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Skuteczność terapii innowacyjnych w onkologii dziecięcej w świetle wyników postępowania przeciwnowotworowego w najczęstszych nowotworach wieku dziecięcego

Rozprawa doktorska w dziedzinie nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

Promotor

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Spis treści

Wykaz skrótów

ALCL - anaplastic large cell lymphoma (wielkokomórkowy chłoniak anaplastyczny)

ALL - acute lymphoblastic leukemia (ostra białaczka limfoblastyczna)

AML - acute myeloid leukemia (ostra białaczka szpikowa)

ANLL - acute nonlymphoblastic leukemia (ostra białaczka nie-limfoblastyczna)

BFM - Berlin-Frankfurt-Münster

CAR-T - chimeric antigen receptor T-cell

CRS - cytokine release syndrome (zespół uwalniania cytokin)

EBV - Epstein-Barr virus (wirus Epstein-Barr)

EFS - event-free survival (przeżycie wolne od zdarzeń)

EuroNet-PHL - European Network-Paediatric Hodgkin Lymphoma Study Group

GUS - Główny Urząd Statystyczny

HSCT - hematopoietic stem cell transplantation (przeszczepienie komórek macierzystych krwi)

ICANS - immune effector cell-associated neurotoxicity syndrome (zespół neurotoksyczności związany z komórkami efektorowymi układu odpornościowego)

IL - interleukina

NOPHO - the Nordic Society of Pediatric Hematology and Oncology

OS - overall survival (przeżycie całkowite)

PD-1 - programmed cell death protein 1

pEFS - probability of event-free survival (prawdopodobieństwo przeżycia wolnego od zdarzeń)

pOS - probability of overall survival (prawdopodobieństwo przeżycia całkowitego)

pRFS - probability of relapse-free survival (prawdopodobieństwo przeżycia wolnego od wznowy)

RFS - relapse-free survival (przeżycie wolne od wznowy)

T-LL - T-lymphoblastic lymphoma (chłoniak limfoblastyczny T-komórkowy)

Rozdział 1. Wstęp

Pomimo dynamicznego rozwoju medycyny oraz powszechnej dostępności do opieki zdrowotnej w Polsce, nowotwory pozostają jedną z najczęstszych przyczyn zgonów u dzieci, stanowiąc czwartą co do częstości przyczynę zgonu w grupie wiekowej 0-19 lat oraz drugą, po przyczynach zewnętrznych (wypadki komunikacyjne, urazy, utonięcia czy zatrucia) w grupie wiekowej 1-19 lat (raport GUS 2021). Rocznie w Polsce rozpoznaje się około 1100-1200 nowych przypadków nowotworów w populacji pediatrycznej. Z łączną zapadalnością wynoszącą 15 nowych przypadków na 100 000 dzieci, nowotwory wieku dziecięcego stanowią grupę chorób rzadkich [1].

Spośród wszystkich rodzajów nowotworów rozpoznawanych i leczonych u dzieci, najczęściej diagnozowane są białaczki (27%), nowotwory ośrodkowego układu nerwowego (20%) oraz chłoniaki (15%). Spośród guzów litych zlokalizowanych poza centralnym układem nerwowym najczęstszy jest nerwiak zarodkowy (neuroblastoma), stanowiący 8% wszystkich rozpoznań onkologicznych [2,3]. Podstawą leczenia nowotworów złośliwych u dzieci pozostaje wielolekowa chemioterapia, w poszczególnych przypadkach uzupełniona leczeniem operacyjnym, radioterapią oraz przeszczepieniem komórek macierzystych krwi, a w ostatnich latach również immunoterapią. Wprowadzenie multimodalnej terapii jako standardu leczenia doprowadziło do znaczącej poprawy wyników leczenia. Na przestrzeni ostatnich 30 lat śmiertelność z powodu nowotworów w Polsce zmniejszyła się z 5,56 zgonów na 100 000 dzieci do 2,61 na 100 000, co stanowi jeden z najlepszych wyników w zakresie poprawy przeżywalności wśród wszystkich krajów europejskich [4].

Znacząca poprawa wyników leczenia jest rezultatem rozwoju schematów terapeutycznych, jak również doskonalenia leczenia wspierającego, m.in. skuteczniejszego leczenia powikłań infekcyjnych oraz stosowania leków ograniczających toksyczność chemioterapii. Wprowadzenie wysokospecjalistycznych metod diagnostycznych, takich jak cytometria przepływowa, a w późniejszym okresie również diagnostyka molekularna, umożliwiło identyfikację markerów biologicznych, będących czynnikami prognostycznymi odpowiedzi na leczenie. Ponadto ocena jakościowa i ilościowa poszczególnych markerów dała możliwość monitorowania odpowiedzi na leczenie na poziomie molekularnym. Umożliwiło to personalizację ścieżki terapeutycznej pacjenta w zależności występowania typowego dla danego nowotworu profilu czynników prognostycznych oraz oceny odpowiedzi na leczenie.

W przypadku pacjentów z grup wysokiego ryzyka, w tym dzieci z nowotworami pierwotnie opornymi oraz wznową, przez wiele lat standardem leczenia była intensywna, multimodalna terapia, której podstawę stanowiła wysokodawkowa chemioterapia. Ze względu na wysoką toksyczność, a także obserwowaną w niektórych typach nowotworów niepełną skuteczność klasycznej chemioterapii, już w latach 60-ych XX wieku rozpoczęto badania nad alternatywnymi metodami leczenia, w tym nad zastosowaniem terapii opartych na odpowiedzi immunologicznej pacjenta [5]. Rozwój wiedzy o funkcjonowaniu układu odpornościowego umożliwił identyfikację mechanizmów ucieczki immunologicznej, prowadzących do transformacji nowotworowej, a następnie wzrostu i rozprzestrzeniania się komórek nowotworowych w organizmie. Równoległy rozwój metod inżynierii biomedycznej, w tym

edycji genów oraz produkcji przeciwciał monoklonalnych, umożliwił celowaną immunoterapię w poszczególnych typach nowotworów.

Jednym z pierwszych przykładów leczenia opartego na modulacji odpowiedzi immunologicznej było zastosowanie allogenicznego przeszczepienia komórek macierzystych krwi w leczeniu wybranych grup pacjentów z białaczką [6]. Metoda ta zapoczątkowała nową erę w leczeniu, torując trzy nowe ścieżki w terapii nowotworów: wykorzystanie komórek macierzystych, immunomodulację i immunoterapię, oraz zastosowania terapii celowanych [6]. Wszystkie trzy wspomniane strategie terapeutyczne zostały w pełni zaadaptowane w leczeniu ostrej białaczki limfoblastycznej, stając się integralnymi elementami współczesnych schematów terapeutycznych. Pomimo imponującej poprawy wyników leczenia w ALL na przestrzeni ostatnich 50 lat, aż 15-20% pacjentów leczonych wg współczesnych protokołów doświadcza wznowy choroby. Co więcej, rokowanie u dzieci ze wznową jest niekorzystne, a pięcioletnie przeżycie całkowite wynosi 30-50% po pierwszej wznowie, i jedynie < 20% po kolejnych [7]. Niezadawalające wyniki leczenia doprowadziły do wprowadzenia przeciwciał bispecyficznych (blinatomumab) oraz koniugatów przeciwciało-lek (inotuzumab ozogamycyny) do terapii wznowy ALL lub choroby pierwotnie opornej. Wstępne wyniki badań klinicznych z zastosowaniem obu cząsteczek wykazały wysokie odsetki remisji całkowitej oraz poprawę krótkoterminowych wyników leczenia [8-10]. Niemniej jednak, dane długoterminowe wskazywały na ograniczenia w zakresie trwałości odpowiedzi [11-13]. Przełomem w leczeniu ALL był rok 2017, kiedy amerykańska Agencja Żywności i Leków zatwierdziła pierwszą terapię CAR-T do leczenia pierwotnie opornej ostrej białaczki limfoblastycznej

