. CD was first described by Benjamin Castleman in 1954 as angiofollicular lymph node hyperplasia limited to one nodal group, now classified as unicentric Castleman disease (UCD) [1]. Similar lesions involving several nodal groups simultaneously, described by Gaba et al. in 1974, usually accompanied by systemic symptoms, are now diagnosed as multicentric Castleman disease (MCD) [2]. In the 1980s, MCD diagnosis was correlated with immune deficiencies, mainly related to human immunodeficiency virus (HIV) infection. Since then, HIV-associated MCD cases and those unrelated to HIV infection (HIV–) have been systematically distinguished [3]. MCD has been also associated with POEMS syndrome (polyneuropathy, organomegaly, endocrine disorders, monoclonal gammopathy and skin lesions). The discovery of a direct link between HIV-associated MCD and human

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herpesvirus 8 (HHV-8/KSHV, Kaposi sarcoma herpesvirus) infection was a breakthrough in our understanding of the pathogenesis of CD. It has been shown that HHV-8 replicating in plasmablastic cells in germinal centres stimulates the secretion of both viral and human IL-6 and many other pro-inflammatory proteins, which leads to characteristic histopathological (HP) lesions in the structure of lymph nodes [4]. The discovery of the key role of HHV-8 in the pathogenesis of HIV-associated MCD and the potential role of monoclonal plasma cells in the pathogenesis of POEMS-MCD contributed to the reclassification of CD. The Castleman Disease Collaborative Network (CDCN) has proposed a classification system distinguishing the disease into 1) CD of known or suspected aetiology, i.e. related to HHV-8 infection (HHV8-MCD) often coinciding with HIV infection; 2) cases associated with POEMS (POEMS-MCD); and 3) idiopathic MCD (iMCD) HHV8(–)/HIV(–) of unknown aetiology [5, 6]. First described in 2010 in Japan, TAFRO syndrome (thrombocytopenia, ascites, reticulin bone marrow fibrosis, renal failure, and organomegaly) has now been recognised as a variant of idiopathic MCD, i.e. iMCD-TAFRO [7]. Patients who do not meet the diagnostic criteria of TAFRO syndrome are classified as having idiopathic multicentric Castleman disease, not otherwise specified (iMCD-NOS) [6].

The incidence of Castleman disease in the general population is unknown. In the United States, approximately 4,750 new cases of CD are diagnosed annually, usually UCD (80–90%). In multicentric disease, 30–60% of cases are idiopathic [6, 8]. The best prognosis is for unicentric disease (UCD). In these cases, surgical resection of the lesion (the treatment of choice) results in long-term disease-free survival (DFS) in more than 90% of patients [9]. The prognosis of idiopathic MCD depends on the severity of clinical symptoms. Before the anti-IL6 antibody era, 5-year overall survival (OS) was 55–77% [10]. The introduction of anti-IL6/IL-6R therapy has significantly improved the quality of life of many patients and prolonged overall survival, especially in the mild form of MCD. Yet the prognosis in the severe disease, including patients with TAFRO syndrome, is still unsatisfactory [11, 12]. The treatment of HIV-associated MCD patients with a combination of antiretroviral therapy and rituximab has been a great success, increasing 5-year OS from 33% to 90% [13].

Pathogenesis of idiopathic multicentric Castleman disease

CD is believed to result from the dysregulation of the immune system due to the excessive secretion of pro-inflammatory cytokines. The current pathogenesis model assumes that lymph node hyperplasia and characteristic HP lesions result from the response to an excess of cytokines, mainly IL-6. For several years, the involvement of three main cytokine-stimulating mechanisms has been considered: 1) autoimmune inflammation hypothesis; 2) paraneoplastic syndrome with ectopic cytokine secretion hypothesis; and 3) cytokine stimulation by viruses other than HHV-8 hypothesis [5, 14]. The relationship between CD and infection with viruses other than HHV-8, including Epstein-Barr virus (HHV-4, human herpesvirus 4) and other viruses from the *Herpesviridae* family, has not been confirmed [5, 15].

The hypothesis suggesting an autoimmune background highlights the role of germline mutations in immune system genes (innate immunity) and the production of abnormal antibodies. In the lymph nodes, autoantibodies stimulate antigen presenting cells to secrete cytokines [IL-1/tumour necrosis factor alpha (TNF-α)], and presumably could activate yet unknown (MCD-related) cells to overproduce IL-6 and other pro-inflammatory cytokines. In a feedback loop, interleukin 6 may stimulate inflammatory cells to excessive secretion of cytokines (in a kind of vicious circle) [5]. Many autoimmune diseases that develop in consequence of unclear, complex immune system disorders leading to inflammatory response in various tissues and organs, including rheumatoid arthritis (RA), Sjögren's syndrome, systemic lupus erythematosus (SLE), and myasthenia gravis, may coexist with similar histopathological features of lymph nodes as in CD.

According to the currently accepted criteria, several inflammatory and autoimmune diseases have to be excluded in order to diagnose iMCD. Importantly, the presence of antibodies can result from MCD activity only. In approximately 30% of iMCD cases, anti-nuclear antibodies (ANA), anti-Sjögren-syndrome-related antigen A antibodies (SS-A), anti-platelet or anti-erythrocyte antibodies may be present without the diagnosis of another autoimmune disease [6, 16].

An alternative hypothesis suggests that CD is a paraneoplastic syndrome. Somatic mutations lead to the formation of a small pool of monoclonal stromal cells secreting pro-inflammatory cytokines in the lymph nodes, which become a 'trigger mechanism' that drives cytokine production further. Next Generation Sequencing studies have shown that platelet-derived growth factor beta (PDGFRβ) mutations are present in lymph nodes in nearly 20% of patients with UCD, and the mutations are probably present in the stromal cells (CD45-negative cells) [17]. Analogically to the paraneoplastic POEMS syndrome, in which monoclonal plasmocytes constituting only a small percentage of bone marrow cells are responsible for the excessive secretion of vascular endothelial growth factor (VEGF) and, consequently, to systemic disorders, it has also been shown that patients diagnosed with CD have a higher risk of cancer compared to the general population of a similar age (19% vs. 6%) [5, 16].

To explain the mechanisms underlying the pathogenesis of MCD, whole-exome sequencing (WES) studies have been performed, which allowed the selection of five unfavourable prognostic genes. Among these, *NCOA4* (nuclear receptor coactivator 4), also known as *ARA70* (androgen

receptor-associated protein 70), encoding nuclear receptor coactivator 4 protein (NCOA4), is of particular interest.

Abnormal expression and function of NCOA4 has been associated with carcinogenesis. L261F mutation in the *NCOA4* gene appears to be highly specific for the idiopathic form of MCD. NCOA4 dysfunction may play an important role in the pathogenesis of CD through its association with MAP kinase (mitogen-activated protein kinase), which controls one of the most important pathways activating IL-6 [18]. Recent studies have suggested that excessive activation of T cells and mTOR protein kinase (mammalian target of rapamycin), associated with the intracellular PI3K/AKT/mTOR signalling pathway, may play a key role in the pathogenesis of iMCD. Early attempts to use mTOR inhibitors in the therapy are promising, and a study with sirolimus is underway in patients resistant to anti-IL6 antibodies [19].

Cytokines and their role in the pathogenesis of Castleman disease

A key role in the development of CD is attributed to IL-6, a multifunctional cytokine secreted by lymphocytes, macrophages, monocytes, fibroblasts, mesothelial cells, mesangial cells, endothelial cells, as well as numerous neoplastic cells.

To exert biological activity, IL-6 requires binding to the IL-6R receptor (gp-80, glycoprotein-80) on the cell surface (mIL-6R, *IL-6* membrane receptor) or in a soluble form (sIL-6R, soluble IL-6 receptor). Binding with the receptor results in a conformational change of glycoprotein-130 (gp-130), followed by autophosphorylation of gp-130-associated JAK kinase (Janus activated kinase), and phosphorylation of gp-130. The sequence of events leads to the activation of two signal pathways: STAT-3 (signal transducer and activator of transcription 3) and MAPK. In the nucleus, STAT-3 binds to the promoters of the acute phase inflammatory response genes. IL-6 plays an important role in inflammation and regulation of the immune response.

Specifically, it: 1) stimulates B cells and plasma cells to produce immunoglobulins (polyclonal hypergammaglobulinemia); 2) together with IL-1, it activates T cells and antigen-presenting macrophages during acute phase reaction; 3) it participates in switching between the innate response (infiltration of neutrophils) and the specific response (infiltration of monocytes and lymphocytes); 4) it stimulates angiogenesis by upregulation of VEGF; and 5) it is involved in the production of acute phase proteins in the liver, including haptoglobin, C-reactive protein (CRP), and hepcidin (decreased production of albumin, iron blocking for haemoglobin) [5, 20]. IL-6 presumably plays an important role in the development of autoimmune disorders associated with CD (haemolytic anaemia and immune thrombocytopenia) by stimulating $CD5^+$ B cells to produce autoantibodies. An excess of IL-6 disrupts the ratio between Th17 cells (excess of pro-inflammatory helper cells) and Treg (regulatory lymphocytes protecting from autoimmunity). The other key cytokines include IL-1, which plays a more significant role in the pro-inflammatory cascade of events than does IL-6. A frequently observed elevated VEGF level is responsible for increased angiogenesis and permeability of blood vessels.

In cases of concomitant POEMS and MCD (POEMS-MCD), excessive VEGF secretion may stimulate the production of IL-6. In other cases (POEMS-like symptoms without the presence of monoclonal plasmocytes), the inverse mechanism is proposed, with stimulation of VEGF secretion by IL-6 [5, 14]. Recently, excessive activation of $CD8^+$ T cells, VEGF-A and the PI3K/AKT/mTOR pathway has been confirmed in patients resistant to IL-6 blockade [19].

Diagnosis, pathomorphology

HP image of the enlarged lymph nodes is characteristic, but not specific to CD. Similar or even identical lesions are described in virtually all enlarged lymph nodes in patients with RA, in approximately 30% of patients with SLE, and in many other inflammatory and infectious diseases [16]. The ultimate diagnosis requires a comparison between the HP images and clinical symptoms, and the results of laboratory workups with the simultaneous exclusion of inflammation, autoimmune, and neoplastic diseases.

In 2017, the panel of CDCN experts released the first unified diagnostic criteria of iMCD. According to this, two major criteria must be met: typical HP image, and lymph node enlargement ≥1 cm in the short axis in at least two locations. These two major criteria should be accompanied by at least two out of 11 minor criteria (including at least one laboratory criterion). At the same time, it is necessary to exclude all syndromes and diseases overlapping with iMCD (Table I) [6]. There are four HP variants of CD: 1) hyaline vascular (HV), characterised by sclerosis of blood vessels in atrophic germinal centre, concentrically surrounded by small lymphocytes in enlarged mantle zone; 2) plasma cell (PC) with expanded germinal centres and proliferating plasma cells; and 3) mixed variant (MV) with HV and PC features and plasmablastic cell (PB) variant present only in HIV-associated MCD [6].

Clinical presentation

The idiopathic form of MCD most often affects men aged 40–60 (median 50 years). The plasma cell variant is observed in 40% of patients, mixed in 40%, and the least common (<20%) is the hyaline vascular variant (characteristic for UCD). The most common symptoms of a relatively mild form of iMCD include progressive malaise, fatigue, exercise intolerance, general symptoms (fever, night sweats, weight loss), usually moderate lymphadenopathy (sometimes spontaneous regression of lesions), and

Table I. Consensus diagnostic criteria for idiopathic multicentric Castleman disease (iMCD) according to Castleman Disease Collaborative Network (CDCN) (2017) [6]

MAJOR CRITERIA (both must be met)
1. Histopathological confirmation of lesions typical for CD, based on lymph node examination
2. Lymphadenopathy ≥1 cm in the short axis, in at least ≥2 areas
MINOR CRITERIA (at least two of these 11 must be met, including at least one laboratory criterion)
Laboratory criteria
1. CRP >10 mg/L or ESR >15 mm/h
2. Anaemia, Hb <12.5 g/dL for men and <11.5 g/dL for women
3. Thrombocytopenia, PLT <150 G/L or thrombocytosis, PLT >400 G/L
4. Hypoalbuminemia (albumin <3.5 g/dL)
5. Renal failure (eGFR <60 mL/min/1.73 m^2) or proteinuria (total protein >150 mg/24 h or >10 mg/100 mL)
6. Polyclonal hypergammaglobulinemia (IgG >1,700 mg/dL)
Clinical criteria
7. General symptoms: night sweats, fever >38°, weight loss, fatigue (≥2 CTCAE)
8. Enlargement of spleen and/or liver
9. Fluid retention: oedema, ascites, pleural/pericardial effusions
10. Eruptive cherry haemangiomatosis or follicular lesions: violaceous papules
11. Lymphocytic interstitial pneumonia
EXCLUSION CRITERIA (it is necessary to exclude all entities resembling iMCD)
1. Infectious diseases
HHV-8 (LANA-1 negative in IHC, PCR negative)
EBV-lymphoproliferative disease
CMV, toxoplasmosis, HIV, active tuberculosis
2. Autoimmunological diseases (diagnosis based on applicable criteria for a given disease; presence of autoantibodies is not a criterion that excludes iMCD)
SLE, RA, Still's disease, juvenile idiopathic arthritis, autoimmune lymphoproliferative syndrome
3. Neoplastic diseases
Hodgkin's lymphoma, non-Hodgkin's lymphoma , multiple myeloma, isolated plasmacytoma, follicular dendritic cell sarcoma (FDC sarcoma), POEMS syndrome

CD — Castleman disease; CRP — C-reactive protein; ESR — erythrocyte sedimentation rate; Hb — haemoglobin; PLT — platelets; eGFR — estimated glomerular filtration rate; IgG — immunoglobulin G; CTCAE — Common Terminology Criteria of Adverse Events; HHV-8 — human herpesvirus 8; LANA-1 — latency-associated nuclear antigen; IHC — immunohistochemistry; PCR — polymerase chain reaction; EBV — Ebstein-Bárr virus; CMV — cytomegalovirus; HIV — human immunodeficiency virus; SLE — systemic lupus erythematosus; RA — rheumatoid arthritis; FDC — follicular dendritic cell; POEMS — polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin lesions

hepatosplenomegaly. Approximately 10–20% of patients experience more severe symptoms of systemic inflammation, and in addition to 'fatigue syndrome', also vascular leakage syndrome with clinically significant peripheral oedema, fluid accumulation in body cavities (pleura, peritoneum, or pericardium) or symptoms of organ failure (most commonly kidneys and lungs) [11, 16, 21, 22]. In rare cases, the symptoms can be life-threatening.

The diagnostic criteria for severe iMCD have recently been defined by the CDCN. These include organ damage, most commonly kidney disease (eGFR <30 mL/min, creatinine >3.0 mg/dL), respiratory failure (interstitial lung inflammation or fibrosis), increased fluid retention (oedema, ascites, pleural or pericardial effusion), severe anaemia (haemoglobin ≤8 g/dL) and significant deterioration in performance status [Eastern Cooperative Oncology Group (ECOG) ≥2]. A diagnosis requires at least two of these symptoms (Table II). Usually in these cases significant laboratory abnormalities are present, such as high CRP level (≥100 g//dL), hypoalbuminaemia (≤2.0 g/dL), and thrombocytopenia (≤100 g/L) [23].

TAFRO syndrome is a unique form of iMCD. It was first described in 2010 in Japan as a syndrome consisting of thrombocytopenia, ascites, reticulin bone marrow fibrosis, renal dysfunction, and organomegaly (TAFRO = **t**hrombocytopenia, **a**scites, **fi**brosis, **r**enal, **o**rganomegaly). It is presumed that the spectrum of cytokines involved in the development of TAFRO syndrome may differ slightly from the

Table II. Diagnostic criteria of severe idiopathic multicentric Castleman disease (iMCD) according to Castleman Disease Collaborative Network (CDCN) (2018) [23]

Severe iMCD symptoms
1. ECOG ≥2
2. Grade IV renal failure (eGFR <30 mL/min, creatinine >3.0 mg/dL)
3. Ascites and/or oedema and/or pleural/pericardial effusion
4. Haemoglobin ≤8.0 g/dL
5. Respiratory failure, shortness of breath, symptoms of interstitial lung inflammation or fibrosis

The diagnosis of severe iMCD requires at least two of these five symptoms; ECOG — Eastern Cooperative Oncology Group/ECOG performance status; eGFR — estimated glomerular filtration rate

classical cytokines associated with iMCD-NOS. This explains the thrombocytosis and hypergammaglobulinemia typical for the diagnosis of iMCD-NOS, and the thrombocytopenia and normal or only slightly elevated levels of immunoglobulins observed in TAFRO syndrome. The latter is usually associated with a severe course of disease and a poor prognosis [12].

CD might be associated with paraneoplastic pemphigus (PNP), a very rare symptom often associated with UCD, and symptoms resembling POEMS syndrome, including polyneuropathy, present in approximately 2% of patients [21]. Typical laboratory abnormalities include normocytic anaemia, thrombocytosis or TAFRO-associated thrombocytopenia, high CRP and erythrocyte sedimentation rate (ESR), elevated fibrinogen, polyclonal gammopathy, decreased albumin level, as well as increased IL-6 and VEGF. Bone marrow examination often shows an increased percentage of polyclonal plasmacytes. In the peripheral blood, autoantibodies against erythrocytes and platelets, as well as antinuclear antibodies, are often present [6].

Treatment of idiopathic multiple Castleman disease based on guidelines of CDCN

The idiopathic form of MCD may be asymptomatic, with mild or moderate systemic symptoms, or immediately life-threatening ‘cytokine storm’ syndrome leading to multiorgan failure. The therapeutic approach to iMCD varies due to the vast array of symptoms observed in the course of the disease. The first attempt to systematise the diagnostic and therapeutic recommendations was recently made by the CDCN expert panel. In 2016–2018, based on a few published studies and case reports (344 patients) and analysis of the treatment regimens used (479 regimens), the CDCN developed a consensus on three key issues for iMCD. The diagnosis criteria for the severe form of iMCD were defined (Table II), treatment algorithms were proposed (Table III), and treatment response evaluation criteria were developed (Table IV) [23].

Table III. Treatment recommendations for idiopathic multicentric Castleman disease (iMCD) according to Castleman Disease Collaborative Network (CDCN) (2018) [23]

iMCD-NOS/iMCD-TAFRO	
Mild	**Severe**
First-line therapy	
Siltuximab ± prednisone Tocilizumab ± prednisone Rituximab* ± prednisone	Siltuximab ± HD methylprednisolone Tocilizumab ± HD methyprednisolone ± cyclosporine**
Second-line therapy	
Rituximab ± prednisone ± immunomodulator (thalidomide) Thalidomide ± prednisone ± cyclophosphamide*** Lenalidomide	CVP ± rituximab CHOP ± rituximab
Second-line drugs	
Bortezomib Cyclosporine Sirolimus Anti-IL-1	

*According to CDCN recommendations, first-line rituximab is recommended when anti-IL-6/IL-6R antibodies are unavailable, or when short-term anti-CD20 therapy is considered as an alternative to long-term anti-IL6 therapy; **combination of tocilizumab, HD-steroids, cyclosporine recommended by Japanese research group in iMCD-TAFRO therapy; ***TCP regimen [31] with an immunomodulatory drug in second-line of treatment or in first-line when anti IL-6/IL-6R are unavailable; NOS — not otherwise specified; TAFRO — thrombocytopenia, ascites, fibrosis, renal, organomegaly; IL-1 — interleukin 1; HD — high dose; CVP — cyclophosphamide, vincristine, prednisone; CHOP — cyclophosphamide, adriamycin, vincristine, prednisone

Treatment of mild iMCD

Patients who do not meet the criteria of severe iMCD (Table II), without obvious organ dysfunction, in relatively good general condition, with moderate severity of IL-6-dependent inflammatory symptoms (fatigue, anaemia, impaired exercise tolerance, peripheral oedema) may require treatment due to their deteriorating quality of life, expressed not only by the disturbance in performing daily activities, but also by limitations in their professional life. Clinically significant lymphadenopathy is a rare indication for treatment initiation. Based on the results of a phase II study (the only randomised, double-blind, placebo-controlled study), the CDCN recommends (category 1) first-line treatment with an anti-interleukin 6 (anti-IL-6) monoclonal antibody, siltuximab (11 mg/kg i.v. every three weeks) [23]. The safety of the drug was confirmed by the results of long-term observation in a previous phase I/II study, in which the median duration of treatment was 5.1 (range 3.4–7.2) years, with a median of 81 cycles administered (range 49–129). In this study, as many as 74% of patients

Table IV. Response criteria according to Castleman Disease Collaborative Network (CDCN) (2018) [23]

Complete response (1 +2 +3)	1. Laboratory workup: Hb, CRP albumins, eGFR	2. Assessment of nodal lesions according to Cheson	3. Evaluation of systemic symptoms: fatigue, anorexia, fever, body weight*
CR	Normal	CR	Complete resolution of symptoms
PR	>50% improvement from baseline applies to all parameters	PR	Improvement in all symptoms (but not complete resolution)
SD	Improvement <50% or deterioration <25% of all parameters	Does not meet CR, PR or PD criteria	Improvement in at least one symptom (but not all)
PD	Deterioration of >25% of one parameter	PD	Worsening of at least one symptom assessed at least twice four weeks apart

*Symptom Severity Score assessed by Common Terminology Criteria of Adverse Events (CTCAE) version 4, improvement in fatigue, appetite and fever symptoms is ≥1 grade CTC reduction from baseline, weight gain ≥5% increase to baseline values; Hb — haemoglobin; CRP — C-reactive protein; eGFR — estimated glomerular filtration rate; CR — complete remission; PR — partial remission; SD — stable disease; PD — progressive disease

stayed on treatment for at least four years [24, 25]. The phase II study included 79 patients (iMCD) randomised to siltuximab or a placebo arm. In the siltuximab group, 34% nodal regression responses (complete or partial remission maintained for at least 18 weeks) were achieved, but no remission was observed in the placebo group. Benefit from the administration of siltuximab, defined as a significant improvement or relief of symptoms related to the disease, was observed in 60% of patients [26].

Data analysis and clinical experience indicate that the greatest benefit from the administration of anti-IL-6 treatment is achieved in patients with severe cytokine-dependent symptoms, defined as the presence of laboratory abnormalities (high CRP, ESR, fibrinogen, hypergammaglobulinemia, significant anaemia, hypoalbuminemia). Final analysis for the predictive model of response to siltuximab identified four important parameters: elevated fibrinogen levels, CRP, hypergammaglobulinemia, and decreased haemoglobin levels. The level of IL-6 alone did not influence the response [27]. The expected effect of siltuximab on nodal regression requires time, as this drug has no direct cytotoxic effect. In the phase II study, mean time to obtain overall response rate (ORR), defined as CR or PR, was five months [26]. It is recommended that in the early stages of treatment, biochemical parameter monitoring (CRP, ESR, albumin) and the improvement or resolution of disease-related symptoms should be used to evaluate treatment response. Nodal remission evaluation (using diagnostic imaging) should not be performed earlier than three months after treatment initiation [14, 23]. Patients who benefit from siltuximab treatment should continue the treatment, because there is a risk of relapse after treatment cessation. In a small group of patients receiving the drug for a long time, the intervals between doses were safely extended from three to six weeks, but the final effect of such a procedure requires longer observation. Many years of follow-up of patients receiving siltuximab did not reveal any significant treatment-related complications. The most frequently observed side effects include lipid disorders (hypertriglyceridemia, hypercholesterolaemia), a slight decrease in platelet count, and itching [25]. Based on the results of the phase II study, siltuximab has been approved for the treatment of iMCD in the United States and the European Union.

When siltuximab is unavailable, anti-interleukin 6 receptor monoclonal antibody (anti-IL-6R), tocilizumab (8 mg/ /kg b.w. i.v. every two weeks), is recommended by the CDCN as an alternative first-line treatment (category 2A). This drug has similar safety and efficacy profiles to siltuximab, but the evidence for its effectiveness is not supported by a randomised controlled trial. In a small, prospective, single-arm study (35 patients with iMCD), 86% of patients who continued treatment for at least five years benefited from tocilizumab. However, the effectiveness of the drug is determined here only by the percentage of patients who continued treatment (86%), and objective response criteria were not shown [28]. The drug has been approved for the treatment of iMCD in Japan. It is not recommended to initiate anti-IL-6 (siltuximab) or anti-IL-6R (tocilizumab) therapy solely based on IL-6 levels. In the phase II study with siltuximab, several cases of patients with low or normal IL-6 levels who responded to treatment, as well as cases of patients with high levels of IL-6 without response to the antibody, were observed [26]. IL-6 concentration was not considered a criterion of response to the treatment because both siltuximab and tocilizumab can cause false elevations in IL-6 levels even up to 24 months after the final dose of the drug [14, 23, 29].

Depending on the clinical situation, anti-IL-6 therapy can be administered in combination with corticosteroids. Prednisone (1 mg/k b.w. for 4–8 weeks) should be considered with a gradual dose reduction (category 2B). It is possible to use higher doses of steroids depending on the clinical need. It is not recommended to administer corticosteroids in monotherapy because, despite their initially high effectiveness (46% ORR), extended treatment is associated with a high rate of progression or requires a change of therapy due to side effects (54% failures) [23].

If anti-IL6/anti-IL6R antibodies are unavailable, the alternative first-line treatment should consist of anti-CD20 monoclonal antibody, rituximab (375 mg/m^2 i.v., 4–8 doses), most often in combination with steroids (category 2B). Rituximab can be administered initially in weekly infusions (four doses) or at 2–3 weekly intervals (6–8 doses) until the major systemic symptoms (e.g. general symptoms, fever, malaise) resolve. According to the CDCN recommendations, rituximab is proposed as the first-line drug for patients who are considering the possibility of short--term therapy (4–8 cycles) as an alternative to long-term anti-IL-6 therapy [23]. Long-term maintenance treatment with rituximab is not recommended.

There is no conclusive evidence for the efficacy of rituximab in patients with idiopathic MCD, as the evaluation of its efficacy is mainly based on the experience with HIV+/HHV-8+ MCD. The results of a retrospective analysis comparing the efficacy of siltuximab to that of rituximab for iMCD patients indicate that the anti-IL6 (siltuximab)--terated group had a significantly higher CR rate (p =0.034) and progression-free survival (PFS). It is worth noting that in the siltuximab group, approximately 75% of objective responses (ORR), defined as regression of nodal lesions, were achieved. This is a much higher percentage compared to the registration phase II study (34% ORR) [22]. The discrepancy in the obtained results is explained by the fact that the patients enrolled to the phase II study had less severe systemic symptoms, which made the placebo arm possible, while patients from the retrospective analysis constituted the 'real life' population presenting a typical broad spectrum of symptoms. This observation seems to confirm that anti-IL-6 efficacy is directly correlated with the severity of inflammatory symptoms.

