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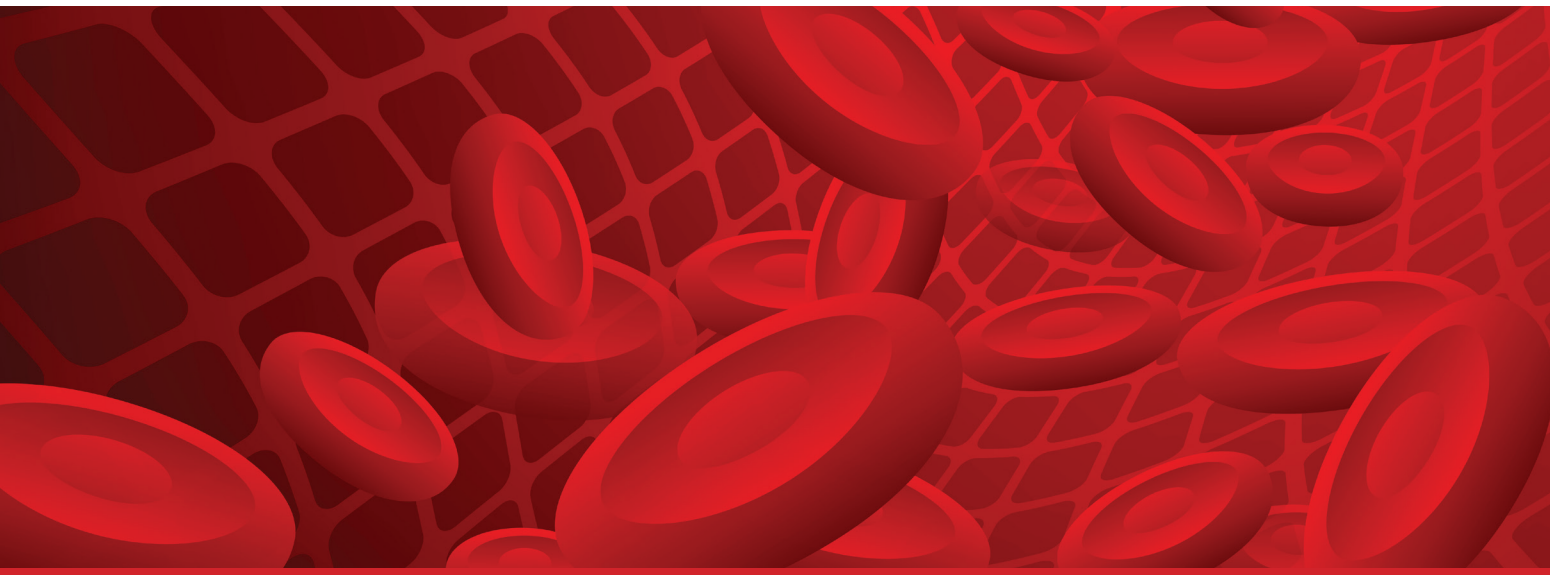

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21-0724.001.002

Jedyna terapia celowana dla pacjentów z przewlekłą anemią w przebiegu MDS

PRZYWRACA PRAWIDŁOWE DOJRZEWANIE KOMÓREK LINII ERYTROIDALNEJ

Wskazanie do stosowania u dorosłych pacjentów:

(konieczne spełnienie wszystkich warunków)[1]

- z anemią zależną od transfuzji
- w przebiegu MDS bardzo niskiego, niskiego i średniego ryzyka wg IPSS-R
- z obecnością pierścieniowatych syderoblastów
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Tabela1: Dane dotyczące zmniejszania dawki w przypadku MDS

Aktualna dawka	Zmniejszenie dawki
1,75 mg/kg	1,33 mg/kg
1,33 mg/kg	1mg/kg
1mg/kg	0,8 mg/kg

Jeżeli u pacjenta wystąpią utrzymujące się działania niepożądane stopnia 3 lub wyższego związane z leczeniem, leczenie powinno zostać opóźnione do momentu poprawy w zakresie toksyczności lub powrotu do stanu wyjściowego. Po opóźnieniu podania dawki pacjenci powinni ponownie rozpocząć leczenie od poprzedniej dawki lub zmniejszoną dawką zgodnie z wytycznymi dotyczącym i zmniejszania dawki. *Pominięcie dawek:* W przypadku pominięcia lub opóźnienia w stosunku do zaplanowanego podania leku pacjentowi należy jak najszybciej podać produkt leczniczy Reblozyl, a dawkowanie powinno być kontynuowane zgodnie z zaleceniami, z odstępem co najmniej 3 tygodni pomiędzy dawkami. *Pacjenci, u których nastąpiła utrata odpowiedzi:* Jeżeli u pacjentów nastąpi utrata odpowiedzi na leczenie z zastosowaniem produktu leczniczego Reblozyl, należy ocenić czynniki przyczynowe (np. epizod krwawienia). Jeżeli typowe przyczyny utraty odpowiedzi hematologicznej zostaną wykluczone, należy rozważyć zwiększenie dawki w sposób opisany powyżej dla konkretnego wskazania leczniczego. *Przerwanie stosowania produktu leczniczego*: Stosowanie produktu leczniczego Reblozyl należy przerwać, jeżeli u pacjenta nie nastąpi zmniejszenie zależności od transfuzji po 9 tygodniach leczenia (3 dawkach) przy maksymalnym poziomie dawki, jeżeli nie stwierdzono innych przyczyn wyjaśniających niepowodzenie odpowiedzi (np. krwawienie, zabieg chirurgiczny, inne choroby współistniejące)lub w przypadku wystąpienia niedopuszczalnej toksyczności. Szczególne populacje: *Pacjenci w podeszłym wieku*: Dostosowanie dawki początkowej nie jest wymagane w przypadku produktu leczniczego Reblozyl. *Pacjenci z zaburzeniami czynności wątroby* Dostosowanie dawki początkowej nie jest wymagane w przypadku pacjentów, u których poziom bilirubiny całkowitej (BIL) wynosi > górnej granicy normy (GGN) i (lub) aminotransferazy alaninowej (ALT) lub aminotransferazy asparaginianowej (AST) wynosi < 3 x GGN. Nie można sformułować konkretnych zaleceń dotyczących dawkowania w przypadku pacjentów z ALT lub AST ≥ 3xGGN lub uszkodzeniem wątroby stopnia ≥3 wg CTCAE z powodu braku danych. *Pacjenci z zaburzeniami czynności nerek:* Dostosowanie dawki początkowej nie jest wymagane w przypadku pacjentów z zaburzeniami czynności nerek o nasileniu łagodnym do umiarkowanego [szacunkowy współczynnik przesączania kłębuszkowego (ang. *estimated glomerular filtration rate*, eGFR) od < 90 do ≥ 30 ml/min/1,73 m^2]. Nie można sformułować konkretnych zaleceń dotyczących dawkowania w przypadku pacjentów z ciężkimi zaburzeniami czynności nerek (eGFR <30 ml/min/1,73 m^2) z powodu braku danych klinicznych. Pacjenci z zaburzeniami czynności nerek w punkcie wyjściowym powinni być uważnie monitorowani pod kątem czynności nerek w ramach leczenia standardowego. *Dzieci i młodzież* Stosowanie produktu leczniczego Reblozyl u dzieci i młodzieży we wskazaniu zespoły mielodysplastyczne nie jest właściwe. Sposób podawania: Podanie podskórne. Po rekonstytucji roztwór produktu leczniczego Reblozyl należy wstrzyknąć podskórnie w górną część ramienia, udo lub brzuch. Należy obliczyć dokładną całkowitą objętość sporządzonego roztworu wymaganego dla pacjenta i pobrać ją powoli z jednodawkowej fiolki/fiolek do strzykawki. Zalecana maksymalna objętość produktu leczniczego w miejscu wstrzyknięcia wynosi 1,2 ml. Jeśli wymagane jest podanie więcej niż 1,2 ml, całkowita objętość powinna zostać podzielona na oddzielne wstrzyknięcia o podobnej objętości i podawana w różne miejsca. Jeśli wymagane są wielokrotne wstrzyknięcia, do każdego wstrzyknięcia podskórnego należy użyć nowej strzykawki i igły. Z fiolki należy podać nie więcej niż jedną dawkę. Jeśli roztwór produktu leczniczego Reblozyl został schłodzony po rekonstytucji, należy go wyjąć z lodówki 15–30 minut przed wstrzyknięciem, aby umożliwić osiągnięcie temperatury pokojowej. Zwiększy to komfort wstrzyknięcia. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą. Ciąża. **Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania** Identyfikowalność: W celu poprawienia identyfikowalności biologicznych produktów leczniczych należy czytelnie zapisać nazwę i numer serii podawanego produktu. Podwyższone ciśnienie krwi: W kontrolowanych badaniach klinicznych dotyczących MDS i β-talasemii u pacjentów leczonych luspatercept wykazano średni wzrost skurczowego i rozkurczowego ciśnienia krwi o 5 mm Hg w stosunku do wartości wyjściowej. Ciśnienie krwi należy monitorować przed każdym podaniem luspatercept. W przypadku utrzymującego się nadciśnienia lub zaostrzenia występującego wcześniej nadciśnienia pacjenci powinni otrzymywać leczenie nadciśnienia zgodnie z aktualnymi wytycznym i klinicznymi. Zawartość sodu Ten lek zawiera mniej niż 1 mmol (23 mg) sodu na dawkę, to znaczy lek uznaje się za „wolny od sodu". **Działania niepożądane:** Zarys profilu bezpieczeństwa: *Zespoły mielodysplastyczne:* Najczęściej zgłaszanymi działaniami niepożądanymi u pacjentów przyjmujących produkt leczniczy Reblozyl (u co najmniej 15% pacjentów) były zmęczenie, biegunka, osłabienie, nudności, zawroty głowy, ból pleców i ból głowy. Najczęściej zgłaszanymi działaniami niepożądanymi stopnia 3 lub wyższego (u co najmniej 2% pacjentów) były między innymi omdlenie/stan przedomdleniowy, zmęczenie, nadciśnienie tętnicze i osłabienie. Najczęściej zgłaszanymi ciężkimi działaniami niepożądanymi (u co najmniej 2% pacjentów) były zakażenie układu moczowego, ból pleców i omdlenie. Osłabienie, zmęczenie, zawroty głowy i ból głowy występowały częściej w trakcie pierwszych 3 miesięcy leczenia. Przerwanie leczenia z powodu działania niepożądanego nastąpiło u 2,0% pacjentów leczonych luspaterceptem. Działaniami niepożądanymi prowadzącymi do przerwania leczenia w grupie leczonej luspaterceptem były zmęczenie i ból głowy. Tabelaryczny wykaz działań niepożądanych Poniższa Tabela 2 zawiera dane dotyczące najwyższej częstotliwości występowania każdego działania niepożądanego, które zaobserwowano i zgłaszano w dwóch przed rejestracyjnych badaniach dotyczących MDS i β-talasemii. Działania niepożądane wymieniono poniżej według klasyfikacji układów i narządów i preferowanych terminów. Częstość występowania z definiowano w następujący sposób: 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) i bardzo rzadko(<1/10 000)

Tabela 2. Działania niepożądane u pacjentów leczonych produktem leczniczym Reblozylwprzypadku MDS

Klasyfikacja układów i narządów	Preferowany termin	Częstość (wszystkie stopnie)
Zakażenia i zarażenia pasożytnicze	zapalenie oskrzeli	Bardzo często
	zakażenie dróg moczowych	Bardzo często
	zakażenie górnych dróg oddechowych	Często
	grypa	Często
Zaburzenia układu immunologicznego	nadwrażliwość*	Często
Zaburzenia metabolizmu i odżywiania	hiperurykemia	Często
Zaburzenia układu nerwowego	zawroty głowy	Bardzo często
	ból głowy	Bardzo często
	omdlenie/stan przedomdleniowy	Często
Zaburzenia ucha i błędnika	zawroty głowy pochodzenia błędnikowego lub pozycyjne zawroty głowy pochodzenia błędnikowego	Często
Zaburzenia naczyniowe	nadciśnienie tętnicze~	Często
	zdarzenia zakrzepowo-zatorowe§	Często
Zaburzenia układu oddechowego, klatki piersiowej i śródpiersia	duszność	Bardzo często
Zaburzenia żołądka i jelit	biegunka	Bardzo często
	nudności	Bardzo często
Zaburzenia mięśniowo-szkieletowe i tkanki łącznej	ból pleców	Bardzo często
	bóle stawów	Często
	ból kości	Często
Zaburzenia ogólne i stany w miejscu podania	zmęczenie	Bardzo często
	osłabienie	Bardzo często
	reakcje w miejscu wstrzyknięcia#	Często

*Nadwrażliwość obejmuje obrzęk powiek, nadwrażliwość na lek, obrzęk twarzy, obrzęk około oczodołowy, obrzęk twarzy, obrzęk naczynioruchowy, obrzęk warg, wysypkę polekową. ~Reakcja nadciśnieniowa obejmuje samoistne nadciśnienie, nadciśnienie i przełom nadciśnieniowy. #Reakcje w miejscu wstrzyknięcia obejmują rumień w miejscu wstrzyknięcia, świąd w miejscu wstrzyknięcia, obrzęk w miejscu wstrzyknięcia i wysypkę w miejscu wstrzyknięcia. §Zdarzenia zakrzepowo-zatorowe obejmują zakrzepicę żył głębokich, zakrzepicę żył wrotnych, udar niedokrwienny i zatorowość płucną. Opis wybranych działań niepożądanych: *Ból kości*: Ból kości zgłoszono u 19,7% pacjentów z β-talasemią leczonych luspaterceptem (placebo 8,3%) oraz u 2,6% pacjentów z MDS leczonych luspaterceptem (placebo 3,9%). U pacjentów z β-talasemią leczonych luspaterceptem ból kości występował najczęściej w okresie pierwszych 3 miesięcy (16,6%) w porównaniu z miesiącami 4–6 (3,7%). Większość zdarzeń (41/44 zdarzeń) miała stopień nasilenia 1–2, przy czym 3 zdarzenia miały stopień nasilenia 3. Jedno z 44 zdarzeń było poważne, a 1 zdarzenie doprowadziło do przerwania leczenia. *Bóle stawów*: Bóle stawów zgłoszono u 19,3% pacjentów z β-talasemią leczonych luspaterceptem (placebo 11,9%) oraz u 5,2% pacjentów z MDS leczonych luspaterceptem (placebo 11,8%). W grupie pacjentów z β-talasemią leczonych luspaterceptem bóle stawów doprowadziły do przerwania leczenia u 2 pacjentów (0,9%). *Nadciśnienie tętnicze*: U pacjentów leczonych luspaterceptem wystąpił średni wzrost skurczowego i rozkurczowego ciśnienia krwi o 5 mm Hg w stosunku do punktu wyjściowego, którego nie zaobserwowano u pacjentów przyjmujących placebo. Nadciśnienie zgłoszono u 8,5% pacjentów z MDS leczonych luspaterceptem (placebo 9,2%) oraz u 8,1% pacjentów z β-talasemią leczonych luspaterceptem (placebo 2,8%). W grupie pacjentów z MDS zdarzenia o stopniu nasilenia 3 zgłoszono u 5 pacjentów (3,3%) leczonych luspaterceptem i u 3 pacjentów (3,9%) przyjmujących placebo. Nie przerwano leczenia w przypadku żadnego z pacjentów z powodu nadciśnienia. W grupie pacjentów z β-talasemią zdarzenia o stopniu nasilenia 3 zgłoszono u 4 pacjentów (1,8%) leczonych luspaterceptem (0,0% placebo).Nie przerwano leczenia w przypadku żadnego z pacjentów z powodu nadciśnienia. *Nadwrażliwość*: Reakcje typu nadwrażliwości (w tym obrzęk powiek, nadwrażliwość na lek, opuchlizna twarzy, obrzęk okołooczodołowy, obrzęk twarzy, obrzęk naczynioruchowy, obrzęk warg,wysypka polekowa) zgłoszono w przypadku 4,6% pacjentów z MDS (2,6% placebo) oraz 4,5% pacjentów z β-talasemią leczonych luspaterceptem (1,8% placebo). W badaniach klinicznych wszystkie zdarzenia miały stopień nasilenia 1/2. W grupie pacjentów z β-talasemią leczonych luspaterceptem nadwrażliwość doprowadziła do przerwania leczenia u 1 pacjenta (0,4%). *Reakcje w miejscu wstrzyknięcia*: Reakcje w miejscu wstrzyknięcia (w tym rumień w miejscu wstrzyknięcia, świąd w miejscu wstrzyknięcia, obrzęk w miejscu wstrzyknięcia i wysypka w miejscu wstrzyknięcia)zgłoszono w przypadku 3,9% pacjentów z MDS (placebo 0,0%) i u 2,2% pacjentów z β-talasemią otrzymujących luspatercept (placebo 1,8%). W badaniach klinicznych wszystkie zdarzenia miały stopień nasilenia 1, a żadne nie doprowadziło do przerwania leczenia. *Zdarzenia zakrzepowo-zatorowe*: Zdarzenia zakrzepowo-zatorowe (w tym zakrzepica żył głębokich, zakrzepica żył wrotnych, udar niedokrwienny i zatorowość płucna) wystąpiły u 3,6% pacjentów z β-talasemią otrzymujących luspatercept (placebo 0,9%). Wszystkie zdarzenia zgłoszono u pacjentów, którzy zostali poddani splenektomii, i u których występował co najmniej jeden inny czynnik ryzyka. Nie zaobserwowano różnic w zakresie zdarzeń zakrzepowo-zatorowych pomiędzy grupami otrzymującymi miluspatercept i placebo w przypadku pacjentów z MDS. *Immunogenność* W badaniach klinicznych dotyczących MDS analiza 260 pacjentów z MDS, którzy byli leczeni luspaterceptem i kwalifikowali się do oceny obecności przeciwciał przeciw luspaterceptowi, wykazała, że 23 (8,8%) pacjentów z MDS uzyskało dodatni wynik badania pod kątem obecności przeciwciał przeciw luspaterceptowi wytworzonych w toku leczenia, w tym u 9 (3,5%) pacjentów z MDS stwierdzono przeciwciała neutralizujące przeciw luspaterceptowi. W badaniach klinicznych dotyczących β-talasemi i analiza 284 pacjentów z β-talasemią, którzy byli leczeni luspaterceptem i kwalifikowali się do oceny obecności przeciwciał przeciw luspaterceptowi, wykazała, że 4 (1,4%) pacjentów z β-talasemią uzyskało dodatni wynik badania pod kątem obecności przeciwciał przeciw luspaterceptowi wytworzonych w toku leczenia, w tym u 2 (0,7%) pacjentów z β-talasemią stwierdzono przeciwciała neutralizujące przeciw luspaterceptowi. Stężenie luspaterceptu w surowicy wykazywało tendencję malejącą w obecności przeciwciał neutralizujących. Nie odnotowano ciężkich systemowych reakcji nadwrażliwości u pacjentów, u których występowały przeciwciała przeciw luspaterceptowi. Nie występował związek pomiędzy reakcjami typu nadwrażliwości lub reakcjami w miejscu wstrzyknięcia a obecnością przeciwciał przeciw luspaterceptowi.

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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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EDITORIAL

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Progress in molecular profiling: new recommendations in myelodysplastic syndromes

Jan Styczyński

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Myelodysplastic syndromes (MDS) are highly heterogeneous disorders characterised by the presence of ineffective hematopoiesis with peripheral blood cytopenias, dysplastic changes in ≥10% of cells in one or more myeloid lineages, and a variable risk of progression to acute myeloid leukemia (AML) [1].

With the rapid development of molecular biology over recent years, significant progress has been made in understanding the genetic characteristics of MDS. Although molecular tests are not included in the diagnostic standard in MDS, many studies have confirmed the predictive and prognostic impact of a number of mutations in patients with this diagnosis. Such knowledge highlights a clear path towards the development of targeted therapies. Today, numerous trials are running all over the world aimed at the use of targeted therapies in higher risk MDS. Additionally, significant progress has been made in therapy of lower risk MDS, especially for those who are transfusion-dependent.

In this issue of "Acta Haematologica Polonica", Mądry et al. [2] present new Polish recommendations for the diagnostics of MDS. This will be followed by recommendations on MDS treatment to appear in the next issue of "Acta Haematologica Polonica" [3]. These recommendations are in line with our journal's policy of international cooperation in hematology [4–10].

Authors' contributions

JS — sole author.

Conflict of interest

Nothing to disclose.

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Ethics

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

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Diagnosis of myelodysplastic syndromes in Poland: Polish Adult Leukemia Group (PALG) 2021 recommendations

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Abstract

Myelodysplastic syndromes (MDS) are a heterogeneous group of neoplastic diseases of the hematopoietic cells manifested by ineffective hematopoiesis and a tendency to transform into acute myeloid leukemia. MDS should be considered in the differential diagnosis of cytopenia, especially in the elderly. This article presents the recommendations of MDS experts of the Polish Adult Leukemia Group (PALG) for the diagnosis of myelodysplastic syndromes. We present current classifications and prognostic indices, as well as diagnostic examinations recommended for MDS: cytological, histopathological, immunophenotypic, cytogenetic and molecular tests. The aim of the study is to implement up-to-date knowledge about myelodysplastic syndromes into routine clinical practice, from the diagnosis of cytopenia to the specific diagnosis and prognosis in MDS patients.

Key words: myelodysplastic syndromes, diagnosis, recommendations

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Introduction

Myelodysplastic syndromes (MDS) are neoplastic diseases with a variable clinical course originating from hematopoietic stem cells (HSCs). A characteristic feature of MDS is ineffective hematopoiesis manifested by dysplasia of bone marrow and peripheral blood cells, peripheral cytopenia, and an increased risk of transformation into acute myeloid leukemia (AML).

Epidemiology

It is estimated that the incidence rate of newly diagnosed MDS is 4.5/100,000/year. Usually the disease affects the elderly. The median adult age at diagnosis is 70–75 years, whilst according to the Polish MDS Registry it is 70 years [1, 2]. In the age bracket of 70–74, the incidence is 16.6/100,000/year; in the age bracket of 75–79 years it is 25.7/100,000/year; and this increases to over 36/100,000/year in people aged 80 and over [3]. MDS in children is very rare, with a median age at diagnosis of 6.8 years. In the population of people under 30 years, the incidence rate of newly diagnosed MDS is 0.1/100,000/year. Patients <50 years of age constitute 9% of all MDS cases. The disease is more prevalent among men, with a male to female ratio 1.1–1.4:1 and this ratio increases with age.

The number of MDS cases is probably underestimated due to the lack of a proper diagnosis of cytopenia in the elderly.

Clinical manifestation

The clinical manifestation of MDS is not characteristic, with symptoms most often resulting from cytopenia. There are features of anemia, thrombocytopenia, infections (usually bacterial and fungal) resulting from granulocytopenia and dysfunction of neutrophils. Hepato- and splenomegaly is rare. General symptoms (weight loss, fever, sweating) are also not common and occur in higher risk MDS patients or during leukemic transformation. Autoimmune diseases are detected in c.19–28% of MDS patients [4], and the most common include hypothyroidism (12%), rheumatoid arthritis (3%), autoimmune thrombocytopenia (3%), psoriasis (2%), ulcerative colitis (1%), and vasculitis (1%). Polymyalgia rheumatica (PMR), skin ulcers, iritis, myositis, peripheral neuropathy, Sweet's syndrome, and pericarditis are slightly less common. Pure red cell anemia (PRCA), large granular lymphocytic leukemia (LGL) and nocturnal paroxysmal hemoglobinuria (PNH) are associated with MDS and they are also found more often than in the general population.

It is worth adding that patients with MDS, especially after the age of 65, are more likely to develop cardiovascular events (CVE), including myocardial infarction, heart failure, and arrhythmia. Within three years of MDS diagnosis, CVE have occurred in 73.2% of patients, compared to 54.5% of 1.4 million people receiving medical services for other reasons (Medicare, USA) [5].

Diagnosis

The primary examination is complete blood count (CBC) with differential (features of dyshematopoiesis, percentage of blasts). The anemia is usually macrocytic, less commonly normocytic or microcytic, and the number of reticulocytes is usually not increased. Cytopenia is a prerequisite for MDS diagnosis. In the differential diagnosis of cytopenia, it is recommended to perform basic tests, such as: iron metabolism parameters, vitamin B_{12} and folic acid levels, direct antiglobulin test (DAT), renal and hepatic function, and exclusion of viral diseases [hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV)].

Another examination is a bone marrow aspiration with cytomorphological, histopathological, cytochemical (Prussian blue staining to detect sideroblasts) and cytogenetic tests.

Cytomorphological bone marrow examination is decisive in assessing the percentage of blasts and the features of dysplasia, both qualitatively and quantitatively [6]. The most common dysplastic features found in patients with MDS are set out in Table I. The coexistence of cytopenia with morphological changes typical for MDS or an increased percentage of blasts allow for an MDS diagnosis. Histopathology should be routinely performed in all patients, and is particularly helpful in assessing bone marrow cellularity, fibrosis, megakaryocyte dysplasia, atypical localization of immature precursors (ALIP), and blast rates in cases where cytomorphology is not fully reliable. In 80% of patients, there is increased bone marrow (BM) cellularity, while in the remaining 20%, BM is normo- or hypocellular.

The diagnosis of MDS is also possible in patients with no obvious signs of dysplasia or increased blast percentage, if specific cytogenetic changes are present, i.e. –7 or del(7q), del(5q), and (17q) or del(17p), –13 or del(13q), del(11q), del(12p) or t(12p), del(9q), idic(X) (q13), t(11; 16) (q23; p13.3), t(3; 21) (q26.2; q22.1), t(1; 3) (p36.3; q21.1), t(1; 3) (p36.3; q21.1), t(2; 11) (p21; q23), inv(3) (q21q26.2), t(3; 3) (q21; q26.2), t(6; 9) (p23; q34). Cytogenetic abnormalities such as +8, del (20q) and –Y are not considered specific for MDS. Molecular and immunophenotypic tests by flow cytometry are complementary and not mandatory. MDS diagnostic criteria are set out in Table II [7].

The initial diagnostic workup for MDS is set out in Table III, while Figure 1 shows MDS diagnostic algorithm.

Differential diagnosis

The first priority in the differential diagnosis of MDS is to exclude primary extramedullary causes of cytopenia,

Table I. Marrow dysplastic features

Erythroid lineage	Cell nucleus: • polyploid shapes • intranuclear bridges • internuclear bridges • tabs • multinuclear forms • megaloblastic forms • nuclear hypersegmentation • pyknosis Cytoplasm: • ring-shaped sideroblasts • vacuolization • uneven staining of cytoplasm • basophilic spotting • Howell-Jolly bodies (fragments of a disintegrated nucleus) • Pappenheimer bodies Asynchronous maturation of nucleus in relation to cytoplasm
Granulocyte lineage	Too small or too large precursor forms Cell nucleus: • atypical nuclear shape • biplane cell nuclei (pseudo Pelger-Huët) • nuclear hypersegmentation Cytoplasm: • reduction of cytoplasm granularity • irregular granularity distribution • pseudo-Chédiak-Higashi granules • Auer rods • vacuoles in cytoplasm Asynchronous maturation of nucleus in relation to cytoplasm
Megakaryocytic lineage	Micromegakaryocytes (7–15 µm) One or two-lobed cell nuclei Lack of synchronization between maturation of nucleus and cytoplasm Multinuclear forms Agranular or hypogranular platelets

other hematopoietic malignancies, as well as PRCA, PNH, or bone marrow metastases. In the case of a typical family or personal medical history, especially in younger people, congenital myeloid neoplasms should be excluded. The causes leading to cytopenia/dysplasia are set out in Table IV.

Pre-MDS states

Some healthy people have somatic mutations/cytogenetic abnormalities typical for myeloid neoplasms. The lack of cytopenia and other features of MDS allows for the diagnosis of clonal hematopoiesis with indeterminate potential (CHIP). In the case of cytopenia without other features of MDS or dysplasia without cytopenia and other features of MDS, idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of undetermined significance (IDUS) are diagnosed, respectively. On the other hand, if cytopenia is revealed without a detectable cause and molecular or cytogenetic abnormalities specific for MDS, a clonal cytopenia of undetermined significance (CCUS) should be diagnosed [8].

It has not been determined whether the acquired mutations in CHIP are the result of aging or an early stage of cancer. Some patients may develop MDS in the future (0.5–1%/year). In people with CHIP, especially with mutations in epigenetic factors, an increased incidence of coexistent cardiovascular diseases has been found. The differential diagnosis of CHIP, CCUS, IDUS, and ICUS conditions is set out in Table V.

Principles of cytogenetic testing in MDS

According to the current criteria for the diagnosis of MDS, cytogenetic testing is still one of the basic diagnostic and prognostic examinations.

Considering cytogenetic abnormalities, only the deletion of the long arm of chromosome 5 defines a distinct histoclinical entity — MDS with an isolated 5q deletion. In the current World Health Organization (WHO) classification, the scope of the concept of 'isolated' has been extended; this category still includes del(5q) as the only aberration, but also del(5q) with one additional aberration, other than the unfavorable chromosome 7 aberrations, e.g. del(7q) and monosomy 7 (–7) [9]. Classic cytogenetic testing in MDS should be performed before commencement of any treatment, because, for example, treatment with steroids may inhibit cell division and make it impossible to put the right diagnosis. The sample should be bone marrow, as there may be an insufficient number of CD34+ cells in the peripheral blood. The culture of the marrow cells of patients suffering from MDS is a short-term, 24- or 48-hour culture without mitogen (stimulator of cell proliferation). Chromosomes are analyzed after G-banding by trypsin with Giemsa (GTG) staining. In MDS, as a rule, at least 20 metaphases are analyzed. If it is difficult to obtain 20 metaphases, a few cells with a clonal aberration are sufficient to determine the result (clonal aberration is defined as the presence of a given trisomy or a structural aberration in at least two cells, or a given monosomy in at least three cells). On the other hand, the lack of aberration does not allow the completion of the test before the analysis of 20 cells. In about half of the karyotypes, chromosomal aberrations are found, most often –5/del (5q), –7/del(7q), +8, del(20q), –Y. The most common abnormalities are set out in Table VI [10].

Table II. Minimal diagnostic criteria of myelodysplatic syndrome (MDS)

MDS can be diagnosed when both preliminary criteria (A) and at least one criterion B are met

A. Preliminary criteria (both must be met)

1. Persistent (≥4 months) peripheral blood cytopenia* affecting ≥1 cell lineage: erythroid, neutrophilic, megakaryocytic (in the case of excess blasts or cytogenetic changes associated with MDS, the diagnosis can be made immediately)
2. Exclusion of other causes of cytopenia/dysplasia**

B. MDS-specific criteria (major; at least one must be met)

1. Dysplasia in ≥10% of cells of a given cell lineage: erythroid, neutrophilic, megakaryocytic***
2. In at least 15% ring-shaped sideroblasts or ≥5% ring-shaped sideroblasts with *SF3B1* mutation
3. From 5% to 19% myeloblasts in bone marrow cytology (or 2–19% myeloblasts in peripheral blood)
4. Typical chromosome abnormalities confirmed by conventional cytogenetics or FISH****

C. Additional criteria — for patients meeting both criteria A but no criteria B, in the case of typical clinical manifestation, e.g. transfusion-dependent macrocytic anemia ≥2 additional criteria (C) must be met in order to diagnose MDS. Regular bone marrow biopsies may allow the final diagnosis

1. Abnormalities in the histological examination and/or immunohistochemistry of trepanobioptates supporting the diagnosis of MDS***
2. Abnormalities in the immunophenotypic test of bone marrow cells, in the form of numerous aberrant MDS phenotypes that indicate the monoclonal nature of erythroid and/or myeloid lineages
3. Proof of the clonality of myeloid lineage cells confirmed in a molecular test by finding somatic mutations typical for MDS*****

*Cytopenia defined as values below the specific reference ranges in a given laboratory; **in rare cases, MDS may coexist with other causes of cytopenia; ***examples: clusters of atypical localization of immature precursors (ALIP), clusters of CD34+ blasts, immunohistochemically confirmed dysplastic micromegakaryocytes (≥10% dysplastic megakaryocytes); ****chromosome abnormalities typical of MDS, e.g. del(5q), –7 indicate the diagnosis of MDS even in the absence of morphological changes; *****the presence of numerous typical mutations (e.g. *SF3B1*) increases the likelihood of MDS diagnosis or the development of MDS in future; FISH — fluorescence *in situ* hybridization

The 5q deletion covering the 5q31 (*EGR1*)–5q33.1 (*RPS14*) region and appearing alone or accompanied by a single additional aberration defined above is associated with a favorable prognosis.

Older men may experience a loss of the Y chromosome unrelated to MDS. Therefore, in this group, –Y is assigned to MDS when it occurs in more than 70% of metaphases, while in younger men the threshold is 30%. A complex karyotype (at least three independent autosomal chromosome aberrations, i.e. autosomes) is always unfavorable. It is often accompanied by the loss of ≥1 copy of *TP53* gene or its mutation. The monosomal karyotype (at least two autosomal monosomies or at least one autosomal monosomy and at least one autosome structural aberration) is always unfavorable, and according to some authors it is worse than the complex karyotype.

If metaphasal plates are not obtained, or the number of metaphasal planes obtained, which also does not contain clonal aberrations, is suboptimal (<20), or they are unreadable, FISH with the MDS probe panel [for –5/del(5q), –7/del(7q), +8, del(17p)/*TP53*, del(20q), and possibly –Y] should be performed. Some of these probes can be used together, which speeds up FISH diagnostics. In total, 100–200 interphasic nuclei are routinely analyzed under fluorescence microscopy. It is believed that FISH detects cytogenetic abnormalities in 15% of patients with normal karyotype tested using the classical method [11].

Immunophenotyping

The WHO classification of hematopoietic neoplasms indicates that flow cytometry (FC) is not a sufficient diagnostic method for MDS in the absence of definitive cytomorphological and/or cytogenetic criteria. However, it points out that the aberrant immunophenotypic features show a strong correlation with this diagnosis [12].

Immunophenotype abnormalities described as typical MDS are shown in 6.4% of cytopenia cases without marked dysplasia in cytological examination, however, around one third of these cases present with typical cytogenetic changes.

The European LeukemiaNet (ELN) Working Group for Flow Cytometry in MDS (IMDSFlow) proposed guidelines for the use of this method in the integrated diagnosis of MDS [13]. For screening purposes, a mini-panel (so-called Ogata score) with four parameters (one point for each) can be used [12]:

- increased CD34+ progenitors rate among all BM nucleated cells (>2%);
- reduced CD34+/CD19+ and/or CD34+ CD10+ B-cell precursors rates among all CD34+ cells;
- altered expression of CD45 on myeloblasts relative to lymphocytes (≤4 or ≥7.8);
- reduced granularity (SSC, side scatter channel) on granulocytes in relation to lymphocytes (≤6).

