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UNIWERSYTET
MIKOŁAJA KOPERNIKA
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Metabolizm żelaza w świetle stanu jego przeładowania u dzieci z ostrymi białaczkami lub poddawanych transplantacji komórek krwiotwórczych

Rozprawa na stopień doktora nauk medycznych

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Panu prof. dr. hab. n. med. Janowi Styczyńskiemu

dziękuję za motywację i nieocenioną pomoc

wniesioną w powstanie tej rozprawy doktorskiej.

SPIS TREŚCI

WYKAZ STOSOWANYCH SKRÓTÓW

AL	ostra białaczka (acute leukemia)
ALL	ostra białaczka limfoblastyczna (acute lymphoblastic leukemia)
ALT	aminotransferaza alaninowa (alanine aminotransferase)
AML	ostra białaczka szpikowa (acute myeloid leukemia)
AST	aminotransferaza asparaginianowa (aspartate aminotransferase)
CRP	białko C-reaktywne (C-reactive protein)
EFS	przeżycie wolne od choroby (event-free survival)
HCT	przeszczepienie komórek krwiotwórczych (hematopoietic cell transplantation)
KKCz	koncentrat krwinek czerwonych (packed red blood cells)
LDH	dehydrogenaza mleczanowa (lactate dehydrogenase)
LPI	labilna pula żelaza w surowicy (labile plasma iron)
NTBI	żelazo niezwiązane z transferyną (non-transferin bound iron)
OS	czas przeżycia całkowitego (overall survival)
sHJV	rozpuszczalna forma hemojuweliny (soluble hemojuvelin)
TIBC	całkowita zdolność wiązania żelaza (total iron-binding capacity)
RI	częstość występowania wznowy choroby (relapse incidence)

WYKAZ PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

I. Łęcka M, Czyżewski K, Dębski R, Wysocki M, Styczyński J. **Impact of ferritin serum concentration on survival in children with acute leukemia: a long-term follow-up.** *Acta Haematologica Polonica.* 2021; 52:54–60. doi:10.5603 /AHP. 2021.000

MNiSW: 100

II. Łęcka M, Słomka A, Albrecht K, Żekanowska E, Romiszewski M, Styczyński J. **Unbalance in iron metabolism in childhood leukemia converges with treatment intensity: biochemical and clinical analysis.** *Cancers.* 2021; 13:1–12. doi:10.3390 /cancers13123029

IF: 6,57
MNiSW: 140

III. Styczyński J, Słomka A, Łęcka M, i in. **Soluble hemojuvelin and ferritin: potential prognostic markers in pediatric hematopoietic cell transplantation.** *Cancers.* 2023; 15:1–14.doi:10.3390/cancers15041041

IF: 5,20
MNiSW: 200

IV. Słomka A, Łęcka M, Styczyński J. **Hepcidin in children and adults with acute leukemia or undergoing hematopoietic cell transplantation: a systematic review.** *Cancers.* 2022; 14:1–17.doi:10.3390/cancers14194936

IF: 5,20
MNiSW: 140

WSTĘP

Terapia dzieci w trakcie leczenia przeciwnowotworowego lub poddawanych przeszczepieniu komórek krwiotwórczych (HCT) w okresach mielosupresji, wiąże się z częstymi transfuzjami preparatów krwi. Jedna jednostka koncentratu krwinek czerwonych (KKCz) zawiera ok 200 mg żelaza. Stan przeładowania obserwowany jest już po ok 10-20 przetoczeniach. W rezultacie dochodzi do zaburzeń funkcjonowania nie tylko narządów wewnętrznych takich jak serce, płuca czy wątroba, ale również wzrasta ryzyko powikłań infekcyjnych.

Ferrytyna jest najczęściej stosowanym markerem przeciążenia organizmu żelazem. Wadą tego parametru jest jednak niska swoistość. Hyperferrytynemia koreluje z ilością transfuzji KKCz [1,2,3,4,5]. Dane literaturowe dotyczące pacjentów dorosłych pokazują zależność między stężeniem ferrytyny, a czasem przeżycia w grupie pacjentów po HCT. W grupie pacjentów leczonych z powodu zespołu mielodysplastycznego hyperferrytynemia wiąże się z wzrostem ryzyka transformacji białaczkowej [6,7]. Badania te dotyczą głównie populacji osób dorosłych, brakuje danych odnoszących się do dzieci leczonych z powodu ostrych białaczek czy poddawanych HCT.

W literaturze pojawiają się dane dotyczące możliwości oceny innych, prawdopodobnie bardziej precyzyjnych parametrów metabolizmu żelaza. Hepcydyna odkryta w 2000 r., jest białkiem ostrej fazy produkowanym głównie w hepatocytach. Odgrywa kluczową rolę w regulacji metabolizmu żelaza [8]. Parametrem modulującym stężenie hepcydyny jest hemojuwelina [9,10]. Rola hepcydyny w populacji osób dorosłych leczonych z powodu białaczek nie jest jasna. Dostępne są pojedyncze prace ukazujące związek pomiędzy stężeniem hepcydyny w odniesieniu do etapu leczenia onkologicznego, które sugerują istotny wzrost stężenia hepcydyny na początku terapii oraz spadek wraz z remisją choroby [11,12]. Brakuje prac opisujących wykorzystanie hepcydyny i hemojuweliny w grupie pacjentów pediatrycznych leczonych z powodu ostrych białaczek lub poddawanych HCT.

Odkrycie zaburzeń w metabolizmie żelaza u pacjentów onkologicznych pozwala szukać możliwości diagnostycznych i terapeutycznych w białkach regulujących gospodarkę żelaza takich jak hepcydyna [13,14].

ZAŁOŻENIA I CEL PRACY

Rozprawa doktorska oparta jest na cyklu czterech prac opublikowanych w recenzowanych czasopismach w latach 2021-2023. Prace zostały ułożone w ciągu chronologicznym: począwszy od analizy retrospektywnej, następnie prospektywnej analizy laboratoryjnej i klinicznej, następnie cykl zakończono przeglądem systematycznym.

Głównym celem pracy było wykazanie znaczących zburzeń w obrębie badanych parametrów gospodarki żelaza u dzieci leczonych z powodu białaczki lub podawanych transplantacji szpiku kostnego.

Uzasadnieniem do realizacji przeprowadzonych badań były ubogie dane literaturowe opisujące wykorzystanie parametrów metabolizmu żelaza w grupie pacjentów pediatrycznych leczonych z powodu AL czy po HCT.

Cele szczegółowe zrealizowano w poszczególnych publikacjach:

1. publikacja I: ocena wpływu stężenia ferrytyny na odległe wyniki leczenia onkologicznego lub przeszczepiania komórek krwiotwórczych u dzieci z ostrymi białaczkami.
2. publikacja II: określenie roli parametrów metabolizmu żelaza takich jak ferrytyna, hepcydyna, NTBI, LPI w stanach przeładowania żelaza u dzieci leczonych z powodu ostrych białaczek lub poddawanych HCT
3. publikacja III: ocena wartości prognostycznej parametrów metabolizmu żelaza u dzieci leczonych z powodu ostrych białaczek lub poddawanych HCT
4. publikacja IV: dokonanie przeglądu i analizy dostępnej literatury naukowej dotyczącej roli hepcydyny w grupie pacjentów z ostrymi białaczkami lub poddawanych HCT

STRESZCZENIA OPUBLIKOWANYCH PRAC

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

Obecnie większość dzieci z ostrą białaczką można wyleczyć. Przeładowanie żelazem związane z licznymi transfuzjami preparatów krwinek czerwonych w trakcie leczenia onkologicznego i związane z nim powikłania pozostają problemem. Hiperferrytynemia jest obserwowana w tej grupie pacjentów. Celem pracy była ocena wartości prognostycznej ferrytyny w surowicy na długoterminowe wyniki leczenia u dzieci leczonych z powodu AL. Badanie retrospektywne objęło 71 pacjentów leczonych w latach 2005-2011 z powodu ALL (n=54) lub AML (n=17). Analizowano 4 parametry tj. stężenie ferrytyny w surowicy, aktywność ALT, stężenie LDH i CRP. Za marker przeładowania żelazem uznano stężenie ferrytyny w surowicy >1000 µg/l. U 52,1% pacjentów wykazano przeładowanie organizmu żelazem. Stężenie ferrytyny w surowicy korelowało z aktywnością ALT (p = 0,001) i stężeniem CRP (p = 0,012). Łącznie 19 (26,76%) pacjentów zmarło podczas obserwacji. Stężenie ferrytyny było wyższe u pacjentów z AML w porównaniu z ALL. Stwierdzono istotną różnicę w długoterminowych wynikach leczenia w odniesieniu do wysokich stężeń ferrytyny u pacjentów z/bez HCT. W obu grupach pacjenci z wyższymi stężeniami ferrytyny mieli gorsze przeżycia całkowite (OS), gorsze przeżycia wolne od choroby (EFS) oraz większą częstość

nawrotów choroby (RI). Wykazano, że stężenie ferrytyny w surowicy >1000 µg/l jest niekorzystnym markerem prognostycznym przeżycia u dzieci z ostrą białaczką leczonych chemioterapią z HCT lub bez HCT.

Publikacja II: **Unbalance in iron metabolism in childhood leukemia converges with treatment intensity: biochemical and clinical analysis.**

W grupie pacjentów pediatrycznych leczonych z powodu AL lub poddawanych HCT analizowano parametry w kontekście przeładowania żelazem. Do badania prospektywnego włączono 85 dzieci, które podzielono na 4 grupy. Pierwszą grupę stanowiły dzieci bez chorób szpiku i układu czerwonokrwinkowego (grupa kontrolna, n=18), kolejne to dzieci z rozpoznaną białaczką de novo (n= 18), dzieci po zakończonym leczeniu intensywnym białaczki (n=25), dzieci po transplantacji komórek krwiotwórczych (n=21). Przeanalizowano 14 parametrów, które podzielono na 3 grupy: parametry odnoszące się do zasobów żelaza w organizmie (NTBI, LPI, żelazo, transferryna, TIBC, ferrytyna, lekkie i ciężkie łańcuchy ferrytyny), białka regulujące wchłanianie żelaza i jego uwalnianie z tkanek (hepcydyna, rozpuszczalna hemojuwelina, rozpuszczalna ferroportyna-1) oraz białka regulujące erytropoetyczną aktywność szpiku kostnego (erytroferon, erytropoetyna, rozpuszczalny receptor transferyny). Wykazano obecność NTBI i LPI w grupie badanej. U dzieci po HCT najistotniejsze odchylenia stwierdzono w parametrach tj. żelazo, ferrytyna, hepcydyna. Na podstawie badania stwierdzono, że zaburzenia metabolizmu żelaza nasilają się wraz z intensywnością leczenia onkologicznego.

Publikacja III: **Soluble hemojuvelin and ferritin: potential prognostic markers in pediatric hematopoietic cell transplantation.**

Przeładowanie żelazem jest częstym i zagrażającym życiu powikłaniem terapii prowadzonej u pacjentów z ostrymi białaczkami lub poddawanych HCT. W grupie pacjentów pediatrycznych przeanalizowano wartość prognostyczną 12 parametrów metabolizmu żelaza: żelazo, transferryna, TIBC, ferrytyna, łańcuchy ciężkie i lekkie ferrytyny, hepcydyna, sHJV, rozpuszczalna ferroportyna-1, erytroferon, erytropoetyna i rozpuszczalny receptor transferryny. Badanie prospektywne objęło 137 dzieci, w tym grupę badaną stanowili pacjenci z AL po intensywnym leczeniu (n=50) oraz po HCT (n=32). Wyniki grupy badanej odniesiono do 55 pacjentów (dzieci bez chorób szpiku i układu czerwonokrwinkowego oraz dzieci w momencie rozpoznania AL). Mediana czasu obserwacji wyniosła 2,2 roku. Po raz pierwszy wykazano, że wysokie stężenie ferrytyny i niskie stężenie rozpuszczalnej formy hemojuweliny ma niekorzystny wpływ na OS i EFS u dzieci po HCT.

Publikacja IV: **Hepcidin in children and adults with acute leukemia or undergoing hematopoietic cell transplantation: a systematic review.**

Rola hepcydyny w ostrej białaczce czy po przeszczepieniu komórek krwiotwórczych jest niejasna. Celem pracy było podsumowanie dostępnych badań obserwacyjnych. Przegląd systematyczny przygotowano na podstawie wzorca PRISMA. Wyszukiwanie elektroniczne objęło dostępne prace do dnia 31.03.2022 r. w trzech bazach danych: PubMed, Scopus i Web of Science Core Collection. Spośród 3607 zidentyfikowanych tytułów, 13 badań opublikowanych w latach 2008–2021 spełniło kryteria włączenia. W badaniach najczęściej stosowano test immunoenzymatyczny (ELISA) w celu określenia stężenia hepcydyny w surowicy krwi, wyniki były porównywane między grupą badaną a kontrolną. Zarówno u dzieci, jak i dorosłych z AL i po HCT stężenie hepcydyny było wysokie niezależnie od fazy choroby. Wykazano, że leczenie AL i HCT znacząco wpływa na stężenie hepcydyny. Ponad to, stężenie hepcydyny może być markerem prognozującym wyniki liczenia po HCT. Ograniczeniem badania był brak standaryzacji metod laboratoryjnych oznaczania stężenia hepcydyny. Rozbieżności w średnich stężeniach hepcydyny i metodach laboratoryjnych jej oznaczeń utrudniały interpretację i porównanie wyników.

PODSUMOWANIE I WNIOSKI

Przeprowadzone badania, których wyniki opisano w publikacjach wykazały zaburzenia metabolizmu żelaza w badanych grupach pacjentów pediatrycznych z ostrymi białaczkami lub poddawanych przeszczepieniu komórek krwiotwórczych.

Analiza retrospektywna (publikacja I) potwierdziła problem hyperferrytynemii u dzieci leczonych z powodu ostrych białaczek. Stężenie ferrytyny >1000 μg/L obserwowano u 37/ 71 pacjentów. Pacjenci z wyższym stężeniem ferrytyny mieli gorsze przeżycie całkowite (OS) i większą częstość nawrotu choroby (RI).

W publikacji II opisano zaburzenia metabolizmu żelaza w badanej grupie pacjentów pediatrycznych. Potwierdzono obecność toksycznych frakcji żelaza (NTBI, LPI) w grupach badanych, w grupie kontrolnej nie wykryto NTBI. W grupie po HCT uzyskano największą ilość przetoczeń KKCz oraz najwyższe stężenia żelaza, ferrytyny i hepcydyny. Wyniki zostały potwierdzone na większej grupie pacjentów (publikacja III). Dodatkowo wykazano, że wysokie stężenie ferrytyny i niskie stężenie rozpuszczalnej formy hemojuweliny ma niekorzystny wpływ na OS i EFS u dzieci po HCT.

Praca przeglądowa podsumowała dostępne dane literaturowe dotyczące hepcydyny w grupie pacjentów z ostrymi białaczkami lub po HCT.

Wyniki publikacji wchodzących w skład niniejszej rozprawy doktorskiej pozwalają na wyciągnięcie wniosków:

1. przeładowanie organizmu żelazem z powodu licznych transfuzji preparatów krwinek czerwonych jest problemem terapeutycznym u dzieci leczonych z powodu ostrych białaczek

2. stężenie ferrytyny w surowicy >1000 µg/L jest niekorzystnym czynnikiem prognostycznym przeżycia u dzieci z ostrymi białaczkami leczonych chemioterapią z HCT oraz bez przeszczepienia

3. zburzenia metabolizmu żelaza nasilają się wraz z intensywnością leczenia onkologicznego w grupie pacjentów pediatrycznych z ostrymi białaczkami
   - toksyczne frakcje żelaza (NTBI, LPI) narastają u dzieci z ostrymi białaczkami w trakcie chemioterapii oraz po HCT
   - wysokie stężenie ferrytyny i niskie stężenie rozpuszczalnej formy hemojuweliny ma niekorzystny wpływ na OS i EFS u dzieci po HCT

4. hepcydyna jest nowoczesnym parametrem metabolizmu żelaza, który odzwierciedla stan przeładowania organizmu żelazem, jej stężenie koreluje ze stężeniem ferrytyny
   - stężenie hepcydyny wykazuje zmienność w zależności od etapu leczenia onkologicznego
   - możliwość wykorzystania hepcydyny jako markera przeładowania organizmu żelazem wymaga standaryzacji oznaczeń i dalszych badań

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STRESZCZENIE W JĘZYKU POLSKIM

Słowa kluczowe: żelazo, przeładowanie żelazem, ferrytyna, hepcydyna, białaczka, transplantacja szpiku kostnego, dzieci

WSTĘP

Współczesna onkologia dysponuje nowoczesnymi terapiami, dzięki czemu obserwujemy ciągły wzrost skuteczności leczenia dzieci z ostrymi białaczkami lub poddawanych przeszczepieniu komórek krwiotwórczych (HCT). Problemem wciąż pozostają powikłania w trakcie leczenia, jak i te odległe. Konsekwencją licznych przetoczeń preparatów krwinek czerwonych (KKCz) w okresach mielosupresji jest przeładowanie organizmu żelazem. Powoduje to nie tylko zaburzenia w funkcjonowaniu narządów wewnętrznych, ale również zwiększa ryzyko infekcji. Dane literaturowe opisujące zaburzenia w metabolizmie żelaza u dzieci leczonych z powodu AL lub poddawanych HCT są skąpe i wskazują na ferrytynę jako najszerzej używany marker przeładowania żelazem. Odkrycie białek regulujących gospodarkę żelaza tj. hepcydyna pozwala szukać nowych możliwości diagnostycznych i terapeutycznych.

CEL

Rozprawa doktorska jest zbiorem cyklu 4 publikacji obejmujących tematykę przeładowania żelazem. Celem pracy było wykazanie zaburzeń w metabolizmie żelaza u dzieci leczonych z powodu ostrych białaczek oraz poddawanych przeszczepieniu komórek krwiotwórczych.

OMÓWIENIE OPUBLIKOWANYCH PRAC

Publikacja pt. **Impact of ferritin serum concentration on survival in children with acute leukemia: a long-term follow-up** (publikacja I) opisuje wyniki badania retrospektywnego, które objęło 71 dzieci leczonych z powodu ostrych białaczek (ALL, AML) w latach 2005-2011. Przeanalizowano liczbę przetoczeń KKCz oraz stężenie ferrytyny, LDH, aktywność ALT i wartość CRP. Celem badania było wykazanie hyperferrytynemii w badanej grupie pacjentów pediatrycznych oraz ocena wartości prognostycznej ferrytyny na długoterminowe wyniki leczenia. U 52,1 % pacjentów stwierdzono przeładowanie organizmu żelazem. Stężenie ferrytyny było wyższe u pacjentów z AML w porównaniu do ALL. Obserwowano korelację pomiędzy stężeniem ferrytyny a wartością CRP oraz aktywnością ALT. Zarówno w grupie pacjentów po HCT jak i bez przeszczepienia obserwowano zależność między stężeniem ferrytyny a wynikami leczenia. Analiza wyników pokazała, że stężenie ferrytyny w surowicy >1000 µg/L jest niekorzystnym czynnikiem prognostycznym przeżycia u dzieci z ostrymi białaczkami leczonych chemioterapią bez/z HCT.

Praca pt. **Unbalance in iron metabolism in childhood leukemia converges with treatment intensity: biochemical and clinical analysis** (publikacja II) przedstawia wyniki badania prospektywnego przeprowadzonego w latach 2019-2020. Do badania zakwalifikowano 85 dzieci,

wyodrębniono 3 grupy: ostre białaczki de novo, po zakończeniu leczenia intensywnego z powodu ostrej białaczki oraz po przeszczepieniu komórek krwiotwórczych oraz grupę kontrolną (n=18). Przeanalizowano 14 parametrów metabolizmu żelaza, w tym ferrytynę, hepcydynę, hemojuwelinę, NTBI, LPI i inne. Wykazano zaburzenia metabolizmu żelaza w badanej grupie pacjentów pediatrycznych. Wykryto obecność toksycznych frakcji żelaza w badanych grupach pacjentów. Wykazano, że zaburzenia metabolizmu żelaza nasilają się wraz z intensywnością leczenia.

W artykule pt. **Soluble hemojuvelin and ferritin: potential prognostic markers in pediatric hematopoietic cell transplantation** (publikacja III) zaprezentowano wyniki badania prospektywnego przeprowadzonego na grupie 137 pacjentów pediatrycznych. Grupę badaną stanowiły dzieci leczone z powodu AL lub poddawane HCT. Potwierdzono występowanie zaburzeń w metabolizmie żelaza u dzieci z AL lub po HCT oraz zależność ich od intensywności leczenia onkologicznego. Wykazano, że podwyższone stężenie ferrytyny i obniżone stężenie hemojuweliny jest czynnikiem prognostycznie negatywnym na przeżycie u dzieci po HCT.