First-line anti-IL-6/ anti-IL-6R treatment failure is observed in approximately 40–50% of patients. In these cases, it is recommended to use immunomodulatory or immunosuppressive drugs, possibly in combination with steroids and rituximab (category 2B). The most important immunomodulatory drug, thalidomide, is effective in monotherapy as well as in combination with rituximab. It lowers IL-6 expression and shows anti-angiogenic activity by lowering VEGF [23, 30]. In 2019, the results of a prospective phase II study were published. The study aimed to evaluate the effectiveness of the TCP regimen based on the use of oral immunochemotherapy (TCP: thalidomide 100 mg daily for two years, oral cyclophosphamide 300 mg/m^2 on days 1, 8, 15, and 22 in a 28-day cycle for one year, prednisone 1 mg/kg b.w. on days 1, 2, 8, 9, 15, 16, 22, 23 in a 28-day cycle for one year). A high percentage of objective responses (48% ORR) was achieved in the group of previously untreated patients, with good treatment tolerance. Based on these results, TCD regimen can be considered as a first-line treatment, especially in countries where siltuximab or tocilizumab are unavailable [31].

The recommendations for the third and subsequent lines of treatment are not strictly defined. According to the CDCN, if iMCD does not meet the severe form criteria, classic chemotherapy regimens should be avoided [23]. It is recommended to use other immunomodulatory or immunosuppressive drugs, because their effectiveness is comparable to classic chemotherapy (ORR 69%), but with significantly lower toxicity. The use of lenalidomide, bortezomib, cyclosporin A, or an IL-1β receptor antagonist anakinra (superior control of IL-1 over IL-6) should be considered [14, 22, 23, 32–34]. The efficacy of the mTOR pathway antagonist sirolimus has also been reported. A study is currently underway to assess the efficacy of sirolimus in patients after treatment failure with anti-IL6 (NCT03933904) [19].

Treatment of severe iMCD

It is estimated that the severe form of iMCD accounts for about 10–20% of all CD cases. Clinically significant organ dysfunction (renal failure, massive oedema and exudates, respiratory failure, poor general condition) can be life-threatening. This group of patients requires urgent use of high doses of steroids. It is recommended (category 1) to administer methylprednisolone at a dose of 500 mg/day simultaneously with siltuximab, which in this situation may be administered once a week for the first month of intensive care (11 mg/kg b.w. at days 1, 8, 15, 22). The patients who benefit from the treatment should continue siltuximab therapy in the standard regimen (every three weeks). At the same time, it is recommended to gradually reduce the doses of steroids until their complete discontinuation as soon as possible. An alternative option is to use tocilizumab (category 2A). Due to the high risk of mortality in severe iMCD, it should be remembered that not all patients will respond to the treatment quickly. Some will not respond to the combination of anti-IL-6 with steroids. Careful daily monitoring of the patient's condition and laboratory parameters to evaluate the response is recommended. If the general condition deteriorates, or no improvement is observed after a week of siltuximab administration, conventional cytostatics in multidrug regimens typically used to treat non-Hodgkin's lymphoma, multiple myeloma or haemophagocytic lymphohistiocytosis (HLH) should be administered [23].

The most commonly used regimens include: CVP ± R (cyclophosphamide, vincristine, prednisone ± rituximab), CHOP ± R (cyclophosphamide, adriamycin, vincristine, prednisone/dexamethasone ± rituximab), VDT-ACE-R (bortezomib, dexamethasone, thalidomide, adriamycin, cyclophosphamide, etoposide, rituximab), and HLH-scheme (etoposide, prednisone/dexamethasone, cyclosporine). Experience with the administration of these regimens is based on small groups of patients, and the evaluation of their effectiveness comes from retrospective analyses and case reports [23, 35]. Attempts to perform autologous or allogenic transplantation are casuistic and, according to

the recommendations of CDCN experts, should be considered only as a last resort therapy (with the exception of POEMS-MCD with bone lesions as an indication for autologous transplantation) [14, 23].

Severe iMCD often meets the criteria for TAFRO syndrome. In such cases, the CDCN recommends the same procedure as for iMCD-NOS. It seems that cyclosporine may play an important role in the therapy of TAFRO, especially in cases of recurrent thrombocytopenia and ascites. The Japanese research group recommends the use of tocilizumab in combination with cyclosporine and high doses of steroids in patients with TAFRO syndrome [36]. The effectiveness of other immunosuppressants, including calcineurin inhibitor (tacrolimus) and mTOR inhibitor (sirolimus), has also been described [37, 38].

Treatment response evaluation

The criteria for evaluation of treatment response were standardised by the CDCN in 2017 (Table IV). Comprehensive evaluation of treatment response includes: 1) laboratory evaluation of inflammatory markers including four parameters: haemoglobin (Hb), CRP, albumin, and eGFR; 2) regression of nodal lesions according to modified Cheson criteria [39]; and 3) assessment of clinical symptoms (fatigue, eating disorders, fever, body weight) according to the National Cancer Institute Common Terminology Criteria of Adverse Events scale [version 4 of the National Cancer Institute — Common Toxicity Criteria (NCI–CTC) of adverse events (AE)]. CR requires normalisation of all biochemical parameters, PR is defined as improvement ranging from 50% to 99% of baseline, while stable disease (SD) is defined as an improvement of less than 50% of baseline or deterioration of any of the parameters <25%. Progressive disease (PD) is defined as a deterioration of any of the parameters by >25% of baseline. Relief of all disease-related symptoms or improvement in their severity (but not complete resolution) as assessed by the Common Terminology Criteria of Adverse Events (CTCAE) scale indicates CR or PR in the assessment of systemic symptoms. In this case, progression means the deterioration of one of the symptoms observed on at least two consecutive visits, four weeks apart [23].

CDCN Project, NCT02817997

As part of the CDCN-supported project (NCT02817997), each patient can register their medical history at http://www.cdcn.org/accelerate. The purpose of the registry is to obtain demographic, clinical and laboratory data for further research into understanding this rare disease [14].

Authors' contributions

BO — concept and design of study, manuscript preparation and verification; JRJ — manuscript preparation and verification; KDCZ — manuscript preparation and verification; JW — manuscript preparation and verification. All authors approved the final version of this article.

Conflict of interest

BO — advisory role: EUSA Pharma. The other authors have no conflict of interest to declare.

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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 prediction models for two different peripheral stem cell collection protocols in autologous patients: how to avoid errors in calculating total blood volume to process?

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Abstract

Introduction: Calculating accurate blood volume to process is a critical practice in apheresis planning; therefore, researchers try to develop dedicated prediction models. In this analysis, we have attempted to compare three algorithms for two different apheresis collection protocols.

Methods: In a retrospective study, we have analyzed 137 apheresis procedures performed on 100 autologous patients. Apheresis procedures were performed with the Spectra Optia apheresis device with two protocols: mononuclear cell collection (MNC) and continuous mononuclear cell collection (cMNC). Three algorithms: a model based on mean collection efficiency (CE2), a linear regression model, and a power regression model were validated by plotting collected $CD34^+$ cell dose versus predicted $CD34^+$ cell dose.

Results: All models showed high predictability for MNC procedure, a high correlation of predicted $CD34^+$ yield and actual $CD34^+$ yield (R^2 =0.9547; 0.9487; 0.9474 for CE2-based model, linear and power regression model, respectively). In contrast, alteration between models for the cMNC procedure was greater (R^2 =0.8049, 0.7970, and 0.8169) with a higher number of overpredictions. Further analysis revealed that for low $CD34^+$ precounts blood volume to process, calculated with the three models, differ significantly up to fivefold times.

Conclusions: Utilizing regression models may lead to calculation errors, which can affect undercollection, repetition of apheresis, or even mobilization failure. Contrary to regression models, the model based on mean CE2 gave the most accurate prediction both for MNC and cMNC procedures. Although new prediction algorithms are created, this simple formula remains a reliable tool that promotes careful planning of apheresis, thus improving patient safety.

Key words: algorithm, apheresis, cMNC, MNC, Optia, peripheral blood stem cells

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Introduction

Understanding the principles of the apheresis technique is crucial for performing an efficient and safe peripheral blood stem cell (PBSC) collection. Optimizing all steps of the mobilization process, that is, preparation of patient, timing of the collection, and collection itself has been of interest to all haematology professionals involved in

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hemopoietic stem cell transplantation (HSCT). PBSC collection aims to gather a sufficient number of cells for HSCT with minimizing the risk of adverse events associated with the procedure. It indicates performing apheresis at the right time with the application of a minimal number of procedures [1]. The kinetics of $CD34^+$ cell increase in patients mobilized with granulocyte colony-stimulating factor (G-CSF), combined with chemotherapy, is difficult to predict. Numerous investigators focus on the timing of apheresis, that is, initiation of the procedure. It is widely approved to start apheresis with $CD34^+$ cells circulating in blood at a minimal level of 10–20 cells in microliter [2–5]. A very strong correlation between CD34 precount and yield ($CD34^+$ cells/kg of body weight) enables to determine a minimal $CD34^+$ precount required for the collection of a minimal target dose and, in consequence, decreasing the number of unnecessary procedures [6–8]. This scheme, however, in which a fixed blood volume is processed through a separator, seems to be insufficient considering the difference in the patient's height and body weight.

A need for an individualized approach to a patient led to a development of different algorithms, which determine blood volume to process through a blood separator to reach a particular $CD34^+$ cell target. Calculations based on the mean collection efficiency (CE2) (with an assumption that the $CD34^+$ cell level in blood is equal throughout the whole procedure) provided a simple and fast method for calculating blood volume required to be processed [9–13]. This method enables to reduce citrate toxicity, improves quality control, and promotes best practices by enabling benchmarking [12]. Furthermore, this algorithm was modified by decreasing the calculated CE to decrease the number of underestimated procedures, which could result in mobilization failure [14, 15].

An alternative method, first introduced by Mitterer et al. in the mid-1990s, applies a linear regression model [16]. A plot with $CD34^+$ cell precount against $CD34^+$ cells collected per 10 L whole blood volume processed is used for creating equations to determine blood volume to process and predicted $CD34^+$ yield [17, 18].

To increase accuracy, these models can be applied for stratified groups, that is, healthy donors, patients with different diagnoses [18]. Furthermore, the regression model could be tested for a nonlinear correlation. It leads to creating many models, but a question arises which model should be chosen? In this article, we try to compare the utility of these models and assess their accuracy. Additionally, to enlarge the extent of research, we build each model for two different PBSC collection protocols. We also observe the efficacy of the mononuclear cell collection (MNC) protocol and continuous mononuclear cell collection (cMNC) protocol.

Methods

Patients and apheresis

A retrospective study was performed on 100 consecutive patients, 137 peripheral blood stem cell collections, completed in a single center between May 2015 and November 2017. Information was collected on patient age, sex, diagnosis, weight, height, peripheral $CD34^+$ precount before the procedure ($CD34^+$pre), total blood volume (TBV) processed and yield — number of $CD34^+$ cells collected per kg of recipient body weight ($CD34^+$/kg body weight). Patient data are summarized in Table I.

All procedures were performed with Spectra Optia apheresis device with two protocols: MNC (n =77) and cMNC (n =60); the latter was introduced in 2014. Peripheral venous access was used whenever the nursing staff was able to insert cannulas (18 G–16 G) palpably; otherwise, a central venous catheter (13 F) was inserted before apheresis. A median inlet flow rate of 39 mL/min (range, 27–53 mL/min) was used for MNC and 50 mL//min (range, 27–77 mL/min) was used for cMNC. An initial 12:1 inlet:anticoagulant (AC) ratio was used for both protocols. It was necessary to decrease the ratio in a few MNC procedures where clothing in collection line was observed.

The CD34 target dose was 8×10^6 cells/kg of body weight for multiple myeloma patients and 4×10^6 cells/kg of body weight for lymphoma patients. Total blood volume to the process was roughly estimated by the operator based on previous experiences.

Collection efficiency (CE2) was calculated only with the pre-apheresis $CD34^+$ count. The mean CE2 value was used for formulating a collection efficiency-based prediction model.

$$CE2 = \frac{CD34^+/\mu L\ prod \times vol}{CD34^+/\mu L\ pre \times TBV\ processed}$$

$$CE1 = \frac{CD34^+/\mu L\ prod \times vol}{\frac{CD34^+/\mu L\ pre + CD34^+/\mu L\ post}{2} \times TBV\ processed}$$

Prediction models and validation

The purpose of each model is to determine blood volume to process knowing the $CD34^+$ pre. To validate their accuracy, created formulas were used for calculation of the predicted $CD34^+$ yield based on the actual blood volume processed (TBV processed). The predicted $CD34^+$ yield was compared to the actual $CD34^+$ yield. Trend lines were set to cross point 0; 0 to eliminate an error where the points lie close to the trend line but are located in the underprediction or overprediction area of the validation graph.

Model based on the mean collection efficiency (CE2)

Blood volume to process can be calculated by equation:

Table I. Patient characteristics

Variable	MNC	cMNC
Number of patients	n =50	n =50
Sex (male, female)	30, 20	32, 18
Age, median (range)	61 (23–69)	60 (26–70)
Donor body weight [kg], median (range)	70 (42–110)	74 (49–114)
Height, median (range)	168 (148–190)	170 (149–190)
Donor TBV [mL], median (range)	4555 (3004–6470)	4693 (3112–6470)
Diagnosis		
Multiple myeloma	40	36
Hodgkin lymphoma	1	6
Mantle cell lymphoma	3	3
Diffuse large B-cell lymphoma	2	4
Peripheral T-cell lymphoma	1	1
Follicular lymphoma	1	0
Burkitt lymphoma	1	0
Blastic plasmacytoid dendritic cell neoplasm	1	0
Mobilization regimen		
Cytarabine + G-CSF	35	36
Cytarabine + G-CSF + plerixafor	2	1
DCEP + G-CSF	4	3
R-DHAP + G-CSF	1	2
ICE + G-CSF	1	2
Dexa-BEAM + G-CSF	1	1
DHAP + G-CSF	2	5
R-GDP + G-CSF	1	0
IGEV + G-CSF	1	0
DCEP + G-CSF + plerixafor	1	0
G-CSF + plerixafor	1	0
Venous access		
Peripheral access	37	29
Central venous catheter	13	21

MNC — mononuclear cell collection; cMNC — continuous mononuclear cell collection; TBV — total blood volume; G-CSF — granulocyte colony-stimulating factor; DCEP — dexamethasone, cyclophosphamide, etoposide, and cisplatin; R-DHAP — dexamethasone, cytarabine, cisplatin, rituximab; ICE — ifosfamide, carboplatin, etoposide; Dexa-BEAM — dexamethasone, carmustine, etoposide, cytarabine, melphalan; DHAP — dexamethasone, cytarabine, cisplatin; R-GDP — rituximab, gemcitabine, cisplatin, dexamethasone, rituximab; IGEV — ifosfamide, gemcitabine, vinorelbine, prednisone

$$\textit{blood volume to process [mL]} = \frac{CD34^{+}\,target\left[\frac{cells}{kg\ body\ weight}\right] \times body\ weight\ [kg]}{CD34^{+}\,pre\left[\frac{cells}{mL}\right] \times CE2\ [\%]}$$

Predicted CD34^{+} dose [cells/kg body weight] can be calculated by equation:

$$CD34^{+}\,predicted\left[\frac{cells}{kg\ body\ weight}\right] = \frac{TBV\ processed\ [mL] \times CD34^{+}\,pre\left[\frac{cells}{mL}\right] \times CE2\ [\%]}{body\ weight\ [kg]}$$

Regression model

A plot with CD34^{+} collected/TBV processed [cells/kg body weight/mL] against CD34^{+}pre enables creation of linear and power estimation curves, which are elucidated with equations $y = ax + b$ and $y = cx^{n}$. This regression model allows for the formation of the following formulas.

Linear regression:

$$\textit{blood volume to process [mL]} = \frac{CD34^{+}\,target\left[\frac{cells}{kg\ body\ weight}\right]}{CD34^{+}\,pre\left[\frac{cells}{mL}\right] \times a + b}$$

$$predicted\ CD34^{+}\left[\frac{cells}{kg\ body\ weight}\right] = TBV\ processed\ [mL] \times \left(CD34^{+}\,pre\left[\frac{cells}{mL}\right] \times a + b\right)$$

Power regression:

$$\textit{blood volume to process [mL]} = \frac{CD34^{+}\,target\ [cells/kg\ body\ weight]}{c \times \left(CD34^{+}\,pre\left[\frac{cells}{mL}\right]\right)^{n}}$$

$$CD34^{+}\ predicted\left[\frac{cells}{kg\ body\ weight}\right] = TBV\ processed\ [mL] \times c \times \left(CD34^{+}\,pre\left[\frac{cells}{mL}\right]\right)^{n}$$

CE — collection efficiency; TBV processed — actual blood volume processed during collection; vol. — product volume; CD34^{+}/µL pre — patient pre-apheresis CD34^{+} blood cell count; CD34^{+}/µL post — patient post-apheresis CD34^{+} blood cell count; CD34^{+}/µL prod — product CD34^{+} cell count.

Statistical analysis

For patient characteristics and device performance, descriptive statistics was used. Results are presented as median (min, max). The Spearman's rank correlation coefficient was used to determine a relationship between the variables. The Wilcoxon signed-rank test assessed the hemoglobin loss after the procedure. Mann–Whitney U test compared the differences between protocol parameters (STATISTICA, version 13, StatSoft).

Results

MNC vs. cMNC protocol

Safety

The default setting of 12:1 inlet:anticoagulant (AC) ratio and the same blood volume processed expectedly did not contribute to difference in the volume of ACD(A) infused to patients: MNC 973 mL (337, 1543), cMNC 894 mL (451, 1600) (Table II). In both procedures, only mild adverse events occurred occasionally, and they were mitigated by oral supplementation and calcium gluconate in saline intravenous drip infusion.

Conversely, a significant difference was observed in platelet loss, higher for the MNC protocol by 24.2% (−5.7 to 60.9), lower for the cMNC protocol 12.2% (−17.3 to 38.3). The platelet CE2 confirms higher platelet attrition for the MNC protocol 21.9% (9.1–47.5) versus 15.9% (10.2–21.3) for cMNC. The better performance of the cMNC protocol is a result of lower packing factor (lower g force), implemented in the Spectra Optia centrifuge. Thicker buffy coat in the cMNC protocol allows for more selective separation in terms of platelet product contamination.

In contrast, the level of hemoglobin in patient does not change for the MNC protocol after the procedure $p = 0.47$, but it decreases significantly after the collection procedure performed with the cMNC disposable set procedure $p = 0.009$ (Table III). Again, this is a result of the technical solution implemented in the Spectra Optia centrifuge. The manufacturer recommends in the cMNC protocol to set the collection preference setting to collect product containing approximately 5% RBC. The Htc level we reached in our center is 3.0% (0.7–5.6), which is significantly higher than in the MNC setup (Htc 1.4% (0.8–3.7)). We indeed collected more RBC in the collection bag with the cMNC protocol 4.94 mL (1.9–14.03) than with MNC 3.96 mL (1.40–13.34).

The difference between the procedures is also distinguishable in cell concentration. White blood count (WBC) and granulocyte count in 1 µL of product was higher when performed with a new procedure. Despite this fact, the granulocyte percentage and overall number of granulocytes did not differ.

Performance

The time and effective collection time (average flow rate) differed favorably for the cMNC procedure (Table II). The manufacturer recommends not to exceed the blood flow rate of 62 mL/min for the MNC procedure to maintain the desired packing factor in the centrifuge, which elsewise could lead to reduced collection efficiency. The new protocol allows us to perform the procedure at higher inlet flows equally effective. However, with the application of this new procedure, we reached blood inlet flow above 62 mL/min only in seven cases, which was caused by vascular access limitations. With peripherally inserted cannulas, it is difficult to exceed the blood flow rate of 60 mL/min. Moreover, the blood flow rate may be limited by the recommended maximum anticoagulant infusion rate of 1.2 mL/min/kg body weight. The higher effective collection time, obtained with the cMNC procedure, can be explained by lower sensitivity to blood flow changes.

Collection efficiency CE2 calculated with $CD34^+$ precount alone was significantly higher for the MNC protocol. The different efficiency for both protocols is also confirmed by other useful and widely utilized parameters, that is, collection rate (Table II). Although the $CD34^+$ precount did vary between the two cohorts, the performance parameters can be compared between the two groups of patients as long as the processed TBV is similar [19]. In our study, CE2 is independent of $CD34^+$ precount ($p = 0.095$ for MNC and $p = 0.281$ for cMNC), which hereby supports this reasoning.

Regression analysis

The regression models showed a strong and very strong correlation between $CD34^+$ precount and cells collected per 1 L of blood volume processed both for MNC and cMNC protocol (Figure 1). This supports the use of $CD34^+$ precount for the calculation of blood volume to process. For the MNC protocol, the linear and power model did not show differences resulting in similar R^2. Interestingly, for the cMNC protocol, power regression showed a better correlation ($R^2 = 0.9122$ vs. $R^2 = 0.7925$).

Validation

We observed a high correlation of predicted $CD34^+$ yield and actual $CD34^+$ yield for the MNC protocol for all three algorithms: based on the mean CE2, linear, and power regression (Figure 2A–C). R^2 was equal to 0.9547, 0.9487, and 0.9474 respectively. It confirms high reproducibility and stability of the procedure.

During the cMNC procedure validation, we noticed differences between prediction algorithms. The power regression model and CE2-based one gave similar results regarding $R^2 = 0.8049$ and 0.8169 (Figure 2D, F), respectively, but the relationship between the predicted and collected $CD34^+$ yield in the linear model contributed to a poorer correlation ($R^2 = 0.7970$, Figure 2E). Moreover, a comparison of validation outcomes for MNC and cMNC procedures reveals that overall the accuracy of cMNC prediction models is lower. The final yield is less predictive suggesting lower stability of the procedure or lesser device automation.

Table II. Patient pre-apheresis haematology, procedure performance

Variable	NMC		cMNC		*p*
	n	Median (range)	n	Median (range)	
Preapheresis haematology					
CD34 [/µL]	77	41.0 (2.5–1500.0)	60	110.5 (8.0–1106.0)	0.010
WBC [$\times 10^9$/L]	77	20.78 (5.05–100.02)	60	22.26 (4.00–65.12)	NS
PLT [$\times 10^9$/L]	72	75 (37–233)	60	80 (41–282)	NS
Granulocytes [$\times 10^9$/L]	77	16.62 (3.27–93.41)	60	17.58 (2.71–55.00)	NS
Safety					
PLT loss [%]	66	24.2 (–5.7–60.9)	56	12.2 (–17.3–38.3)	0.002
PLT CE1 [%]	66	21.9 (9.1–47.5)	56	15.9 (10.2–21.3)	<0.001
ACD infused	65	973 (377–1543)	59	894 (451–1600)	NS
Procedure					
TBV processed	77	2.20 (1.10–3.00)	60	2.15 (1.30–3.20)	NS
Whole blood processed [mL]	77	10162 (4218–16175)	60	9806 (4930–17603)	NS
Procedure time [min]	77	261 (137–366)	60	203 (115–497)	<0.001
effective collection time (average flow rate) [mL/min]	77	39 (27–53)	60	50 (27–77)	<0.001
Product					
Total collect volume pump [mL]	77	260 (100–580)	60	177 (104–295)	<0.001
Htc [%]	77	1.4 (0.8–3.7)	60	3.0 (0.7–5.6)	
WBC [$\times 10^9$/L]	77		60	260.805 (56.580–713.97)	<0.001
PLT [$\times 10^9$/L]	77		60	636 (255–2426)	NS
Granulocytes [$\times 10^9$/L]	77		60	71.790 (11.76–310.97)	<0.001
Granulocytes [%]	77		60	29.523 (0.702–75.41)	NS
Granulocyte content [$\times 10^9$]	76	12.9 (0.3–55.1)	59	13.5 (0.2–41.7)	NS
MNC prod [$\times 10^9$/L]	77	98.44 (31.470–166.27)	60	133.655 (23.540–341.74)	<0.001
MNC [%]	77	50.77 (24.26–94.66)	60	50.44 (14.07–87.88)	NS
Neutrophils [$\times 10^9$/L]	77	46.88 (2.000–152.80)	60	63.905 (11.76–274.54)	<0.001
Recipient $CD34^+$/kg	77	3.10 (0.31–61.90	60	7.06 (0.40–48.30)	0.027
CD34+ total [$\times 10^9$]	77	201.24 (21.94 –5320.08)	60	567.94 (35.84–4103.72)	0.023
CD34+ [cells/µL]	77	1133.0 (93.8–22167.0)	60	2961.5 (128.0–22063.0)	<0.001
RBC content [mL]	77	3.96 (1.40–13.34)	60	4.94 (1.9–14.03)	0.001
Performance					
CD34 CE2 [%]	77	58.82 (31.55–157.38)	60	51.26 (11.27–100.20)	0.003
CD34 CE1 [%]	0	–	31	57.36 (28.69–107.58)	–
MNC CE1 [%]	77	81.8 (28.1–233.5)	60	69.7 (9.2–253.4)	0.011
Collection rate [mL/kg]	77	71.93 (27.47–260.00)	60	59.50 (23.39–204.55)	0.027
Throughput [Cr/min]	77	0.2790 (0.1122–0.9886)	60	0.2970 (0.0629–0.6796)	NS

MNC — mononuclear cell collection; cMNC — continuous mononuclear cell collection; WBC — white blood count; NS — not significant; PLT — platelets; TBV — total blood volume; RBC — red blood count; CE — collection efficiency

If we further analyze the linear regression validation plot for cMNC, we notice that the estimated and real $CD34^+$ yields deflect from an ideal trend line toward lower yields. To further investigate models for each protocol, we plotted predicted blood volume to process to target 8 $\times 10^6$ $CD34^+$/ /kg body weight, which is the most common case (Figure 3). For $CD34^+$ precount above 100 $CD34^+$/µL, the calculated blood volume to process is comparable for all models. The

Table III. Hemoglobin loss (Wilcoxon signed-rank test)

Variable	MNC		cMNC	
	n	Median (range)	n	Median (range)
Hb pre	70	9.6 (8.0–15.1)	55	10.0 (7.9–13.2)
Hb post	70	9.6 (7.1–14.9)	55	9.7 (7.6–12.4)
p	0.47		0.009	

MNC — mononuclear cell collection; cMNC — continuous mononuclear cell collection; Hb — hemoglobin