A total score of ≥2 points allows the diagnosis of low-risk MDS with a specificity of 92–98%. The disadvantage

Table III. Diagnostic tests used in myelodysplatic syndrome (MDS)

Test	Material/assessment recommendations	Purpose	
CBC (differential)	Peripheral blood	Cytopenia and dysplasia of one or more cell lineages Assessment of blasts rate	Required
Bone marrow aspiration biopsy	Bone marrow Assessment of at least 500 cells	Assessment of bone marrow cellularity and E:M lineage ratio Assessment of blasts rate — percentage of nucleated cells regardless of dominant erythroid lineage Dysplasia of one or more hematopoietic cell lineages	Required
	Ring-shaped sideroblasts — Prussian blue staining Assessment of 100 erythroblasts	Assessment of ring sideroblasts rate	Required in LR-MDS
Bone marrow aspiration biopsy (histopathology)	Biopsy ≥1 cm (formalin) • IHC minimal panel: CD34+, CD117/KIT, CD42b or CD61, tryptase • Additional panel: CD3, CD14, CD20	Assessment of BM cellularity, blasts rate and dysplasia features	Required
Cytogenetic analysis	Bone marrow (heparin) ≥5 mL Blood — conditionally, in absence of BM material	Detection of acquired chromosomal abnormalities that may allow establishment of final diagnosis and prognosis	Required
FISH	Bone marrow/peripheral blood	Detection of targeted abnormalities in the case of failure of standard cytogenetic method	Recommended
Flow cytometry — immunophenotyping	Bone marrow (EDTA) ≥2 mL	Detection of abnormalities in erythroid lineage, immature cells of myeloid lineage, mature granulocytes, monocytes, immature and mature cells of lymphoid lineage	Recommended
Molecular testing	Bone marrow (EDTA) ≥2–5 mL	Detection of somatic mutations	Recommended
	Skin, hair follicles	Detection of somatic mutations	Recommended

BM — bone marrow; CBC — complete blood count; EDTA — ethylenediaminetetraacetic acid; FISH — fluorescence *in situ* hybridization; IHC — immunohistochemistry; LR-MDS — low-risk MDS

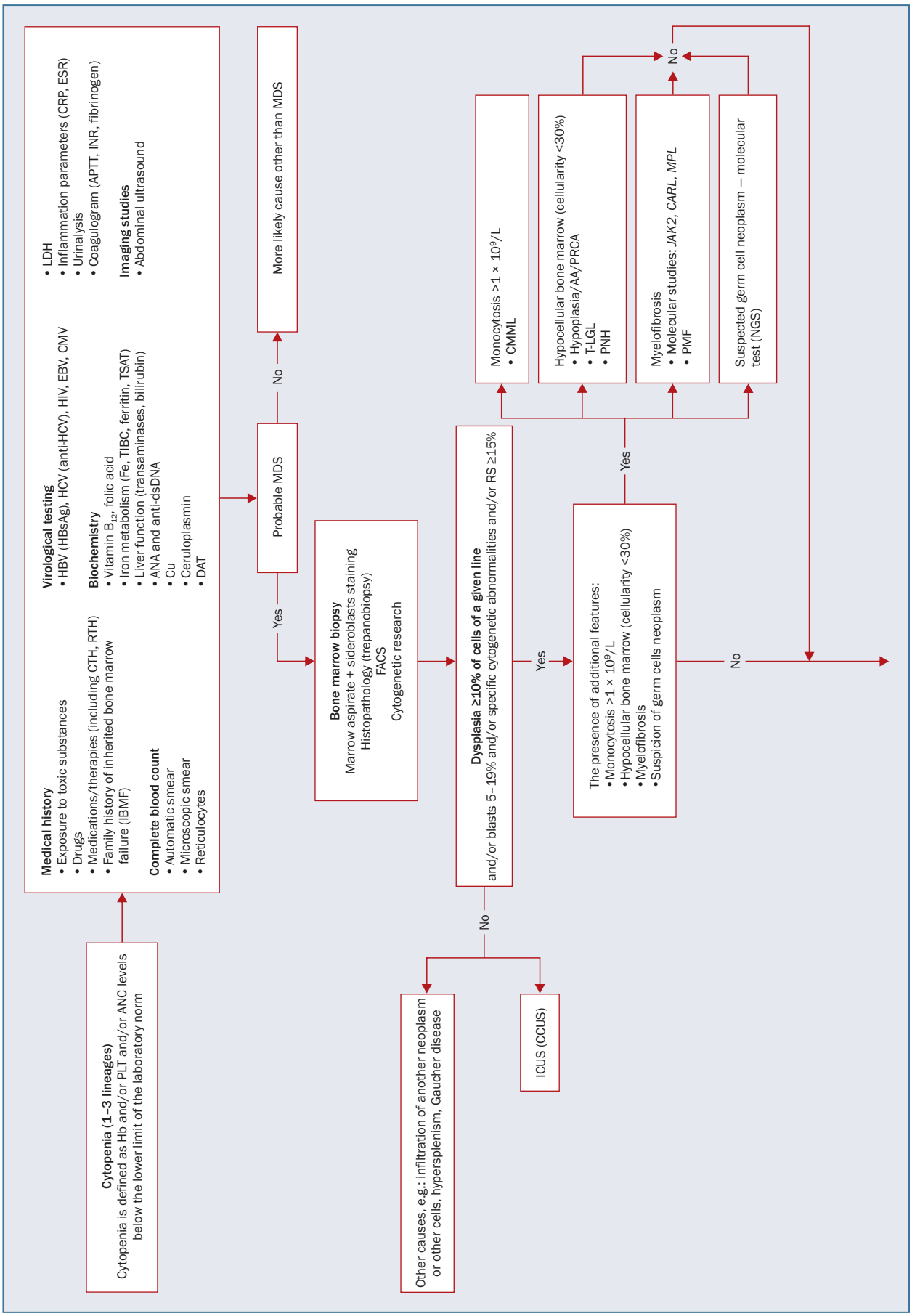
of the panel is the low sensitivity (44–71%) resulting, among others, from reduced blasts rate due to peripheral blood contamination in bone marrow aspirate sample. Reduction in the B-cell progenitors rate is sometimes found in the elderly, and MDS blasts may aberrantly fail to express CD34 [14].

In conclusion, flow cytometry testing in the diagnosis of MDS is useful:

- in cases of cytopenia without marked dysplasia and cytogenetic/molecular changes. The abnormalities found in FC may then indicate MDS or, in their absence, another cause of detected abnormalities;
- in MDS patients with single-lineage dysplasia in cytology examination, and with alterations of other lineages in FC. These changes may have a prognostic value or suggest incorrect classification of MDS (re-assessment of dysplasia in lineage disturbed in FC is strongly recommended).

Molecular testing in MDS

Thanks to the use of techniques based on next-generation gene sequencing, molecular disorders in the course of MDS have been found in c.80–90% of patients. Some of them have been shown to occur in MDS with a specific phenotype, but the most important observations concern the significance of the mutations for survival prognosis and transformation into AML, as well as treatment response. So


Cytopenia (1–3 lineages)
Cytopenia is defined as Hb and/or PLT and/or ANC levels below the lower limit of the laboratory norm
Medical history
• Exposure to toxic substances
• Drugs
• Medications/therapies (including CTH, RTH)
• Family history of inherited bone marrow failure (IBMF)
Complete blood count
• Automatic smear
• Microscopic smear
• Reticulocytes
Virological testing
• HBV (HBsAg), HCV (anti-HCV), HIV, EBV, CMV
Biochemistry
• Vitamin B12, folic acid
• Iron metabolism (Fe, TIBC, ferritin, TSAT)
• Liver function (transaminases, bilirubin)
• ANA and anti-dsDNA
• Cu
• Ceruloplasmin
• DAT
• LDH
• Inflammation parameters (CRP, ESR)
• Urinalysis
• Coagulogram (APTT, INR, fibrinogen)
Imaging studies
• Abdominal ultrasound
Probable MDS
Yes
No
More likely cause other than MDS
Bone marrow biopsy
Marrow aspirate + sideroblasts staining
Histopathology (trepanobiopsy)
FACS
Cytogenetic research
Dysplasia ≥10% of cells of a given line
and/or blasts 5–19% and/or specific cytogenetic abnormalities and/or RS ≥15%
No
Other causes, e.g.: infiltration of another neoplasm or other cells, hypersplenism, Gaucher disease
ICUS (CCUS)
Yes
The presence of additional features:
• Monocytosis >1 × 10⁹/L
• Hypocellular bone marrow (cellularity <30%)
• Myelofibrosis
• Suspicion of germ cells neoplasm
No
Yes
Monocytosis >1 × 10⁹/L
• CMML
Hypocellular bone marrow (cellularity <30%)
• Hypoplasia/AA/PRCA
• T-LGL
• PNH
Myelofibrosis
• Molecular studies: JAK2, CARL, MPL
• PMF
Suspected germ cell neoplasm — molecular test (NGS)
No

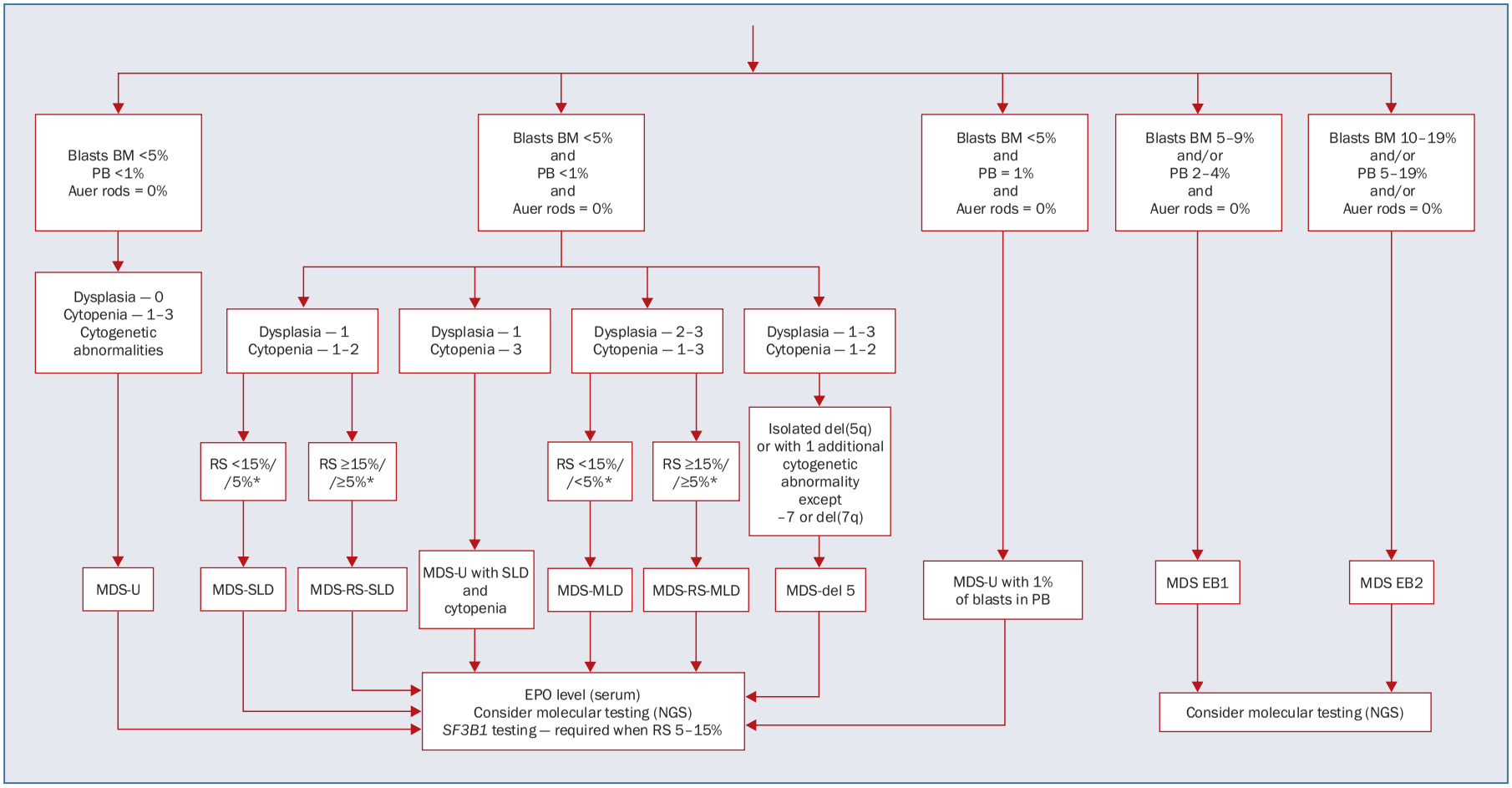


Figure 1. Diagnostic algorithm of myelodysplatic syndromes (MDS); *if mutation *SF3B1* (splicing factor 3b subunit 1) is present; AA — aplastic anemia; ANA — anti-nuclear antibodies; ANC — absolute neutrophil count; anti-dsDNA — anti-double stranded DNA; anti-HCV — anti-hepatitis C virus test; APTT — activated partial thromboplastin time; BM — bone marrow; CCUS — clonal cytopenia of undetermined significance; CTH — chemotherapy; CMML — chronic myelomonocytic leukemia; CMV — citomegalovirus; CRP — C-reactive protein; EB — excess blasts; EBV — Epstein-Bárr virus; EPO — erythropoietin; ESR — erythrocyte sedimentation rate; FACS — fluorescence-activated cell sorting; Hb — hemoglobin; HBsAg — hepatitis B surface antigen; HBV — hepatitis B virus; HCV — hepatitis C virus; HIV — human immonodeficiency virus; ICUS — idiopathic cytopenia of undetermined significance; INR — international normalized ratio; LDH — lactate dehydrogenase; MDS-del 5 — multilineage dysplasia; NGS — next-generation sequencing; PB — peripheral blood; PLT — platelets; PMF — primary myelofibrsis; PNH — nocturnal paroxysmal hemoglobinuria; PRCA — pure red cell leukemia; RS — ring sideroblasts; RTH — radiotherapy; SLD — single lineage dysplasia; TIBC — total iron-binding capacity; T-LGL — T-cell large granular lymphocytic leukemia; TSAT — transferrin saturation; U — unclassified

Table IV. Differential diagnosis of cytopenia/dysplasia

Diagnosis	Bone marrow/morphology		Other tests (serum)
Vitamin B_{12} deficiency	Megaloid forms of erythroid and granulocytic lineages, irregular shapes of erythroblast nuclei, Howell-Jolly bodies, neutrophil nucleus hypersegmentation, macrocytosis, pancytopenia, decreased number of reticulocytes, hypercellular cell bone marrow		↓ vitamin B_{12} ↓ holotranscobalamin ↑ methylmalonic acid (MMA) ↑ homocysteine ↑ LDH ↑ bilirubin
Folic acid deficiency	Similar to vitamin B_{12} deficiency		↓ folic acid ↑ homocysteine MMA in normal range
Eating syndromes	Anorexia	Hypocellular bone marrow, possible 'gelatinous transformation' of bone marrow and/or cell necrosis, more numerous histiocytes, peripheral blood acanthocytes, anemia, leukopenia, less often thrombocytopenia	↑ cholesterol ↑ ALT ↓ Mg, Zn, P, K, Na ↓ estrogen, testosterone ↓ fT3, fT4
	Copper deficiency	Pancytopenia, vacuolization of cytoplasm of erythroid and granulocytic lineages, ring-shaped sideroblasts	↓ Cu
Exposure to heavy metals (including lead Pb, Hg, Cd, As)	Hypocellular bone marrow, ring-shaped sideroblasts, pancytopenia, basophilic spotting		↑ Pb, Hg, Cd, As
Alcohol abuse	Vacuolization of cytoplasm of erythroid and granulocytic lineages, ring-shaped sideroblasts, hyperplasia of erythroid lineage, hypocellular bone marrow, macrocytosis, pancytopenia, symptoms resolve after alcohol withdrawal;stomatocytes, acanthocytes are present in liver cirrhosis		↑ serum Fe ↑ GGTP
Cytostatics	Most cytostatics	After higher doses — hypoplasia Regeneration of megakaryocytic and erythroid lineages usually precedes regeneration of granulocytic lineage After low doses — megaloblastic regeneration, dyserythropoiesis	
	Hydroxyurea, cyclophosphamide, methotrexate, azathioprine	Macrocytosis	
Anticonvulsants, antithyroid drugs, antibiotics		Bone marrow hypoplasia, dysplasia of granulocytic and megakaryocytic lineages, mono-, duo-, pancytopenia	
Isoniazid, linezolid		Sideroblastic anemia	
Trimethoprim/sulfamethoxazole, tacrolimus, mycophenolate mofetil, azathioprine		Hypersegmentation of neutrophil nuclei, dyserythropoiesis	
Steroids		Granulocytic dysplasia with high doses of corticosteroids (resolves 1–4 weeks after discontinuation) ↑ neutrophils, ↓ lymphocytes	
G-CSF		Granulocyte lineage: toxic granules, 'left shift', vacuolization of cytoplasm, abnormal lobulization of neutrophil nuclei, ↑ neutrophils	

→

Table IV (cont.). Differential diagnosis of cytopenia/dysplasia

Diagnosis	Bone marrow/morphology		Other tests (serum)
Infections	HIV	Dysplasia (especially aggravated during antiretroviral therapy), megaloblastic reaction, cytopenia, macrocytosis	Serological tests, PCR
	Parvovirus B19	Hypoplasia of erythroid lineage, presence of single, giant basophils	PCR — parvovirus B19
	HBV, HCV	Lymphocyte clusters, reactive plasmocytes, dyserythropoiesis, cytopenia (more often thrombocytopenia and anemia)	↑ bilirubin ↑ AST, ALT, ALP, INR Serological tests, PCR
	EBV, CMV	Possible cytopenias, monocytoid cells, activated lymphocytes, possible development of HLH, NHL	Serological tests, PCR
	Leishmaniasis	Pancytopenia, dysplasia, fibrosis, hemophagocytosis, iron deposits	
	Bacterial	Neutrophils: toxic granules, Doehle bodies	↑ CRP, culture
	Tuberculosis	Cytopenias (mainly anemia), numerous macrophages, granular lymphocytes, granulomas in bone marrow	Culture, PCR
Autoimmune diseases	SLE, RA	Erythroid and megakaryocytic lineage dysplasia, cytopenia	Antinuclear antibodies, rheumatoid factor, ↑ ESR
	Autoimmune hemolytic anemia (AIHA)	Anemia, slight erythroid lineage dysplasia	Positive DAT, ↑ bilirubin, ↑ LDH, ↓ haptoglobin
	Primary immune thrombocytopenia (ITP)	Possible dysplasia of megakaryocytic lineage (micromegakaryocytes) ↑ MPV ↑ PDW, giant platelets	Antiplatelet antibodies
	Autoimmune neutropenia (including Felty's syndrome)	Neutropenia, possible dysplasia, possible T-cell clone	Anti-neutrophilic antibodies, splenomegaly, rheumatoid factor
	Hypothyroidism (Hashimoto's disease)	Possibility of erythroid lineage hypoplasia, anemia, macrocytosis, ↓ reticulocytes	TSH, fT3, fT4, anti-TG antibodies, anti-TPO antibodies
Chronic idiopathic neutropenia		Normal bone marrow or hypoplasia of granulocytic lineage, possible lymphopenia	
Post-transplant	Liver	Possible trilinear dysplasia, macrocytosis of erythrocytes	
	allo-HCT	Hypoplastic bone marrow, dyserythropoiesis, risk of t-MDS/t-AML	
Aplastic anemia		Hypocellular bone marrow, mainly lymphocytes present, anemia, duo- or pancytopenia, ↓ reticulocytes	
HLH		Cytopenia, hemophagocytosis and to a lesser extent dysplasia	↑ ferritin, TG ↓ fibrinogen ↑ sCD25 Imaging tests — splenomegaly
PNH		Cytopenia, hypo-/normocellular bone marrow	FACS: ↓ FLAER, CD 55, CD59 on granulocytes, monocytes and erythrocytes, ↑ bilirubin
LGL		Neutropenia, pancytopenia, possibility of hypoplastic bone marrow, infiltration of clonal T or NK lymphocytes in bone marrow, granular lymphocytes in peripheral blood	Possibility of a positive rheumatoid factor, imaging tests: splenomegaly
Congenital bone marrow failure syndromes (e.g. Fanconi anemia)		Cytopenias	Typical genetic abnormalities

→

Table IV (cont.). Differential diagnosis of cytopenia/dysplasia

Diagnosis	Bone marrow/morphology	Other tests (serum)
Other hematopoietic malignancies (aCML, PMF, CMML)	1–3 linear dysplasia, fibrosis, anemia, thrombocytopenia Monocytosis >1 × 10^9/L (CMML) or immature forms of granulocyte lineage >10% peripheral blood WBC (aCML) or erythroblasts (PMF)	*JAK2* V617F, *MPL*, *CALR* genes mutations imaging tests — spleno- and/or hepatomegaly
Congenital sideroblastic anemia (e.g. XLSA)	Erythroid lineage dysplasia, ring-shaped sideroblasts, Pappenheimer bodies, ↓ MCV	Typical genetic abnormalities (e.g. *ALAS2* gene mutation) ↑ ferritin, hepatosplenomegaly

↓ — reduced value; ↑ — increased value; aCML — atypical chronic myeloid leukemia; ALAS2 — aminolaevulinic acid synthetase 2; allo-HCT — allogeneic hematopoietic cell transplantation; anti-TG — anti-thyroglobulin antibodies; ALP — alkaline phosphatase; ALT — alanine aminotransferase; anti-TPO — anti-thyreoperoxidase antibodies; As — arsenic; AST — aspartate aminotransferase; CD — cadmium; CMML — chronic myelomonocytic leukemia; Cu — cuprum; DAT — direct antiglobulin test; ESR — erythrocyte sedimentation rate; FACS — flow cytometric immunophenotypic testing; Fe —ferrum; FLAER — alexa 488 proaerolysin variant; fT3 — free triiodothyronine; fT4 — free thyroxine; G-CSF — granulocyte colony-stimulating factor; GGTP — gamma-glutamyltranspeptidase; Hg — mercury; HIV — human immonodeficiency virus; HLH — hemophagocytic lymphohistiocytosis; INR — international normalized ratio; ITP — primary immune thrombocytopenia; K — potassium; LDH — lactate dehydrogenase; LGL — large granular lymphocytic leukemia; line E — erythroid lineage; line G — granulocytic lineage; line M — megakaryocytic lineage; MCV — mean corpuscular volume; Mg — magnesium; MMA — methylmalonic acid; MPV — mean platelet volume; Na — calcium; NAIH — autoimmune hemolytic anemia; P — phosphorus; Pb — plumbum; PCR — polymerase chain reaction; PDW — platelet distribution width; PMF — primary myelofibrosis; PNH — paroxysmal nocturnal hemoglobinuria; RA — rheumatoid arthritis; sCD25 — soluble interleukin 2 receptor; SLE — systemic lupus erythematosus; TG — triglycerides; t-MDS/t-AML — therapy-related MDS/therapy-related AML; XLSA — X-linked sideroblastic anemia; TSH — thyroid-stimulating hormone; Zn — zinc

Table V. Pre-myelodysplatic syndrome (MDS) conditions

Idiopathic cytopenia of undetermined significance (ICUS)	Idiopathic dysplasia of undetermined significance (IDUS)	
Cytopenia (s) in CBC* No apparent causes of cytopenia Failure to meet criteria for MDS diagnosis No genetic abnormalities typical for MDS** Absence or presence of dysplasia in <10% of cells of a given hematopoietic lineage <5% blasts***	No cytopenia in CBC Failure to meet criteria for MDS diagnosis No genetic abnormalities typical of MDS** Dysplasia features in ≥10% of cells of a given hematopoietic lineage <5% blasts***	ICUS + IDUS — pre-MDS conditions without genetic abnormalities typical of MDS
Clonal cytopenia of undetermined significance (CCUS)	**Clonal hematopoiesis of indeterminate potential (CHIP)**	
Cytopenia (s) in CBC* No apparent causes of cytopenia Failure to meet criteria for MDS diagnosis Genetic abnormalities typical for MDS (one or more)** Absence or presence of dysplasia in <10% of cells of a given hematopoietic lineage <5% blasts***	No cytopenia in CBC Failure to meet criteria for MDS diagnosis Genetic abnormalities typical for MDS (one or more)** Dysplasia features in <10% of cells of a given hematopoietic lineage <5% blasts***	CCUS + CHIP — pre-MDS conditions with genetic abnormalities typical of MDS
ICUS + CCUS — pre-MDS conditions with cytopenias	IDUS + CHIP — pre-MDS conditions without cytopenias	

*Any grade cytopenia lasting more than four months after excluding other causes; **abnormalities identified by G-banding by trypsin with Giemsa (GTG) cytogenetics, fluorescence *in situ* hybridization (FISH) or by molecular biology. In the case of sequencing methods, a variant allele frequency (VAF) of at least 2% is considered to be diagnostic for pre-MDS conditions; for diagnosis of MDS, VAF should be ≥10%; ***blasts found in peripheral blood and/or bone marrow smears; CBC — complete blood count

Table VI. Commonest cytogenetic abnormalities in myelodysplatic syndrome patients (acc. to Schanz et al. [10])

Cytogenetic abnormalities	Incidence [%]
Single abnormalities:	
• del(5q)	6.4
• +8	4.7
• –Y	2.2
• del(20q)	1.7
• –7	1.6
• del(11q)	0.7
• del(12p)	0.6
• del(7q)	0.5
• i(17q)	0.4
• +19	0.4
• inv(3)/t(3q)/del(3q)	0.4
• +21	0.3
• der(1;7)	0.3
• –13/del(13q)	0.3
• –21	0.3
• –X	0.3
• Other single	5.8
Double abnormalities:	
• including del(5q)	1.6
• including –7/del(7q)	1.2
• including other abnormalities	3.4
Complex abnormalities:	
• 3 aberrations	2.1
• >3 aberrations	7.0
Independent clones	0.9
Clonal evolution	13.4

far, the occurrence of sequence variants has been demonstrated in as many as 181 genes, but only in 79 of them was it repeatable [15]. Two of the largest studies, involving a total of 1,682 patients, tested 104–111 genes and significant mutations were found in 43–47 genes [16, 17].

The most common mutations in MDS are detected in genes responsible for the following functions:

- **pre-mRNA splicing.** Sequence variants in *SF3B1* (splicing factor 3b subunit 1), *SRSF2* (serine and arginine rich splicing factor 2), *U2AF1* (U2 small nuclear RNA auxiliary factor 1) and *ZRSR2* (zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2) genes are among the most frequently reported disorders regulating splicing in MDS. They are found in almost 70% of patients, and are the most specific for MDS. *SF3B1* is the only gene whose sequence variants are included in the 2016 WHO revised classification, as well as in the 2020 proposal for a new MDS subtype with the *SF3B1* mutation [18]. The sequence variants in *SF3B1* gene is closely related to the presence of ring sideroblasts and is helpful in defining the myelodysplastic syndrome with ring sideroblasts (MDS-RS) subtype classification. Alterations of *SF3B1* gene occur proportionally more frequently in lower-risk patients according to the Revised International Prognostic Scoring System (IPSS-R). Sequence variants in *SRSF2* gene are indicative of an unfavorable course of MDS, with an increased risk of leukemic transformation;
- **epigenetic DNA modification.** Sequence variants in *TET2* (ten eleven translocation-2) gene are found quite frequently, in 19–25% of MDS cases, but are also present in other myeloid neoplasms. The presence of mutation predicts a good response to azacytidine, which, however, is not associated with an increase in survival time. Sequence variants in the DNMT3A (DNA-methyltransferase 3 alpha) gene occur mainly in elderly patients with a frequency of 3–8% in MDS. They are found in all of the marrow cells. The presence of mutation is associated with shorter survival;
- **chromatin modification.** Sequence variants in *ASXL1* (associated sex combs-like 1) gene are among the more frequent molecular changes in MDS, occurring in 15–20% of patients. Their presence in MDS adversely affects overall survival and is associated with a higher probability of transformation into AML. Sequence variants in *EZH2* (enhancer of zeste homolog 2) gene occur in 5–10% of myelodysplastic syndromes and are a marker of a poor prognosis;
- **DNA repair (*ATM, DLRE1C, FANCL, BRCC3*)**, as well as
- **cohesive complex (*CTCF, RAD21, STAG2, SMAC1A*);**
- ***RAS* signaling pathway (*KRAS, NRAS, CBL, NF1, PTPN11*);**
- **transcription factors (*RUNX1, GATA, TP53, CEBPA, BCOR, NCOR, PHF6, ETV6*).** Sequence variants in *TP53* gene (tumor protein 53) occur in 5–14% of higher risk MDS according to IPSS-R and in 27% of t-MDS cases. Their presence is associated with a poor survival prognosis. The presence of sequence variants in *TP53* gene in MDS with del (5q) results in lower lenalidomide efficacy and rapid transformation to AML in this group of patients. Bernard et al. [19] showed that an unfavorable prognosis concerns patients with a biallelic *TP53* mutation, usually the dominant clone. On the other hand, patients with the monoallelic *TP53* mutation, which usually occurs within the subclone, do not have a significantly worse prognosis compared to patients without *TP53* mutation [19]. Sequence variants in *RUNX1* (runt-related transcription factor 1) gene are found in approximately 10–15% of MDS cases, usually in advanced disease, and adversely affect overall survival. They are more common in secondary MDS (22–50%) and often coexist with *RAS* gene mutations;

Table VII. 2016 World Health Organization (WHO) classification of myelodysplastic syndromes (source [9])

Entity		Number of dysplastic lineages	Cytopenias*	Ring sideroblasts [%]	Blasts in bone marrow (BM) and peripheral blood (PB) [%]
MDS with single lineage dysplasia, MDS-SLD		1	1 or 2	<15/<5**	BM <5, PB <1, without Auer rods
MDS with multilineage dysplasia, MDS-MLD		2 or 3	1–3	<15/<5**	BM <5, PB <1, without Auer rods
MDS with ring sideroblasts (MDS-R)	MDS-RS-SLD	1	1 or 2	≥15/≥5**	BM <5, PB <1, without Auer rods
	MDS-RS-MLD	2 or 3	1–3	≥15/≥5**	BM <5, PB <1, without Auer rods
MDS with isolated del(5q)***		1–3	1–2	None or any	BM <5, PB <1, without Auer rods
MDS with excess blasts-MDS-EB	MDS-EB1	0–3	1–3	None or any	BM 5–9 or PB 2–4, without Auer rods
	MDS-EB2	0–3	1–3	None or any	BM 10–19 or PB 5–19 or Auer rods
MDS unclassifiable, MDS-U	With 1% blasts in PB	1–3	1–3	None or any	BM <5, PB = 1 without Auer rods
	Pancytopenia with unilineage dysplasia	1	3	None or any	BM <5, PB <1, without Auer rods
	Without dysplasia with a cytogenetic alteration defining MDS****	0	1–3	None or any	BM <5, PB <1, without Auer rods
	Del (5q) and pancytopenia	1–3	3	None or any	BM <5, PB <1,without Auer rods
Refractory cytopenia of childhood		1–3	1–3	None	BM <5, PB <2

*Cytopenias are defined as follows: hemoglobin (Hb) <10.0 g/dL, platelet count <100 × 10^9/L, absolute neutrophil count <1.8 × 10^9/L. Myelodysplatic syndrome (MDS) can rarely present with mild anemia or thrombocytopenia above the indicated values. Peripheral blood monocytes <1 × 10^9/L; **presence of *SF3B1* gene mutation; ***presence of cytogenetic del (5) abnormalities ± additional aberration without chromosome 7 abnormalities; ****presence of typical cytogenetic abnormalities; BM — bone marrow; PB — peripheral blood

- **signal transduction factors (*JAK2, FLT3, KIT, MLP, GNAS*).** In more than 10% of patients, sequence variants are found in six genes: *TET2*, *SF3B1*, *ASXL1*, *SRSF2*, *DNMT3A* and *RUNX1*, in 8–12% of patients in two genes *U2AF1* and *TP53,* and in 5–10% of patients in four genes: *EZH2*, *ZRSR2*, *STAG2*, *NRAS.*

WHO classification 2016, 2020

In the current modification of the 2016 classification, all types of syndromes are preceded by the name MDS [9] (*see* Table VII). Specific subtype without excess blasts and 5q deletion is primarily determined by the number of dysplastic cell lineages, and not by cytopenia.

Based on the data of 3,479 patients, of whom 795 had *SF3B1* gene mutation, an additional group of experts of the International Working Group for the Prognosis of MDS (IWG PM) proposed in 2020 to distinguish a new MDS subtype, i.e. MDS with *SF3B1* mutation (Table VIII). The authors showed that the presence of *SF3B1* gene mutation without ring sideroblasts (RS) had significant prognostic value. Patients with refractory anemia with ring sideroblasts (RARS) or refractory cytopenia with multilineage dysplasia (RCMD-RS) subtype with the *SF3B1* mutation live statistically significantly longer than patients with RARS or RCMD without *SF3B1* mutation. The effect of *SF3B1* mutation on survival is significant only in the group with blasts rate <5% in BM and <1% in peripheral blood without deletion 5q, monosomy 7 and chromosome 3 disorders, as well as in patients without *EZH2* and *RUNX1* mutations [18].

Congenital myeloid neoplasms

Myeloid neoplasms in which the predisposing genetic defect is inherited or arises *de novo* at an early stage of embryogenesis and is present in all (including terminal) cells in the body are known as myeloid neoplasms with germline predisposition (MNGP) or hereditary myeloid malignancy syndromes (HMMSs). Most often they concern genes such as: *CEBPA*, *DDX41*, *RUNX1*, *ANKRD26*, *ETV6*, *GATA2*, *SRP72*, and *SAMD9*. The exact frequency of these diseases is unknown, but it is estimated that they affect 1–5% of myeloid neoplasms. MNGP/HMMS can occur in as isolated abnormality or with concomitant diseases (including other neoplasms — lymphoid or solid tumors) or as part of complex syndromes [20–22].

The possibility of MNGP/HMMS should always be considered in patients with MDS and the following features:

Table VIII. Myelodysplatic syndromes (MDS) with *SF3B1* gene mutation: International Working Group (IWG) 2020 diagnostic criteria (source [18])

Peripheral blood cytopenia defined as reduction of parameters below laboratory standards in at least one cell lineage: erythroid, neutrophil and platelets
Presence of somatic mutation of *SF3B1* gene
Isolated erythroid or multilineage dysplasia*
Blasts in bone marrow <5% and in peripheral blood <1%
Failure to meet criteria for diagnosis of other diseases: MDS with isolated 5q deletion, MDS/MPN-RS-T and other MDS/MPN as well as primary myelofibrosis and other myeloproliferative neoplasms according to WHO 2016 classification
Normal karyotype or cytogenetic abnormalities with exception of: del(5q), chromosome 7 monosomy, inv(3) or 3q26 abnormalities, composite karyotype (≥3 abnormalities)
Coexistence of other somatic mutations except RUNX1 and/or EZH2**

*Presence of ring sideroblasts is not required; **presence of *JAK2* V617F, *CALR* or *MPL* mutation strongly suggests MDS/MPN-RS-T (a myelodysplastic/myeloproliferative neoplasm with an excess of ring sideroblasts and thrombocythemia); WHO — World Health Organization

- clinical symptoms or other diseases suggesting one of the congenital syndromes [20];
- ≥2 cases of MDS/AML in a family;
- ≥2 cases of aplastic anemia/unexplained cytopenia in a family;
- congenital syndrome diagnosed in relative;
- pathogenic mutation in the gene associated with congenital myeloid neoplasms, in particular:
  - *CEBPA* biallelic mutation (10% of cases have a germinal mutation),
  - *GATA2* mutation and/or chromosome 7 monosomy in a very young individual (most cases affect children and adolescents),
  - *RUNX1* mutation,
  - *DDX41* mutation;
- patients with and/or family history of multiple cancers (with the exception of chronic lymphocytic leukemia);
- related bone marrow donor of a patient with diagnosed congenital syndrome.

In addition, the possible presence of MNGP/HMMS should always be considered if:

- unexplained cytopenias are found;
- there are difficulties with hematopoietic cells mobilization.

Testing procedure for suspected MNGP

Testing of germline DNA from fibroblast cultures obtained by skin biopsy is the gold standard for MNGP/HMMS diagnostics. Other sources of DNA used include:

- skin cells examined immediately after skin specimen collection (no culture);
- hair follicles, nails (small amount of material);
- buccal swab/saliva (material quick and easy to collect, but possible contamination with blood and cancer cells).

Germinal DNA testing should also be performed in relatives and potential related donors of hematopoietic cells. In this situation, fibroblast culture is also the gold standard.