oraz wznowy ALL [7]. Terapia CAR-T wykorzystuje genetycznie zmodyfikowane autologiczne limfocyty T, wykazujące ekspresję chimerycznego receptora przeciwko antygenom obecnym na komórkach nowotworowych. Badania kliniczne wykazały, że jednokrotna infuzja CAR-T indukuje remisję całkowitą u 70-90% pacjentów w grupie dzieci, u których kolejne linie leczenia okazywały się nieskuteczne. Długoterminowe obserwacje wykazały, że CAR-T może indukować trwałą remisję w ALL opornej na leczenie lub wznowie choroby [7,14]. Wyzwaniem pozostaje zapobieganie i leczenie powikłań związanych z terapią CAR-T, takich jak zespół uwalniania cytokin oraz neurotoksyczność leczenia, w tym zespół neurotoksyczności związany z komórkami efektorowymi układu odpornościowego.

Wprowadzenie nowoczesnych metod leczenia, w tym immunoterapii oraz terapii CAR-T, a także szczegółowa stratyfikacja do grup ryzyka istotnie poprawiły wyniki leczenia, zwłaszcza u pacjentów z grup wysokiego ryzyka. Pomimo obiecujących wyników leczenia z zastosowaniem immunoterapii, metoda ta wiąże się ze zmienną trwałością odpowiedzi oraz specyficznym profilem działań niepożądanych. Zapobieganie i leczenie toksyczności związanych z immunoterapią, a także poprawa w zakresie trwałości odpowiedzi, wymagają szczegółowej wiedzy na temat mechanizmów działania układu odpornościowego, modyfikowanych ścieżek odpowiedzi immunologicznej oraz długoterminowych konsekwencji tej metody leczenia. Uwzględniając ograniczenia immunoterapii, nadchodząca era leczenia ma za zadanie ustalić optymalne połączenie klasycznej chemio- i radioterapii z terapiami komórkowymi, immunoterapią oraz metodami inżynierii genetycznej, w

celu ustalenia schematów leczenia charakteryzujących się optymalną odpowiedzią kliniczną oraz immunologiczną [15].

Rozdział 2. Cele pracy

Celem ogólnym projektu jest ocena skuteczności terapii innowacyjnych w onkologii dziecięcej w świetle wyników postępowania przeciwnowotworowego w najczęstszych nowotworach wieku dziecięcego.

Celami szczegółowymi są:

1. Ocena skuteczności terapii ostrej białaczki limfoblastycznej metodami CAR-T oraz odpowiedzi immunologicznej w leczeniu CAR-T w stosunku do wyników terapii w okresie wcześniejszym, analizowanych metodą retrospektywną.
2. Ocena skuteczności terapii ostrej białaczki szpikowej z wykorzystaniem przeszczepienia komórek macierzystych krwi.
3. Ocena skuteczności leczenia chłoniaka Hodgkina z ograniczeniem radioterapii w wybranych grupach pacjentów z dobrą odpowiedzią na leczenie oraz zastosowania przeciwciał monoklonalnych anty-CD30 w połączeniu z inhibitorami punktów kontrolnych PD-1 w leczeniu wznowy choroby.
4. Analiza wyników leczenia chłoniaków nieziarniczych T/NK-komórkowych z zastosowaniem indywidualizacji ścieżki terapii u pacjentów z rzadkimi typami chłoniaków.
5. Ocena skuteczności terapii neuroblastoma, z wykorzystaniem przeciwciał monoklonalnych dinutuximab-beta, poprzedzonych przeszczepieniem komórek krwiotwórczych.

Rozdział 3. Metodyka

Projekt zrealizowany został na podstawie analizy statystycznej wyników terapii w najczęstszych nowotworach wieku dziecięcego u pacjentów w wieku 0-19 lat, leczonych w Klinice Pediatrii, Hematologii i Onkologii Szpitala Uniwersyteckiego nr 1 im dr. A. Jurasza w Bydgoszczy w okresie od stycznia 1990 do grudnia 2024. Szczegółowej analizie zostały poddane wyniki leczenia z zastosowaniem terapii innowacyjnych, takich jak przeciwciała monoklonalne, w tym koniugaty przeciwciało-lek oraz przeciwciała bispecyficzne, inhibitory punktów kontrolnych, terapie komórkowe oraz CAR-T. Analiza została przeprowadzona na podstawie retrospektywnych danych pacjentów leczonych w latach 1990-2021, a także prospektywnych danych pacjentów leczonych w latach 2022-2024. Projekt został zatwierdzony przez Komisję Bioetyczną Collegium Medicum Uniwersytetu Mikołaja Kopernika w Bydgoszczy (KB 577/2021).

Do analizy włączeni zostali pacjenci leczeni z powodu najczęstszych nowotworów wieku dziecięcego: ostrej białaczki limfoblastycznej, ostrej białaczki szpikowej, chłoniaka Hodgkina, chłoniaków nieziarniczych oraz neuroblastoma. Z powyższych grup chorych wyłączeni zostali pacjenci z niekompletnymi danymi medycznymi oraz utraceni w okresie obserwacyjnym po zakończeniu leczenia. Rozpoznanie nowotworu opierało się na wynikach badań histopatologicznych oraz mielogramach wykonanych w Szpitalu Uniwersyteckim nr 1 w Bydgoszczy. W okresie od roku 2004 dla białaczek i chłoniaków, a od roku 1996 dla neuroblastoma, rozpoznanie było w każdym przypadku weryfikowane w krajowym ośrodku referencyjnym.

Analizie poddano dane dotyczące bezpośrednio pacjentów, takie jak płeć i wiek w momencie rozpoznania, jak również wyniki badań

laboratoryjnych z uwzględnieniem kluczowych markerów biochemicznych dla danych typów nowotworów, badań obrazowych, stopień zaawansowania nowotworu, grupa ryzyka, zastosowane leczenie z podziałem na poszczególne protokoły terapeutyczne oraz zastosowane w leczeniu modalności, takie jak leczenie operacyjne, chemioterapia, radioterapia, immunoterapia, przeszczepienie komórek macierzystych krwi.

Parametrami określającymi odpowiedź na leczenie i ocenianymi w analizowanych nowotworach były odsetek pacjentów osiągających remisję całkowitą, odsetek chorych z progresją w toku leczenia oraz z wystąpieniem wznowy. Remisja całkowita i progresja była określana według definicji specyficznych dla każdego typu nowotworu zawartych w poszczególnych protokołach terapeutycznych. Wznowa była określana jako ponowne wykrycie utkania nowotworowego po osiągnięciu remisji całkowitej. Zdarzenie definiowane było jako brak osiągnięcia remisji w czasie określonym w poszczególnych protokołach terapeutycznych, progresja choroby, wznowa lub śmierć z jakiejkolwiek przyczyny. Przeżycie całkowite określane było jako czas od rozpoznania do śmierci z jakiejkolwiek przyczyny. Przeżycie wolne od zdarzeń określane było jako czas od rozpoznania do wystąpienia zdarzenia, a przeżycie wolne od wznowy jako czas od rozpoznania do wystąpienia wznowy.