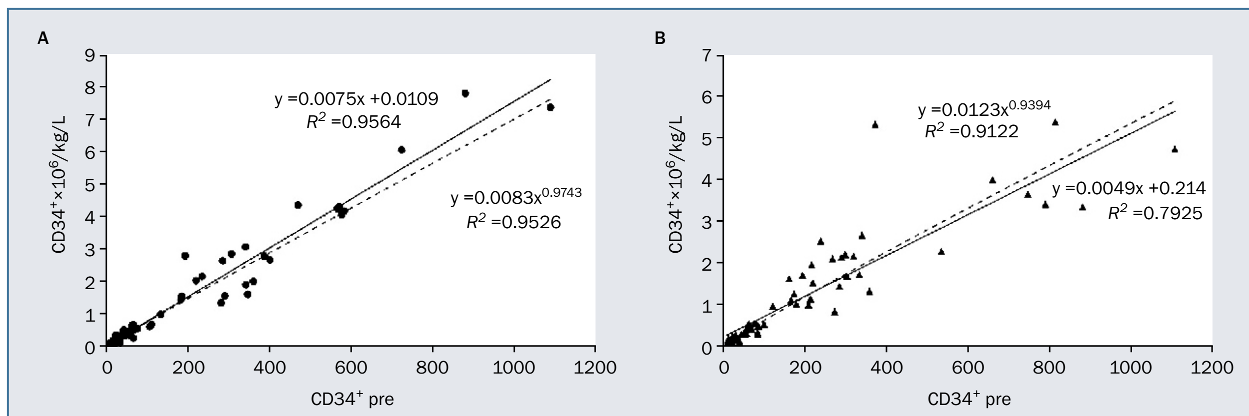

Figure 1. Regression model curves. Solid lines represent the linear model, dotted lines represent power regression model: mononuclear cell collection (MNC) protocol (**A**) and continuous mononuclear cell collection (cMNC) protocol (**B**)

Figure 2A–F. Validation plots for mononuclear cell collection (MNC) and continuous mononuclear cell collection (cMNC) protocols. Collected versus predicted CD34+ dose. The white area of the graph represents undercollection, that is, less CD34+ cells were collected than the model predicted. The grey area represents overcollection, more CD34+ cells were collected than the model predicted. Overcollection also implicates that the model has underestimated blood volume to process, which would enable to obtain the desired CD34+ target dose

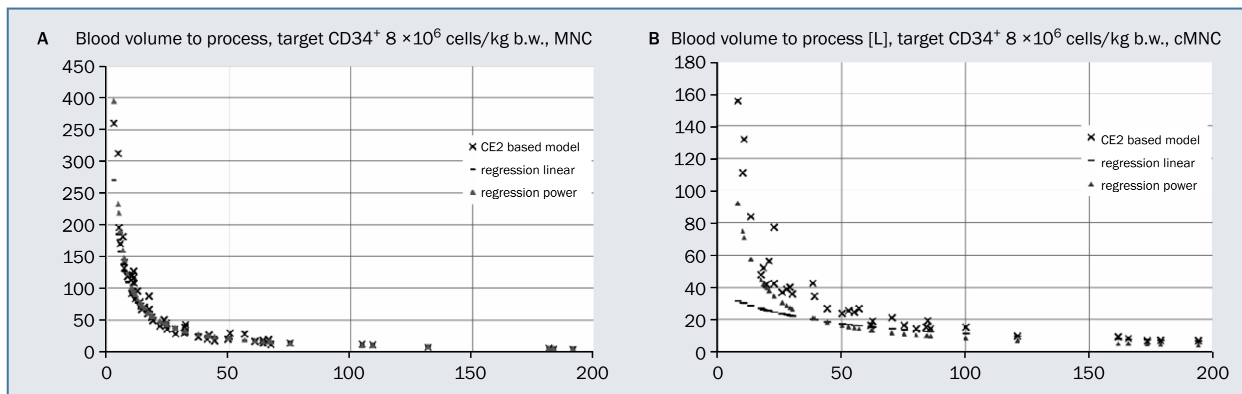

Figure 3A, B. Predicted blood volume to process [L] versus CD34$^+$ pre. For the continuous mononuclear cell collection (cMNC) protocol, the differences in predicted volume increase significantly with decreasing CD34$^+$ pre

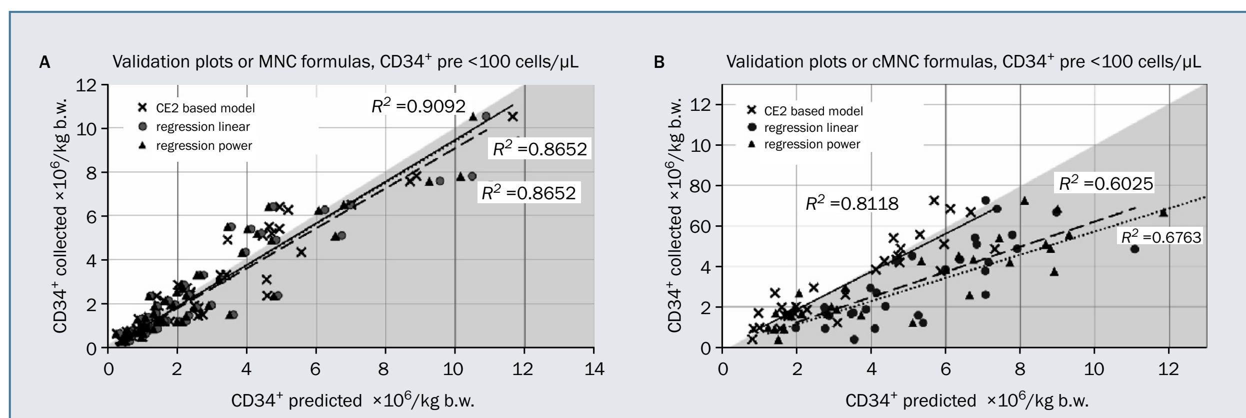

Figure 4A, B. Validation plots for mononuclear cell collection (MNC) and continuous mononuclear cell collection (cMNC) protocols for CD34$^+$ pre <100 cells/μL. Collected versus predicted CD34$^+$ dose. The white area of graph represents underprediction, the gray area represents overcollection. Solid, dashed, and dotted trend lines denote CE2-based model, linear regression, and power regression, respectively. MNC models give analogous results. For the cMNC procedure, only the CE2-based model contributes to a good correlation where neither under-collections or overcollections are significantly dominant

difference rises below 100 CD34$^+$/μL, and the difference in the values is from twofold even up to fivefold.

Validation CD34$^+$ precount <100 cells/μL

Again, by generating a validation plot we examined the accuracy of models for collections with CD34$^+$ precount below 100 CD34$^+$/μL (Figure 4). Similar to blood volume to process (Figure 3A), models for the MNC procedure, based on the mean CE2, linear, and power regression give comparable results (Figure 4A). A very good correlation, which is almost close to ideal, is observed for trend lines. The coefficient of determination equals 0.9092, 0.8652, 0.8652, respectively. For the cMNC protocol, the three models give different results (Figure 4B). The linear and power regression models show a low correlation, where R^2=0.6025, R^2=0.6763 with most points lying in the overprediction part of the validation graph. Only the model based on the mean CE2 proves a good correlation R^2=0.8118 with trend line lying close to ideal.

Underestimation and overestimation of blood volume to process

An algorithm can underpredict the target dose, which means that the predicted dose will be lower than actual. It can also overpredict the dose; therefore, the predicted dose will be higher than the collected one. Overprediction also means that the algorithm underestimates blood volume to process. This may result in the repetition of apheresis or even mobilization failure. We enumerated the number of underestimations and overestimations of blood volume to process to study the safety of each prediction model (Figure 5).

MNC models again showed consistent results with slightly more underestimations than overestimations (Figure 5A–C). cMNC regression models underestimated blood volume to process in most cases (Figure 5E, F). Only the mean CE2 model gave a similar number of underestimations and overestimations (Figure 5D).

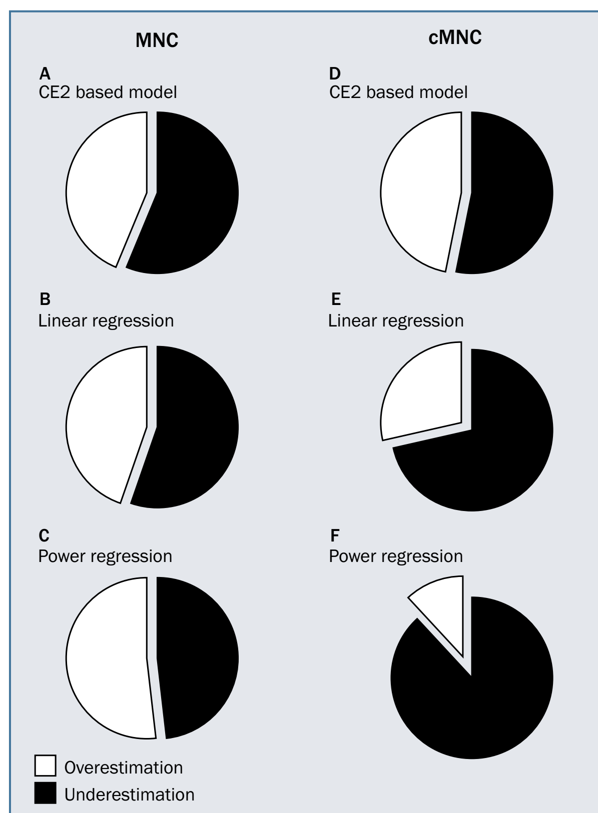

Figure 5A–F. The white color represents the number of procedures that overestimated blood volume to process and the gray color represents underestimation. The latter situation is particularly dangerous because of the need for apheresis repetition and mobilization failure risk; MNC — mononuclear cell collection; cMNC — continuous mononuclear cell collection

Discussion

MNC versus cMNC

Safety and performance

The relative safety and simplicity of peripheral stem cell collection rendered blood separators widely utilized in hematopoietic stem cell transplantation. Although severe adverse events happen rarely [20], professionals constantly pay attention to the safety of a procedure, particularly when introducing a new apheresis protocol [12]. Although some data about the new cMNC protocol have been published [11, 15, 21], we have carefully examined patient blood parameters to ensure safety.

The anticoagulant consumption did not differ, which made the new procedure as safe as the MNC in terms of anticoagulation optimization. This is not surprising as the device manages anticoagulation identically, and settings for both protocols are managed equally.

Hemoglobin and platelet count are often below the reference level in mobilized patients after chemotherapy treatment. Shlenke et al. [22] considered hemoglobin (Hg) level above 9 g/dL safe and platelet level above 30 $\times 10^9$/L satisfactory for performing a safe apheresis. By selecting a proper Spectra Optia parameter setup and the appropriate collection set, transfusion can be avoided. The MNC protocol will be suited for patients with critically low hemoglobin levels, and the cMNC protocol will be beneficial in individuals with critically low platelet count. This is a very important conclusion that illustrates how understanding of apheresis principles impacts the planning of collection procedures.

The occurrence of adverse events after the infusion of PBSC is related to the number of granulocytes in the graft. It is stressed that high-quality apheresis product is needed for transplantation [23]. As the granulocyte content between two protocols did not differ, we conclude that the new protocol again fulfills acceptance criteria and is safe.

The mean CE2 value of 56.0% for MNC and 47.6% for the cMNC protocol correspond to the published ones [11, 12, 15, 17, 21, 24, 25]. The consistency of Spectra Optia performance results, revealed by different authors, supports repeatability of devices. Consequently, our findings may have a relevant impact on the work of apheresis units, which utilize prediction algorithms to calculate blood volume to process.

Accuracy of prediction models

A reliable algorithm for determining blood volume to process should enable us to collect enough cells for a transplantation and prevent unnecessary long procedures. Prediction models for MNC procedures were comparable and suggest high reproducibility of the procedure. Performance variables support this conclusion as they are similar to those published by other authors [12, 15, 17, 21, 24–26].

The most significant differences between the algorithms were observed in the cMNC protocol. Which model should be therefore utilized for clinical practice? Regression models showed either a worse correlation (particularly for $CD34^+$ precount below 100 cells/μL) or significant overprediction. The regression models are based on an entire range of $CD34^+$ precounts. If the correlation is not ideally linear, high values can increase discrepancy. This observation is consistent with observations made by other authors who note that $CD34^+$ precount >200 cells/μL is considered as an outlier [27]. This data, coming from patients called super mobilizers, affected the model, resulting in overprediction. This can be especially dangerous for poor mobilizers when underestimation of blood volume to process can increase probability of mobilization failure.

In contrast to the MNC protocol, where all models were accurate, for the cMNC, only the mean CE2-based model was proven to be reliable. It contributed to a good correlation during the validation and did not show overestimation or underestimation.

Which algorithm to choose?

The authors have considered the regression model a good tool for determining blood volume to process. However, they have also reported that the prediction algorithm was not followed unreservedly as there was a risk of collecting an insufficient number of CD34$^+$ cells [17]. This approach did not enable to reveal the number of undercollections, but it was reasonable in terms of patient safety. The CE2-based model, applied and validated in the MNC protocol, did not differ from the regression models. When the model was applied in the cMNC protocol, the most accurate results were obtained.Hence, we suggest utilization of this model for clinical practice.

As was said in the introduction, many models and approaches have been presented and examined, but there is no publication which would compare these models. Further studies in the field of prediction models are required. Despite limitations of retrospective studies, this analysis has provided a safe way to notice hazardous inaccuracy of regression models. With a low CD34$^+$ precount, the calculated blood volume to process differed fivefold between models. The CE2-based model is simple, but yet no one has developed a model which would significantly change prediction accuracy. Although WBC precount also correlates with CD34$^+$ yield, it does not increase multiple regression model (data not shown).

In conclusion, the CE2 model is a simple method, yet reliable for all CD34$^+$ precounts. Moreover, it can be easily adjusted by constrained CE2 decrease to avoid undercollections. The utility and simplicity of this method should promote accurate planning of apheresis and procedure parameters among apheresis unit operators, which will improve patients' safety.

Authors' contributions

RM — conception and design of the work, acquisition, analysis, interpretation of data for the work, preparation of the manuscript, final approval of the version to be published; OG, AS, AP, MC, PS — substantial contribution to the drafting and editing of the manuscript; KB, MN, KZ — acquisition, analysis of technical data for the work, revising the work critically for statistical analysis; AW — revising the work critically for important intellectual content, final approval of the version to be published.

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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

Neurological presentation predicting immune thrombotic thrombocytopenic purpura outcome

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Abstract

Introduction: Immune thrombotic thrombocytopenic purpura (iTTP) is a rare disorder caused by acquired autoantibodies to a disintegrin and metalloprotease with thrombospondin-1 motifs (ADAMTS-13) that normally cleaves von Willebrand factor macromolecules. It is manifested by microangiopathic hemolytic anemia, systemic microvascular thrombi formation, and subsequent end-organ ischemia (renal and neurological manifestations). Early diagnosis and management resulted in improving the survival rate. This is a retrospective study conducted to describe the clinical characteristics of patients diagnosed with iTTP, their survival, and prognostic factors affecting it.

Methods: We included adult patients who met the diagnostic criteria of iTTP between 2016 and 2019. Based on PLASMIC score for TTP, our patients ranged from 6 to 7. ADAMTS-13 testing was not done because of financial issues.

Results: A total of 21 patients were included in this study. The median age of the studied patients was 30.45 years, and 81% of them were female. The most common clinical feature was fever (57.1%), followed by bleeding manifestations (52.4%), neurological manifestations (47.6%), renal impairment (42.9%), and cardiac manifestations (9.5%). There were a total of 4 deaths (19.04%). The overall survival was correlated significantly with neurological manifestations and PLASMIC scores (p =0.02, 0.012, respectively).

Conclusions: Our report reinforces that iTTP is not mandatory to be presented with classic pentad. Using PLASMIC score could help in the diagnosis and prediction of survival, and we strongly suggest that the absence of neurological manifestations results in better overall survival. Therapeutic plasma exchange should be started as soon as possible once iTTP is suspected. Rituximab has an important role in improving treatment outcomes.

Key words: ADAMTS13, rituximab, thrombotic microangiopathy, thrombotic thrombocytopenic purpura

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Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening thrombotic microangiopathy associated with a severe deficiency in ADAMTS13 (a disintegrin and metalloprotease with thrombospondin-1 motifs; 13th member of the family), the enzyme that cleaves the ultra-large von Willebrand factor (vWF) multimers in human's plasma [1]. ADAMTS-13 deficiency is caused by a mutational defect in the *ADAMTS-13* gene (congenital TTP) or immune-mediated (iTTP) by circulating autoantibodies against ADAMTS-13 that may be associated with a prior immune "trigger" such as pregnancy, history of infection, systemic disorders, and drugs [2]. iTTP is more common in the 4th decade, with female to male ratio of 3:2 [3]. TTP must be suspected and confirmed by laboratory testing following its clinical

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presentation. The "classical" pentad that includes thrombocytopenia, MAHA, fever, renal failure, and neurological manifestations is uncommon. Gastrointestinal symptoms, such as vomiting, diarrhea, and abdominal pain are frequent; however, myocardial ischemia, pancreatitis, and less commonly, a pulmonary injury may also occur [3, 4].

Advanced age, cardio-vascular system affection, and high lactate dehydrogenase (LDH) values have been correlated with high mortality risk. Management of iTTP involves proceeding safely and quickly initiating the immunomodulating agents targeting the lymphocytes responsible for autoantibodies production alongside a treatment backbone of therapeutic plasma exchange (TPEx), which helps to replete ADAMTS-13 and remove vWF and autoantibodies [5]. Untreated iTTP is associated with mortality as high as 90%; with TPEx, mortality can be reduced to 20% [6]. ISTH guidelines recommend using corticosteroids in addition to TPEx for treatment of first acute episode and relapses of iTTP. Meanwhile, adding rituximab will be effective in the treatment of known comorbid autoimmune disease [7].

Recently, caplacizumab (anti-vWF nanobody), blocks the adhesion of platelets to vWF and prevents the formation of microvascular thrombi [8]. It is recommended to be used early at the time of an acute TTP event. However, it neither corrects the level of plasma ADAMTS-13 nor does it suppress the autoantibodies to ADAMTS-13. Thus, other immunosuppressive therapies use steroids and rituximab that are still required to control the underlying disease process [7–9].

In this study, we demonstrate the basic clinic-pathological features of TTP patients emphasizing the pattern and outcome of the disease.

Material

Patients and methodology

All data from iTTP patients referred to Oncology Center and Mansoura University from 2016 to 2019 were analyzed. We diagnosed our cases based on negative coombs test hemolytic anemia and found the presence of schistocytes in peripheral blood film stained with Leishman stain, acute peripheral thrombocytopenia ($<150 \times 10^3/mm^3$), reticulocyte counting by brilliant cresyl blue staining plus or minus the rest of the characteristic pentad. A workup including history, physical examination, radiological studies, serum LDH, liver function, renal function test, viral marker, antinuclear antibody, anti-double-stranded DNA antibody to exclude secondary causes of iTTP as drugs, pregnancy, autoimmune diseases, malignancies, HIV, and other infections. ADAMTS-13 testing was not done because of financial issues.

We retrospectively applied the PLASMIC score to stratify the cases (Table I). The PLASMIC score is a seven-item score with one point is assigned for each item. The risk stratification categories are as follows: low-risk (0–4), intermediate-risk (5), and high-risk (6–7). Internal and external validation studies revealed that the frequency of severe ADAMTS-13 deficiency is 0–4%, 5–24%, and 62–82% in all risk categories, respectively [10].

Table I. PLASMIC scoring system

Variables	Points
Platelet count $<30 \times 10^9$/L	1
Hemolysis present:	1
• reticulocyte count >2.5%, or	
• haptoglobin undetectable, or	
• indirect bilirubin >2.0 mg/dL	
No active cancer	1
No history of solid organ or stem cell transplant	1
Mean corpuscular volume (MCV) <90	1
International normalized ratio (INR) <1.5	1
Creatinine <2.0 mg/dL	1

Treatment response was defined according to international recommendations as a complete response (CR), which was defined as the absence of any clinical manifestations and normalization of platelet count for ≥2 days. No doubling of platelet count after four days of standard intensive therapy and persistently high LDH levels was considered as a refractory disease. Relapse was the reappearance of clinical features of iTTP for at least 2 days [11].

Statistical analysis

Data were analyzed on a personal computer running SPSS21 for Windows (Statistical Package for Social Scientists) Release 18. A 2-tailed *p*-value of <0.05 was statistically significant. For descriptive statistics of qualitative variables, the frequency distribution procedure was run with the calculation of the number of cases and percentages. For descriptive statistics of quantitative variables, mean, standard deviation (SD), and range were used to describe the central tendency and dispersion as appropriate. The overall survival was estimated through the Kaplan-Meier test.

Results

This study was conducted on 21 patients. The mean age of the studied patients was 30.45 years, and 81% of them were female. The most common clinical feature was fever (57.1%), followed by bleeding manifestations (52.4%), neurological manifestations (47.6%) in form of headache, slurred speech, seizures, confusion up to coma, renal impairment (42.9%), and cardiac manifestations (9.5%) in form of arrhythmia and myocardial infarction. The mean values at presentation were consistent with microangiopathic hemolytic anemia (Table II).

Table II. Basic descriptive date of studied group

Parameters		Mean ± SD
Age (years)		30.38 ± 9
Haematological parameters	WBC ×10⁹/L	10.42 ± 6.58
	Hb [g/dL]	7.31 ± 1.43
	Platelets ×10⁹/L	33.9 ± 24.11
	Reticulocyte count [%]	5.5% ± 2.6%
	Schistocytes cells [%]	6.14% ± 2.26%
Liver function test	SGPT	44.9 ± 38.12
	SGOT	64.14 ± 60.9
	Total bilirubin [mg/dL]	2.81 ± 1.05
	Serum albumin [mg/dL]	3.62 ± 0.71
Renal function test	Serum creatinine [g/dL]	3.21 ± 3.12
LDH [U/L]		1966.52 ± 941.13
		No. [%]
Clinical presentations	Fever	12 (57.1%)
	Bleeding manifestation	11 (52.4%)
	Neurological manifestation	10 (47.6%)
	Renal impairment	9 (42.9%)
	Cardiac manifestation	2 (9.5%)
PLASMIC score	6	12 (57.1%)
	7	9 (42.9%)

SD – standard deviation; WBC – white blood cells; Hb – hemoglobin; SGPT – serum glutamic-pyruvic transaminase; SGOT – serum glutamic-oxaloacetic transaminase; LDH – lactate dehydrogenase

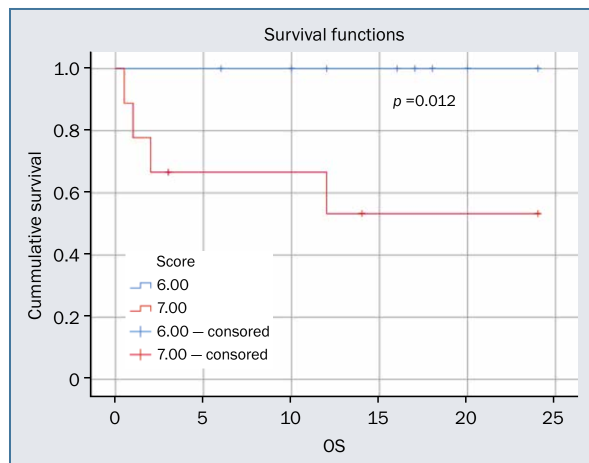

Figure 1. Effect of PLASMIC score on overall survival (OS) of studied cases

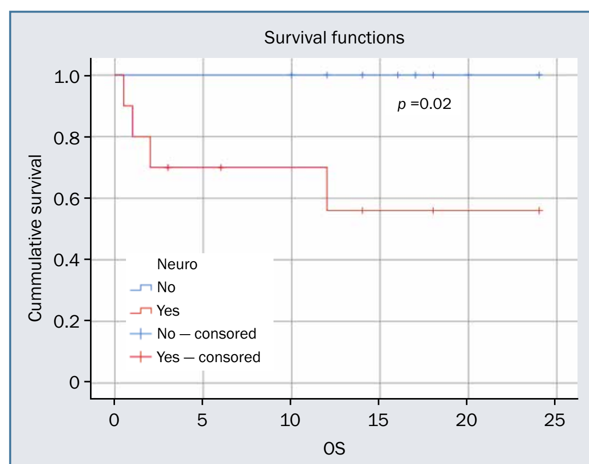

Figure 2. Effect of neurological (neuro) manifestations on overall survival (OS) of studied cases

The patients were administered with steroids and TPEx for treatment, and the average number of sessions was 15 (range 2–45 sessions). Seven patients required rituximab, 6 of them received it at presentation, and it was administered as the second line in one patient; 47.62% of patients required cyclosporine as second or third line. There were 4 deaths (19.04%), 2 of them were related to relapse and the other 2 were secondary to refractoriness to therapy.

The mean overall survival of the studied patients was 19.84 months [95% (confidence interval) CI: 16.41–23.55]. The patients presented with a PLASMIC score 6 and the absence of neurological problems were associated with better overall survival compared to other groups with significant p =0.012, 0.02, respectively compared with other groups as shown figures 1 and 2, while overall survival insignificantly correlated with age >30 years (p =0.1), fever (p =0.42), and renal impairment (p =0.38).

Discussion

Thrombotic thrombocytopenic purpura's diagnosis is challenging secondary to its variable clinical presentations, which could overlap with other thrombotic microangiopathies and the unavailability of ADAMTS13 tests [12]. Many diagnostic scores are available such as the PLASMIC score, which is considered a useful application; as it has been able to diagnose TTP associated with ADAMTS-13 deficiency practically and statistically accurate, it's of low-cost, fast, especially in developing countries [10]. Other scores as the French registry-based score (Table III) and the one proposed by Bentley et al. are also available [13, 14].

In this study, we review the clinical presentations and laboratory tests for TTP. We also used the PLASMIC score to retrospectively assess our patients.

Our data revealed that females represented 81% of the studied cases, with a mean age of 30.45 ±9 years. The most

Table III. The French score — modified

Variables	Points
Platelet count <3 ×10^{10}/L	1
Creatinine >2.26 mg/dL	1
Reactive antinuclear antibodies	1

common feature in our study was fever (57.1%), followed by bleeding manifestations (52.4%), neurological manifestations (47.6%) in form of headache, slurred speech, seizures, confusion up to coma, renal impairment (42.9%), and lastly cardiac manifestations (9.5%).

Meanwhile, an Omanian study conducted on 38 TTP patients revealed that the patients' mean age was 36 years, 66% of patients were females. Their clinical presentation was: fever (63%), renal impairment (50%), bleeding (31%), fits (21%), high blood pressure reading (21%), headache (15%), confusion (15%), postpartum presentation (15%) and stroke (7.9%) [15].

In comparison to the review from Oklahoma TTP-HUS Registry that studied 78 patients with acquired TTP, gastrointestinal symptoms were found recorded in 69%, weakness in 63%, bleeding or purpura in 54%, major neurological findings including (coma, stroke, transient focal abnormalities, and seizures) were recorded in 41% of patients, while minor neurological findings including (confusion and headache) were found in 26% of patients and fever with chills in 10% [16, 17].

Focusing on neurological and renal affection in TTP patients, their pathogenesis is mainly caused by the deposition of hyaline thrombi widely with fibroblastic infiltration and endothelial overlay found in the terminal arterioles and capillaries of multi-organs. Pre-occlusive pseudo-aneurysmal dilatation may be one of the mechanisms. These mechanisms explain the severity of these events in TTP patients and their consideration as independent risk factors for prognosis [18].