Prognostic factors

Prognostic and predictive scores for MDS patients have been published, including responses to immunosuppressive therapy, responses to treatment with erythropoiesis stimulating proteins or intensive chemotherapy. The most important is the International Prognostic Scoring System (IPSS) published in 1997 by Greenberg et al. and its modified 2012 version known as IPSS-R (see Table IX) [23, 24]. The International Prognostic Scoring System (IPSS) index stratifies patients depending on BM blasts percentage, cytogenetic abnormalities, and the number of peripheral blood cytopenia. IPSS risk stratification correlates with overall survival and transformation time to acute myeloid leukemia. The IPSS index is of significant clinical importance as it helps to qualify patients for a given type of treatment. The modified IPSS-R International Scoring System based on the analysis of data from 7,012 patients introduces five cytogenetic groups (with 18 subgroups of karyotype variants) and new, more detailed ranges for BM blasts rate, hemoglobin concentration, and the number of platelets and neutrophils. A limitation of the discussed indices is the lack of consideration of patient-related factors such as age, general condition and comorbidities. These factors are particularly important in qualifying for allotransplantation of hematopoietic cells. Della Porta et al. [25] have developed an index of comorbidities specific for MDS patients.

In 2005, Malcovati et al. [26] demonstrated that the dependence on red blood cell (RBC) transfusion assessed at any time point during the disease (defined as the necessity to transfuse ≥1 RBC within 8 weeks) is an important factor associated with shorter survival. Adverse prognostic factors in patients with MDS also include: increased ferritin and LDH and hypoalbuminemia. In recent years, much attention has been paid to molecular studies in MDS patients, which have been discussed in detail above. Bejar et al. [27] showed that the presence of a mutation of at least one of the following genes: *TP53*, *EZH2*, *ETV6*, *RUNX1* or *ASXL1* causes the patient to move to the next prognostically less favorable group according to IPSS. This is of particular importance in low-risk patients according to IPSS, as it allows to qualify them earlier for more aggressive treatment [27]. It seems that the implementation of molecular test results in MDS prognostic indices is an inevitable process, and additional research is still ongoing.

Table IX. Prognostic indices used in patients with myelodysplastic syndromes (sources [23, 24])

International Prognostic Scoring System (IPSS)					
Score	**0**	**0.5**	**1.0**	**1.5**	**2.0**
Blasts in BM (%)	<5	5–10	–	11–20	21–30
Karyotype	Normal; –Y del(5q) del(20q)	Other abnormalities	Complex (≥3 abnormalities) chromosome 7 aberrations	–	–
Cytopenias*	0–1	2–3	–	–	–
Risk groups					
Low	Intermediate-1	Intermediate-2		High	
0 points	0.5–1 points	1.5–2 points		≥2.5 points	
Median overall survival					
Low risk	Intermediate-1	Intermediate-2		High	
5.7 years	3.5 years	1.2 years		0.4 years	
Mean time to transformation into AML (25% of the group)					
Low risk	Intermediate-1	Intermediate-2		High	
9.4 years	3.3 years	1.1 years		0.2 years	

Revised International Prognostic Scoring System (IPSS-R)							
Score	**0**	**0.5**	**1**	**1,5**	**2**	**3**	**4**
Karyotype	–Y del(11q)		Normal del(5q) del(12p) del(20q) 2 abnormalities including del(5q)		del(7q) +8 +19 i(17q) others (1 abnormality) others (2 abnormalities)	–7 inv(3) t(3q) del(3q) 2 abnormalities (including –7/del(7q) 3 abnormalities	>3 abnormalities
Blasts in BM [%]	≤2		>2–<5		5–10	>10	
Hemoglobin [g/dL]	≥10		8–<10	<8			
Platelets [× 10^9/L]	≥100	50–<100	<50				
Neutrophils (×10^9/L)	≥0.8	<0.8					

Risk groups				
Very low	Low	Intermediate	High	Very high
≤1.5 points	>1.5–3.0 points	>3.0–4.5 points	>4.5–6.0 points	>6.0 points
Proportion of patients at specified risk				
Very low	Low	Intermediate	High	Very high
19%	38%	20%	13%	10%

→

Table IX (cont.). Prognostic indices used in patients with myelodysplastic syndromes (sources [23, 24])

Revised International Prognostic Scoring System (IPSS-R)				
Median overall survival				
Very low risk	Low risk	Indirect risk	High risk	Very high risk
8.8 years	5.3 years	3 years	1.6 years	0.8 years
Mean time to transformation into AML (25% of group)				
Very low risk	Low risk	Intermediate risk	High risk	Very high risk
NR	10.8 years	3.2 years	1.4 years	0.73 years

*Hemoglobin (Hb) <10 g/dL; neutrophils <1,8 × 10^9/L; platelets <100 × 10^9/L; AML — acute myeloid leukemia; BM — bone marrow; IPSS — International Prognostic Scoring System; NR — not reached

Authors' contributions

Conception and design: KM, JDT. Manuscript writing, final approval of the manuscript: all authors.

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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Hemophagocytic lymphohistiocytosis: what's new in old diagnostic and clinical criteria?

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Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a condition of overexpressed inflammatory response resulting in hypercytokinemia, macrophages infiltration and subsequent multiple organ failure. Without treatment, it leads to death. The main etiological factors include: viral, bacterial and parasitic infections, malignancies and autoinflammatory diseases. The main clinical manifestations are: high fever ≥38 °C, lymphadenopathy, splenomegaly, and hepatomegaly. Central nervous system involvement occurs in 30–70% of cases. Less common symptoms include: dyspnea, cough, arrhythmias, jaundice, peripheral edema, rashes, albinism and diarrhea. The picture of the disease seen in laboratory tests consists of: duopenia, hypofibrinogenemia (<150 mg/dL) high D-dimers level, and hyperferritinemia. Other abnormalities include hypertriglyceridemia, elevated liver enzymes, hyperbilirubinemia, hypoalbuminemia and hyponatremia. Diagnostics include: laboratory tests, histopathological examination, lumbar puncture, radiological imaging, functional test and genetic checking. It is important to rule out factors mimicking HLH. Some of the old, well-known criteria are of less relevance nowadays. The aim of the therapy is immunosuppressive, immunomodulatory and anti-cytokine treatment, using the HLH-2004 protocol. In secondary HLH, elimination of the causative agent is critical. In primary HLH, or relapse of secondary forms, allogeneic transplantation is the only curative treatment. The prognosis is uncertain.

Key words: hemophagocytic syndrome, hypercytokinemia, macrophage activation syndrome

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Introduction

Definition

Hemophagocytic lymphohistiocytosis (HLH) is a condition of specific hyperinflammation that directly leads to death without prompt treatment. It should not be classified as a disease, but rather as a certain set of clinical conditions that lead to an overexpressed inflammatory response resulting in infiltration of internal organs by macrophages and subsequent multiorgan failure [1].

Pathophysiology

In congenital syndromes, there is a defect in the production of certain proteins responsible for the correct course of the granule-dependent cytotoxicity mechanism. These cytotoxic granules contain granzymes and protein-perforin, which are released at the immunological synapse between infected cells and cytotoxic lymphocytes and NK cells. As a result of the defect described above, a paralysis of the killing cells occurs and, consequently, a vicious circle is set in motion — the helper lymphocytes, stimulated by the trigger, instead of stimulating the NK cells and cytotoxic lymphocytes, cause their inhibition and dysfunction. However, they stimulate production of interferon gamma (IFN-γ) and stimulate macrophages, which together with INF-γ fuel the so-called 'cytokine storm' leading to even greater paralysis of cytotoxic lymphocytes and NK cells, which cannot cope with elimination of infectious agents. Under the influence

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of INF-γ and macrophages, infiltration of organs occurs, leading to organ dysfunction. The cytokines that play a key role in hyperinflammation include interleukin (IL)-6, IL-10, INF-γ, as well as IL-1, IL-8, IL-12, IL-18, and tumor necrosis factor alpha (TNF-α) [1, 2].

For acquired forms of HLH, the exact pathogenesis is not fully understood. It seems that viruses and other infectious agents block the function of NK and cytotoxic lymphocytes. It has been observed, e.g. in the case of Epstein-Bárr virus (EBV)-acquired HLH, that NK and cytotoxic lymphocytes are directly infected (instead of B lymphocytes) and destroyed by the virus. This infection stimulates helper lymphocytes and results in the production of INF-γ, proinflammatory cytokines, which in turn activate macrophages and stimulate the secretion of TNF-α, IL-6, plasminogen activator, and ferritin.

A cascade of these reactions leads to the defined metabolic abnormalities and clinical manifestations of HLH [2].

Clinical picture

Usually, the first symptoms of HLH occur in the form of full-blown syndrome resembling sepsis, but there are hemophagocytic syndromes with an initially sparse symptomatic course. The primary forms usually occur at a young age, while the acquired form affects children older than 2 years. There are rare exceptions, however, and congenital forms of HLH are sometimes revealed even in teenagers [3, 4].

Most patients present with high, non-remitting fever, ≥38°C, poorly responsive to medication as a result of proinflammatory cytokines. Exceptions are premature infants, whose temperature may be even lower than 36.6°C. Other clinical features include splenomegaly (97% of patients) and hepatomegaly (>50% of patients) with or without laboratory signs of liver dysfunction. Very often, generalized or local lymphadenopathy (effect of hemophagocytosis in lymph nodes) is observed in patients. Kidney involvement (edemas, dysuria, anuria) and pulmonary infiltration (dyspnea, cough, decreased saturation), or skin rash can be seen in hemophagocytic lymphohistiocytosis–macrofage activation syndrome (HLH-MAS). 30–73% of patients develop central nervous system (CNS) involvement. This usually accompanies the generalized form of the disease, but sometimes it is the only symptom of HLH. Clinical symptoms include seizures (mainly in young children), positive meningeal signs, ataxia (in older children), opisthotonus and cranial nerve palsy. It seems that patients without neurological symptoms have changes in imaging studies and vice versa — patients with multiple neurological changes do not always have visible abnormalities in radiological imaging. Less frequent clinical manifestations of hemophagocytic syndrome include: arrhythmia [mainly in the course secondary to Kawasaki disease (KD)] or chronic bloody diarrhea (XLP-2), albinism or pseudoalbinism — typical for primary HLH such as Hermansky-Pudlak syndrome (HPS), Griscelli syndrome (GS II), and Chediak-Higashi syndrome (CHS) [3, 4–6].

The disease presentation seen in laboratory tests includes:

1) Duopenia (decreased morphology values affecting two cell lines e.g. thrombocytopenia and anemia, or granulocytopenia and thrombocytopenia *etc.*) resulting from bone marrow immunosuppression under the influence of proinflammatory cytokines (TNF-α, INF-γ). This occurs in 80% of patients. The exceptions are patients with MAS, in whom in the early stages of the disease morphological parameters may be normal. It is now considered that a decreasing platelet count is a very sensitive marker of HLH and its recurrence [2, 7];
2) Hyperferritinemia is another fairly important marker of the syndrome. A value of above 500 µg/L may suggest hemophagocytic syndrome. However, it should be remembered that ferritin, which is an acute phase protein as well as a marker of iron deficiency and iron overload, will not be very sensitive in the context of patients after transplantation, multiple transfusions or liver disease. Although in the international HLH criteria a ferritin value >500 µg/L is given, it is currently accepted that a value >2,000 µg/L is relevant for the diagnosis of HLH;
3) Hypofibrinogenemia (<150 mg/dL) resulting mainly from hepatic dysfunction and INF-γ-induced increase in plasminogen activator is a quite sensitive parameter of the syndrome activity. While monitoring the coagulation system, it is also important to pay attention to rising D-dimers, which are often significantly elevated in active HLH;
4) Hyperglyceridemia (>3 mmol/L or 265 mg/dL) resulting from inhibition of lipoprotein lipase by TNF-α, is another component of the diagnostic criteria. In the context of recent guidelines, its diagnostic value is no more relevant.

Less common laboratory criteria include elevated liver enzymes [alanine aminotransferase (ALT), and aspartate aminotransferase (AST)], hyperbilirubinemia, hypoalbuminemia, and hyponatremia (may be a marker of CNS involvement or apparent as a result of hypertriglyceridemia) [8, 9].

The diagnostic criteria for HLH are presented in Table I.

Cytological and histopathological confirmation

One important diagnostic criteria for HLH is hemophagocytosis. This can be found in the bone marrow, liver, spleen, lymph nodes and cerebrospinal fluid (CSF), and is usually checked in bone marrow and CSF. In the past, biopsies of lymph nodes, liver and even spleen were also performed. Due to safety concerns, the latter two tests are not performed nowadays. It should be remembered that in the early stages of the disease, hemophagocytosis may not be present in in bone marrow (BM), and in about

Table I. Diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH) (based on HLH-2004 protocol)

The diagnosis can be confirmed by:
A. Detection of mutations (*PRF1, UNC13D, Munc18-2, Rab27a, STX11, SH2D1A or BIRC4*)
or
B. When five of the following eight criteria are met:
1. Fever >38.5°C
2. Splenomegaly
3. Duopenia:
• **Hb <9 g/L (in neonates Hb <10 g/L)**
• **PLT <100 × 10^3/mL**
• **GR < 1 × 10^3/mL**
4. Hypertriglyceridemia >265 mg/dL (>3 mmol/L) and/or hypofibrinogenemia <150 mg/dL
5. Hemophagocytosis in bone marrow/lymph nodes/ /liver*
6. Low/no NK cell activity
7. Ferritin >500 µg/L
8. Elevated sCD25 levels

*Liver biopsy for material collection is not currently recommended; Hb — hemoglobin; PLT — platelets; GR — granulocytes

40% of patients it is not present at all in bone marrow examination.

BM biopsy should therefore be performed several times, as changes may appear over time. In addition to hemophagocytosis, normal cellularity is observed in the bone marrow of patients with primary HLH, sometimes with an elevated red cell line. In contrast, in HLH-MAS, there is an increased percentage of granulocytic lineage in the marrow. In addition, a follow-up bone marrow examination can rule out proliferative disease as well as Leishmaniasis infection (amastigotes present in microscopic examination). Where there is a suspicion of Leishmaniasis, a bone marrow sample should be taken for polymerase chain reaction (PCR) testing, because tests from peripheral blood often give false negative results. Until recently, it was thought that a diagnosis should only be made if there was a positive history of travel to the Mediterranean region. Nowadays, due to the widespread distribution of the parasite (e.g. Germany, the Netherlands), this test is considered mandatory for diagnostic screening [10].

Another very important diagnostic test is lumbar puncture with collection of CSF. It is mandatory to examine the fluid for pleocytosis, protein, glucose, lactates and microbiological examination. In most cases, a predominance of lymphocytes is observed in the CSF smear, while hemophagocytosis alone is described in only 39% of cases. Lumbar punctures should be repeated in the absence of baseline fluid changes, as in the case of bone marrow examination. During disease monitoring, it is important to perform follow-up lumbar punctures, in which signs of disease retreatment are usually seen earlier than on imaging studies. In primary forms of HLH, CSF lesions are more commonly present, with no abnormalities on CNS magnetic resonance imaging (MRI) [6, 11].

Less frequently performed tests include microscopic examination of the hair, performed when congenital immunodeficiency running with albinism is suspected.

Imaging studies

Radiological imaging is a very important part of diagnostics. Magnetic resonance imaging (MRI) is the gold standard to exclude central nervous system involvement. Lesions typical of HLH are: symmetric periventricular infiltrates, sometimes cerebellar lesions, usually not involving the thalamus or brainstem, not enhancing in T1-dependent sequence. As previously mentioned, there are patients with clinical symptoms without MRI changes, as well as patients without neurological symptoms who do have lesions on imaging studies. It is important that diagnostic imaging and lumbar puncture should be performed before the start of therapy. When monitoring the disease, if the neurological symptoms disappear, it is not necessary to repeat the imaging examinations, but it is crucial to control the cerebrospinal fluid by lumbar puncture. It should also be kept in mind that abnormalities seen on imaging studies may be a result of chemotherapy. Examples include changes consistent with posterior reversible encephalopathy (PRES), associated with steroid therapy and cyclosporine A (CsA) [6, 11].

In addition, abdominal and lymph node ultrasound, cardiac ultrasound and chest radiography are performed in every patient. In justified cases (where there is a strong suspicion of HLH secondary to malignancy), the diagnostics should be extended.

Functional tests

Functional tests provide great support in the diagnosis of hemophagocytic syndrome, but it should be emphasized that they are performed only in a few highly specialized laboratories.

The primary test is the degranulation assay. This evaluates the process of exocytosis that occurs in cytotoxic cells by assessing the expression of CD170a and CD170b antigens on activated cells. Abnormalities of the degranulation process are an indication to look for some congenital form of HLH. For example, a degranulation defect and decreased cytotoxicity test results may indicate defects of *MUNC, STX11* and *STXPB2* genes (FHL-3, FHL-4, FHL-5) responsible for the production of proteins involved in maturation and fusion of exocytic granules with the cell membrane. Impaired degranulation is also observed in Griscelli syndrome and Chediak-Higashi syndrome.

The cytotoxic assay allows examination of the cytotoxic capacity of NK cells. It defines cytotoxicity as the number of leukemic cell line K562 cells killed by cytotoxic cells. This test is performed using radioactive chromium or fluorescent dye and propidium iodide. It is performed by flow cytometry. Its reduced values suggest a congenital defect. The test result distinguishes patients with a reduced number of NK cells without loss of cytotoxicity from patients with normal or reduced number of NK cells and reduced cytotoxic function.

Another important test is the evaluation of NK cell activity. Transiently reduced NK cell counts may accompany acquired forms of HLH due to overuse. In patients who enter remission of the disease, NK counts return to normal. In the congenital forms, usually NK counts and activity are permanently reduced. However, it should be remembered that there are forms of HLH with normal numbers and activity of NK cells [12, 13].

Intracellular expression of perforin (a glycoprotein that has the ability to incorporate and form channels in the cytoplasmic membrane of target cells, inducing of apoptosis in the target cell) is assessed by flow cytometry. This test can be very helpful when congenital forms are suspected. Abnormal expression of perforin, with reduced cytotoxicity test values and normal degranulation values, may suggest a genetic form of FHL2 (*PRF1*).

An important test that should be performed in males is expression of X-linked inhibitor of apoptosis protein (XIAP) and SLAM (signaling lymphocytic activation molecule)-associated protein (SAP). If there is an abnormal result, primary HLH (XLP-1, XLP-2) can be suspected.

The concentration of soluble receptor for IL-2 (sCD25) shows the activation level of T lymphocytes. This is a sensitive marker of hemophagocytic syndrome and is one of the diagnostic criteria (sCD25 level >2,400 U/mL). Apart from time of diagnosis, a follow-up test can be performed after about two weeks — the marker should decrease within treatment [5, 7, 12].

Genetic testing

A genetic test is not always needed to make the diagnosis of hemophagocytic syndrome. Sometimes, it is sufficient that the patient only meets the diagnostic criteria (Table I) for the disease. Immunological and genetic tests are necessary to rule out congenital HLH. The commonly used Sanger test is a quick and easy way to rule out basic mutations that are already known. The disadvantage of this method, however, is that it only targets previously known genetic alterations. In contrast to traditional methods, next generation sequencing (NGS) allows for the inspection of almost the entire genome, and the detection of new mutations responsible for HLH. This method has limitations including high cost, but in the future it will certainly become a more common tool, and it is useful in patients suspected of having HLH or other immunodeficiencies [13, 14].

Secondary hemophagocytic syndromes — diagnostic tips

This group includes acquired HLH, which develops due to other factors such as infection, drugs, neoplasms, or connective tissue disease.

EBV-HLH

The most common causes of acquired HLH are viral infections and among them EBV. This infects mainly B-lymphocytes, controlled by NK-lymphocytes and cytotoxic lymphocytes. The development of EBV-HLH depends on age, stage of disease, and number and function of NK and cytotoxic lymphocytes.

EBV-HLH during primary infection tends to occur in younger children, in contrast to lymphoproliferative disease or chronic EBV infection, which is usually seen in older children and adolescents, and tends to occur during the reactivation phase of infection. Typical features on physical examination include jaundice, skin rashes, breathing difficulties, and neurological disturbances (seizures). In laboratory tests, high levels of lactate dehydrogenase and hyponatremia and high values of liver enzymes can be observed. Most patients have bone marrow and/or lymph node involvement, and CSF examination reveals elevated pleocytosis and protein levels. The CNS may also be involved. In contrast to other forms of HLH, there is an increased soluble IL-2 receptor (sCD25) with normal or slightly decreased NK cell activity. The presence of EBV-HLH does not exclude lymphoma [15, 16].

HLH secondary to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

HLH can also be triggered by other infectious factors including coronavirus disease 2019 (COVID-19). Recent reports suggest that the hypercytokinemia caused by the novel Coronavirus infection, COVID-19, has significant similarities with the laboratory and clinical findings of HLH. Due to the rapid deterioration of patients' general status, secondary hemophagocytic lymphohistiocytosis (sHLH) needs a timely diagnosis for the initiation of life-saving treatment [17]. Similarly to the clinical symptoms of sHLH, the majority of patients with COVID-19 present with high fever, cough, dyspnea, myalgia or fatigue [18]. As in HLH patients, COVID-19 patients present with several laboratory abnormalities i.e. thrombocytopenia, lymphocytopenia, elevated D-dimer, serum ferritin, lactate dehydrogenase (LDH), C-reactive protein (CRP) and IL-6 levels [19, 20].

Multisystem inflammatory syndrome in children (MIS-C)

Recent reports suggest a new COVID-19-related clinical syndrome, with significant hyperinflammation and similarities to KD, which can be found in children [21].

This multisystem inflammatory syndrome in children (MIS-C) can appear at any time, although it most commonly occurs 1–6 weeks following infection, and may overlap with an acute respiratory COVID-19 presentation [22, 23]. Clinical symptoms can be similar to KD with coronary artery aneurysms and extracardiac manifestations. Apart from typical symptoms associated with KD (i.e. hand and foot inflammation/swelling, rash, mucous membrane changes/strawberry tongue, conjunctivitis, lymphadenopathy) [24], MIS-C can present with significant gastrointestinal manifestations (vomiting, diarrhea and severe abdominal pain), neurological involvement, hyperferritinemia, and cardiogenic shock leading to multiorgan failure. It is very important to differentiate between MIS-C and sHLH because the treatment of Multisystem Inflammatory Syndrome differs and mainly involves, immunoglobulin (IVIG), corticosteroids, anakinra, tocilizumab and remdesivir in PCR positive patients [21].

Secondary hemophagocytic syndrome associated with malignancies

HLH associated with malignant neoplasms is more common in adults, although pediatric cases have been described. It is important to distinguish HLH occurring during tumor onset or relapse from hemophagocytic syndrome occurring in remission of the disease and associated with chemotherapy. In the former, HLH is caused by the tumor itself. Presumably, tumor cells (e.g. lymphoma) secrete the interleukins, INF-γ, IL-6, which are involved in the development of HLH. The soluble receptor for IL-2 (sCD25) is a marker of both HLH and certain lymphomas. It is also important to remember that EBV is both a 'trigger' for HLH and a cause of some lymphoid proliferations. In the pediatric population, T-cell lymphomas are the most common cause of secondary HLH.

In HLH associated with chemotherapy, immunosuppressive therapy and infections [especially bacterial, cytomegalovirus (CMV), EBV and fungal infections] are triggering factors. The greatest difficulty in the diagnosis of patients is the fact that most symptoms of HLH (fever, pancytopenia, coagulation abnormalities, high inflammatory markers) are also present in malignancy. Genetic testing may be helpful in diagnosis. For example, certain defects resulting in the development of XLP1 should be associated with a higher predisposition to B-cell lymphoma in boys, especially after EBV exposure, as opposed to XLP-2 which is not associated with lymphoma [16, 25].

Table II. Classification criteria for macrophage activation syndrome (MAS) [26–31]

Patient with fever, suspected of having idiopathic juvenile arthritis (SJIA)
Meets criteria for macrophage-MAS activation syndrome when:
• ferritin >684 μg/L
And two of the following:
• PLT <181 × 10^9/L
• AST > 48 U/L
• TG >156 mg/dL
• Fibrinogen ≤360 mg/dL

PLT — platelets; AST — aspartate aminotransferase; TG — triglycerides

Macrophage activation syndrome

The definition of MAS refers to hemophagocytic syndromes complicating connective tissue diseases. Excessive activation of T lymphocytes and macrophages leads to a condition of hyperinflammation, with symptoms of cytopenia, hepatic dysfunction, coagulation disorders and hyperferritinemia. Typically, MAS does not occur at the time of diagnosis, but rather at a later stage, and only in 25% is it concurrent. Approximately 7–17% of patients develop a full-blown macrophage activation syndrome, while a mild, subclinical syndrome is seen in more than 60% of cases. A very characteristic feature is the improvement of rheumatological symptoms at the onset of HLH. The clinical picture of the disease includes: high fever, generalized lymphadenopathy, edema, and hepatosplenomegaly. It resembles sepsis. In addition, a hemorrhagic rash can be found on physical examination, and in the more advanced stage of the disease, nose and gastrointestinal bleeding is possible. In addition, CNS symptoms such as seizures, behavioral disturbances, and even coma are quite common. Cerebrospinal fluid examination reveals high pleocytosis with moderately elevated protein level. Renal infiltration is quite common and is associated with a high mortality rate. Approximately 25% of patients present with pulmonary infiltration and thus respiratory distress, dyspnea, cough and even features of respiratory failure. In laboratory tests, a very characteristic feature of MAS is a rapid decrease in the erythrocyte sedimentation rate (ESR) value with still high CRP. Additionally, in biochemical parameters there is a significant increase in transaminases with a very slight increase in bilirubin and slight hypoalbuminemia. In coagulogram, massive abnormalities including intravascular coagulation have been described. Another very characteristic marker of MAS is a very high ferritin level (>5,000 μg/L). Apart from that, hypertriglyceridemia, high LDH levels, and low sodium levels are observed in laboratory tests. The diagnosis of MAS poses many problems due to overlapping symptoms

of rheumatic diseases and the hemophagocytic syndrome itself. There are numerous diagnostic criteria dedicated to the diagnosis of MAS. Some of the most recent are shown in Table II [26–31].

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MSB — sole author.

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

Modeling congenital dyserythropoietic anemia in genetically modified mice

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Abstract

Congenital dyserythropoietic anemias (CDAs) are a group of inherited disorders distinguished by ineffective production of red blood cells and peculiar abnormalities in the precursors from which red blood cells arise. The identification of disease-causing mutations and CDA-associated genes is rapidly improving the accuracy of diagnosis, aided by the growing accessibility of next-generation sequencing. Currently, it is much easier to identify the morphological abnormalities and classify different CDA types; however, a range of suitable, experimentally tractable models is needed in order to understand the pathogenic mechanisms at the molecular level.

This review explains the basic concepts related to CDAs, covers different genetically modified mouse lines that are available for CDA researchers, and highlights the challenges inherent to modeling human disease in another species.

Key words: congenital dyserythropoietic anemia, knockout mouse, erythropoiesis

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Erythropoiesis

Erythropoiesis is a 'finely-crafted' cellular differentiation and maturation program that begins with hematopoietic stem cells (HSCs) and eventually leads to the production of mature red blood cells (RBCs) [1, 2]. In this multi-step process, HSCs differentiate into common myeloid progenitors that give rise to either a granulocyte-monocyte progenitor (GMP) or a megakaryocytic-erythroid progenitor (MEP). MEPs can differentiate into megakaryocytes or continue the erythropoietic path by becoming burst-forming unit-erythroid progenitors (BFU-E). Further maturation of these committed erythroid precursor cells (EPCs) into mature RBCs is called 'terminal erythropoiesis'. RBC production is regulated by several cytokines, post-translational modifications of histones, translational factors, cofactors, and miRNAs at different stages of differentiation. Extrinsic cytokines, erythropoietin (EPO) and the stem cell factor (SCF), are known to play important roles in the early stages of erythroid differentiation.

Acquisition of EPO receptors (EPOR) at the BFU-E stage is vital for cell survival and progression through the colony-forming unit-erythroid stage (CFU-E) [3, 4]. Erythroid maturation transpires within erythroblastic islands located in the bone marrow of adults. The fetal liver functions as the main site of erythropoiesis during gestation. In both cases, the process is very similar at the cellular level, and is referred to as 'definitive erythropoiesis'. At a very early stage of embryonic development, a different form of so-called 'primitive erythropoiesis' takes place directly in the bloodstream, without the formation of erythroblastic islands [5].

This review is mainly focused on definitive erythropoiesis, unless indicated otherwise. Each erythroblastic island consists of nearly 30 erythroblasts at different maturation stages, surrounding a central macrophage. The central macrophage interacts with erythroblasts, provides instructions for their proliferation and differentiation, and

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supplies them with iron [6]. EPCs undergo morphological changes from proerythroblasts to basophilic (baso-E), polychromatic (poly-E), and orthochromatic erythroblasts (ortho-E). This phase is marked by increased hemoglobin production, cell size reduction, and nuclear condensation, resulting in nuclear extrusion known as 'enucleation' [7]. Enucleated erythroblasts, called reticulocytes, are released into the bloodstream, where the final maturation stage takes place. Reticulocytes lose the Golgi apparatus, ribosomes, endoplasmic reticulum (ER), and mitochondria. In some mammals, including humans, the reticulocyte loses an additional 20–30% of the cell surface to attain a biconcave shape [8, 9].

The lifecycle of human erythrocytes is c.110–120 days, and about 1% of cells are recognized as senescent and removed from the circulation daily. Therefore, the bone marrow should produce the required number of erythrocytes and release them into the circulation on a daily basis. In cases where the production, differentiation, or maturation of erythrocytes is impaired, this results in an abnormal number of RBCs. If this impairment is permanent, a state of chronic anemia develops. Investigation of genetic factors that lead to various forms of anemia aids the diagnosis and treatment of these disorders, while simultaneously expanding the knowledge of the mechanisms that control erythropoiesis.

This review is focused on congenital dyserythropoietic anemias (CDAs) that are caused by mutations in genes involved in terminal erythropoiesis. The scarcity of samples from CDA patients hampers functional studies. Differentiation of patient-derived induced pluripotent stem cells into erythroid precursors can partially overcome this limitation. However, erythropoiesis is influenced by specific niches of the bone marrow, fetal liver, and spleen. *In vivo* models that can be experimentally manipulated are needed to explain how CDA-associated proteins function, which of their roles are intrinsic to erythropoietic precursors, and which aspects of the disease depend on genetic background, age or erythropoietic stress. To aid progress in this field, we summarize the efforts and challenges of modeling CDA in genetically modified mice.

Congenital dyserythropoietic anemias

CDAs are a diverse group of rare genetic disorders characterized by inefficient erythropoiesis with distinct morphological features in the late erythroblasts that result in cytopenia. CDAs primarily affect the differentiation-proliferation process of terminal erythropoiesis. Characteristic morphological anomalies in the late erythroblasts, accompanied by the presence of binucleated or multinucleated erythroid cells, were one of the original diagnostic features of CDAs [10, 11].

However, these features are not uniquely linked to CDAs, and can also be seen in patients with erythropoietic stress, premature birth, and iron deficiency. Thus, diagnosing CDAs poses a challenge. Some of the symptoms manifested in patients with CDAs are jaundice, splenomegaly, anemia, reduced hemoglobin concentration, and suboptimal reticulocyte response for the degree of anemia [12]. CDAs are a heterogeneous group of hypoproliferative anemias that can be classified into five categories based on specific histological findings and genetics: CDA 1, 2, 3, transcription-factor-related CDAs, and CDA variants [13, 14]. These divisions are useful in clinical practice and reflect differences in the pathogenic mechanisms, although cross-talk between molecular pathways and gene products from separate CDA classes does occur (Figure 1A).

CDA type 1

CDA 1 is an autosomal recessive disorder associated with severe or moderate anemia, which is mostly macrocytic. CDA 1 is also characterized by relative reticulocytopenia and congenital disabilities, which include chest deformity, short stature, and skeletal abnormalities of distal limbs [15]. At the late erythroblast stage, 2.4–10% of the cells are bi-nucleated and the nuclei are often at different stages of maturation [16, 17]. Thin internuclear chromatin bridges are present in between the nuclei. Adjacent erythroblasts may be connected by cytoplasmic bridges and internuclear chromatin, which is observed in c.79% of those affected [18]. The most striking morphological feature of CDA 1 is the appearance of the nucleus under the electron microscope. This is described as "spongy" or "Swiss-cheese" due to the structure of heterochromatin, which is denser than normal. Nuclear-membrane-lined cytoplasmic intrusions may also be present.

Two genes associated with CDA 1 have been identified so far: *CDAN1* and *CDIN1* (C15orf41) [19, 20]. Bi-allelic mutations account for almost 90% of cases [19]. The coincidence of homozygous and compound heterozygous null mutations has not been observed, suggesting that the loss of either is lethal. Chromatin reassembly, DNA replication, and DNA repair are postulated to be the primary functions of codanin-1 [16]. It is also possible that some aspect of intracellular transport of proteins is disrupted by codanin-1 mutations, as the intermediate erythroblasts of CDA 1 show the aberrant distribution of heterochromatin protein-1 (HP-1a) in the Golgi apparatus. In intermediate erythroblasts, codanin-1 is partly colocalized with SEC23B, the protein mutation responsible for CDA 2, indicating a defect in the intracellular transport pathway in both CDA 1 and CDA 2 [21]. In non-erythroid cells, transcription factor E2F1, the main regulator of G1/S transition, directly initiates codanin-1 transcription and increases its levels during the S phase [22]. Furthermore, a cell-cycle regulated codanin-1 binds the anti-silencing factor-1 (ASF1), which acts on the assembly and disassembly of the nucleosome.