Punktami końcowymi dla poszczególnych typów nowotworów było 5-letnie prawdopodobieństwo przeżycia całkowitego, przeżycia wolnego od zdarzeń oraz przeżycia wolnego od wznowy. Krzywe przeżycia oraz prawdopodobieństwo przeżycia całkowitego, przeżycia wolnego od zdarzeń i przeżycia wolnego od wznowy obliczane były za pomocą metody Kaplan-Meiera i porównywane za pomocą testu log-rank. Wszystkie

uzyskane dane były objęte analizą statystyczną pod kątem ich wpływu na punkty końcowe. Zastosowano analizę jednoczynnikową w celu oceny istotności statystycznej poszczególnych czynników prognostycznych. Zmienna była uznana za istotną statystycznie jeśli $p<0,05$. Zmienne istotne statystycznie w analizie jednoczynnikowej zostały włączone do analizy wieloczynnikowej. Obliczenia statystyczne zostały przeprowadzone za pomocą programu MedCalc 20.100 (MedCalc Software, Mariakerke, Belgium).

Projekt został zrealizowany w systemie kolejnych opracowań tematów, a wyniki analiz dla poszczególnych typów nowotworów zostały przedstawione w cyklu publikacji.

Rozdział 4. Wnioski

1. Na podstawie przeprowadzonych w ramach projektu analiz wykazano, że w latach 1976-2018 wyniki leczenia w ALL uległy istotnej poprawie. Zastosowanie terapii CAR-T u pacjentów z pierwotnie oporną ALL lub wznową choroby umożliwiło uzyskanie remisji całkowitej u pacjentów z niepowodzeniem standardowego leczenia drugiej linii. Monitorowanie odpowiedzi immunologicznej oraz liczby komórek CAR-T umożliwia identyfikację pacjentów z grupy ryzyka wystąpienia powikłań leczenia, w tym CRS i ICANS. Infuzja oraz ekspansja komórek CAR-T powoduje również odpowiedź immunologiczną niezależnie od wystąpienia reakcji niepożądanych.
2. Wyniki leczenia w AML uległy znaczącej poprawie w ciągu ostatnich 30 lat. Wprowadzenie przeszczepienia allogenicznych komórek krwi w grupie wysokiego ryzyka poprawiło 5-letnie prawdopodobieństwo przeżycia całkowitego oraz przeżycia wolnego od zdarzeń. W dalszych badaniach konieczna jest współpraca międzynarodowa ze względu na niewielką liczbę pacjentów pediatrycznych z AML.
3. Wprowadzenie oceny odpowiedzi na leczenie w toku terapii chłoniaka Hodgkina umożliwiło zmniejszenie liczby pacjentów leczonych z zastosowaniem radioterapii. Ograniczenie radioterapii do wybranych grup pacjentów daje możliwość redukcji długoterminowych powikłań bez wpływu na wyniki leczenia. Leczenie wznowy z zastosowaniem przeciwciał monoklonalnych anty-CD30 w połączeniu z inhibitorami punktów kontrolnych PD-1 może stanowić alternatywę dla standardowego leczenia drugiej linii.

4. Pięcioletnie prawdopodobieństwo przeżycia całkowitego w anaplastycznym chłoniaku wielkokomórkowym jest wyższe niż w T-komórkowym chłoniaku limfoblastycznym. W terapii chłoniaków nieziarniczych związanych z infekcją wirusem Epstein-Barr pacjenci mogą donieść korzyść z zastosowania cytotoksycznych limfocytów T specyficznych dla EBV.
5. W terapii neuroblastoma leczenie pacjentów z grupy wysokiego ryzyka z wykorzystaniem przeciwciał monoklonalnych dinutuximab-beta, poprzedzonych przeszczepieniem komórek krwiotwórczych poprawiło z 5-letnie pOS z 0,0% do 41,1%. W grupie pacjentów ze wznową choroby przeszczepienie komórek macierzystych krwi wiązało się z istotną statystycznie poprawą przeżycia całkowitego.
6. Stwierdzono, że w badanym okresie wyniki leczenia nowotworów u dzieci uległy istotnej poprawie, co wynikało z wprowadzania unowocześnianych międzynarodowych programów terapeutycznych oraz terapii innowacyjnych, w tym terapii komórkowych i immunoterapii.

Rozdział 5. Streszczenie

Pomimo dynamicznego rozwoju medycyny oraz powszechnej dostępności do opieki zdrowotnej w Polsce, nowotwory pozostają jedną z najczęstszych przyczyn zgonów u dzieci, stanowiąc drugą przyczynę zgonów w grupie wiekowej 1-19 lat. Ze względu na wysoką toksyczność, a także obserwowaną w niektórych typach nowotworów niepełną skuteczność klasycznej chemioterapii i radioterapii, już w latach 60-ych XX wieku rozpoczęto badania nad alternatywnymi metodami leczenia, w tym nad zastosowaniem terapii opartych na odpowiedzi immunologicznej pacjenta. Aktualnie terapie innowacyjne, w tym immunoterapia coraz częściej stanowią integralny element leczenia w onkologii dziecięcej.

Powyższy projekt badawczy ma na celu ocenę skuteczności terapii innowacyjnych w onkologii dziecięcej w najczęstszych nowotworach wieku dziecięcego. Projekt zrealizowany został na podstawie analizy statystycznej wyników terapii u pacjentów w wieku 0-19 lat, leczonych w Klinice Pediatrii, Hematologii i Onkologii Szpitala Uniwersyteckiego nr 1 im dr. A. Jurasza w Bydgoszczy w okresie od stycznia 1990 do grudnia 2024. Szczegółowej analizie zostały poddane wyniki leczenia z zastosowaniem terapii innowacyjnych, takich jak przeciwciała monoklonalne, w tym koniugaty przeciwciało-lek oraz przeciwciała bispecyficzne, inhibitory punktów kontrolnych, terapie komórkowe oraz terapia CAR-T. Wyniki poszczególnych analiz przedstawione zostały w formie 7 publikacji dołączonych do rozprawy doktorskiej.

Immunoterapia znacząco poprawiła wyniki u pacjentów z ostrą białaczką limfoblastyczną z grupy wysokiego ryzyka. Zastosowanie terapii CAR-T u pacjentów z pierwotnie oporną ALL lub wznową choroby umożliwiło

uzyskanie remisji całkowitej u pacjentów z niepowodzenia standardowego leczenia drugiej i następnych linii. Monitorowanie odpowiedzi immunologicznej oraz liczby komórek CAR-T umożliwia identyfikację pacjentów z grupy ryzyka powikłań leczenia.

Wyniki leczenia w AML uległy znaczącej poprawie w ciągu ostatnich 30 lat. Wprowadzenie allogenicznego przeszczepienia komórek krwi w grupie pacjentów z AML wysokiego ryzyka poprawiło 5-letnie prawdopodobieństwo przeżycia całkowitego oraz przeżycia wolnego od zdarzeń. W dalszych badaniach konieczna jest współpraca międzynarodowa ze względu na niewielką liczbę pacjentów pediatrycznych z AML.