We retrospectively stratified our iTTP patients based on the PLASMIC score, and all of them were categorized as a high-risk group, 12 patients (57.1%) were with a score of 6 and 9 patients (42.9%) were with a score of score 7. The PLASMIC score was developed and validated by a study conducted on 214 cases in the Harvard Thrombotic Microangiopathies Research Collaborative Registry [10]. In addition, the 100% diagnostic accuracy for PLASMIC score was confirmed by a study conducted in eight patients with iTTP, 4 cases with ADAMTS-13 activity <10%, had a PLASMIC score of 6. The other 4 had ADAMTS-13 activity >10%, had a <6 score. Also, the PLASMIC score predicted accurately their response to TPEx and the risk of long-term poor outcomes [19].

However, Prevel and his colleagues [5] provide evidence that the diagnosis of iTTP based on clinical scores aimed at predicting a severe ADAMTS-13 deficiency may be less accurate among older patients as organ damage in older patients following microthrombi formation may develop earlier than in younger patients, leading to earlier clinical manifestations with older age. In contrast, the organs of younger patients could tolerate microthrombi and ischemia, which leads to more aggressive cytopenias.

Steroids and TPEx were used for treatment, and the average number of sessions was 15; (range 2–45 sessions). Seven patients required rituximab, 6 of them received it at presentation, and it was administrated as the second line in one patient, 47.62% of patients required cyclosporine as the second or third line. There were 4 deaths (19.04%), 2 of them were related to relapse and the other 2 were secondary to refractoriness to therapy. However, we noticed that using rituximab as the first line in addition to TPEx and steroids improved the survival of our patients who had neurological manifestation at presentation.

Rituximab is a monoclonal IgG1κ antibody, which is commonly used in treatment regimens for iTTP. Rituximab can prevent relapse and reduce mortality. Its half-life is about 2 to 3 weeks but ultimately depends on the underlying treated condition, presence of TPEx, and CD20^{+} lymphocyte load [20, 21]. A prospective study by Carden et al. 2020 [22] conducted on three patients studying the role of rituximab in iTPP, found that rituximab can eliminate all circulating CD20^{+} B and T cells within 24 hours for each patient, despite uninterrupted daily TPEx through multiple mechanisms, including antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and apoptosis.

Data revealed that the mean overall survival of our patients was 19.84 months (95%CI: 16.41–23.55). Patients presented with PLASMIC score 7 and absence of neurological manifestations were associated with better overall survival with significant p =0.012 and 0.02, respectively, compared with the other groups, while the overall survival insignificantly correlated with age, fever and renal impairment (p =0.1, 0.42, and 0.38, respectively). Similarly, it was reported in a study from the UK TTP Registry involving 292 patients that 24% of the patients who presented with a reduced Glasgow Coma Score were significantly associated with shorter overall survival compared to the other groups (p <0.0001) [23].

A study conducted by Prevel et al. [5] on 411 patients showed that for patients aged more than 60 years, renal impairment; cardiac involvement, and total TPEx volume were independently associated with mortality. That is why physicians should be aware of atypical clinical presentations and for immediate intervention once suspected, which could improve the prognosis in iTTP patients.

Conclusion

The high cost of ADAMTS-13 testing makes it infeasible to do it for all patients in our country, and that is why we retrospectively applied the PLASMIC score trying to validate

it in our community. A high PLASMIC score indicates immediate TPEx without losing time waiting for ADAMTS-13 level, while a low score, synchronized with low clinical probability, justifies further search for an alternate diagnosis in order to avoid overuse and associated complications of TPEx. We also aimed to focus the spot on neurological presentation of TTP patient correlating with worse survival and this may be put into consideration as a risk factor during deciding the treatment plan.

Authors' contributions

SE, EJ, RSS, YS — equally participated in collecting clinical, radiological, and statistical analysis. MIM, MAG — interpreted laboratory data. All authors — contributed to the study design, participated in writing and editing the final version of the manuscript, read and approved the final manuscript.

Conflict of interest

On behalf of all authors, the corresponding author states that there is 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

VIA MEDICA

Impact of ferritin serum concentration on survival in children with acute leukemia: a long-term follow-up

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Abstract

Introduction: Nowadays, a significant number of children with acute leukemia can be cured. Iron overload, related to blood transfusions and its long-term complications, remains a problem. Elevated ferritin concentration is often observed in this group.

The aim of this study was to evaluate the prognostic value of serum ferritin on long-term outcomes in children treated for acute leukemia.

Material: We studied 71 patients treated for acute lymphoblastic (ALL) or myeloblastic (AML) leukemia between 2005 and 2011. Serum ferritin concentration, serum transaminases activity, lactic dehydrogenase and C-reactive protein levels (CRP) were analysed. Serum ferritin >1,000 μg/L was considered to be a marker of iron overload.

Results: Thirty-seven patients (52.1%) had iron overload. Ferritin serum concentration correlated with alanine aminotranferase activity (p =0.001) and CRP concentration (p =0.012). A total of 19 (26.76%) patients died during follow-up. Ferritin level was higher in patients with AML vs. ALL. There was a significant difference in long-term outcomes with respect to high ferritin concentrations, both in patients undergoing haematopoietic cell transplantation (HCT) and in the non-HCT group.

Conclusions: In both groups, patients with higher ferritin concentrations had worse overall and event-free survivals and a higher relapse incidence. Ferritin concentration >1,000 μg/L was the strongest determinant of long-term treatment outcome. Ferritin serum concentration >1,000 μg/L is an adverse prognostic marker of survival in children with acute leukemia treated with chemotherapy with or without HCT.

Key words: ferritin, iron, leukemia, children, haematopoietic cell transplantation

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Introduction

In recent years, a great deal of progress has been made in treating acute leukemia in children. Nonetheless, treatment is still complicated by significant morbidity and mortality. With improved diagnostic procedures, intensification of therapy, and effective treatment of infections, the prognosis for children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) has improved [1–3]. However, a major challenge has arisen regarding long-term complications including iron overload and its sequelae [4].

Serum ferritin is usually used to detect iron overload. It is a sensitive parameter, albeit of low specificity because it can be elevated in a variety of inflammatory states, as well as other clinical entities including sickle cell anaemia [5], haemophagocytic lymphohistiocytosis and macrophage activation syndrome [6]. It is also a surrogate marker for cytokine release syndrome [7] and neuroblastoma [8]. The incidence of hyperferritinemia increases with the

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number of transfusions. One red blood cell (RBC) unit administered to correct anaemia contains 200–250 mg of iron which is being delivered to the patient. Iron overload can occur after 10–20 RBC transfusions. It is universally recognised that iron overload is a risk factor for organ and metabolic complications. Dysfunction of the heart and liver are commonly observed [9].

Serum ferritin concentration has been shown to be a strong predictor of survival after allogeneic haematopoietic cell transplantation (allo-HCT) [5, 8, 10, 11]. Apart from acute leukemia patients, iron overload resulting from transfusion-dependent conditions is frequently observed in patients with myelodysplastic syndromes (MDS). In that group, elevation of serum ferritin is associated with a high risk of leukemic transformation [12].

Data regarding the impact of serum ferritin concentration on treatment in children with acute leukemia or undergoing HCT is scarce. Thus, the objective of this study was to evaluate the prognostic value of serum ferritin concentration on the long-term treatment results in children with acute leukemia who were undergoing intensive chemotherapy with or without HCT.

Material

Study design

In this retrospective single centre study, all patients treated in our department for acute leukemia between 2005 and 2011 who were tested for ferritin serum concentration were included. Children undergoing multiagent chemotherapy with or without subsequent HCT were qualified for long-term follow-up. This study was approved by the Local Bioethical Committee (169/2020; 31 March 2020).

Collection of data

Serum ferritin concentration, serum transaminases activity, and C-reactive protein (CRP) levels were analysed among the study participants. In all cases, ferritin concentration was measured at least four months after the diagnosis of leukemia. In cases of multiple testing, the highest concentration was taken into account. Serum ferritin >1,000 μg/L was considered to be a marker of iron overload. The values of CRP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) obtained at closest proximity to the day of ferritin concentration testing were also analysed. In most cases, the time interval between these tests was four days or less.

Treatment of leukemia

Children with *de novo* ALL were treated according to the ALL-IC-2002 protocol. Children with relapsed ALL were treated according to the ALL-REZ BFM 2002 protocol. Children with *de novo* AML were treated according to the AML-BFM-2004 protocol. Children with relapsed AML were treated according to the IDA-FLAG/FLAG protocol.

Transplant procedures

Children were qualified for HCT according to chemotherapy protocols. The conditioning regimen before transplantation was either myeloablative (MAC) or reduced intensity conditioning (RIC). MAC was based on total body irradiation (TBI), busulfan or treosulfan. RIC was based on fludarabine or busulfan at doses of ≤8 mg/kg/cycle. For prophylaxis of graft-versus-host disease (GvHD), cyclosporine A (CsA) and short-term methotrexate (MTX) were used. Patients from alternative donors [i.e. matched unrelated donor (MUD), mismatched unrelated donor (MMUD), or haploidentical donor] received anti-thymocyte globulin (ATG).

Definitions

The primary endpoint was overall survival (OS). Additional endpoints were event-free survival (EFS) and relapse incidence (RI). An event was defined as relapse or death from any cause. EFS was defined as survival without evidence of relapse or progression. Relapse was considered in the presence of >5% bone marrow (BM) blasts and/or the reappearance of the underlying disease. OS was analysed for non-HCT patients as the time from the start of chemotherapy to death from any cause, or until the end of follow-up; OS for transplanted patients was calculated from the day of allo-HCT to death from any cause or until the end of follow-up. Death from any cause was regarded as an event for OS, while relapse and death from any cause were considered to be events for EFS. RI was estimated by considering a relapse or the reappearance of the underlying disease as events of interest, and death without relapse as a competing event.

Statistical analysis

The Mann-Whitney U-test was used for non-categorical comparisons and Chi-square or Fisher exact test for categorical comparisons. Correlations between laboratory parameters were analysed with Spearman rho coefficient. OS, EFS and RI were calculated with the Kaplan-Meier curves method, and differences between the curves were compared by log-rank test. Mean survival was determined by Kaplan-Meier method. The Cox regression model was used to calculate treatment outcomes for risk factors, and hazard ratios (HR) were calculated with 95% confidence interval (95%CI). All the tests were two-sided. Statistical significance was defined as p <0.05. Am SPSS25 (IBM, Armonk, NY, USA) statistical package was used.

Results

Demographics

Our study included 71 patients with acute leukemia, 43 boys and 28 girls with a median age of 9 (range 1–19.7)

Table I. Patient characteristics

Characteristics	Total [%]	HCT (n = 34)	Non-HCT (n = 37)	*p*
Age (years)				
Median, range [years]	9.4 (1.1–19.7)	11.2 (2.5–19.7)	6.6 (1.1–19.7)	
<10 vs. >10	36 (50.7):35 (49.3)	13 (38.2):21 (61.8)	23 (62.2):14 (37.8)	0.205
Gender				
Male:female	43 (60.6):28 (39.4)	23 (67.6):11 (32.4)	20 (54.1):17 (45.9)	0.245
Type of leukemia				
ALL:AML	54 (76.1):17 (23.9)	18 (52.9):16 (47.1)	36 (97.3):1 (2.7%)	<0.001
Ferritin [µg/L]	461 (4–8,500)	1,060 (14–8,500)	284 (15–2,110)	0.002
ALT [U/mL]	20 (6–1,172)	33 (9–769)	14 (6–1,172)	<0.001
AST [U/mL]	30 (11–554)	40 (13–415)	26 (11–554)	0.054
CRP [mg/mL]	7 (<5–374)	10 (<5–146)	5 (<5–374)	0.215
LDH [U/mL]	297 (5–4,705)	341 (113–2,815)	5 (257–4,705)	0.473

ALL — acute lymphoblastic leukemia; AML — acute myeloblastic leukemia; ALT — alanine aminotransferase; AST — aspartate aminotransferase; CRP — C-reactive protein; LDH — lactate dehydrogenase

years. The primary disease in these patients was ALL in 54, and AML in 17 (Table I). The total number of patients who underwent HCT was 34, comprising 32 allo-HCT and two auto-HCT. In 19 patients a MUD, and in 13 a matched sibling donor (MSD) transplant was performed. In one patient, auto-HCT was followed by allo-HCT; this patient was categorised as allo-HCT. The stem cell source for HCT was peripheral blood (19 patients) and bone marrow (15 patients).

Ferritin concentration

The median value of highest serum ferritin concentrations was 2,307 µg/L (range: 33–8,500 µg/L) and 708.44 µg/L (range: 14–7,440 µg/L) in patients with AML and ALL, respectively. In 37/71 patients, serum ferritin was >1,000 µg/L. Ferritin serum concentration correlated with ALT activity (Spearman's rho coefficient 0.41; *p* =0.001), and CRP concentration (rho 0.32; *p* =0.012), but not with AST activity or LDH concentration.

Survival of patients

Overall, 52 (73%) patients were alive at the end of the study in 2020. Mean survival was 9.0 years (95%CI =7.8–10.1). Probability of overall survival (pOS) of all patients at 5 years was 0.79 ±0.05, and at 10 years it was pOS =0.63 ±0.10; the 5-year EFS was 0.70 ±0.06, and the 5-year RI was 0.19 ±0.05.

Splitting the analysis into subgroups with respect to the highest serum ferritin concentrations, the values of probability of OS, EFS and RI are set out in Table II. Patients with a higher ferritin concentration, regardless of its cut-off value, had worse overall survival and a higher incidence of relapses.

Table II. 5-year treatment outcomes with respect to serum ferritin concentration

Ferritin cut-off concentration [µg/L]	Patients	OS	EFS	RI
Ferritin 500				
<500	36	0.89 ±0.05	0.86 ±0.06	0.06 ±0.04
≥500	35	0.68 ±0.08	0.52 ±0.09	0.35 ±0.09
p-value		0.008	0.003	0.006
Ferritin 1,000				
<1,000	49	0.90 ±0.04	0.86 ±0.05	0.09 ±0.04
≥1,000	22	0.55 ±0.11	0.32 ±0.10	0.52 ±0.13
p-value		<0.001	<0.001	<0.001
Ferritin 1,500				
<1,500	54	0.85 ±0.05	0.82 ±0.05	0.12 ±0.04
≥1,500	17	0.59 ±0.12	0.31 ±0.12	0.51 ±0.15
p-value		<0.001	<0.001	<0.001
Ferritin 2,000				
<2,000	58	0.83 ±0.05	0.79 ±0.05	0.13 ±0.04
≥2,000	13	0.62 ±0.14	0.25 ±0.13	0.59 ±0.185
p-value		0.001	<0.001	<0.001
Total	71	0.79 ±0.05	0.70 ±0.06	0.19 ±0.05

OS — overall survival; EFS — event-free survival; RI — relapse incidence

Long-term outcomes and differences between HCT and non-HCT patients

Patients undergoing HCT (n =34) vs. non-HCT (n =37) had significant differences in long-term outcomes, although in both groups those with a higher ferritin concentration had worse survival. The outcomes for a ferritin concentration threshold value of 1,000 µg/L are shown (Figure 1A–I). The OS values for non-HCT patients were insignificant compared to those with higher vs. lower ferritin (Figure 1B), but EFS values were significantly lower for those with high ferritin (Figure 1E). This was caused by the higher relapse incidence (Figure 1H). For HCT patients, all outcomes were significantly worse in patients with a high ferritin concentration (Figure 1C, F, I).

Risk factor analysis

We conducted univariate analysis of factors contributing to overall survival including the following parameters: age of patient (<10 vs. >10 years), primary diagnosis (ALL vs. AML), ferritin serum concentration (<1,000 vs. >1,000 µg/ /L), and HCT treatment (non-HCT vs. HCT) (Table III). Adverse risk factors with a *p* <0.1 value were included into multivariate analysis: diagnosis of AML, ferritin concentration >1,000 µg/L, and treatment with HCT. The only significant risk factor in multivariate analysis was ferritin concentration >1,000 µg/L, with a 7.1-fold hazard risk for death. Analogically, ferritin concentration >1,000 µg/ /L was the only significant risk factor for EFS and RI with a 4.3-fold and a 6.9-fold hazard risk, respectively, for adverse events and leukemic relapse (Tables IV and V).

Discussion

Our analysis shows that iron overload is often observed in paediatric patients with acute leukemia who are undergoing intensive chemotherapy with or without HCT. Furthermore, we have shown an adverse prognostic value of ferritin serum concentration. This parameter can be used as a marker negatively influencing overall survival, EFS and relapse incidence in children after treatment for acute leukemia, particularly those undergoing HCT. Previous data has confirmed the predictive power of serum ferritin concentration for survival after allo-HCT and showed that elevated ferritin is associated with an increased risk of relapse [10].

Univariate and multivariate analysis showed that high ferritin serum concentrations were correlated with

Table III. Univariate and multivariate risk factors analysis for overall survival (OS)

Parameter	Characteristics	5-year OS	Univariate analysis		Multivariate analysis	
			HR (95%CI)	*p*	HR (95%CI)	*p*
Age	<10 years	0.82 ±0.06	1	0.339	–	–
	>10 years	0.75 ±0.08	1.6 (0.7–3.9)		–	
Diagnosis	ALL	0.83 ±0.05	1	0.076	1	0.762
	AML	0.65 ±0.12	2.3 (0.9–5.7)		1.2 (0.4–3.1)	
Ferritin	<1,000 µg/L	0.90 ±0.04	1	<0.001	1	<0.001
	>1,000 µg/L	0.55 ±0.11	6.8 (2.8–20)		7.1 (2.6–20)	
Treatment	Non-HCT	0.92 ±0.05	1	0.009	1	0.165
	HCT	0.65 ±0.08	5.5 (1.6–18)		2.4 (0.7–10)	

HR — hazard ratio; 95%CI — 95% confidence interval; ALL — acute lymphoblastic leukemia; AML — acute myeloblastic leukemia; HCT — haematopoietic cell transplantation

Table IV. Univariate and multivariate risk factors analysis for event-free survival (EFS)

Parameter	Characteristics	5-year EFS	Univariate analysis		Multivariate analysis	
			HR (95%CI)	*p*	HR (95%CI)	*p*
Age	<10 years	0.79 ±0.07	1	0.110	–	–
	>10 years	0.58 ±0.09	2.0 (0.8–4.1)		–	
Diagnosis	ALL	0.77 ±0.06	1	0.044	1	0.753
	AML	0.47 ±0.12	2.4 (1.1–5.5)		1.3 (0.4–2.8)	
Ferritin	<1,000 µg/L	0.86 ±0.05	1	<0.001	1	0.003
	>1,000 µg/L	0.32 ±0.10	6.8 (2.8–16)		4.3 (1.6–12)	
Treatment	Non-HCT	0.89 ±0.05	1	0.002	1	0.111
	HCT	0.48 ±0.09	4.9 (1.9–13)		2.5 (0.8–7.3)	

HR — hazard ratio; 95%CI — 95% confidence interval; ALL — acute lymphoblastic leukemia; AML — acute myeloblastic leukemia; HCT — haematopoietic cell transplantation

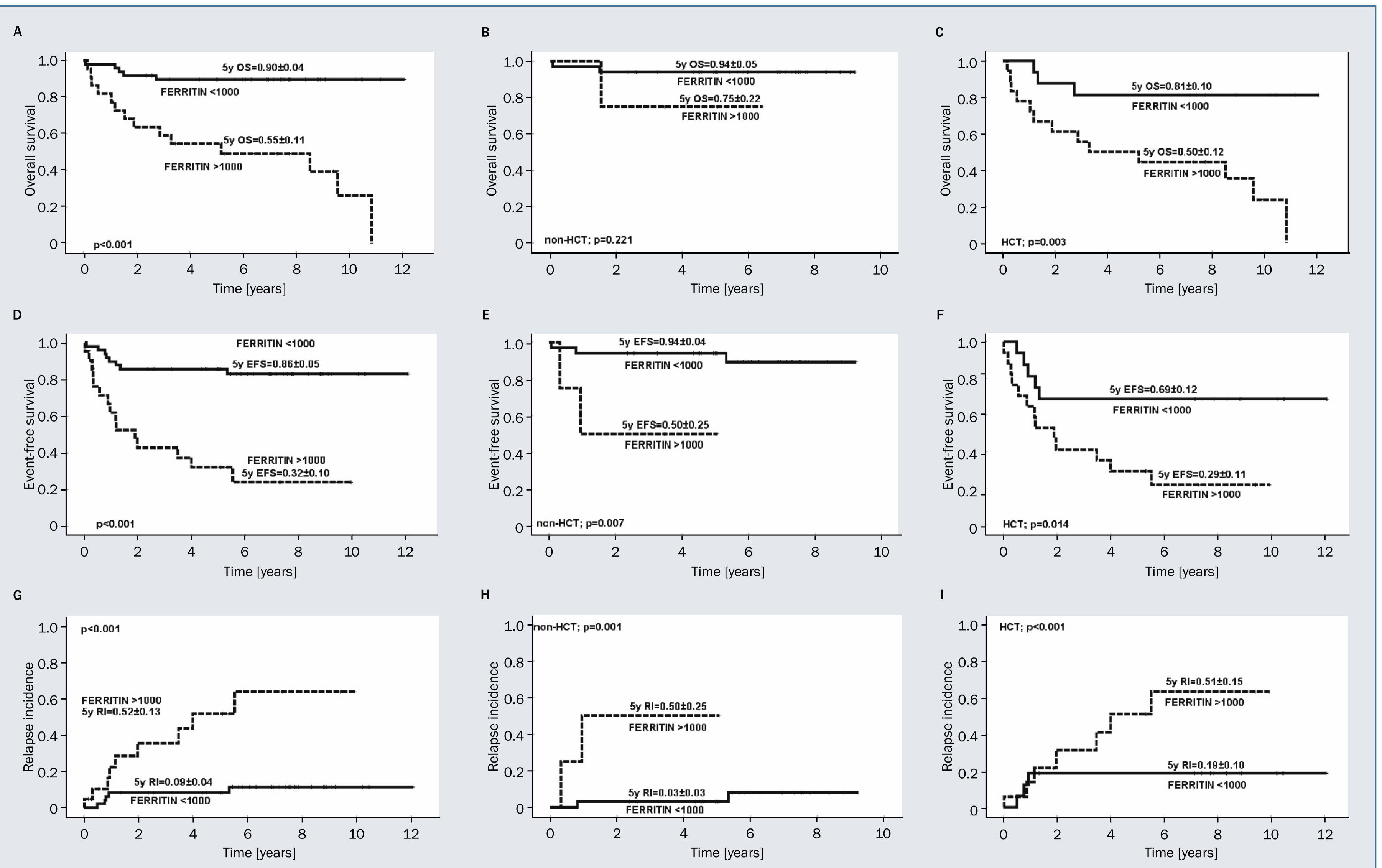

Figure 1. Long-term outcomes [overall survival (OS; A–C), event-free survival (EFS; D–F), and relapse incidence (RI; G–I)] with respect to ferritin serum concentration. Results for all patients (A, D, G), non-haematopoietic cell transplantation (HCT) (B, E, H) and HCT patients (C, F, I) are shown with respect to ferritin concentration of 1,000 µg/L; 5y — 5-year

Table V. Univariate and multivariate risk factors analysis for relapse incidence

Parameter	Characteristics	5-year RI	Univariate analysis		Multivariate analysis	
			HR (95%CI)	*p*	HR (95%CI)	*p*
Age	<10 years	0.11 ±0.05	1	0.174	–	–
	>10 years	0.29 ±0.09	2.1 (0.7–5.9)		–	
Diagnosis	ALL	0.14 ±0.05	1	0.188	–	–
	AML	0.37 ±0.13	2.1 (0.7–6.2)		–	
Ferritin	<1,000 µg/L	0.09 ±0.04	1	<0.001	1	<0.001
	>1,000 µg/L	0.52 ±0.13	6.7 (2.2–21)		6.9 (2.3–21)	
Treatment	Non-HCT	0.08 ±0.05	1	0.039	1	0.536
	HCT	0.33 ±0.09	3.1 (1.1–10)		1.6 (0.4–5.9)	

HR — hazard ratio; 95%CI — 95% confidence interval; ALL — acute lymphoblastic leukemia; AML — acute myeloblastic leukemia; HCT — haematopoietic cell transplantation

decreased survival. This finding aligns with previous data suggesting iron overload to be strongly correlated with a poor prognosis in patients with MDS or after HCT [12]. Data on children undergoing non-HCT treatment for leukemia and other types of malignancy is inconsistent, with iron overload rates ranging from 24–90% of children (according to [13]), and the clinical consequences of iron overload with respect to risk groups require additional research. As yet, there are no clear guidelines in terms of recommendations for iron overload screening.

Our study has several limitations. It was a retrospective study, meaning that other possible factors influencing iron metabolism could not be taken into account [14]. Also, the impact of disease stage and other possible therapy complications [15–19] were not analysed. The validity of the assessment of ferritin as an indicator related to the clinical course of the disease after treatment in paediatric leukemia is debatable, because serum ferritin might normalise during follow-up in some children [20]. Another concern is the measurement of parameters in different time intervals of blood samples. The number of administered transfusions also was not taken into consideration. Our group of patients was heterogeneous in terms of disease stage and donor type.

Based on our results, we propose the monitoring of ferritin and iron concentrations in all patients with acute leukemia, especially those after the consolidation phase of chemotherapy and those undergoing HCT. Ferritin level >1,000 µg/L should be taken into account as a significant prognostic factor of death.

Conclusion

In conclusion, a high ferritin concentration is an adverse prognostic factor for overall survival and event-free survival, and contributes to a higher relapse incidence in children after treatment for acute lymphoblastic or myeloblastic leukemia.

Authors' contributions

JS, MŁ — study design and manuscript writing; MŁ, RD, KC, JS — collection and analysis of data. All authors — critical review 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; and Uniform requirements for manuscripts submitted to biomedical journals.

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ORIGINAL RESEARCH ARTICLE

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Ferric reducing ability of plasma: a potential oxidative stress marker in stored plasma

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Abstract

Introduction: The ferric reducing ability of plasma (FRAP) assay is used for measuring the antioxidant capacity. FRAP is proportional to the molar concentration of the antioxidant capacity. This study attempts to analyze the possibilities of FRAP as an indicator of oxidative stress.

Methods: Blood was drawn from male Wistar rats and stored for 20 days at 4°C in citrate phosphate dextrose adenine 1. The rats were divided into two groups: controls and experimentals. The experimentals were added with antioxidants – L-carnitine, curcumin, vitamin C (VC), and caffeic acid of varying concentrations – 10, 30, and 60 mM (n =5 for each group). Plasma was isolated from these samples at regular intervals (every 5 days), and FRAP and protein were assayed. Results were analyzed by two-way ANOVA, using GraphPad prism 6. FRAP was maintained in controls.

Results: VC (ascorbic acid) was the most potent antioxidant in terms of FRAP during storage compared with the above antioxidants. This study emphasizes the use of FRAP as a potential marker of oxidative stress in plasma of stored blood.

Conclusion: FRAP can be utilized as a reliable marker for determining the antioxidant capacity.