Publikacja pt **Hepcidin in children and adults with acute leukemia or undergoing hematopoietic cell transplantation: a systematic review** (publikacja IV) jest przeglądem systematycznym, którego celem była analiza dostępnej literatury naukowej dotyczącej roli hepcydyny w grupie pacjentów z ostrymi białaczkami lub poddawanych HCT. Z 3607 publikacji wyodrębniono zgodnie z kryteriami włączenia 13 prac. Tylko 4 badania odnosiły się do dzieci. Opisano wyższe stężenia hepcydyny w grupie pacjentów z ostrymi białaczkami lub po HCT w porównaniu do grup kontrolnych oraz zmienność poziomu hepcydyny w zależności od etapu leczenia onkologicznego. Dodatkowo, analiza ujawniła brak standaryzacji metod w oznaczeniach laboratoryjnych hepcydyny.

PODSUMOWANIE

Każda z przedstawionych prac uzupełnia skąpe dane dotyczące zaburzeń w metabolizmie żelaza u dzieci leczonych z powodu ostrych białaczek lub poddawanych przeszczepieniom komórek krwiotwórczych. Wyniki przeprowadzonych badań pokazują jednoznacznie konsekwencję licznych transfuzji KKCz jaką jest przeładowanie organizmu żelazem w badanej grupie pacjentów pediatrycznych. Intensywność leczenia onkologicznego wpływa na zaburzenia w metabolizmie żelaza. Ocena stężenia ferrytyny oraz hepcydyny odzwierciedla stan przeładowania żelazem. Niezależnie od przedstawionych wniosków potencjalne wykorzystanie parametrów takich jak hepcydyny, hemojuweliny, NTBI czy LPI w pracy klinicznej wymaga dalszych badań na większej grupie pacjentów.

STRESZCZENIE W JĘZYKU ANGIELSKIM/ SUMMARY

Key words: iron, iron overload, ferritin, hepcidin, leukemia, hemopoietic cell transplantation, children

INTRODUCTION

Modern therapies in pediatric oncology result in increasing effectiveness of treatment of children with acute leukemia (AL) or undergoing hematopoietic cell transplantation (HCT). Patients experiencing myelosuppression are in need of multiple transfusions of red blood cells. That leads to iron overload and its long-term complications. Data describing disorders in iron metabolism of children treated for AL or undergoing HCT is scarce. Discovery of hepcidin, a new iron regulatory protein may open up diagnostic and therapeutic possibilities.

RESULTS

The results of the study were published in a series of four publications. The aim of the study was to analyze disorders in iron metabolism of children treated for acute leukemias and undergoing hematopoietic cell transplantation.

The article **Impact of ferritin serum concentration on survival in children with acute leukemia: a long-term follow up** describes the results of a retrospective study that included 71 children treated for acute leukemia (ALL, AML) between 2005 and 2011. The number of RBC transfusions and serum ferritin concentration, serum transaminases activity, lactic dehydrogenase and C-reactive protein levels (CRP) were analyzed. The aim of the study was to evaluate the prognostic value of serum ferritin on long-term outcomes. Iron overload was observed in 52.1% of patients. Children treated for AML have had higher ferritin level compared to ALL. There was a correlation between ferritin concentration and alanine aminotransferase activity and CRP concentration. Both in the group of patients after HCT and without transplantation, there were differences in long-term outcomes with respect to high ferritin concentrations. Analysis of the results showed that serum ferritin concentration >1000 μg/L is an adverse prognostic marker of survival in children with acute leukemia treated with chemotherapy without/ with HCT.

In a paper entitled **Unbalance in iron metabolism in childhood leukemia converges with treatment intensity: biochemical and clinical analysis** results of prospective study conducted in 2019-2020 are presented. 85 children were qualified for the study, three groups were distinguished: de novo acute leukemia, after completion of intensive treatment for acute leukemia and after hematopoietic cell transplantation and a control group (n=18). 14 parameters of iron metabolism were analyzed, including ferritin, hepcidin, hemojuvelin, NTBI, LPI and others. Iron metabolism disorders were demonstrated in the studied group of pediatric patients. The presence of toxic iron fractions was detected in the studied patient groups. It has been shown that disturbances in iron metabolism increase with the intensity of treatment.

The article **Soluble hemojuvelin and ferritin: potential prognostic markers in pediatric hematopoietic cell transplantation** describes the results of a prospective study conducted on a group of 137 pediatric patients. The study group included children treated for AL or undergoing HCT. The occurrence of disorders in iron metabolism in children with AL or after HCT and their dependence on the intensity of oncological treatment were confirmed. It has been shown that increased ferritin levels and decreased hemojuvelin levels are negative prognostic factors for survival in children after HCT.

The purpose of systematic review **Hepcidin in children and adults with acute leukemia or undergoing hematopoietic cell transplantation** was to summarize the observational studies on hepcidin in patients treated for acute leukemia or undergoing hematopoietic cell transplantation. From 3687 publications, 13 papers were identified in accordance with the inclusion criteria. Only 4 studies focused on children. Higher hepcidin concentrations have been described in the group of patients with acute leukemia or after HCT compared to control groups, as well as variability of hepcidin levels depending on the stage of oncological treatment. Additionally, the analysis revealed a lack of standardization of methods in laboratory determinations of hepcidin.

CONCLUSIONS

Each of the presented studies complements the scarce data on iron metabolism disorders in children treated for acute leukemia or undergoing hematopoietic cell transplantation. The results of this study clearly show that the consequence of numerous RBC transfusions is the overload of the body with iron in the studied group of pediatric patients. The intensity of oncological treatment affects disorders in iron metabolism. The assessment of ferritin and hepcidin levels reflects the state of iron overload. Regardless of the presented conclusions, the potential use of parameters such as hepcidin, NTBI or LPI in clinical work requires further research on a larger group of patients.

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KB 169/2020 Bydgoszcz, 31.03.2020 r.

Działając na podstawie art.29 ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz.U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), rozporządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz.U. Nr 47 poz.480) oraz Zarządzenia Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(skład podano w załączeniu), na posiedzeniu w dniu **31.03.2020 r.** przeanalizowała wniosek, który złożył kierownik badania:

prof. dr hab. n. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Szpital Uniwersytecki nr 1 w Bydgoszczy

z zespołem w składzie

- lek. Monika Łęcka, dr n. med. Monika Pogorzała, dr n. med. Robert Dębski,

w sprawie badania:

„Badanie wpływu stężenia ferrytyny na wyniki leczenia pacjentów leczonych z powodu chorób nowotworowych lub poddawanych przeszczepieniu komórek krwiotwórczych."

Po zapoznaniu się ze złożonym wnioskiem i w wyniku przeprowadzonej dyskusji oraz głosowania Komisja podjęła:

Uchwałę o pozytywnym zaopiniowaniu wniosku

w sprawie przeprowadzenia badań w zakresie określonym we wniosku pod warunkiem uzyskania zgody osób badanych na przetwarzanie danych osobowych w celach naukowych, a w przypadku braku takiej zgody, analizowania jedynie danych zanonimizowanych, pozbawionych danych personalnych (zgodnie z RODO). Zgoda obejmuje tylko dane z dokumentacji uczestników badania z okresu od 01.01.2004r. do 31.12.2011 r.

Zgoda obowiązuje od daty podjęcia uchwały (31.03.2020 r.) do końca 2020 r.

Wydana opinia dotyczy tylko rozpatrywanego wniosku z uwzględnieniem przedstawionego projektu; każda zmiana i modyfikacja wymaga uzyskania odrębnej opinii

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:
prof. dr hab. n. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Szpital Uniwersytecki nr 1 w Bydgoszczy

Uniwersytet Mikołaja Kopernika w Toruniu
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Ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, tel.(052) 585-35-63, fax.(052) 585-38-11

KB 608/2019

Bydgoszcz, 25.06.2019 r.

Działając na podstawie art.29 Ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz.U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), zarządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz.U.Nr 47 poz.480) oraz Zarządzeniem Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(skład podano w załączeniu), na posiedzeniu w dniu **25.06.2019 r.** przeanalizowała wniosek, który złożył kierownik badania:

prof. dr. hab. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Szpital Uniwersytecki nr 1 w Bydgoszczy

z zespołem w składzie

- dr n. med. Krzysztof Czyżewski, lek. med. Monika Łęcka,

w sprawie badania:

„Metabolizm żelaza w świetle stanu jego przeładowania (hyperferrytynemii) u dzieci z ostrymi białaczkami lub poddawanych transplantacji komórek krwiotwórczych."

Po zapoznaniu się ze złożonym wnioskiem i w wyniku przeprowadzonej dyskusji oraz głosowania Komisja podjęła

Uchwałę o pozytywnym zaopiniowaniu wniosku

w sprawie przeprowadzenia badań, w zakresie określonym we wniosku pod warunkiem:

- poinformowania uczestników badania w tym również uczestników stanowiących grupę kontrolną, o celu oraz zakresie badań i uzyskania od nich, lub w przypadku osób małoletnich, ich rodziców/opiekunów prawnych osobnej, pisemnej, świadomej zgody na udział w badaniu, zgodnie z obowiązującymi przepisami, datowanej najpóźniej na moment rozpoczęcia badania a nie wcześniej niż data uzyskania z Komisji Bioetycznej zgody na takie badanie;
- UWAGA! W przypadku małoletnich, którzy ukończyli 16 lat życia oraz młodszych małoletnich, którzy są w stanie z rozeznaniem wypowiedzieć się, co do swojego udziału w badaniu obowiązuje również konieczność uzyskania zgody od tych małoletnich;
- zapewnienia, że osoby uczestniczące w eksperymencie badawczym nie są ubezwłasnowolnione, nie są żołnierzami służby zasadniczej, nie są osobami pozbawionymi wolności, nie pozostają w zależności służbowej, dydaktycznej lub innej z prowadzącym badanie;
- UWAGA! Uczestnicy badania stanowiący grupę kontrolną nie mogą być rekrutowani spośród studentów lub pracowników Collegium Medicum w Bydgoszczy.
- zachowania tajemnicy wszystkich danych, w tym danych osobowych pacjentów, umożliwiających ich identyfikację w ewentualnych publikacjach;

- sugerujemy uzyskanie podpisu uczestnika badania pod informacją o badaniu, lub sporządzenie formularza informacji i świadomej zgody na udział w badaniu na jednej kartce.

Jednocześnie informujemy, iż „Zgoda na udział w badaniu" winna zawierać m.in.: imię i nazwisko badanej osoby; Nr historii choroby pacjenta (L.ks.gł. Oddziału/Poradni) oraz datę i podpis badanej osoby, a także klauzule, że uczestnik badania wyraża zgodę na przetwarzanie danych osobowych dotyczących realizacji tematu badawczego, z wyjątkiem publikacji danych osobowych.

Kierownik badania zobowiązany jest do przechowywania wszystkich dokumentów dotyczących badania przez okres dwudziestu lat.

Zgoda obowiązuje od daty posiedzenia (25.06.2019 r.) do końca 2021 r.

Wydana opinia dotyczy tylko rozpatrywanego wniosku z uwzględnieniem przedstawionego projektu; każda zmiana i modyfikacja wymaga uzyskania odrębnej opinii. Wnioskodawca zobowiązany jest do informowania o wszelkich poprawkach, które mogłyby mieć wpływ na opinię Komisji oraz poinformowania o zakończeniu badania.

Od niniejszej uchwały podmiot zamierzający przeprowadzić eksperyment medyczny, kierownik zakładu opieki zdrowotnej, w której eksperyment medyczny ma być przeprowadzony, mogą wnieść odwołanie do Odwoławczej Komisji Bioetycznej przy Ministrze Zdrowia, za pośrednictwem Komisji Bioetycznej przy Collegium Medicum im. L. Rydygiera w Bydgoszczy, w terminie 14 dni od daty otrzymania niniejszej Uchwały.

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:
prof. dr. hab. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
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Uniwersytet Mikołaja Kopernika w Toruniu

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Ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, tel.(052) 585-35-63, fax.(052) 585-38-11

KB 608/2016 Bydgoszcz, 24.09.2019 r.

Działając na podstawie art.29 Ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz. U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), zarządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz. U. Nr 47 poz.480) oraz Zarządzeniem Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(której skład podano w załączeniu) na posiedzeniu w dniu **24.09.2019 r.** przeanalizowała prośbę o wyrażenie zgody na:

- rozszerzenie zespołu badawczego o wykonawców badania z Kliniki Onkologii, Hematologii Dziecięcej, Transplantologii Klinicznej i Pediatrii; Uniwersyteckie Centrum Kliniczne, Warszawski Uniwersytet Medyczny w składzie: prof. dr hab. med. Michał Matysiak, dr n. med. Michał Romiszewski, dr n. med. Monika Pogorzała,

którą złożył:

prof. dr. hab. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Szpital Uniwersytecki nr 1 w Bydgoszczy

w sprawie badania:

„Metabolizm żelaza w świetle stanu jego przeładowania (hyperferrytynemii) u dzieci z ostrymi białaczkami lub poddawanych transplantacji komórek krwiotwórczych. "

Po zapoznaniu się ze złożonym dokumentem i w wyniku przeprowadzonej dyskusji oraz głosowania jawnego Komisja przyjęła do wiadomości podane informacje i wyraża zgodę na powyższe pod warunkami określonymi w uchwale Komisji podjętej w dniu 25.06.2019 r.

Zgoda na kontynuowanie przedmiotowego badania obowiązuje do końca 2021 r.

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:

prof. dr. hab. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
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KB 608/2019

Bydgoszcz, 15.03.2022 r.

Działając na podstawie art.29 ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz.U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), rozporządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz.U. Nr 47 poz.480) oraz Zarządzenia Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(której skład podano w załączeniu) na posiedzeniu w dniu **15.03.2022 r.** przeanalizowała prośbę o:

- przedłużenie terminu prowadzenia badań do końca 2023 roku
- rozszerzenie zespołu badawczego o: dr n. med. Małgorzatę Kubicką, dr n. med. Beatę Kuryło-Rafińską, dr n. med. Barbarę Tejza, dr hab. n. med. Grażynę Gadomską, prof. UMK, dr Małgorzatę Michalską.

którą złożył:

prof. dr hab. n. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Collegium Medicum w Bydgoszczy, UMK w Toruniu

w sprawie badania:

„Metabolizm żelaza w świetle stanu jego przeładowania (hyperferrytynemii) u dzieci z ostrymi białaczkami lub poddawanych transplantacji komórek krwiotwórczych".

Po zapoznaniu się ze złożonym dokumentem i w wyniku przeprowadzonej dyskusji oraz głosowania jawnego Komisja przyjęła do wiadomości podane informacje i wyraża zgodę na powyższe pod warunkami określonymi w uchwale Komisji podjętej w dniu 25.06.2019 r. oraz w ewentualnych aneksach do tejże uchwały.

Zgoda na kontynuowanie przedmiotowego badania obowiązuje do końca 2023 r.

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:

prof. dr hab. n. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Collegium Medicum w Bydgoszczy, UMK w Toruniu

OŚWIADCZENIA WSPÓŁAUTORÓW

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Toruń, dnia 29.11.2023 r.

Monika Łęcka
Katedra Pediatrii, Hematologii i Onkologii
Uniwersytet Mikołaja Kopernika
Collegium Medicum im. L. Rydgiera w Bydgoszczy

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika

Oświadczenie autora

Niniejszym oświadczam, że w pracy oryginalnej pt. Impact of ferritin serum concentration on survival in children with acute leukemia: a long term follow- up. *Acta Haematologica Polonica*. 2021; 52:54-60 mój wkład w powstanie pracy polegał na opracowaniu koncepcji pracy, zebraniu i interpretacji danych oraz przygotowaniu publikacji wraz z jej weryfikacją i ostatecznym zatwierdzeniem.
Mój udział w powstaniu pracy wynosi 60 %.

Oświadczam, ze mój wkład w powstanie pracy oryginalnej pt. Unbalance in iron metabolism in childhood leukemia converges with treatment intensity: biochemical and clinical analysis. *Cancers*. 2021;13:1-12 polegał na udziale zebraniu i analizie danych oraz przygotowaniu manuskryptu wraz z jego weryfikacją i zatwierdzeniem.
Mój udział w powstaniu pracy wynosi 50 %.

Oświadczam, ze mój wkład w powstanie pracy oryginalnej pt. Hepcidin in children and adults with acute leukemia or undergoing hematopoietic cell transplantation: a systematic review. *Cancers*. 2022;14:1–17 polegał na dokonaniu przeglądu dostępnej literatury, wyodrębnieniu publikacji spełniających założenia pracy, analizie uzyskanych danych oraz na przygotowaniu tabel i manuskryptu wraz z weryfikacją i ostatecznym zatwierdzeniem pracy.
Mój udział w powstaniu pracy wynosi 40 %.

Oświadczam, że mój wkład w powstanie pracy oryginalnej pt. Soluble hemojuvelin and ferritin: potential prognostic markers in pediatric hematopoietic cell transplantation. *Cancers*. 2023;15:1–14 polegał na zebraniu i analizie danych oraz przygotowaniu manuskryptu wraz z jego weryfikacją i zatwierdzeniem.
Mój udział w powstaniu pracy wynosi 10 %.

Monika Łęcka

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Toruń, dnia 29.11.2023 r.

prof. dr hab. n. med. Jan Styczyński
Katedra Pediatrii, Hematologii i Onkologii
Uniwersytet Mikołaja Kopernika
Collegium Medicum im. L. Rydgiera w Bydgoszczy

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika

Oświadczenie o współautorstwie

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Robert Dębski

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ORIGINAL RESEARCH ARTICLE

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

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Abstract

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

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

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

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

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

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

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Introduction

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

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

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

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

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

Material

Study design

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

Collection of data

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

Treatment of leukemia

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

Transplant procedures

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

Definitions

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

Statistical analysis

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

Results

Demographics

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

Table I. Patient characteristics

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

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

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

Ferritin concentration

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

Survival of patients

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

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

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

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

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

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

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

Risk factor analysis

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

Discussion

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

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

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

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

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

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

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

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

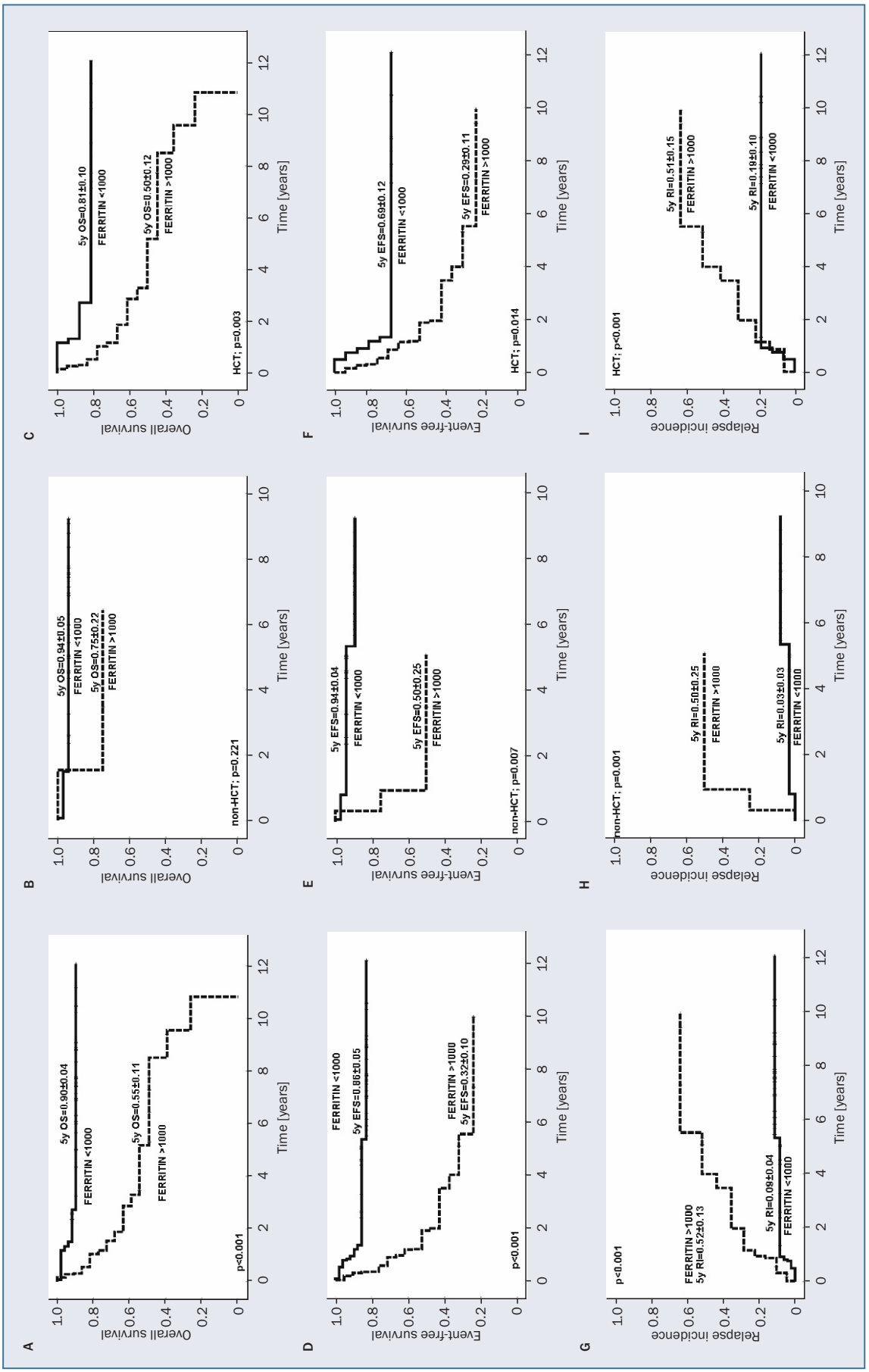

Figure 1. Long-term outcomes [overall survival (OS; **A–C**), event-free survival (EFS; **D–F**), and relapse incidence (RI; **G–I**)] with respect to ferritin serum concentration. Results for all patients (**A**, **D**, **G**), non-haematopoietic cell transplantation (HCT) (**B**, **E**, **H**) and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

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

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

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

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

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

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

Conclusion

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

Authors' contributions

JS, MŁ — study design and manuscript writing; MŁ, RD, KC, JS — collection and analysis of data. All authors — critical review and final approval.