Wprowadzenie oceny odpowiedzi na leczenie w toku terapii chłoniaka Hodgkina umożliwiło zmniejszenie liczby pacjentów leczonych z zastosowaniem radioterapii. Ograniczenie radioterapii do wybranych grup pacjentów daje możliwość redukcji długoterminowych powikłań bez wpływu na wyniki leczenia. Leczenie wznowy z zastosowaniem przeciwciał monoklonalnych anty-CD30 w połączeniu z inhibitorami punktów kontrolnych PD-1 może stanowić alternatywę dla standardowego leczenia drugiej linii.

Pięcioletnie prawdopodobieństwo przeżycia całkowitego w anaplastycznym chłoniaku wielkokomórkowym jest wyższe niż w T-komórkowym chłoniaku limfoblastycznym. W terapii chłoniaków nieziarniczych związanych z infekcją wirusem Epstein-Barr pacjenci mogą donieść korzyść z zastosowania cytotoksycznych limfocytów T specyficznych dla EBV.

W terapii neuroblastoma leczenie pacjentów z grupy wysokiego ryzyka z wykorzystaniem przeciwciał rnonoklonalnych dinutuximab-beta,

poprzedzonych przeszczepieniem komórek krwiotwórczych poprawiło z 5-letnie pOS z 0,0% do 41,1%. W grupie pacjentów ze wznową choroby HSCT wiązało się z istotną statystycznie poprawą przeżycia całkowitego.

Na podstawie przeprowadzonych w ramach projektu analiz wykazano, że w badanym okresie wyniki leczenia nowotworów u dzieci uległy istotnej poprawie, co wynikało z wprowadzania unowocześnianych międzynarodowych programów terapeutycznych oraz terapii innowacyjnych, w tym terapii komórkowych i immunoterapii.

Summary

Despite the dynamic development and broad access to healthcare in Poland, malignant diseases remains one of the leading causes of death among children, ranking as the second most common in the 1-19 age group. Due to the high toxicity and the incomplete efficacy of conventional chemotherapy and radiotherapy observed in certain types of cancer, research into alternative treatment modalities, including approaches based on the patient's immune response, was initiated as early as in the 1960s. Currently, innovative therapies, including immunotherapy, are increasingly becoming an integral part of treatment in pediatric oncology.

This project aimed to evaluate the effectiveness of innovative therapies for selected childhood cancers in pediatric oncology. The project was conducted through statistical analysis of treatment outcomes in children aged 0-19 years treated at the Department of Pediatrics, Hematology and Oncology, University Hospital No. 1 in Bydgoszcz between January 1990 and December 2024. The analysis focused on the outcomes of innovative therapies, such as monoclonal antibodies, including antibody-drug conjugates and bispecific antibodies, immune checkpoint inhibitors, cellular therapies, and CAR-T cells. The results of individual analyses are presented in seven publications attached to the doctoral dissertation.

Immunotherapy has significantly improved outcomes in high-risk ALL patients. The use of CAR-T therapy in patients with primary refractory ALL or relapsed disease enabled complete remission in cases where standard second-line and subsequent treatments had failed. Monitoring immune response and CAR-T cell number allows for the identification of patients at risk of treatment-related complications. In the analyzed group, the

fourth day after CAR-T cell infusion was associated with elevated plasma levels of most analyzed cytokines, which temporally correlated with the onset of CRS and ICANS.

Treatment outcomes in AML have significantly improved over the past 30 years. The introduction of allogeneic hematopoietic stem cell transplantation in high-risk patients improved both 5-year overall survival and event-free survival. Further research should require international collaboration due to the small number of pediatric AML patients.

The introduction of response-adapted treatment evaluation in Hodgkin lymphoma therapy has enabled a reduction in the number of patients receiving radiotherapy. Limiting radiotherapy to selected patient groups allows for a reduction in long-term complications without compromising treatment outcomes. Treatment of relapsed disease with anti-CD30 monoclonal antibodies in combination with PD-1 checkpoint inhibitors may serve as an alternative to standard second-line therapy.

The five-year overall survival probability in anaplastic large cell lymphoma is higher than in T-cell lymphoblastic lymphoma. For rare lymphoma types, there are no established treatment guidelines. In EBV-associated non-Hodgkin lymphomas, patients may benefit from treatment with EBV-specific cytotoxic T lymphocytes.

In neuroblastoma therapy, high-risk patients treated with dinutuximab-beta following hematopoietic stem cell transplantation showed an improvement in 5-year overall survival from 0.0% to 41.1%. Among patients with relapsed disease, stem cell transplantation was statistically associated with improved overall survival.

Treatment outcomes for pediatric malignancies have significantly improved over the study period, mainly due to the introduction of new international treatment protocols, and innovative therapies, including cellular therapies and immunotherapy.

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Changing risk factors in childhood acute lymphoblastic leukemia: experience from Kujawsko-Pomorski region 1976–2018

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Abstract

Introduction: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Risk factors in childhood ALL have changed during recent decades, mostly due to treatment personalization.

The aim of this study was to analyze therapy results and prognostic factors in childhood ALL in the Kujawsko-Pomorski region of Poland between 1976 and 2018.

Material and methods: Data from 495 patients (0–18 years old) diagnosed with ALL from the Kujawsko-Pomorski region between 1976 and 2018 was analyzed. Prognostic factors were analyzed separately in specific therapeutic groups, which were defined by several therapy protocols.

Results: Prognostic factors have changed over the course of consecutive therapeutic periods. Between 1976 and 1988 (the first and second therapeutic protocols), central nervous system involvement was the most important risk factor. During the third therapeutic period, an unsatisfactory treatment response on days 8 and 14 was related to a poor outcome. In 1995–2002, the risk factors were hepatomegaly, splenomegaly, lymph nodes involvement, and unsatisfactory therapy response on days 15 and 33. Between 2002 and 2011, immunophenotype other than 'common' and hemoglobin level at diagnosis were the risk factors, and a lack of BCR-ABL aberration was related to better therapy results. During the final analyzed period (2011–2018), failure to achieve remission on day 33 was a risk factor, and patients classified as non-high risk group and those aged <6 years had better outcomes.

Conclusions: The changing profile of risk factors in ALL has reflected progress in ALL therapy, with the gradual elimination of factors related to poor outcomes, mostly due to modifications in treatment and the development of diagnostic methods as well as therapy monitoring.

Key words: acute lymphoblastic leukemia, prognostic factors, risk factors, children, therapeutic era

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and represents more than 20% of all malignancies in patients aged 0–18 years. Each year, c.200–220 children are diagnosed with ALL in Poland [1]. Therapy outcomes have improved significantly over recent decades — the probability of five-year overall survival has increased from 31% in 1975 to c.85% with current therapy protocols [2, 3]. The identification of prognostic factors was undoubtedly one of the milestones in ALL therapy: the presence of risk factors enabled risk group stratification and therapy adjustment. Patients with factors related to a poor outcome have received more intensive treatment, whereas in children with more favorable features, treatment has been modified to avoid severe toxicity and short-term as well as long-term side effects [4].

Prognostic factors in ALL can be divided into three groups: factors related to patient characteristics, factors related to disease features, and factors related to treatment response. Age at diagnosis, race and sex are prognostic factors related to patient characteristics. Factors related to disease include leukocytes count at diagnosis, blasts immunophenotyping, chromosomal aberrations in blast cells, and the presence of extramedullary infiltrations. Prognostic factors related to therapy include response to treatment on days 8, 15 and 33 and the presence of minimal residual disease (MRD) at later timepoints [5].