Key words: plasma, antioxidants, FRAP, blood storage

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Introduction

Ferric reducing ability of plasma (FRAP) is an assay that is used for measuring the antioxidant power. This assay is based on the reduction of a Fe^{3+} complex of tripyridyltriazine ($Fe(TPTZ)^{3+}$) to $Fe(TPTZ)^{2+}$ which is intensely in blue color at low pH. Excess Fe^{3+} is utilized and $Fe(TPTZ)^{2+}$ is the rate-limiting factor. Thus the color formation reflects the reducing ability of the sample [1, 2]. However, FRAP was developed to give a more biologically relevant overview than individual biomarkers of oxidative stress (OS). Antioxidants (endogenous and exogenous) together provide protection against reactive oxygen species (ROS) than individual compounds. Therefore overall antioxidant capacity, such as FRAP, gives a cumulative effect of all the antioxidants present than individual components. FRAP is the only assay that measures the antioxidants directly when compared with other assays that measure the inhibition of free radicals. FRAP is directly proportional to the concentration of the electron-donating antioxidants [3]. FRAP can be used as a single test for the estimation of total antioxidant capacity of blood. FRAP describes the prooxidant–antioxidant equilibrium better than other assays [4]. FRAP does not measure thiol antioxidants and the reduction of ferric ions [5, 6]. However, FRAP has gained importance as it is simple, cost-effective, straightforward, fast, and highly reproducible compared with other tests of total antioxidant capacity [4].

During storage of blood, OS is induced which causes irreversible damage that limits its shelf life [7]. OS represents an imbalance between the ROS produced and the biological system's ability to counteract or detoxify the ROS or repair the resulting damage caused [8]. Blood and its components are stored in different storage solutions. The most commonly used storage solution is citrate phosphate dextrose adenine 1 (CPDA-1). Blood and its components possess an innate antioxidant system that helps in

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protecting itself against the ROS [9]. Since plasma holds all the blood's cellular components in suspension, it provides an overview of the OS microenvironment over storage.

Free radicals are highly unstable molecules that can cause OS, triggering cellular damage. Antioxidants combat these free radicals, thereby providing a protective effect [10]. An antioxidant is defined as "any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" [11]. Various studies have reported the beneficial effects of antioxidants (L-carnitine [LC], curcumin [Cu], vitamin C [VC]) in blood storage solutions [12–22].

LC (L-3 hydroxy-4-*N*-*N*-*N*-trimethylaminobutyrate) is one of the nutrient-derived, non-enzymatic antioxidants, which plays an important role in fatty acid turnover. LC, the biologically active stereoisomer, is an endogenous compound derived from the diet or synthesized in the liver from lysine and methionine. It acts as an antioxidant that reduces metabolic stress in cells, thus reducing OS [21, 23].

Cu (1,7-*bis*(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) or diferuloylmethane, a component of *Curcuma longa* (turmeric), possesses antioxidant activity and free radical scavenging activity. Cu increases intracellular glutathione (GSH) and regulates antioxidant enzymes. It also protects oxyhemoglobin from nitrite-induced oxidation [12, 22].

VC or ascorbic acid is a cofactor for at least eight enzymatic reactions. Ascorbic acid acts as a reducing agent. The oxidized forms of VC are semidehydroascorbic acid and dehydroascorbic acid. Ascorbate is maintained in its reduced state by glutathione and NADPH-dependent reactions [24, 25].

Caffeic acid (CA; 3,4-dihydroxycinnamic acid) and its conjugates (chlorogenic acid and caftaric acid) are powerful antioxidants [26]. They are ubiquitous in nature, found in almost every plant. Thus, there is a high potential to utilize this antioxidant [27]. They prevent the formation of mutagenic and carcinogenic compounds as they inhibit the N-nitrosation reactions [28].

Studies have reported the use of FRAP to determine the antioxidant capacity of various extracts [29–33]. However, FRAP as an OS marker during blood storage has not been reported. Thus, this study attempts to analyze the possibilities of FRAP as an indicator of OS.

Methods

Animal care and maintenance was in accordance with the ethical committee regulations (841/b/04/CPCSEA).

Blood sampling

Animals were lightly anesthetized with ether and restrained in dorsal recumbancy as described earlier [34]. In brief, the syringe needle was inserted just below the xyphoid cartilage and slightly to the left of the midline. Of note, 4–5 mL of blood was carefully aspirated from the heart into 5 mL polypropylene collection tubes with CPDA-1 (sodium dihydrogen orthophosphate 2.22 g/L, citric acid 3.27 g/L, sodium citrate 26.3 g/L, dextrose 31.9 g/L, and adenine 0.27 g/L) [35].

Experimental design

Blood was drawn from 65 male *Wistar* rats (4 months old) and divided into two groups: controls and experimentals. The experimentals were added with antioxidants — LC, Cu, VC, and CA of varying concentrations — 10, 30, and 60 mM and n =5 for each group and stored for 20 days at 4°C. Plasma was isolated from whole blood at regular intervals (every 5 days) and assayed for FRAP (Figure 1).

Plasma separation

Plasma was isolated from 1 mL whole blood in microcentrifuge tubes by centrifuging in a fixed angle rotor for 20 min at 1,000 g. The plasma was removed and stored at –20°C for further assays [36].

Ferric reducing ability of plasma

The FRAP assay was performed as described by Benzie and Strain [2]. In brief, sample was added to freshly prepared FRAP reagent (300 mM acetate buffer [pH 3.6], 10 mM TPTZ, and 20 mM $FeCl_3$). The reaction mixture was incubated for 5 min at 37°C and absorbance was read at 593 nm. FRAP was determined by using the extinction coefficient of 21,250 $mM^{-1}cm^{-1}$.

Protein estimation

Protein was determined in the plasma by the method of Lowry et al. [37], using bovine serum albumin (BSA) as the standard.

Statistical analyses

Results are represented as mean ± standard error (SE). Values between the groups (storage period) and subgroups (antioxidants) were analyzed by two-way ANOVA and were considered significant at $p < 0.05$. Bonferroni post-test was performed for FRAP using GraphPad Prism 6 software.

Results

Results are represented as 1) changes during the storage in all groups with day 0 and 2) changes between different concentrations against control on a particular day.

FRAP was maintained during storage in controls. Changes in FRAP were significant in all experimental groups.

L-carnitine

FRAP increased by 85% and 52% on days 10 and 20, respectively, against day 0 in LC 10. Decrements of 80%

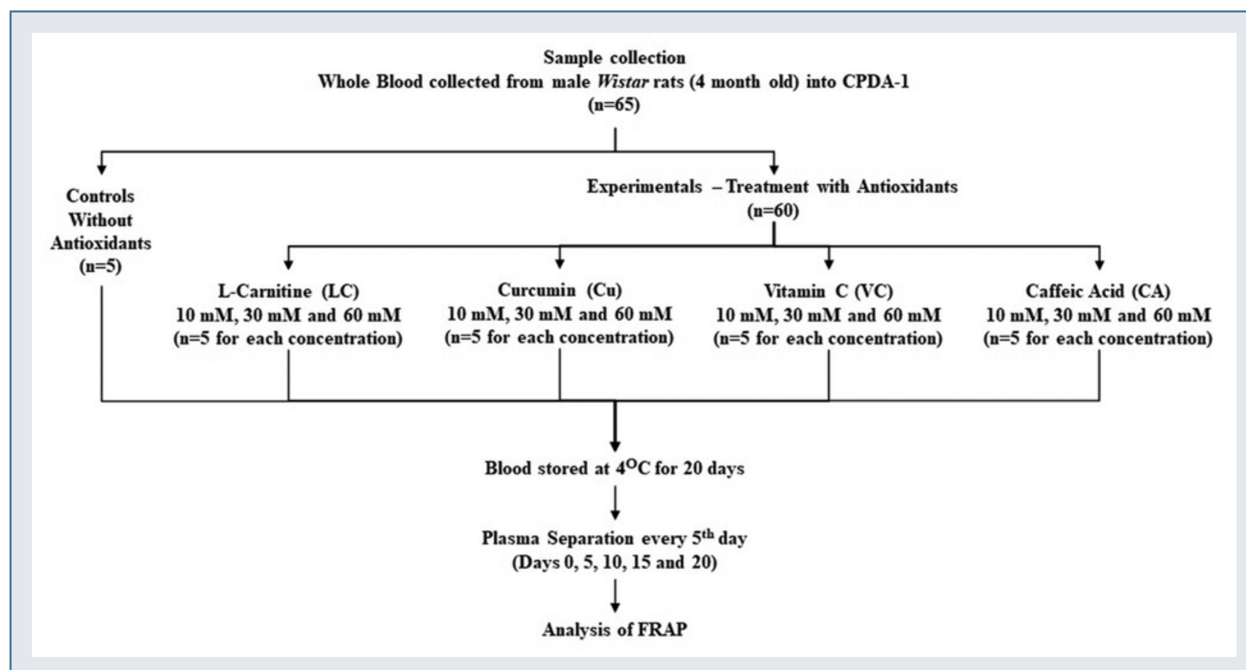

Figure 1. Experimental design; CPDA-1 — citrate phosphate dextrose adenine 1; FRAP — ferric reducing ability of plasma

were observed in LC 30 and LC 60 on all days when compared with day 0.

Increments of 100% and 55% were observed on days 10 and 20, respectively, in LC 10 against their controls. FRAP increased by 73% on day 0, while it decreased by 73% (days 5 and 20), and 65% (days 10 and 15) in LC 30 and LC 60 with controls (Figure 2).

Curcumin

FRAP increased by 51% in Cu 10 on day 10 and 100% in Cu 30 from day 10 to day 20, and Cu 60 on days 5, 10, 15, and 20 against day 0. Changes in FRAP were insignificant in Cu 10 against controls. FRAP elevated by twofold on days 10, 15, and 20 in Cu 30 with respect to controls. Elevations of one-fold (days 5 and 15) and two-fold (days 10 and 20) were also observed in Cu 60 (Figure 3).

Vitamin C

FRAP levels decreased by 63% (day 10), 37% (day 15), and 55% (day 20) against day 0 in VC 10. Elevation of 42% was observed on day 5, whereas decrements of 44%, 22%, and 27% were observed on days 10, 15, and 20 in VC 30. FRAP elevated by 13-fold (days 5 and 10), 16-fold (day 15), and 1-fold (day 20) in VC 60. FRAP increased by twofold on days 0 and 5 in VC 10 against their respective controls. Increments of threefold (day 0), fourfold (day 5), onefold (day 10), and twofold (days 15 and 20) were observed in VC 30 against controls. FRAP also elevated by threefold on days 5, 10, and 15 in VC 60 (Figure 4).

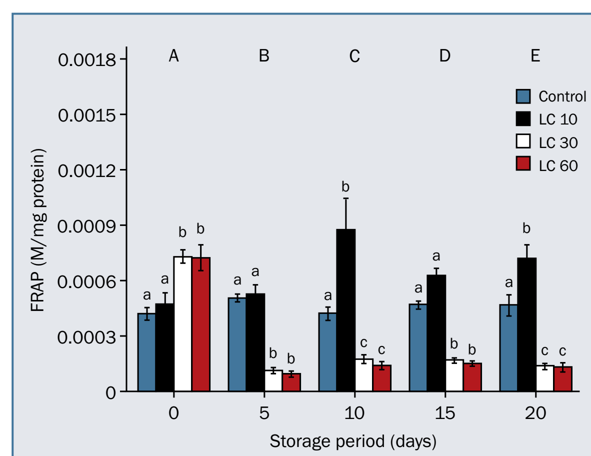

Figure 2. Effect of L-carnitine on ferric reducing ability of plasma (FRAP) during storage. LC 10 = L-carnitine 10 mM, LC 30 = L-carnitine 30 mM, and LC 60 = L-carnitine 60 mM. Values are mean ± standard error (SE) of five animals per group. Two-way ANOVA was performed between the groups and subgroups to analyze FRAP, followed by Bonferroni post-test, using GraphPad Prism 6 software. Changes between the groups (storage period) are represented in upper case. Changes within the groups (treatment–antioxidant concentrations) are represented in lower case. Those not sharing the same letters are significantly different; A, B, C, D, E — changes between the groups (storage days); a, b, c — changes within the groups (treatment-antioxidant concentrations on a particular day)

Caffeic acid

FRAP was maintained in CA samples throughout the storage period. A decrement of 32% was observed in CA 10 on day

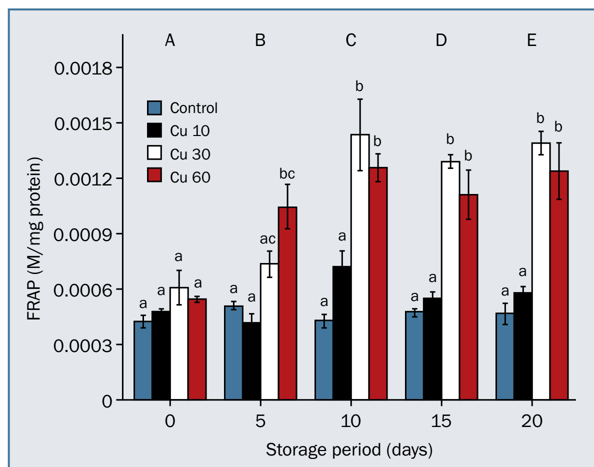

Figure 3. Effect of curcumin on ferric reducing ability of plasma (FRAP) during storage. Cu 10 = curcumin 10 mM, Cu 30 = curcumin 30 mM, and Cu 60 = curcumin 60 mM. Values are mean ± standard error (SE) of five animals per group. Two-way ANOVA was performed between the groups and subgroups to analyze FRAP, followed by Bonferroni post-test, using GraphPad Prism 6 software. Changes between the groups (storage period) are represented in upper case. Changes within the groups (treatment–antioxidant concentrations) are represented in lower case. Those not sharing the same letters are significantly different; A, B, C, D, E — changes between the groups (storage days); a, b, c — changes within the groups (treatment-antioxidant concentrations on a particular day)

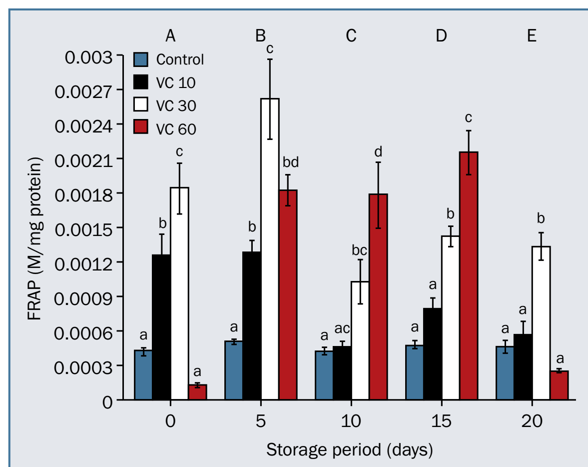

Figure 4. Effect of vitamin C on ferric reducing ability of plasma (FRAP) during storage. VC 10 = vitamin C 10 mM, VC 30 = vitamin C 30 mM, and VC 60 = vitamin C 60 mM. Values are mean ± standard error (SE) of five animals per group. Two-way ANOVA was performed between the groups and subgroups to analyze FRAP, followed by Bonferroni post-test, using GraphPad Prism 6 software. Changes between the groups (storage period) are represented in upper case. Changes within the groups (treatment–antioxidant concentrations) are represented in lower case. Those not sharing the same letters are significantly different; A, B, C, D, E — changes between the groups (storage days); a, b, c, d — changes within the groups (treatment-antioxidant concentrations on a particular day)

20 when compared with day 0. FRAP increased by 100% on days 0, 5, 10, and 15, and 56% on day 20 in CA 10 against controls. Increments of twofold were observed on all days in CA 30. FRAP also elevated by threefold (days 0 and 10) and twofold (days 5, 15, and 20) in CA 60 (Figure 5).

Discussion

FRAP is proportional to the molar concentration of antioxidants present. An increase in FRAP value is usually a desirable phenomenon as it proves a better protection against OS [38]. FRAP assay depends on the reduction of the TPTZ complex (Fe^{3+} to Fe^{2+}) by a reductant (plasma constituents) at low pH. The Fe^{2+} complex results in the blue coloration that can be detected at 593 nm [8, 39].

FRAP was maintained in controls over the storage period, indicating that the innate antioxidant system present in plasma can combat the OS induced during storage.

LC is an effective antioxidant as it possesses radical scavenging (superoxides, hydrogen peroxide), metal chelating activity, and great reducing power [40]. It contributes to the antioxidant defense by 1) directly scavenging free radicals, 2) preventing the formation of free radicals, 3) maintaining the redox state of cells, and 4) activating vitagens [41]. LC also stabilizes the energy balance across cell membranes and enhances carbohydrate metabolism, along with maintaining the cell volume and fluid balance [42], thus protecting the erythrocyte membrane. LC reduces OS as it increases the antioxidant activity and sulfhydryls while it reduces lipid peroxidation [43]. LC at 10 mM is more beneficial than at 30 and 60 mM in terms of FRAP. LC at 10 mM may be the optimum concentration to maintain the antioxidant capacity.

Cu (phenolic chain-breaking antioxidant) donates hydrogen atoms from the phenolic group or through the central methylenic hydrogen. This is responsible for the antioxidant property of Cu [44, 45]. Cu at higher concentrations upregulates the antioxidant enzyme activity and reduces lipid peroxidation and protein oxidation [12]. Thus FRAP was directly proportional to the concentration of Cu.

VC reduces metal ions (such as iron) that are present in the active sites of mono- and dioxygenases. It acts as a co-substrate rather than a coenzyme [46]. Ascorbate also assists in the regeneration of α-tocopherol from the α-tocopheryl radical. It reacts with radicals to form an intermediate radical (ascorbate radical) of low reactivity. [47]. VC at all concentrations upregulated FRAP. This can be attributed to VC's potent ferric reducing ability. It reduces Fe^{3+} similar to hydroxylamine [48].

CA protects α-tocopherol in low-density lipoprotein [26]. CA and its analogs are antioxidants with multiple mechanisms that include free radical scavenging and metal ion

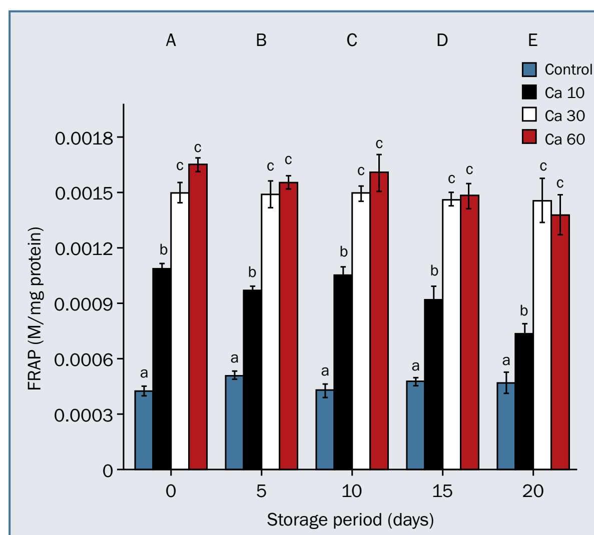

Figure 5. Effect of caffeic acid on ferric reducing ability of plasma (FRAP) during storage. CA 10 = caffeic acid 10 mM, CA 30 = caffeic acid 30 mM, CA 60 = caffeic acid 60 mM. Values are mean ± SE of five animals per group. Two-way ANOVA was performed between the groups and subgroups to analyze FRAP, followed by Bonferroni post-test, using GraphPad Prism 6 software. Changes between the groups (storage period) are represented in upper case. Changes within the groups (treatment–antioxidant concentrations) are represented in lower case. Those not sharing the same letters are significantly different; A, B, C, D, E — changes between the groups (storage days); a, b, c — changes within the groups (treatment-antioxidant concentrations on a particular day)

chelation, and they inhibit free radical and lipid hydroperoxide formation [49]. CA increased FRAP at all concentrations and hence FRAP was proportional to the concentration of CA. This can be attributed to CA's potent free radical scavenging, metal chelating property, and its effective reducing power. It has a greater reducing power than the standard compounds such as butylated hydroxytoluene, butylated hydroxyanisole, trolox, α-tocopherol, *etc.* [26].

Conclusion

FRAP is a potential marker of OS in plasma of stored blood as it reflects the antioxidant capacity and has a positive correlation with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [29]. Thus, FRAP can be utilized as a reliable marker for determining the antioxidant capacity. VC (ascorbic acid) was the most potent antioxidant in terms of FRAP during storage, with respect to the above antioxidants (VC > CA > Cu > LC).

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Authors' contributions

CH — performed the research, analyzed the data, and prepared the manuscript. VR — designed the study and edited the manuscript.

Conflict of interest

The authors have no conflict of interest (personal or financial) to disclose.

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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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SHORT COMMUNICATION

VIA MEDICA

Intravascular lung coagulopathy by COVID-19

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Introduction

Coronavirus disease 2019 (COVID-19), caused by a new coronavirus (SARS-CoV-2, severe acute respiratory syndrome coronavirus 2), is a highly contagious disease that first appeared in Wuhan, China, in December 2019 [1]. On 30 January 2020, the World Health Organisation (WHO) declared that the COVID-19 outbreak constituted a public health emergency of international concern (PHEIC) [2, 3]. On 11 March 2020, the WHO officially declared a pandemic [3].

Most infected patients have mild symptoms including fever, fatigue, and cough [1, 4]. However, severe cases can progress rapidly and develop adult respiratory distress syndrome (ARDS) with coagulopathy [1, 4]. The latter is already described as one of the most important independent poor prognostic factors [5, 6]. Thus, the objective of this investigation was to analyse the coagulation alterations produced by COVID-19, and assess whether they present different characteristics to the coagulopathy present in other pathologies.

Methods

From the Haemostasis and Coagulation Laboratory, we selected 15 consecutive patients who had D-dimer values at least four times higher than normal (0.1–0.5 μg/mL), positivity by polymerase chain reaction (PCR) of SARS-CoV-2, and pneumonia on chest radiography. All of them were admitted during March 2020 to the Mostoles University Hospital (Madrid), which cares for a population of approximately 169,000 patients. During the months of March and April, the number of new patients with COVID-19 pneumonia admitted to the Hospital was 515 and 358, respectively.

All patients, coinciding with a scheduled extraction, had a blood sample drawn to determine basic coagulation parameters such as the prothrombin time ratio (PT ratio), activated partial thromboplastin time ratio (APTT ratio), thrombin (TT), D-dimer and fibrinogen. In addition, the von Willebrand factor (vVF:Ag and vWF:Rco), blood group ABO, natural anticoagulants such as antithrombin (AT), protein C and protein S, were analysed, and coagulation factors II, V, VII, VIII, IX, X, XI, and XII were dosed. All of the determinations were made using STA Compact MAX and R MAX analysers, along with their original reagents (Diagnostica Stago, Saint-Denis, France). A clot solubility assay in 5M urea was performed as a factor XIII deficiency screening method. The determination of lactate dehydrogenase, C-reactive protein (Cobas Roche with its original reagents) and ferritin (ECLIA electrochemiluminescence) were also included in the analysis.

In addition, we collected demographic data of the patients (age and sex), the number of hospital admission days at the time of sample extraction, the occurrence of thrombotic events such as pulmonary thrombosis diagnosed by imaging test [angiography computed tomography (angio-CT)], and data showing if they were previously receiving antithrombotic prophylaxis. Moreover, the severity of the patients' infectious picture, the appearance of other related complications, and information determining if eventually the patient improved or died, were also acquired.

Results

Out of the 15 patients included in the study, seven were women and eight were men, with a median age of 63 years (range 30–86). Median days of hospitalisation at the time of sample collection was 6 (range 1–12) (Table I). All patients presented pneumonia confirmed by chest radiography from admission; 11 of them required invasive mechanical ventilation in the Intensive Care Unit. Before admission, none of them were on anticoagulant treatment. Prophylactic low-molecular-weight heparin (LMWH) was prescribed at standard doses (40 mg of enoxaparin per day) from the moment of admission, except for two patients who empirically received anticoagulant doses because they presented D-dimer levels 20 times higher than normal (0.1–0.5 μg/mL); and one who did not receive it due to thrombocytopenia (22 $\times 10^3$/μL platelets).

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Table I. Clinical characteristics of patients

Parameter	Value
Age	Median 63 years (range 30–86)
Sex	7 (46.7%) female 8 (53.3%) male
Thrombotic complications diagnosed by imaging test	6 (60%) pulmonary thrombosis 1 (6.7%) ischaemic stroke 1 (6.7%) myocardial infarction
Haemorrhagic complications	0 (0%)
Clinical evolution	7 (46.7%) death 5 (33.3%) hospital discharge 3 (20%) ICU

ICU — Intensive Care Unit

10 of the 15 patients underwent angio-CT after observing the elevation of D-dimer, and six of the 10 (60%) had peripheral pulmonary thrombosis. As previously discussed, 5/6 patients were receiving prophylactic LMWH at standard doses prior to diagnosis. The remaining patient did not receive it, because at the time of admission he had thrombocytopenia of 22 $\times 10^3$/μL, presenting a thrombotic event 48 hours after it. This patient, who was diagnosed with essential thrombocythemia, presented a multiple ischaemic stroke with subsequent haemorrhagic transformation, as well as an acute myocardial infarction.

Subsequently, eight patients died as a result of COVID-19 lung infection, five were definitively discharged, and two remain in long-stay hospitals for recovery at the time of writing this review (Table I).

D-dimer values at the time of the study were between 2.39 and 172.33 μg/mL (median 16.39 μg/mL); significantly higher in relation to the DD value on admission day (median 0.86 μg/mL, range 0.47–13.19 μg/mL). The platelet count was within normality in 80% of the patients and slightly decreased in the rest (>80 $\times 10^3$/μL). The PT ratio was also normal, except for one patient in whom the determination after the admission figure was normalised. Fibrinogen was above the upper limit of normality in 85.7% of patients (12/14), while in the rest it was normal, and the APTT ratio was normal or shortened. The TT was normal, except in three patients due to the effect of LMWH (Table II).

None of the patients included in the study met DIC criteria. No significant decrease in AT, PC and PS was observed. Only one patient had a slightly decreased determination of AT (76%) and PC (64%), and another of coagulative PS (51%). In both patients, the determination was made in the context of a pulmonary thrombosis diagnosed by angio-CT, so the decrease was not measurable given the consumption of coagulation factors in the acute thrombotic event.

A highly increased factor VIII and vWF (medians of 267% and 466.5% respectively) were found in all patients. The blood group was tested in 14 patients, with nine of them being from blood group A, four from group O, and one from group B. No significantly higher values of factor VIII or vWF were observed in the non-O groups compared to the O group.

We did not observe a significant decrease in the rest of the coagulation factors, with the exception of four patients who presented a minimal decrease in factors II and V, and one patient in factor XII (Table II).

Discussion

The main finding of our study was that there is no factor consumption, nor a decrease in natural anticoagulants, while all the patients presented a factor VIII and a vWF greatly increased, reflecting the state of hypercoagulability they presented, although this assertion is just a hypothesis that requires further investigation.