Conflict of interest

None.

Financial support

None.

Ethics

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

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Unbalance in Iron Metabolism in Childhood Leukemia Converges with Treatment Intensity: Biochemical and Clinical Analysis

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Simple Summary: In children undergoing therapy for acute leukemia or after hematopoietic cell transplantation, the following iron metabolism parameters were analyzed in the context of iron overload: (1) parameters measuring functional and storage iron pools: non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) levels, iron, transferrin, total iron-binding capacity, ferritin, ferritin heavy and light chains; (2) proteins regulating iron absorption and its release from tissue stores: hepcidin, soluble hemojuvelin, soluble ferroportin-1; (3) proteins regulating the erythropoietic activity of bone marrow: erythroferrone, erythropoietin, soluble transferrin receptor. It has been shown that the occurrence of NTBI and LPI in the circulation and the intensification of disturbances in iron metabolism were associated with the intensity of anti-leukemic treatment and were the highest in the transplant group followed by the acute leukemia after treatment and de novo groups. In patients after transplantation, the most significant changes were found in NTBI, LPI, iron, ferritin, hepcidin, and ferroportin-1 levels.

Abstract: Objective: The aim of this study was to evaluate non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) levels and other parameters of iron metabolism in children undergoing therapy for acute leukemia or after hematopoietic cell transplantation (HCT), in the context of iron overload. Patients: A total number of 85 children were prospectively included into four groups: controls, acute leukemia de novo, acute leukemia after intensive treatment, and after HCT. Methods: The following iron metabolism parameters were analyzed: (1) parameters measuring functional and storage iron pools: NTBI, LPI, iron, transferrin, total iron-binding capacity, ferritin, ferritin heavy and light chains; (2) proteins regulating iron absorption and its release from tissue stores: hepcidin, soluble hemojuvelin, soluble ferroportin-1; (3) proteins regulating the erythropoietic activity of bone marrow: erythroferrone, erythropoietin, soluble transferrin receptor. Results: Intensive treatment of leukemia in children was associated with the presence of serum NTBI and LPI, which was the highest in the HCT group followed by the acute leukemia after treatment and de novo groups. In patients after HCT, the most significant changes were found in NTBI, LPI, iron, ferritin, hepcidin, and ferroportin-1 levels. Conclusions: The occurrence of NTBI and LPI in the circulation and the intensification of disturbances in iron metabolism were associated with the intensity of the anti-leukemic treatment.

Keywords: children; acute leukemia; hematopoietic cell transplantation; iron metabolism

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1. Introduction

Iron overload is a common secondary complication in patients treated for acute leukemia or undergoing hematopoietic cell transplantation (HCT), resulting from frequent red blood cell transfusions [1–3]. Each milliliter of transfused red cells contains 0.8 mg iron [4], and thus multiple transfusions contribute to rapid iron accumulation. Transfusional iron overload increases the risk of infectious complications and the proliferation of malignant cells [5], associated with an increased risk of veno-occlusive disease (also known as hepatic sinusoidal obstruction syndrome), incidence of graft-versus-host disease, non-relapse mortality, and reduced overall survival [6]. The evidence of the deleterious effect of iron burden on the outcomes of transplantation comes mainly from studies analyzing serum ferritin as a marker for iron overload.

In the case of iron overload, the iron-binding ability of transferrin is highly exceeded, and thus non-transferrin-bound iron (NTBI) appears in the blood [1,7]. NTBI usually rises markedly with the increase in transferrin saturation up to 70%, whereas a highly reactive Fe^{2+} species of NTBI referred to as labile plasma iron (LPI) increases simultaneously [1,7]. Therefore, the risk of an imbalance in the entire iron homeostasis should be taken into account. The toxicity of iron results from the Fe^{2+} forms of iron, which are highly reactive and cause rapid oxidant damage of proteins and DNA, permanently changing the structure of proteins and genetic material [7,8]. This process is mainly caused by the excess of NTBI and its fraction LPI [2].

NTBI is a toxic, low-molecular weight fraction of iron, which is usually detectable in iron-overloaded patients. NTBI can also be present in patients during cytotoxic chemotherapy, contributing to organ damage following chemotherapy [9]. NTBI possibly reflects a significant disturbance in iron utilization which is related to its release from dying cells in bone marrow and possibly other tissues, as well as liver toxicity and decreased transferrin production [10,11]. NTBI has a heterogenous character related to the degree, duration, and etiology of iron overload. LPI is the most toxic fraction of NTBI components and represents deleterious, organ-penetrating redox-active forms of iron. It is a labile and chelatable form that can induce tissue iron overload and permeate into organs, causing their damage [12,13]. LPI itself, more than NTBI, can provide a direct insight into chelatable iron which is not explained by other diagnostic tests of iron metabolism such as ferritin and transferrin saturation [12,14].

Iron metabolism is also regulated by a number of proteins. The principal regulator of iron homeostasis is hepcidin, a 25-amino acid protein secreted primarily by hepatocytes [15]. Hepcidin regulates iron absorption and release from tissue stores by targeting ferroportin and downregulates the entry of iron into the plasma by downregulating ferroportin, the sole cellular iron exporter [16]. Hepcidin itself is also subject to complex molecular control involving, among other factors, hemojuvelin (HJV), which, depending on its form, has opposite functions. Membrane HJV stimulates hepcidin synthesis. Still, the blood-circulating form (sHJV) significantly reduces its production [17,18]. The role of circulating ferroportin (FNP-1) remains a subject that is undergoing intense research, both in its release mechanisms from the cell membrane and its biological functions. It is known that the membrane form of this protein is a transporter involved in the transfer of iron from the cells to the bloodstream, which is degraded by the action of hepcidin. The concentration of FNP-1 in the blood seems to reflect the amount of the membrane form of this protein, just as the soluble transferrin receptor (sTfR) demonstrates the expression of the membrane receptor for transferrin.

Erythroferrone (ERFE) is the main regulator of hepcidin synthesis. Increased ERFE suppresses hepcidin synthesis, leading to mobilization of cellular iron stores and its use in heme and hemoglobin synthesis. Overproduction of ERFE and suppressing hepcidin can cause iron overload, even in non-transfused patients. ERFE can be regarded as a biomarker of ineffective erythropoiesis and a possible therapeutic target [19].

Due to frequent red blood cell transfusions, patients with acute leukemia or undergoing HCT are at increased risk of iron overload and its consequences. However, the precise

mechanisms behind these processes are far from being understood. They are even less known in pediatric leukemia patients and children after HCT. Thus, the purpose of this prospective study was to evaluate NTBI and LPI levels in children undergoing therapy for acute leukemia or after engraftment post-HCT and find relations between them and other parameters of iron metabolism, in the context of iron overload.

2. Materials and Methods

Study design. Pediatric patients with acute leukemia or after HCT, qualifying for the study, were analyzed for parameters of iron metabolism at a specific time point, in the context of blood transfusions and iron overload.

Patients. A total number of 85 patients (45 boys and 40 girls), with a median age of 7 (range 0–19) years, treated in two pediatric hematology and oncology centers between June 2019 and July 2020, were included into the study. Patients were enrolled into 4 groups: controls (group I), acute leukemia de novo (group II), acute leukemia after intensive treatment (group III), and patients after HCT (group IV) (Table 1). Serum samples were obtained at admission in group I, at the time of diagnosis in group II, within one month after consolidation therapy in group III, and one month after HCT in group IV. Children in groups III and IV were, after finalizing the respective periods of treatment, in an overall good condition, without signs of severe infection. Children with leukemia de novo were treated according to the AIEOP-BFM-ALL-2017 protocol or the AML-BFM-2019 protocol. Patients in group III completed intensive multiagent chemotherapy, usually complicated with frequent hematological adverse events and blood transfusions; during inclusion to the study, they were on oral maintenance chemotherapy. Patients who underwent HCT qualified according to the chemotherapy protocols. They were diagnosed with acute lymphoblastic leukemia (ALL, n = 6), acute myeloblastic leukemia (AML, n = 8), and other diagnoses including myelodysplastic syndrome (MDS, n = 1), severe aplastic anemia (SAA, n = 1), severe congenital neutropenia (SCN, n = 1), anaplastic large B-cell lymphoma (ALCL, n = 1), Ewing sarcoma (ES, n = 1), and neuroblastoma (NBL, n = 2), who underwent allogeneic transplantations in 18 (including 17 from matched unrelated donors, and 1 from family donor) cases and autologous ones in another 3. All transplantations were performed after myeloablative conditioning, except in 2 patients with SAA/SCN, who received reduced-intensity conditioning.

Table 1. Patient characteristics.

Characteristics	Total (%) (n = 85)	Group I (n = 18)	Group II (n = 21)	Group III (n = 25)	Group IV (n = 21)
Age (years)					
Median (range)	7 (0–19)	8 (2–16)	7 (0–17)	5 (1–19)	8 (1–19)
Years: <10 vs. >10	59 (69.5):26 (30.5)	11 (61.1):7 (38.9)	16 (76.2):5 (23.8)	23 (92.0):2 (8.0)	9 (42.8):12 (57.2)
Gender					
male/female (%)	45 (52.9):40 (45.1)	8 (44.4):10 (55.6)	12 (57.2):9 (48.8)	13 (52.0):12 (48.0)	12 (57.2):9 (42.8)
Diagnosis					
ALL (%)	48 (56.4)	0	19 (90.4)	23 (92.0)	6 (28.6)
AML (%)	12 (0.1)	0	2 (9.5)	2 (8.0)	8 (38.1)
Other (%)	25 (0.3)	18 (100.0)	0	0	7 (33.3)
HCT (%)	21 (0.2)	0	0	0	21 (100)
PRBC transfusions					
>5 units (%)		0	2 (9.5)	23 (92.0)	21 (100)
>10 units (%)		0	1 (4.8)	11 (44.0)	19 (90.4)
>20 units (%)		0	0	2 (8.0)	15 (71.4)

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; HCT, hematopoietic cell transplantation; PRBC, packed red blood cell concentrate.

The control group was composed of healthy children with no history of any transfusions or hematological disorders. Overall, 60 (70.5%) children were transfused with concentrates of packed red blood cells (PRBC) (all patients in groups III and IV, none in group I, and 14/21 in group II). Patients received a median of 5 (range: 0–99) units of PRBC (including median of 1 unit in group II, median of 9 units in group III, and median of 23 units in group IV). At the time of analysis, 6/85 (7.1%) patients died.

Collection of samples. Venous blood samples were collected from each participant under fasting conditions and placed into serum tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Blood samples were allowed to clot for 30 min at room temperature and then were centrifuged for 20 min at 2000× *g* at room temperature. Serum was collected and stored at −80 °C until analyses. Serum samples from hemolyzed blood were excluded.

Iron metabolism parameters. To assess iron metabolism as comprehensively as possible, 14 laboratory parameters were analyzed in this study. They included three categories of markers: (1) parameters measuring functional and storage iron pools (NTBI, LPI, iron, transferrin, total iron-binding capacity (TIBC), ferritin, ferritin heavy chain (FTH1), and ferritin light chain (FTL)); (2) proteins regulating the absorption of iron and its release from the tissue stores (hepcidin (25-amino acid isoform), soluble hemojuvelin (sHJV), and soluble ferroportin-1 (sFNP-1)); (3) proteins regulating the erythropoietic activity of bone marrow (erythroferrone (ERFE), erythropoietin (EPO), and soluble transferrin receptor (sTfR)).

Determination of NTBI and LPI. Serum NTBI and LPI levels were determined at Savyon Diagnostics Ltd. (Ashdod, Israel) using fluorescence-based assays FeROS™eLPI and FeROS™LPI kit, respectively. The results are given in LPI units. NTBI and LPI were considered positive at ≥0.2 LPI units. The results below this value were regarded negative.

Reagents. Determination of hepcidin (Intrinsic Hepcidin IDx™ ELISA Kit, ICE-007, Intrinsic Life Sciences, La Jolla, CA, USA), sHJV (ELISA Kit for Hemojuvelin (HJV), CEB995Hu, Cloud-Clone Corp., Katy, TX, USA), sFNP-1 (Human SLC40A1/Ferroportin-1 (Sandwich ELISA) ELISA Kit, LS-F33705, LifeSpan BioSciences, Inc., Seattle, WA, USA), ERFE (Intrinsic Erythroferrone IE™ ELISA Kit, ERF-001, Intrinsic Life Sciences, La Jolla, CA, USA), EPO (Erythropoietin Human ELISA, RAF013R, BioVendor-Laboratorni medicina a.s., Brno, Czech Republic), sTfR (sTfR Human ELISA (soluble Transferrin Receptor), RD194011100, BioVendor-Laboratorni medicina a.s., Brno, Czech Republic), FTH1 (Human FTH1/Ferritin Heavy Chain (Sandwich ELISA) ELISA Kit, LS-F26901, LifeSpan BioSciences, Inc., Seattle, WA, USA), FTL (Human FTL/Ferritin Light Chain (Sandwich ELISA) ELISA Kit, LS-F26902, LifeSpan BioSciences, Inc., Seattle, WA, USA), and transferrin (Transferrin Human ELISA Kit, EHTF, Thermo Fisher Scientific, Waltham, MA, USA) levels was conducted using a highly specific and sensitive enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. ELISA kits used in our study and their precise characterizations, including assay range, detection limit, and intra- and inter-assay precision, are shown in Table 2. Coefficients of variation (CVs) for all kits were lower than 10% for intra-assay and lower than 15% for inter-assay. Investigators who performed all the study's assays were blind to clinical characteristics and patients' outcomes.

Table 2. The main features of enzyme-linked immunosorbent assay (ELISA) kits used in the current study.

Kit	Manufacturer	Assay Range	Detection Limit	Intra-Assay Coefficient of Variation (CV, %)	Inter-Assay Coefficient of Variation (CV, %)
Intrinsic Hepcidin IDx™ ELISA Kit (ICE-007)	Intrinsic Life Sciences (La Jolla, CA, USA)	5–250 ng/mL	2.5 ng/mL	2.4–4.0	2.6–3.8
Intrinsic Erythroferrone IE™ ELISA Kit (ERF-001)		0.16–10 ng/mL	0.02 ng/mL	4.7–6.7	7.0–14.9
ELISA Kit for Hemojuvelin (HJV) (CEB995Hu)	Cloud-Clone Corp. (Katy, TX, USA)	12.35–1000 ng/mL	4.93 ng/mL	<10	<12
Erythropoietin Human ELISA (RAF013R)	BioVendor-Laboratorni medicina a.s., (Brno, Czech Republic)	1.6–100 mIU/mL	0.14 mIU/mL	6.2	4.3
sTfR Human ELISA (soluble Transferrin Receptor) (RD194011100)		0.05–2 µg/mL	0.002 µg/mL	6.8	6.3
Transferrin Human ELISA Kit (EHTF)	Thermo Fisher Scientific (Waltham, MA, USA)	1.029–750 ng/mL	1 ng/mL	<10	<12
Human SLC40A1/Ferroportin-1 (Sandwich ELISA) ELISA Kit (LS-F33705)	LifeSpan BioSciences, Inc. (Seattle, WA, USA)	0.156–10 ng/mL	0.094 ng/mL	<8	<10
Human FTH1/Ferritin Heavy Chain (Sandwich ELISA) ELISA Kit (LS-F26901)		50–1000 pg/mL	1 pg/mL	<9	<10
Human FTL / Ferritin Light Chain (Sandwich ELISA) ELISA Kit (LS-F26902)		100–2500 pg/mL	1 pg/mL	<10	<12

Clinical parameters. Other iron status markers, including serum iron, ferritin, and TIBC, were measured using standard methods at the central hospital laboratory. Laboratory markers of inflammation, i.e., C-reactive protein (CRP) and procalcitonin (PCT), were also measured at the central hospital laboratory.

Statistical analysis. The Wilcoxon U test and Mann–Whitney U test were used for non-categorical comparisons and the chi-square or Fisher exact test for categorical comparisons. The statistical level of significance was a 2-tailed p-value of < 0.05. The analysis was performed using the statistical package SPSS 25.0 (IBM, Armonk, NY, USA).

3. Results

3.1. NTBI/LPI and Other Parameters Measuring Functional and Storage Iron Pools

We first addressed whether NTBI and LPI are present in the blood of leukemic patients and the control group. NTBI was detected in all three patient groups, with the highest frequency in children after HCT (group IV), and this iron fraction was not found in the control group of healthy children (Table 3). The difference in incidence of NTBI between these groups (IV vs. controls) was statistically significant ($p = 0.012$). LPI was detected in the blood of all groups. Nevertheless, its presence was most common in children after HCT; this fraction was found in almost half of the group. The incidence of LPI in patients after HCT was significantly higher compared to controls ($p = 0.018$).

Table 3. Differences between parameters of iron metabolism.