The aim of this study was to analyze therapy results and the significance of prognostic factors in childhood ALL in the Kujawsko-Pomorski region between 1976 and 2018.

Material and methods

Design of study

In this study, data from 495 patients (0–18 years old) diagnosed with ALL from the Kujawsko-Pomorski region of Poland between 1976 and 2018 was analyzed. Children were treated in the Department of Children's Hematology and Oncology of Antoni Jurasz University Hospital in Bydgoszcz. Prognostic factors were analyzed separately in specific therapeutic groups, which were defined by several therapy protocols.

Definitions

Treatment response was assessed on days 8, 14/15 and 28/33. Prednisone good response (PGR) was defined as absolute blast count in peripheral blood <1,000/μL on day 8 of therapy. Prednisone poor response (PPR) was defined as absolute blast count in peripheral blood ≥1,000/μL on day 8. MRD was calculated as blast cells count according to cells immunophenotyping. Patients stratified to the standard risk (SR) group should have MRD <0.1% on day 14/15 to remain in the SR group. In a case of MRD between 0.1% and 10%, they were stratified to the intermediate risk group, and in a case of MRD above 10% they were stratified to the high risk group. Response definition on day 28/33 was divided into three groups, based on blast count in the bone marrow:

- **M1** <5% of blasts in representative bone marrow with sufficient cellularity and signs of regeneration of normal myelopoiesis;
- **M2** 5 <25% of blasts in representative bone marrow with sufficient cellularity and signs of regeneration of normal myelopoiesis;
- **M3** ≥25% of blasts in representative bone marrow with sufficient cellularity and signs of regeneration of normal myelopoiesis.

Hepatomegaly and splenomegaly was defined as enlargement of liver and spleen above the value normal for the patient's age. Central nervous system (CNS) involvement was defined as clinical or imaging findings of CNS disease and the presence of blasts on cytospin preparation in cerebrospinal fluid. Complete remission (CR) was achieved when the following criteria were fulfilled on day 33 of therapy: <5% blast cells (M1) in representative bone marrow with sufficient cellularity and signs of regeneration of normal myelopoiesis; ≤5 nucleated cells/μL and no evidence of blasts in cytospin and no evidence of leukemic infiltrates as evaluated clinically and by imaging; and a pre-existing mediastinal mass must have decreased to at least one third of the initial tumor volume.

Treatment protocols

According to therapy protocols, patients were divided into six groups:

1. 1976–1983 — MEMPHIS V–VII (56 patients) [6];
2. 1983–1988 — BFM-83 (33 patients) [7];
3. 1988–1995 — NOPHO-86 (81 patients) [8];
4. 1995–2002 — BFM-90 (96 patients) [7] and New York I–II (19 patients) [9];
5. 2002–2011 — ALL-IC-2002 (115 patients) [10];
6. 2011–2018 — ALL-IC-2009 (95 patients) [11].

Risk factors

Prognostic factors analyzed in the entire group included age at diagnosis, sex, CNS involvement, lymph nodes involvement, mediastinal mass, splenomegaly >4 cm, hepatomegaly >4 cm, risk group according to the Berlin–Frankfurt–Munster (BFM) protocol, leukocyte count at diagnosis, hemoglobin (Hgb) level at diagnosis, and treatment response (GPR vs. PPR) on day 8 of therapy.

From 1990 onwards, additional prognostic factors were analyzed: blasts morphology according to the French–American–British (FAB) classification; blasts immunophenotyping; and treatment response on days 14/15 and 28/33. From 1996 onwards, chromosomal aberrations BCR-ABL, TEL-AML1, MLL-AF4, and the presence of hypodiploidy or hyperdiploidy were evaluated.

Table I. Prognostic factors analyzed in respective therapeutic groups

Group	Years	Prognostic factors
1	1976–1983	Age at diagnosis, sex, leukocyte count and hemoglobin level at diagnosis, extramedullary involvement (liver, spleen, CNS, lymph nodes, mediastinal mass)
2	1983–1988	Age at diagnosis, sex, leukocyte count, hemoglobin level at diagnosis, extramedullary involvement (liver, spleen, CNS, lymph nodes, mediastinal mass), treatment response on day 8
3	1988–1995	Age at diagnosis, sex, leukocyte count and hemoglobin level at diagnosis, extramedullary involvement (liver, spleen, CNS, lymph nodes, mediastinal mass), treatment response on day 8, count of blast cells in bone marrow on days 14 and 28
4a/4b	1995–2002	Age at diagnosis, sex, leukocyte count and hemoglobin level at diagnosis, extramedullary involvement (liver, spleen, CNS, lymph nodes, mediastinal mass), treatment response on day 8, count of blast cells in bone marrow on days 15 and 33, blast immunophenotyping, FAB classification, hypodiploidy, BCR-ABL rearrangement
5	2002–2011	Age at diagnosis, sex, leukocyte count, platelets number and hemoglobin level at diagnosis, extramedullary involvement (liver, spleen, CNS, lymph nodes, mediastinal mass), treatment response on day 8, count of blast cells in bone marrow on days 15 and 33, MRD on day 15, blast immunophenotyping, FAB classification, hypodiploidy, hyperdiploidy, BCR-ABL, TEL-AML1 and MLL-AF4 rearrangement, risk group
6	2011–2018	Age at diagnosis, sex, leukocyte count, platelets number and hemoglobin level at diagnosis, extramedullary involvement (liver, spleen, CNS, lymph nodes, mediastinal mass), treatment response on day 8, count of blast cells in bone marrow on days 15 and 33, MRD on day 15, blast immunophenotyping, FAB classification, hypodiploidy, hyperdiploidy, BCR-ABL, TEL-AML1 and MLL-AF4 rearrangement, risk group

CNS – central nervous system; FAB – French–American–British; MRD – minimal residual disease

Risk factors analyzed in the respective therapeutic groups are set out in Table I.

Statistical methods

The probability of overall survival (pOS), probability of event-free survival (pEFS), and probability of relapse-free survival (pRFS) were calculated with the Kaplan–Meier method, and compared by log-rank test. An 'event' was defined as relapse, death or secondary malignancy. Cox regression model was used to calculate univariate and multivariate analysis of prognostic factors. Factors with p-value <0.1 in univariate analysis were included into the multivariate model. Odds ratio (OR) was calculated with 95% confidence interval.

Results

For each therapeutic group, pOS, pEFS and pRFS were calculated. Risk factors of death, event and relapse were analyzed separately in each group. Results of multivariate analysis are shown in Tables II, III and IV.

Group 1

Group 1 includes patients treated between 1976 and 1983 according to the St. Jude Memphis therapeutic protocol. 5-year pOS was 19.6% (±5.3%). None of the evaluated factors achieved statistical significance in pOS analysis. 5-year pEFS was 7.4% (±3.4%). Event occurred in 92.9% of patients. The only factor with a significant impact on pEFS in univariate analysis was CNS involvement. Relapse occurred in 80.4% of patients and the 5-year pRFS was 11.2% (±4.9%). In both univariate and multivariate analysis, CNS involvement had a significant impact on pRFS and was related to a more than 20-fold increased risk of relapse. Other important adverse prognostic factors included mediastinal mass and Hgb level <8 g/dL at diagnosis.