These findings are in contrast to findings reported in other studies about coagulopathy in infectious diseases. As observed in our study, the alterations found in patients with severe SARS-CoV-2 infection are different from the coagulopathy found in disseminated intravascular coagulation (DIC) [7]. This finding is reflected in the constant observation that platelet counts and fibrinogen concentrations do not decrease significantly in patients with COVID-19, despite the marked increase in D-dimer concentrations [8, 9].

However, given the sparse knowledge that we still have about the SARS-CoV-2 infection, we believe that the analytical alterations do not offer a good reflection of the alterations at the local level. Whether microthrombosis exists at this level is beyond the scope of this study.

We do not have a real incidence of thrombotic events. In our study, six patients were diagnosed with pulmonary thrombosis after requesting imaging tests. Some of the remaining patients may also have it, but it is an event that has not been confirmed. At the present time, a retrospective study is underway, organized by Dr. Llamas Sillero, Head of Haematology Department of Fundacion Jimenez Diaz University Hospital (Madrid), and endorsed by the Madrid Society of Haematology, which aims to make an accurate estimation of them in the Community of Madrid.

Thus, the concept of pulmonary intravascular coagulopathy (CIP) seems correct to us [9] for this condition with activation of coagulation located in the lung, where an environment of hypercoagulability occurs, not caused directly by the virus, but rather secondary to three triggers:

1) inflammation, both produced by cellular damage caused by cells damaged by the virus (type II pneumocytes), as well as secondary to innate immunity. This inflammation would activate coagulation through tissue factor expressed by macrophages, neutrophils,

Table II. Laboratory parameters

Parameter	Reference range	n	Median (min–max)	< lower limit Reference	> upper limit Reference
LDH	135–225 U/L	15	479 (227–768)	0 (0%)	15 (100%)
Ferritin	50–220 ng/mL	15	1,544 (394–6,230)	0 (0%)	15 (100%)
CRP	0–5 mg/L	15	239.4 (0.7–598.8)	0 (0%)	14 (93.3%)
Platelets	150–450 $\times 10^3$/µL	15	294 (81–426)	3 (20%)	0 (0%)
PT ratio	0.9–1.25	15	1.12 (1–1.31)	0 (0%)	1 (6.7%)
APTT ratio	0.86–1.18	15	0.93 (0.8–1.04)	2 (13.3%)	0 (0%)
Fibrinogen	200–400 mg/dL	14	535.5 (201–848)	0 (0%)	12 (85.7%)
D-dimer on admission	0.1–0.5 µg/mL	13	0.86 (0.47–13.2)	0 (0%)	11 (84.6%)
D-dimer at time of study		15	16.39 (2.39–172.3)	0 (0%)	15 (100%)
TT	16–21 s	12	18.75 (16.4–27.1)	0 (0%)	3 (25%)
Factor II	70–120% (95%)	15	104 (66–129)	2 (13.3%)	1 (6.7%)
Factor V	70–120% (95%)	15	113 (52–122)	2 (13.3%)	5 (33.3%)
Factor VII	55–170% (112.5%)	15	99 (55–183)	0 (0%)	3 (20%)
Factor X	70–120% (95%)	15	106 (77–135)	0 (0%)	4 (26.7%)
Factor VIII	60–150% (105%)	15	267 (128–383)	0 (0%)	15 (100%)
vWF:Ag	50–150% (100%)	12	406.5 (255–420)	0 (0%)	12 (100%)
vWF:Rco	50–200% (125%)	10	236 (from 156 to >200)	0 (0%)	7 (46.7%)
Factor IX	60–150% (105%)	15	151 (107–225)	0 (0%)	8 (53.3%)
Factor XII	60–150% (105%)	15	101 (35–139)	1 (6.7%)	0 (0%)
Factor XIII*		15	Negative 15 (100%)		
AT III	80–120% (100%)	15	115 (76–135)	1 (6.7%)	5 (33.3%)
PC	70–130% (100%)	15	99 (64–181)	1 (6.7%)	3 (20%)
Free PS	M: 70–148% (109%)	8	109.5 (87–179)	0 (0%)	1 (12.5)
	F: 50–134 (92%)	7	90 (67–129)	0 (0%)	0 (0%)
Functional PS	M: 77–143% (110%)	8	94.5 (89–137)	0 (0%)	0 (0%)
	F: 55–123 (89%)	7	64 (51–125)	1 (14.3%)	1 (14.3%)
APC ratio	≥2.9	13	3.58 (3.27–4.34)	0 (0%)	

*Clot solubility assay in urea 5M; LDH — lactate dehydrogenase; CRP — C-reactive protein; PT — prothrombin time; APTT — activated partial thromboplastin time; TT — thrombin time; vWF — von Willebrand factor; Ag — antigen; Rco — ristocetin cofactor; AT — antithrombin; PC — protein C; PS — protein S; f; APC — activated protein C ratio

endothelium, platelets and extracellular vesicles, phosphatidyl serine, free DNA, damage-associated molecular patterns (DAMPs), extracellular neutrophil traps (NETs) or platelet activation;

2) the endothelial lesion, which loses its protective layer and alters its regulatory function of fibrinolysis in order to avoid the formation of thrombi on its surface, and its regulatory function of vascular permeability. In addition, there is direct endothelial damage from the virus since the endothelium, like type II pneumocytes, also has angiotensin-converting enzyme (ACE2R) receptors [10];
3) hypoxia that causes cellular damage and regulates coagulation and fibrinolysis through hypoxia-inducible transcription factors (HIFs) [11].

The role of immunothrombosis in containing bacteria is well known, but its role in viral infections is not well understood [9]. In any case, when it occurs excessively, as is happening in many of the patients with SARS-CoV-2 pneumonia, it can be more harmful than the virus itself.

Conclusions

After analysing the results of the referred determinations, we can establish:

- classical DIC does not occur in COVID-19 associated coagulopathy (CAC). There is no consumption of factors, nor a decrease in natural anticoagulants. There is a significant increase in factor VIII and vWF, due to extensive endothelial injury, which seems to play a leading role in pulmonary microthrombosis, and in injury to other affected organs (heart, kidneys, small intestine);

- the elevation of D-dimer suggests that there is local coagulation activation in the lung, where fibrinolysis is increased, but with an overall balance in favour of thrombosis;
- what analytics 'globally' reflects does not seem to represent what is happening at the local level. In the places where microthrombosis is taking place, it is very likely that the levels of factors and anticoagulants are different from those found in this study, which points to the idea of a different haemostatic microenvironment.

Regarding the anticoagulation in these patients, given the extensive endothelial injury, we suggest that heparin, which apart from being an anticoagulant can also protect the endothelium [8] which is especially affected in this pathology, would be, given the data available so far, the chosen anticoagulant drug for both thromboprophylaxis and for the treatment of established venous thromboembolism (VTE) [8, 12], at least in the acute moment. It will be interesting to observe the outcomes of patients who, for various reasons, have been treated with direct anticoagulants.

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Authors' contributions

BFJ — study design; LMM, BFJ — selecting patients, analysing variables, writing article.

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

VIA MEDICA

Recurrent and isolated central nervous system blast crisis in chronic myelogenous leukemia

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Introduction

Chronic myelogenous leukemia (CML) is the commonest myeloproliferative neoplasm encountered and it is characterized by the hallmark translocation t(9;22) or *BCR-ABL1* transcripts. The natural history of the disease has three phases: the chronic phase (CP) followed by progressive transformation to the accelerated or blast phase. The defining criteria for the blast phase are the presence of at least 20% blasts in peripheral blood or bone marrow or extramedullary proliferation of blasts [1]. Infiltration of the central nervous system as the sole site of extramedullary blast crisis is exceptional and deserves attention.

Clinical vignette

We report the case of a 42-year-old male who was diagnosed as a case of chronic myelogenous leukemia in chronic phase (CML-CP) in August 2016. At the time of diagnosis, hemoglobin (Hb) was 11.2 g/dL, platelet counts were normal, and white blood cell counts were 120 × 10^3/µL with left shift, 7% basophils, and 5% blasts. Bone marrow cytogenetics confirmed the presence of *BCR-ABL1* transcript in all metaphases. Clinically, the patient had splenomegaly 3 cm below the left costal margin. He was started on imatinib therapy. He responded well to therapy achieving major molecular response (MMR) at the end of 1 year (*BCR-ABL1* by reverse-transcriptase polymerase chain reaction [RT-PCR] = 0.015%). However, in November 2018 he presented with severe frontal headache and vomiting. Magnetic resonance imaging (MRI) brain revealed chronic small vessel ischemia and nonspecific demyelination. Bone marrow studies showed a normocellular bone marrow. Flow cytometry of cerebrospinal fluid (CSF) was suggestive of pre-B cell lymphoblasts. He was thus diagnosed as CML in central nervous system (CNS) blast crisis and managed with triple intrathecal chemotherapy, which included methotrexate (12 mg), cytosine arabinoside (30 mg), and dexamethasone (4 mg), a total of four doses in a month along with cranial radiotherapy. The patient responded well to the treatment without residual sequelae and was started on the second-generation tyrosine kinase inhibitor (TKI), tablet dasatinib 70 mg twice a day and was being worked up for bone marrow transplantation After 2 months, he again presented with CNS relapse and complained of acute onset ptosis and diplopia in the absence of fever, headache, seizure, or any trauma. Ophthalmologic examination revealed cranial nerve III palsy with pupillary sparing. Haematological parameters were Hb 10.0 g/dL, TLC 3,200/ /µL, and platelet count 177 × 10^3/µL. Bone marrow study revealed cellular reactive marrow without an increase in blasts. MRI brain showed nonspecific white matter changes. The CSF study showed markedly elevated white blood cell (WBC) count of 270/µL with 90% blasts (Figure 1); the total protein was 56 mg/dL with raised globulins, glucose was 55 mg/dL, and adenosine deaminase and lactate dehydrogenase levels were normal. CSF flow cytometry this time again was consistent with pre-B lymphoblasts (positive for CD10, CD19, CD79a, HLA-DR and negative for CD13, CD33, CD117, cyto MPO). He was again started on triple intrathecal chemotherapy and cranial radiotherapy. However, he had progressive deterioration in his neurological status and succumbed to his illness after two cycles of chemotherapy. Postmortem could not be performed due to unwillingness by family members.

Discussion

Extramedullary blast crisis in CML is rare and CNS involvement is a rarity that is noted in only 5–10% of patients [2]. Our patient had two episodes of isolated CNS blast crisis, despite having achieved haematologic and molecular

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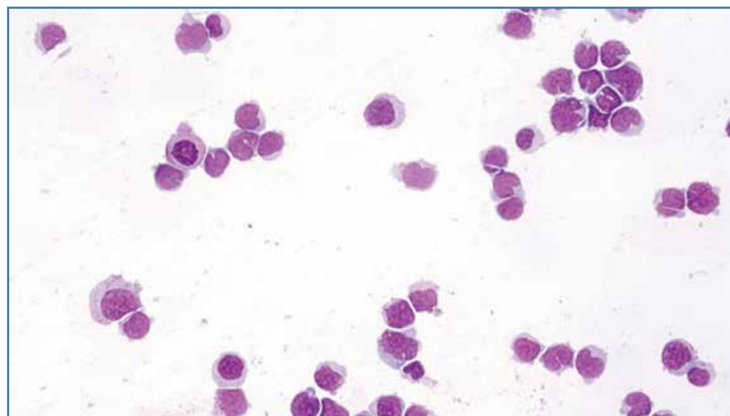

Figure 1. LG stained cytospin smears of cerebrospinal fluid. Markedly cellular smears showing atypical mitosis, numerous blasts which have high N:C ratio, a large nucleus with open chromatin, and prominent nucleoli 400 ×

remission. The most plausible explanation for extramedullary relapse is the poor penetration of imatinib through the blood–brain barrier, hence CNS serves as the sanctuary site leading to relapses. Second-generation TKIs such as dasatinib, nilotinib, and bosutinib are more effective in treating CNS disease due to better penetration through the blood–brain barrier [3]. Unfortunately, our patient relapsed even with dasatinib treatment. Rare case reports of isolated CNS relapse with dasatinib therapy like our patient have been reported [4, 5].

Systemic chemotherapy, which includes daily cytarabine 200 mg/m^2 and daunorubicin 40 mg/m^2 have been used for treatment in CML blast crisis. So far, allogeneic hematopoietic stem-cell transplantation is still the only way to cure CML and is one of the first treatment options for children with chronic myelogenous leukemia [6].

Despite advances in molecular disease detection and monitoring, there is still a need to identify the risk factors leading to CNS relapses. Unfortunately, CSF study is not included in the CML diagnostic workup or monitoring protocols, unlike other acute leukemias and lymphomas.

Through this case, we intend to highlight the fact and also caution physicians that one should be aware of the possibility of CNS relapse in CML patients on imatinib even if they have achieved haematological and cytogenetic remission. Hence, a high index of suspicion, prompt CSF studies, and aggressive management are key to salvaging CML patients.

Authors' contributions

AS, SS — interpreted patient data regarding the disease; AS — performed the analysis of CSF and flow cytometry and critically revised the manuscript and improved the intellectual content; SS — prepared the draft manuscript.

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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Tasigna® *Nilotynib*

Postać, skład: Kapsułka twarda. Jedna kapsułka zawiera 50 mg, 150 mg lub 200 mg nilotynibu (w postaci jednowodnego chlorowodorku) oraz substancje pomocnicze, w tym laktozę jednowodną, odpowiednio – 39,03 mg, 117,08 mg lub 156,11 mg na kapsułkę. **Wskazania:** Produkt leczniczy Tasigna jest wskazany w leczeniu dorosłych pacjentów oraz dzieci i młodzieży z nowo rozpoznaną przewlekłą białaczką szpikową (CML) w fazie przewlekłej z chromosomem Philadelphia. Produkt leczniczy Tasigna 200 mg jest wskazany ponadto w leczeniu dorosłych pacjentów z CML z chromosomem Philadelphia w fazie przewlekłej lub fazie akceleracji, w przypadku oporności lub nietolerancji na uprzednie leczenie, w tym leczenie imatynibem. Nie są dostępne dane dotyczące skuteczności stosowania u pacjentów z CML w przełomie blastycznym. Produkt leczniczy Tasigna jest wskazany w leczeniu dzieci i młodzieży z CML z chromosomem Philadelphia w fazie przewlekłej, w przypadku oporności lub nietolerancji na uprzednie leczenie, w tym leczenie imatynibem. **Dawkowanie:** Leczenie należy kontynuować tak długo, jak długo przynosi ono korzyść kliniczną dla pacjenta lub do czasu wystąpienia niemożliwych do zaakceptowania działań toksycznych. W razie pominięcia dawki, pacjent nie powinien przyjmować dodatkowej dawki, ale kolejną przepisaną dawkę przyjąć o zwykłej porze. *Dawkowanie u dorosłych pacjentów z CML z chromosomem Philadelphia:* Zalecana dawka produktu leczniczego: – 300 mg dwa razy na dobę u pacjentów z nowo rozpoznaną CML w fazie przewlekłej, – 400 mg dwa razy na dobę u pacjentów z CML w fazie przewlekłej lub w fazie akceleracji, z nietolerancją lub opornością na uprzednie leczenie. Dla dawki 300 mg podawanej dwa razy na dobę dostępne są kapsułki twarde po 150 mg. *Dawkowanie u dzieci i młodzieży z CML z chromosomem Philadelphia:* Dawkowanie u dzieci i młodzieży jest ustalane indywidualnie i zależy od powierzchni ciała pacjenta (mg/m² pc.). Zalecana dawka nilotynibu wynosi 230 mg/m² pc. dwa razy na dobę, w zaokrągleniu do najbliższej wielokrotności dawki 50 mg (nie przekraczając maksymalnej dawki pojedynczej wynoszącej 400 mg) (patrz Tabela 1). Aby uzyskać żądaną dawkę, można łączyć kapsułki twarde Tasigna o różnej mocy. Brak jest doświadczenia w leczeniu dzieci w wieku poniżej 2 lat. Nie ma danych dotyczących dzieci w wieku poniżej 10 lat z nowo rozpoznaną chorobą i istnieją ograniczone dane dotyczące dzieci w wieku poniżej 6 lat z opornością na imatynib lub nietolerancją imatynibu.

Tabela 1. Schemat dawkowania nilotynibu w dawce 230 mg/m² pc. dwa razy na dobę u dzieci i młodzieży

Powierzchnia ciała	Dawka w mg (dwa razy na dobę)	Powierzchnia ciała	Dawka w mg (dwa razy na dobę)
Do 0,32 m²	50 mg	0,98-1,19 m²	250 mg
0,33-0,54 m²	100 mg	1,20-1,41 m²	300 mg
0,55-0,76 m²	150 mg	1,42-1,63 m²	350 mg
0,77-0,97 m²	200 mg	≥1,64 m²	400 mg