Parameters	Controls (Group I)	Acute Leukemia de Novo (Group II)	Acute Leukemia after Intensive Chemotherapy (Group III)	After HCT (Group IV)	p-Value
PRBC transfusions (units) median (range)	0 (0–0)	1 (0–10)	9 (2–35)	23 (6–99)	I vs. II; $p < 0.001$ I vs. III; $p < 0.001$ I vs. IV; $p < 0.001$ II vs. III; $p < 0.001$ II vs. IV; $p < 0.001$ III vs. IV; $p < 0.001$
NTBI positive (number)	0/18 (0%)	3/21 (14.3%)	3/25 (12.0%)	6/21 (28.6%)	I vs. II; $p = 0.459$ I vs. III; $p = 0.058$ I vs. IV; $p = 0.012$ II vs. III; $p = 0.272$ II vs. IV; $p = 0.079$ III vs. IV; $p = 0.359$
LPI positive (number)	2/18 (11.1%)	5/21 (23.8%)	6/25 (24.0%)	10/21 (47.6%)	I vs. II; $p = 0.820$ I vs. III; $p = 0.398$ I vs. IV; $p = 0.018$ II vs. III; $p = 0.666$ II vs. IV; $p = 0.063$ III vs. IV; $p = 0.062$
Serum iron (mg/dL) median (range)	69.2 (20.10–97.40)	125.05 (40.40–259.00)	103.6 (10.00–236.70)	128.40 (41.90–265.40)	I vs. II; $p = 0.001$ I vs. III; $p = 0.007$ I vs. IV; $p = 0.001$ II vs. III; $p = 0.424$ II vs. IV; $p = 0.754$ III vs. IV; $p = 0.295$
Transferrin (ng/mL) median (range)	32.11 (10.94–750.00)	32.95 (7.59–750.00)	38.00 (12.16–260.30)	23.36 (6.57–94.54)	I vs. II; $p = 0.481$ I vs. III; $p = 0.109$ I vs. IV; $p = 0.371$ II vs. III; $p = 0.316$ II vs. IV; $p = 0.076$ III vs. IV; $p = 0.011$

Table 3. *Cont.*

Parameters	Controls (Group I)	Acute Leukemia de Novo (Group II)	Acute Leukemia after Intensive Chemotherapy (Group III)	After HCT (Group IV)	*p*-Value
TIBC (µg/L) median (range)	356.00 (292.00–404.00)	276.50 (125.00–329.00)	262.50 (183.00–328.00)	234.00 (128.00–434.00)	I vs. II; $p < 0.001$ I vs. III; $p < 0.001$ I vs. IV; $p < 0.001$ II vs. III; $p = 0.316$ II vs. IV; $p = 0.004$ III vs. IV; $p = 0.004$
Ferritin (µg/L) median (range)	27.40 (11.00–73.30)	238.50 (14.20–1660.00)	739.00 (26.40–5278.00)	3670.00 (51.10–12,000.00)	I vs. II; $p < 0.001$ I vs. III; $p < 0.001$ I vs. IV; $p < 0.001$ II vs. III; $p = 0.069$ II vs. IV; $p < 0.001$ III vs. IV; $p < 0.001$
FTH1 (pg/mL) median (range)	16.45 (0.54–70.05)	24.45 (1.00–137.90)	18.81 (0.50–132.00)	22.76 (1.00–309.40)	I vs. II; $p = 0.091$ I vs. III; $p = 0.481$ I vs. IV; $p = 0.528$ II vs. III; $p = 0.275$ II vs. IV; $p = 0.345$ III vs. IV; $p = 0.930$
FTL (pg/mL) median (range)	94.65 (41.09–571.80)	129.30 (44.45–363.00)	113.30 (3.86–286.10)	117.00 (20.40–301.70)	I vs. II; $p = 0.547$ I vs. III; $p = 0.990$ I vs. IV; $p = 0.918$ II vs. III; $p = 0.372$ II vs. IV; $p = 0.372$ III vs. IV; $p = 0.991$
Hepcidin (ng/mL) median (range)	30.61 (14.55–468.20)	158.50 (21.69–738.60)	106.60 (17.26–383.20)	278.30 (22.15–1000.00)	I vs. II; $p = 0.001$ I vs. III; $p = 0.013$ I vs. IV; $p < 0.001$ II vs. III; $p = 0.087$ II vs. IV; $p = 0.031$ III vs. IV; $p < 0.001$
sHJV (ng/mL) median (range)	65.58 (49.02–91.47)	52.77 (27.33–88.23)	57.95 (24.78–136.80)	40.62 (18.34–98.41)	I vs. II; $p = 0.029$ I vs. III; $p = 0.025$ I vs. IV; $p < 0.001$ II vs. III; $p = 0.635$ II vs. IV; $p = 0.051$ III vs. IV; $p = 0.007$
FNP (pg/mL) median (range)	76.46 (41.94–251.90)	80.03 (29.12–924.70)	74.20 (38.57–618.20)	110.00 (37.69–1250.00)	I vs. II; $p = 0.872$ I vs. III; $p = 0.990$ I vs. IV; $p = 0.212$ II vs. III; $p = 0.921$ II vs. IV; $p = 0.213$ III vs. IV; $p = 0.310$
Erythroferrone (ERFE) (ng/mL) median (range)	0.83 (0.30–10.00)	1.29 (0.23–10.00)	0.69 (0.14–10.00)	1.26 (0.13–10.00)	I vs. II; $p = 0.290$ I vs. III; $p = 0.191$ I vs. IV; $p = 0.837$ II vs. III; $p = 0.024$ II vs. IV; $p = 0.279$ III vs. IV; $p = 0.494$

Table 3. *Cont.*

Parameters	Controls (Group I)	Acute Leukemia de Novo (Group II)	Acute Leukemia after Intensive Chemotherapy (Group III)	After HCT (Group IV)	*p*-Value
EPO (mIU/mL) median (range)	5.26 (0.45–12.73)	44.85 (1.81–100.00)	12.73 (5.31–100.00)	12.32 (3.10–100.00)	I vs. II; $p < 0.001$ I vs. III; $p < 0.001$ I vs. IV; $p < 0.001$ II vs. III; $p = 0.004$ II vs. IV; $p < 0.001$ III vs. IV; $p = 0.372$
STfR (µg/mL) median (range)	0.12 (0.07–0.29)	0.11 (0.09–0.52)	0.11 (0.08–0.71)	0.09 (0.07–0.32)	I vs. II; $p = 0.340$ I vs. III; $p = 0.918$ I vs. IV; $p = 0.020$ II vs. III; $p = 0.480$ II vs. IV; $p = 0.024$ III vs. IV; $p = 0.012$
CRP (mg/L) median (range)	0.49 (0.16–2.34)	6.68 (0.37–156.50)	0.50 (0.16–36.37)	3.51 (0.22–297.54)	I vs. II; $p < 0.001$ I vs. III; $p = 0.739$ I vs. IV; $p < 0.001$ II vs. III; $p < 0.001$ II vs. IV; $p = 0.580$ III vs. IV; $p < 0.001$
PCT (ng/mL) median (range)	0.02 (0.02–0.10)	0.10 (0.01–2.82)	0.05 (0.00–0.36)	0.16 (0.02–11.47)	I vs. II; $p < 0.001$ I vs. III; $p = 0.002$ I vs. IV; $p < 0.001$ II vs. III; $p = 0.005$ II vs. IV; $p = 0.669$ III vs. IV; $p = 0.002$

PRBC, packed red blood cell concentrate; NTBI, non-transferrin-bound iron; LPI, labile plasma iron; sHJV, soluble hemojuvelin; sFNP-1, soluble ferroportin-1; ERFE, erythroferrone; EPO, erythropoietin; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; FTH, ferritin heavy chain; FTL, ferritin light chain; CRP, C-reactive protein; PCT, procalcitonin; HCT, hematopoietic cell transplantation.

Then, we analyzed other parameters measuring functional and storage iron pools (iron, ferritin, transferrin, TIBC, FTH1, and FTL). The highest levels of iron and ferritin were found in patients after HCT, which corresponded to the highest values of the number of PRBC transfusions in this group of patients. Consequently, TIBC in patients after HCT or after chemotherapy for acute leukemia was lower than in healthy children ($p < 0.001$, for each group). The transferrin concentration was significantly decreased after HCT, when compared to patients after standard chemotherapy for acute leukemia ($p = 0.011$). On the other hand, FTH1 and FTL levels were not statistically different between the groups.

3.2. Proteins Regulating Absorption of Iron and Its Release from the Tissue Stores

Differences in hepcidin and related protein levels (soluble hemojuvelin, sHJV; soluble ferroportin-1, FNP-1) were then assessed between the analyzed groups. Serum hepcidin was highly increased after HCT in comparison to the other groups. Additionally, the median concentration of FNP-1 was over 30% higher after HCT than in the other groups; however, the difference was not significant. The concentration of sHJV was highest in healthy children and gradually decreased after chemotherapy for acute leukemia ($p = 0.025$) and after HCT ($p < 0.001$ for control group; $p = 0.007$ for acute leukemia group). These results are summarized in Table 3.

3.3. Proteins Regulating Erythropoietic Activity of Bone Marrow

Subsequently, the relationship of bone marrow activity markers (EPO, ERFE, STfR) and phases of treatment of patients was investigated. The median EPO concentration was the highest in untreated patients with acute leukemia (group II) ($p = 0.004$ in comparison to group III, and $p < 0.001$ in comparison to other groups). On the other hand,

no significant differences between groups were found for ERFE and STfR concentrations, although these values were also higher in the de novo acute leukemia group. The ERFE concentration in HCT patients was almost doubled in comparison to the group of patients after chemotherapy for acute leukemia.

4. Discussion

In this study, we analyzed 14 biochemical parameters of iron metabolism in children after intensive chemotherapy of acute leukemia or after hematopoietic cell transplantation, when compared to healthy pediatric controls or children newly diagnosed with acute leukemia. We showed a variety of significant abnormalities in iron metabolism in these patients. The intensity of the pathology increased with the clinical pathology and intensity of treatment administered to these patients. Since treatment in these groups of patients was based on intensive multiagent chemotherapy, the most frequent adverse events included hematological consequences such as anemia, neutropenia, and thrombocytopenia. Frequent red cell transfusions are a standard approach in order to ensure patient safety.

The main findings of our study can be summarized in three aspects. Firstly, abnormalities in iron metabolism parameters are dependent on the intensity of treatment, being highest in children after HCT. Secondly, the intensity of chemotherapy results in an increase in NTBI and LPI, the most toxic forms of iron, occurring simultaneously with increased serum iron, ferritin, hepcidin, and ferroportin concentrations. Additionally, thirdly, based on our results, we can hypothesize the logical pathway of interactions between parameters in patients exposed to frequent blood transfusions due to leukemia or HCT.

Results obtained in children might vary from those in adults, as the intensity of multiagent chemotherapy in pediatric protocols is usually much higher, and the profile of hematological toxicity is increased in this population [20]. This possibly results in more frequent transfusions of red cell concentrates, a higher risk of iron overload, and long-term sequelae, including lower overall survival [21–24]. Still, there are no comparative studies on iron metabolism abnormalities in children vs. adults undergoing chemotherapy/transplantation. One can expect that similar changes in iron metabolism parameters can occur in both age groups; however, it is anticipated that their intensity is deeper in children due to more intensive chemotherapy, complications, and blood transfusions.

This study was designed in order to cover gaps in the literature on pediatric patients. We determined the presence of NTBI and LPI simultaneously with other iron metabolism markers developing during chemotherapy in children undergoing anti-leukemic treatment or HCT. The measurement of NTBI and LPI in patients undergoing intensive multiagent chemotherapy for acute leukemia or HCT is of clinical relevance for both diagnosis and therapy as it could serve as a strong and reliable new indicator of iron overload. Possibly, it could also confirm the association between free iron and early toxicity and subsequent complications. Finally, these markers can be future potential targets for the use of iron chelators [10].

All results obtained in our study can be grouped together in a pathophysiological network of changes in the expression of iron metabolism parameters with logical interactions resulting from clinical activity (Figure 1). The complexity of childhood leukemia therapy and hematopoietic cell transplantation affects the occurrence of disturbances in iron metabolism. Obviously, frequent blood transfusions lead to iron overload, and this is reflected in the high total levels of this element in the blood as well as the very high levels of ferritin. This results in the appearance of extremely toxic NTBI and LPI in the circulation, which may additionally be released from combinations with other blood compounds, as a result of chemotherapy administration. High iron levels and the presence of an increased inflammatory response induce hepatic hepcidin synthesis. We hypothesized that, at the same time, the high concentrations of iron led to a decrease in the release of HJV from cell membranes, which resulted in a reduction in the concentrations of sHJV, leading to a disturbance in the hepcidin–sHJV axis. Both HCT and chemotherapy affect the increased synthesis of EPO, which is a powerful regulator of the synthesis of ERFE and hepcidin.

Despite the high levels of EPO, there is no stimulation of ERFE synthesis and no inhibition of hepcidin synthesis. EPO is unable to break the potency of two cardinal stimulators of hepcidin synthesis, namely, high blood iron levels and severe inflammation. An interesting observation was made regarding the relationship between hepcidin and its receptor FPN-1. The relationship between membrane FPN-1 and its serum form is not fully understood; however, the relationship is directly proportional. Although we did not find a statistically significant difference in the concentration of sFNP-1 between the subgroups, its highest concentration (with a median value almost 50% higher in comparison to the other groups) was recorded in group IV. This may be related to hyperferremia, which is known to enhance FNP expression. Thus far, only a few clinical studies have assessed the concentration of serum FNP-1. Our study is the first to use an ultra-sensitive enzyme immunoassay to evaluate the circulating pool of this protein. Still, the mechanism responsible for the lack of a negative feedback loop between hepcidin and sFPN-1 in hemato-oncological patients deserves further research.

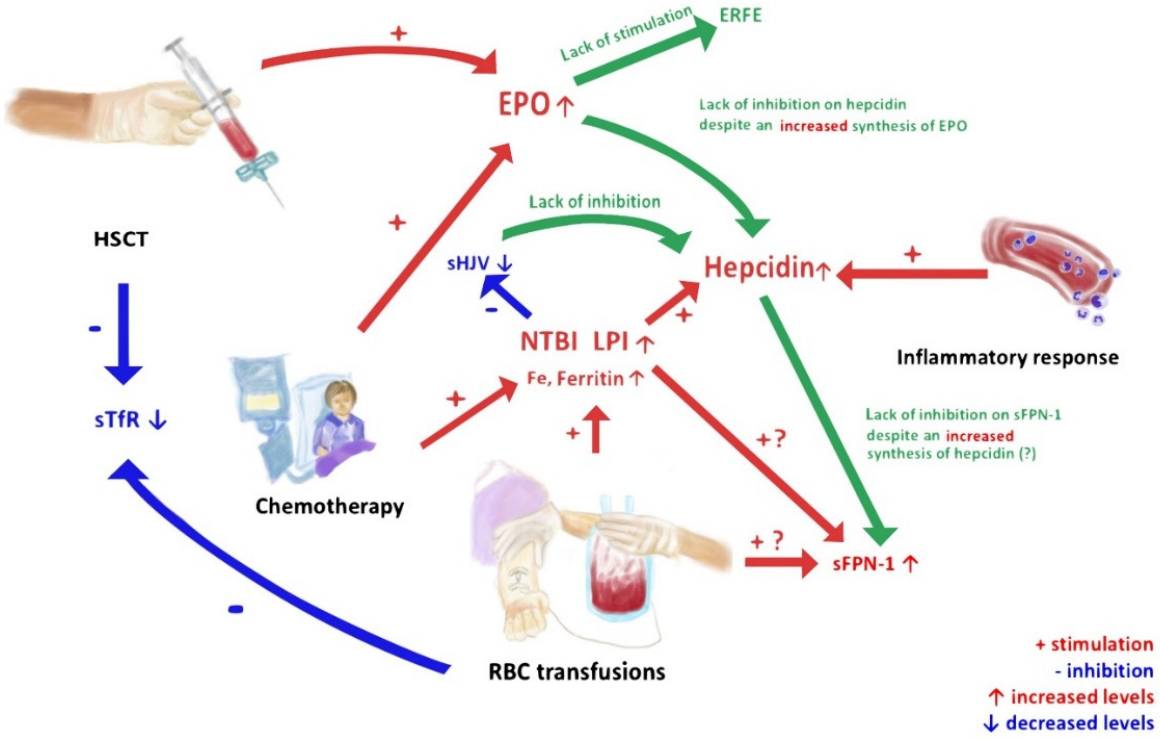

Figure 1. Flow chart of impact of anti-leukemic therapy and blood transfusions on mechanisms of iron overload metabolism.

The ferritin concentration progressively increased from groups I to IV. However, simultaneously, there were no significant differences in FTH and FTL chains. This discrepancy is a consequence of the structure and function of these proteins. Ferritin is composed of 24 heavy (H) and light (L) subunits, which, depending on the place of cellular synthesis, form a protein with different proportions between the subunits. FTL and FTH are subject to different translational and post-translational regulations. Various factors including cellular iron concentrations, the ongoing inflammatory process, and oxidative stress regulate the expression of FTL and FTH, often modifying it to a significantly different extent [25,26]. FTL and FTH fulfill different roles in iron metabolism. L subunits are responsible for iron storage and mineralization, while H subunits have a ferroxidase activity [27]. Hence, the precise relationships between these subunits, molecular regulations of their levels, their ability to form multimeric ferritin, and clinical significance are still matters of scientific debate.

Our study has some limitations. Although a large number of parameters were tested, and, in many cases, high statistical significance was found, the number of patients in each

group is still not large enough to obtain a strong statistical power of this study. Additionally, this study did not analyze the differences in age groups: children vs. adults.

5. Conclusions

We showed an imbalance in iron metabolism, possibly increasing with the intensity of treatment with standard anti-leukemic chemotherapy and hematopoietic cell transplantation. In particular, the presence of NTBI and LPI increased with the intensity of antimalignant treatment. This mostly affected children after transplantation. Iron metabolism abnormalities highly corresponded to the number of blood transfusions.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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Article

Soluble Hemojuvelin and Ferritin: Potential Prognostic Markers in Pediatric Hematopoietic Cell Transplantation

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Simple Summary: In children after intensive chemotherapy for acute leukemias (AL) or undergoing hematopoietic cell transplantation (HCT), the prognostic impact of 12 serum iron metabolism parameters was analyzed. With a median follow-up of 2.2 years, high levels of ferritin and low levels of soluble hemojuvelin (sHJV) had an adverse prognostic impact on overall survival (OS) and event-free survival (EFS) in children after HCT. If these patients were combined with those with AL after intensive chemotherapy, the results were confirmed for OS and EFS, both for ferritin and sHJV. For the first time, we have shown the prognostic effect of sHJV on the outcome of HCT. Further studies are required to confirm our preliminary findings in a larger sample of patients.

Abstract: Objective: Iron overload (IO) is a common and life-threatening complication resulting from the therapy of AL and HCT patients. This study aimed to evaluate the prognostic value of 12 serum biomarkers of iron metabolism in pediatric patients treated for AL or undergoing HCT. Patients: Overall, 50 patients with AL after intensive treatment and 32 patients after HCT were prospectively included in the study. AL patients at diagnosis and healthy controls served as reference groups. Methods: The impact of the following 12 serum iron metabolism parameters on the outcome of AL/HCT patients was analyzed: iron, transferrin (Tf), total iron-binding capacity (TIBC), ferritin, ferritin heavy chains (FTH1), ferritin light chains (FTL), hepcidin, soluble hemojuvelin (sHJV), soluble ferroportin-1 (sFPN1), erythroferrone (ERFE), erythropoietin (EPO), and soluble transferrin receptor (sTfR). Results: With a median follow-up of 2.2 years, high levels of ferritin and low levels of sHJV had an adverse prognostic impact on OS and EFS in children after HCT. If these patients were combined with those with AL after intensive chemotherapy, the results were confirmed for OS and EFS both for ferritin and sHJV. Conclusions: Among the 12 analyzed serum parameters of iron metabolism, increased levels of ferritin and decreased levels of sHJV had an adverse prognostic impact on survival in children after HCT. More data are needed to clarify the relationship between ferritin, sHJV, and mortality of AL children after intensive chemotherapy, and more extensive prospective studies are required to prove sHJV predictivity.

Keywords: children; acute leukemia; hematopoietic cell transplantation; iron metabolism; ferritin; hemojuvelin

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1. Introduction

Acute leukemias (AL) are the most common malignancy affecting children [1–3]. The outcomes of children with AL treated with chemotherapy has considerably improved over

time, with 5-year survival rates reaching, nowadays, almost 90% for acute lymphoblastic leukemia (ALL) and 70% for acute myeloid leukemia (AML) [2,4]. The current challenge for clinicians is to minimize treatment-related adverse effects [1–4].

Apart from chemotherapy, hematopoietic cell transplantation (HCT) belongs to basic methods in treating hematological malignancies, several solid tumors, and various non-malignant diseases [5,6]. Since HCT is usually based on high-dose chemotherapy or chemo-radiotherapy, the risk of complications is even higher than in standard chemotherapy. Thus, multiple blood transfusions are common episodes for children with malignancies or undergoing HCT. Iron overload (IO) due to repeated red blood cell (RBC) transfusions develop rapidly [7,8] and increases the risk of infections, hepatic dysfunction, and veno-occlusive disease (VOD) after HCT. For this reason, recognizing pathomechanisms responsible for IO, its relationship with prognosis in oncologically ill children, and its relationship with their therapy deserves an intensive search for explanation.

The biochemical markers of iron metabolism in standard clinical practice include serum iron, ferritin, and transferrin levels. Several studies showed that elevated serum ferritin levels significantly affect complications and overall survival (OS) in patients with AL or undergoing HCT [8–17]. Data on respective pediatric populations have confirmed that elevated serum ferritin levels confer an adverse prognosis [18–23]. Modern iron status indicators like hepcidin and hemojuvelin (HJV) might be the background for future research. Hepcidin is the main protein that controls iron homeostasis, by limiting both intestinal iron absorption and macrophage iron release [24]. HJV is expressed in the liver, skeletal muscle, and heart, and may be released from those tissues into circulation as a form of soluble hemojuvelin (sHJV). Although skeletal muscles are the source of sHJV, this form does not participate in iron metabolism [25,26]. HJV seems to play a role in iron absorption and is essential for hepcidin expression [27,28].

Apart from ferritin and hepcidin, the clinical impact of other iron metabolism parameters on treatment outcomes in pediatric populations with cancers is largely unknown. Most of these parameters' roles in patients with AL or treated with HCT have not been analyzed before. In this study, we aimed to evaluate the prognostic value of 12 serum biomarkers of iron metabolism in pediatric patients treated for AL or undergoing HCT. To the best of our knowledge, this is the first study to examine iron metabolism in these patient groups on such a large scale. Thanks to a rigorous methodological approach and an extensive biochemical analysis, we were able to pioneer the demonstration that sHJV could become a valuable tool in predicting adverse events in children with AL, after chemotherapy or after HCT. Although our study is more in the nature of a pilot, its results can also serve as a platform for planning future studies. In addition, the precise mechanism responsible for releasing HJV from cells and the molecular mechanisms controlling its serum levels need to be better understood.

2. Patients and Methods

Study design. In this prospective two-center study in Poland (Bydgoszcz, Warsaw), pediatric patients diagnosed and treated for acute leukemia (AL) or undergoing hematopoietic cell transplantation (HCT) between June 2019 and December 2021 were tested for serum levels of iron metabolism parameters. The impact of serum iron metabolism parameters on the short-term outcomes of antileukemic therapy or HCT was analyzed. The Local Bioethical Committee approved the study (608/2018; 25 June 2019).