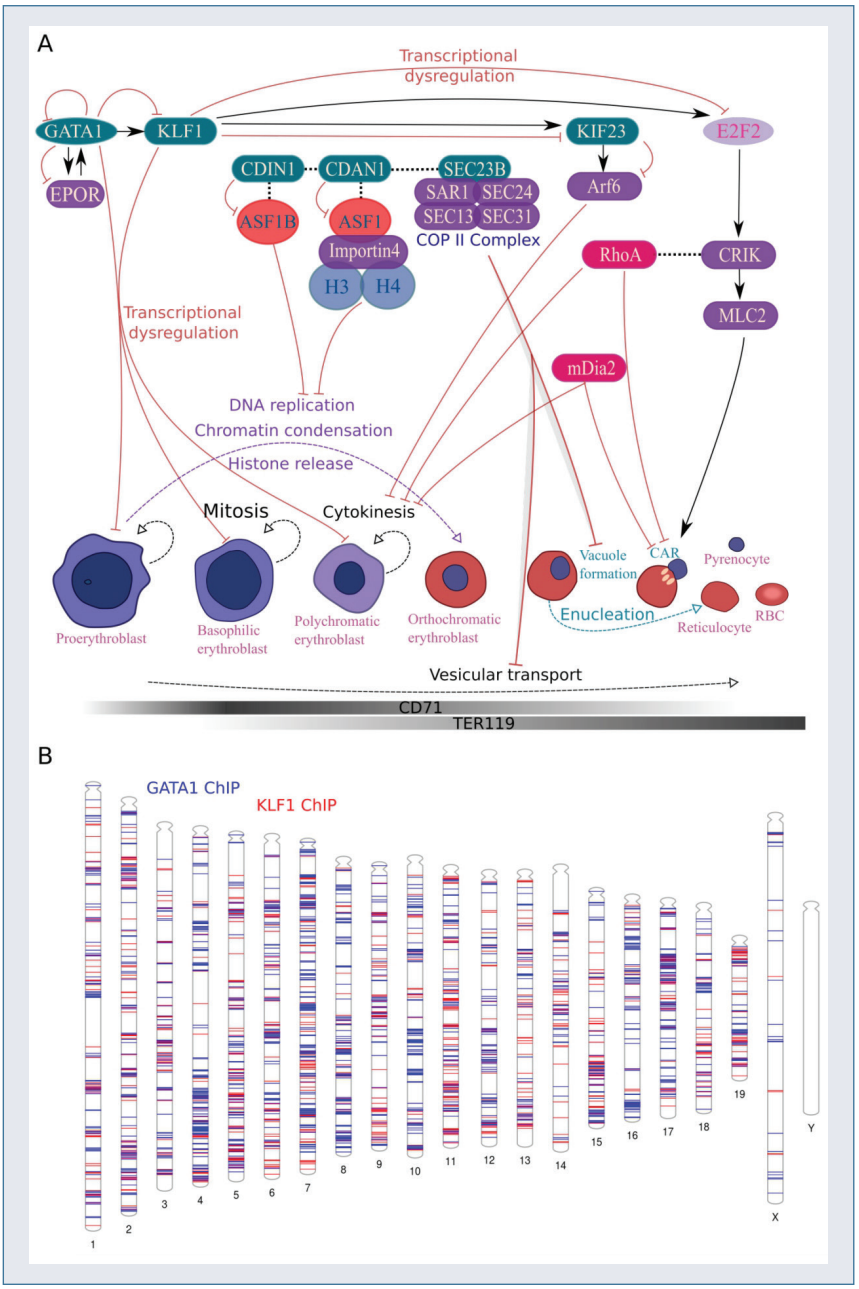

Figure 1. Proteins involved in pathogenesis of congenital dyserythropoietic anemia (CDA) participate in terminal erythropoiesis: **A.** Multiple cellular processes are required for maturation from rapidly dividing proerythroblasts to mature red blood cells (RBCs), including activation of erythropoietic lineage gene expression program, multiple rounds of DNA synthesis, and mitotic division followed by cytokinesis that occurs up to basophilic erythroblast stage, ongoing vesicular transport of membrane proteins, including transferrin receptor CD71 and marker of mature erythrocytes (TER119). Progressive chromatin condensation is accompanied by release of histones from nucleus, nuclear polarization, formation of large vacuoles between nucleus and reticulocyte, and enucleation. Known CDA proteins (cyan blue), functioning as transcription factors (GATA1 and KLF1), interact with histone chaperones (CDAN1, CDIN1), engage COPII complex (SEC23B), and participate in cytokinesis (KIF23). Other proteins that are inactivated in mouse models which mimic CDA III are represented in pink. Solid lines with full arrowheads pointing from upstream to downstream molecules signify functional dependence; dashes indicate known biochemical interactions, dashes with empty arrowheads depict key cellular events, and red lines with blunt stop-ends represent pathways and processes potentially disrupted by CDA-causing mutations. Diagram is simplified and organized around topics discussed herein; **B.** CDA causing mutations in GATA1 and KLF1 must cause pleiotropic effects as these factors bind multiple genomic locations in erythropoietic precursors. GATA1 ChIP-seq peaks confirmed by ChIP-Chip (blue) and KLF1 ChIP-seq peaks in E14.5 fetal liver erythroblasts (red), based on two example studies visualized in PhenoGram [53[Supp GATA1peaks.xls], 54[Table S3], 55]. Actual impact of these mutations needs to be assessed individually, as only some binding sites correspond to genomic regions that regulate transcription, and some mutations may lead to ectopic binding

Codanin-1 is a negative regulator of ASF1 and a part of the ASF1-H3-H4-importin-4 cytosolic complex [23]. In the nucleus, codanin-1 dissociation from the complex allows ASF1 to bind other histone chaperones and helps transport histones for chromatin assembly [16]. CDIN1 is localized in either the cytosol or the nucleus, suggesting a dual function of the protein. Both CDAN1 and CDIN1 are enriched in the nucleoli of erythropoietic cells [24], and nucleolar abnormalities are a recognized feature of CDA 1.

Interestingly, CDIN1 is homologous to restriction endonucleases and a high throughput protein interaction survey has indicated that it interacts with ASF1B [20, 25], adding to the hypothesis that DNA replication and chromatin assembly are hampered in CDA 1. *Cdan1* knockout mice die at an early embryonic stage of development (E6.5) [26]. There is no information on *Cdin1* knockout mice in the literature, but The International Mouse Phenotyping Consortium (www.mousephenotype.org) lists the homozygous *Cdin1*$^{em1(IMPC)J}$ allele as lethal at embryonic day 9.5. Only some of the known mutations in these proteins alter erythropoiesis and lead to CDA 1.

One hypothesis is that sub-lethal mutations primarily affect the rapidly dividing and maturing erythropoietic precursors. Multiple cycles of chromatin remodeling are necessary during the subsequent cell divisions, as well as for the final condensation of the nucleus. Nuclear condensation might require the eviction of specific histones from the DNA [9]. Caspase 3 activity-dependent lamin B cleavage and chromatin condensation induce the release of major histones through a nuclear opening [27]. It is conceivable that CDA 1 erythroblasts fail to evict histones H3 and H4 efficiently enough to prepare for enucleation. Recent analysis of *Asf1b* knockout mice has confirmed the involvement of this gene in erythropoiesis, but the phenotype is relatively mild, with a slight reduction of RBC count and increase of hemoglobin content and mean corpuscular volume (MCV). Persistent embryonic globin expression in adults and enlarged spleen likely result from the activation of compensatory erythropoiesis [28].

CDA type 2

CDA 2 is the most frequent form of CDAs [17], clinically characterized by varying degrees of normocytic anemia, with normal or slightly increased reticulocyte number [10, 29]. CDA 2 patients usually suffer from jaundice and splenomegaly due to the destruction of cell membranes in RBCs, which might result in the release of hemoglobin from disrupted cells. CDA 2 patients show a wide spectrum of clinical symptoms; about 10% of cases are asymptomatic, whereas 20% of them are dependent on blood transfusions [30, 31]. CDA 2 has distinct erythroid hyperplasia, with hypercellularity in the bone marrow just as CDA 1. The most striking morphological feature of CDA 2 is the presence of binucleated cells with both nuclei at the same stage of erythroid maturation. Morphologically, the diagnosis of CDA 2 is definite when at least 10% of erythroblasts are binucleated, with over 2% of the cells having a fragmented nucleus and irregular chromatin distribution in the cytoplasm [17, 32]. A discontinuous double membrane is present in the mature CDA 2 erythroblasts in electron microscopy, which is likely formed by large vesicles or cisterns of the ER that are positioned just below the plasma membrane [16].

SEC23B, the causative gene of CDA 2, encodes the cytoplasmic COPII (coat protein) component, which has a role in the secretory pathway of eukaryotic cells [33]. The COPII complex is responsible for the transport of correctly folded cargo from the ER to the Golgi apparatus after secretory cargo accumulation and membrane deformation [34]. Missense mutations in *SEC23B* are responsible for 52% of CDA 2 cases, nonsense mutations for 20%, and intronic for 13%. The remaining cases are caused by small insertions or deletions. CDA 2 is inherited in an autosomal recessive manner. Nonetheless, only one mutated allele of *SEC23B* has been found in 13% of cases, suggesting that a second unidentified mutation is present in the non-coding, regulatory region of the gene [35]. Occasionally, patients are diagnosed with CDA 2 based on bone marrow and biochemical analysis, but no *SEC23B* mutation is involved, as indicated by linkage exclusion.

This finding suggests that there might be another gene involved in CDA 2 [36]. Clinical symptoms vary depending on the compound heterozygosity of the mutations, such as missense and nonsense mutations [37]. In CDA 2 patients, no homozygous or compound heterozygous null mutations have been found, indicating that the absence of SEC23B is lethal. The expression of *SEC23B* is ubiquitous, but the effects of the known mutations of this gene are limited to the erythroid lineage. This phenotype could be explained by the hypothesis that SEC23B expressed in the hematopoietic system participates in terminal erythropoiesis in humans [33, 38], playing a role in vesicle trafficking by correctly transporting erythroid-specific cargoes from the ER to the Golgi apparatus with the help of the COPII complex [39].

Additionally, impairment of cytokinesis in CDA 2 erythroblasts suggests a possible primary function of SEC23B in the midbody assembly [39]. The presence of two hypomorphic alleles manifests in mild CDA 2 symptoms and is accompanied by increased expression of highly homologous SEC23A that could potentially compensate for the reduced expression of SEC23B [39]. The pathophysiology of CDA 2 is particularly challenging to establish due to the lack of a reliable animal model. *Sec23b*-deficient mice (*Sec23b*$^{gt/gt}$) die shortly after birth, due to pancreas and salivary gland degeneration. Surprisingly, they do not display any obvious signs of anemia [40]. The conditional *Sec23b* knockout specific to erythropoietic cells is viable, yet it does not develop anemia [41]. Moreover, fetal

liver cells (FLCs) isolated from *Sec23b*$^{gt/gt}$ donor mice were equally competent as wild-type FLCs in re-establishing erythropoiesis when transplanted to previously irradiated acceptor mice [41].

This discrepancy between human and mouse phenotypes could be explained by the difference in the temporal expression pattern of SEC23B and SEC23A proteins during erythroblast maturation in the two species [38]. The expression of SEC23B is maintained from the proerythroblast throughout the orthochromatic erythroblast stage in both species. SEC23A protein level drops sharply in transition from polychromatic to early orthochromatic erythroblast in humans, but not in mice.

This notion is supported by a comparative mouse and human mRNA expression study that revealed that human *SEC23A* mRNA level begins to decline at the early basophilic stage, and falls to undetectable levels in late basophilic erythroblasts, while the mouse gene continues to be expressed [42]. An intriguing possibility is that the disparate phenotypes and relative expression level differences of SEC23A and SEC23B that are observed between humans and mice could be a sign of an evolutionary shift in function between these two closely related proteins [41].

It has been recently reported that double conditional (*Sec23a*$^{fl/fl}$*Sec23b*$^{fl/fl}$*Mx1-cre*) knockout mice die *in utero*, presenting symptoms of anemia. This confirms that compensation by *Sec23a* is the reason why *Sec23b* knockouts are not anemic [43].

CDA type 3

CDA 3 has an autosomal dominant inheritance pattern, and is the rarest of the three classical types of CDA [16]. Most CDA 3 patients studied to date have belonged to just two families, one American, the other Swedish [44–46]. The clinical symptoms in CDA 3 patients are usually mild, showing no or moderate anemia, normal or slightly increased MCV, slight relative reticulocytopenia, hemolysis, and jaundice [47]. Typically, splenomegaly does not occur. On the cellular level, erythroid hyperplasia, altered morphology of erythroid cells visible under light microscopy, and the presence of giant multinucleated erythroblasts in the bone marrow are the characteristic features of CDA 3. Electron microscopy reveals the presence of autophagic vacuoles, clefts with heterochromatic, iron-laden mitochondria, and myelin figures in the cytoplasm of the erythroblasts [48]. Additionally, some CDA 3 patients from the Swedish family had disrupted vision, degeneration of the macula, as well as angioid streaks in the retina. Additionally, some patients have developed monoclonal gammopathy and multiple myeloma [45]. *KIF23*, the gene associated with the autosomal dominant inheritance of CDA 3, encodes a kinesin-superfamily molecule, mitotic kinesin-like protein 1 (MKLP1) [49, 50]. MKLP1 is a mitotic protein that interacts with adenosine diphosphate-ribosylation factor 6 (ARF6) and is essential for cytokinesis [49]. The ARF6-MKLP1 complex mediates the interaction between the cell membrane and the microtubule bundle at the cleavage furrow [49]. Reducing expression of ARF6 leads to the formation of binucleated and multinucleated cells, which is a striking morphology of CDA 3 erythroblasts. Transfection of GFP-MKLP1 harboring a CDA 3-specific mutation (P916R) in HeLa cells results in the presence of binucleated cells and the failure of cytokinesis. Wild-type GFP-MKLP1 does not cause this effect [45]. Impaired cytokinesis of erythropoietic precursors could explain the presence of giant multinucleated erythroblasts in the bone marrow of CDA 3 patients [45]. A mouse line created to replicate the exact CDA 3 mutation found in human patients has a P909R substitution in the *Kif23* gene [51]. Neither heterozygous nor homozygous mutant mice showed any symptoms of dyserythropoiesis, suggesting that this mutation is not as deleterious for mouse erythropoietic precursor cells as it is for their human counterparts. Complex alternative splicing of *KIF23/Kif23* that happens in both species, in conjunction with a hypothetical influence of the *KIF23* c.2747C>G, p.P916R mutation on splicing of *KIF23* mRNA in human erythropoietic precursors, has been proposed as a possible explanation for this discrepancy [51].

Transcription factor-related CDAs

Mutations in KLF1 and GATA1, two transcription factors (TFs) involved in erythropoiesis [52], cause CDA IV and X-linked thrombocytopenia associated with dyserythropoietic anemia (XLTDA), respectively. The common feature of TF-related disorders is that the expression of many TF target genes is affected, making the exact pathogenic mechanism difficult to elucidate. The case of KLF1 and GATA1 is no different, as each of them is known to bind several thousand sites in the genome (Figure 1B) [53–55]. Even though only a few hundred of these are associated with experimentally confirmed target genes, the task of evaluating all the potential consequences of disease-causing mutations is daunting.

There have only been eight recorded CDA 4 patients [56–60]. CDA 4 is distinguished by severe hemolytic anemia, normal or slightly increased reticulocyte count, and elevated levels of fetal hemoglobin. Hypercellular bone marrow contains immature erythroid progenitors with binucleated or multinucleated cells. Electron microscopy confirms CDA 4 when an engulfed nuclear membrane, marked heterochromatin, and atypical cytoplasmic inclusions are seen in cells of the erythroid lineage [56, 57]. CDA 4 is inherited in an autosomal dominant manner, and caused by mutations in the erythroid-specific transcription factor 1 gene (*KLF1*). KLF1 is involved in terminal erythropoiesis

and essential for the transition of hemoglobin expression from the fetal to the adult form [58]. Recently, some of the molecular consequences of CDA 4-specific mutation have been deciphered.

Erythroid cells differentiated from induced pluripotent stem cells (IPSCs) derived from a CDA 4 patient presented deregulated expression of genes linked to erythroid identity, involved in membrane transport, iron-utilization, cytokinesis, cell-surface receptors, and cell-cycle regulators [60]. KLF1 mutation E325K alters the sequence of the second zinc-finger, a crucial DNA binding domain. Another CDA 4-causing mutation, G973A results in high fetal hemoglobin expression and a lack of erythroid cell surface markers CD44 and aquaporin 1 (AQP1) in circulating erythrocytes and erythroblasts [56, 57]. However, it is still unclear exactly which KLF1 target genes are responsible for dyserythropoiesis in CDA 4.

One approach to this problem involves analyzing the impact of different genetic mutations or posttranslational modifications on KLF1 activity towards specific targets and correlating the observed changes in transcriptional profiles with the severity of the cellular phenotypes [61–63]. Comparison of homozygous loss-of-function mutations to hypomorphic alleles and haploinsufficiency caused by heterozygous frameshift or nonsense mutations should be performed within a frame of a consistent experimental system *in vitro*, while the most interesting or clinically relevant mutations could be modelled *in vivo*.

The need for new accurate models is evident in light of the documented discrepancies between human disease and the phenotypes of different *Klf1* mutant mouse lines. Complete loss of *Klf1* in mice causes severe anemia that leads to embryonic lethality and a cellular phenotype that recapitulates beta-thalassemia, rather than CDA 4 [64, 65]. In line with this observation, no human patients with a homozygous null *KLF1* mutation were known until a surprising recent report of severe neonatal hemolytic anemia with jaundice and kernicterus, due to compound heterozygous loss-of-function mutations in *KLF1* [66]. Decreased binding affinity of the CDA 4-related KLF1 E325K mutant to the consensus sequence (NCNCNCCCN) in the promoters of several target genes, including HBB, AQP1, and CD44 [67–71]. proves that, in addition to its dominant effect, it is also a partial loss-of-function mutation. Of the existing murine KLF1 mutant lines, the *Nan* strain had the highest potential to mimic CDA 4. *Nan* mice were created by N-ethyl-N-nitrosurea (ENU) mutagenesis [72–74] and carry an amino acid substitution (E339D) that affects the exact same position in the conserved, DNA-binding ZF2 domain as the human E325K mutation [75].

Surprisingly, *Nan* mice present a phenotype that resembles hereditary spherocytosis (HS) more than CDA [74]. It is unclear whether the interspecies differences in KLF1 functions or the opposing biophysical properties of the amino acids introduced by the human E325K and mouse E339D substitutions are responsible for the difference in erythropoietic phenotypes. In support of the latter, homology modeling suggests that *Nan* KLF1 has a higher affinity for the consensus binding motif than wild-type KLF1 [75]. Transcriptomic analysis of erythroid progenitors isolated from *Nan* mice reveals that only 52% of down-regulated, and 18% of upregulated, genes are known KLF1 targets [76, 77].

Prominent *Nan* KLF1 binding to ectopic sites in chromatin immunoprecipitation (ChIP-seq) experiments further highlights the complex consequences of semi-dominant KLF1 mutations [76]. Despite nearly four decades having passed since its appearance on the scene, the *Nan* model continues to yield general insights applicable to both HS and CDA 4.

XLTDA is caused by certain mutations in GATA1, a crucial regulator of erythroid and megakaryocyte differentiation. The exact phenotype differs substantially depending on the mutation. The symptoms include a tendency for bleeding, mild-to-severe anemia, and macro-thrombocytopenia with hypogranulated platelets. Anemia may improve with age. Some patients experience incidents of severe hemorrhage, and require transfusions [78]. Characteristic changes in the bone marrow of XLTDA patients include a decreased number of megakaryocytes that contain cytoplasmic vacuoles but lack platelet membrane demarcation, and dyserythropoiesis that is present in a subset of patients with GATA1 mutations.

The function of GATA1 in erythropoiesis has been extensively studied for several decades, and can be viewed as a model for comprehensive analysis of the role that a single gene plays in a complex biological process. Patients with GATA1 mutations may present with symptoms of beta-thalassemia, Diamond-Blackfan anemia, or CDA. Many of the GATA1 target genes are involved in erythropoiesis. In the context of dyserythropoietic anemia, GATA1 regulates the expression of *SEC23B* [79]. However, the significance of this fact might differ in humans and mice, given the previously outlined interspecies differences in the expression of *SEC23A*. Several mouse models that phenocopy the effects of human GATA1 mutations are available and have been thoroughly reviewed [80]. Complete *GATA1* knockouts die at an early embryonic stage due to severe defects in primitive and definitive fetal erythropoiesis [81]. Only the models best suited to studying GATA1 dependent dyserythropoietic anemia will be briefly discussed here.

Gata1$^{neo\Delta HS}$ is an allele obtained by targeted disruption of upstream regulatory sequence with a floxed neomycin resistance cassette. As a result, GATA1 expression is reduced 4–5-fold. Only 8% of the expected number of males is born, and they have severe anemia. Half of these mice die within 48 hours. Binucleate primitive erythrocytes are present in peripheral blood smears from both E11.5 and

E13.5 embryos. Dyserythropoietic features disappear around 4–5 weeks, suggesting that compensatory mechanisms are able to overcome the reduced expression of GATA1. Colony assays reveal a normal number of erythroid progenitors and a defect in their maturation [82, 83]. *Gata1*$^{\Delta IE}$ mice that lack the erythroid-specific first exon can also be used to study GATA1-dependent CDA. Hemizygous male embryos are severely anemic and die by E12.5, but conditional knockouts obtained using this allele have nucleated erythrocytes present in the peripheral blood [84]. Finally, a transgenic *Gata1*^{V205G} mouse line which expresses a mutated GATA1 that no longer interacts with FOG1 (Friend-of-GATA1) can rescue the complete *Gata1* knockout [85]. Only mice with high transgene expression are viable and suffer from thrombocytopenia and megakaryocyte defects. Surprisingly, the *Gata1* knockouts rescued by crossing to the *Gata1*^{V205G} transgenic mice do not have anemia, even though the V205G mutation targets the very same amino acid that is mutated in patients with severe dyserythropoietic anemia who carry the V205M substitution.

Syndromic forms of CDA

Apart from being a stand-alone condition, CDA is also present in the context of three unrelated genetic disorders. Majeed syndrome is caused by recessive mutations of lipin2 (*LPIN2*). Its manifestations include microcytic dyserythropoietic anemia, inflammatory dermatosis, and osteomyelitis. Erythropoiesis in the bone marrow is increased and 25% of erythroblasts are bi- or trinucleated [86]. LPIN2 is essential for lipid metabolism and affects phosphatidate phosphatase activity. Lpin2 knockout mice are viable and replicate many features of Majeed syndrome, including mild anemia with decreased levels of hemoglobin and reduced RBC volume [87].

A homozygous E138K mutation in COX4I2 causes exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis. *COX4I2* encodes the cytochrome c oxidase (COX) subunit 4 isoform 2, a structural component of the mitochondrial oxidative chain COX complex. The most severe symptoms include dysfunction and degenerative changes of the pancreas, hepatosplenomegaly, episodic jaundice, and life-threatening anemia that requires transfusions. Erythropoietic defects associated with this disorder include the presence of bi- and multinucleated erythroblasts, megaloblastic changes, and erythroid hyperplasia [88]. Surprisingly, *Cox4i2*$^{-/-}$ mice have normal pancreatic function, RBC count, and hematocrit and hemoglobin concentrations. Reduced airway responsiveness, progressive lung dysfunction, and reduced body mass and grip strength in females are the main findings in these mice [89].

There are two possible general classes of explanations for the stark difference between the human disease and the mouse model. The first is a typical interspecies difference in gene function. Since COX4I2 is a facultative subunit of the COX complex, expressed only in some tissues, it is possible that tissue-specific expression of its close paralog, COX4I1, differs between humans and mice. In support of this line of reasoning, *Cox4i2* mRNA expression level in the mouse pancreas is very low [89].

The second possibility is that the E138K mutation leads to more than just a functional loss of COX4I2. Perhaps the presence of the mutated protein prevents the activation of a putative compensatory mechanism that is triggered in *Cox4i2*$^{-/-}$ mice.

Aside from these possible explanations, it should be pointed out that there are only a few known cases of exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis, all of which come from just two families inhabiting two neighboring villages [88]. Is it possible that an undetected mutation, closely linked with the *COX4I2* locus, is responsible for some of the symptoms? The disease-causing mutation maps to an interval on chromosome 20, between markers D20S101 and D20S195. Only 12 of the 117 open reading frames (ORFs) present in this interval have been sequenced. Among others, an interesting candidate is *BCL2L1*, the closest genomic neighbor of *COX4I2* located on the opposite strand. Less than 20 kbp separates the 3' ends of both genes. Several members of the BCL2 family are involved in various aspects of erythropoiesis. *BCL2L1* codes for the BCL-XL (BCL-X) protein that is critically important for erythropoiesis [90–92]. Given the improved availability and the reduced cost of next generation sequencing, it is now feasible to sequence all the remaining genes in this interval to rule out any additional mutations.

Mevalonate kinase deficiency is caused by mutations in the *MVK* gene. Most of the 60+ known mutations cause hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) or mevalonic aciduria (MA), while some patients suffer from a skin condition known as porokeratosis. A compound heterozygous mutation carrier (310M/Y116H), with symptoms intermediate between HIDS and MA, also displays features of CDA [93]. Homozygous *Mvk* knockout mice are not viable, but heterozygous knockouts survive and are a good model of HIDS [94]. Interestingly, some of the *Mvk*$^{+/-}$ mice also display bone marrow hypocellularity, neutropenia and basophilic stippling of circulating RBCs, which indicates that hematological disturbances can be caused by *Mvk* gene dose reduction alone, and not just a specific point mutation.

Conclusions

Systematic progress in the identification of causative genes for the most common CDA types facilitates diagnosis and offers insights into the pathogenic mechanisms; however, the various cellular and molecular mechanisms of dyserythropoiesis are only partially understood.

Table I. Congenital dyserythropoietic anemia types and existing animal models

CDA type	Gene	Mouse model	Erythropoietic phenotype of model
CDA 1	*CDAN1* (AR)	***Cdan1***$^{-/-}$	Embryonic lethal (E6.5)
	CDIN1 (AR)	***Cdin1***$^{-/-}$ [em1(IMPC)J]*	Embryonic lethal (E9.5)
	Unknown	***Asf1b***$^{-/-}$	Mild dyserythropoiesis, persistent embryonic globin, increased fraction of immature EPCs in BM
CDA 2	*SEC23B* (AR)	***Sec23b***$^{-/-}$	Lethal (P0–P1), no anemia
		Sec23b$^{fl/-}$ ***(EpoR-cre***$^{Tg/+}$***)***	Viable, no anemia
	KIF23 (AD)	***Kif23***$^{P909R/+}$	Mice viable, active, no CDA or signs of any disease
		Kif23$^{P909R/\ P909R}$	
CDA 3	Unknown	***mDia2***$^{-/-}$	Lethal (E11.5) anemia, bi- and multinuclear embryonic EBs
		mDia2$^{fl/fl}$ ***(Mx1-cre)***	Defects in BM EBs
		RhoA$^{f/f}$ ***(EpoR-cre***$^{Tg/+}$***) (Mx1-cre)***	Enlarged primitive RBCs, poikilocytic, often multinucleated, failed definitive erythropoiesis, deletion with *EpoR-cre* is lethal (E16.5)
CDA 4	*KLF1* (AD)	***Klf1***$^{-/-}$	Embryonic lethal, severe anemia
		Nan	Hereditary spherocytosis
XLTDA	*GATA1* (XR)	***Gata1***$^{-/-}$	Lethal (E10.5–11.5), severe anemia
		Gata1^{05}	Embryonic lethal, severe anemia
		Gata1^{V205G} ***(Tg)***	Rescues *Gata1*^{05}, anemia, binucleate primitive RBCs
		Gata1$^{neo\Delta HS}$	Dyserythropoiesis, anemia
?	*VPS4A*	***Vps4a***$^{-/-}$ [tm1b(EUCOMM)Hmgu]*	Embryonic lethal (E18.5)
Majeed syndrome	*LPIN2*	***Lpin2***$^{-/-}$	Mild anemia, lower Hb concentration and MCV
EPIDACH	*COX4I2* (AR)	***Cox4i2***$^{-/-}$	Airway and lung defects, no erythropoietic (or pancreatic) phenotype
Mevalonate kinase deficiency	*MVK* (AR/CH)	***Mvk***$^{-/-}$	Embryonic lethal, heterozygotes survive, mild dyserythropoiesis
		Mvk$^{+/-}$	

*Public consortium allele type in square brackets; AR — autosomal recessive; EPCs — erythroid precursor cells; BM — bone marrow; AD — autosomal dominant; CDA — dyserythropoietic anemia; EBs — erythroblasts; RBCs — red blood cells; XR — X-linked recessive; Tg — transgenic line; Hb — haemoglobin; MCV — mean corpuscular volume; CH — compound heterozygous; EPIDACH — exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis

The complication which arises in studying the mechanism of terminal erythropoiesis in CDAs is the lack of a mouse model. Mouse models which have been generated to date (Table I) die during gestation or shortly after birth. Some of the mouse lines made to replicate specific human mutations do not develop CDA. Nevertheless, they might provide interesting information regarding the interspecies differences in erythropoiesis. Occasionally, a significant disparity between the mouse phenotype and the human disease might warrant re-evaluation of the initial findings, as in the case of *Cox4i2*.

Of the currently available models, the *Gata1*$^{neo\Delta HS}$ and the conditional *RhoA* and *mDia2* (*Diap3*) knockouts are likely to have the greatest impact on CDA research. The

first is close to recapitulating CDA naturally occurring as part of XLTDA, while the other two replicate several features of CDA 3, such as the presence of binucleated and giant multinucleated erythroblasts, due to a defect in cytokinesis [95, 96]. The complete knockouts of each of these genes in mice are lethal.

In the case of *RhoA*$^{fl/fl}$, inactivation with erythroid-specific *EpoR-cre*$^{Tg/+}$ still leads to embryonic lethality (E16.5) but it is late enough to analyze the defects of fetal, definitive erythropoiesis. Inducible inactivation with the *Mx1-cre* allows the study of the function of RhoA in adult erythropoiesis but requires complicated hematopoietic stem cell transplantation experiments into irradiated mice, and leads to defects not only in the erythroid lineage but also in the neutrophils, monocytes, and platelets [97].

Human CDA-causing mutations affecting either the *RHOA* or the *DIAP3* genes have not been identified so far, and therefore the status of the corresponding knockout mouse lines as faithful models of an actual human CDA, or simply as useful tools for replicating some aspects of the disease, is yet to be determined. Despite being instrumental in the above-mentioned studies, the inducible *Mx1-cre* system presents recognized challenges in itself [98]. Perhaps an inducible *EpoR-cre* line could be developed to improve temporal and cellular resolution of phenotypic characterization of the conditional gene knockouts in erythropoiesis.

The symptoms and hematological findings resembling CDA that are seen in certain patients do not fit any of the classical CDA types. Sporadic cases of unknown genetic background also exist. The establishment of the Congenital Dyserythropoietic Anemia Registry [99] is bound to accelerate the identification of novel CDA-associated genes and increase the need for new *in vivo* models. In fact, the first such gene is *VPS4A* [100, 101], a regulator of the ESCRT-III complex. Two probands have heterozygous *de novo* mutations R284W and G203E, and a third carries a homozygous A28V mutation.

Multinuclear erythroblasts in the bone marrow and circulating RBCs displaying transferrin receptor at the surface indicate that defects in cell division and endosomal vesicle trafficking are the main culprits. The gain-of-function or dominant-negative character of the heterozygous mutations is evident from the inheritance mode. It is more difficult to anticipate the degree of functional loss of VPS4A in the proband carrying the homozygous mutation, based on the phenotype and genetics alone. Homozygous knockout mice (*Vps4a*$^{tm1b(EUCOMM)Hmgu}$) die *in utero* (IMPC).

Due to the high throughput nature of these experiments, only four female and two male homozygous mutants have been observed. At E18.5, embryos were unresponsive to tactile stimuli, and were of abnormal size and abnormal body wall morphology. Given the complete penetrance of embryonic lethality of the knockout mice, it is likely that the human CDA-causing A28V mutation leads to only a partial loss of VPS4A function.

Undoubtedly, efforts aimed at developing animal models better suited for CDA research are increasing the understanding of the pathophysiology of this disease, which will eventually lead to new therapeutic strategies.

Authors' contributions

RK and PK wrote the review together.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

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

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New therapeutic options for hairy cell leukemia

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Abstract

Hairy cell leukemia (HCL) is a rare B-cell lymphoproliferative disorder characterized by pancytopenia, splenomegaly and increased susceptibility to infections. In 2011, *BRAF* gene mutation was identified in almost all the patients with the classical type of HCL. The purine analogs cladribine and pentostatin induce long-term remission in the majority of patients, and they remain the standard treatment for this type of leukemia.

However, more than half of patients in complete response relapse over the long term, with a quarter of them relapsing within the first five years.

Recently, new drugs have been developed and have demonstrated efficacy in refractory or relapsed HCL. The immunotoxin Moxetumomab pasudotox was registered for HCL in 2018. The BRAF kinase inhibitors vemurafenib and dabrafenib, as well as the Bruton kinase inhibitor ibrutinib, are also proven highly effective in clinical trials.

Key words: BRAF, dabrafenib, hairy cell leukemia, ibrutinib, moxetumomab pasudotox, purine analogs, rituximab, vemurafenib

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Introduction

Hairy cell leukemia (HCL) is a rare B-cell lymphoproliferative disorder [1, 2]. The classical type is characterized by pancytopenia, splenomegaly, general symptoms and increased susceptibility to infections. Rarer clinical presentations and manifestations include lymphadenopathy, skin lesions, osteolytic bone changes, and leukemic infiltrates in the gastrointestinal tract and liver [3–5]. HCL gets its name from the characteristic villous cytoplasmic projections. The classic phenotype of HCL consists of high expression of surface immunoglobulin, with high co-expression of CD20, CD22, CD11c, CD103 and CD25, CD123 and CD200. The *BRAF* V600E mutation is almost universally present in classical HCL [6]. HCL incidence is estimated to be 0.3 cases per 100,000 people per year [7, 8]. The median age at diagnosis is 58 years. HCL accounts for 2–3% of all adult leukemias [1, 2]. There is a strong male predominance, with a male-to-female ratio of 4 to 1.

In 2008, the World Health Organization (WHO) reclassified HCL and distinguished a variant of hairy cell leukemia (HCLv), which it then included as a provisional entity within the spectrum of 'splenic B-cell leukemia/lymphomas unclassifiable' [9–11]. HCLv is characterized by leucocytosis with lymphocytosis, cytopenias without monocytopenia, lymphoid cells of relatively large size with prominent nucleoli, atypical HCL immunophenotype, and resistance to conventional HCL therapy. BRAF mutation is not detected. Mutations of the immunoglobulin heavy chain (IGVH) are seen in two thirds of cases with a preferential VH4-34 family usage [9].

This article presents new therapeutic options for patients with hairy cell leukemia.

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Standard therapies

As with chronic lymphocytic leukemia (CLL) and indolent lymphomas, in asymptomatic HCL patients, the strategy applied is one of deferred treatment to progression (watch and wait) [1, 2]. The indications for treatment initiation are anemia [hemoglobin (Hb) <11 g/dL] not due to other causes, thrombocytopenia [platelets (PLT) <100,000/μL], neutropenia [absolute neutrophil count (ANC) <1,000 μL], symptomatic splenomegaly, recurrent infections, or systemic symptoms. Only 10% of patients with newly diagnosed HCL have no indication to start treatment [1]. Cladribine (2-CdA, 2-chlorodeoxyadenosine) and pentostatin (DCF, deoxycoformycin) are recommended for first-line treatment [1, 2]. Their introduction to HCL therapies in 1990 was a breakthrough in the treatment of this group of patients, and allowed for long-term remissions in over 90% of patients [12, 13]. Both drugs interfere with DNA synthesis in resting and proliferating cells, disrupting their metabolism and inducing apoptosis. Both drugs also show a strong myelosuppressive effect and cause long-term immunosuppression [14].

So far, no randomized study directly comparing the efficacy of 2-CdA and DCF therapy has been conducted. However, both drugs seem to be similarly effective [14]. In addition, 2-CdA can be given as second-line treatment in patients with primary DCF resistance. In practice, 2-CdA is used more often, mainly due to easier dosing and lower drug toxicity, especially nephrotoxicity [15]. Purine analogs induce long-term complete response (CR) in over 70% of patients with classic HCL, with a median response duration of 8–10 years [16, 17]. Patients who achieve a CR without minimal residual disease (MRD) have a longer disease-free survival (DFS). Purine analogs can be used in second-line treatment, but the response rate is lower and response duration shorter. The median duration of the second response to purine analogs is 2–9 years [18–23].

Rituximab monotherapy is not very effective [24]. In patients with early relapse (within 12–18 months), it is recommended to combine 2-CdA with rituximab [25, 26]. Such treatment allows CR to be obtained in 89–100% of patients with a 5-year progression-free survival (PFS) reaching 100%, and a 3-year risk of recurrence of about 7% [25].

Another treatment option for relapsed patients is fludarabine (administered orally at 40 mg/m^2 for 5 days) or bendamustine (70–90 mg/m^2 on days 1 and 2) in combination with rituximab (375 mg/m^2) on the first day of the cycle, every 28 days, for up to four cycles [27, 28]. Splenectomy is considered in patients with refractory, massive splenomegaly (>10 cm below the costal arch) with minor bone marrow involvement, or in pregnant women who have not responded to treatment with alpha interferon (IFN-α) [29].