Dorośli pacjenci z CML w fazie przewlekłej z chromosomem Philadelphia, którzy byli leczeni nilotynibem w terapii pierwszego rzutu i którzy uzyskali trwałą głęboką odpowiedź molekularną (MR4,5): Można rozważyć zakończenie leczenia u spełniających kryteria dorosłych pacjentów z CML w fazie przewlekłej z chromosomem Philadelphia (Ph+), którzy byli leczeni nilotynibem w dawce 300 mg dwa razy na dobę przez co najmniej 3 lata, jeśli głęboka odpowiedź molekularna utrzymuje się przez minimum jeden rok bezpośrednio przed zakończeniem leczenia. Zakończenie leczenia nilotynibem powinno być inicjowane przez lekarza posiadającego doświadczenie w leczeniu pacjentów z CML (patrz ChPL). U spełniających kryteria pacjentów, którzy zakończą leczenie nilotynibem, konieczne jest comiesięczne monitorowanie poziomu transkryptów BCR-ABL i morfologii krwi z rozmazem przez jeden rok, następnie co 6 tygodni w drugim roku, a później co 12 tygodni. Monitorowanie poziomu transkryptów BCR-ABL musi być wykonywane za pomocą ilościowego testu diagnostycznego zwalidowanego dla pomiaru poziomu odpowiedzi molekularnych w Skali Międzynarodowej (IS) o czułości przynajmniej MR4,5 (BCR-ABL/ABL ≤0,0032% IS). U pacjentów, którzy utracą MR4 (MR4=BCR-ABL/ABL ≤0,01%IS), ale nie utracą MMR (MMR=BCR-ABL/ABL ≤0,1%IS) w fazie bez leczenia, poziom transkryptów BCR-ABL należy monitorować co 2 tygodnie aż do chwili, gdy poziom BCR-ABL powróci do zakresu pomiędzy MR4 a MR4,5. Pacjenci, którzy utrzymają poziom BCR-ABL pomiędzy MMR a MR4 w minimum 4 kolejnych pomiarach mogą wrócić do pierwotnego harmonogramu monitorowania. Pacjenci, którzy utracą MMR, muszą wznowić leczenie w ciągu 4 tygodni od stwierdzenia utraty remisji. Leczenie nilotynibem należy wznowić w dawce 300 mg dwa razy na dobę lub w zmniejszonej dawce wynoszącej 400 mg raz na dobę, jeśli przed zakończeniem leczenia u pacjenta zmniejszono dawkę. U pacjentów wznawiających leczenie nilotynibem należy monitorować poziom transkryptów BCR-ABL co miesiąc aż do ponownego stwierdzenia MMR, a następnie co 12 tygodni (patrz ChPL). *Dorośli pacjenci z CML w fazie przewlekłej z chromosomem Philadelphia, którzy uzyskali trwałą głęboką odpowiedź molekularną (MR4,5) podczas leczenia nilotynibem po wcześniejszym leczeniu imatynibem:* Można rozważyć zakończenie leczenia u spełniających kryteria dorosłych pacjentów z CML w fazie przewlekłej z chromosomem Philadelphia (Ph+), którzy byli leczeni nilotynibem przez co najmniej 3 lata, jeśli głęboka odpowiedź molekularna utrzymuje się przez minimum jeden rok bezpośrednio przed zakończeniem leczenia. Zakończenie leczenia nilotynibem powinno być inicjowane przez lekarza posiadającego doświadczenie w leczeniu pacjentów z CML (patrz ChPL). U spełniających kryteria pacjentów, którzy zakończą leczenie nilotynibem, konieczne jest comiesięczne monitorowanie poziomu transkryptów BCR-ABL i morfologii krwi z rozmazem przez jeden rok, następnie co 6 tygodni w drugim roku, a później co 12 tygodni. Monitorowanie poziomu transkryptów BCR-ABL musi być wykonywane za pomocą ilościowego testu diagnostycznego zwalidowanego dla pomiaru poziomu odpowiedzi molekularnych w Skali Międzynarodowej (IS) o czułości przynajmniej MR4,5 (BCR-ABL/ABL ≤0,0032% IS). Pacjenci z potwierdzoną utratą MR4 (MR4=BCR-ABL/ABL ≤0,01%IS) w fazie bez leczenia (dwa kolejne pomiary wykonywane w odstępie co najmniej 4 tygodni wykazujące utratę MR4) lub utratą większej odpowiedzi molekularnej (MMR=BCR-ABL/ABL ≤0,1%IS) muszą wznowić leczenie w ciągu 4 tygodni od stwierdzenia utraty remisji. Leczenie nilotynibem należy wznowić w dawce 300 mg lub 400 mg dwa razy na dobę. U pacjentów wznawiających leczenie nilotynibem należy monitorować poziom transkryptów BCR-ABL co miesiąc aż do ponownego stwierdzenia wcześniej występującej większej odpowiedzi molekularnej lub MR4, a następnie co 12 tygodni (patrz ChPL). *Dostosowanie lub zmiana dawki:* Jeśli wystąpi toksyczność dotycząca układu krwiotwórczego (neutropenia, trombocytopenia) niezwiązana z chorobą podstawową – białaczką – może być konieczne zaprzestanie podawania leku Tasigna przez pewien czas i (lub) zmniejszenie podawanej dawki: Szczegółowe informacje dotyczące dostosowania dawki u pacjentów z neutropenią i trombocytopenią – patrz ChPL. Jeśli wystąpi umiarkowana lub ciężka, klinicznie istotna toksyczność niedotycząca układu krwiotwórczego, należy przerwać podawanie produktu leczniczego, a pacjenci powinni być monitorowani i odpowiednio leczeni. Jeśli uprzednio podawana dawka wynosiła 300 mg dwa razy na dobę u dorosłych pacjentów z nowo rozpoznaną CML w fazie przewlekłej lub 400 mg dwa razy na dobę u dorosłych pacjentów z CML w fazie przewlekłej lub w fazie akceleracji z opornością na imatynib lub nietolerancją imatynibu, lub 230 mg/m² pc. dwa razy na dobę u dzieci i młodzieży, podawanie leku można wznowić w dawce 400 mg raz na dobę u pacjentów dorosłych oraz w dawce 230 mg/m² pc. raz na dobę u dzieci i młodzieży, gdy objawy toksyczności ustąpią. Jeśli wcześniej podawano dawkę 400 mg raz na dobę u pacjentów dorosłych lub 230 mg/m² pc. raz na dobę u dzieci i młodzieży, leczenie należy zakończyć. Jeżeli jest to klinicznie uzasadnione, należy rozważyć ponowne zwiększenie dawki do dawki początkowej 300 mg dwa razy na dobę u dorosłych pacjentów z nowo rozpoznaną CML w fazie przewlekłej lub do 400 mg dwa razy na dobę u dorosłych pacjentów z CML z opornością lub nietolerancją na leczenie imatynibem, w fazie przewlekłej lub w fazie akceleracji, lub do 230 mg/m² pc. dwa razy na dobę u dzieci i młodzieży. Zwiększona aktywność lipazy w surowicy. Jeśli nastąpi zwiększenie aktywności lipazy do stopnia 3-4, dawkę u pacjentów dorosłych należy zmniejszyć do 400 mg podawanych raz na dobę lub przerwać podawanie leku. U dzieci i młodzieży leczenie należy przerwać do czasu, gdy wartości aktywności lipazy powrócą do stopnia ≤1. Następnie, jeśli uprzednio stosowana dawka wynosiła 230 mg/m² pc. dwa razy na dobę, leczenie można wznowić w dawce 230 mg/m² pc. raz na dobę. Jeśli uprzednio stosowana dawka wynosiła 230 mg/m² pc. raz na dobę, leczenie należy zakończyć. Aktywność lipazy w surowicy należy oznaczać co miesiąc lub jeśli zaistnieją wskazania kliniczne (patrz punkt 4.4). Zwiększone stężenie bilirubiny i zwiększona aktywność aminotransferaz wątrobowych. Jeśli nastąpi zwiększenie stężenia bilirubiny do stopnia 3-4 oraz zwiększenie aktywności aminotransferaz wątrobowych do stopnia 3-4 u pacjentów dorosłych, dawkę należy zmniejszyć do 400 mg podawanych raz na dobę lub przerwać podawanie leku. W przypadku zwiększenia stężenia bilirubiny stopnia ≥2 lub zwiększenia aktywności aminotransferaz wątrobowych stopnia ≥3 u dzieci i młodzieży, leczenie należy przerwać do czasu, gdy wartości powrócą do stopnia ≤1. Następnie, jeśli uprzednio stosowana dawka wynosiła 230 mg/m² pc. dwa razy na dobę, leczenie można wznowić w dawce 230 mg/m² pc. raz na dobę. Jeśli uprzednio stosowana dawka wynosiła 230 mg/m² pc. raz na dobę, a powrót do stopnia ≤1 trwa dłużej niż 28 dni, leczenie należy zakończyć. Stężenie bilirubiny i aktywność aminotransferaz wątrobowych należy oznaczać co miesiąc lub jeśli zaistnieją wskazania kliniczne. *Szczególne populacje pacjentów: Osoby w podeszłym wieku:* Około 12% pacjentów z nowo rozpoznaną białaczką w fazie przewlekłej biorących udział w badaniu III fazy i około 30% pacjentów z CML w fazie przewlekłej i w fazie akceleracji z opornością lub nietolerancją imatynibu, biorących udział w badaniu klinicznym II fazy było w wieku 65 lat lub starszych. Nie zaobserwowano większych różnic w bezpieczeństwie stosowania i skuteczności pomiędzy pacjentami w wieku 65 lat i starszymi, a osobami dorosłymi w wieku 18 do 65 lat. *Zaburzenia czynności nerek:* Nie przeprowadzono badań klinicznych z udziałem pacjentów z zaburzeniami czynności nerek. Ponieważ nilotynib i jego metabolity nie są wydalane z moczem, nie przewiduje się zmniejszenia całkowitego klirensu u pacjentów z zaburzeniami czynności nerek. *Zaburzenia czynności wątroby:* Zaburzenia czynności wątroby mają niewielki wpływ na farmakokinetykę nilotynibu. U pacjentów z zaburzeniami czynności wątroby nie jest konieczna modyfikacja dawki, jednakże podczas ich leczenia należy zachować ostrożność. *Zaburzenia serca:* Wykluczano z udziału w badaniach klinicznych pacjentów z z istotnymi zaburzeniami serca (np. z przebytym niedawno zawałem mięśnia sercowego, zastoinową niewydolnością serca, niestabilną dusznicą lub klinicznie istotną bradykardią). Należy zachować ostrożność u pacjentów z istotnymi zaburzeniami serca. Podczas leczenia nilotynibem zgłaszano przypadki zwiększenia całkowitego stężenia cholesterolu w surowicy. Przed rozpoczęciem leczenia nilotynibem, a także po 3 i 6 miesiącach od rozpoczęcia leczenia należy wykonać lipidogram, który należy powtarzać co najmniej raz w roku podczas długotrwałego leczenia. Podczas leczenia nilotynibem zgłaszano przypadki zwiększenia stężenia glukozy we krwi (patrz ChPL). Przed rozpoczęciem leczenia nilotynibem należy oznaczyć stężenie glukozy we krwi i monitorować je podczas leczenia. *Dzieci i młodzież:* Określono bezpieczeństwo stosowania i skuteczność produktu leczniczego Tasigna u dzieci i młodzieży z CML w fazie przewlekłej z chromosomem Philadelphia, w wieku od 2 do mniej niż 18 lat (patrz punkt 4.8 i 5.2). Brak jest doświadczenia u dzieci w wieku poniżej 2 lat oraz u dzieci i młodzieży z CML z chromosomem Philadelphia w fazie akceleracji lub w fazie przełomu blastycznego. Nie ma danych dotyczących dzieci w wieku poniżej 10 lat z nowo rozpoznaną chorobą i istnieją ograniczone dane u dzieci w wieku poniżej 6 lat z opornością na imatynib lub nietolerancją imatynibu. Sposób podawania: Lek Tasigna należy przyjmować dwa razy na dobę co około 12 godzin i nie należy przyjmować go z posiłkiem. Kapsułki twarde należy połykać w całości, popijając wodą. Nie należy spożywać żadnych pokarmów na 2 godziny przed przyjęciem dawki i przynajmniej jedną godzinę po przyjęciu dawki leku. Pacjenci, którzy nie są w stanie połknąć kapsułek twardych, mogą rozpuścić zawartość każdej kapsułki twardej w jednej łyżeczce do herbaty musu jabłkowego (przecieru jabłkowego) i natychmiast zażyć. Nie wolno używać objętości większej niż zawartość jednej łyżeczki do herbaty przecieru jabłkowego ani żadnego innego pokarmu niż przecier jabłkowy. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub którąkolwiek substancję pomocniczą (patrz ChPL). **Środki ostrożności/Ostrzeżenia:** Zahamowanie czynności szpiku: Leczenie nilotynibem wiąże się z wystąpieniem trombocytopenii, neutropenii i niedokrwistości (stopień 3-4 wg skali toksyczności National Cancer Institute Common Toxicity Criteria). Zaburzenia występują częściej u pacjentów z CML i opornością lub nietolerancją imatynibu, a zwłaszcza u chorych w fazie akceleracji CML. W pierwszych dwóch miesiącach leczenia badanie morfologii krwi należy wykonywać co dwa tygodnie, a później co miesiąc lub zgodnie ze wskazaniami klinicznymi. Zahamowanie czynności szpiku jest zwykle przemijające, a właściwym postępowaniem jest wstrzymanie podawania leku Tasigna na pewien czas lub zmniejszenie dawki. Wydłużenie odstępu QT: Wykazano, że nilotynib może powodować zależne od stężenia wydłużenie repolaryzacji komór serca, skutkujące wydłużeniem odstępu QT w powierzchniowym zapisie EKG u pacjentów dorosłych oraz u dzieci i młodzieży. W badaniu III fazy z udziałem pacjentów z nowo rozpoznaną CML w fazie przewlekłej, otrzymujących 300 mg nilotynibu dwa razy na dobę, w stanie stacjonarnym, średnia zmiana odstępu QTcF od poziomu początkowego wyniosła 6 msec. U żadnego z pacjentów nie obserwowano QTcF >480 msec. Nie odnotowano żadnego przypadku zaburzeń rytmu typu „torsade de pointes". W badaniu fazy II u pacjentów nietolerujących imatynibu lub z CML oporną na imatynib, w fazie przewlekłej i w fazie akceleracji, otrzymujących 400 mg nilotynibu dwa razy na dobę, zaobserwowane średnie zmiany odstępu QTcF od poziomu początkowego w stanie stacjonarnym wynosiły odpowiednio 5 i 8 milisekund. U mniej niż 1% pacjentów obserwowano QTcF >500 milisekund. W badaniach klinicznych nie obserwowano przypadków występowania zaburzeń rytmu typu „torsade de pointes". W badaniu zdrowych ochotników, narażonych w stopniu porównywalnym z narażeniem pacjentów, średnia zmiana odstępu QTcF od poziomu początkowego, uzyskana w badaniu z placebo wynosiła 7 milisekund (wskaźnik sercowy CI ± 4 milisekundy). U żadnego z ochotników nie zaobserwowano QTcF >450 milisekund. Ponadto w czasie trwania badań nie zaobserwowano występowania klinicznie istotnej arytmii. Nie zaobserwowano zwłaszcza przypadków częstoskurczu komorowego typu „torsade de pointes" (utrwalonego i nieutrwalonego). Do istotnego wydłużenia odstępu QT może dojść, gdy nilotynib jest niewłaściwie przyjmowany z silnymi inhibitorami CYP3A4 i (lub) lekami, które mogą wydłużać odstęp QT, i (lub) z pokarmem. Współistniejąca hipokaliemia lub hipomagnezemia mogą dodatkowo nasilić to działanie. W razie wydłużenia odstępu QT może wystąpić u pacjentów ryzyko zgonu. Należy zachować ostrożność stosując lek Tasigna u pacjentów z wydłużeniem odstępu QTc lub u których występuje znaczne ryzyko wydłużenia odstępu QTc, takich jak: – pacjentów z wrodzonym wydłużeniem odstępu QT; – pacjentów z niewyrównanymi lub znaczącymi chorobami serca, w tym z niedawno przebytym zawałem mięśnia sercowego, zastoinową niewydolnością serca, niestabilną dusznicą bolesną lub klinicznie istotną bradykardią; – pacjentów przyjmujących leki przeciwarytmiczne lub inne substancje powodujące wydłużenie odstępu QT. Zaleca się ścisłe kontrolowanie wpływu leczenia na wydłużenie odstępu QTc oraz wykonanie badania EKG przed rozpoczęciem leczenia nilotynibem i zawsze, gdy istnieją wskazania kliniczne. Przed podaniem leku Tasigna należy skorygować istniejącą hipokaliemię i hipomagnezemię, a następnie okresowo kontrolować stężenie potasu i magnezu podczas leczenia. Nagły zgon: U pacjentów z CML w fazie przewlekłej lub w fazie akceleracji, z nietolerancją lub opornością na leczenie imatynibem, z chorobą serca w wywiadzie lub z istotnymi kardiologicznymi czynnikami ryzyka, odnotowano niezbyt częste przypadki (0,1 do 1%) nagłych zgonów. U pacjentów tych, oprócz procesu nowotworowego, współistniały często inne choroby, jak również przyjmowali oni inne leki. Czynnikiem sprzyjającym mogły być zaburzenia repolaryzacji komór. Nie zgłaszano żadnych przypadków nagłego zgonu w badaniu III fazy z udziałem pacjentów z nowo rozpoznaną CML w fazie przewlekłej. Zatrzymanie płynów i obrzęk: W badaniu III fazy z udziałem pacjentów z nowo rozpoznaną CML, niezbyt często (0,1 do 1%) obserwowano ciężkie postaci zatrzymania płynów związanego z przyjmowaniem leku, takie jak wysięk opłucnowy, obrzęk płuc i wysięk osierdziowy. Podobne zdarzenia występowały w doniesieniach po wprowadzeniu leku do obrotu. Należy uważnie badać przypadki niespodziewanego, szybkiego przyrostu masy ciała. Jeżeli w trakcie leczenia nilotynibem wystąpią objawy ciężkiego zatrzymania płynów, należy ustalić ich etiologię i odpowiednio leczyć pacjenta (instrukcja postępowania w przypadku toksyczności niedotyczącej układu krwiotwórczego, patrz „Dawkowanie"). Zdarzenia dotyczące układu sercowo-naczyniowego: Zdarzenia dotyczące układu sercowo-naczyniowego były zgłaszane w randomizowanym badaniu III fazy z udziałem pacjentów z nowo rozpoznaną CML oraz opisywano je w doniesieniach po wprowadzeniu produktu do obrotu. W tym badaniu klinicznym z medianą czasu trwania terapii wynoszącą 60,5 miesięcy, zdarzenia stopnia 3-4 dotyczące układu sercowo-naczyniowego obejmowały chorobę zarostową tętnic obwodowych (1,4% i 1,1% odpowiednio po dawce 300 mg i 400 mg nilotynibu dwa razy na dobę), chorobę niedokrwienną serca (2,2% i 6,1% odpowiednio po dawce 300 mg i 400 mg nilotynibu dwa razy na dobę) i udary niedokrwienne mózgu (1,1% i 2,2% odpowiednio po dawce 300 mg i 400 mg nilotynibu dwa razy na dobę). Należy poradzić pacjentom, by w razie wystąpienia ostrych, przedmiotowych i podmiotowych objawów zdarzeń sercowo-naczyniowych natychmiast zgłaszali się po pomoc medyczną. Podczas stosowania nilotynibu należy oceniać stan układu krążenia pacjenta oraz występowanie czynników ryzyka chorób sercowo-naczyniowych. W przypadku pojawienia się w/w czynników ryzyka, należy zastosować odpowiednie leczenie zgodnie ze standardowymi wytycznymi (instrukcje postępowania w przypadku wystąpienia toksyczności niedotyczącej układu krwiotwórczego, patrz „Dawkowanie"). Reaktywacja wirusowego zapalenia wątroby typu B: U pacjentów będących przewlekłymi nosicielami wirusa zapalenia wątroby typu B dochodziło do reaktywacji zapalenia wątroby po otrzymaniu przez nich inhibitorów kinazy tyrozynowej BCR-ABL. Niektóre przypadki prowadziły do ostrej niewydolności wątroby lub piorunującego zapalenia wątroby, a w konsekwencji do przeszczepienia wątroby lub zgonu pacjenta. U pacjentów należy wykonać badania pod kątem zakażenia wirusem HBV przed rozpoczęciem leczenia nilotynibem. Przed rozpoczęciem leczenia u pacjentów z dodatnim wynikiem badania serologicznego w kierunku wirusowego zapalenia wątroby typu B (w tym u pacjentów z aktywną chorobą) i w przypadku pacjentów z dodatnim wynikiem badania w kierunku zakażenia wirusem HBV w trakcie leczenia należy skonsultować się z ekspertami ds. chorób wątroby i leczenia wirusowego zapalenia wątroby typu B. Nosiciele wirusa HBV, którzy wymagają leczenia nilotynibem, powinni być poddawani ścisłej obserwacji pod kątem objawów podmiotowych i przedmiotowych aktywnego zakażenia wirusem HBV w trakcie całego okresu leczenia i przez kilka miesięcy po jego zakończeniu (patrz punkt ChPL). Szczególne monitorowanie dorosłych pacjentów z CML Ph+ w fazie przewlekłej, którzy uzyskali trwałą głęboką odpowiedź molekularną: *Kryteria zakończenia leczenia:* Zakończenie leczenia można rozważyć u spełniających kryteria pacjentów, u których potwierdzono ekspresję typowych transkryptów BCR-ABL, e13a2/b2a2 lub e14a2/b3a2. U pacjentów muszą występować typowe transkrypty BCR/ABL umożliwiające ilościowe określenie BCR-ABL, ocenę głębokości odpowiedzi molekularnej i stwierdzenie ewentualnej utraty remisji molekularnej po zakończeniu leczenia nilotynibem. *Monitorowanie pacjentów, którzy zakończyli leczenie:* Konieczne jest częste monitorowanie poziomów transkryptów BCR-ABL u pacjentów spełniających kryteria zakończenia leczenia przy użyciu ilościowego testu diagnostycznego zwalidowanego dla pomiaru poziomu odpowiedzi molekularnej o czułości przynajmniej MR4,5 (MR4,5=BCR-ABL/ABL ≤0,0032% IS). Poziom transkryptów BCR-ABL musi być oceniony przed i w trakcie przerywania leczenia (patrz ChPL). Utrata większej odpowiedzi molekularnej (MMR=BCR-ABL/ABL ≤0,1%IS) u pacjentów z CML, którzy otrzymywali nilotynib w leczeniu pierwszego lub drugiego rzutu, lub potwierdzona utrata MR4 (dwa kolejne pomiary w odstępie co najmniej 4 tygodni wykazujące utratę MR4 (MR4=BCR-ABL/ABL ≤0,01%IS)) u pacjentów z CML, którzy otrzymywali nilotynib w leczeniu drugiego rzutu spowodują konieczność wznowienia leczenia w ciągu 4 tygodni od stwierdzenia utraty remisji. W fazie bez leczenia może wystąpić nawrót molekularny, a dane dotyczące długoterminowych wyników nie są jeszcze dostępne. Dlatego bardzo ważne jest, by prowadzić częste monitorowanie poziomów transkryptów BCR-ABL i morfologii krwi z rozmazem, aby wykryć ewentualną utratę remisji (patrz ChPL). U pacjentów, którzy nie osiągną MMR po trzech miesiącach od wznowienia leczenia, należy wykonać badanie na obecność mutacji domen kinazy BCR-ABL. Badania laboratoryjne i kontrolne: *Stężenia lipidów we krwi:* W badaniu III fazy z udziałem pacjentów z nowo rozpoznaną CML, u 1,1% pacjentów leczonych nilotynibem w dawce 400 mg podawanej dwa razy na dobę wystąpiło zwiększenie stopnia 3-4 stężenia całkowitego cholesterolu; nie obserwowano jednak zwiększenia stężenia cholesterolu stopnia 3-4 w grupie otrzymującej dawkę 300 mg nilotynibu dwa razy na dobę. Zaleca się, by stężenie lipidów oceniać przed rozpoczęciem leczenia nilotynibem, po 3 i 6 miesiącach od rozpoczęcia leczenia oraz co najmniej raz w roku podczas długotrwałej terapii. Jeśli konieczne będzie podanie inhibitora reduktazy HMG-CoA (leku zmniejszającego stężenie lipidów), przed rozpoczęciem leczenia należy zapoznać się z ChPL produktu Tasigna, ponieważ niektóre inhibitory reduktazy HMG-CoA są także metabolizowane z udziałem CYP3A4. *Stężenie glukozy we krwi:* W badaniu III fazy z udziałem pacjentów z nowo rozpoznaną CML, u 6,9% i 7,2% pacjentów leczonych nilotynibem w dawce wynoszącej odpowiednio 400 mg i 300 mg dwa razy na dobę wystąpiło zwiększenie stężenia glukozy we krwi stopnia 3-4. Zaleca się, by przed rozpoczęciem leczenia nilotynibem ocenić stężenie glukozy we krwi, a w razie wystąpienia wskazań klinicznych monitorować stężenie glukozy we krwi także podczas leczenia. Jeśli wyniki badań uzasadniają wprowadzenie leczenia, lekarze powinni przestrzegać lokalnie obowiązujących standardów postępowania i wytycznych dotyczących terapii. Interakcje z innymi lekami: Należy unikać podawania leku Tasigna z lekami, które są silnymi inhibitorami CYP3A4 (w tym, ale nie tylko, ketokonazol, itrakonazol, worykonazol, klarytromycyna, telitromycyna, rytonawir). Jeżeli konieczne jest leczenie którymkolwiek z wyżej wymienionych leków, zaleca się w miarę możliwości przerwanie leczenia nilotynibem. Jeżeli przerwanie leczenia nie jest możliwe, wskazana jest dokładna obserwacja, czy nie występuje wydłużenie odstępu QT. Równoczesne stosowanie nilotynibu z lekami, które są silnymi induktorami CYP3A4 (np. fenytoina, ryfampicyna, karbamazepina, fenobarbital i ziele dziurawca zwyczajnego) prawdopodobnie zmniejsza narażenie na nilotynib w stopniu mającym znaczenie kliniczne. Dlatego też pacjentom przyjmującym nilotynib należy dobierać do stosowania leki słabiej indukujące CYP3A4. Wpływ pokarmu: Pokarm zwiększa dostępność biologiczną nilotynibu. Leku Tasigna nie wolno przyjmować w czasie posiłków. Lek należy przyjmować dwie godziny po posiłku. Nie należy przyjmować pokarmu w ciągu przynajmniej jednej godziny od przyjęcia dawki leku. Należy unikać spożywania soku z grejpfrutów i innych pokarmów, o których wiadomo, że hamują działanie CYP3A4. Pacjenci, którzy nie są w stanie połknąć kapsułek twardych, mogą rozpuścić zawartość każdej kapsułki twardej w jednej łyżeczce do herbaty przecieru jabłkowego i natychmiast zażyć. Nie wolno używać objętości większej niż zawartość jednej łyżeczki do herbaty przecieru jabłkowego ani żadnego innego pokarmu niż przecier jabłkowy. Zaburzenia czynności wątroby: Zaburzenia czynności wątroby mają niewielki wpływ na farmakokinetykę nilotynibu. Podawanie pojedynczych dawek 200 mg nilotynibu spowodowało zwiększenie AUC odpowiednio o 35%, 35% i 19% u pacjentów z lekkimi, umiarkowanymi i ciężkimi zaburzeniami czynności wątroby, w porównaniu do grupy kontrolnej pacjentów z prawidłową czynnością wątroby. Przewidywana wartość C_{max} nilotynibu w stanie stacjonarnym zwiększyła się odpowiednio o 29%, 18% i 22%. Z badań klinicznych wykluczono pacjentów, u których aktywność aminotransferazy alaninowej (AlAT) i (lub) aminotransferazy asparaginianowej (AspAT) była 2,5-krotnie większa niż górna granica normy (lub 5-krotnie, jeśli było to związane z chorobą) i (lub) u których stężenie bilirubiny całkowitej przekraczało 1,5-krotnie górną granicę normy. Nilotynib jest metabolizowany głównie w wątrobie. Dlatego u pacjentów z zaburzeniami czynności wątroby może dojść do zwiększonego narażenia na nilotynib i należy ich leczyć z zachowaniem ostrożności. Lipaza w surowicy: Zaobserwowano zwiększenie aktywności lipazy w surowicy. U pacjentów, którzy przebyli zapalenie trzustki należy zachować ostrożność. Jeśli zwiększeniu aktywności lipazy towarzyszą objawy w obrębie jamy brzusznej, leczenie nilotynibem należy przerwać i rozważyć wykonanie odpowiednich badań diagnostycznych w celu wykluczenia zapalenia trzustki. Całkowite wycięcie żołądka: Dostępność biologiczna nilotynibu może być zmniejszona u pacjentów po zabiegu całkowitego wycięcia żołądka. Należy rozważyć częstsze kontrole tych pacjentów. Zespół rozpadu guza: Ze względu na możliwość wystąpienia zespołu rozpadu guza (TLS, ang. tumour lysis syndrome), przed rozpoczęciem leczenia nilotynibem zaleca się skorygowanie klinicznie istotnego odwodnienia oraz leczenie zmniejszające podwyższone stężenie kwasu moczowego. Laktoza: Kapsułki twarde leku Tasigna zawierają laktozę. Lek nie powinien być stosowany u pacjentów z rzadko występującą dziedziczną nietolerancją galaktozy, niedoborem laktazy (typu Lapp) lub zespołem złego wchłaniania glukozy-galaktozy. Dzieci i młodzież: U dzieci obserwowano, częściej niż u dorosłych, odchylenia w wynikach badań laboratoryjnych, tj. przemijające zwiększenie w stopniu łagodnym do umiarkowanego aktywności aminotransferaz wątrobowych i stężenia bilirubiny całkowitej, wskazujące na zwiększone ryzyko hepatotoksyczności u dzieci i młodzieży (patrz ChPL).