Patients. Inclusion and exclusion criteria. A total of 137 patients (69 boys and 68 girls), with a median age of 8 (range 3–18) years, were included in the study and were divided into four groups. Patients with newlydiagnosed acute leukemia (group II), patients immediately after the phase of intensive consolidation chemotherapy being on maintenance therapy (group III), and patients one month after allogeneic HCT (group IV) were considered qualified (Table 1). The control group (group I) included healthy children without hematological disorders and a history of any blood transfusions. Exclusion criteria included severe infection in groups I, III, and IV; no exclusion criteria were applied

for patients newly diagnosed with AL (group II). Children after intensive chemotherapy (group III) and after HCT (group IV) qualified for follow-up analysis. Children with acute lymphoblastic leukemia (ALL) were treated according to protocol AIEOP-BFM-ALL-2017. Children with acute myeloid leukemia (AML) were treated according to protocol AML-BFM-2019. Patients undergoing HCT were treated according to respective chemotherapy protocols (or supportive therapy in cases of non-malignant diagnoses), followed by specific conditioning therapy before transplant. All but two patients received myeloablative conditioning. Patients in group IV were diagnosed with AML (n = 14), ALL (n = 8), and other diagnoses (n = 10), including severe aplastic anemia (SAA, n = 3), neuroblastoma (NBL, n = 3), myelodysplastic syndrome (MDS, n = 1), severe congenital neutropenia (SCN, n = 1), anaplastic large B-cell lymphoma (ALCL, n = 1), and Ewing sarcoma (ES, n = 1). Overall, 27 patients received allogeneic (5 from a family donor and 22 from a matched unrelated donor) or autologous (n = 5) transplantations. Table 1 summarizes the clinical and demographic characteristics of the patients included in the study. The four groups did not differ significantly in terms of age and sex. As expected from the study's design, the groups differed in clinical diagnosis and the number of patients transfused with packed red blood cell concentrate (PRBC).

Table 1. The demographic and clinical characteristics of patients included in the study, stratified according to clinical diagnosis and healthy controls.

	Total (%) (n = 137)	**Group I (n = 19)**	**Group II (n = 36)**	**Group III (n = 50)**	**Group IV (n = 32)**	***p*-Value**
		Controls	**AL at Diagnosis**	**AL after Chemotherapy**	**After HCT**	
Median age (range) years	8.0 (3.0–18)	10 (4.2–15)	8.0 (3–18)	8.7 (4–17.9)	8.0 (3–17.9)	0.234
Age < 10 vs. >10 years (%)	78 (57%): 59 (43%)	7 (37%): 12 (63%)	21 (58%): 15 (42%)	33 (66%): 17 (34%)	17 (53%): 15 (47%)	0.173
Sex Male: Female (%)	69 (50%): 68 (50%)	8 (42%): 11 (58%)	17 (47%): 19 (53%)	27 (54%): 23 (46%)	17 (53%): 15 (47%)	0.798
Diagnosis						
ALL (%)	88	0	33	47	8	<0.001
AML (%)	20	0	3	3	14	<0.001
Other (%)	29	19	0	0	10	<0.001
HCT	32	0	0	0	32	<0.001
Patients after PRBC transfusions	110	0	28	50	32	<0.001

Abbreviations: AL, acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; HCT, hematopoietic cell transplantation; PRBC, packed red blood cells concentrate.

Overall, 110 (80.3%) children were transfused with concentrates of packed red blood cells (PRBC) (all patients in groups III and IV, 28/36 in group II, and none in group I). Patients received a median of 5 (range: 0–99) units of PRBC (including a median of 1 unit in group II, a median of 10 units in group III, and a median of 23 units in group IV). At the time of analysis, 10/137 (7.3%) patients died (1 in group II, 1 in group III, and 8 in group IV).

Transplant procedures. Children were qualified for transplantation according to specific protocols for malignant or non-malignant diseases [5,6]. Transplantation was preceded by a myeloablative (MAC) or reduced intensity (RIC) conditioning regimen. MAC was based on total body irradiation (TBI) or chemotherapy with treosulfan or busulfan. RIC was based on chemotherapy with fludarabine or busulfan at doses $\leq$8 mg/kg/cycle. Prophylaxis of graft-versus-host disease (GVHD) was done with cyclosporine A (CsA) and short-term use of methotrexate (MTX) [29]. Patients with transplants from alternative

donors (matched unrelated donor, MUD; haploidentical donor; MMUD, mismatched unrelated donor) received in vivo T-cell depletion with anti-thymocyte globulin (ATG).

Laboratory markers of iron metabolism. Overall, 12 serum laboratory parameters of iron metabolism were analyzed. The collection of samples, reagents, and laboratory tests were as described previously [18]. Three categories of iron metabolism markers were included: (1) parameters determining functional and storage iron pool (iron, total iron-binding capacity (TIBC), transferrin, ferritin, ferritin heavy (FTH1) and light chain (FTL)); (2) proteins contributing to the absorption of iron and its release from tissues stores (hepcidin, soluble ferroportin-1 (sFNP-1), and soluble hemojuvelin (sHJV)); and (3) proteins determining the erythropoietic activity of the bone marrow (erythropoietin (EPO), erythroferrone (ERFE), and soluble transferrin receptor (sTfR)).

Laboratory markers of inflammation. C-reactive protein (CRP) and procalcitonin (PCT) serum levels were analyzed using standard laboratory methods at hospital laboratories.

Statistical analysis. The study's primary endpoint was the overall survival (OS), determined in landmark analysis. Additional endpoints included event-free survival (EFS) and relapse incidence (RI). All endpoints were calculated from the day of inclusion to analyze iron metabolism parameters. OS was interpreted as the time from inclusion to death, regardless of the cause or last day of follow-up. Death was regarded as an event for OS; relapse and death were considered as events for EFS. Relapse was considered as the presence of >5% bone marrow (BM) blasts and/or the reappearance of the underlying disease. RI was estimated to consider relapse or reappearance of the underlying disease as an event of interest, and death without relapse as a competing event. The event was defined as relapse or death from any cause. EFS was defined as survival without evidence of relapse or progression. Mean survival was analyzed using the Kaplan-Meier method. Values of overall survival (OS), event-free survival (EFS), and relapse incidence (RI) were calculated using the Kaplan-Meier method, and the log-rank test compared differences between the Kaplan-Meier curves. This method was used to correlate each potential laboratory or clinical prognostic factor with survival in univariate analysis. Multivariate risk factor analyses of treatment outcomes were done with the Cox regression model. The factors with p-values < 0.1 in univariate analyses were then fitted together and dropped one at a time, in a backward stepwise manner, using the likelihood ratio test at a 0.05 level, until all factors in the model were significant. A final check was made to ensure that no excluded factors would improve the fit. Hazard ratios (HR) and 95% confidence interval (95% CI) were determined. The chi-square or Fisher's exact tests were used for categorical comparisons, while the Kruskal-Wallis test and Mann-Whitney U-test were used for non-categorical comparisons. All the tests were two-sided. A p-value < 0.05 was regarded as statistically significant. The SPSS28 (IBM, Armonk, NY, USA) statistical package was used.

3. Results

3.1. Comparison of Laboratory Markers of Iron Metabolism between Four Subgroups of Children

In the first stage of our analysis, we compared serum levels of iron metabolism parameters between four pediatric patient subgroups: (1) healthy controls (group I), (2) AL at diagnosis (group II), (3) AL after chemotherapy (group III), and (4) children after HCT (group IV). As noted in the previous manuscript section, iron metabolism parameters had been grouped into three categories to facilitate the analysis of as many as 12 laboratory parameters, which allowed for a wide-ranging and all-encompassing analysis of iron metabolism in the enrolled patients. Overall, the results of this part of our study are consistent with those presented by us previously [18]. That is, the imbalance in iron metabolism, illustrated by changes in serum levels of iron metabolism parameters, which converges with the intensity of the treatment implemented in pediatric patients. Firstly, several parameters determining functional and storage iron pool, including TIBC and serum levels of transferrin and ferritin, confirmed that the degree of iron overload may depend on the treatment modalities and is dominant in pediatric patients post-HCT (Supplementary Table S1). Secondly, the analysis of serum levels of iron metabolism parameters contributing to the

absorption of iron and its release from tissues confirmed intensified iron overload post-HCT, as reflected by high and low serum levels of hepcidin and sHJV in this group of pediatric patients, respectively (Supplementary Table S1). Lastly, serum erythropoietin levels were lower post-HCT than in other patient groups (Supplementary Table S1). Collectively, our current results support the original hypothesis of a relationship between therapy and disturbances in iron metabolism. Changes in iron metabolism were also associated with worsening inflammatory reactions and multiple PRBC transfusions (Supplementary Table S1).

3.2. Impact of Iron Metabolism Parameters on Therapy Outcomes

At the end of the study, 6 patients relapsed (3/50 in group III and 3/32 in group IV), and 10 died (1/36 in group II, 1/50 in group III, and 8/32 in group IV). The number of patients with events was 12 (1/36 in group II, 3/50 in group III, and 8/32 in group IV). Control patients (group I) were not analyzed for outcomes.

Overall survival (OS). Median follow-up was 2.5 years (range: 0.5–3.1) for AL patients at diagnosis (group II), 2.5 years (range: 1.2–3.2) for AL after intensive chemotherapy (group III), and 2.2 years (range: 0.1–3.2) for patients after HCT (group IV). The OS of the patients was calculated in univariate analysis for each analyzed serum parameter for iron metabolism, dichotomized by a median value for each parameter. We analyzed the OS in patients after HCT (group IV) and additionally in a combination of these patients with AL after chemotherapy (group III), to increase the sensitivity of our analysis. Moreover, these groups seem to be the most similar among the populations we analyzed concerning clinical and laboratory characteristics. Only for ferritin and sHJV were the differences between OS significant (Table 2); thus, these two laboratory parameters were used in further univariate and multivariate analyses. Lower levels of serum ferritin (<2000 μg/L) and higher levels of sHJV (>40 μg/L) contributed to better OS (Figure 1). Additionally, we calculated the ferritin/sHJV (FER/sHJV) ratio. Patients with a ratio < 100 had significantly better OS. The median FER/sHVJ ratio was 78 (range, 11–330) for survivors and 126 (range, 87–341) for those who died ($p < 0.001$). Combining the two study groups (III and IV) into one confirmed that the previous OS analysis is better when lower serum ferritin and higher sHJV are observed (Table 2; Figure 1).

Table 2. Univariate analysis for overall survival (OS) for patients after HCT and patients after HCT combined with those with AL after chemotherapy.

	HCT Patients			**HCT and AL after Chemotherapy**		
Parameter	**Below Median**	**Above Median**	***p***	**Below Median**	**Above Median**	***p***
Serum iron	0.69 ± 0.12	0.81 ± 0.09	0.413	0.86 ± 0.05	0.91 ± 0.05	0.549
Transferrin	0.80 ± 0.10	0.70 ± 0.11	0.501	0.90 ± 0.05	0.87 ± 0.05	0.731
TIBC	0.69 ± 0.12	0.81 ± 0.10	0.336	0.83 ± 0.07	0.91 ± 0.04	0.187
Ferritin	0.97 ± 0.03	0.59 ± 0.11	**0.012**	0.97 ± 0.03	0.79 ± 0.07	**0.008**
FTH1	0.79 ± 0.11	0.72 ± 0.10	0.767	0.86 ± 0.05	0.90 ± 0.04	0.571
FTL	0.75 ± 0.11	0.74 ± 0.11	0.968	0.86 ± 0.06	0.90 ± 0.04	0.567
Hepcidin	0.81 ± 0.10	0.69 ± 0.11	0.349	0.92 ± 0.03	0.80 ± 0.08	0.066
sHJV	0.56 ± 0.13	0.93 ± 0.06	**0.012**	0.75 ± 0.07	0.97 ± 0.02	**<0.001**
Ferritin/sHJV ratio	0.93 ± 0.06	0.56 ± 0.13	**0.012**	0.96 ± 0.02	0.65 ± 0.11	**<0.001**
FNP	0.81 ± 0.10	0.68 ± 0.12	0.393	0.91 ± 0.04	0.85 ± 0.06	0.315

Table 2. *Cont.*

Parameter	HCT Patients			HCT and AL after Chemotherapy		
	Below Median	Above Median	*p*	Below Median	Above Median	*p*
Erythroferrone	0.75 ± 0.11	0.74 ± 0.11	0.730	0.87 ± 0.05	0.90 ± 0.04	0.707
EPO	0.75 ± 0.11	0.74 ± 0.11	0.908	0.85 ± 0.06	0.93 ± 0.04	0.317
sTfR	0.75 ± 0.11	0.74 ± 0.11	0.989	0.86 ± 0.06	0.89 ± 0.04	0.570
CRP	0.86 ± 0.09	0.67 ± 0.11	0.207	0.94 ± 0.04	0.83 ± 0.06	0.075
PCT	0.81 ± 0.10	0.68 ± 0.12	0.353	0.95 ± 0.04	0.83 ± 0.06	0.073
PRBC transfusions	0.82 ± 0.09	0.67 ± 0.12	0.316	0.95 ± 0.04	0.83 ± 0.06	0.063

Abbreviations: sHJV, soluble hemojuvelin; sFNP-1, soluble ferroportin-1; ERFE, erythroferrone; EPO, erythropoietin; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; FTH1, ferritin heavy chain; FTL, ferritin light chain; CRP, C-reactive protein; PCT, procalcitonin; HCT, hematopoietic cell transplantation; PRBC, packed red blood cell concentrate.

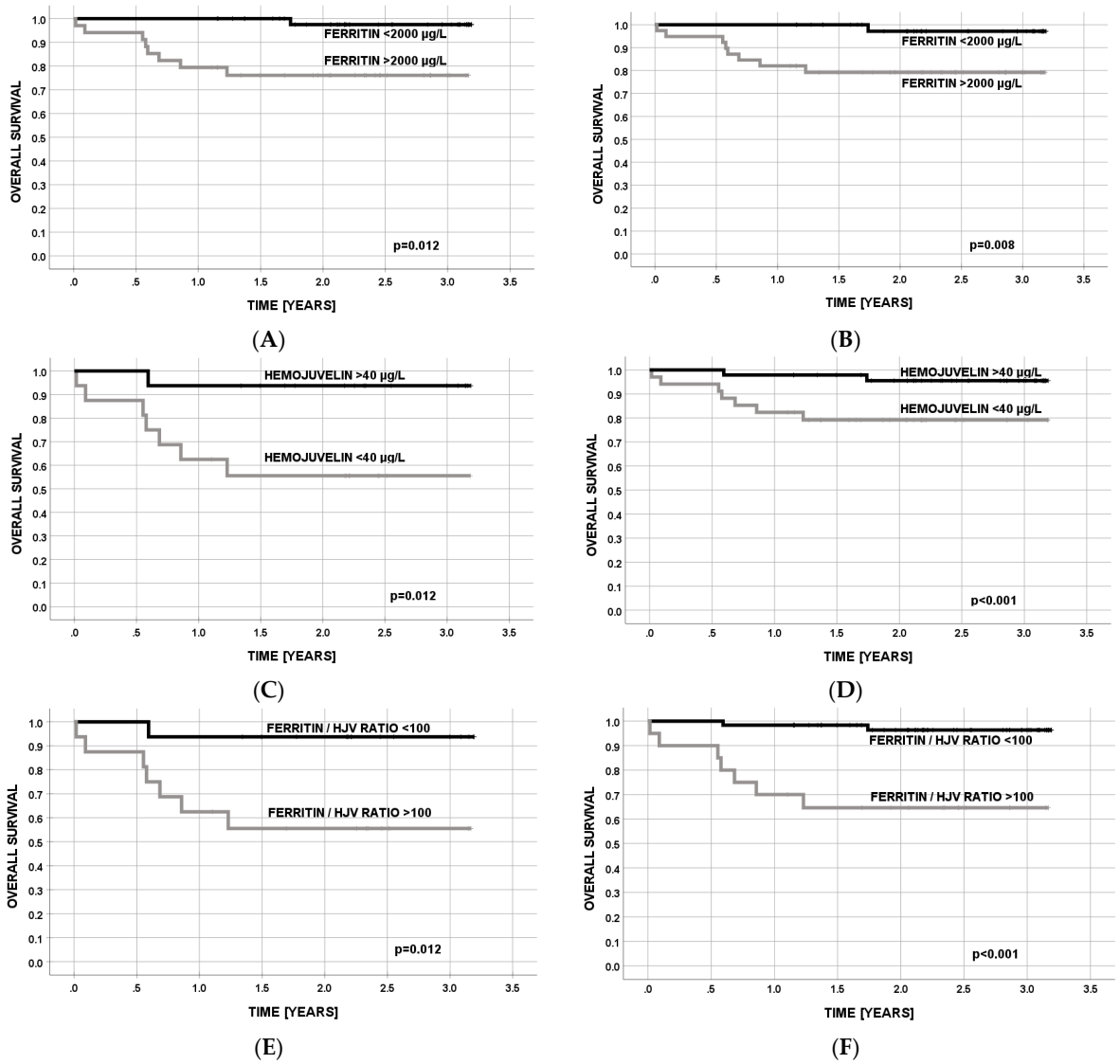

Figure 1. Overall survival (OS) for patients after HCT (**A**,**C**,**E**) and patients after HCT combined with those with AL after chemotherapy (**B**,**D**,**F**) concerning serum levels of ferritin and sHJV levels and ferritin/sHJV ratio.

Event-free survival (EFS). EFS was calculated in univariate analysis with the same approach for each analyzed parameter of iron metabolism, dichotomized by a median value of each parameter. Similar to OS, the analysis of EFS was done for post-HCT patients (group IV) and HCT patients combined with those from group III (AL after chemotherapy). We demonstrated that the differences between EFS were significant for ferritin and sHJV (Table 3; Figure 2) in both HCT and HCT patients combined with AL patients after chemotherapy. For this reason, these two laboratory parameters were used in the next part of the statistical analysis (univariate and multivariate analysis). Lower serum levels of ferritin (<2000 μg/L) and higher levels of sHJV (>40 μg/L) contributed to better EFS (Table 3; Figure 2) in pediatric populations. Patients with the FER/sHJV ratio < 100 also had significantly better EFS (Table 3; Figure 2).

Table 3. Univariate analysis for event-free survival (EFS) for patients after HCT and patients after HCT combined with those with AL after chemotherapy.

	HCT Patients			HCT and AL after Chemotherapy		
Parameter	**Below Median**	**Above Median**	***p***	**Below Median**	**Above Median**	***p***
Serum iron	0.69 ± 0.12	0.81 ± 0.10	0.426	0.85 ± 0.05	0.88 ± 0.05	0.681
Transferrin	0.80 ± 0.10	0.70 ± 0.11	0.518	0.90 ± 0.05	0.84 ± 0.05	0.440
TIBC	0.69 ± 0.12	0.81 ± 0.10	0.323	0.83 ± 0.07	0.88 ± 0.04	0.460
Ferritin	1.00 ± 0.00	0.59 ± 0.11	**0.016**	0.93 ± 0.04	0.79 ± 0.06	**0.046**
FTH1	0.78 ± 0.11	0.72 ± 0.11	0.745	0.84 ± 0.06	0.88 ± 0.05	0.535
FTL	0.75 ± 0.11	0.74 ± 0.11	0.951	0.85 ± 0.07	0.88 ± 0.06	0.610
Hepcidin	0.81 ± 0.10	0.69 ± 0.12	0.337	0.89 ± 0.04	0.80 ± 0.08	0.200
sHJV	0.56 ± 0.13	0.94 ± 0.06	**0.018**	0.75 ± 0.07	0.94 ± 0.03	**0.016**
Ferritin/sHJV ratio	0.94 ± 0.06	0.56 ± 0.13	**0.018**	0.94 ± 0.03	0.64 ± 0.10	**<0.001**
FNP	0.81 ± 0.10	0.68 ± 0.12	0.455	0.88 ± 0.05	0.85 ± 0.06	0.754
Erythroferrone	0.75 ± 0.11	0.74 ± 0.11	0.850	0.88 ± 0.05	0.85 ± 0.06	0.797
EPO	0.74 ± 0.11	0.75 ± 0.11	0.992	0.83 ± 0.05	0.95 ± 0.05	0.203
sTfR	0.75 ± 0.11	0.74 ± 0.11	0.990	0.88 ± 0.05	0.85 ± 0.06	0.781
CRP	0.86 ± 0.09	0.67 ± 0.11	0.246	0.90 ± 0.05	0.83 ± 0.06	0.321
PCT	0.81 ± 0.10	0.68 ± 0.12	0.440	0.93 ± 0.04	0.80 ± 0.06	0.098
PRBC transfusions	0.82 ± 0.09	0.67 ± 0.12	0.370	0.90 ± 0.05	0.83 ± 0.06	0.308

Abbreviations: sHJV, soluble hemojuvelin; sFNP-1, soluble ferroportin-1; ERFE, erythroferrone; EPO, erythropoietin; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; FTH1, ferritin heavy chain; FTL, ferritin light chain; CRP, C-reactive protein; PCT, procalcitonin; HCT, hematopoietic cell transplantation; PRBC, packed red blood cell concentrate.