Group 2

The second group was treated between 1983 and 1988 according to the BFM-83 therapeutic protocol. 5-year pOS was 54.5% (±8.7%) and pEFS was 53.2% (±8.8%). CNS involvement was a risk factor of death and event in univariate and multivariate analysis of both parameters. Additionally, age <1 year and >6 years at diagnosis had a significant impact on pOS; patients of this age had a 3-fold higher risk of death during this therapeutic era. Relapse occurred in 12 patients (36.4%) and 5-year pRFS was 61.0% (±9.2%). None of the analyzed factors achieved statistical significance in either univariate or multivariate analysis of pRFS.

Group 3

Between 1988 and 1995, patients were treated according to the NOPHO-86 protocol. In this group, 5-year pOS was 58.0% (±5.5%) and 37 children died during the observational period, which represented 45.7% of the entire group. The most important prognostic factor on pOS was treatment response on day 8. Patients with PPR at this timepoint had a 3-fold higher risk of death. In univariate analysis also Hgb level <8 g/dL had a significant impact on pOS, although this effect was not shown in multivariate analysis. 5-year pEFS was 51.9% (±5.6%). In univariate analysis, therapy response on days 8 (PPR) and 14 (M3) as well as Hgb level

Table II. Multivariate analysis of prognostic factors for probability of overall survival

Group	Years	Prognostic factors	OR* (95% CI)	*p*
1	1976–1983	No parameter reached statistical significance	–	–
2	1983–1988	CNS involvement	10 (1.7–64)	$p = 0.010$
		Age at diagnosis <1 year or >6 years	3.8 (1.1–13)	$p = 0.033$
3	1988–1995	Treatment response on day 8 (PPR)	3.1 (1.6–6.2)	$p = 0.001$
4a	1995–2002	Risk group – HR	5.6 (2.21–14)	$p < 0.001$
		Hepatomegaly	4.6 (1.7–12)	$p = 0.002$
		Treatment response on day 33 (bone marrow morphology – M2)	10 (1.03–96)	$p = 0.047$
4b	1995–2002	No parameter reached statistical significance	–	–
5	2002–2011	Immunophenotype other than 'common ALL'	3.1 (1.2–8.2)	$p = 0.019$
		Lack of BCR-ABL arrangement	0.1 (0.02–0.3)	$p < 0.001$
6	2011–2018	Risk group – non-HR	0.2 (0.1–0.5)	$p < 0.001$

*Odds ratio (OR) >1 means an increased risk of failure; CI – confidence interval; CNS – central nervous system; PPR – prednisone poor response; HR – high risk; ALL – acute lymphoblastic leukemia

Table III. Multivariate analysis of prognostic factors for probability of event-free survival

Group	Years	Prognostic factors	OR* (95% CI)	*p*
1	1976–1983	CNS involvement	8.3 (1.6–43.7)	$p = 0.012$
2	1983–1988	CNS involvement	8.6 (1.5–49)	$p = 0.015$
3	1988–1995	Treatment response on day 14 (M3)	2.6 (1,2–5.9)	$p = 0.018$
4a	1995–2002	Risk group – HR	3.5 (1.5–7.9)	$p = 0.003$
		Splenomegaly	2.9 (1.3–6.3)	$p = 0.008$
4b	1995–2002	Lymph nodes involvement	4.2 (1.3–13)	$p = 0.011$
		Treatment response on day 15 (M2)	23 (1.6–100)	$p = 0.022$
5	2002–2011	Hgb <8 g/dL at diagnosis	2.3 (1.1–4.8)	$p = 0.028$
6	2011–2018	Failure to achieve CR on day 33	10.7 (1.0–114)	$p = 0.049$
		Age <6 at diagnosis	0.2 (0.1–0.9)	$p = 0.031$

*Odds ratio (OR) >1 means an increased risk of failure; CI – confidence interval; CNS – central nervous system; HR – high risk; Hgb – hemoglobin; CR – complete remission

Table IV. Multivariate analysis of prognostic factors for probability of relapse-free-survival

Group	Years	Prognostic factors	OR* (95% CI)	*p*
1	1976–1983	CNS involvement	34 (4.2–270)	$p = 0.001$
		Mediastinal mass	4.9 (1.04–23)	$p = 0.044$
		Hgb <8 g/dL at diagnosis	2.8 (1.1–7)	$p = 0.029$
2	1983–1988	No parameter reached statistical significance	–	–
3	1988–1995	Treatment response on day 8 (PPR)	1.8 (0.8–4.2)	$p = 0.019$
4a	1995–2002	Splenomegaly	5.0 (1.7–14)	$p = 0.002$
		T-cell immunophenotyping	4.3 (1.4–13)	$p = 0.009$
4b	1995–2002	Treatment response on day 15 (M2)	7.7 (1.04–56)	$p = 0.042$
5	2002–2011	Hgb >8 g/dL at diagnosis	3.9 (1.5–10.4)	$p = 0.007$
6	2011–2018	Failure to achieve remission on day 33	24 (1.4–402)	$p = 0.027$
		Age <6 at diagnosis	0.1 (0.01–0.7)	$p = 0.027$

*Odds ratio (OR) >1 means an increased risk of failure; CI – confidence interval; CNS – central nervous system; Hgb – hemoglobin; PPR – prednisone poor response;

lower than 8 g/dL had significant impacts on pEFS, but in multivariate analysis only therapy response on day 14 was related to a worse outcome and doubled the risk of event. 5-year pRFS was 66.2% (±6.9%). The only risk factor related to pRFS was therapy response on day 8.

Group 4

Group 4 was divided into two subgroups due to different therapeutic protocols: the BFM-90 protocol (group 4a) and the NEW YORK I–II protocol (group 4b).

In group 4a, 5-year pOS was 77.9% (±4.3%) and 5-year pEFS was 68.8% (±4.7%). The most important prognostic factor in both pOS and pEFS was treatment response on day 8, which was correlated with a 10-fold increased risk of event and a more than 3-fold higher risk of death in patients with PPR. Other factors that achieved statistical significance in univariate analysis in pOS and pEFS were risk group, hepatomegaly or splenomegaly at diagnosis, leukocyte count at diagnosis >20,000/μL, blasts phenotype, and treatment response on days 15 and 33 (bone marrow classified as M2). Relapse occurred in 16 children (16.7%) and mean time to relapse was 2.5 years. Among factors significant in univariate analysis, only T-cell blasts phenotype and splenomegaly proved significant in multivariate analysis.

In group 4b, 5-year pOS was 73.7% (±10.1%). None of the analyzed factors had an impact on pOS. 5-year pEFS was 68.4% (±10.7%). Involvement of lymph nodes and treatment response on day 15 had significant impacts on pEFS. Relapse was observed in five cases (26.3%) and four patients in this group died. Treatment response on day 15 was the only prognostic factor related to pRFS.

Group 5

Group 5 included 115 patients treated between 2002 and 2009 according to the IC-BFM 2002 protocol. 5-year pOS was 79.1% (±3.8%). Mean OS was 7.4 years [95% confidence interval (CI): 6.8–7.8 years). The most important factor with a significant negative impact on patient pOS was the presence of BCR-ABL fusion gene [as a result of translocation t(9;22)]; children with this mutation had a more than 7-fold lower pOS. In univariate analysis, hepatomegaly and splenomegaly at diagnosis had a significant impact on pOS as well. 5-year pEFS was 71.1% (±4.2%). Relapses occurred in 27 (23.5%) children and 5-year pRFS was 79.3% (±3.9%). Only Hgb <8 g/dL at diagnosis had a significant impact on both pEFS and pRFS, with a 2.3-fold higher risk of event and an almost 4-fold higher risk of relapse in patients with this feature.