New drugs

In recent years, the effectiveness of several new drugs has been demonstrated in patients with the classic form of HCL. These include the immunotoxin moxetumomab pasudotox (Moxe, Lumoxiti™), targeting the CD22 antigen, BRAF kinase inhibitors (vemurafenib and dabrafenib), as well as the Bruton kinase (BTK) inhibitor ibrutinib [14]. These drugs have been studied mainly in patients who are refractory to purine analogs. Table I presents the characteristics of the new drugs showing clinical activity in HCL patients [30–36].

Moxetumomab pasudotox

Moxetumomab pasudotox (Moxe) is a recombinant immunotoxin that consists of an anti-CD22 monoclonal antibody and *Pseudomonas* PE38 exotoxin [37]. The CD22 antigen is a transmembrane protein present only on B lymphocytes. This antigen is seen in increased numbers on HCL leukemic cells. Moxetumomab pasudotox binds to CD22-expressing cells followed by internalization of the drug-CD22 complex. The active form of PE38 exotoxin is released, which inactivates elongation factor 2 (EF-2). This in turn results in protein synthesis inhibition and, consequently, cell apoptosis. The amount of both normal lymphocytes and leukemic cells decreases over a short period of time. About six months after the end of therapy, the amount of normal B lymphocytes returns to baseline values. The efficacy of Moxe was assessed in phase I and phase III trials in patients with refractory and recurrent HCL [30–33].

In the phase I study, Moxe was used in 26 patients at increasing doses of 5, 10, 20, 30 μg/kg, 40 μg/kg, and 50 μg/kg administered on days 1, 3, and 5 of each 28-day treatment cycle [30]. The treatment was well tolerated, and no dose-limiting toxicity was observed. Two patients developed moderate hemolytic uremic syndrome (HUS). In 10 patients (38%), the development of drug-neutralizing antibodies was observed during the use of Moxe. A response was achieved in 86% of patients, including 13 (46%) CR. Lesser efficacy was observed in patients who had undergone splenectomy and with massive splenomegaly. In the next analysis, the study group was increased by 21 patients who received a dose of 50 μg/kg [31]. A total of 33 patients received this dose. As in the previous analysis, 88% of patients responded to the treatment, and 64% achieved a CR of a mean duration of 42.4 months. The duration of CR was significantly longer in 11 patients without minimal residual disease (MRD) (42.1 months) than in MRD-positive patients (13.5 months) (p <0.001). The most common adverse drug reactions observed were: peripheral edema (52.5%), nausea (35.0%), infusion-related reactions (25.0%), hypoalbuminemia (21.3%), and increased transaminases (21.3%).

Table I. New drugs active in hairy cell leukemia (acc. to [30–36])

Drug	Drug's mechanism of action	Drug dosage	Drug effectiveness	Side effects	Literature
Moxetumomab pasudotox (Lumoxiti™)	After association with cell, drug-CD22 complex is internalized and active form of exotoxin is released	0.04 mg/kg on days 1, 3 and 5 of each 28-day cycle, up to 6 cycles, disease progression or drug-induced toxicity	Follow-up* 24.6 months ORR 75% CR 41% CR MRD(–) 34% PR 34% PFS 71.7 months	Edema, peripheral nausea, infusion reactions, hypoalbuminemia, increase in transaminases, hemolytic uremic syndrome, capillary leak syndrome	[30–33]
Vemurafenib (Zelboraf®)	BRAF serine-threonine kinase inhibitor caused mutations in *BRAF* gene in codon 600	240–960 mg twice a day orally for 16/18 weeks	Follow-up* 23 months ORR 96% CR 35% PR 62% PFS 9 months	Skin rash, skin sensitivity to light, joint pain and inflammation, fever, increase in transaminases and creatinine, QT prolongation, ocular reactions, skin tumors	[34]
Dabrafenib (Tafinlar®)	BRAF serine-threonine kinase inhibitor caused mutations in *BRAF* gene in codon 600	150 mg twice a day orally for 12 weeks	Follow-up* 64 months ORR 80% CR 30% CR MRD(–) 10% PR 50% PFS 14 months	Skin rash, skin sensitivity to light, joint pain and inflammation, fever, increase in transaminases and creatinine, QT prolongation, ocular reactions, skin tumors	[35]
Ibrutinib (Imbruvica®)	Bruton's kinase inhibitor	420, 840 mg 1 pc, orally until disease progression or unacceptable toxicity	Follow-up* 3.5 years ORR 36% after 48 weeks of treatment	Diarrhea, weakness, muscle and joint pain, nausea, hemorrhagic diathesis, cytopenia, hypertension, atrial fibrillation	[36]

*Follow-up — observation time; ORR — overall response rate; CR — complete remission; MRD — minimal residual disease; PR — partial response; PFS — progression-free survival

The results obtained in the phase I study were confirmed by a phase III study in 80 patients with refractory and recurrent HCL [37]. Moxe was administered at a dose of 40 µg/kg on days 1, 3, and 5 every 28 days for up to 6 cycles. Patients who had previously received at least two lines of treatment, including one with a purine analog, were included in the study. The mean follow-up was 16.7 months. Hematological remission was achieved by 80% of patients, and CR in 41%. In the group of patients with CR, 85% of patients did not show MRD in immunohistochemical tests. The most common adverse events were peripheral edema (39%), nausea (35%), fatigue (34%), and headache (33%). Hemolytic uremic syndrome occurred in 7.5%, and capillary leak syndrome in 8.8% of patients. These symptoms resolved after discontinuation of therapy. The long-term results of this study with a median follow-up of 24.6 months were recently presented [33]. The median for prior lines of treatment was three lines, and 49% of patients were refractory to purine analogs. The CR rate was 41%. Long-term CR (>180 days) was achieved by 36% of patients and of over 360 days by 33%. In 27 patients with CR (82%) no minimal residual disease was found. The median PFS was 71.7 months, and 61% of patients with CR had no relapse by 60 months. No treatment-related death was observed.

In 2018, Moxe was approved by the Food and Drug Administration (FDA) for the treatment of patients with refractory and relapsed HCL who have received at least two systemic therapies, including one with a purine analog [37]. The recommended dose is 0.04 mg/kg. The drug should be administered as a 30-minute intravenous infusion on days 1, 3 and 5 of each 28-day cycle, after the patient has been hydrated and supplied with antiallergic drugs. Patients should continue treatment for a maximum of six cycles or until disease progression or signs of unacceptable drug toxicity. In special circumstances, treatment may be discontinued earlier if the patient has achieved CR. FDA also

allows repeated treatment with Moxe within 3–12 years after completing the first treatment. Yurkiewicz et al. [38] analyzed the cases of three patients who achieved at least PR after the first Moxe therapy. In two of the three patients treated with Moxe a hematological response was achieved, and in one CR was confirmed by bone marrow examination. One patient did not respond to treatment. No serious side effects were observed during the second treatment with Moxe. The combined treatment of Moxe with rituximab remains in the first phase of clinical trials [39].

BRAF kinase inhibitors

The identification of the *BRAF* V600E kinase mutation in 2011 as the pathogenetic cause of classical HCL allowed for the introduction of new drugs for HCL therapy [6, 40–42]. The mutation causes constitutive activation of the MAP kinase pathway and determines the survival of the leukemic cells. In 2014, the BRAF kinase inhibitors, vemurafenib and dabrafenib, began to be used in patients with refractory and recurrent HCL [40]. Another reason for choosing BRAF inhibitors are patients with contraindications to purine analogs, agranulocytosis and active infection [2]. BRAF kinase inhibitors have turned out to be effective in the treatment of classic HCL, but CR is achieved only in some patients, and median time to relapse after cessation of vemurafenib is just 14 months [34, 42].

Vemurafenib

Vemurafenib (Zelboraf®) was originally approved for the treatment of malignant melanoma. In 2015, Tiacci et al. [34] published the results of a phase II clinical trial involving 54 patients with relapsed or refractory BRAF positive HCL. The study was conducted in Italy (28 patients) and in the USA (26 patients). The drug was administered at a dose of 960 mg twice daily for 16–18 weeks. The overall response rates (ORR) were 96% and 100% after 8 and 12 weeks, in the Italian and in the American study, respectively. The CR rates were 35% and 42% in the two trials. After a median follow-up of 23 months, the Italian median relapse-free survival was 19 months for patients who achieved CR, and 6 months for those with partial response (PR). Patients who required dose reduction due to toxicity obtained similar results (CR 60%, PR 40%). Splenectomy or the number of prior treatments did not correlate with the quality of response. However, splenectomy was associated with shorter progression-free survival (PFS) and time to treatment (6 vs. 11 months).

In 2020, Libers et al. [42] presented retrospective data of 27 patients treated with different doses of BRAF inhibitors (vemurafenib, dabrafenib) outside clinical trials, in seven different European centers. The analysis of variable doses (range: vemurafenib: 240–1,920 mg per day; dabrafenib: 150–300 mg per day) and treatment durations (median 3.8 months, range: 1.7–19.9) addressed the question of whether individual approach had an impact on time to next treatment (TTT). All patients obtained complete hematological response. Based on the available 18 samples of bone marrow biopsies, six patients achieved CR and 12 achieved PR. The analysis also showed that neither the dose nor duration of treatment, or the number of previous lines, had any effect on the final response. Similarly to other studies, patients who achieved CR had a significantly longer PFS than those who achieved PR (19.8 vs. 11.4 months respectively). In addition, it has been shown that vemurafenib increases platelet count >100 G/L after two weeks, the number of neutrophils >1.5 G/L after four weeks, and an improvement in red blood cell parameters hemoglobin (Hg) >11 g/dL after eight weeks [42]. The best results were obtained during the first two relapses, with PFS 10.9 and 12.1 months. The duration of remission after successive cycles of vemurafenib was significantly shorter (median 3.4 months) [42]. An early, retrospective analysis indicated that lower doses of the drug (2 × 240 mg per day) are as effective as higher doses (2 × 960 mg per day) [41]. However, patients who received higher doses of BRAF inhibitors (vemurafenib >480 mg and dabrafenib >150 mg) had a significantly longer treatment-free survival (14.6 months vs. 9.4 months) than patients receiving lower doses.

In summary, it seems that the highest, well-tolerated doses of BRAF inhibitors should be used in order to obtain the best response.

Vemurafenib is well-tolerated. The most common drug-related adverse events are grade 1/2 and include skin rash, photosensitivity, arthritis, fever, elevated liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGTP)] and renal function tests. A temporary dose reduction and symptomatic treatment relieve the symptoms [34, 42]. Vemurafenib may also accelerate the growth of secondary skin cancers. The risk of developing a new tumor may be dose-related [34, 42–44].

The effectiveness of BRAF inhibitors is higher when combined with rituximab [45]. Tiacci et al. recently published the results of a study with vemurafenib at a dose of 960 mg, given twice daily for eight weeks combined with eight doses of rituximab 375 mg/m^2 over 18 weeks [45]. Thirty patients with refractory or recurrent HCL were enrolled to the study, including 10 patients resistant to chemotherapy, five patients resistant to rituximab, and seven patients resistant to BRAF inhibitors. 26 patients achieved CR (87%), with 17 of them (65%) having an MRD negative response. Median PFS was 78% (median follow-up was 37 months). Lack of MRD and no prior use of BRAF inhibitors correlated with a longer PFS.

In another study, the utility of targeted therapy for nine patients (three treatment-naive patients with severe neutropenia and active infection) was reported. Vemurafenib was administered at 240–480 mg twice daily and

combined with rituximab in seven patients. Vemurafenib was given for 38–140 days. In spite of lower doses of vemurafenib than those in previous studies, therapy with the BRAF inhibitor was successful in achieving a remission in all patients. Our observations are compatible with these results [46, 47]. Recently, we reported the cases of four relapsed patients with classical HCL who were treated with vemurafenib combined with rituximab after the failure of at least three lines of therapy including 2-CdA and Moxe [46]. Two patients achieved MRD negative CR, and a third achieved a hematologic response. The fourth patient died due to severe infection.

To overcome resistance to the BRAF inhibitor, vemurafenib was also administered together with the MEK inhibitor, cobimetinib [48]. Caesar et al. [48] presented a case report of a patient resistant to purine analogs who was treated with cobimetinib, initially at a dose of 20 mg/ /day, in combination with vemurafenib 240 mg twice a day. After four months, the cobimetinib dose was increased to 60 mg daily for 21 days. The cycles were repeated every four weeks. At 12 months, the patient remains well and asymptomatic with continued combination therapy.

It should be emphasized that retreatment with vemurafenib may also be effective in relapsed patients [34]. However, the duration of response after retreatment is shorter than after the first treatment. Liebers et al. [42] observed rapid hematological improvement after repeated drug use in most patients. Our observations indicate that vemurafenib in combination with rituximab shows therapeutic efficacy, and this includes patients previously treated with Moxe [46].

Dabrafenib

Dabrafenib (Tafinlar®) is another oral BRAF inhibitor used in patients with relapsed classic HCL. Tiacci et al. [35] presented the results of 10 patients with relapsed HCL treated with dabrafenib, including two previously treated with vemurafenib. The drug was administered at a dose of 150 mg twice a day for 12 weeks. 60% of patients required a dose reduction to 50–100 mg twice a day due to adverse drug events. Most of them returned to the initial dose of 150 mg twice daily after appropriate symptomatic treatment. Response was obtained in 80% of patients, including CR in 30% and PR in 50%. The increase in platelet count was the fastest (after c. 15 days), then in neutrophils (after an average of 35 days), and finally in hemoglobin (after 51 days). After a follow-up period of 14–79 months (median 64) from the start of treatment, survival in this group was 90%. One patient still remains in CR 60 months after the end of treatment, and two relapsed at 14 and 15 months. As with vemurafenib, the most common adverse events were grade 1–2. These included joint pain, facial flushing, skin changes, asymptomatic QT prolongation, and increased levels of transaminases and pancreatic enzymes.

Kreitman et al. [49] assessed the effectiveness of treatment with dabrafenib at a dose of 150 mg twice a day in combination with the MEK inhibitor trametinib 2 mg once a day. The drug was used in 43 patients with relapsed and refractory HCL. Response to treatment was achieved in 78% of patients, including 49% of patients achieving CR. MRD negative CR was found in 15% of patients, and MRD positive CR in 34%. PFS and OS at 12 months were 97.6%.

Ibrutinib

Ibrutinib (Imbruvica®) is a BTK inhibitor widely used in CLL and other B-cell lymphomas [50]. Ibrutinib works by modulating the signaling pathway from the B-cell receptor (BCR). In a multicenter phase II study, ibrutinib was used in 28 patients with newly diagnosed and relapsing classic HCL and in nine patients with HCLv [48]. The *BRAF* V600E mutation was found in 20 patients. Ibrutinib at a dose of 420 mg/day was used in 24 patients, and in 13 patients at 840 mg/day. After an initial assessment of the response to treatment and analysis of drug-related complications after 32 weeks, the original dose of 840 mg/day was reduced to 420 mg/day. The median follow-up time was 3.5 years for all patients. Fifteen patients are still receiving ibrutinib, and 22 patients have had the therapy discontinued. The response rate at 32 weeks was 24% and was 36% at 48 weeks. At week 32, one patient had achieved CR, eight PR, 21 stable disease (SD), and three patients had progressed. Treatment was discontinued in four patients, three due to non-response and one due to death. During a longer follow-up, seven patients achieved CR including three CR MRD negative, and 13 achieved PR, and 10 SD. The median PFS was not reached, and the PFS over 36 months was estimated to be 73%. The median OS was 69 months. There were no differences in PFS and OS between the histological subtypes or the dose of ibrutinib. A better response was seen in younger patients. However, no differences were found in ORR, PFS and OS in patients with classic HCL and HCLv. Hematological complications included anemia (43%), thrombocytopenia (41%), and neutropenia (32%). Of the 13 neutropenic patients, only four developed fever. Among the non-hematological adverse events, diarrhea, weakness, myalgia, nausea, and infection of the upper respiratory tract were observed. Hypertension, atrial fibrillation, palpitations, sinus bradycardia, and heart failure have also been reported as in other lymphoproliferative malignancies. Treatment was discontinued in seven patients due to cardiac complications (decreased ejection fraction, arrhythmias), hypersensitivity to ibrutinib, thrombocytopenia and neutropenia, as well as a diagnosis of colorectal cancer. Five patients died, three from pneumonia and two from disease progression.

The obtained results indicate that ibrutinib is an effective drug in patients with HCL after multiple treatment lines. The study also showed that, as with treatments for

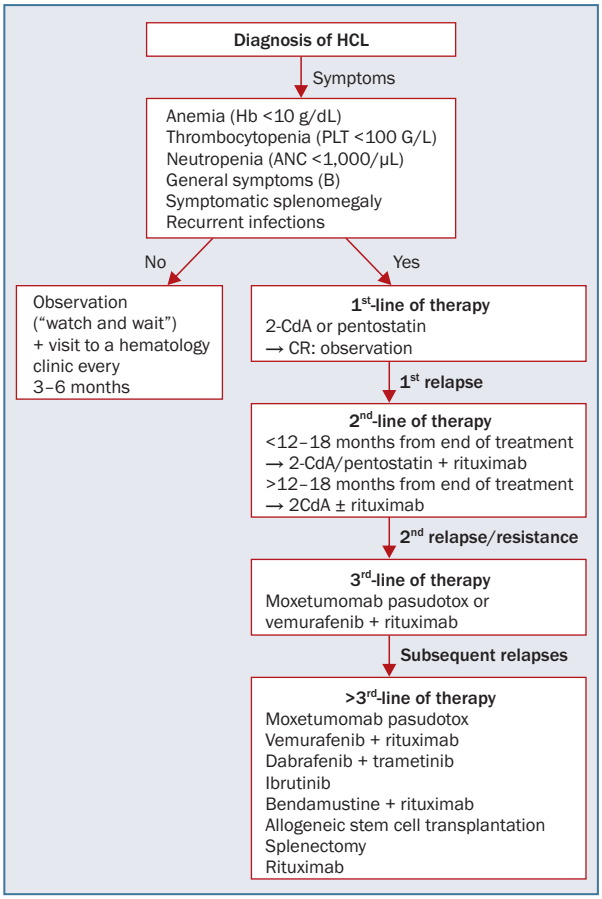

Figure 1. Proposed treatment regimen of hairy cell leukemia (HCL) in different stages of disease (acc. to [14]); Hb — hemoglobin; PLT — platelets; ANC — absolute neutrophil count; 2-CdA — (2-chlorodeoxyadenosine) cladribine; CR — complete response

other B-cell cancers, longer treatment duration may be required to obtain an optimal response. Drug-induced complications were similar to those in patients treated for other hematological malignancies. Dose-related drug toxicity has not been reported.

HCL variant

The HCL variant (HCLv) is a rare B-cell neoplasm with different immunophenotypic and molecular characteristics compared to classic HCL [9, 36, 51, 52]. HCLv is less common than the classical form of HCL. The incidence is 0.2 cases per 100,000 people and 2% of all leukemias [7]. As in the classical form, patients with HCLv present with splenomegaly, but the marrow is cell-rich and easily aspirated, in contrast to classical HCL. Moreover, there is no BRAF mutation [9]. So far, there are no widely recognized recommendations for the treatment of HCLv patients. Choosing the optimal treatment for this group of patients is still a major challenge due to the unsatisfactory results of treatment with purine analogs. Less than half of patients achieve a response, which is most often a short-term partial remission [9, 51]. It is also rare to achieve a CR. Possible therapeutic options include primarily immunochemotherapy, but also splenectomy or radiotherapy. Due to the low effectiveness of purine analogs, they should not be used as monotherapy [9, 51]. Rituximab can be used as monotherapy or in combination with 2-CdA or bendamustine, especially in patients in a fairly good general condition without significant internal diseases. Rituximab monotherapy may reduce cytopenia and tumor size before the administration of immunochemotherapy. The combination of 2-CdA or bendamustine with rituximab caused a marked improvement in treatment outcomes, including the possibility of achieving CR and MRD negativity [53, 54]. This antibody can also be used as a consolidation treatment after splenectomy.

Splenectomy is another therapeutic option in patients with HCLv [2]. Among 19 patients who underwent splenectomy, 13 (74%) achieved a hematological response, and the median response duration was 4 years [9]. In the elderly, and in those who do not qualify for more intensive treatment, radiotherapy of the spleen may be considered as symptomatic treatment of hypersplenism [55]. Rituximab used after splenectomy may also be an effective treatment method [56]. Another treatment option is Moxe. In the studies published so far, only a few patients have received treatment [30, 32]. None achieved CR, but several achieved PR. Promising results were also obtained in patients treated with ibrutinib [36, 52].

Summary

Hairy cell leukemia (HCL) is a rare B-cell lymphoproliferative disorder characterized by pancytopenia, splenomegaly, general symptoms and increased susceptibility to infections. Until now, the standard treatment has been based on purine analogs, often in combination with rituximab. In recent years, new drugs have been developed that are effective in purine analog-resistant and relapsed patients, often after multiple lines of treatment. In addition to the previously available interferon-α and rituximab, the immunotoxin Moxetumomab pasudotox has recently been registered for HCL. BRAF kinase inhibitors (vemurafenib and dabrafenib) as well as the BTK inhibitor ibrutinib are also highly effective in clinical trials. Although these drugs are less effective than 2-CdA and pentostatin, they play an important role in the treatment of relapsed/refractory patients and patients unsuitable for purine nucleoside analogs (PNA).

BRAF inhibitors induce a high response rate in classical HCL. They are also well tolerated and can be used in patients with neutropenia and co-infection. Their therapeutic activity increases when used in combination with rituximab or a MEK inhibitor. Moxe and ibrutinib are clinically active in both HCL and HCLv, but their use is associated with the risk of serious complications such as hemolytic uremic syndrome and capillary leak syndrome. Moxe

induces deep remission, including a significant percentage of complete remissions with negative MRD. Unlike BRAF inhibitors, the responses in patients treated with ibrutinib are longer. However, this drug should be used protractedly until progression or unacceptable toxicity. It should be emphasized that, except for Moxe, other drugs active in HCL patients have not yet been approved for treatment of this disease and are used 'off-label'.

Despite the high effectiveness of the new targeted drugs, it is important to remember other therapeutic options that will cancel the activity effective in previously treated patients, such as the combination of bendamustine with rituximab or pegylated IFN-α [25]. The optimal sequence in which to use these drugs in patients with HCL requires further research. Figure 1 presents the current recommendations for the treatment of HCL at different stages of the disease.

Authors' contributions

All authors participated equally in the design, editing and approval of this work.

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

Atypical immunophenotype of chronic lymphocytic leukemia

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Abstract

Assessment of the immunophenotype plays a crucial role in the diagnostic process of chronic lymphocytic leukemia (CLL). The expression of CD5, CD19 and CD23 antigens with a concomitant reduction or lack of surface immunoglobulin expression as well as CD22 and CD79b antigens is the basic part of CLL diagnosis. A significant diagnostic challenge is atypical CLL with cells devoid of CD5 or CD23 antigens. The assessment of additional antigens in flow cytometry, especially the CD200 glycoprotein, may facilitate the process of differential diagnosis of atypical CLL from other B-cell lymphoproliferative neoplasms. The results of current studies analyzing the influence of atypical CLL on prognosis are inconclusive. The analysis of a large group of patients with atypical CLL is difficult because of the rare occurrence of CD5(–) or CD23(–) CLL and the misdiagnosis of this disease as other B-cell lymphoproliferative neoplasms.

The following paper aims to show how important it is to include atypical CLL in the diagnostic process of this disease and to re-standardize the commonly used immunophenotypic scales for its diagnosis.

Key words: CLL, atypic CLL, CD5 antigen, CD23 antigen, CD200 antigen

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Introduction

Chronic lymphocytic leukemia (CLL) is a lymphatic system neoplasm characterized by a proliferation of small, mature lymphocytes and their accumulation in peripheral blood, bone marrow and lymphatic organs [1, 2]. CLL is the most common lymphoid malignancy in Western Europe and North America. The cumulative incidence of CLL is 4.2 per 100,000 people.

The disease mainly affects the elderly, and is twice as common in males as in females. The heterogenic course of the disease encourages the search for prognostic factors which would aid the selection of the most effective individual therapy for each patient. In clinical practice, there is remarkable morphological, cytogenetic and immunophenotypic differentiation of leukemia cells in CLL patients [3–7]. Taking into consideration the phenotype of neoplastic lymphocytes in CLL, a classification has been made that differentiates the classical from the atypical form of the disease.

The typical form is characterized by both the expression of antigens CD5, CD19, CD23 and the lack of expression of immunoglobulins and antigens CD22 and CD79b [8, 9]. The atypical form however, differs from the typical one in the expression of one or fewer surface antigens and, at the same time, without any criteria met for a diagnosis of another B-cell lymphoproliferative disorder. Interestingly, a different CLL phenotype can affect the clinical course of the disease, the duration of progression-free survival, and overall survival. Furthermore, an association between CLL's

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immunophenotype and some specific cytogenetic disorders has been suggested, which may also affect prognosis [5, 10]. Therefore, researching new markers of the disease, or modifying the interpretation of existing ones, is crucial in clinical practice. The significance of these studies is emphasized in the light of current knowledge showing that 13% of primary diagnoses of lymphoproliferative disorders are altered during the diagnostic process.

The aim of this article was to summarize the data concerning the types of CLL with atypical immunophenotype, as well as to discuss a methodology that could be useful for differential diagnosis from other B-cell lymphoproliferative disorders.

Assessment of immunophenotype in CLL

In recent years, the Matutes Score, and its subsequent modification the Moreau Score, have been used as the basis for immunophenotypic diagnosis of CLL, as well as for differential diagnosis including CLL and other B-cell neoplasms (Table I). The Matutes Score includes typical CLL markers such as CD5, CD23, CD22, FMC7 and SmIg [11], while the Moreau Score additionally encompasses CD79b expression without expression of CD22 [12]. Achieving at least 4 points in both the Matutes and Moreau Scores is characteristic for the immunophenotype in typical CLL. If the score is 3 points or less, this indicates a diagnosis of a B-cell neoplasm other than CLL.

However, none of the classifications mentioned enables an appropriate differential diagnosis of the atypical form of CLL from other B-cell lymphoproliferative disorders.

CD5-negative CLL

CD5 antigen is a protein expressed on the surface of normal T lymphocytes and in a subset of B-cells known as B-1a [13]. Although the role of CD5 is unknown, the expression of this antigen has a major impact on the pathogenesis of some disorders. Population of CD5-positive (CD5+) B lymphocytes associated with the immune system overreaction has been found in some autoimmune diseases such as rheumatoid arthritis and lupus erythematosus [14, 15].

Expression of CD5 is a crucial marker in differential diagnosis of B-cell lymphoproliferative disorders, particularly CLL [14]. The presence of this antigen on the surface of neoplastic cells in flow cytometry is one of the most important elements of the diagnostic process of CLL. This protein is observed in 95% of CLL patients. CLL without expression of the CD5 antigen was first discovered in the 1990s [14]. In the light of this, making a differential diagnosis of CD5-negative (CD5–) CLL and other CD5– lymphoproliferative disorders such as splenic marginal zone lymphoma, lymphoplasmatic lymphoma, hairy cell leukemia, or follicular lymphoma, seems to pose a significant challenge [14].

Table I. Score for a typical chronic lymphocytic leukemia (acc. to [11, 12])

Marker	Matutes Score
CD5	+
CD23	+
FMC7	–
CD22	Weak expression/–
SmIg	Weak expression
	Moreau Score
CD5	+
CD23	+
FMC7	–
CD79b	Weak expression/–
SmIg	Weak expression

smIg – surface membrane immunoglobulin

Interestingly, the results of recent studies indicate differences between the clinical course of CD5– CLL and CD5+ CLL. Recently, Cartron et al. presented the results of a clinical analysis of 42 patients with CD5– CLL compared to a group of 79 patients with typical CD5+ CLL. In the CD5– cohort, newly diagnosed patients presented lymphadenopathy less frequently, but splenomegaly more frequently [14]. Efsathiou et al. [15] showed that in patients with CD5-negative CLL, lymphadenopathy, splenomegaly and autoimmune hemolytic anemia were observed less frequently compared to patients with CD5+ CLL. Moreover, the disease was less advanced among patients with newly diagnosed CD5– CLL, and their median survival was significantly longer (97.2 vs. 84 months) [15].

However, a correlation between the occurrence of an atypical immunophenotype and a better prognosis has not been confirmed in other studies. Romano et al. analyzed a cohort of 400 CLL patients including 13 patients with a CD5– phenotype. In this study, no significant differences in clinical course and survival were observed, regardless of the immunophenotype of CLL cells [16]. Furthermore, Kurec et al. [17] showed that newly diagnosed patients with CD5– CLL had a lower level of hemoglobin, a higher disease stage in Rai's classification, and a worse prognosis (five-year survival rate among CD5– and CD5+ patients: 55% and >90%, respectively). Only autoimmune complications were observed less frequently in the CD5– CLL cohort [17]. However, the authors noted that the increased rate of these complications in the group of patients with typical CLL might be associated with a higher percentage of patients with a more advanced stage of the disease in their cohort [17].

A crucial aspect of the diagnostic process in CLL is to set an unambiguous definition, determining the exact percentage of neoplastic CD5+ cells that would allow for a diagnosis of an atypical CLL. So far, in most publications,

authors have assumed that the presence of less than 5% of leukemic cells with CD5 antigen allows for a diagnosis of CD5– CLL [14, 15, 17].

However, Friedman et al. [18] analyzed the correlation between mean fluorescence intensity of the CD5 antigen in a population of leukemic cells and a clinical course of the disease in a group of 423 patients suffering from CLL. It was shown that high MFI rate correlates with longer progression-free survival.

Therefore, it appears worth considering not only the expression of CD5 protein in CLL, but also its intensity.

CD23-negative CLL

The CD23 antigen is a surface glycoprotein and one of the most valuable markers used in the identification of neoplastic CLL cells. It is the low-affinity receptor for IgE, and is found on resting mature B cells as well as some activated ones. CD23 takes part in the process of activation and proliferation of normal B lymphocytes [19]. Moreover, it plays an important role in the pathogenesis of B-CLL. It has been proven that higher expression of its isotypes, CD23a and CD23b, results in having a protective and proliferation stimulating effect on neoplastic B-cells, respectively [20]. Co-expression of CD23, CD5 and CD19 forms a basis of classical CLL diagnosis. The presence of CD5 and CD19, combined with the absence of CD23 however, is characteristic for MCL [21].

Keeping in mind the existence of atypical immunophenotype in both disorders (CD23– in CLL and CD23+ in MCL), Barna et al. [22] undertook to establish a threshold of CD23 expression and MFI in differential diagnosis of the aforementioned diseases. In their study, they observed a correlation between high levels of CD23 expression (>92.5%) and/or high MFI (>44.5) and a diagnosis of CLL. At the same time, a lower expression and lower MFI were found to correlate with a more frequent MCL diagnosis. Expression oscillating between 30% and 92.5% and MFI <44.5 were observed in both CLL and MCL. The authors themselves pointed out that in these cases it is essential to include cytogenetic evaluation so that the final diagnosis can be made [22].

The potential connection between the levels of CD23 expression and the prognosis for, as well as the survival rate of, patients suffering from CLL, is drawing increasing attention. Yet so far, the results are ambiguous [10, 19, 23, 24].

Jurisic et al.'s study [19] can serve as an example. They analyzed a group of 77 patients newly diagnosed with CLL. Their analysis focused on finding a possible correlation between the level of expression of CD23 antigen and the clinical course of the disease. A correlation between a lower level of CD23 expression and the amount of peripheral blood lymphocytes was observed. Nevertheless, this correlation was found only in patients whose lymphocytosis exceeded 100×10^9/l. Furthermore, the group of patients whose CD23 expression was over 40% achieved a longer overall survival compared to those whose CD23 expression was below 40% (92.8 months vs. 35.3 months). Moreover, patients suffering from CLL with a higher CD23 expression also achieved longer progression-free survival, which is an important prognostic factor [19].

Furthermore, Kriston et al. [10] found that coexistence of low CD23 expression and high CD20 and CD38 expression correlated significantly with the presence of trisomy 12, which is a crucial poor prognosis factor. As the authors suggest, the reduction of CD23 isotypes expression, along with trisomy 12, can even be regarded as a first step to Richter's transformation [10].

Despite the growing interest in this subject, there remains insufficient data to enable a detailed description of the correlation between CD23 expression and CLL clinical course. The abovementioned articles unequivocally depict the correlation's presence, yet it is vital to continue research into the effects of diverse CD23 expression, as well as other typical CLL antigens.

Differentiating atypical CLL forms from other B-cell neoplasms on basis of immunophenotype

Due to diagnostic problems in the process of differentiating atypical CLL forms from other lymphoproliferative disorders such as MCL or HCL, the necessity to include new antigens in immunophenotyping has been strongly emphasized in recent years.

The surface glycoprotein CD200 is a critically important antigen in differentiating atypical forms of CLL (especially CD23– CLL) and MCL. In El Din Fouad et al.'s study [26], all patients suffering from CLL with an atypical immunophenotype tested positive for CD200 expression. Moreover, some authors consider the lack of CD200 expression in mature B-cell neoplasms, together with a CD5 presence, as being sufficient to exclude CLL from differential diagnosis [25]. The confirmation of CD200 antigen's significance in differential diagnosis, including atypical CLL, can be seen in the results of studies in which 840 patients in total were observed. In all CLL cases, CD200 expression was confirmed. Conversely, only 10% of patients diagnosed with mantle cell lymphoma (MCL) presented CD200 expression [26]. Likewise, in Lesesve et al.'s study [26], among 69 patients diagnosed with CLL, 83% had CD200+ expression. Moreover, the authors also analyzed CD160 antigen expression as a helpful tool in differential diagnosis of neoplasms deriving from B-cell lymphocytes.

Li et al. in turn described the role of CD43 and CD180 antigens in the process of differentiating atypical CLL from other lymphoproliferative disorders deriving from mature B-cells. CD5– and CD23– CLL forms were given

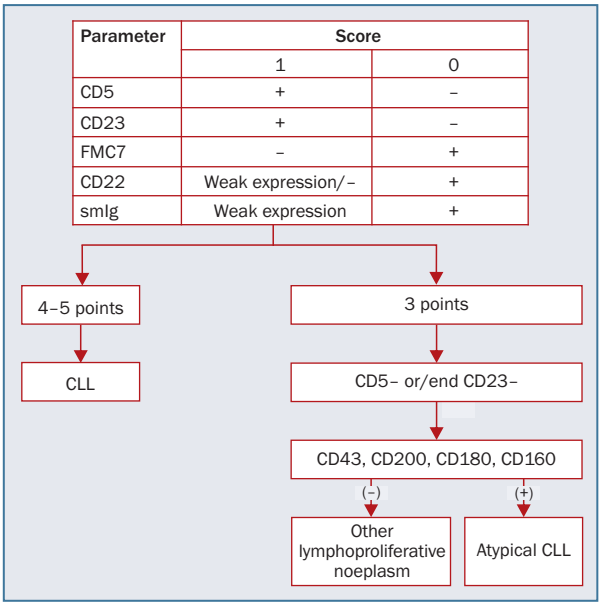

Figure 1. Possible diagnostic pattern in patients with atypical chronic lymphocytic leukemia (CLL) suspicion (based on Matutes Score [11], Table I); smIg — surface membrane immunoglobulin

Table II. Differential diagnosis of atypical CD5– and CD23– chronic lymphocytic leukemia (CLL) from other B-cell lymphatic system neoplasms

Lymphatic B-cell neoplasm	Antigens					
	CD19 CD20 CD22	CD23	CD5	CD10	CD11c	CD43
CLL	+	+	+	–	–/+	+
CD5– CLL	+	+	–	–	–/+	+
CD23– CLL	+	–	+	–	–/+	+
LPL	+	–	–	–	–/+	+/–
MCL	+	–	+	–/+	–	+
FL	+	–	–	+/–	–	–
SMZL	+	–	–	–	+/–	+/–
HCL	+	–	–	–	+	–
DLBCL	+	–	–/+	–/+	–	–

LPL — lymphoplasmacytic lymphoma; MCL — mantle cell lymphoma; FL — follicular lymphoma; SMZL — splenic B-cell marginal zone lymphoma; HCL — hairy cell leukemia; DLBCL — diffuse large B-cell lymphoma

particular attention. Through modification of the Moreau Score (excluding CD5 and CD23 and including CD43 and CCD180 at the same time), the researchers managed to achieve greater sensitivity in detecting CD5–/CD23– CLL of up to 79.4%, compared to 41.2% of cases detected using the classical Moreau Score [12].