Relapse incidence (RI). In univariate analysis, RI of patients was calculated with the same approach for each analyzed parameter of iron metabolism, dichotomized by a median value of each parameter. Notwithstanding the low number of relapses, no significant differences were found for a single group of HCT patients (group IV) nor when we combined HCT patients with AL patients after chemotherapy (group III). Results for serum ferritin, sHJV and FER/sHJV ratio are shown in Table 4.

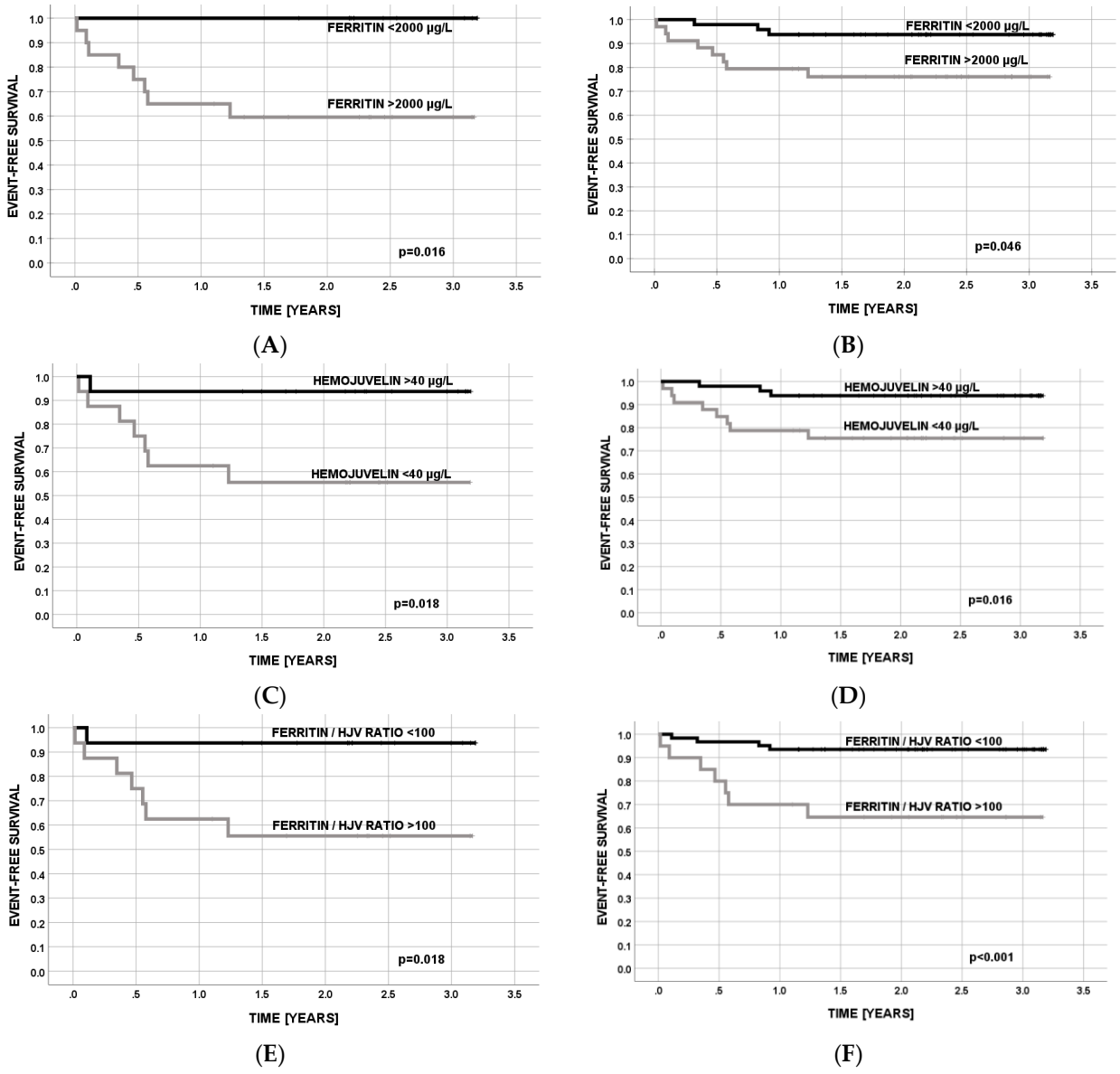

Figure 2. Event-free survival (EFS) for patients after HCT (**A**,**C**,**E**) and patients after HCT combined with those with AL (**B**,**D**,**F**) concerning serum levels of ferritin and sHJV levels and ferritin/sHJV ratio.

Table 4. Univariate analysis for relapse incidence (RI) in patients after HCT and patients after HCT combined with those with AL after chemotherapy.

	HCT Patients			HCT and AL after Chemotherapy		
Parameter	**Below Median**	**Above Median**	***p***	**Below Median**	**Above Median**	***p***
Ferritin	0.00 ± 0.00	0.15 ± 0.08	0.162	0.93 ± 0.04	0.92 ± 0.04	0.881
sHJV	0.13 ± 0.08	0.06 ± 0.06	0.686	0.90 ± 0.05	0.94 ± 0.03	0.595
Ferritin/sHJV ratio	0.06 ± 0.06	0.13 ± 0.08	0.612	0.93 ± 0.03	0.90 ± 0.07	0.597

Abbreviations: sHJV, soluble hemojuvelin; AL, acute leukemia; HCT, hematopoietic cell transplantation.

3.3. Risk Factor Analysis

Transplant-related risk factors. In the subsequent statistical analysis stage, we verified which HCT-related factors may influence overall survival (OS) and event-free survival

(EFS) in our pediatric patients. In univariate analysis of transplant risk factors, both for OS and EFS, no parameter was found to be significant in the Kaplan-Meier method and log-rank test analysis (Table 5).

Table 5. Univariate analyses for overall survival (OS) and event-free survival (EFS) for HCT patients.

Parameter	Characteristics	Overall Survival (OS)	*p*	Event-Free Survival (EFS)	*p*
Sex	Male	0.65 ± 0.12	0.166	0.65 ± 0.17	0.183
	Female	0.87 ± 0.09		0.86 ± 0.09	
Age	<10	0.71 ± 0.11	0.544	0.71 ± 0.11	0.493
	>10	0.80 ± 0.10		0.80. ± 0.10	
Diagnosis	AL	0.76 ± 0.09	0.764	0.76 ± 0.09	0.722
	Other	0.80 ± 0.13		0.80 ± 0.13	
Disease status	CR1	0.85 ± 0.08	0.109	0.85 ± 0.087	0.093
	Other	0.60 ± 0.16		0.60 ± 0.15	
Transplant	First	0.80 ± 0.08	0.571	0.80 ± 0.08	0.627
	Second	0.67 ± 0.19		0.67 ± 0.19	
Donor	Sibling	0.80 ± 0.18	0.880	0.80 ± 0.18	0.824
	Unrelated	0.77 ± 0.08		0.77 ± 0.08	
CMV serostatus	Negative	0.75 ± 0.22	0.961	0.75 ± 0.22	0.969
	Positive	0.76 ± 0.08		0.76 ± 0.08	
Conditioning intensity	Reduced	0.50 ± 0.20	0.083	0.50 ± 0.20	0.129
	Myeloablative	0.84 ± 0.07		0.84 ± 0.07	
TBI	TBI	1.00 ± 0.00	0.249	1.00 ± 0.00	0.249
	Chemotherapy	0.71 ± 0.08		0.71 ± 0.08	
Acute GVHD	<II°	0.74 ± 0.09	0.640	0.74 ± 0.09	0.583
	≥II°	0.78 ± 0.14		0.78 ± 0.14	
Chronic GVHD	None/limited	0.73 ± 0.09	0.249	0.73 ± 0.09	0.249
	Extensive	0.83 ± 0.5		0.83 ± 0.5	

Abbreviations: HCT, hematopoietic cell transplantation; AL, acute leukemia; CR1, first complete remission; CMV, cytomegalovirus; TBI, total body irradiation; GVHD, graft-versus-host disease.

Overall survival (OS). In univariate analysis of iron metabolism parameters contributing to OS, as calculated using the Kaplan-Meier method (Table 2, Figure 1), serum ferritin, sHJV, and their ratio were significant. Since the ratio depended on two other parameters, only ferritin and sHJV were included in the multivariate analysis model (Table 6). Due to non-significance, no additional transplant-related risk factor was included (Table 5). Both iron metabolism parameters were significant for OS: the hazard risk was 3.5 (95% CI = 1.3–28) for a higher value of serum ferritin levels and 12 (95% CI = 1.8–90) for a lower value of sHJV levels in serum. The significance of these two parameters was also confirmed when two groups were combined into one (group III and group IV).

Table 6. Multivariate analysis for overall survival (OS) in patients after HCT and patients after HCT combined with those with AL after chemotherapy.

	HCT Patients			HCT and AL after Chemotherapy		
Parameter	**Characteristics**	**HR (95% CI)**	***p*-Value**	**Characteristics**	**HR (95% CI)**	***p*-Value**
Ferritin	<2000 µg/L	1	0.035	<2000 µg/L	1	0.048
	>2000 µg/L	3.5 (1.3–28)		>2000 µg/L	15.8 (1.1–250)	
sHJV	>40 µg/L	1	0.006	>40 µg/L	1	0.026
	<40 µg/L	12 (1.8–90)		<40 µg/L	6.5 (1.2–31)	

Abbreviations: AL, acute leukemia; HCT, hematopoietic cell transplantation; sHJV, soluble hemojuvelin.

Event-free survival (EFS). Also, in univariate analysis of iron metabolism parameters contributing to EFS, as calculated using the Kaplan-Meier method (Table 2, Figure 1), serum ferritin, sHJV, and their ratio were significant. Again, only serum ferritin and sHJV were included in the multivariate analysis (Table 7). Due to non-significance, no additional transplant-related risk factor was included (Table 5). Both iron metabolism parameters were significant for survival: the hazard risk (HR) was 24 (95%CI = 1.1–120) for a higher value of serum ferritin and 8.0 (95%CI = 1.2–82) for a lower value of sHJV levels in serum in both HCT patients alone and when we combined them with those with AL after chemotherapy (Table 6).

Table 7. Multivariate analysis for event-free survival (EFS) in patients after HCT and patients after HCT combined with those with AL after chemotherapy.

	HCT Patients			HCT and AL after Chemotherapy		
Parameter	**Characteristics**	**HR (95% CI)**	***p*-Value**	**Characteristics**	**HR (95% CI)**	***p*-Value**
Ferritin	<2000 µg/L	1	0.049	<2000 µg/L	1	0.041
	>2000 µg/L	24 (1.1–120)		>2000 µg/L	4.2 (1.1–16)	
sHJV	>40 µg/L	1	0.043	>40 µg/L	1	0.026
	<40 µg/L	8.0 (1.2–82)		<40 µg/L	2.5 (1.2–9.2)	

Abbreviations: AL, acute leukemia; HCT, hematopoietic cell transplantation; sHJV, soluble hemojuvelin.

4. Discussion

Although it is well known that iron overload is a life-threatening complication of HCT, the precise mechanism behind it is far from understood. In this study, we attempted to assess comprehensively the ability of iron metabolism parameters to predict short-term mortality in pediatric HCT patients. As far as we know, we are the first to demonstrate that pediatric patients with low soluble hemojuvelin (sHJV) serum levels one-month post-HCT have worse survival than those with high sHJV serum levels. We also confirmed that hyperferritinemia (serum ferritin >2000 µg/L) is unequivocally associated with reduced survival of HCT pediatric patients.

The Kaplan-Meier curve revealed that HCT pediatric patients with serum sHJV levels <40 µg/L had shorter survival than those with serum sHJV levels >40 µg/L. In addition, univariate and multivariate analyses confirmed the association between low serum sHJV levels and poor overall survival (OS) and event-free survival (EFS). These associations were also established when HCT patients were combined with children diagnosed with acute leukemia (AL) undergoing intensive chemotherapy. This new data from our observational study unambiguously indicates that sHJV plays a primary role in controlling systemic iron metabolism. Most previous studies have investigated the clinical utility of hepcidin as a potential HCT patient outcome marker [30,31]. Somewhat surprisingly, we did not observe the relationship between serum hepcidin levels and short-term mortality of HCT pediatric patients; however, it should be noted that, compared with our study, the two previous studies [30,31] reported the association between pre-HCT hepcidin levels and patient outcome in adults. We focused solely on evaluating such relationships in young

patients, as our understanding of them in this age group is limited. While our finding might depend upon the low sample size, it might also indicate that the time of blood collection determines the role of hepcidin and other laboratory markers of iron metabolism in appraising HCT patient evolution.

Current experimental and clinical study data support the hypothesis that HJV has a role in iron metabolism by controlling hepcidin synthesis [26,32–34]. The soluble form of this protein, measured in the present study, suppresses hepcidin synthesis and can lead to iron overload [35–37]. High serum sHJV levels have previously been reported in patients with congenital dyserythropoietic anemia type I [33] and thalassemia [34], but their levels in patients after HCT are not widely known. Our previous study illustrated that serum levels of sHJV after HCT in the pediatric population are significantly lower than those observed in healthy children [18]. Low levels of plasma sHJV have also been described in patients with nonalcoholic fatty liver disease (NAFLD) associated with iron overload [38]. While these results are not truly comparable to ours, due to the incompatible etiopathophysiology of the described disorders, they nevertheless may be helpful to understand the mechanism of sHJV-associated mortality. The reasons that low serum sHJV levels are associated with a high mortality rate in HCT pediatric patients remain unknown. Nonetheless, cell culture and animal studies suggest that body iron load negatively regulates sHJV release from cells [35,39,40]. A few clinical studies have shown that sHJV negatively correlates with serum ferritin levels [34] and is low in iron-overload patients [38]. Thus, low serum sHJV levels may result from the significant iron overload observed in post-HCT pediatric patients, as evidenced by hyperferritinemia, among other indicators. There is more to be known about sHJV. The activity of this protein is modulated by sophisticated machinery, involving, inter alia, neogenin (NEO1) and matriptase-2 (TMPRSS6) [26,32]. There would be significant research value in a study that assesses the relationship among sHJV, NEO1, TMPRSS6, and both short- and long-term mortality of HCT patients. Ideally, such a complex study would measure these iron metabolism markers both pre- and post-transplantation.

Our study confirmed that pediatric patients, after HCT or diagnosed with AL after intensive chemotherapy, develop iron overload (IO), a life-threatening, yet common, complication in these groups of patients. This condition is demonstrated in everyday practice with high serum ferritin levels, resulting from frequent packed red blood cell (PRBC) transfusions. This exacerbates systemic iron stores. The relationship between repeated transfusions, hyperferritinemia, and poor outcomes for HCT and AL patients is extensively discussed in the literature. In turn, the effect of PRBC transfusions on sHJV, the second crucial parameter of iron metabolism predicting OS and EFS in our cohort, is barely understood and practically unexplained. Since the serum levels of HJV are regulated by iron stores in the body [35,39,40], the evaluation of such interdependence appears to be tremendously advantageous for explaining the mechanisms in which sHJV is involved in the pathophysiology of IO. All of our patients, after HCT and with AL after intensive chemotherapy, received PRBC transfusions. This, consequently, led to IO, reflected in prominent hyperferritinemia, among other issues. Interestingly, these patients were characterized by low levels of sHJV, particularly in children after transplantation. The relationship between PRBC transfusions and sHJV levels remains a matter for further intensive research, especially in light of previous results that suggest lower sHJV levels in patients with β-thalassemia major who are receiving PRBC, as compared with untransfused β-thalassemia intermedia individuals [34].

Our study has several limitations that should be considered when interpreting the results. First, we included a relatively small group of patients in the study, but this number was sufficient to confirm our previous hypothesis that there is a relationship between the intensity of treatment in pediatric post-HCT and AL patients and the severity of iron metabolism imbalance. Moreover, the incorporation of as many as 12 different parameters of iron metabolism puts this work at the forefront of studies of iron metabolism and pediatric hematology. Although our study only examined the relationship between the parameters of iron metabolism and short-term mortality of post-HCT or chemotherapy

pediatric patients, we could still demonstrate that a fairly sturdy interdependence exists between serum ferritin and sHJV and short-term mortality in this patient population.

5. Conclusions

This observational study met its primary objective of evaluating the interconnection between iron metabolism parameters and short-term mortality of pediatric patients undergoing HCT or receiving chemotherapy. It has confirmed that post-treatment sHJV serum levels are linked to this outcome and has explored the possibility of sHJV involvement in controlling iron metabolism. The soluble form of HJV might be a promising biomarker of short-term outcomes for post-HCT or post-chemotherapy pediatric patients; however, more studies are warranted to confirm this dependence. Future studies should aim to determine which molecular mechanism may be engaged in the higher mortality experienced by patients with low serum sHJV levels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers15041041/s1, Table S1: Differences between parameters of iron metabolism.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of Collegium Medicum Bydgoszcz, code 608/2019, on 25 June 2019, annexed on 15 March 2022.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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cancers

MDPI

Systematic Review

Hepcidin in Children and Adults with Acute Leukemia or Undergoing Hematopoietic Cell Transplantation: A Systematic Review

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Simple Summary: In this systematic review, we summarized the observational studies on hepcidin in patients treated for acute leukemia or undergoing hematopoietic cell transplantation (AL/HCT). Thanks to the rigorous methodology used, we were able to trace the available literature conscientiously and draw the following conclusions: (1) in both children and adults with AL and qualified for HCT, hepcidin levels are high regardless of the phase of the disease or iron resources;, (2) AL therapy, and HCT in particular, may affect hepcidin levels, but the data, especially for children, are fragmented; (3) pre-HCT hepcidin levels may help predict post-HCT outcomes; (4) there is a need to standardize the determination of hepcidin levels in the clinical setting. We find a very large discrepancy in the reported mean and median hepcidin levels, both in healthy subjects and in AL. This significantly hinders the interpretation and comparison of the results.

Abstract: Objectives: The association between hepcidin and acute leukemia (AL) or hematopoietic cell transplantation (HCT) in children and adults remains obscure. We aimed to assess this potential relationship through a systematic review of observational studies. Methods: An electronic search of three databases, including PubMed, Scopus, and Web of Science Core Collection, was performed up to 31 March 2022. Two independent reviewers assessed the search results according to predetermined inclusion and exclusion criteria, following PRISMA guidelines. Results: Of the 3607 titles identified, 13 studies published between 2008 and 2021 met the inclusion criteria. Most studies included a moderate number of participants and controls and used enzyme-linked immunosorbent assay (ELISA) to determine serum hepcidin levels. The principal findings: (1) serum hepcidin levels in patients with AL or undergoing HCT are increased compared to controls, regardless of the patient's age and the phase of disease treatment; (2) AL therapy and HCT significantly influence serum hepcidin levels; (3) serum hepcidin may predict a worse outcome in patients with AL and post-HCT. Conclusions: This systematic review provides an overview of observational studies that deal with the association of hepcidin with AL and HCT. Although disturbances in iron metabolism are common in AL and HCT, and hepcidin seems to play a cardinal role in their modulation, more extensive research is needed.

Keywords: hepcidin; iron overload; ferritin

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1. Introduction

Acute leukemias (AL) are belligerently progressive neoplasms of the bone marrow, characterized by clonal expansion of immature and highly undifferentiated hematopoietic cells [1,2]. In conformity with the 2020 Global Cancer Statistics study, leukemias were diagnosed worldwide in over 474,000 patients with over 311,000 mortalities [3]. Despite

substantial progress in AL patient management, these diseases remain a grievous clinical concern in pediatric and adult groups [4,5].

It is becoming increasingly clear that iron overload is an exceptionally influential component of the pathophysiology of AL, and, as recent studies show, this over-abundance is a fundamental factor that may negatively affect the outcome of patients [6,7]. Although iron overload is observed in some patients with AL at diagnosis [8,9], the leading causes of this phenomenon are frequent blood transfusions [10,11] during chemotherapy treatment [12–15]. Notably, many investigations revealed a close relationship between iron overload—primarily defined as hyperferritinemia—and poor prognosis in patients undergoing hematopoietic cell transplantation (HCT) [8,16,17]. This was also confirmed in a meta-analysis of 25 studies with 4545 patients undergoing HCT, which demonstrated that pre-transplantation hyperferritinemia has a negative prognostic role and is associated with decreased overall survival and progression-free survival, as well as a higher incidence of non-relapse mortality and bloodstream infections [18].

Due to the extreme toxicity of iron in the human body, an immeasurably precise mechanism operates to control its levels inside and outside of cells. Many proteins are involved in this machinery, but hepcidin is of crucial importance. Produced by the hepatocytes, this small 25-amino acids protein inhibits iron absorption and releases from tissue resources by degrading ferroportin, the sole known cellular iron exporter [19]. Considering the paramount role of hepcidin in iron metabolism and the disturbances noted in AL and after HCT, it is understandable that researchers were looking for the role of hepcidin in the course of diseases.