Group 6

In group 6, data from children treated according to the ALL IC-BFM2009 protocol was analyzed. 5-year pOS was 90.7% (±3.4%). Mean OS was 4.1 years (95% CI: 2.7–6.5 years). In univariate analysis, only hypodiploidy had a significant impact on pOS. 5-year pEFS was 86.6% (±4.1%). Among prognostic factors related to lower pEFS, only Hgb level at diagnosis <8 g/dL was statistically significant. Relapses occurred in nine patients and 5-year pRFS was 90.1% (±3.6%). In univariate analysis, patients who did not achieve remission on day 33 had a more than 35-fold higher risk of relapse (data not shown). Other factors related to a worse pRFS were hepatomegaly, splenomegaly, and age <10 years at diagnosis.

Discussion

Decades of research into childhood ALL have resulted in the identification of several clinical and laboratory features which have had significant impacts on therapy outcomes. The best-known factors include age, leukocyte count at diagnosis, immunophenotype and chromosomal abnormalities in blasts, and response to initial therapy. The presence of prognostic factors has enabled risk group stratification and led to therapy intensification in patients at risk of treatment failure [4, 12]. The present data reflects improvements in therapy outcomes in childhood ALL as well as developments in diagnostic methods achieved due to international collaboration and great research effort.

During the first two analyzed periods, CNS involvement was one of the most important factors related to a poor outcome. Patients with CNS involvement had a 34-fold higher risk of relapse in the period 1976–1984 ($p = 0.001$) and a 10-fold higher risk of death between 1983 and 1988 ($p = 0.010$). This impact was also observed in international therapy protocols analysis, and resulted in the introduction of CNS prophylaxis and the introduction of the administration of high doses of methotrexate, which improved 5-year pEFS from 9% to 36% [6]. In other research, CNS prophylaxis with intrathecal methotrexate and cranial irradiation reduced the risk of CNS relapse from 32.5% to 1.4% after hematological remission [13]. Further efforts have been made towards limiting the side effects of CNS prophylaxis, and currently only a strictly limited group of patients who are at the highest risk of CNS relapse are treated with cranial irradiation.

Another feature early identified as a risk factor was age at diagnosis. Infants, especially in the first year of life, have significantly worse outcomes compared to children aged between one and six. In our analysis, patients aged <1 year treated between 1983 and 1988 had significantly lower pOS ($p = 0.033$) and pEFS ($p = 0.082$). This effect is caused by the different leukemia biology in this particular group and the high risk of long-term side effects [14]. The answer for issues related to infant ALL was the development of dedicated therapy protocols, conducted by three large collaborative groups – the Children's Oncology Group (COG), the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), and the Interfant Study Group [14].

Infant-dedicated protocols, as well as previous observations, resulted in better understanding of infant ALL genetic background and the identification of risk factors in this group, and provided necessary information about treatment toxicity [14].

The role played by Hgb concentration at diagnosis is unknown, with a significant impact of Hgb <8/dL on EFS and RFS. This phenomenon was also reported by Schrappe et al. [15] in their analysis of BFM-90 protocol results. This is difficult to explain, but hypothetically it might correspond to marrow blasts involvement or cellular sensitivity.

One of the most important prognostic factors in childhood lymphoblastic leukemia is early response to treatment. It has been proved that the hematological response to prednisone on day 8 of therapy, and the bone marrow response at later timepoints, have crucial impacts on long-term outcomes [2, 16, 17]. In our study, this effect was mostly seen in children treated in the period 1988–1995, when PPR was related to a more than 3-fold higher risk of death ($p = 0.001$) and an almost 2-fold higher risk of relapse ($p = 0.019$). Due to this observation, patients with PPR were stratified into a high risk group with therapy intensification, which led to an improvement in therapy outcome in this particular group [18]. Furthermore, in our analysis, the response to treatment was one of the most important risk factors during subsequent therapeutic periods [4, 19]. That resulted in the development of diagnostic methods related to therapy response assessment, and the implementation of MRD monitoring. This in turn enabled the early identification of patients at risk of relapse, even at times when the disease seems to be in remission. Moreover, it allows us to reduce therapy in standard-risk patients with a low level of MRD [20].

Genetic aberrations in blast cells proved to be crucial to the proper understanding of ALL biology and therapy response. One of the first genetic aberrations identified as a risk factor was the BCR-ABL mutation, and patients with this feature were thus stratified into a high risk group [21]. In our cohort, genetic diagnostics become available in 1996. In the period 2001–2011, a lack of the BCR-ABL mutation was the most important factor related to a better pOS ($p <0.001$). Unsatisfactory therapy results in this group resulted in treatment modification, with the introduction of targeted therapy with tyrosine kinase inhibitors (TKI), which have dramatically improved patients' outcomes. The success of TKI drove further research into targeted therapy in childhood ALL [21].

Conclusions

Prognostic factors in ALL have changed during the last few decades, and the development of diagnostic methods have led to a better understanding of the underlying causes of the disease. Medicine has become more aware of ALL's genetic background, and this has triggered further research in the field of genetic diagnostics and contributed to its accessibility. Furthermore, the changing landscape of risk factors in ALL has reflected sustained progress in ALL therapy, with the gradual elimination of features related to poor outcomes, mostly due to modifications in treatment and developments in diagnostic methods as well as therapy monitoring.

The modern era of immunotherapy and treatment focused on molecular pathways facilitates a more targeted approach, with new opportunities regarding the high risk group of patients [22]. Moreover, targeted therapy has had a great impact on treatment toxicity reduction in specific subgroups. New therapy protocols should bring answers regarding the efficiency and side effects of novel therapies in ALL.

Authors' contributions

JS — data collection and interpretation, statistical analysis, description of results. ED, AJG — data collection and interpretation, statistical analysis. NB, AK, SK, KC, MRP, RD, MP, BT, PK, JC, ME, AM, AD, AU, EG, KJ, EW, DK, MŁ, MA, SW, OG, ST, MM, MD, MK, BKR, ED, AM — data collection and interpretation. JS — thesis draft, critical review and important intellectual content, acceptance of final version for publication.

Conflict of interest

The authors declare no conflict of interest.

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None.

Ethics

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

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SHORT COMMUNICATION

VIA MEDICA

Impact of CAR-T therapy for outcomes in primary refractory acute lymphoblastic leukemia

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of malignancy in children. Improvements in ALL therapy have led to significant progress in outcomes, so that currently more than 85% of children with ALL survive for five years or longer after diagnosis [1]. Unfortunately, relapses or refractory disease remain one of the main reasons for therapy failure. Patients who do not achieve remission after the induction phase are considered a very high-risk group, with an unfavorable prognosis [2]. Since the early 2010s, patients not responding to initial treatment have been treated with intensified chemotherapy, with allogeneic hematopoietic stem cells transplantation (allo-HSCT) as the treatment of choice [2]. However, this treatment is associated with severe toxicity and potentially life-threatening complications [1, 2].

During the last decade, several new therapeutic options for children with high-risk ALL have been developed, such as monoclonal antibodies, drug-antibody conjugates, and chimeric antigen receptor T-cells (CAR-T) [3].