Romano et al. [16] analyzed the immunophenotype of patients suffering from CD5– CLL. The results showed expression of surface glycoproteins, such as CD95, CD69, CD23, CD25, CD80 and CD20, distinct from a typical one, which might be applicable, while differentiating between CD5– CLL and other lymphoproliferative disorders devoid of the CD5 antigen [16]. Figure 1 and Table II illustrate an example of the diagnostic pattern in the process of differential diagnosis of CD5– and CD23– CLL from other lymphoproliferative B-cell neoplasms.

Summary

Immunophenotyping is an indispensable element of the CLL diagnostic process. So far, no pathognomonic factor enabling us to determine a diagnosis has been found. In addition, attempts are still being made to establish an international agreement as to the markers essential to determine a diagnosis of CLL.

In the light of the studies pertaining to diagnostic difficulties in lymphoproliferative disorders, including CLL, it seems crucial to verify the existing scales and definitions in regards to the immunophenotype. Finding a correlation between these aspects and the clinical course of the neoplasm is also of paramount importance.

Authors' contributions

All authors participated equally in creating, editing and accepting this article.

Conflict of interest

None.

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Ethics

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

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VIA MEDICA

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Mean platelet volume and other hematological parameters in pediatric migraine

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Abstract

Introduction: According to our hypothesis, the mean platelet volume (MPV) value in migraine patients should be lower than in healthy individuals due to recurrent neuroinflammation during headache attacks. Therefore, this study aimed to identify differences between MPV and other hemogram parameters between pediatric patients with migraine and a healthy control group.

Material and methods: This study included a total of 80 people, 40 patients and 40 control subjects. Those with hematological diseases, using alcohol or smoking, with malignancy, pregnancy, or with any chronic disease were excluded from the study.

Results: Individuals in the control group were chosen to have the same age and same sex as the patient group ($p = 0.98$). The patient group had statistically significantly higher platelet counts than the control group ($p = 0.043$). MPV was lower in the patient group compared to the control group ($p = 0.001$), but platelet distribution width was higher in the patient group compared to the control group ($p = 0.034$)

Conclusions: Migraine patients in the pediatric period were shown in our study to have low MPV values and elevated platelet counts due to recurrent neuroinflammation. This result shows that inflammation especially plays a vital role in migraine pathophysiology.

Key words: pediatric migraine, platelets, hematological parameters, mean platelet volume

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Introduction

Headache is a health problem known from ancient times, and nearly everyone experiences it at least once in their lifetime. It is the commonest cause of presenting to emergency services. The International Headache Society (IHS) has divided headaches into three groups: primary, secondary, and other headaches [1]. Migraine, recurrent headache attacks with stress-type pain, comprises most primary headache classes [2]. The most commonly suggested hypothesis for migraine pathophysiology is the trigeminovascular pathway hypothesis [3]. According to this hypothesis, vasodilation in the meningeal arteries causes the activation of inflammatory neuropeptides and inflammation in the brain tissue, activating the trigeminal system and initiating a vicious cycle. As a result, there is ongoing inflammation in the brain tissue. Neurogenic inflammation causes the release of vasoactive peptides such as neurokinin A, substance P from trigeminal and parasympathetic fibers. These vasoactive peptides release pro-inflammatory mediators such as prostanoids and bradykinin, causing vasodilation and extravasation. Thus, the event cycle of migraine occurs.

It is well known that lymphocytes and other leukocytic serial cells play roles in inflammation. Studies have been

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performed recently showing variations related to inflammation of another hematological parameter, mean platelet volume (MPV). MPV is a standard hematological parameter demonstrating platelet functions. It can be easily measured with hemogram tests. Platelets that are more active in metabolic and enzymatic terms and with thrombogenic potential have a larger volume, while they have smaller volumes in situations related to chronic inflammation [4]. Due to the association with neurogenic inflammation, studies have been performed investigating whether there is a correlation between migraine and hemogram parameters. Some studies have identified significant correlations between migraine and MPV, while others have not [5, 6]. The common denominator of these studies is that they have generally been performed in adult patients. There have been few studies with pediatric patients [7].

According to our hypothesis, the MPV value should be lower in migraine patients than in healthy individuals due to recurrent neuroinflammation during headache attacks. Therefore, this study aimed to identify differences between MPV and other hemogram parameters between pediatric patients with migraine and a healthy control group.

Material and methods

This study included a total of 80 children, with 40 patients and 40 control subjects. The patient group was chosen from patients (6–18 years) who attended the Pediatric Neurology Clinic at Mustafa Kemal University School of Medicine, Hatay, Turkey with headache and who received a migraine diagnosis according to the IHS diagnostic criteria [1] (Table I). The control group was chosen from healthy children attending the hospital in the same distributions of gender and age. Those with iron, vitamin B_{12} or folic acid deficiency, with anemia or other hematological diseases, who were using alcohol or smoking, with any malignancy, pregnancy, with any chronic disease (e.g. hypertension, diabetes, pulmonary, renal, liver diseases), with acute or chronic inflammatory disease, or using antibiotics were excluded from the study.

In our study, children's age, gender, ferritin, serum iron levels, iron-binding capacity, vitamin B_{12}, folic acid and hemogram data were obtained from hospital files. In addition, the two groups were compared in terms of hemoglobin (Hb), white blood cell (WBC) count, platelets (PLT), neutrophil (Neu), lymphocyte (Lym), monocyte (Mon), MPV, platelet haematocrit (PCT), platelet distribution width (PDW) values, and neutrophil/lymphocyte (N/L), neutrophil/monocyte (N/M) and lymphocyte/monocyte (L/M) ratios.

For the study, blood samples were taken from arm veins in tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at room temperature. Samples were studied within two hours. Complete blood count, thyroid hormones, routine blood biochemistry, and C-reactive protein results were recorded.

Table I. Migraine diagnostic criteria

Migraine without aura	Migraine with aura
A. At least five attacks [1] fulfilling criteria B–D	A. At least two attacks fulfilling criteria B and C
B. Headache attacks lasting 4–72 h (untreated or unsuccessfully treated) [2, 3]	B. One or more of following fully reversible aura symptoms: 1. visual 2. sensory 3. speech and/or language 4. motor 5. brainstem 6. retinal
C. Headache has at least two of following four characteristics: a) unilateral location b) pulsating quality c) moderate or severe pain intensity d) aggravation by, or causing avoidance of, routine physical activity (e.g. walking or climbing stairs)	C. At least three of following six characteristics: 1. at least one aura symptom spreading gradually over ≥5 minutes 2. two or more aura symptoms occurring in succession 3. each individual aura symptom lasting 5–60 minutes [1] 4. at least one aura symptom being unilateral [2] 5. at least one aura symptom positive [3] 6. aura accompanied by, or followed within 60 minutes by, a headache
D. During headache, at least one of following: a) nausea and/or vomiting b) photophobia and phonophobia	D. Not better accounted for by another ICHD-3 diagnosis
E. Not better accounted for by another ICHD-3 diagnosis	

ICHD-3 – 3rd edition of the International Classification of Headache Disorders

Analysis of data used the IBM SPSS Statistics Version 21 program. Categoric measures were given as number and percentages, while fixed measures used mean ± standard deviation (if necessary, median, min-max). The Kolmogorov-Smirnov test was used to determine whether data had parametric distribution or not. Investigating data with parametric distribution used the *t*-test, while non-parametric data was compared with the Mann-Whitney U test. Statistically, values of $p = 0.005$ and lower were accepted as significant.

Results

Eighty children were included in the study. Both the patient and the control group included 40 people, with 14 boys and 26 girls in each. The age range in the patient group was 6–18 years, while the age range in the control group was

Table II. Ages and genders of patient and control groups

Parameter	Patients	Controls	*p*
Age	12.92 ± 3.04	12.8 ± 3.07	0.98
Gender	N (%)	N (%)	
Male	14 (35)	14 (35)	
Female	26 (65)	26 (65)	

6–17 years. There was no significant difference between the patient and control groups regarding age (p = 0.98). The age intervals and gender distributions of both groups are set out in Table II.

The hemogram parameters and N/L, N/M and L/M ratios were compared in the patient and control groups (Table III). While the white blood cell value was slightly higher in the patient group, the hemoglobin value was higher in the control group. Still, there was no significant difference between the patient and control groups regarding hemoglobin and white blood cells (p = 0.181, p = 0.761). The patient group had statistically significantly higher platelet counts than the control group. While the mean platelet count in the patient group was 311.75 ± 82.79 [mean ± standard deviation (SD)], it was 275.52 ± 74.21 (mean ± SD) in the control group (p = 0.043). When the specific parameters of platelets i.e. MPV, PDW and PCT, were compared, MPV was lower in the patient group than the control group (p = = 0.001), PDW was higher in the patient group than the control group (p = 0.034), and there was no difference between the two groups in terms of PCT value (p = 0.525). We did not identify any significant difference between the other hemogram parameters. Similarly, the N/L, N/M, L/M and (P/L) ratios were compared between the two groups. The most remarkable difference for these ratio comparisons was for the P/L ratio, but no comparison provided statistically significant results (Table III).

Discussion

Platelets are known to play roles in thrombosis, angiogenesis and inflammation. MPV is a good marker of platelet activation. There is an increase in MPV values in thrombotic and cardiovascular diseases, while a fall occurs in inflammatory situations [4]. Many studies performed in recent years have shown a correlation between platelets and inflammation [8–14]. A common feature of these studies is that they were performed for chronic inflammatory diseases emerging outside the central nervous system (CNS). Among CNS diseases, the correlation between migraine and MPV has mainly been investigated [6, 7, 15]. In these studies, MPV values in migraine cases were higher [15], or not different from [6] those in the control group. Our study identified a statistically significant difference between the MPV values in the patient and control groups (p = 0.001).

Table III. Comparison of hemogram parameters

Parameter	WBC	Hb	PLT	Neu	Lym	Mon	N/L	N/M	P/L	L/M	MPV	PCT	PDW
Patients	7.9 ± 2.3	13.1 ± 1.2	311.7 ± ± 82.7	4.3 ± 1.6	2.8 ± 1.2	0.5 ± 0.17	1.75 ± ± 0.89	9.31 ± ± 4.49	123.5 ± ± 49.1	5.95 ± 2.4	9.5 ± 0.88	0.291 ± ± 0.07	16.12 ± ± 1.87
Controls	7.3 ± 16	13.2 ± 0,8	275.5 ± ± 74.2	4.1 ± 1.6	2.5 ± 0.4	0.48 ± ± 0.15	1.75 ± 0.9	8.97 ± ± 3.35	111.9 ± ± 33.7	5.62 ± ± 1.79	10.2 ± 0.9	0.282 ± ± 0.054	15.99 ± ± 0.31
p	0.181	0.761	**0.043**	0.698	0.132	0.545	0.977	0.706	0.224	0.499	**0.001**	0.525	**0.034**

WBC – white blood cell; Hb – hemoglobin; PLT – platelets; Neu – neutrophil; Lym – lymphocyte; Mon – monocyte; N/L – neutrophil/lymphocyte; N/M – neutrophil/monocyte; P/L – platelets/lymphocyte; L/M – lymphocyte/monocyte; MPV – mean platelet volume; PCT – platelet haematocrit; PDW – platelet distribution width

This difference was due to the patient group's MPV value being lower than the control group. The previous studies were performed with adult migraine patients. A study of pediatric migraine patients by Poyrazoğlu et al. in 2020 [7] compared a 56-person patient group to a 45-person control group, but found no significant difference between the groups.

In our study, unlike previous studies, the low MPV values in migraine patients were linked to recurrent neuroinflammation during migraine attacks in the brain. Studies investigating MPV association with inflammation have identified that the MPV values were lower than control groups. Two different studies of patients with inflammatory bowel disease showed low MPV and elevated platelet counts [8, 9].

Similarly, our study identified the platelet count as significantly higher compared to the control group. This suggests that the results obtained in our study could be related to continuing inflammation in the brain. Studies obtaining different results about migraine and MPV may be explained by performing studies in adult groups and by results being affected by age and gender differences between the patient and control groups. In addition to many hormonal and immunological agents playing a role in the maturation of thrombopoietic cells [16], thrombopoietin release levels differ between the genders. There are higher levels of thrombopoietin secretion in males, especially [17]. Our study set up our patient and control groups to have exactly the same numbers of male and female individuals in order to prevent MPV ratios from being affected by external factors. Additionally, we were very careful to ensure that the individuals in the control group were the same age as those in the patient group ($p = 0.98$). In both groups, individuals were chosen from among children without anemia and with serum iron, ferritin, folate, vitamin B_{12} and TSH levels within normal limits.

It is known that leukocyte series cells in blood play a leading role in pro-inflammatory and anti-inflammatory mechanisms [18]. Many studies have shown the correlation between L/M, N/M, N/L and P/L ratios and systemic inflammation [19–22]. These studies showed that different ratios were more valuable, but a ratio with a typical value could not be shown. In our study, significant differences were not identified for any of these ratios in the patient and control groups. This result shows that these ratios should be investigated on a disease basis, and that different diseases may provide different results.

Conclusions

Migraine patients in the pediatric period in our study revealed low MPV values and elevated platelet counts due to recurrent neuroinflammation. This shows that inflammation especially plays a vital role in migraine pathophysiology.

Authors' contributions

YA — sole author.

Conflict of interest

The author declares no conflict of interest.

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Ethics

All the procedures performed in studies involving human participants followed institutional and/or national research committees' ethical standards and the 1964 Helsinki Declaration. The study protocol was approved by the Mustafa Kemal University School of Medicine Ethics Committee (2021/23).

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Role of tumor necrosis factor-alpha–308 G/A and interleukin-10 promotor–592 C/A polymorphisms in adult immune thrombocytopenic purpura

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Abstract

Introduction: Immune thrombocytopenia (ITP) pathogenesis has been related to cytokine imbalance, which is controlled genetically with gene polymorphisms. The correlation of the interleukin (IL)-10 gene and tumor necrosis factor alpha (TNF-α) polymorphisms with ITP susceptibility has been previously investigated, but the association with clinical and prognostic parameters remains unclear.

Material and methods: To investigate the relation between IL-10-592 C/A and TNF-α-308 G/A gene polymorphisms and their clinical significance in adult patients with ITP.

This study was conducted on 40 ITP patients and 40 control individuals. The IL-10-592 C/A polymorphism was genotyped by the polymerase chain reaction-restriction fragment length polymorphism method and the TNF- α-308 G/A polymorphism by amplification refractory mutation system analysis.

Results: The TNF-α-308 G/A polymorphism was significantly associated with low platelet count, wet purpura, higher bleeding score, higher incidence of complications, and lack of response to steroid therapy. The IL-10-592 C/A polymorphism was not significantly associated with any of these parameters.

Conclusion: We found a significant association between the TNF-α-308 G/A polymorphism and several clinical parameters, which suggests a probable role in the prognosis among adult ITP patients.

Key words: immune thrombocytopenic purpura, polymorphism, interleukin 10 promotor gene, tumor necrosis factor-alpha gene, prognosis

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Introduction

Immune thrombocytopenia (ITP) is an acquired thrombocytopenia characterized by a platelet count of fewer than 1003 × 10^9/L, and it is caused by elevated destruction of antibody-sensitized platelets [1, 2]. This disorder occurs in both adults and children, showing a bimodal incidence with a peak in childhood and another peak in young adults. The pathology in childhood and adult ITP may be basically different, as evidenced by the incidence of chronic ITP in adults [3]. Most children have a self-limited disease, whereas ITP is mostly a chronic disorder in adults.

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Recently, the pathophysiology of this disease has become better understood. The human immune system is known to play an important role, including antibodies, cytokines, antigen presenting cells, as well as T and B lymphocytes [4]. Understanding the important role of genetic factors in autoimmune diseases has led to the conduction of many studies. Consequently, many single-nucleotide polymorphisms (SNPs) with potential clinical significance have been identified. Some immune-related genes have been studied as candidates for the genes susceptible to ITP, including the polymorphisms of inflammatory cytokine genes which have been correlated with ITP [5]. These cytokines include tumor necrosis factor alpha (TNF-α), beta (TNF-β), interferon (IFN)-alpha (IFN-α), beta (IFN-β), gamma (IFN-γ), interleukin (IL)-1α, β, and IL-10 based on the presence of well-defined SNPs associated with autoimmune diseases.

It should be noted that risk susceptibility studies cannot be anticipated from one ethnic group to another due to the popular variability of the relative frequency of different alleles among different populations [6]. Hence, to evaluate the possible role of cytokine genes in addition to their polymorphisms in adult ITP, we investigated the allelic and genotypic frequencies of cytokine gene polymorphisms known to be correlated to autoimmunity and inflammation (IL-10-592 and TNF-α-308) in Egyptian patients with ITP and healthy controls. Additionally, we evaluated the possible role of these polymorphisms in relation to the risk of ITP development, disease prognosis, and response to steroid therapy.

TNF gene locus is located within the class III region of the human major histocompatibility complex (MHC) on chromosome 6 (6p21.31) [7]. Several SNPs have been identified in the human *TNF-α* gene promoter region, such as TNF-α 308 (G/A) polymorphism. TNF-α is a pleiotropic cytokine produced mainly by macrophages and T-cells which has a range of inflammatory and immunomodulatory activities [8]. The polymorphisms of TNF-α promoter are correlated with high levels of TNF-α and have been evaluated as a risk factor for the development of numerous diseases [8–10].

To the best of our knowledge, the role of the TNF-α-308 G/A polymorphism in the development and progression of ITP has not been previously studied among adult populations. Many polymorphism studies have demonstrated a relation between IL-10 and various immunological disorders [11, 12]. According to previous studies, IL-10 SNPs may have an important effect on the immune function through regulating the activities of natural killer (NK) T-cells, and macrophages, thus affecting disease progression [13]. Several SNPs of IL-10, including −1081, −819, and −592, may be linked to ITP [14].

The aim of our study was to investigate the role of the SNPs of IL-10-592 and TNF-α-308 genes on the susceptibility, severity, and outcomes of ITP. In addition, we compared our data to other published data.

Materials and methods

Our study comprised 40 adult ITP patients and 40 age- and sex-matched healthy Egyptian subjects (control group). This study was jointly conducted by the Hematology Department at the Medical Research Institute, and the Clinical and Chemical Pathology Department at the Faculty of Medicine, Alexandria University, Alexandria, Egypt. Patients with primary ITP and aged 18 and older were included in the study. Patients under 18 and those with proven secondary ITP [as cases initiated by or associated with infections due to human immunodeficiency virus (HIV-associated) or hepatitis C virus associated secondary ITP] were excluded. Moreover, patients with accompanying autoimmune disorders such as systemic lupus erythematosus (SLE) were excluded. Relatives or members from consanguineous families were excluded as patients or controls to ensure more accurate allele segregation frequency. The study protocol was approved by the Ethical Committee of Alexandria University. All patients enrolled in the study signed informed written consent after an explanation of the nature, steps and aim of the study before enrolment.

ITP diagnosis was made on the basis of history, detailed physical examination, complete blood count to determine the presence of thrombocytopenia ($<100 \times 10^9$/L) with otherwise normal red cells and leukocytes, and examination of the peripheral smear which should exclude other causes of thrombocytopenia [15]. Other tests were done if there was a suspicion of a disease other than ITP. Serological tests for infectious causes and autoimmune diseases were performed for antinuclear antibodies, HIV, hepatitis C virus (HCV), and *Helicobacter pylori* (*H. pylori*) infection. A detailed questionnaire was carried out including demographic characteristics, duration of disease, bleeding symptoms, life-threatening bleeding if patients had clinically significant symptoms of severe bleeding such as intracranial bleeding, internal hemorrhage, and gastrointestinal or genitourinary bleeding, history of preceding viral infection, and recent medication use. This questionnaire was distributed among patients and answered during history taking. Physical examination included an examination for the presence of lymph nodes as well as the examination of abdominal organs. Severity of bleeding was also assessed using a specific bleeding score from grade 1 to grade 4 [16]. Newly diagnosed patients received either prednisone at 1 mg/kg bodyweight daily for 4 weeks to the maximum and then tapered [17], or a high-dose of dexamethasone (40 mg orally per day for 4 days) with no taper [18]. For the majority of included patients, we recommended a high dose of dexamethasone to avoid prolonged exposures to oral prednisone (which can be associated with significant toxicities). The response to immune thrombocytopenia treatment was evaluated [19] as follows: Complete response (CR): any platelet count of

at least 100 × 10^9/L and absence of bleeding; response (R): any platelet count of at least 30 × 10^9/L and at least a two-fold increase in the baseline count and absence of bleeding. No response: any platelet count less than 30 × 10^9/L or less than a two-fold increase in the baseline platelet count or bleeding; Loss of CR: platelet count below 100 × 10^9/L or bleeding; Loss of R: platelet count below 30 × 10^9/L or less than a two-fold increase in the baseline platelet count or bleeding. Time to response: the duration between starting the treatment and the achievement of CR or R.

Investigations

Full blood count was done using an ADVIA 2120 automated cell counter (Siemens Healthcare Diagnostics, USA).

Genomic DNA was extracted from all K2EDTA peripheral blood samples by the column method using a DNA extraction kit (Pure Link Genomic DNA Mini Kit, Cat no K182001, Invitrogen™ by Thermo Fisher Scientific).

Genotyping was performed following the genomic DNA amplification by amplification refractory mutation system — polymerase chain reaction (ARMS-PCR) technique for the promoter site TNF-α-308 (G/A) SNP using the following primers:

- Forward primer F: 5'CTGCATCCCCGTCTTTCTCC-3'and;
- Reverse primer 1 (wild allele): 5'-ATAGGTTTTGAGGGGCATCG-3';
- Reverse primer 2 (mutant allele): 5'-ATAGGTTTTGAGGGGCATCA-3';

Genotyping using PCR-restriction fragment length polymorphism (PCR-RFLP) was performed for the IL-10--592 (C/A) SNP using restriction enzyme RsaI. The following primers were used:

- Forward primer: 5' GGT GAG CAC TAC CTG ACT AGC 3';
- Reverse primer: 5' CCT AGG TCA CAG TGA CGT GG 3';
- Amplification was performed using an HVD S24 thermal cycler (Quanta Biotech, UK).

PCRs were performed in a final volume of 50 µL using approximately 200 ng of template genomic DNA which was done using 10 pmol of each of the primers, 1.5 mmol/L $MgCl_2$, 400 mmol/L of each dNTP, 1 U Taq polymerase, and 1 × PCR buffer. The PCR amplification consisted of an initial denaturation step at 96°C for 3 min, followed by 30 cycles of 94°C for 45 s, 55°C for 2 min (for TNF-α-308 G/A), 62°C for 45 s for IL-10-592-C/A, 72°C for 2 min for TNF-α-308 G/A, and 55 s for IL-10-592-C/A, as well as a final step at 72°C for 3 min. The amplified products were applied to gel electrophoresis in a 2% agarose gel, visualized upon staining with ethidium bromide in reference to a molecular weight marker, and detected using ultraviolet (UV) light (UVP dual intensity trans--illuminator).

RFLP analysis was done using a restriction enzyme RsaI for IL-10-592 C/A genotyping by mixing 10 µL of PCR product with 1 µL of RsaI enzyme, 2 µL green buffer, and 17 µL nuclease free water. This mixture was incubated at 37°C for one day then heated at 65°C for 20 minutes. Then, DNA fragments were detected by 2% agarose gel electrophoresis.

Genotypes were determined as follows: IL-10-592 gene CC [412 base pair (bp)], AC (412, 236, 176 bp), and AA (236, 176 bp) (Figure 1), and TNF-α-308 gene 863bp (Figure 2). Ten percent of the samples were amplified twice for verification of genotyping results.

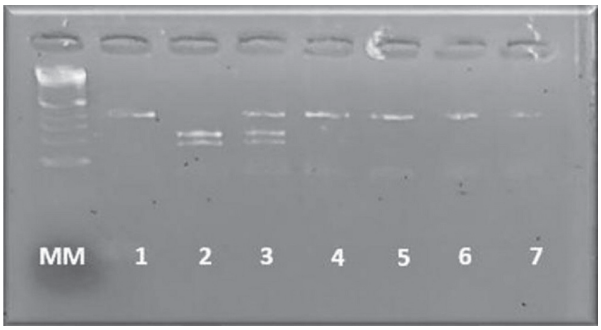

Figure 1. Agarose gel electrophoresis of genomic polymerase chain reaction (PCR) for interleukin (IL)-10-592 (C/A) polymorphism after restriction by RsaI enzyme
MM: molecular weight marker
Lanes 1, 4, 5, 6, 7: showing one band 413 base pair (bp), representing homozygous C/C genotype
Lane 2: shows two bands, 236 bp and137 bp, representing homozygous A/A genotype
Lane 3: showing three bands; 413 bp, 236 bp, and 137 bp, representing heterozygous C/A genotype

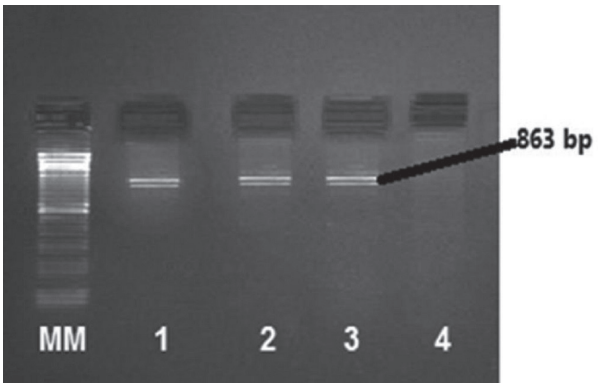

Figure 2. Detection of amplified fragments of tumor necrosis factor-alpha (TNF-α)-308 (G/A) by ARMS-PCR on 2% agarose gel electrophoresis
Lane MM: 100 base pair (bp) molecular weight marker
Lanes 1, 2: heterozygous (G/A) genotype as both G and A alleles--specific bands are present
Lanes 3, 4: homozygote (G/G) genotype as G allele-specific band is only present

Table I. Characteristics of studied subjects

Parameter	Cases (n = 40)	Control (n = 40)	*p*
Sex			
Male	12 (30%)	10 (25%)	0.617
Female	28 (70%)	30 (75%)	
Age (years)			
Median (min–max)	33.5 (18–72)	34 (18–70)	0.326
Mean ± SD	34.3 ± 12.6	36.7 ± 13	
Hb [mg/dL]			
Median (min–max)	11.7 (5.1–15.8)	12.6 (11.5–15)	0.002*
Mean ± SD	11.8 ± 1.7	12.8 ± 1.1	
WBCs [× 10^3/mm^3]			
Median (min–max)	7.4 (4.3–12.9)	7.1 (4.2–10.4)	0.533
Mean ± SD	7.8 ± 2.2	7.5 ± 1.7	
PLT [× 10^3/mm^3]			
Median (min–max)	19 (3–90)	268 (160–400)	<0.001*
Mean ± SD	23.5 ± 19.8	270.5 ± 72.5	
Clinical presentation			
Mucocutaneous bleeding	28 (70%)	–	–
Vaginal	7 (17.5%)	–	
Accidental discovery	5 (12.5%)	–	

*Statistically significant at $p \leq 0.05$; *p* – *p* value for comparing between studied groups; SD – standard deviation; Hb – hemoglobin; WBCs – white blood cells; PLT – platelets

Table II. Comparison between studied groups according to tumor necrosis-alpha-(TNF-α)-308 and interleukin (IL)-10-592 polymorphisms

Polymorphism	Cases (n = 40)	Controls[R] (n = 40)	OR (95%CI)	*p*
TNF-α-308				
GG	37 (92.5%)	40 (100%)	0.17 (0.008–3.34)	0.077
GA	3 (7.5%)	0 (0%)	5.9 (0.3–119)	0.077
AA	0 (0%)	0 (0%)	–	–
Allele				
G	77 (96.3%)	80 (100%)	–	0.080
A	3 (3.8%)	0 (0%)	–	
IL-10-592				
CC	31 (77.5%)	29 (72.5%)	1.307 (0.473–3.609)	0.606
CA	8 (20%)	10 (25%)	0.750 (0.261–2.153)	0.592
AA	1 (2.5%)	1 (2.5%)	1.000 (0.060–16.56)	1.000
Allele				
C	70 (87.5%)	68 (85%)	1.235 (0.501–3.048)	0.646
A	10 (12.5%)	12 (15%)	0.810 (0.328–1.997)	

[R]reference group; *p* – *p* value for comparing between studied groups; OR – odds ratio; CI – confidence interval

All statistical calculations were done using SPSS statistical programs (version 24.0 SPSS, Inc., Chicago, IL, USA). Data was statistically described in terms of mean ± standard deviation (± SD) and range or frequencies (number of cases) and percentages when appropriate. Descriptive statistics were done for quantitative data as minimum and maximum of the range as well as mean ± SD for quantitative parametric data, while it was done for qualitative data as number and percentage. Inferential analyses were done for quantitative variables using the unpaired *t*-test in cases of two dependent groups with parametric data for comparing categorical data, the chi-square test (χ^2) was performed. The Mann-Whitney test was used to compare two unrelated samples. Association was expressed as odds ratios (OR) with 95% confidence intervals (CIs). P values of less than 0.05 were considered statistically significant.

Results

The current study included 40 adults with ITP: 12 males (30%) and 28 females (70%) with a female-to-male ratio of 2.8:1 and ages ranging from 18 to 72 years with a mean of 34.3 ± 12.6. Also, the study included 40 age- and sex-matched healthy controls and adolescents with a mean age of 36.7 ± 12.9 years. Among the 40 ITP cases, it was found that ITP incidence was higher in females than males, especially during the childbearing period. The characteristics of the studied subjects are set out in Table I.

Distribution of cytokine genotype frequencies in studied population

A total of 40 adult patients with ITP and 40 hematologically normal volunteers were analyzed for the presence of TNF-α-308 G/A and IL-10-592 (C/A) polymorphisms. The

Table III. Association between tumor necrosis-alpha-(TNF-α)-308 and interleukin (IL)-10-592 genotypes with different parameters in case group (n = 40)

	TNF-α-308		IL-10-592		
	GG (n = 37)	GA (n = 3)	CC (n = 31)	CA (n = 8)	AA (n = 1)
Sex					
Male	10 (27%)	2 (66.7%)	8 (25.8%)	3 (37.5%)	1 (100%)
Female	27 (73%)	1 (33.3%)	23 (74.2%)	5 (62.5%)	0 (0%)
χ^2 (p)	χ^2 = 2.076 (0.209)		χ^2 = 2.696 (^{MC}p = 0.259)		
Age (years)					
Median (min–max)	33 (18–72)	34 (26–39)	33 (18–72)	38 (19–70)	26
Mean ± SD	34.4 ± 13)	33 ± 6.6	33.7 ± 11.7	37.5 ± 16.5	
Test of sig. (p)	U = 53.50 (0.923)		H = 0.550 (0.760)		
Platelets					
Median (min–max)	20 (3–90)	6 (5–8)	20 (3–90)	19 (7–85)	8
Mean ± SD	24.9 ± 19.9	6.3 ± 1.5	22.8 ± 18.5	28.3 ± 25.3	
Test of sig. (p)	U = 15.0* (0.035*)		H = 1.160 (0.560)		
Wet purpura					
No	33 (89.2%)	0 (0%)	25 (80.6%)	8 (100%)	0 (0%)
Yes	4 (10.8%)	3 (100%)	6 (19.4%)	0 (0%)	1 (100%)
χ^2 (p)	χ^2 = 15.290* (0.004*)		χ^2 = 4.834 (^{MC}p = 0.073)		
Bleeding score					
Median (min–max)	3 (0–8)	8 (8–12)	3 (0–12)	3.5 (0–6)	5
Mean ± SD	3.6 ± 2.1	9.3 ± 2.3	4.3 ± 2.6	2.8 ± 2.4	
Test of sig. (p)	U = 2.0* (0.001*)		H = 1.706 (0.426)		
Fate of ITP patients					
No complications	37 (100%)	0 (0%)	28 (90.3%)	8 (100%)	1 (100%)
Intracranial hemorrhage and death	0 (0%)	2 (66.7%)	2 (6.5%)	0 (0%)	0 (0%)
Septic arthritis and death	0 (0%)	1 (33.3%)	1 (3.2%)	0 (0%)	0 (0%)
χ^2 (p)	χ^2 = 19.442* (<0.001*)		χ^2 = 4.100 (^{MC}p = 1.000)		
Steroid treatment response					
Complete response	6 (16.2%)	0 (0%)	5 (16.1%)	1 (12.5%)	0 (0%)
Response	1 (2.7%)	0 (0%)	1 (3.2%)	0 (0%)	0 (0%)
No response	1 (2.7%)	0 (0%)	3 (9.7%)	0 (0%)	0 (0%)
No treatment	2 (5.4%)	0 (0%)	1 (3.2%)	1 (12.5%)	0 (0%)
Loss of complete response	16 (43.2%)	0 (0%)	13 (41.9%)	2 (25.0%)	1 (100%)
Loss of response	11 (29.7%)	1 (33.3%)	8 (25.8%)	4 (50.0%)	0 (0%)
χ^2 (p)	χ^2 = 10.229* (0.034*)		χ^2 = 10.099 (0.725)		

*Statistically significant at p ≤ 0.05; *p* – *p* value for association between different categories; $\chi 2$ – chi square test; MC – Monte Carlo; F – ANOVA test; U – Mann-Whitney test; H – Kruskal-Wallis test; SD – standard deviation; ITP – immune thrombocytopenia

distribution of the TNF-α-308 G/A genotype frequency among the studied population is set out in Table II. Among the included ITP cases, 92.5% showed G/G genotype and 7.5% showed (G/A) genotype. Among controls, 100% showed G/G genotype and none showed (G/A) genotype. Patients with ITP showed an increased frequency of the G/A genotype compared to controls, but this relation was not statistically significant (p = 0.077). Allele A exhibited an increased frequency among cases but was not statistically significant (p = 0.08). Moreover, it was found that none of the patients or the controls had AA genotype of TNF-α-308 (G/A).

The IL-10-592 (C/A) genotype frequency among the studied population is set out in Table II. Among ITP patients, 77.5% showed C/C genotype, 20% showed C/A genotype, and 2.5% showed A/A genotype. Among controls, 72.5% showed C/C genotype, 25% showed C/A genotype, and 2.5% showed A/A genotype. This relation was not statistically significant (p = 0.893).

Relationship between genotypes, clinical parameters, and response to treatment

There were no significant differences between different TNF-α-308 (G/A) and IL-10-592 (C/A) genotypes in various clinical features including gender and age (p >0.5). Regarding the relation between different genotypes and the hematological profile, no statistically significant difference was detected between the TNF-α-308 (G/A) gene polymorphism and hemoglobin concentration or white blood cell count. However, a statistically significant difference was detected between this polymorphism and platelet count (U = 15, p = 0.035). In addition, there was no statistically significant association between different IL-10-592 (C/A) genotypes and the hematological profile (Table III).

The three detected ITP cases with TNF-α (G/A) genotype developed wet purpura (100%), while from the seven cases with (G/G) genotype, only six (12.8%) developed wet purpura (p = 0.004). Hence, there was a statistically significant association between TNF-α-308 polymorphism and wet purpura. Bleeding score was higher among cases with (G/A) genotype (p = 0.0001), showing a statistically significant association between different genotypes of ITP cases regarding the bleeding score. However, no statistical significance was found between IL-10-592 (C/A) polymorphism and wet purpura or the bleeding score (p >0.5) (Table III).