Studies published to date, also by our team, suggest that hepcidin levels are high in both children and adults with AL [20,21]. Most studies, however, focus on assessing hepcidin levels in HCT patients as a predictor of adverse events such as infections, acute graft-versus-host disease (aGVHD), and poor overall survival [22–24]. That notwithstanding, the disorganized data on the relationship between hepcidin, AL, and HCT may cause an earnest misinterpretation of the role of hepcidin in the pathophysiology and prognosis of the disease process or the post-transplant patient response.

To clarify this issue, we performed a systematic review of studies reporting the association between hepcidin levels, AL, and HCT. As far as we are aware, our study is the first that exclusively focuses on the assessment of hepcidin in these patient groups.

2. Materials and Methods

2.1. Search Methodology

We conducted a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Supplementary Table S1) [25] and prospectively registered the review on PROSPERO (identifier: CRD42022323952).

We comprehensively searched the PubMed, Scopus, and Web of Science Core Collection electronic databases to identify studies published until 31 March 2022 (date of the last search), with detailed search terms for: “hepcidin”, “leukemia”, and “hematopoietic stem cell transplantation”. The full PubMed search strategy is shown in Supplementary Table S2 and was appropriately translated for the other two databases. There were no restrictions on language or publication type. We also hand-searched the bibliographies of all the included studies and relevant review articles to identify any remaining studies. Duplicate studies were manually deleted using Zotero, version 6.0.4 (Corporation for Digital Scholarship, Vienna, VA, USA).

2.2. Study Selection

Two reviewers (A.S. and M.Ł.) made the initial selection of the studies based on titles and abstracts. Next, we obtained the full texts of the studies that seemed to fulfill the inclusion criteria for evaluation. Any discrepancies were resolved by consensus, referring back to the studies, in consultation with a third reviewer (J.S.).

2.3. Inclusion Criteria

Studies were deemed eligible if they met the following criteria: (1) were observational (case-control, cohort, or cross-sectional studies) [26] and (2) included original data relevant to measuring hepcidin levels in pediatric and adult AL patients or those undergoing HCT. Stages of disease or treatment modalities were not the criteria for excluding the study from the systematic review. We did not set a minimum number of patients as a criterion for a study's inclusion; however, case reports were not included in the systematic review.

2.4. Exclusion Criteria

Studies were excluded if they included patients with diseases other than AL—unless patients with AL constituted only a part of the study group. When AL patients comprised only a portion of the study group, we included such studies in the systematic review. For example, if the study group included patients with AL and myelodysplastic syndromes (MDS), the study was included in the current systematic review. Other exclusion criteria were insufficient data on hepcidin (e.g., lack of numerical values of hepcidin levels) and studies published in non-English languages. Clinical trials, reviews, case reports, editorials, comments, position articles, guidelines, chapters of books, conference proceedings, and nonhuman studies were also excluded.

2.5. Data Extraction

Relevant data from the included studies were extracted by two reviewers (M.Ł. and J.S.). From each eligible study, data were captured on the following: general study information (first author, year of publication, study design, and study location), participant characteristics (sample size, age, sex, diagnosis, and therapeutic modalities), details relating to the assessment of hepcidin (type of biospecimen, measurement time with corresponding detection method, and hepcidin levels), and, lastly, each study's main findings. All data were extracted from the published studies; we did not contact the corresponding authors to collect further information.

2.6. Quality Assessment

We assessed the methodological quality of the included studies by using the Newcastle–Ottawa (NOS) scale for case-control and cohort studies [27], adapted for cross-sectional studies [28]. The NOS score is recommended for assessing the quality of nonrandomized studies [29]. As originally developed, NOS consists of eight items in three domains: selection (total score 4), comparability (total score 2), and exposure for case-control studies or outcome for cohort studies (total score 3). The highest total score is nine points. The NOS, as adapted for cross-sectional studies, consists of seven items in three domains: selection (total score 5), comparability (total score 2), and outcome (total score 3), with the highest total score being ten points. A total score of 3 or less was considered low quality, 4 to 6 was considered moderate quality, and 7 to 9 (10 for cross-sectional studies) was deemed high quality [30]. Any discrepancies in the quality assessment were discussed and resolved by the two reviewers (A.S. and M.Ł.).

3. Results

3.1. Literature Search

Our systematic search identified 3607 unique citations. Of these, 3439 (95%) were found in Scopus, 106 (3%) were found in Web of Science, and 62 (2%) were found in PubMed. No additional studies were identified through our hand search of references from published studies, relevant reviews, and previous meta-analyses.

After adjusting for duplicates, the searches provided a total of 2949 citations, of which 2802 were excluded based on abstract and title. One hundred forty-seven articles underwent full-text review, and one hundred thirty-four were excluded. The remaining thirteen met all inclusion criteria [20–22,24,31–39]. There was no disagreement about any of the studies selected for final inclusion in the systematic review. Most studies were excluded

because they were irrelevant to the current study subject (n = 45). A full list of excluded studies and reasons for exclusion is available in Supplementary Table S3. Due to the study design's high heterogeneity, we did not use formal meta-analysis techniques. The flow of study selection is reported in Figure 1.

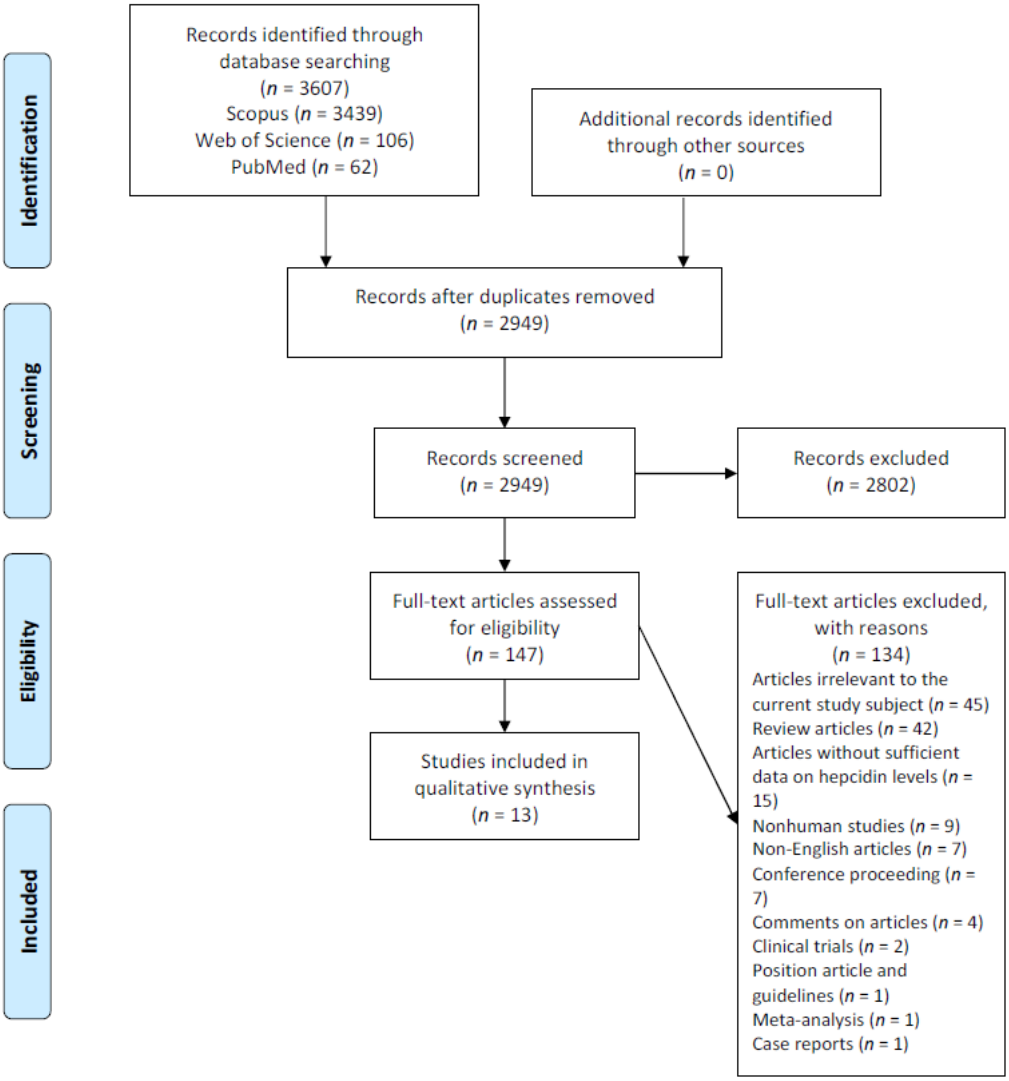

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

3.2. Characteristics of the Included Studies

The characteristics of the studies included in the review are given in Table 1 for case-control studies and in Table 2 for cohort and cross-sectional studies. The studies were published between 2008 and 2021 with 92% (n = 12) of the studies published on or before 2020 [20,22,24,31–39]. Out of the thirteen studies [20–22,24,31–39], three were conducted in Japan [22,24,31], two in Germany [32,38], two in China [20,34], and one each in the United States [33], Brazil [35], Egypt [36], Turkey [37], Indonesia [39], and Poland [21]. Eleven studies were single-center [20,22,24,31–37,39] while two were multicenter [21,38]. We included seven case-control studies [20,21,31,32,34,36,37], four cohort studies [22,24,35,38] and two cross-sectional studies [33,39]. Eleven studies were fully published journal articles [20–22,24,32–34,36–39]; one study was published as a brief report [31], and one as a letter to the editor [35].

Table 1. Characteristics of the seven case-control studies included in a systematic review.

First Author, Year	Region of Origin	Number of Patients	Mean or Median Age of Patients [Years]	Clinical Diagnosis	HCT	Hepcidin Assay	Type of Biological Material	Mean or Median Hepcidin Levels in Patients	Number of Controls	The Mean or Median Age of Controls [Years]	Mean or Median Hepcidin Levels in Controls	Main Study Results
Kanda et al., 2008 [31]	Asia (Japan)	31 (W: ND M: ND)	51	13 AML, 8 NHL, 3 MDS, 3 ATL, 2 ALL, 1 HL, 1 MM	Yes (5 autologous; 26 allogeneic)	LC/ESI-MS/MS	Blood (serum)	42.8 ng/mL (one week pre-HCT) 232.5 ng/mL (one week post-HCT)	17 (W:1 M:16)	31	19.05 ng/mL	(1) hepcidin levels were higher in patients than in controls (2) hepcidin levels increased until the first week post-HCT, then decreased to the fourth week after HCT
Eisfeld et al., 2011 [32]	Europe (Germany)	42 (W:19 M: 23)	57	AML	Yes (42 allogeneic)	ELISA (Intrinsic Hepcidin IDx™ ELISA Kit, Intrinsic Life Sciences, La Jolla, CA, USA)	Blood (serum)	358 ng/mL (ten days pre-HCT) 398 ng/mL (three months post-HCT	21 (W:15 M:6)	57	52.1 ng/mL	(1) hepcidin levels were higher in patients than in controls (2) pre- and post-HCT hepcidin levels were similar
Cheng et al., 2012 [20]	Asia (China)	32 (W:13 M:19)	Three groups of patients *: A—29 (n = 10) B—33 (n = 15) C—34 (n = 7)	AL †	No	ELISA (Uscn Life Science Inc, Wuhan, China)	Blood (serum)	A—343.447 ng/mL B—523.758 ng/mL C—486.176 ng/mL (all before treatment) 685.633 ng/mL (before treatment; n = 20) 485.438 ng/mL (during complete or partial remission; n = 20)	11 (W:4 M:7)	35	141.098 ng/mL	(1) hepcidin levels were higher in patients than in controls, regardless of patients' iron storage (2) hepcidin levels decreased during complete or partial remission
Chen et al., 2013 [34]	Asia (China)	57 (W: 26 M: 31)	49	27 HCT (due to hematologic tumors), 18 liver transplantation, 12 kidney transplantation	Yes (27 autologous or allogeneic)	ELISA (DRG Instruments GmbH, Marburg, Germany)	Blood (serum)	38.31 ng/mL (pretransplant) 51.82 ng/mL (one week before transplantation in the high-hepcidin group; n = 19) ‡ 129.60 ng/mL (one week after transplantation in the high-hepcidin group; n = 19)	50 (W:ND M: ND)	ND	18.70 ng/mL	(1) hepcidin levels were higher in patients than in controls (2) hepcidin levels increased until the first week after transplantation, then decreased to the fourth week after transplantation (3) pretransplant hepcidin as a biomarker of invasive fungal disease

Table 1. *Cont.*

First Author, Year	Region of Origin	Number of Patients	Mean or Median Age of Patients [Years]	Clinical Diagnosis	HCT	Hepcidin Assay	Type of Biological Material	Mean or Median Hepcidin Levels in Patients	Number of Controls	The Mean or Median Age of Controls [Years]	Mean or Median Hepcidin Levels in Controls	Main Study Results
Ragab et al., 2016 [36]	Africa (Egypt)	40 (W: 13 M: 27)	Two groups of patients #: I—5 (n = 20) II—5 (n = 20)	ALL	No	ELISA (EIAab® Human Hepcidin ELISA kit, EIAab Science INC, Wuhan, China)	Blood (serum)	Group I: 387.6 ng/mL (at diagnosis) 221.5 ng/mL (after remission) Group II: 181.9 ng/mL (during maintenance therapy)	20 (W:6 M:14)	6	69.8 ng/mL	(1) hepcidin levels were higher in patients than in controls, regardless of the stage of the disease (2) hepcidin levels decreased during remission (3) hepcidin levels were the lowest during maintenance therapy
Yavuz et al., 2017 [37]	Asia (Turkey)	58 (W: 26 M: 32)	10	28 sarcomas, 11 ALL, 10 solid tumors, 9 lymphomas $	No	ELISA (DRG Instruments GmbH, Marburg, Germany)	Blood (serum)	Sarcomas 34.51 ng/mL (at diagnosis) 17.92 ng/mL (at remission) Lymphomas 24.83 ng/mL (at diagnosis) 21.13 ng/mL (at remission) ALL 58.45 ng/mL (at diagnosis) 50.81 ng/mL (at remission) Solid tumors 43.82 ng/mL (at diagnosis) 35.60 ng/mL (at remission)	17 (W: 8 M:9)	9	6.98 ng/mL	(1) hepcidin levels were higher in patients than in controls, regardless of the stage of the disease

Table 1. *Cont.*

First Author, Year	Region of Origin	Number of Patients	Mean or Median Age of Patients [Years]	Clinical Diagnosis	HCT	Hepcidin Assay	Type of Biological Material	Mean or Median Hepcidin Levels in Patients	Number of Controls	The Mean or Median Age of Controls [Years]	Mean or Median Hepcidin Levels in Controls	Main Study Results
Łęcka et al., 2021 [21]	Europe (Poland)	67 (W: 30 M: 37)	7	21 AL de novo, 25 AL after intensive treatment, 21 HCT €	Yes (3 autologous; 18 allogeneic)	ELISA (Intrinsic Hepcidin IDx™ ELISA Kit, Intrinsic Life Sciences, La Jolla, CA, USA)	Blood (serum)	158.50 ng/mL (AL de novo) 106.60 ng/mL (AL after intensive therapy) 278.30 ng/mL (one month post-HCT)	18 (W:10 M: 8)	8	30.61 ng/mL	(1) hepcidin levels were higher in patients than in controls, regardless of the stage of the disease (2) hepcidin levels were the highest post-HCT

* Patients were divided into three groups (A, B, C) according to the degree of the extracellular iron (EI) store and the value of the intracellular iron (II) store. † FAB criteria: 2 M1, 4 M2, 5 M3, 6 M4, 3 M5, 12 ALL. ‡ High-hepcidin group = patients with serum hepcidin levels greater than 40 ng/mL. We wrote 'one week before transplantation' on purpose, not 'one week before HCT' because the patient group included those who underwent HCT and those who underwent liver and kidney transplants. # Patients were divided into two groups (I, II): group I (newly diagnosed ALL; $n = 20$), group II (patients with ALL in the maintenance phase of therapy; $n = 20$). $ Sarcoma (11 Ewing's sarcoma, 11 osteosarcoma, 6 rhabdomyosarcoma); solid tumors (4 neuroblastoma, 3 central nervous system tumors, 2 Wilms tumor, 1 hepatoblastoma); lymphoma (5 NHL, 4 HL). € HCT group: 8 acute myeloid leukemia, 6 acute lymphoblastic leukemia, 2 neuroblastoma, 1 myelodysplastic syndrome, 1 severe aplastic anemia, 1 severe congenital neutropenia, 1 anaplastic large B-cell lymphoma, 1 Ewing sarcoma. Abbreviations: AL = acute leukemia; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ATL = adult T-cell leukemia; ELISA = enzyme-linked immunosorbent assay; HL = Hodgkin lymphoma; HCT = hematopoietic cell transplantation; LC/ESI-MS/MS = liquid chromatography-electrospray ionization tandem mass spectrometry; M = man; MDS = myelodysplastic syndrome; MM = multiple myeloma; ND = not determined; NHL = non-Hodgkin lymphoma; W = woman.

Table 2. Characteristics of the four cohort studies and the two cross-sectional studies included in a systematic review.

First Author, Year	Region of Origin	Number of Patients	Mean or Median Age of Patients [Years]	Clinical Diagnosis	HCT	Hepcidin Assay	Type of Biological Material	Mean or Median Hepcidin Levels in Patients	Follow-Up	Main Study Results
Kanda et al., 2009 [22]	Asia (Japan)	55 (W: 28 M: 27)	47 (whole cohort) Two groups of patients *: Low-hepcidin—47.5 ($n = 38$) High-hepcidn—47 ($n = 17$)	23 AML, 9 MDS/ MPN, 9 NHL, 8 ALL, 5 ATL, 1 HL	Yes (47 allogeneic)	LC/ESI-MS/MS	Blood (serum)	21.6 ng/mL (pre-HCT)	100 days post-HCT	(1) high hepcidin levels ($\geq$ 50 ng/mL) are associated with an increased risk of bacterial infection post-HCT (2) hepcidin as a biomarker of bacterial infection post-HCT
Armand et al., 2011 [33]	North America (USA)	48 (ND)	47	29 AML, 11 ALL, 8 MDS	Yes (48 allogeneic)	MALDI-TOF MS	Blood (plasma or serum) † Urine	59 ng/mL (blood, pre-HCT, $n = 39$) 110 ng/mg creatinine (urine, $n = 33$)	-	(1) blood hepcidin levels are correlated to other iron parameters

Table 2. *Cont.*

First Author, Year	Region of Origin	Number of Patients	Mean or Median Age of Patients [Years]	Clinical Diagnosis	HCT	Hepcidin Assay	Type of Biological Material	Mean or Median Hepcidin Levels in Patients	Follow-Up	Main Study Results
Naoum et al., 2016 [35]	South America (Brazil)	25 (W: 15 M: 10)	46	13 MM, 8 ML, 3 AL, 1 seminoma	Yes (25 autologous)	ELISA (DRG Instruments GmbH, Marburg, Germany)	Blood (serum)	25.1 ng/mL (before the start of conditioning) 40.0 ng/mL (before SC infusion) 39.1 ng/mL (on engraftment)	engraftment [#]	(1) hepcidin levels were higher before SC infusion and on engraftment than before the start of conditioning
Sakamoto et al., 2017 [24]	Asia (Japan)	166 (W: 74 M: 92)	49.5 (whole cohort) Two groups of patients [‡]: Low-hepcidin—47 (n = 83) High-hepcidn—51 (n = 83) [‡]	103 MMa, 63 LM	Yes (166 allogeneic)	SELDI-TOF MS	Blood (serum)	7.8 ng/mL [$] 35.0 ng/mL (pre-HCT)	46.8 months (median)	(1) high hepcidin levels ($\geq$35 ng/mL) are associated with lower overall survival post-HCT (2) high hepcidin levels are associated with a lower incidence of platelet engraftment post-HCT
Wermke et al., 2018 [38]	Europe (Germany)	112 (W: 47 M:65)	62 (whole cohort) Two groups of patients [€]: eLPI µmol/L $\leq$ 0.4—62 (n = 85) eLPI µmol/L > 0.4—62 (n = 27)	90 AML, 22 MDS	Yes (112 allogeneic)	ELISA (DRG Instruments GmbH, Marburg, Germany)	Blood (serum)	77 ng/mL (whole cohort; pre-HCT) eLPI $\leq$ 0.4 µmol/L: 70 ng/mL (pre-HCT) 64 ng/mL (on the day of HCT) 81 ng/mL (on day 21 post-HCT) eLPI > 0.4 µmol/L: 103 ng/mL (pre-HCT) 83 ng/mL (on the day of HCT) 127 ng/mL (on day 21 post-HCT)	373 days (median)	(1) hepcidin levels were higher in the eLPI > 0.4 µmol/L group pre-HCT and on day 21 post-HCT

Table 2. *Cont.*

First Author, Year	Region of Origin	Number of Patients	Mean or Median Age of Patients [Years]	Clinical Diagnosis	HCT	Hepcidin Assay	Type of Biological Material	Mean or Median Hepcidin Levels in Patients	Follow-Up	Main Study Results
Wande et al., 2020 [39]	Asia (Indonesia)	48 (W: 17 M: 31)	Three groups of patients: induction phase—6.8 (n = 16) consolidation phase—9.7 (n = 16) maintenance phase—7.8 (n = 16)	48 ALL	No	ELISA (Bioassay Technology Laboratory, Jiaxing, China)	Blood (serum)	7.545 ng/mL (induction phase) 1.728 ng/mL (consolidation phase) 0.210 ng/mL (maintenance phase)	-	(1) hepcidin levels vary depending on disease state

* Patients were divided into two groups: low-hepcidin group (hepcidin levels lower than 50 ng/mL; n = 38), high-hepcidin group (hepcidin levels greater than 50 ng/mL; n = 17). † In the materials and methods section, the authors reported that they measured plasma levels of hepcidin, but the results report the levels of hepcidin in serum samples. ‡ Patients were divided into two groups: low-hepcidin group (hepcidin levels lower than 35 ng/mL; n = 83), high-hepcidin group (hepcidin levels greater than 35 ng/mL; n = 83). # Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of at least 0.5×10^9/L. $ In the results section, the authors presented the hepcidin levels in the healthy volunteers; however, this group was not described in materials and methods, and there are no details regarding this analysis; hence, we arbitrarily concluded that the study is a cohort. € Patients were divided into two groups: eLPI (enhanced labile plasma iron) $\leq$ 0.4 µmol/L (n = 85), eLPI > 0.4 µmol/L (n = 27). Abbreviations: AL = acute leukemia; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ATL = adult T-cell leukemia; ELISA = enzyme-linked immunosorbent assay; HL = Hodgkin lymphoma; HCT = hematopoietic cell transplantation; LC/ESI-MS/MS = liquid chromatography-electrospray ionization tandem mass spectrometry; LM = lymphoid malignancies; M = man; MALDI-TOF MS = matrix assisted laser desorption/ionization time-of-flight mass spectrometry; MDS = myelodysplastic syndrome; ML = malignant lymphoma; MM = multiple myeloma; MMa = myeloid malignancies; ND = not determined; NHL = non-Hodgkin lymphoma; SELDI-TOF MS = surface enhanced laser desorption/ionization time-of-flight mass spectrometry; SC = stem cell; W = woman.