Należy monitorować czynność wątroby (stężenie bilirubiny i aktywność aminotransferaz wątrobowych) raz na miesiąc lub w zależności od wskazań klinicznych. Postępowanie w przypadku zwiększonego stężenia bilirubiny i aktywności aminotransferaz wątrobowych polega na czasowym wstrzymaniu podawania nilotynibu, zmniejszeniu dawki i (lub) zakończeniu leczenia nilotynibem. Długoterminowe skutki przewlekłego leczenia nilotynibem u dzieci i młodzieży są nieznane. W badaniu z udziałem dzieci i młodzieży z CML u pacjentów leczonych nilotynibem wykazano opóźnienie wzrostu (patrz punkt 4.8). W populacji dzieci i młodzieży leczonych nilotynibem zaleca się uważne monitorowanie wzrostu. Kobiety w wieku rozrodczym / Antykoncepcja: W trakcie leczenia nilotynibem i przez okres do dwóch tygodni po zakończeniu leczenia konieczne jest stosowanie przez kobiety w wieku rozrodczym wysoce skutecznej metody antykoncepcji. Ciąża: Brak danych dotyczących stosowania nilotynibu u kobiet w okresie ciąży lub są one ograniczone. Leku Tasigna nie należy stosować w okresie ciąży, chyba że stan kliniczny kobiety wymaga leczenia nilotynibem. Jeżeli lek jest stosowany w czasie ciąży, pacjentkę należy poinformować o ryzyku dla płodu. Jeśli kobieta leczona nilotynibem rozważa zajście w ciążę, można rozważyć zakończenie leczenia na podstawie kyteriów kwalifikujących do odstawienia leczenia opisanych powyżej. Istnieją ograniczone dane dotyczące ciąż u pacjentek podczas próby remisji bez leczenia (ang. treatment-free remission, TFR). W przypadku planowania ciąży w fazie TFR pacjentka musi być poinformowana o potencjalnej potrzebie wznowienia leczenia nilotynibem podczas ciąży. Karmienie piersią: Nie wiadomo czy nilotynib przenika do mleka ludzkiego. Na podstawie dostępnych danych toksykologicznych dotyczących zwierząt stwierdzono przenikanie nilotynibu do mleka. Ponieważ nie można wykluczyć zagrożenia dla noworodków i (lub) niemowląt, kobiety nie powinny karmić piersią podczas leczenia produktem Tasigna i przez 2 tygodnie po przyjęciu ostatniej dawki produktu. Płodność: Badania na zwierzętach nie wykazały wpływu na płodność u samców i samic szczura. Pacjenci, u których występują zawroty głowy, zmęczenie, zaburzenia widzenia lub inne objawy niepożądane mogące wpływać na zdolność prowadzenia pojazdów lub obsługiwania maszyn, powinni unikać wykonywania tych czynności, aż do momentu ustąpienia działań niepożądanych. **Działania niepożądane: Tasigna 50 mg i 200 mg:** Dane przedstawione poniżej dotyczą narażenia na nilotynib łącznej liczby 737 dorosłych pacjentów z nowo rozpoznaną CML Ph+ w fazie przewlekłej (n=279), leczonych zalecaną dawką 300 mg dwa razy na dobę w randomizowanym badaniu III fazy oraz dorosłych pacjentów z CML w fazie przewlekłej (n=321) i w fazie akceleracji (n=137), z opornością lub nietolerancją imatynibu leczonych zalecaną dawką 400 mg dwa razy na dobę, w ramach wieloośrodkowego, otwartego badania klinicznego II fazy. Przedstawiono również informacje o bezpieczeństwie stosowania pochodzące z dwóch badań z zakończeniem leczenia produktem leczniczym Tasigna oraz z prospektywnego nieinterwencyjnego badania z udziałem dorosłych pacjentów z CML w fazie przewlekłej, z opornością lub nietolerancją imatynibu, z dwuletnim okresem obserwacji (n=507). Dorośli pacjenci z nowo rozpoznaną CML w fazie przewlekłej: Mediana czasu trwania narażenia na lek wyniosła 60,5 miesięcy (zakres 0,1-70,8 miesiąca). Najczęstszymi (≥10%) niehematologicznymi działaniami niepożądanymi były wysypka, świąd, ból głowy, nudności, uczucie zmęczenia, łysienie, bóle mięśni i bóle w nadbrzuszu. Większość z tych działań niepożądanych miała przebieg lekki lub umiarkowanie ciężki. Zaparcie, suchość skóry, osłabienie, skurcze mięśni, biegunkę, bóle stawów, ból brzucha, wymioty i obrzęki obwodowe obserwowano rzadziej (<10% i ≥5%), miały one przebieg lekki lub umiarkowanie ciężki, poddawały się leczeniu i na ogół nie było konieczne zmniejszenie dawki. Związane z leczeniem objawy toksyczności hematologicznej obejmują zahamowanie czynności szpiku kostnego: trombocytopenię (18%), neutropenię (15%) i niedokrwistość (8%). Do biochemicznych działań niepożądanych leku należą: zwiększenie aktywności aminotransferazy alaninowej (24%), hiperbilirubinemia (16%), zwiększenie aktywności aminotransferazy asparaginianowej (12%), zwiększenie aktywności lipazy (11%), zwiększenie stężenia bilirubiny we krwi (10%), hiperglikemia (4%), hipercholesterolemia (3%) i hipertrójglicerydemia (<1%). Wysięki opłucnowe i osierdziowe wystąpiły, niezależnie od związku przyczynowego, odpowiednio u 2% i <1% pacjentów przyjmujących nilotynib w dawce 300 mg dwa razy na dobę. Krwawienie z przewodu pokarmowego zgłaszano, niezależnie od związku przyczynowego, u 3% tych pacjentów. Średnia zmiana odstępu QTcF od poziomu początkowego w stanie stacjonarnym wyniosła 6 msec. U żadnego z pacjentów nie obserwowano bezwzględnych wartości QTcF >500 msec podczas leczenia badanym lekiem. Zwiększenie QTcF powyżej 60 msec względem stanu początkowego odnotowano u <1% pacjentów podczas leczenia badanym lekiem. Nie odnotowano żadnego przypadku nagłego zgonu lub zaburzeń rytmu typu „torsade de pointes" (przemijających lub utrwalonych). W żadnym momencie leczenia nie obserwowano zmniejszenia średniej frakcji wyrzutowej lewej komory (LVEF) względem stanu początkowego. U żadnego z pacjentów nie stwierdzono LVEF <45% podczas leczenia, ani bezwzględnego zmniejszenia LVEF o ponad 15%. U 10% pacjentów przerwano leczenie z powodu wystąpienia działań niepożądanych. *Dorośli pacjenci z CML w fazie przewlekłej lub w fazie akceleracji z opornością na imatynib lub nietolerancją imatynibu:* Dane przedstawione poniżej dotyczą narażenia na nilotynib 458 dorosłych pacjentów z CML, w fazie przewlekłej (n=321) oraz w fazie akceleracji (n=137), z opornością lub nietolerancją imatynibu, leczonych w ramach wieloośrodkowego, otwartego badania II fazy, zalecaną dawką 400 mg dwa razy na dobę. Najczęściej zgłaszanymi (≥10%) niehematologicznymi objawami niepożądanymi związanymi ze stosowaniem leku były wysypka, świąd, nudności, zmęczenie, ból głowy, wymioty, bóle mięśni, zaparcie i biegunka. Nasilenie większości wyżej wymienionych działań niepożądanych było lekkie do umiarkowanego. Łysienie, kurcze mięśni, zmniejszenie łaknienia, bóle stawów, ból brzucha, bóle kości, obrzęki obwodowe, osłabienie, ból w nadbrzuszu, suchość skóry, rumień i ból kończyn obserwowano rzadziej (<10% i ≥5%) i były o nasileniu lekkim do umiarkowanego (stopień 1 lub 2). Przerwanie leczenia z powodu działań niepożądanych związanych z zastosowaniem leku nastąpiło u 16% pacjentów w fazie przewlekłej (CP) i 10% pacjentów w fazie akceleracji (AP). Związane z leczeniem objawy toksyczności hematologicznej obejmują zahamowanie czynności szpiku kostnego: trombocytopenię (31%), neutropenię (17%) i niedokrwistość (14%). U <1% pacjentów przyjmujących lek Tasigna wystąpiły wysięki opłucnowe i osierdziowe oraz powikłania wskutek zatrzymania płynów. U ≤1% pacjentów zaobserwowano niewydolność serca. Krwawienie z przewodu pokarmowego i krwawienie w ośrodkowym układzie nerwowym zgłoszono u odpowiednio 1% i ≤1% pacjentów. U mniej niż 1% pacjentów obserwowano QTcF dłuższe niż 500 milisekund. Nie zaobserwowano przypadków częstoskurczu komorowego typu „torsade de pointes" (przemijającego lub utrwalonego). Poniżej przedstawiono niehematologiczne objawy niepożądane (z wyjątkiem zmian w wynikach badań laboratoryjnych) zaobserwowane u co najmniej 5% pacjentów leczonych nilotynibem w dawce 300 mg dwa razy na dobę w randomizowanym badaniu III fazy. Działania niepożądane pogrupowano zgodnie z częstością występowania według następującej konwencji: bardzo często (≥1/10), często (≥1/100 do <1/10), niezbyt często (≥1/1 000 do <1/100), rzadko (≥1/10 000 do <1/1 000), bardzo rzadko (<1/10 000) i częstość nieznana (nie może być określona na podstawie dostępnych danych). ***Bardzo często:*** ból głowy, nudności, ból w nadbrzuszu, wysypka, świąd, łysienie, bóle mięśniowe, zmęczenie, zaparcie[1], biegunka[1], wymioty[1]. ***Często:*** jadłowstręt, zaparcia[2], biegunka[2], wymioty[2], ból brzucha, niestrawność, suchość skóry, rumień, ból stawów, kurcze mięśni, ból kości, ból kończyn, osłabienie, obrzęki obwodowe. Następujące działania niepożądane zgłaszano u dorosłych pacjentów w badaniach klinicznych leku Tasigna służących za podstawę dla zatwierdzonych wskazań, z częstością mniejszą niż 5%. Uwzględniono również bardzo częste działania niepożądane polegające na zmianach w wynikach badań laboratoryjnych, nieuwzględnione w wykazie niehematologicznych działań niepożądanych. Te działania niepożądane podano uwzględniając znaczenie kliniczne. ***Bardzo często:*** hipofosfatemia (w tym zmniejszenie stężenia fosforu we krwi), hiperbilirubinemia (w tym zwiększenie stężenia bilirubiny we krwi), zwiększenie aktywności aminotransferazy alaninowej, zwiększenie aktywności aminotransferazy asparaginianowej, zwiększenie aktywności lipazy, zwiększenie stężenia lipoproteiny cholesterolu (w tym o małej i dużej gęstości), zwiększenie stężenia cholesterolu całkowitego, zwiększenie stężenia trójglicerydów we krwi. ***Często:*** zapalenie mieszków włosowych, zakażenie górnych dróg oddechowych (w tym zapalenie gardła, zapalenie błony śluzowej części nosowej gardła, zapalenie błony śluzowej nosa), zapalenie płuc, brodawczak skóry, leukopenia, eozynofilia, neutropenia z gorączką, pancytopenia, limfopenia, zaburzenia równowagi elektrolitowej (w tym hipomagnezemia, hiperkaliemia, hipokaliemia, hiponatremia, hipokalcemia, hiperkalcemia, hiperfosfatemia), cukrzyca, hiperglikemia, hipercholesterolemia, hiperlipidemia, hipertriglicerydemia, depresja, bezsenność, lęk, zawroty głowy, neuropatia obwodowa, niedoczulica, parestezje, krwawienie do oka, obrzęk wokół oczu, swędzenie oka, zapalenie spojówek, suchość oka (w tym zespół suchego oka), układowe zawroty głowy, dławica piersiowa, zaburzenia rytmu serca (w tym blok przedsionkowo-komorowy, trzepotanie serca, dodatkowe skurcze, tachykardia, migotanie przedsionków, bradykardia), kołatanie serca, wydłużenie odstępu QT w elektrokardiogramie, niewydolność serca*, nadciśnienie tętnicze, uderzenia gorąca, zwężenie tętnic obwodowych, duszność, duszność wysiłkowa, krwawienie z nosa, kaszel, dysfonia, zapalenie trzustki, uczucie dyskomfortu w jamie brzusznej, rozdęcie jamy brzusznej, zaburzenia smaku, wzdęcia, zaburzenia czynności wątroby, nocne poty, wyprysk, pokrzywka, nadmierna potliwość, siniaki, trądzik, zapalenie skóry, (w tym alergiczne, złuszczające i trądzikopodobne), ból mięśniowo-szkieletowy w klatce piersiowej, ból mięśniowo-szkieletowy, ból pleców, ból w boku, ból szyi, osłabienie mięśni, częstomocz, niewydolność nerek*, ból w klatce piersiowej (w tym ból w klatce piersiowej niezwiązany z sercem), ból, gorączka, dyskomfort w klatce piersiowej, złe samopoczucie, zmniejszenie stężenia hemoglobiny, zwiększenie aktywności amylazy we krwi, zwiększenie aktywności fosfatazy zasadowej we krwi, zwiększenie aktywności gamma-glutamylotransferazy, zwiększenie aktywności fosfokinazy kreatyninowej we krwi, zmniejszenie masy ciała, zwiększenie masy ciała, zwiększenie stężenia insuliny we krwi, zmniejszenie stężenia globulin. ***Niezbyt często:*** zakażenie dróg moczowych, zapalenie żołądka i jelit, zapalenie oskrzeli, zakażenie wirusem opryszczki, kandydozy (w tym kandydozy jamy ustnej), nadpłytkowość, leukocytoza, nadczynność tarczycy, niedoczynność tarczycy, odwodnienie, zwiększenie łaknienia, dna, dyslipidemia, krwotok wewnątrzczaszkowy, udar niedokrwienny, przemijający napad niedokrwienny, zawał mózgu, migrena, utrata przytomności (w tym omdlenia), drżenie, zaburzenia koncentracji, przeczulica, zaburzenia widzenia, niewyraźne widzenie, krwawienie do spojówek, zmniejszona ostrość wzroku, obrzęk powieki, fotopsja, przekrwienie (twardówki, spojówki, oka), podrażnienie oka, zawał mięśnia sercowego, choroba wieńcowa, szmer sercowy, wysięk osierdziowy, sinica, przełom nadciśnieniowy, miażdżyca zarostowa tętnic obwodowych, chromanie przestankowe, zwężenie tętnic kończyn, krwiak, miażdżyca, obrzęk płuc, wysięk opłucnowy, choroba śródmiąższowa płuc, ból opłucnowy, zapalenie opłucnej, ból gardła i krtani, podrażnienie gardła, krwotok z przewodu pokarmowego, krew w kale, owrzodzenie jamy ustnej, refluks żołądkowo-przełykowy, zapalenie błony śluzowej jamy ustnej, ból przełyku, suchość w jamie ustnej, zapalenie żołądka, wrażliwość zębów, toksyczny wpływ na wątrobę, toksyczne zapalenie wątroby, żółtaczka, złuszczająca się wysypka, wysypka polekowa, ból skóry, wybroczyny, obrzęk twarzy, sztywność mięśniowo-szkieletowa, obrzęk stawów, bolesne oddawanie moczu, nagłe oddawanie moczu, częste oddawanie moczu w nocy, ból piersi, ginekomastia, zaburzenia erekcji, obrzęk twarzy, obrzęk nóg, choroby grypopodobne, dreszcze, odczucie zmian w temperaturze ciała (w tym uczucie gorąca, uczucie zimna), zwiększenie aktywności dehydrogenazy mleczanowej we krwi, zmniejszenie stężenia glukozy we krwi, zwiększenie stężenia mocznika we krwi. ***Częstość nieznana:*** posocznica, ropień podskórny, ropień odbytu, czyrak, grzybica stóp, reaktywacja wirusowego zapalenia wątroby typu B, brodawczak jamy ustnej, paraproteinemia, nadwrażliwość, wtórna nadczynność przytarczyc, zapalenie tarczycy, hiperurycemia, hipoglikemia, dezorientacja, splątanie, amnezja, dysforia, epizod mózgowo-naczyniowy, obrzęk mózgu, zapalenie nerwu wzrokowego, letarg, zaburzenia czucia, zespół niespokojnych nóg, obrzęk tarczy nerwu wzrokowego, chorioretinopatia, podwójne widzenie, światłowstręt, opuchnięcie oka, zapalenie powiek, ból oka, alergiczne zapalenie spojówek, choroby powierzchni oka, zaburzenia słuchu, ból ucha, szumy uszne, zaburzenia czynności komór, zapalenie osierdzia, zmniejszenie frakcji wyrzutowej, wstrząs krwotoczny, niedociśnienie tętnicze, zakrzepica, nadciśnienie płucne, świszczący oddech, ból części ustnej gardła, perforacja wrzodów żołądka i jelit, krwotok zaotrzewnowy, krwawe wymioty, wrzód żołądka, wrzodziejące zapalenie przełyku, niedrożność przepuszczająca jelit, zapalenie jelita cienkiego i okrężnicy, guzy krwawnicowe, przepuklina rozworu przełykowego, krwotok odbytniczy, zapalenie dziąseł, zastój żółci, powiększenie wątroby, rumień wielopostaciowy, rumień guzowaty, owrzodzenie skóry, zespół erytrodyzestezji dłoniowo-podeszwowej, wybroczyny punktowe, nadwrażliwość na światło, pęcherze, torbiele skórne, rozrost gruczołów łojowych, zanik skóry, przebarwienia skóry, złuszczanie skóry, nadmierna pigmentacja skóry, przerost skóry, hiperkeratoza, łuszczyca, zapalenie stawów, krwiomocz, nietrzymanie moczu, nieprawidłowe zabarwienie moczu, stwardnienie piersi, nadmiernie obfite krwawienie miesiączkowe, obrzęk brodawek, obrzęk miejscowy, zwiększenie stężenia troponiny, zwiększenie stężenia nieskoniugowanej bilirubiny we krwi, zmniejszenie stężenia insuliny we krwi, zmniejszenie stężenia peptydu C, zwiększenie stężenia hormonu przytarczyc we krwi.

** Szacunkowa częstość występowania opiera się na danych z prospektywnego nieinterwencyjnego badania z udziałem dorosłych pacjentów z CML w fazie przewlekłej z opornością lub nietolerancją imatynibu, z dwuletnim okresem obserwacji (n=507)*

Klinicznie istotne lub ciężkie odchylenia od normy wartości parametrów oznaczanych w rutynowych badaniach hematologicznych lub biochemicznych – patrz ChPL (tabela 5). **Tasigna 150 mg:** Dane przedstawione poniżej dotyczą narażenia na nilotynib 279 dorosłych pacjentów z nowo rozpoznaną CML Ph+ w fazie przewlekłej, leczonych 300 mg nilotynibu dwa razy na dobę w randomizowanym badaniu III fazy. Przedstawiono również informacje o bezpieczeństwie stosowania pochodzące z badań z zakończeniem leczenia u pacjentów z CML, którzy byli leczeni nilotynibemw terapii pierwszego rzutu. Mediana czasu trwania narażenia na lek wyniosła 60,5 miesięcy (zakres 0,1-70,8 miesiąca). Najczęstszymi (≥10%) niehematologicznymi działaniami niepożądanymi były wysypka, świąd, ból głowy, nudności, uczucie zmęczenia, łysienie, bóle mięśni i bóle w nadbrzuszu. Większość z tych działań niepożądanych miała przebieg lekki lub umiarkowanie ciężki. Zaparcie, suchość skóry, osłabienie, skurcze mięśni, biegunkę, bóle stawów, ból brzucha, wymioty i obrzęki obwodowe obserwowano rzadziej (<10% i ≥5%), miały one przebieg lekki lub umiarkowanie ciężki, poddawały się leczeniu i na ogół nie było konieczne zmniejszenie dawki. Związane z leczeniem objawy toksyczności hematologicznej obejmują zahamowanie czynności szpiku kostnego: trombocytopenię (18%), neutropenię (15%) i niedokrwistość (8%). Do biochemicznych działań niepożądanych leku należą: zwiększenie aktywności aminotransferazy alaninowej (24%), hiperbilirubinemia (16%), zwiększenie aktywności aminotransferazy asparaginianowej (12%), zwiększenie aktywności lipazy (11%), zwiększenie stężenia bilirubiny we krwi (10%), hiperglikemia (4%), hipercholesterolemia (3%) i hipertrójglicerydemia (<1%). Wysięki opłucnowe i osierdziowe wystąpiły, niezależnie od związku przyczynowego, odpowiednio u 2% i <1% pacjentów przyjmujących nilotynib w dawce 300 mg dwa razy na dobę. Krwawienie z przewodu pokarmowego zgłaszano, niezależnie od związku przyczynowego, u 3% tych pacjentów. Średnia zmiana odstępu QTcF od poziomu początkowego w stanie stacjonarnym wyniosła 6 msec. U żadnego z pacjentów nie obserwowano bezwzględnych wartości QTcF >500 msec podczas leczenia badanym produktem leczniczym. Zwiększenie QTcF powyżej 60 msec względem stanu początkowego odnotowano u <1% pacjentów podczas leczenia badanym produktem leczniczym. Nie odnotowano żadnego przypadku nagłego zgonu lub zaburzeń rytmu typu „torsade de pointes" (przemijających lub utrwalonych). W żadnym momencie leczenia nie obserwowano zmniejszenia średniej frakcji wyrzutowej lewej komory (LVEF) względem stanu początkowego. U żadnego z pacjentów nie stwierdzono LVEF <45% podczas leczenia, ani bezwzględnego zmniejszenia LVEF o ponad 15%. U 10% pacjentów przerwano leczenie z powodu wystąpienia działań niepożądanych. Poniżej przedstawiono niehematologiczne objawy niepożądane (z wyjątkiem zmian w wynikach badań laboratoryjnych) zaobserwowane u co najmniej 5% pacjentów leczonych nilotynibem w dawce 300 mg dwa razy na dobę w randomizowanym badaniu III fazy. Działania niepożądane pogrupowano zgodnie z częstością występowania według następującej konwencji: bardzo często (≥1/10), często (≥1/100 do <1/10), niezbyt często (≥1/1 000 do <1/100), rzadko (≥1/10 000 do <1/1 000), bardzo rzadko (<1/10 000) i częstość nieznana (nie może być określona na podstawie dostępnych danych). ***Bardzo często:*** bóle głowy, nudności, ból w nadbrzuszu, wysypka, świąd, ból mięśni, zmęczenie, łysienie. ***Często:*** zaparcie, biegunka, wymioty, ból w jamie brzusznej, niestrawność, suchość skóry, ból stawów, skurcze mięśni, ból kończyn, obrzęki obwodowe, osłabienie. Następujące działania niepożądane zgłaszano u dorosłych pacjentów w badaniach klinicznych leku Tasigna u mniej niż 5% pacjentów. Uwzględniono również bardzo częste (≥1/10) zmiany w wynikach badań laboratoryjnych, nieuwzględnione w wykazie niehematologicznych działań niepożądanych. Te działania niepożądane podano uwzględniając znaczenie kliniczne. ***Bardzo często:*** hipofosfatemia (w tym zmniejszenie stężenia fosforu we krwi), hiperbilirubinemia (w tym zwiększenie stężenia bilirubiny we krwi), zwiększenie aktywności aminotransferazy alaninowej, zwiększenie aktywności aminotransferazy asparaginianowej, zwiększenie aktywności lipazy, zwiększenie stężenia lipoproteiny cholesterolu (w tym o małej i dużej gęstości), zwiększenie stężenia cholesterolu całkowitego, zwiększenie stężenia trójglicerydów we krwi. ***Często:*** zapalenie mieszków włosowych, zakażenie górnych dróg oddechowych (w tym zapalenie gardła, zapalenie błony śluzowej części nosowej gardła, zapalenie błony śluzowej nosa), brodawczak skóry, leukopenia, eozynofilia, limfopenia, cukrzyca, hipercholesterolemia, hiperlipidemia, hipertriglicerydemia, hiperglikemia, zmniejszenie łaknienia, hipokalcemia, hipokaliemia, bezsenność, depresja, lęk, nieukładowe zawroty głowy, niedoczulica, neuropatia obwodowa, swędzenie oczu, zapalenie spojówek, suchość oka (w tym zespół suchego oka), układowe zawroty głowy, dławica piersiowa*, zaburzenia rytmu serca (w tym blok przedsionkowo-komorowy , tachykardia, migotanie przedsionków, dodatkowe skurcze komorowe, bradykardia)*, wydłużenie odstępu QT w zapisie EKG*, kołatanie serca*, zawał mięśnia sercowego*, nadciśnienie tętnicze, uderzenia gorąca, duszność, kaszel, rozdęcie jamy brzusznej, uczucie dyskomfortu w jamie brzusznej, zaburzenia smaku, wzdęcia, zaburzenia czynności wątroby, rumień, nadmierna potliwość, siniaki, trądzik, zapalenie skóry (w tym alergiczne, złuszczające i trądzikopodobne), nocne poty, wyprysk, ból kości, ból pleców, osłabienie mięśni, gorączka, ból w klatce piersiowej (w tym ból w klatce piersiowej niezwiązany z sercem), uczucie dyskomfortu w klatce piersiowej, zmniejszenie stężenia hemoglobiny, zwiększenie aktywności amylazy we krwi, zwiększenie aktywności fosfatazy zasadowej, zwiększenie aktywności gamma-glutamylotransferazy, zwiększenie masy ciała, zwiększenie stężenia insuliny we krwi, zmniejszenie stężenia globulin. ***Niezbyt często:*** pancytopenia, hiperkaliemia, dyslipidemia, dna, udar niedokrwienny, zawał mózgu, migrena, parestezje, obrzęk powiek, fotopsja, krwawienie do spojówek, przekrwienie (twardówek, spojówki, oka), niewydolność serca*, sinica*, chromanie przestankowe, miażdżyca zarostowa tętnic obwodowych, miażdżyca, wysięk opłucnowy, zapalenie trzustki, zapalenie żołądka, wrażliwość zębów, żółtaczka, wysypka polekowa, ból skóry, ból mięśniowo-szkieletowy, ból w boku, zaburzenia erekcji, dreszcze, odczucie zmian w temperaturze ciała (w tym uczucie gorąca, uczucie zimna), złe samopoczucie. ***Częstość nieznana:*** zakażenie wirusem opryszczki, kandydoza jamy ustnej, ropień podskórny, ropień odbytu, grzybica stóp, reaktywacja wirusowego zapalenia wątroby typu B, brodawczak jamy ustnej, paraproteinemia, gorączka neutropeniczna, nadwrażliwość, wtórna nadczynność przytarczyc, hiperurykemia, hipoglikemia, zaburzenia apetytu, amnezja, dysforia, epizod mózgowo-naczyniowy, zwężenie tętnicy podstawnej mózgu, omdlenie, drżenie, letarg, zaburzenia czucia, zespół niespokojnych nóg, przeczulica, obrzęk wokół oczu, zapalenie powiek, ból oka, chorioretinopatia, alergiczne zapalenie spojówek, choroby powierzchni oka, niewyraźne widzenie, zmniejszenie frakcji wyrzutowej*, wysięk osierdziowy*, zapalenie osierdzia*, zaburzenia czynności rozkurczowej*, blok lewej odnogi pęczka Hisa*, krwiak, zwężenie tętnic obwodowych, duszność wysiłkowa, zapalenie opłucnej, krwawienie z nosa, ból części ustnej gardła, wrzód przełyku, wrzód żołądka, ból przełyku, zapalenie błony śluzowej jamy ustnej, suchość w jamie ustnej, zapalenie jelita cienkiego i okrężnicy, guzki krwawnicze, przepuklina rozworu przełykowego, krwotok odbytniczy, zapalenie dziąseł, toksyczne zapalenie wątroby, rumień wielopostaciowy, pokrzywka, pęcherze, torbiele skórne, rozrost gruczołów łojowych, obrzęk twarzy, zanik skóry, przerost skóry, złuszczanie skóry, nadmierna pigmentacja skóry, przebarwienia skóry, hiperkeratoza, łuszczyca, bolesne oddawanie moczu, częstomocz, nieprawidłowe zabarwienie moczu, ginekomastia, stwardnienie piersi, nadmiernie obfite krwawienie miesiączkowe, obrzęk brodawek, obrzęk twarzy, obrzęki miejscowe, zwiększenie stężenia hormonu przytarczyc we krwi, zmniejszenie stężenia insuliny we krwi, zmniejszenie stężenia peptydu C, zmniejszenie masy ciała.

** zgłaszane w badaniu III fazy, w grupie przyjmującej dawki 300 mg dwa razy na dobę i (lub) 400 mg dwa razy na dobę*

Klinicznie istotne lub ciężkie odchylenia od normy wartości parametrów oznaczanych w rutynowych badaniach hematologicznych lub biochemicznych u dorosłych pacjentów – patrz ChPL (tabela 5). Opis wybranych działań niepożądanych: *Nagły zgon:* W badaniach klinicznych z lekiem Tasigna i (lub) w programach leczniczych w ramach tzw. „compassionate use" u pacjentów z CML w fazie przewlekłej lub w fazie akceleracji oraz opornością lub nietolerancją imatynibu zgłaszano niezbyt często (0,1 do 1%) nagłe zgony pacjentów z chorobą serca w wywiadzie lub z istotnymi kardiologicznymi czynnikami ryzyka. *Reaktywacja wirusowego zapalenia wątroby typu B:* Opisywano reaktywację wirusowego zapalenia wątroby typu B powiązaną ze stosowaniem inhibitorów kinazy tyrozynowej BCR-ABL. Niektóre przypadki prowadziły do ostrej niewydolności wątroby lub piorunującego zapalenia wątroby, a w konsekwencji do przeszczepienia wątroby lub zgonu pacjenta (patrz ChPL). Doświadczenie po wprowadzeniu leku do obrotu: Następujące działania niepożądane pochodzą z okresu po wprowadzeniu produktu leczniczego Tasigna do obrotu, na podstawie zgłoszeń spontanicznych, raportów literaturowych, programów rozszerzonego dostępu oraz badań klinicznych innych niż ogólnoświatowe badania rejestracyjne. Ponieważ działania te zgłaszano dobrowolnie w populacji o nieokreślonej liczebności, oszacowanie ich częstości lub ustalenie związku przyczynowo-skutkowego z narażeniem na nilotynib nie zawsze jest możliwe. Bardzo często: u dzieci i młodzieży leczonych nilotynibem wykazano opóźnienie wzrostu. Rzadko: przypadki wystąpienia zespołu rozpadu guza u pacjentów leczonych nilotynibem. Częstość nieznana: u pacjentów leczonych nilotynibem zgłaszano przypadki porażenia twarzy. Dzieci i młodzież: Bezpieczeństwo stosowania nilotynibu u dzieci i młodzieży (w wieku od 2 do <18 lat) z CML z chromosomem Philadelphia w fazie przewlekłej (n=69) analizowano w dwóch badaniach (patrz punkt 5.1 ChPL). U dzieci i młodzieży częstość, rodzaj i nasilenie obserwowanych działań niepożądanych były na ogół zgodne z działaniami niepożądanymi obserwowanymi u osób dorosłych, z wyjątkiem odchyleń w wynikach badań laboratoryjnych, takich jak hiperbilirubinemia (stopnia 3/4: 13,0%) i zwiększenia aktywności aminotransferaz wątrobowych (AspAT stopnia 3/4: 1,4%; AlAT stopnia 3/4: 8,7%), które zgłaszano z większą częstością niż u pacjentów dorosłych. Podczas leczenia należy kontrolować stężenie bilirubiny i aktywność aminotransferaz wątrobowych (patrz punkty 4.2 i 4.4 ChPL). *Opóźnienie wzrostu u dzieci i młodzieży:* W analizie pośredniej dokonanej podczas badania z udziałem dzieci i młodzieży z CML przy medianie ekspozycji równej 33 miesiące w każdej kohorcie (pacjenci z CML Ph+ w fazie przewlekłej z chorobą nowo rozpoznaną lub chorobą oporną na leczenie albo nietolerancją leczenia) opóźnienie wzrostu (zejście o dwa główne kanały centylowe od stanu początkowego) wykazano u 12,1% pacjentów. W populacji dzieci i młodzieży leczonej nilotynibem zaleca się uważne monitorowanie wzrostu (patrz punkt 4.4). 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 (patrz ChPL). **Pozwolenia Komisji Europejskiej na dopuszczenie do obrotu nr:** Tasigna 50 mg – EU/1/07/422/015; Tasigna 150 mg – EU/1/07/422/005-006, EU/1/07/422/009-010, EU/1/07/422/013; Tasigna 200 mg – EU/1/07/422/001-004, EU/1/07/422/007-008, EU/1/07/422/011-012, EU/1/07/422/014. **Kategoria dostępności:** Rpz – lek wydawany z przepisu lekarza do zastrzeżonego stosowania. **Podmiot odpowiedzialny:** Novartis Europharm Limited, Vista Building, Elm Park, Merrion Road, Dublin 4, Irlandia. **Uwaga:** Przed przepisaniem leku należy zapoznać się z pełną informacją o leku. **Pełna informacja o leku jest dostępna w:** Novartis Poland Sp. z o.o., 02-674 Warszawa, ul. Marynarska 15, tel. +48 22 375 4 888. **Opracowano:** 01/2021.

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[1] CML – faza przewlekła i CML – faza akceleracji z opornością lub nietolerancją na imatynib, 400 mg dwa razy na dobę, n=458;
[2] Nowo rozpoznana CML – faza przewlekła, 300 mg dwa razy na dobę, n=279.

Zgodnie z aktualnym Obwieszczeniem Ministra Zdrowia w sprawie wykazu refundowanych leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych, produkt leczniczy Tasigna (nilotynib) 200 mg, 112 kaps., kod EAN: 5909990073535 jest dostępny w ramach programu lekowego „Leczenie przewlekłej białaczki szpikowej ICD-10 C92.1" (załącznik B.14 do ww. Obwieszczenia Ministra Zdrowia). Urzędowa cena zbytu wynosi 10 588,46 zł. Poziom odpłatności dla pacjenta: bezpłatnie.

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TWOI PACJENCI JUŻ NIE POTRZEBOWALI TABLETEK?