Follow-up on ITP patients revealed that the three cases with TNF-α (G/A) genotype died; two of them (66.7%) developed intracranial hemorrhage up to death, and one (33.3%) developed septic arthritis on top of steroid treatment up to death. The cases with (G/G) genotype showed better survival, and did not develop any life-threatening complications, indicating that mortality was higher among ITP cases with (G/A) genotype than those with (G/G) genotype. There was a statistically significant association between TNF-α-308 (G/A) polymorphism and the incidence of death (p = 0.001). Regarding corticosteroid response, two of the cases (66.6%) with (G/A) genotype did not respond to steroids from the beginning, and one case (33.3%) lost the response after achieving it. However, among the cases with (G/G) genotype, six patients (12.7%) achieved complete response, one (2.1%) achieved response, one (2.1%) did not respond to steroids from the beginning, 19 (40.4%) lost complete response after achieving it, 17 (36.2%) lost response after achieving it, and three (6.4%) did not need treatment in the beginning (Table III).

This indicates that the response rate was higher among cases with (G/G) genotype, while the non-response rate was higher among cases with (G/A) genotype. A statistically significant relation was noticed between the TNF-α-308 (G/A) polymorphism and steroid treatment response (p = 0.034). However, there was no statistically significant association between the IL-10-592 (C/A) polymorphism and the fate of patients or the steroid treatment response.

The risk of developing ITP was not associated with either the TNF-α-308 G/A (OR 0.7, 95% CI: 0.275–1.981) or the IL-10-592 (C/A) (OR 1.5, 95% CI: 0.396–5.685) polymorphisms.

Discussion

Novel genes and loci identified may help to explain the biology of ITP and suggest the possibility of new testing for clinical or prognostic biomarkers of the disease or targets for therapy. Based on our present understanding of the pathogenesis and drug responses, many candidate genes can be studied to confirm, or potentially rule out, postulated contributions [20].

We have examined potential associations between some of the clinical parameters of ITP and SNPs within the genes for the previously mentioned inflammatory cytokines. Cytokine genes are polymorphic, which explains the different levels of cytokine production. Moreover, they are related to the regulation of the immune-mediated inflammatory process. Cytokine gene polymorphisms have become a subject of interest because certain alleles of cytokine genes have been correlated with different immunoinflammatory diseases [21].

IL-10 is the most important anti-inflammatory cytokine in the human immune response. IL-10 is a powerful inhibitor of Th1 cytokines, including both IL-2 and IFN-γ [22]. The gene for IL-10 is located in chromosome region 1q31–q32. It includes multiple polymorphisms associated with multiple immune and inflammatory disorders [23].

Concerning TNF-α, many studies have shown that any SNP at the position -308 G/A is associated with multiple inflammatory conditions [24]. Any genetic variability in the production of TNF-alpha after an infectious stimulus could have a significant influence on the degree of inflammatory response which will eventually influence the clinical outcome [25].

Contrary to our results, a previous study [5] investigated polymorphisms of TNF-α (–308) among chronic adult ITP patients in Turkey. The researchers found that the expression of TNF-α (–308) GA phenotype was significantly increased in patients with ITP compared to normal controls and that the presence of GA genotype increased the risk of ITP by 3.1 times (p <0.05). Also in contrast to our results, an Egyptian case-control study aimed at detecting the frequency of TNF-α (–308) G/A gene polymorphism revealed that the frequency of TNF-α (–308) A/A homotype in ITP patients

was significantly higher than that in controls, conferring an almost six-fold increased risk of ITP acquisition. The polymorphic A allele frequency was significantly higher in ITP patients than in controls, conferring an almost two-fold increased ITP risk [26]. Another previous study [27] investigated five SNPs among 84 adult Japanese patients with chronic ITP and 56 race-matched healthy controls, revealing that there was no difference in the distribution of SNPs present at TNF-α (–308) between ITP patients and healthy controls.

A previous Egyptian study [1] reported that the frequency of IL-10 (–592) C/A polymorphic genotype was significantly lower in males, which was similar to our results but not statistically significant. Our results showed that genotypes, alleles, and haplotypes distributions at IL-10--592 polymorphisms were not different between ITP patients and controls. These findings are consistent with those previously reported by Saitoh et al. [28], but inconsistent with the results of Wu et al. [29], who reported that the IL-10–592 C/A genotype was associated with the susceptibility of developing chronic ITP in children.

Our current study showed a clinically significant association between the TNF-α (–308) G/A polymorphic genotype and low platelet count, wet purpura, bleeding score, incidence of mortality, and steroid treatment response. However, there was no statistical association between IL-10 (–592) genotypes and various clinical features. This is contrary to the results of El Ghannam et al. [1], who found that the severity of ITP was significantly associated with IL-10-592 AA genotype.

No statistically significant difference was noted between patients with ITP harboring the normal or polymorphic TNF-α-308 and IL-10 (–592) alleles regarding their age or sex. This is in agreement with the studies conducted by Okulu et al. [30] and El Ghannam et al. [1].

There are some limitations to the present study that need to be addressed. Firstly, the relatively small number of patients and controls requires that larger multicenter studies will be needed in order to confirm our findings. Secondly, it was difficult to examine the long-term influence of these polymorphisms on the functional outcome of ITP, so a longer follow-up may be needed. Our data should therefore be considered to be preliminary, and further confirmatory studies on a wider base are needed. The differences between our study and other studies can be attributed to differences in the ages of participating cases, the sample sizes, the ethnic groups under study, and the methods used.

Conclusions

Our study suggests that TNF-α-308 (G/A) gene polymorphism predicts for some serious complications such as intracranial hemorrhage and non-response to corticosteroid treatment, and other prognostic factors such as the bleeding score and platelet count which may help to identify high-risk patients who should be offered more intensive or alternative lines of treatment from the beginning.

However, the prognostic significance of this polymorphism remains a matter of debate. Larger studies including larger sample size and more sensitive detection techniques will be needed to clarify the prognostic significance of this polymorphism. Our study also has shown no significant association between TNF-α-308 (G/A) and IL-10 (–592) genotypes and alleles frequencies in cases and controls. The risk of developing ITP was not related to the studied polymorphisms. However, the role of other genetic and environmental factors cannot be entirely ruled out. Further studies are required to establish the basis for these observations and their impact on the pathogenesis and progression of, and therapies for, ITP.

Authors' contributions

The data used and/or analyzed during the current study is available from the corresponding author upon reasonable request.

AS and SI were responsible for creating the study protocol. IM and AB were responsible for ethical approval and patient enrolment. AS, SI, SM and AE were responsible for performing genotype analysis. AE and SM were responsible for data acquisition and statistical analysis. SI and IM were responsible for writing the manuscript. AS, AB and IM were responsible for manuscript revision and proofreading.

Conflict of interest

The authors have no conflict of interest to declare that are relevant to the content of this article.

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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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SHORT COMMUNICATION

Ruxolitinib for steroid-refractory acute graft-versus-host disease: case series and literature review

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Introduction

Acute graft-versus-host-disease (aGvHD) is one of the leading causes of mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Despite the availability of different strategies of prophylaxis, ~15% of patients suffer from life threatening grade III–IV aGVHD with severe liver impairment and/or massive diarrhea [1].

Fifty years after the worldwide introduction of allo-HSCT, the use of methylprednisolone (MP) remains the only accepted first-line treatment for aGvHD. Unfortunately, MP is not effective in almost 60% of patients with grade IV disease [2]. Steroid-refractory aGvHD (SR-aGvHD) is associated with a poor outcome, with only a 5–30% survival rate [1]. Despite much work in this field, second-line treatment for SR-aGVHD has yet to be established, and much depends on the experience of the transplant center.

In May 2019, the US Food and Drug Administration (FDA) approved ruxolitinib (RUX), a JAK2 inhibitor for the treatment of SR-aGvHD in adult and pediatric patients aged 12 and above [3]. Approval was based on the results of the REACH2 clinical trial in which therapy of SR-aGvHD with RUX led to significant improvements in outcomes compared to other immunosuppressive therapies [4].

We describe below our experience with RUX in therapy of gut and liver SR-aGvHD through two descriptive cases.

Material and methods

We conducted a selective review of allo-HSCT recipients who received RUX for SR-aGvHD in 2020. All study patients provided written informed consent for therapy.

Results and discussion

Patient 1

A 37-year-old male diagnosed with myelodysplastic syndrome with multilineage dysplasia underwent allo-HSCT from a 10/10 human leukocyte antigen (HLA)-matched unrelated donor in August 2020.

His conditioning regimen included busulfan and cyclophosphamide (BuCy). GvHD prophylaxis consisted of cyclosporine (CsA), methotrexate (Mtx) and rabbit ATG (thymoglobulin). The patient achieved neutrophil and platelet engraftments on days +15 and +13 respectively. The early aplastic post-transplantation period was complicated by fever of unknown origin, with an increase in C-reactive protein (CRP) level up to 274 mg/L (normal range <5 mg/L). On day +14, he developed an erythematous maculopapular rash on >50% of his body surface (grade II aGvHD) and required pulses of MP at 1 mg/kg for a couple of days, with rapid resolution. Due to increased serum creatinine level, CsA was switched to mycophenolate mofetil (MMF).

Several days later, mild watery diarrhea (less than 500 mL/day) appeared. *Clostridioides difficile* infection and other possible causes of diarrhea were carefully excluded. MP at 2 mg/kg/day and oral budesonide were administered due to rapid progression of intestinal symptoms (>1,500 mL of watery stool a day and worsening abdominal pain). The patient was diagnosed with intestinal grade III aGvHD [5]. Five days later, the patient's condition deteriorated and subileus developed. MP was gradually tapered, and tacrolimus (TAC) in continuous infusion was attempted. Two days later, TAC was stopped due to repeated episodes of acute renal failure. RUX was started at 5 mg twice daily from day +28. Stool volume steadily decreased to less than 200 ml on

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day +32 and abdominal cramps significantly diminished. A repeated bone marrow aspiration biopsy performed on day +28 revealed complete remission with full donor chimerism. On day +47, the patient was discharged on maintained doses of oral MP, MMF and RUX (5 mg twice daily).

On day +56, the patient was urgently readmitted to hospital because of progressive pancytopenia probably due to the myelosuppressive effect of RUX. Bone marrow biopsy on day +60 showed normocellular marrow with normal myeloid maturation. Full donor chimerism was documented on reverse-transcriptase polymerase chain reaction (RT-PCR). Cytomegalovirus (CMV) reactivation was excluded. Due to severe thrombocytopenia, MP at 32 mg daily was given. RUX was reduced to 5 mg daily while MMF was maintained. Unfortunately, the patient died several weeks later due to massive bleeding from the upper gastrointestinal tract as a consequence of thrombocytopenia.

Patient 2

A 32-year-old female with myeloid blast crisis of chronic myeloid leukemia (CML) received stem cells from a brother in December 2019. The procedure was performed in active disease after ineffective salvage regimen. Thiotepa, busulfan and fludarabine (TBF) were administered as conditioning. GvHD prophylaxis consisted of CsA and Mtx. An early aplastic phase was uncomplicated. Neutrophil and platelet engraftments occurred on days 12 and 11, respectively. Bone marrow assessment on day +29 revealed complete hematological remission with full donor chimerism. The BCR-ABL(p210) transcript was undetectable. Due to the absence of aGvHD symptoms and the high risk of disease recurrence, donor lymphocyte infusion (DLI) of 1×10^7 CD3 positive cells was administered on day +29. The patient was discharged on tapering doses of CsA.

On day +133 while free of any immunosuppressive therapy, she was urgently readmitted to hospital because of jaundice and dark-coloured urine. Bilirubin concentration was significantly increased to 190 µmol/L (normal range 5–21 µmol/L) with marked elevation of alanine aminotransferase (346 IU/L; normal range <35 IU/L), alkaline phosphatase (169 IU/L; normal range 30–120 IU/L) and gamma-glutamyl transferase (701 IU/L; normal range <38 IU/L). Cholestasis as well as hepatitis B and C reactivations were excluded. The patient was diagnosed with hepatic grade III aGvHD. Skin and gut were free of aGVHD. The patient received MP at 1 mg/kg intravenously for 13 days with a subsequent slow reduction and conversion to oral therapy. Serum bilirubin concentration diminished to 97 µmol/L and remained stable. RUX at 5 mg twice daily was added to oral MP and bilirubin concentration diminished to 37 µmol/L. The dose of RUX was reduced to 7.5 mg per day. Currently, two years after transplantation, the patient remains free of immunosuppressive treatment with no symptoms of GvHD. There was no reactivation of CMV during RUX treatment.

The treatment of SR-aGvHD has remained unsatisfactory for decades. Patients with SR aGVHD are generally treated with different immunosuppressive agents with variable effects [1]. RUX by selective inhibition of JAK1 and JAK2 has been proven to block the action of proinflammatory cytokines signaling through the JAK-STAT pathway such as interleukin-1 and -6, tumor necrosis factor, and interferon-gamma [6]. This results in reduced proliferation of effector T-cells and an increase of regulatory T-cells in the colon and liver, leading to the attenuation of aGvHD symptoms [7].

In the pivotal REACH-1 trial, the administration of RUX in SR aGvHD patients resulted in a nearly 55% overall response rate, including 27% with complete responses at day 28. RUX treatment started from 5 mg twice daily, and the dose was escalated to 10 mg twice daily when no toxicity was present. The highest response rate was observed in skin (61%), then in the upper (45%), and the lower (46%) gastrointestinal tract, and the liver (26%) [8]. An even higher overall response rate (70%) was demonstrated in a study by a Spanish group. The response to RUX was achieved after a median of two weeks of treatment with overall survival for responders of 62% vs. 28% for non-responders [9]. Although RUX has a favourable toxicity profile, the most common side effects concern cytopenias and infectious complications [4, 10]. Anemia and thrombocytopenia were seen in 65% and 62% of patients in the REACH-1 trial, respectively [8]. In another study, of RUX-treated patients, 68% had at least one episode of infection complication. Among infections, CMV and human herpesvirus 6 (HHV-6) viremia were demonstrated in 21% and 11% of patients respectively. Bacteremias were recorded in 42% of patients, whereas no fungal infection was noted [10].

Although intensive immunosuppression reduces the beneficial graft-versus-leukemia effect and may lead to potential recurrence of prior malignancy [11], therapy with RUX has not been associated with higher frequency of hematological relapse [12]. The relapse rate during RUX for SR aGVHD varies between 1.2% and 9.3% depending on the published data [9, 13].

With the increasing use of RUX, a definition of RUX refractoriness should be established. Progression of GvHD after at least 5–10 days of RUX, a lack of improvement in GvHD symptoms ≥14 days of RUX, or a worsening of GvHD after initial improvement, define RUX-refractory aGvHD and necessitate alternative treatment [14].

RUX has not yet been reimbursed for Polish patients with GvHD, and the drug in our cases was obtained through the Managed Access Program of the Novartis company. Our clinical experience is limited to fewer than 15 cases (herewith we have presented two illustrative patients) treated exclusively for hepatic and intestinal manifestations of SR aGvHD. We have never used RUX for patients with SR cutaneous aGvHD, and therefore we cannot judge its efficacy in this patient population.

According to our observations, RUX treatment seems to be more effective in liver involvement than in intestinal, although this is in contrast to the results of the REACH-1 trial where the lowest efficacy was demonstrated for liver manifestation [8]. The latter was also true in a recently published study of patients after haploidentical transplantation [15]. However, more data is needed on particular organ response. Regarding our patient population, the starting dose of RUX varied from 5 mg daily to 5 mg twice daily, and none of the patients received 10 mg twice daily. Most patients, if not all, developed or worsened pancytopenia as a consequence of RUX therapy, other co-used therapies and concomitant bacterial or viral infections.

Based on our limited experience, 10 mg daily of RUX should not be exceeded. Due to the difficult access to RUX in Poland, it is usual to only administer it as a third or further line of therapy when other immunosuppressive agents have failed to achieve a response and the patient remains in a serious condition with concomitant pancytopenia associated with infection. Thus, it seems reasonable to administer RUX at an early stage of SR aGvHD treatment, but this requires a system solution allowing easy access to the drug.

In conclusion, RUX represents a valuable therapeutic option for patients with SR-aGvHD. Our experience is limited to case series. Easier access to RUX leading to its broader use would be required to obtain more conclusive data.

Authors' contributions

AS, GH — planned study, wrote manuscript, analyzed data; KW, AA, AWK, IN — collected data, critical review.

Conflictsof interest

GH — advisory board Novartis, Abbvie, speaker fee Novartis; AS — speaker fee Novartis; KW, AA, AWK, IN — none.

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Informed consent

Informed consent was obtained from all patients participating in this study.

Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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VIA MEDICA

CLINICAL VIGNETTE

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Class switch recombination defect in child with ataxia–telangiectasia with hyper IgM phenotype

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Case report

A 6.5-year-old female child presented with progressive hepatomegaly and generalized lymphadenopathy to the Primary Immunodeficiency Unit of Zagazig University Children's Hospital in Egypt. She had been in good health until the age of one year when she began to exhibit recurrent respiratory tract infections and purulent otitis media. Her parents were consanguineous, and there was a family history that was consistent with her presentation.

At the age of two, she presented with splenomegaly and chronic anemia. Investigations revealed low iron level, low transferrin, and normal metabolic and hemolysis workup. She was treated accordingly with iron supplementation and other supportive management with follow-up.

At the age of three, she developed bilateral ocular telangiectasia, truncal titubation, and disturbed gait. She was evaluated by a pediatric neurology specialist, who diagnosed cerebellar ataxia. She was referred to our Primary Immunodeficiency Unit at this age due to associated recurrent sinopulmonary and gastrointestinal tract infections.

Our initial laboratory workup revealed microcytic hypochromic anemia, high alpha-fetoprotein (AFP) levels, and mild chromosomal breakage induction. The results of an immunological workup revealed low IgG, low IgA, high IgM, and normal IgE. In lymphocyte subsets, CD20 percentage was reduced, but CD3, CD4, CD8, and CD56/16 percentages were normal (Table I). A diagnosis of ataxia–telangiectasia (AT) was made, and monthly intravenous immunoglobulin (IVIg) replacement and prophylactic treatment with trimethoprim–sulfamethoxazole were initiated at the age of three.

Her condition worsened at the age of five, and abnormal findings resulted, including generalized lymphadenopathy and hepatosplenomegaly. Lab and radiology workups were performed to exclude malignancies, autoimmune lymphoproliferative syndrome (ALPS), hemophagocytic lymphohistiocytosis (HLH), and infectious causes. In both bone marrow aspiration and bone marrow biopsy, normal cellularity was found with no malignancy or hemophagocytosis observed. A serology and polymerase chain reaction (PCR) for cytomegalovirus (CMV) showed positive results, and the patient was treated with antiviral therapy (gancyclovir) until complete eradication of the CMV infection, with a slight improvement in her clinical status. Results of repeated viral investigations were all negative. Blood, urine, and cerebrospinal fluid cultures (bacterial and mycobacteria) were sterile.

At the age of six, the patient developed hypersplenism, so a splenectomy was performed with subsequent improvement of pancytopenia. Six months later, hepatomegaly and lymphadenopathy worsened, and fever appeared. Therefore, she was admitted to the hospital once again. A multislice computed tomography (CT) chest, abdomen, and pelvis post intravenous contrast study showed bilateral axillary, supraclavicular, infraclavicular, mediastinal, and mesenteric lymphadenopathy, as well as hepatomegaly. After lymph node excision, the histopathology showed no evidence for malignancy and suggested lymphoproliferative disease of immunodeficiency, including partly disturbed architecture with attenuation of lymphoid follicles and expansion of the interfollicular area by marginal zone cells. The interfollicular area showed an abundance of plasma cells and histiocytes admixed with small lymphocytes, and a few scattered mononuclear cells were seen.

Due to the presence of lymphoproliferative disease and elevated IgM levels, we tested for class switch recombination (CSR) in this patient by evaluating the ability of peripheral blood mononuclear cells to produce a normal level of

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Table I. Laboratory and immunological data of a child diagnosed with HIgM-phenotype of ataxia–telangiectasia with class switch defect (HIgM AT-CSD)

Parameter	Values at diagnosis (age 3 years)	Values at age 6	Reference value
Total WBCs [1,000/μL]	6.6	3.1	5–15
Neutrophil [1,000/μL]	3.21	0.3	1.5–8.5
Lymphocyte [1,000/μL]	2.73	1	2–8
Hb [mg/dL]	10.7	8.3	11.5–15
MCV [fL]	68.9	76.2	75–87
Platelets [1,000/μL]	188	240	150–500
IgM [mg/dL]	1,090	3,300	(Normal value: 19–146)
IgG [mg/dL]	116	238	(Normal value: 453–916)
IgA [mg/dL]	Undetectable	29	(Normal value: 20–100)
IgE [IU/mL]	0.14	0.13	(Normal value: 0–60)
CD3+ [%]	84.7	65	(Normal value: 43–76)
CD4+ [%]	44	53	(Normal value: 23–48)
CD8+ [%]	24.2	10	(Normal value: 14–33)
CD4/CD8 ratio	1.8	5.3	(Normal value: 1.6–6.2)
CD3+ absolute count	2,397/μL	3,217/μL	(Normal value: 900–4,500)
CD4+ absolute count	1,245/μL	2,623/μL	(Normal value: 500–2,400)
CD8+ absolute count	685/μL	495	(Normal value: 300–1,600)
CD20+ [%]	1.7	1.5	(Normal value: 14–44)
CD56–/CD16+ [%]	2.4	2.8	
CD56+/CD16+ [%]	10.4	11.2	
CD56+/CD16– [%]	0.9	5	
Total NK cells [%]	13.7	19	(Normal value: 4–23)
HBe Ag (ECL)	Negative	Negative	
HBc Ag (ECL)	Negative	Negative	
HBs Ab [IU/L]	Negative	Negative	
HCV Ab (Index)	Negative	Negative	
Anti-EBV Ab (Index)	Negative	Negative	
Anti-CMV Ab (COI)	Negative	Negative	
Anti-HSV1+2 Ab (Index)	Negative	Negative	
Anti-HIV Ab (Index)	Negative	Negative	
Toxoplasma IgM (Index)	Negative	Negative	
Rubella IgM (Index)	Negative	Negative	
Alpha-fetoprotein [ng/mL]	99.1	149.9	Up to 8

WBCs — white blood cells; Hb — hemoglobin; MCV — mean corpuscular volume; Ig — immunoglobulin; NK — natural killers; ag — antigen; ECL — electrochemoluminescence assay; HCV — hepatitis C virus; EBV — Epstein-Bárr virus; CMV — cytomegalovirus; HSV1 — herpes simplex virus 1; HIV — human immunodeficiency virus

IgE after being cultured for 12 days at 37°C in a humidified atmosphere with 5% CO_2 in the presence of recombinant interleukin 4 (IL-4) and CD40L. CSR is considered defective if the quantity of IgE produced after stimulation is less than 0.35 IU/mL [1]. As this patient had a class switch recombination defect, we concluded that she had HIgM-phenotype ataxia–telangiectasia with a class switch defect (HIgM AT-CSD).

Discussion

Patients with primary immune deficiency are susceptible to hepatosplenomegaly, a lymphoproliferative complication that is generally caused by infection or immune dysregulation. According to some reports, hepatosplenomegaly is a characteristic feature of AT patients with HIgM profiles [2, 3].

Class switch recombination defect (CSRD) is an immunodeficiency disorder characterized by low levels of serum IgG, IgA, and IgE, with normal or raised levels of IgM [1, 4, 5].

In this AT patient, having high levels of serum IgM in association with low levels of IgG and IgA led to a presumptive diagnosis of a HIgM-phenotype. CSRD was diagnosed based on the absence of IgE production by B-lymphocytes stimulated by IL-4 and anti-CD40, leading to the diagnosis of HIgM-phenotype of ataxia–telangiectasia with class switch defect (HIgM AT-CSD). Mohammadinejad et al. [1] also reported three Iranian girls and a German boy of Turkish origin with high IgM serum levels and a diagnosis of AT and defective CSR processes.

Summary

Whenever there is a disseminated disease, a pediatric immunology service should be consulted as soon as possible to rule out any underlying immunodeficiency disease, and to optimize treatment [6].

Authors' contributions

MA — clinical analysis, writing the manuscript. EGB — clinical analysis, writing the manuscript. TAA — clinical analysis, writing the manuscript. All authors critically revised and approved the 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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‘Sport Hematology’ (‘Hematologia sportowa’) is the only book on the effects of physical activity on peripheral blood ever published in Poland. The data presented in the work is based on the most recent international scientific research on blood and its elements in the context of athletic activity and physical therapy. The book begins with a general outline of the physiology of physical activity and the involvement of the circulatory system at various levels of athletic behavior. Subsequent chapters touch on topics such as post-effort changes in the blood including changes in morphology, plasma, erythrocyte rheology, and biochemistry. There is a chapter on the causes of anemia in athletes as a result of physical activity. A lot of space has been devoted to doping substances’ effects on the blood and the dangers of using them. Another chapter describes new developments in medical treatment that greatly diminish the effects of drastic changes in blood morphology that occur in compound fractures. This publication provides tips on prophylactics and examples of exercises for patients with hematological problems, and guides physicians as to what sports would be recommended for their patients depending on their hematological indices, e.g. marathon running as opposed to walking. The 23 contributors all recommend controlled, moderate training, and warn against putting the body to extreme physical challenges.

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YESCARTA®▼

(aksykabtagen cyloleucel)

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

1. NAZWA PRODUKTU LECZNICZEGO: Yescarta, 0,4-2 $\times 10^8$ komórek, dyspersja do infuzji. **2. SKŁAD JAKOŚCIOWY I ILOŚCIOWY: 2.1 Opis ogólny:** Yescarta (aksykabtagen cyloleucel) to genetycznie zmodyfikowane autologiczne limfocyty T skierowane przeciw CD19 stosowane w immunoterapii. Aby przygotować produkt Yescarta, od pacjenta pobiera się limfocyty T, które są następnie genetycznie modyfikowane w warunkach *ex vivo* metodą transdukcji retrowirusowej w celu uzyskania ekspresji chimerowego receptora antygenowego (ang. *chimeric antigen receptor*, CAR) zawierającego mysi jednołańcuchowy fragment Fv anty-CD19 połączony z domeną kostymulującą CD-28 i domeną sygnalizacyjną CD3-zeta. Żywotne limfocyty CAR-T anty-CD19 są namnażane i z powrotem wprowadzane za pomocą infuzji do organizmu pacjenta, gdzie mogą rozpoznawać i eliminować komórki docelowe prezentujące CD19. **2.2 Skład jakościowy i ilościowy:** Każdy worek do jednorazowej infuzji ze swoistym dla danego pacjenta produktem Yescarta zawiera około 68 ml dyspersji limfocytów CAR-T anty-CD19, co umożliwia otrzymanie docelowej dawki wynoszącej 2×10^6 żywotnych limfocytów CAR-T anty-CD19/kg masy ciała (zakres od 1×10^6 do 2×10^6 limfocytów/kg) z maksymalną liczbą limfocytów CAR-T anty-CD19 wynoszącą 2×10^8. Substancje pomocnicze o znanym działaniu: Każdy worek z produktem Yescarta zawiera 300 mg sodu. Pełny wykaz substancji pomocniczych, patrz punkt 6.1 ChPL. **3. POSTAĆ FARMACEUTYCZNA:** Dyspersja do infuzji. Klarowna do opalizującej dyspersja w kolorze od białego do czerwonego. **4. SZCZEGÓŁOWE DANE KLINICZNE: 4.1 Wskazania do stosowania:** Produkt Yescarta jest wskazany w leczeniu nawrotowego lub opornego na leczenie chłoniaka rozlanego z dużych komórek B (ang. *diffuse large B-cell lymphoma*, DLBCL) i pierwotnego chłoniaka śródpiersia z dużych komórek B (ang. *primary mediastinal large B-cell lymphoma*, PMBCL) u dorosłych pacjentów, którzy uprzednio otrzymali co najmniej dwie linie leczenia systemowego. **4.2 Dawkowanie i sposób podawania:** Produkt Yescarta musi być podawany w kwalifikowanym ośrodku leczniczym przez lekarza mającego doświadczenie w leczeniu złośliwych nowotworów krwi oraz przeszkolonego w zakresie podawania i leczenia pacjentów produktem Yescarta. Na wypadek wystąpienia zespołu uwalniania cytokin (ang. *cytokine release syndrome*, CRS) przed infuzją musi być dostępna przynajmniej 1 dawka tocilizumabu i sprzęt ratunkowy. Ośrodek, w którym odbywa się leczenie musi mieć zapewniony dostęp do dodatkowej dawki tocilizumabu w ciągu 8 godzin od uprzedniego podania każdej dawki. W wyjątkowej sytuacji braku dostępności tocilizumabu uwzględnionym w wykazie produktów leczniczych zagrożonych brakiem dostępności Europejskiej Agencji Leków przed rozpoczęciem infuzji muszą być dostępne odpowiednie, alternatywne leki do leczenia CRS zamiast tocilizumabu. Dawkowanie: Yescarta to produkt przeznaczony wyłącznie do stosowania autologicznego (patrz punkt 4.4). Pojedyncza dawka produktu Yescarta zawiera 2×10^6 żywotnych limfocytów CAR-T na kilogram masy ciała (lub maksymalnie 2×10^8 żywotnych limfocytów CAR-T dla pacjentów o masie ciała równej 100 kg i większej) w około 68 ml dyspersji w worku do infuzji. Należy potwierdzić dostępność produktu Yescarta przed rozpoczęciem limfodeplecji. *Leczenie wstępne (chemioterapia limfodeplecyjna):* W 5., 4. i 3. dniu przed infuzją produktu Yescarta należy zastosować chemioterapię limfodeplecyjną, składającą się z cyklofosfamidu w dawce 500 mg/m^2 podawanego dożylnie i fludarabiny w dawce 30 mg/m^2 podawanej dożylnie. *Premedykacja:* Zaleca się podanie paracetamolu w dawce 500-1000 mg doustnie i difenhydraminy w dawce 12,5 do 25 mg dożylnie lub doustnie (lub równoważnej) na około 1 godzinę przed infuzją produktu Yescarta. Nie zaleca się podawania ogólnoustrojowych kortykosteroidów w ramach profilaktyki, ponieważ mogą mieć wpływ na działanie produktu Yescarta. *Monitorowanie:* Po infuzji pacjentów należy monitorować codziennie przez pierwsze 10 dni po infuzji pod kątem objawów przedmiotowych i podmiotowych potencjalnego CRS, zdarzeń neurologicznych i innych toksyczności. Lekarze powinni rozważyć hospitalizację pacjenta przez pierwsze 10 dni po infuzji lub w momencie pojawienia się pierwszych objawów przedmiotowych lub podmiotowych CRS i (lub) zdarzeń neurologicznych. Po pierwszych 10 dniach od infuzji o monitorowaniu pacjenta zdecyduje lekarz. Pacjentów należy poinformować o konieczności pozostania w pobliżu kwalifikowanej placówki klinicznej przez co najmniej 4 tygodnie po infuzji. Specjalne grupy pacjentów: *Pacjenci z zakażeniem ludzkim wirusem niedoboru odporności (ang. human immunodeficiency virus, HIV), wirusowym zapaleniem wątroby typu B (ang. hepatitis B virus, HBV) oraz wirusowym zapaleniem wątroby typu C (ang. hepatitis C virus, HCV):* Brak doświadczenia klinicznego ze stosowaniem u pacjentów z aktywnym zakażeniem HIV, HBV lub HCV. *Dzieci i młodzież:* Nie określono jeszcze bezpieczeństwa stosowania ani skuteczności produktu leczniczego Yescarta u dzieci i młodzieży w wieku poniżej 18 lat. Dane nie są dostępne. *Osoby w podeszłym wieku:* Nie jest wymagane dostosowywanie dawki u pacjentów w wieku 65 lat i starszych. Skuteczność leczenia była zgodna ze skutecznością leczenia w całej leczonej populacji pacjentów. Sposób podawania: Produkt Yescarta jest podawany drogą infuzji dożylnej. Produktu Yescarta nie wolno naświetlać. NIE używać filtra do deplecji leukocytów. *Środki ostrożności, które należy podjąć przed użyciem lub podaniem produktu leczniczego:* Ten produkt leczniczy zawiera genetycznie zmodyfikowane ludzkie komórki krwi. Fachowy personel medyczny przygotowujący produkt Yescarta powinien stosować odpowiednie środki ostrożności (nosić rękawice i okulary), aby uniknąć potencjalnego przeniesienia chorób zakaźnych. *Przygotowanie do infuzji:* Zweryfikować zgodność tożsamości pacjenta (ang. *identity,* ID) z oznaczeniami na kasecie z produktem Yescarta. Nie wolno wyjmować worka z produktem Yescarta z metalowej kasety, gdy brak zgodności informacji na etykiecie dotyczącej danego pacjenta z danymi pacjenta, dla którego przeznaczony jest ten produkt. Po potwierdzeniu ID pacjenta wyjąć worek z produktem Yescarta z metalowej kasety. Sprawdzić, czy dane pacjenta na etykiecie metalowej kasety są zgodne z danymi na etykiecie worka. Przed rozmrożeniem sprawdzić, czy worek z produktem nie ma jakichkolwiek uszkodzeń. W razie uszkodzenia worka postępować zgodnie z lokalnymi wytycznymi dotyczącymi postępowania z odpadami materiałów pochodzenia ludzkiego (lub niezwłocznie przekazać informację do firmy Kite). Umieścić worek do infuzji w drugim worku. Rozmrozić produkt Yescarta w temperaturze wynoszącej około 37°C w kąpieli wodnej lub metodą suchego rozmrażania do momentu, gdy w worku infuzyjnym nie będzie widocznego lodu. Delikatnie wymieszać zawartość worka, aby rozprowadzić grudki materiału komórkowego. Jeśli nadal widoczne są grudki materiału komórkowego, kontynuować delikatne mieszanie zawartości worka. Powinno to doprowadzić do rozproszenia małych grudek materiału komórkowego. Produktu Yescarta nie należy myć, wirować ani powtórnie odtwarzać zawiesiny na nowych nośnikach przed infuzją. Rozmrażanie powinno trwać około 3 do 5 minut. Po rozmrożeniu produkt Yescarta zachowuje trwałość w temperaturze pokojowej (od 20°C do 25°C) do 3 godzin. Infuzję produktu Yescarta należy jednak rozpocząć w ciągu 30 minut od rozmrożenia. *Podawanie:* Tylko do stosowania autologicznego. Tocilizumab oraz sprzęt ratunkowy powinny być dostępne przed infuzją i podczas monitorowania. W wyjątkowej sytuacji braku dostępności tocilizumabu uwzględnionym w wykazie produktów leczniczych zagrożonych brakiem dostępności Europejskiej Agencji Leków przed rozpoczęciem infuzji muszą być dostępne odpowiednie, alternatywne leki do leczenia CRS zamiast tocilizumabu. Nie wolno używać filtra do deplecji leukocytów. Zaleca się założenie centralnego dostępu żylnego w celu podania produktu Yescarta. Ponownie zweryfikować ID pacjenta w celu potwierdzenia zgodności z oznaczeniami pacjenta na worku z produktem Yescarta. Przed infuzją wypełnić zestaw do infuzji roztworem chlorku sodu (0,154 mmol sodu/ml). Cała zawartość worka z produktem Yescarta powinna zostać podana w infuzji w ciągu 30 minut metodą grawitacyjną lub z użyciem objętościowej pompy infuzyjnej. Delikatnie masować worek podczas infuzji produktu Yescarta, aby zapobiec tworzeniu się grudek materiału komórkowego. Po podaniu w infuzji całej zawartości worka, przepłukać zestaw do infuzji roztworem chlorku sodu (0,154 mmol sodu/ml) z tą samą prędkością infuzji, aby upewnić się, że produkt Yescarta został podany w całości. Instrukcja dotycząca postępowania, przypadkowego narażenia oraz usuwania tego produktu, patrz punkt 6.6 ChPL.