All studies combined, there were a total of 781 patients and 154 controls. Most patients were male [20,21,24,32,34,36–39]. In four studies [37,38,40,41], participants were children and adolescents, with the mean or median age ranging from 5 [36] to 10 [37] years old; all participants in the other studies [20,22,24,31–35,38] were adults, and the mean or median age ranged from 29 [20] to 62 [38] years old. Participants represent heterogeneous populations; however, most studies included patients with acute myeloid leukemia (AML) [20,22,31–33,38]. None of the included studies describe the selection of controls; hence, it is unknown whether the participants were population- or hospital-based controls. Follow-up varied between studies, ranging from 100 days [22] to 46.8 months [24]. One study reported follow-up as documented engraftment [35]. The patient populations were HCT in nine studies [21,22,24,31–35,38]. Five studies were limited to allogeneic HCT [22,24,32,33,38], one study was limited to autologous HCT [35], and the remaining three studies included both autologous and allogeneic HCT [21,31,34]. Only one study included pediatric HCT patients [21].

3.3. Quality Assessment of Studies

We assessed the quality of the thirteen eligible studies according to the NOS (Supplementary Tables S4–S6). We judged four studies [20,21,32,36] to be of high methodologic quality and the remaining nine studies [22,24,31,33–35,37–39] to be of moderate quality. No studies were of low quality. The median NOS score was 6 (range: 5 to 7).

3.4. Hepcidin Assays

All included studies specified the time at which biological samples were obtained. Eight studies have documented pre-transplant hepcidin levels [22,24,31–35,38], six post-transplant hepcidin levels [21,31,32,34,35,38], four before treatment [20,21,36,37], two during treatment [36,39], three at remission [20,36,37], and one after treatment [21]. Five studies had three time points of assessment [20,34,35,38,39]. Data on the collection, preparation, or storage of biological samples were described in five studies [21,22,24,33,37]. For the testing of biological materials, twelve of the studies evaluated hepcidin levels in the blood (serum or plasma) [20–22,24,31,32,34–39] and the other one in blood and urine [33]. Different hepcidin assays were applied in the selected studies. Nine studies used enzyme-linked immunosorbent assay (ELISA) kits from different suppliers [20,21,32,34–39]. However, the most commonly employed kit in the four studies was DRG Instruments GmbH (Marburg, Germany) [34,35,37,38]. The mass spectrometry (MS) methods were also used to measure hepcidin levels in four studies [22,24,31,33]. Most of the selected studies did not provide sufficient data regarding the methods applied for hepcidin measurement. Only one study reported assay range, detection limit, and intra- and inter-assay coefficient of variation (CV) for hepcidin assay [21]. Two studies reported a normal range for serum hepcidin, according to manufacturers [32,38]. Blinding of laboratory personnel to the clinical characteristics and patients' outcomes was reported in one study [21]. For the standardization of the results and straightforward comparisons between studies in this systematic review, the hepcidin levels are presented in nanograms per milliliter (ng/mL) for each study included.

Table 3 compares blood hepcidin levels with a commonly used marker illustrating iron metabolism, i.e., serum ferritin levels. As expected, ferritin levels were very high in the patients in the included studies, which is also related to the number of packed red blood cells (PRBCs) transfused (Table 3).

Table 3. The blood hepcidin levels reported in patients with AL/HCT on the background of serum ferritin levels and packed red blood cells (PRBCs).

First Author, Year	Mean or Median Hepcidin Levels in Patients *	Mean or Median Ferritin Levels in Patients † Reference Range: 1.2–20.0 µg/dL	Mean or Median Units of PRBCs
Kanda et al., 2008 [31]	42.8 ng/mL (one week pre-HCT)	726.3 µg/dL (one week pre-HCT)	ND
	232.5 ng/mL (one week post-HCT)		
Kanda et al., 2009 [22]	21.6 ng/mL (pre-HCT)	664 µg/dL (low hepcidin group)	ND
		1551 µg/dL (high hepcidin group)	
Eisfeld et al., 2011 [32]	358 ng/mL (ten days pre-HCT)	194.5 µg/dL (pre-HCT)	22 (pre-HCT)
	398 ng/mL (three months post-HCT)	226 µg/dL (post-HCT)	30 (post-HCT)
Armand et al., 2011 [33]	59 ng/mL (pre-HCT)	154.9 µg/dL	20 (pre-HCT)
Cheng et al., 2012 [20]	A—343.447 ng/mL B—523.758 ng/mL C—486.176 ng/mL (all before treatment)	A—62. 806 µg/dL B—94.964 µg/dL C—77.381 µg/dL (all before treatment)	ND
	685.633 ng/mL (before treatment)	105.082 µg/dL (before treatment)	
	485.438 ng/mL (during complete or partial remission)	61.437 µg/dL (during complete or partial remission)	
Chen et al., 2013 [34]	38.31 ng/mL (pretransplant) ‡	The authors did not present numerical values for ferritin but observed its high serum levels.	ND
	51.82 ng/mL (one week before transplantation in the high-hepcidin group)		
	129.60 ng/mL (one week after transplantation in the high-hepcidin group)		
Naoum et al., 2016 [35]	25.1 ng/mL (before the start of conditioning)	73.3 µg/dL (before the start of conditioning)	3 (from SC infusion to engraftment)
	40.0 ng/mL (before SC infusion)	78.2 µg/dL (before SC infusion)	
	39.1 ng/mL (on engraftment)	77.8 µg/dL (on engraftment)	
Sakamoto et al., 2017 [24]	35.0 ng/mL (pre-HCT)	69.4 µg/dL (pre-HCT)	
Ragab et al., 2016 [36]	Group I: 387.6 ng/mL (at diagnosis) 221.5 ng/mL (after remission)	Group I: 126.5 µg/dL (at diagnosis) 79.3 µg/dL (after remission)	ND
	Group II: 181.9 ng/mL (during maintenance therapy)	Group II: 60.4 µg/dL (during maintenance therapy)	

Table 3. *Cont.*

First Author, Year	Mean or Median Hepcidin Levels in Patients *	Mean or Median Ferritin Levels in Patients † Reference Range: 1.2–20.0 µg/dL	Mean or Median Units of PRBCs
Yavuz et al., 2017 [37]	Sarcomas 34.51 ng/mL (at diagnosis) 17.92 ng/mL (at remission)	ND	ND
	Lymphomas 24.83 ng/mL (at diagnosis) 21.13 ng/mL (at remission)		
	ALL 58.45 ng/mL (at diagnosis) 50.81 ng/mL (at remission)		
	Solid tumors 43.82 ng/mL (at diagnosis) 35.60 ng/mL (at remission)		
Wermke et al., 2018 [38]	77 ng/mL (whole cohort; pre-HCT)	1731 µmol/L # (whole cohort)	18 (whole cohort)
	eLPI ≤ 0.4 µmol/L: 70 ng/mL (pre-HCT) 64 ng/mL (on the day of HCT) 81 ng/mL (on day 21 post-HCT)	eLPI ≤ 0.4 µmol/L: 1563 µmol/L	eLPI ≤ 0.4 µmol/L: 18
	eLPI > 0.4 µmol/L: 103 ng/mL (pre-HCT) 83 ng/mL (on the day of HCT) 127 ng/mL (on day 21 post-HCT)	eLPI > 0.4 µmol/L: 2425 µmol/L	eLPI > 0.4 µmol/L: 22
Wande et al., 2020 [39]	7.545 ng/mL (induction phase)	135.307 µg/dL (induction phase)	ND
	1.728 ng/mL (consolidation phase)	175.826 µg/dL (consolidation phase)	
	0.210 ng/mL (maintenance phase)	74.977 µg/dL (maintenance phase)	
Łęcka et al., 2021 [21]	158.50 ng/mL (AL de novo)	23.85 µg/dL (AL de novo)	1
	106.60 ng/mL (AL after intensive therapy)	73.9 µg/dL (AL after intensive therapy)	9
	278.30 ng/mL (one month post-HCT)	367.0 µg/dL (one month post-HCT)	23

* The results are partially repeated with those presented in Tables 1 and 2; however, the re-presentation of the hepcidin levels facilitates its comparison with those of ferritin. † The units of the ferritin levels are standardized and presented as micrograms per deciliter (µg/dL). The reference values were based on data from the "*WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations*" (https://www.who.int/publications/i/item/9789240000124 (accessed on 4 July 2022)). ‡ We wrote 'pre-transplant' on purpose, not pre-HCT because the patient group included those who underwent HCT and those who underwent liver and kidney transplants. # The unit in which the authors reported the ferritin levels was left. However, it seems that a mistake was made here (e.g., when calculating the levels for all patients (n = 112), we receive extremely high values, i.e., 1730.0 µmol/L = 76985000.0 µg/dL). Abbreviations: AL = acute leukemia; ALL = acute lymphoblastic leukemia; eLPI = enhanced labile plasma iron; HCT = hematopoietic cell transplantation; ND = not determined; PRBC = packed red blood cells; SC = stem cell.

3.5. Hepcidin Levels in Childhood Leukemia

Three case-control studies [21,36,37] and one cross-sectional study [39] evaluated hepcidin levels in childhood leukemia. The studies included ranged in size from 40 [36] to 67 patients [21] and from 17 [37] to 20 controls [36], for a total number of 213 patients and 55 controls. Most of the included patients were diagnosed with acute lymphoblastic leukemia (ALL). The main message from case-control studies is that hepcidin levels are significantly higher in children with AL than in controls, regardless of the stage of the disease [21,36,37]. Hepcidin levels vary between phases of the disease [21,36,37,39], and

they appear to decrease for cases of childhood leukemia in remission when compared to the levels at the time of diagnosis [36,37]; however, a significant difference was found only in one study [36]. Two studies demonstrated that hepcidin levels are lower during maintenance therapy [36,39]. Particular attention should also be paid to the wide range of serum hepcidin levels in both AL and controls (Table 1), e.g., 58.45 ng/mL [37] to 387.6 ng/mL [36] at the diagnosis of the disease. In the controls, the differences are even more noticeable (ten times lower levels in one study [37] compared to another [36]). This is a supplemental factor, due to which we did not perform a meta-analysis.

3.6. Hepcidin Levels in Adult Leukemia

Four case-control studies [20,31,32,34], four cohort studies [22,24,35,38], and one cross-sectional study [33] were used to evaluate hepcidin levels in adulthood leukemia. The studies included ranged in size from 31 [31] to 166 patients [24] and from 17 [31] to 50 controls [34], for a total number of 568 patients and 99 controls. In the case of studies in adult patients, we found significant heterogeneity in the populations; however, AML seems to be the most frequently diagnosed.

The main message from case-control studies performed in adults with AL is similar to the conclusion involving childhood studies. Hepcidin levels are significantly higher in adults with AL than in the controls, regardless of the stage of the disease or of patients' iron storage [20,31,32,34]. A single study found a decrease in serum hepcidin levels in patients in remission compared to pre-treatment levels [20]. As the remaining studies [22,24,31–33,35,38] largely concerned patients undergoing HCT, we described their results in the next subsection of our systematic review. As in the pediatric population, studies in adult patients and adult controls showed a wide spread of the mean or median of hepcidin levels (Tables 1 and 2).

3.7. Hepcidin Levels in HCT Patients

HCT was performed in adult patients in eight studies (n = 498) [22,24,31–35,38]. In two studies, a significant increase in serum hepcidin levels was observed one week after HCT compared to pretransplant levels, with normalization of the levels one month after transplantation [31,34]. One study found no differences in the serum levels of hepcidin 10 days before HCT compared to the third month afterward [32]. In turn, the lowest level of serum hepcidin was found before the start of conditioning rather than before stem cell (SC) infusion or on engraftment [35]. High serum hepcidin levels before transplantation are also associated with a higher risk of bacterial infections [22], invasive fungal disease [34], and lower overall survival [24] after HCT. The remaining studies found a positive relationship between pre-transplant serum hepcidin levels and other markers of iron metabolism [33,38].

Only one study investigated hepcidin levels in children undergoing HCT (n = 21): it found that one month after transplantation, serum hepcidin levels were significantly higher compared to the levels at diagnosis or after the end of intensive chemotherapeutic treatment [21].

4. Discussion

Iron overload is a common secondary complication in patients treated for AL or undergoing HCT and is caused by frequent packed red blood cell concentrate (PRBC) transfusions. Each milliliter of transfused PRBC contains 0.8 mg of iron [40]; thus, repeated transfusions ponderously contribute to iron accumulation. No other diagnoses in oncology bearing this complication in such an aggrandized grade. Iron overload is the long-term sequelae of blood component therapy, and intensive treatment damages cells, causing clinically relevant homeostasis imbalance. The pathophysiological processes following one another include: PRBC repeated transfusions; iron delivery; ferritin production and storage; iron overload; imbalance in iron regulation; cellular, tissue, and organ toxicity; and finally, organ failure.

There are four significant findings of our study. First, hepcidin increases during intensive chemotherapy of AL, then partially decreases during maintenance therapy or after its completion. Second, in patients undergoing allogeneic HCT, hepcidin increases during the pre-engraftment period and might partially decrease after engraftment. Third, these profiles of serum hepcidin levels seem to be similar in children and adults. Fourth, hepcidin levels correlate with ferritin levels and iron overload status in patients treated for leukemia or undergoing allo-HCT.

From a pathophysiological point of view, hepcidin production in hepatocytes is stimulated by various factors, including iron and inflammatory status, expressed substantially by the upregulation of interleukin-6 (IL-6). The serum levels of hepcidin correlate with the serum levels of ferritin, and both proteins are upregulated by systemic iron overload. In this context, an increase in serum hepcidin levels reflects a regulation mechanism secondary to iron overload. Therefore, it is unsurprising that the ongoing disease process and applied treatment induce inflammation, leading to increased hepcidin synthesis. As such, our observation of high serum hepcidin levels is widely reported in children and adults. New data emerging from our analysis shows that, regardless of the phase of the disease (and thus also of treatment), hepcidin levels remain high in patients compared to controls.

Increased serum hepcidin levels are also the result of the compensation of iron overload. Nevertheless, mechanisms of homeostasis, and return of effective myelopoiesis, including erythropoiesis, cannot cause full utilization of iron excess. In this context, hepcidin in patients treated for AL or undergoing HCT is an ineffective marker of iron overload and metabolism.

No studies directly compare hepcidin levels in children and adults with AL. The profile of hepcidin levels during and after intensive anti-leukemic chemotherapy seems similar and age-independent. However, it is possible that mechanisms of homeostasis and organ abilities to compensate for organ toxicities are better in children than in adults; thus, some improvement, expressed as a decrease in iron overload, ferritin or hepcidin levels, can be expected in the pediatric population [41–43].

Hepcidin levels peaked after the conditioning and pre-engraftment phase in transplanted patients, then decreased during engraftment [44]; however, not in each study [35]. This process clinically correlates with the need for frequent blood transfusions before engraftment, which is not the case afterward due to more efficient myelopoiesis and partial iron utilization.

HCT is a perfect model showing the profile of changes in serum hepcidin levels related to iron overload, followed by effective erythropoiesis, presented in three phases. The first phase, on patients' referral to HCT, reflects the status of patients heavily transfused with PRBC, often in chronic inflammatory status caused by chemotherapy-induced mucositis and possible organ toxicity. Thus, pre-transplant serum hepcidin levels are usually high, at least doubled in most studies, compared to leukemic patients on diagnosis, both in children and adults [21,31,34]. In the second phase, during the conditioning and pre-engraftment phase, the serum hepcidin levels usually increase [34,35], as the intensity of PRBC transfusion is even higher than in non-transplant patients. High hepcidin levels cause a delay in platelet engraftment after HCT [24]. Finally, the third phase starts from the day of engraftment, followed by effective erythropoiesis, resulting in the utilization of iron and decreased ferritin and hepcidin levels. Obviously, it is not possible to utilize all excesses of iron; hence, the status of iron overload persists, causing cellular, tissue, and organ damage impairing their function and leading to worse overall survival [14,24]. In the case of non-transplant leukemic patients, this becomes a two-phasic model. The first phase, during intensive chemotherapy, is characterized by frequent PRBC transfusions and increasing iron overload, followed by increased ferritin and hepcidin levels [21,36]. In the second phase, during maintenance therapy or after cessation of treatment, when effective erythropoiesis is present, the content of iron and levels of ferritin and hepcidin is lowered [36].

The strength of the study is the first systematic review on hepcidin in AL/HCT patients with a new insight into the assessment of serum hepcidin levels in pediatric and adult patients, showing variability in serum profile before, during, and after AL/HCT treatment.

This study has several limitations due to heterogeneity of the studies, relatively low number of patients included in basic studies, lack of studies comparing other novel parameters of iron metabolism, lack of studies comparing children and adults, and lack of long-term analyses of survival outcomes.

5. Conclusions

To the best of our knowledge, our study is the first to summarize the observational studies on hepcidin in AL and HCT. Thanks to the rigorous methodology used, we were able to trace the available literature conscientiously and draw the following conclusions: (1) in both children and adults with AL and qualified for HCT, hepcidin levels are high regardless of the phase of the disease or iron resources; (2) AL therapy, and HCT in particular, may affect hepcidin levels, but the data, especially for children, are fragmented; (3) pre-HCT hepcidin levels may help predict post-HCT outcomes; (4) there is a need to standardize the determination of hepcidin levels in the clinical setting. We find a very large discrepancy in the reported mean and median hepcidin levels, both in healthy subjects and in AL. This significantly hinders the interpretation and comparison of the results.

In conclusion, we can show that the profile of hepcidin levels in patients treated for AL/HCT is presumably similar in children and adults. Hepcidin levels increase relatively quickly with RBC transfusions during intensive chemotherapy for AL or between the start of conditioning and engraftment after HCT. However, in both settings, it tends to decrease: during maintenance therapy for AL or in the post-engraftment phase of HCT. Nevertheless, the homeostasis mechanisms are not efficient enough, and increased hepcidin levels might be a risk factor for overall survival.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers14194936/s1, Table S1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist; Table S2: PubMed search strategy; Table S3: A complete list of excluded studies along with reasons for exclusion; Table S4: The Newcastle–Ottawa Scale (NOS) for case-control studies; Table S5: The Newcastle–Ottawa Scale (NOS) for cohort studies; Table S6: The Newcastle–Ottawa Scale (NOS) for cross-sectional studies.

Author Contributions: A.S. was responsible for the study design and supervision, developed and performed the search strategy, performed full-text literature screening and assessed the quality, extracted the data, analyzed the data and drafted the manuscript, and validated the final version of the manuscript. M.Ł. performed the literature search, performed full-text literature screening and assessed the quality, extracted the data, analyzed the data, drafted tables, and revised the manuscript. J.S. was responsible for the study design and supervision, drafted the manuscript, and validated the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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