4.3 Przeciwwskazania: Nadwrażliwość na substancję czynną lub którąkolwiek substancję pomocniczą wymienioną w punkcie 6.1 ChPL. Należy uwzględnić przeciwwskazania dotyczące chemioterapii limfodeplecyjnej. **4.4 Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania:** Identyfikowalność: Należy bezwzględnie przestrzegać wymogów identyfikowalności produktów leczniczych stosowanych w zaawansowanych terapiach komórkowych. Aby zapewnić identyfikowalność nazwę produktu leczniczego, numer serii oraz imię i nazwisko leczonego pacjenta należy przechowywać przez okres 30 lat po upłynięciu terminu ważności produktu. Ogólne: Produkt Yescarta jest przeznaczony wyłącznie do stosowania autologicznego i nie wolno podawać go innym pacjentom. Przed infuzją: tożsamość pacjenta musi być zgodna z danymi identyfikacyjnymi na worku infuzyjnym i kasecie z produktem Yescarta. Nie należy podawać infuzji produktu Yescarta, gdy informacja na etykiecie dotyczącej danego pacjenta nie jest zgodna z danymi pacjenta, dla którego przeznaczony jest produkt. Pacjentów należy monitorować codziennie przez pierwsze 10 dni po infuzji w celu wykrycia przedmiotowych i podmiotowych objawów potencjalnego CRS, zdarzeń neurologicznych i innych toksyczności. Lekarze powinni rozważyć hospitalizację pacjenta przez pierwsze 10 dni po infuzji lub w momencie wystąpienia pierwszych objawów przedmiotowych/podmiotowych CRS i (lub) zdarzeń neurologicznych. Po pierwszych 10 dniach po infuzji o monitorowaniu pacjenta zdecyduje lekarz. Należy poinformować pacjentów o konieczności pozostawania w pobliżu kwalifikowanego ośrodka leczniczego przez co najmniej 4 tygodnie po infuzji oraz o konieczności uzyskania natychmiastowej pomocy medycznej w razie wystąpienia przedmiotowych lub podmiotowych objawów CRS lub neurologicznych działań niepożądanych. W zależności od stopnia nasilenia danego działania niepożądanego należy rozważyć monitorowanie parametrów życiowych i czynności narządów. Powody odroczenia leczenia: Ze względu na zagrożenia związane z leczeniem produktem Yescarta podanie infuzji należy wstrzymać, jeśli u pacjenta występuje którykolwiek z następujących stanów: utrzymujące się ciężkie działania niepożądane (w szczególności działania dotyczące płuc, serca lub niedociśnienie), w tym związane z wcześniejszymi chemioterapiami; czynne, niekontrolowane zakażenie; czynna choroba przeszczep przeciwko gospodarzowi (ang. *graft-versus-host disease*, GVHD). Badania serologiczne: Przed pobraniem komórek do wytworzenia produktu Yescarta, należy wykonać badania przesiewowe na obecność HBV, HCV i HIV (patrz punkt 4.2). Oddawanie krwi, narządów, tkanek i komórek: Pacjenci leczeni produktem Yescarta nie powinni być dawcami krwi, narządów, tkanek ani komórek do przeszczepienia. Choroba współistniejąca: Pacjenci z czynnym zaburzeniem OUN lub z zaburzeniami czynności nerek, wątroby, płuc lub serca mogą być bardziej podatni na skutki działań niepożądanych opisanych poniżej i wymagają szczególnej uwagi. Pierwotny chłoniak ośrodkowego układu nerwowego (OUN): Brak doświadczenia związanego ze stosowaniem produktu Yescarta u pacjentów z pierwotnym chłoniakiem OUN. W związku z tym nie określono stosunku ryzyka do korzyści leczenia produktem Yescarta dla tej populacji. Zespół uwalniania cytokin: U prawie wszystkich pacjentów wystąpił CRS któregoś stopnia. W przypadku stosowania produktu Yescarta bardzo często stwierdzano ciężkie przypadki CRS, w tym reakcje zagrażające życiu i prowadzące do zgonu, a czas do ich wystąpienia wynosił od 1 do 12 dni (patrz punkt 4.8). O leczeniu CRS decyduje lekarz na podstawie występujących u pacjenta objawów klinicznych oraz zgodnie z algorytmem postępowania w CRS przedstawionym w tabeli 1. W leczeniu CRS o nasileniu umiarkowanym lub ciężkim, związanego z podaniem produktu Yescarta, stosowano terapię opartą na inhibitorze receptora interleukiny 6 (IL-6), takim jak tocilizumab. Ośrodek, w którym odbywa się infuzja produktu Yescarta, musi przystępując do niej dysponować co najmniej 1 dostępną do podania dawką tocilizumabu dla każdego pacjenta. Ośrodek, w którym odbywa się leczenie, musi mieć dostęp do dodatkowej dawki tocilizumabu w ciągu 8 godzin od podania każdej z uprzednich dawek. W wyjątkowej sytuacji braku dostępności tocilizumabu uwzględnionym w wykazie produktów leczniczych zagrożonych brakiem dostępności Europejskiej Agencji Leków ośrodek leczniczy musi mieć dostęp do odpowiednich, alternatywnych leków do leczenia CRS zamiast tocilizumabu. Pacjentów należy codziennie monitorować w kwalifikowanej placówce klinicznej przez co najmniej 10 dni po infuzji pod kątem objawów przedmiotowych i podmiotowych CRS. Po upływie pierwszych 10 dni od infuzji o monitorowaniu pacjentów zdecyduje lekarz. Należy poinformować pacjentów o konieczności pozostawania w pobliżu kwalifikowanej placówki klinicznej przez co najmniej 4 tygodnie po infuzji oraz o konieczności uzyskania natychmiastowej pomocy medycznej w razie wystąpienia objawów przedmiotowych lub podmiotowych CRS. Opracowano algorytmy postępowania mające na celu łagodzenie niektórych objawów CRS występujących u pacjentów przyjmujących produkt Yescarta. Obejmują one stosowanie tocilizumabu lub tocilizumabu z kortykosteroidami w przypadkach CRS o nasileniu umiarkowanym, ciężkim lub zagrażającym życiu, jak przedstawiono w tabeli 1. Pacjentów, u których wystąpi CRS w stopniu 2. lub wyższym (np. niedociśnienie, brak odpowiedzi po podaniu płynów lub hipoksja wymagająca podania dodatkowego tlenu), należy monitorować z wykorzystaniem ciągłej telemetrii kardiologicznej oraz pulsoksymetrii. U pacjentów z ciężkim CRS należy rozważyć wykonanie echokardiogramu w celu oceny czynności serca. W przypadku ciężkiego lub zagrażającego życiu CRS należy rozważyć wdrożenie intensywnej terapii w ramach leczenia wspomagającego. Produktu Yescarta nie należy podawać pacjentom, u których występuje czynne zakażenie lub choroba zapalna, dopóki stany te nie ustąpią. Wiadomo, że CRS jest związany z niewydolnością narządów docelowych (np. wątroby, nerek, serca i płuc). Ponadto w przebiegu CRS może dojść do nasilenia występujących zaburzeń tych narządów. U pacjentów z istotnymi klinicznie zaburzeniami kardiologicznymi należy stosować standardy opieki w stanach krytycznych oraz rozważyć takie środki, jak echokardiografia. W celu rozpoznania CRS należy wykluczyć inne przyczyny ogólnoustrojowej odpowiedzi zapalnej, w tym zakażenie. W razie wystąpienia gorączki neutropenicznej należy dokonać oceny pod kątem zakażenia i zastosować antybiotyki o szerokim spektrum działania, płyny oraz leczenie podtrzymujące zgodnie z istniejącymi wskazaniami medycznymi. W przypadku pacjentów z ciężkim lub nieodpowiadającym na leczenie CRS należy rozważyć możliwość wystąpienia limfohistiocytozy hemofagocytarnej/zespołu aktywacji makrofagów (ang. *haemophagocytic lymphohistiocytosis/macrophage activation syndrome*, HLH/MAS). Produkt Yescarta nadal rozprzestrzenia się i utrzymuje w organizmie po podaniu tocilizumabu oraz kortykosteroidów. Nie zaleca się stosowania antagonistów czynnika martwicy nowotworu (ang. *tumour necrosis factor*, TNF) w leczeniu CRS związanego z zastosowaniem produktu Yescarta. **Tabela 1: Algorytm postępowania w zespole uwalniania cytokin (CRS).** Nasilenie CRS[a]: Stopień 1.: Objawy wymagają zastosowania tylko leczenia objawowego (np. gorączka, nudności, zmęczenie, ból głowy, ból mięśni, złe samopoczucie). *Tocilizumab:* W razie braku poprawy po 24 godzinach postępować jak w stopniu 2. *Kortykosteroidy:* N/A. Stopień 2.: Objawy wymagają umiarkowanej interwencji, po której ustępują. Zapotrzebowanie na tlen wynoszące mniej niż 40% FiO_2 lub hipotensja odpowiadająca na podanie płynów lub małej dawki jednego leku wazopresyjnego, lub toksyczność narządowa 2. stopnia[b]. *Tocilizumab:* Podać tocilizumab[c] w dawce 8 mg/kg dożylnie przez 1 godzinę (nie przekraczać dawki 800 mg). W razie potrzeby podawać tocilizumab co 8 godzin, gdy brak odpowiedzi na dożylne podanie płynów lub rośnie zapotrzebowanie na tlenoterapię. Nie podawać więcej niż 3 dawki w ciągu 24 godzin; maksymalna całkowita liczba dawek to 4 dawki, jeśli brak poprawy klinicznej dotyczącej objawów przedmiotowych i podmiotowych CRS lub jeśli brak odpowiedzi na drugą lub kolejną dawkę tocilizumabu, należy rozważyć zastosowanie alternatywnych sposobów leczenia CRS. *Kortykosteroidy:* Leczenie jak w stopniu 3., gdy brak poprawy w ciągu 24 godzin od podania tocilizumabu. Stopień 3.: Objawy wymagają poważnej interwencji, po której ustępują. Zapotrzebowanie na tlen przekraczające lub równe 40% FiO_2 lub hipotensja wymagająca podania dużej dawki leku wazopresyjnego albo zastosowania wielu leków wazopresyjnych, lub toksyczność narządowa 3. stopnia, albo hipertransaminazemia 4. stopnia. *Tocilizumab:* Leczenie jak w stopniu 2. *Kortykosteroidy:* Podać metyloprednizolon dożylnie w dawce 1 mg/kg dwa razy na dobę lub równoważną dawkę deksametazonu (np. 10 mg dożylnie co 6 godzin). Kontynuować podawanie kortykosteroidów do momentu, gdy zdarzenie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a następnie stopniowo zmniejszać dawkę. Gdy brak poprawy, zastosować leczenie jak w stopniu 4. (poniżej). Stopień 4.: Objawy zagrażające życiu. Pacjent wymaga zastosowania respiratora lub ciągłej hemodializy żylno-żylnej, lub toksyczność narządowa 4. stopnia (z wyjątkiem hipertransaminazemii). *Tocilizumab:* Leczenie jak w stopniu 2. *Kortykosteroidy:* Podawać metyloprednizolon dożylnie w dawce dobowej 1000 mg przez 3 dni, gdy jest poprawa – stosować leczenie jak powyżej. Rozważyć podanie innych leków immunosupresyjnych, gdy brak poprawy lub pogorszenie stanu. N/A = brak/nie dotyczy. *(a) Lee et al 2014. (b) Leczenie neurologicznych działań niepożądanych – patrz Tabela 2. (c) Szczegółowe informacje można znaleźć w charakterystyce produktu leczniczego tocilizumabu.* Neurologiczne działania niepożądane: U pacjentów leczonych produktem Yescarta bardzo często obserwowano ciężkie neurologiczne działania niepożądane, które mogą zagrażać życiu lub prowadzić do zgonu (patrz punkt 4.8). Pacjenci z zaburzeniami OUN w wywiadzie, takimi jak drgawki lub niedokrwienie naczyniowo-mózgowe, mogą być narażeni na większe ryzyko. Zgłaszano przypadki zakończonego zgonem i ciężkiego obrzęku mózgu u pacjentów leczonych produktem Yescarta. Pacjentów należy monitorować pod kątem objawów przedmiotowych i podmiotowych neurologicznych działań niepożądanych (Tabela 2). Po infuzji pacjentów należy monitorować w kwalifikowanej placówce opieki zdrowotnej co najmniej codziennie przez pierwsze 10 dni pod kątem objawów przedmiotowych i podmiotowych toksyczności neurologicznej. Po tych pierwszych 10 dniach o monitorowaniu pacjenta zdecyduje lekarz. Pacjentów należy poinformować o konieczności pozostania w pobliżu kwalifikowanej placówki klinicznej przynajmniej przez 4 tygodnie po infuzji i natychmiastowego zwrócenia się po pomoc medyczną, jeśli wystąpią objawy przedmiotowe lub podmiotowe toksyczności neurologicznej. W zależności od stopnia nasilenia danego działania niepożądanego należy rozważyć monitorowanie parametrów życiowych i wydolności narządów. Pacjentów, u których wystąpi toksyczność neurologiczna stopnia 2. lub wyższego, należy monitorować z wykorzystaniem ciągłej telemetrii kardiologicznej oraz pulsoksymetrii. W razie ciężkiej lub zagrażającej życiu toksyczności neurologicznej należy wdrożyć intensywną terapię wspomagającą. Należy rozważyć podanie leków przeciwdrgawkowych niemających działania uspokajającego w ramach profilaktyki przeciwdrgawkowej zgodnie ze wskazaniami klinicznymi dla działań niepożądanych 2. lub wyższego stopnia. Opracowano algorytmy postępowania mające na celu złagodzenie neurologicznych działań niepożądanych występujących u pacjentów przyjmujących produkt Yescarta. Obejmują one stosowanie tocilizumabu (w razie współistniejącego CRS) i (lub) kortykosteroidów w przypadku umiarkowanych, ciężkich lub zagrażających życiu neurologicznych działań niepożądanych, jak przedstawiono w tabeli 2. **Tabela 2: Algorytmy postępowania dotyczące objawów neurologicznych działań niepożądanych.** Stopień 2.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Gdy brak poprawy w ciągu 24 godzin po podaniu tocilizumabu: podać deksametazon dożylnie w dawce 10 mg co 6 godzin, jeśli pacjent nie przyjmuje już innych kortykosteroidów. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. *Bez współistniejącego CRS:* Podawać deksametazon dożylnie w dawce 10 mg co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. Stopień 3.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Dodatkowo z pierwszą dawką tocilizumabu podać deksametazon dożylnie w dawce 10 mg, powtarzając co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. *Bez współistniejącego CRS:* Podawać deksametazon dożylnie w dawce 10 mg co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. Stopień 4.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Wraz z pierwszą dawką tocilizumabu podać metyloprednizolon dożylnie w dobowej dawce 1000 mg oraz kontynuować podawanie metyloprednizolonu dożylnie w dobowej dawce 1000 mg przez 2 kolejne dni; w przypadku poprawy stosować leczenie jak powyżej. Gdy brak poprawy, rozważyć podanie metyloprednizolonu 1000 mg dożylnie 3 razy na dobę lub alternatywne leczenie[a]. *Bez współistniejącego CRS:* Podawać metyloprednizolon dożylnie w dawce dobowej 1000 mg przez 3 dni, gdy poprawa – stosować leczenie jak powyżej. Gdy brak poprawy, rozważyć podanie metyloprednizolonu 1000 mg dożylnie 3 razy na dobę lub alternatywne leczenie[a]. *(a) Alternatywne leczenie obejmuje (między innymi): anakinrę, siltuksymab, ruksolitynib, cyklofosfamid, immunoglobuliny podawane dożylnie (ang. intravenous immunoglobulin, IVIG) oraz globulinę anty-tymocytarną (ang. anti-thymocyte globulin, ATG).* Zakażenia i gorączka neutropeniczna: Podczas stosowania produktu Yescarta bardzo często obserwowano ciężkie zakażenia (patrz punkt 4.8). Pacjentów należy monitorować pod kątem objawów przedmiotowych i podmiotowych zakażenia przed, w trakcie i po podaniu produktu Yescarta w infuzji oraz zastosować odpowiednie leczenie. Należy zastosować profilaktykę przeciwbakteryjną zgodnie z obowiązującymi standardowymi wytycznymi. Po infuzji produktu Yescarta u pacjentów stwierdzano gorączkę neutropeniczną (patrz punkt 4.8) i może ona występować jednocześnie z CRS. W przypadku wystąpienia gorączki neutropenicznej należy ocenić, czy nie jest to zakażenie, i zastosować antybiotyki o szerokim spektrum działania, płyny oraz leczenie objawowe zgodnie ze wskazaniami medycznymi. Reaktywacja HBV: U pacjentów leczonych lekami skierowanymi przeciwko limfocytom B może dojść do reaktywacji HBV, w niektórych przypadkach prowadzącej do piorunującego zapalenia wątroby, niewydolności wątroby i zgonu. Przed pobraniem komórek do wytworzenia produktu Yescarta należy przeprowadzić badanie przesiewowe w kierunku HBV, HCV i HIV. Przedłużająca się cytopenia: Po chemioterapii limfodeplecyjnej i infuzji produktu Yescarta u pacjentów przez kilka tygodni może występować cytopenia. Odnotowano bardzo częste występowanie przedłużającej się cytopenii stopnia 3. lub wyższego, w tym małopłytkowość, neutropenię i niedokrwistość, po infuzji produktu Yescarta. Należy monitorować wyniki badań morfologii krwi po infuzji produktu Yescarta. Hipogammaglobulinemia: U pacjentów leczonych produktem Yescarta może wystąpić aplazja limfocytów B, prowadząca do hipogammaglobulinemii. Hipogammaglobulinemię obserwowano bardzo często u pacjentów leczonych produktem Yescarta. Należy monitorować stężenie immunoglobulin po leczeniu produktem Yescarta oraz stosować leczenie obejmujące profilaktykę zakażeń, profilaktykę antybiotykową oraz immunoglobulinową terapię zastępczą. Reakcje nadwrażliwości: Po infuzji produktu Yescarta mogą wystąpić reakcje alergiczne. Ciężkie reakcje nadwrażliwości, w tym reakcja anafilaktyczna, mogą być spowodowane obecnością dimetylosulfotlenku (ang. *dimethylsulfoxide*, DMSO) lub resztkowej gentamycyny w produkcie Yescarta. Wtórne nowotwory złośliwe: U pacjentów leczonych produktem Yescarta mogą rozwinąć się wtórne nowotwory złośliwe. Pacjentów należy monitorować przez całe życie pod kątem wtórnych nowotworów złośliwych. W razie pojawienia się wtórnego nowotworu złośliwego należy skontaktować się z firmą w celu uzyskania instrukcji dotyczących pobrania od pacjenta próbek do badań. Zespół rozpadu guza (ang. *tumour lysis syndrome*, TLS): Rzadko obserwowano występowanie TLS, który może mieć ciężką postać. W celu zminimalizowania ryzyka wystąpienia TLS pacjenci ze zwiększonym stężeniem kwasu moczowego lub dużą łączną masą guza powinni otrzymywać allopurynol lub inne leczenie profilaktyczne przed infuzją produktu Yescarta. Pacjentów należy monitorować pod kątem przedmiotowych i podmiotowych objawów TLS, a objawy należy leczyć zgodnie ze standardowymi wytycznymi. Wcześniejsze leczenie terapią anty-CD19: Doświadczenie związane ze stosowaniem produktu Yescarta u pacjentów, którzy wcześniej otrzymali leczenie celowane przeciwko CD19, jest ograniczone. Nie zaleca się stosowania produktu Yescarta, jeśli u pacjenta nastąpił nawrót choroby bez ekspresji CD19 po uprzedniej terapii anty-CD19. Substancje pomocnicze: Ten produkt leczniczy zawiera 300 mg sodu na worek infuzyjny, co odpowiada 15% maksymalnego dziennego dopuszczalnego spożycia sodu, wynoszącego 2 g dla osoby dorosłej, zgodnie z zaleceniami WHO. Oczekuje się, że pacjenci zostaną wpisani do rejestru pacjentów, na podstawie którego prowadzona będzie obserwacja w celu lepszego poznania długoterminowego bezpieczeństwa stosowania i skuteczności produktu Yescarta. **4.8 Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: Dane dotyczące bezpieczeństwa opisane w tym punkcie odzwierciedlają skutki narażenia na produkt Yescarta w badaniu fazy I/II ZUMA-1, w którym 108 pacjentów z nawrotowym/opornym chłoniakiem nieziarniczym (NHL) z limfocytów B otrzymywało limfocyty CAR-T w zalecanej dawce w zależności od masy ciała. Mediana okresu obserwacji wyniosła 27,4 miesiąca. Do najbardziej poważnych i najczęstszych działań niepożądanych należały CRS (93%), encefalopatia (58%) oraz zakażenia (39%). Ciężkie działania niepożądane wystąpiły u 56% pacjentów. Do najczęstszych działań niepożądanych należały: encefalopatia (22%), zakażenia nieswoistymi patogenami (16%), zakażenia bakteryjne (6%), gorączka neutropeniczna (6%), zakażenia wirusowe (5%) i gorączka (5%). Najczęściej występujące działania niepożądane stopnia 3. lub wyższego obejmowały encefalopatię (31%), zakażenia nieswoistymi patogenami (19%), CRS (11%), zakażenie bakteryjne (9%), afazję (7%), zakażenie wirusowe (6%), stan majaczeniowy (6%), hipotensję (6%) i nadciśnienie (6%). Tabelaryczna lista działań niepożądanych: Działania niepożądane zgłaszane w badaniach klinicznych oraz po wprowadzeniu produktu do obrotu przedstawiono poniżej. Działania te są wymienione według klasyfikacji układów i narządów oraz częstości występowania. Częstości występowania określone są w następujący sposób: bardzo często (≥ 1/10), często (≥ 1/100 do < 1/10), niezbyt często (≥ 1/1000 do < 1/100), rzadko (≥ 1/10 000 do < 1/1000) i bardzo rzadko (< 1/10 000). W obrębie każdej grupy o określonej częstości występowania działania niepożądane wymieniono zgodnie ze zmniejszającym się nasileniem. **Tabela 3: Działania niepożądane związane ze stosowaniem produktu Yescarta.** *Zakażenia i zakażenia pasożytnicze:* Bardzo często: zakażenia nieswoistymi patogenami, zakażenie wirusowe, zakażenie bakteryjne. Często: zakażenie grzybicze. *Zaburzenia krwi i układu chłonnego:* Bardzo często: leukopenia, neutropenia, niedokrwistość, małopłytkowość. Często: koagulopatia. *Zaburzenia układu immunologicznego:* Bardzo często: zespół uwalniania cytokin, hipogammaglobulinemia. Często: nadwrażliwość, histiocytoza hemofagocytarna. *Zaburzenia metabolizmu i odżywiania:* Bardzo często: odwodnienie, zmniejszone łaknienie, hipofosfatemia, hiponatremia, zmniejszenie masy ciała. Często: hipokalcemia, hipoalbuminemia. *Zaburzenia psychiczne:* Bardzo często: majaczenia, lęk. Często: bezsenność. *Zaburzenia układu nerwowego:* Bardzo często: encefalopatia, ból głowy, drżenie, zawroty głowy, afazja. Często: ataksja, neuropatia, drgawki, dyskalkulia, mioklonie. Niezbyt często: obrzęk rdzenia kręgowego, zapalenie rdzenia kręgowego, porażenie czterokończynowe. *Zaburzenia serca:* Bardzo często: tachykardia, arytmia. Często: zatrzymanie akcji serca, niewydolność serca. *Zaburzenia naczyniowe:* Bardzo często: niedociśnienie, nadciśnienie. Często: zakrzepica, zespół przesiąkania włośniczek. *Zaburzenia układu oddechowego, klatki piersiowej i śródpiersia:* Bardzo często: kaszel, duszność, hipoksja, wysięk opłucnowy. Często: obrzęk płuc. *Zaburzenia żołądka i jelit:* Bardzo często: biegunka, nudności, wymioty, zaparcia, ból brzucha, suchość w jamie ustnej. Często: dysfagia*. *Zaburzenia skóry i tkanki podskórnej:* Często: wysypka. *Zaburzenia układu mięśniowo-szkieletowego i tkanki łącznej:* Bardzo często: zaburzenia ruchowe, ból kończyn, ból pleców, ból stawów, ból mięśni. *Zaburzenia nerek i dróg moczowych:* Często: niewydolność nerek. *Zaburzenia ogólne i stany w miejscu podania:* Bardzo często: zmęczenie, gorączka, obrzęk, dreszcze. *Badania diagnostyczne:* Bardzo często: zwiększone stężenie aminotransferazy alaninowej, zwiększone stężenie aminotransferazy asparaginowej. Często: zwiększone stężenie bilirubiny. W tabeli 3 ujęto wyłącznie przypadki cytopenii, które spowodowały (I) wystąpienie nowych lub zaostrzenie istniejących następstw klinicznych, (II) wymagały leczenia lub (III) modyfikacji aktualnego leczenia. * Dysfagię zgłaszano w ramach toksycznego działania na układ nerwowy i encefalopatii. Opis wybranych działań niepożądanych: *Zespół uwalniania cytokin:* CRS wystąpił u 93% pacjentów. U jedenastu procent (11%) pacjentów wystąpił CRS stopnia 3. lub wyższego (ciężki, zagrażający życiu oraz prowadzący do zgonu). Mediana czasu do wystąpienia CRS wynosiła 2 dni (zakres od 1 do 12 dni), a mediana czasu trwania 7 dni (zakres od 2 do 29 dni). U dziewięćdziesięciu ośmiu procent pacjentów (98%) doszło do ustąpienia CRS. Najczęściej występujące objawy przedmiotowe i podmiotowe związane z CRS obejmowały gorączkę (83%), niedociśnienie (44%), tachykardię (24%), hipoksję (23%) i dreszcze (20%). Ciężkie działania niepożądane, które mogą być związane z CRS, obejmowały: ostre uszkodzenie nerek, migotanie przedsionków, tachykardię komorową, zatrzymanie akcji serca, niewydolność serca, zespół przesiąkania włośniczek, niedociśnienie, hipoksję i HLH/MAS. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Neurologiczne działania niepożądane:* Neurologiczne działania niepożądane wystąpiły u 67% pacjentów. U trzydziestu dwóch procent (32%) pacjentów wystąpiły działania niepożądane stopnia 3. lub wyższego (ciężkie lub zagrażające życiu). Mediana czasu do wystąpienia działań niepożądanych wynosiła 5 dni (zakres od 1 do 17 dni). Mediana czasu trwania wynosiła 13 dni (zakres: od 1 do 191 dni). U większości pacjentów neurologiczne działania niepożądane ustąpiły, z wyjątkiem 4 pacjentów, u których niepożądane działania neurologiczne utrzymywały się do momentu zgonu; zgony te były spowodowane innymi przyczynami. Najczęstszymi objawami przedmiotowymi i podmiotowymi związanymi z neurologicznymi działaniami niepożądanymi były: encefalopatia (58%), ból głowy (40%), drżenie (31%), zawroty głowy (21%), afazja (18%) i stan majaczeniowy (17%). Zgłaszano ciężkie działania niepożądane, w tym encefalopatię (22%), afazję (4%), stan majaczeniowy (4%) i drgawki (1%), u pacjentów, którym podawano produkt Yescarta. Inne neurologiczne działania niepożądane były zgłaszane rzadziej w badaniach klinicznych i obejmowały dysfagię (5%), zapalenie rdzenia kręgowego (0,2%) i porażenie czterokończynowe (0,2%). W okresie po wprowadzeniu produktu do obrotu zgłoszono wystąpienie obrzęku rdzenia kręgowego, w kontekście neurotoksyczności. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Gorączka neutropeniczna i zakażenia:* Gorączkę neutropeniczną zaobserwowano u 36% pacjentów po infuzji produktu Yescarta. Zakażenia wystąpiły u 39% pacjentów w badaniu ZUMA-1. Zakażenia stopnia 3. lub wyższego (ciężkie, zagrażające życiu lub prowadzące do zgonu) wystąpiły u 26% pacjentów. Zakażenia nieswoistymi patogenami oraz zakażenia bakteryjne i wirusowe stopnia 3. lub wyższego wystąpiły u, odpowiednio, 19%, 9% i 6% pacjentów. Najczęstszym miejscem zakażenia były drogi oddechowe. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Przedłużająca się cytopenia:* Neutropenia (w tym gorączka neutropeniczna), niedokrwistość i małopłytkowość stopnia 3. lub wyższego wystąpiły u, odpowiednio, 80%, 45% i 40% pacjentów. Przedłużająca się (utrzymująca się w dniu 30. lub pojawiająca się w dniu 30. lub później) neutropenia, małopłytkowość i niedokrwistość stopnia 3. lub wyższego wystąpiła u, odpowiednio, 26%, 24% i 10% pacjentów. Utrzymująca się neutropenia, małopłytkowość i niedokrwistość stopnia 3. lub wyższego po dniu 93. wystąpiła u, odpowiednio, 11%, 7% i 3% pacjentów. Wskazówki dotyczące leczenia – patrz punkt 4.4. *Hipogammaglobulinemia:* W badaniu ZUMA-1 hipogammaglobulinemia wystąpiła u 16% pacjentów. Łącznie 33 (31%) ze 108 pacjentów otrzymywało dożylne leczenie immunoglobulinami w momencie przeprowadzania analizy po 24 miesiącach. Wskazówki dotyczące leczenia – patrz punkt 4.4. Immunogenność: Immunogenność produktu Yescarta oceniano za pomocą testu immunoenzymatycznego (ELISA) wykrywającego przeciwciała wiążące FMC63, z których wywodzą się przeciwciała anty-CD19 CAR. U trzech pacjentów przed leczeniem produktem Yescarta badanie przeciwciał anty-FMC63 dało wynik dodatni. Wpływ tych przeciwciał na skuteczność lub bezpieczeństwo stosowania nie był dostrzegalny. Specjalne grupy pacjentów: Doświadczenie dotyczące stosowania produktu Yescarta u pacjentów w wieku 75 lat i starszych jest ograniczone. Na ogół bezpieczeństwo stosowania oraz skuteczność były podobne jak w przypadku pacjentów w wieku ≥ 65 lat i < 65 lat leczonych produktem Yescarta. Wyniki były zgodne z wynikami uzyskanymi w grupach pacjentów z oceną w skali Eastern Cooperative Oncology Group (ECOG) 0 i 1 oraz według płci. Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem *Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, PL-02 222 Warszawa, tel.: +48 22 49 21 301, faks: +48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl.* Działania niepożądane można także zgłaszać za pośrednictwem przedstawiciela podmiotu odpowiedzialnego w Polsce: Gilead Sciences Poland Sp. z o.o., tel.: +48 22 262 87 02 lub e-mail: DrugSafety.Poland@gilead.com. **7. PODMIOT ODPOWIEDZIALNY POSIADAJĄCY POZWOLENIE NA DOPUSZCZENIE DO OBROTU:** Kite Pharma EU B.V., Tufsteen 1, 2132 NT Hoofddorp, Holandia. **8. NUMER POZWOLENIA NA DOPUSZCZENIE DO OBROTU:** EU/1/18/1299/001. **WYDANE PRZEZ:** Komisję Wspólnot Europejskich. **OGÓLNA KATEGORIA DOSTĘPNOŚCI:** Rpz. **9. DATA WYDANIA PIERWSZEGO POZWOLENIA NA DOPUSZCZENIE DO OBROTU I DATA PRZEDŁUŻENIA POZWOLENIA:** Data wydania pierwszego pozwolenia na dopuszczenie do obrotu: 23 sierpnia 2018. **10. DATA ZATWIERDZENIA LUB CZĘŚCIOWEJ ZMIANY TEKSTU CHARAKTERYSTYKI PRODUKTU LECZNICZEGO:** 01/2022. Szczegółowe informacje o tym produkcie leczniczym są dostępne na stronie internetowej Europejskiej Agencji Leków http://www.ema.europa.eu.

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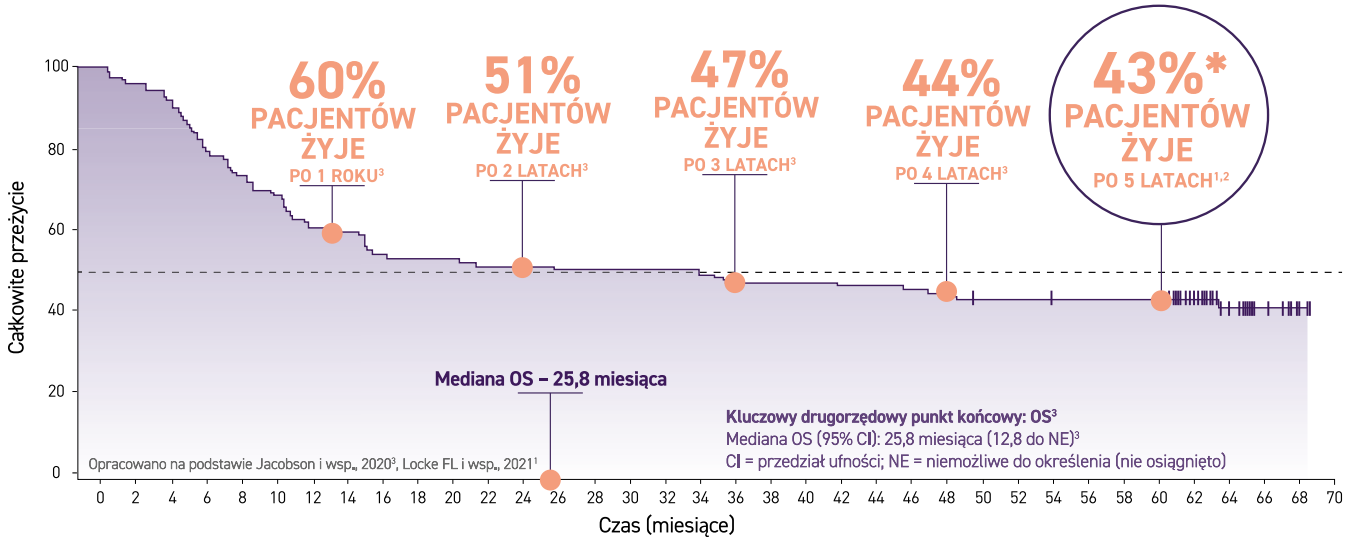
5 LAT TEMU TAKIE PRZEŻYCIA BYŁY MARZENIEM

DZIŚ SĄ RZECZYWISTOŚCIĄ

5-LETNIE CAŁKOWITE PRZEŻYCIE (OS) WYNIOSŁO 43%[1,2]*
w badaniu rejestracyjnym Zuma-1



Pacjenci narażeni																																
101	97	93	80	74	69	61	60	54	53	53	51	51	50	50	50	50	50	47	47	47	46	46	45	44	42	42	41	41	41	41	26	14	6	1	0
(Pacjenci odcięci)																																
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* Wartości szacunkowe wg Kaplana-Meiera dla 5-letniego wskaźnika OS wyniosły 42,6%.

Piśmiennictwo: 1. Locke FL et al. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. N Engl J Med 2021. doi: 10.1056/NEJMoa2116133. 2. Jacobson C et al. Long-term (4- and 5-year) overall survival in ZUMA-1, the pivotal study of axicabtagene ciloleucel in patients with refractory large B-Cell lymphoma (LBCL), Poster 1764 at ASH 2021. 3. Jacobson C et al. Poster presented at: American Society for Hematology Annual Meeting (Virtual); December 5-8, 2020.

PL-YES-0057. Data przygotowania: 02.2022

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