We describe below two children with refractory ALL treated with modern therapeutic options in the pre-CAR-T era, as well as after the introduction of CAR-T therapy in Poland.

Methods

Anonymized data of two patients treated in the Department of Pediatric Hematology and Oncology, Antoni Jurasz University Hospital No. 1 in Bydgoszcz, Poland was collected. The diagnosis was established based on a bone marrow biopsy including cell morphology with French–American–British (FAB) classification and immunophenotyping. Analyzed data included clinical observations, laboratory test results and therapy outcomes. Analysis of blast cells in peripheral blood (PB) and bone marrow (BM) was performed in the Laboratory of Clinical and Experimental Oncology at Antoni Jurasz University Hospital No. 1; molecular analysis of blast cells was performed in the Medical Laboratory of Pediatric Oncology and Hematology at the Central Clinical Hospital of the Medical University of Lodz; flow cytometry monitoring of minimal residual disease (MRD) was performed in the Department of Pediatric Hematology and Oncology in the Central Clinical Hospital of the Medical University of Silesia; and polymerase chain reactions (PCR) MRD were also performed in the Medical Laboratory of Pediatric Oncology and Hematology in the Central Clinical Hospital of the Medical University of Lodz. This study was approved (KB 577/2021) by the Bioethics Committee of the Nicolaus Copernicus University in Toruń.

Results

Patient 1

A 4-month-old boy, previously healthy, was referred to the Department of Pediatric Hematology and Oncology due to hyperleukocytosis (112 G/L), severe anemia (3.9 g/dL) and thrombocytopenia (7 G/L). The child was diagnosed with pro-B ALL with MLL/KMT mutation, without central nervous system (CNS) involvement. Therapy according to the AIEOP-BFM ALL 2017 protocol was initiated. On day 8 of his treatment, the response was assessed as satisfying without blast cells in PB (no prednisone poor response). MRD on day 15 measured by flow cytometry was 9.9%. Bone marrow biopsy performed on day 33 showed 0.6% of

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blast cells in myelogram and PCR MRD 3×10^{-2}. The boy was subsequently stratified to a high risk (HR) group with therapy intensification.

On day 64 of therapy, PCR MRD was still positive ($1 \times \times 10^{-3}$) and increased to 2×10^{-2} on day 118 of treatment. According to the therapy protocol, the boy was eligible for allo-HSCT. Subsequently, he received the IDA-FLAG [fludarabine, high-dose cytarabine (HD-Ara-C), idarubicin, granulocyte colony-stimulating factor (G-CSF)] chemotherapy cycle, which was complicated by sepsis and cardiorespiratory failure. He was treated with broad-spectrum antibiotics and catecholamines, and he responded well to the treatment. Afterwards, he received one cycle (of 28 days) of blinatumomab therapy administered through continuous infusion with a dose of 2 µg/day during the first seven days, followed by a dose of 6 µg/day for the next 21 days. However, after the cycle, PCR MRD increased to 8×10^{-1}. The therapy was switched to HIB chemotherapy according to the ALL IntReALL2010 HR protocol. After three weeks of the next line treatment, there were 79.8% blast cells in the bone marrow. Due to the failure of classical chemotherapy, the patient received two cycles of immunotherapy treatment with monoclonal antibody-drug conjugate anti-CD22 (inotuzumab ozogamicin) at a dose of 0.5 mg in a single infusion, as part of mini-Hyper-CVD [cyclophosphamide, dexamethasone, methotrexate and cytarabine in reduced doses] chemotherapy. The blast cell load in the bone marrow was 40% after the first cycle of inotuzumab and 28% after the second one. However, after the third cycle of immunotherapy, there was another increase of blast cells in the bone marrow, with the percentage reaching 30%. The patient then received another cycle of chemotherapy with clofarabine, cyclophosphamide, etoposide and vincristine. Treatment was complicated with *Candida* sepsis, pneumonia and cardiorespiratory failure, which required treatment in the intensive care unit. Over the next few days, continuous clinical progression of leukemia was observed (progressive hepatosplenomegaly, increased blast cells count in PB). He was qualified for palliative care treatment. He died 14 months after the beginning of the ALL treatment.

Patient 2

A 13-month-old girl, previously healthy, was referred the Department of Pediatric Hematology and Oncology with hyperleukocytosis (268 G/L), severe anemia (3.9 g/dL), and thrombocytopenia (16 G/L). The girl was subsequently diagnosed with pro-B ALL with MLL mutation without CNS involvement. She was treated according to the AIEOP-BFM ALL 2017 protocol. During the induction phase, a poor response to initial treatment was observed (poor prednisone response on 8^{th} day, 74% of blast cells in PB by flow cytometry (FC) MRD on 15^{th} day, 6.6% of blast cells in BM on 33^{rd} day). At the end of the consolidation phase, there were 0.4% of blast cells in BM. Due to an unsatisfactory response, the girl began the first of two scheduled cycles of blinatumomab administered through continuous infusion with a dose of 2 µg/day. The first cycle was interrupted after only two days because of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, but it was resumed immediately with a dose of 3 µg/day after the patient recovered from the infection. After the first cycle of blinatumomab, which lasted a total of 28 days, MRD PCR was 2×10^{-4}. During the second cycle of blinatumomab therapy, she received the drug at a dose of 12.5 µg/day via continuous infusion for 28 days.

However, despite the higher dose of blinatumomab, the MRD PCR increased to 7×10^{-4} after the second cycle. Furthermore, there were no matched related or unrelated donors for allo-HSCT. The patient was eligible for CAR-T therapy. As a bridge therapy during CAR-T preparation, she received immunotherapy with a single infusion of inotuzumab ozogamicin at a dose of 0.4 mg, followed by another infusion at a dose of 0.5 mg one week later. Unfortunately, there was a poor response, as demonstrated by a bone marrow biopsy that showed 47% blast cells. The therapy was switched to HIB cycle according to the IntReALL HR 2010 protocol. The next step was CAR-T infusion which was complicated by grade I cytokine release syndrome (CRS) and autoimmune hypothyroidism. Another complication of the treatment was secondary hypogammaglobulinemia, which has required IVIG substitution continuing up to date. The girl has been in remission since CAR-T infusion (for 19 months now), in a good general condition, and with adequate psychomotor development.

Discussion

Primary refractory childhood ALL occurs in a small subsequent group of patients, and represents 2–3% of all cases. Ten-year overall survival in this group is 32 ± 1%, with a particularly unfavorable survival rate among infants with an *MLL* gene rearrangement [2]. Previously, children with primary refractory ALL have received an intensification of conventional chemotherapy, but the results of this approach have proved unsuccessful due to the high toxicity of the treatment [4]. New therapeutic options based on immunotherapy have significantly improved prognosis in this high-risk group, with particular success demonstrated by CAR-T, which has proved to be a breakthrough in the treatment of patients with primary refractory or relapsed disease.

Blinatumomab has become the first approved bispecific monoclonal antibody for the treatment of pediatric relapsed or refractory B-cell ALL (B-ALL) [4]. Blinatumomab is a monoclonal antibody that simultaneously targets CD3+ of endogenous T-cells and CD19+ B-ALL cells. In phase I/II studies, it has demonstrated complete response (CR) rates of 30% in patients with refractory ALL, and of 48% in those with relapsed disease [5]. In phase III clinical trials in

high-risk relapsed childhood ALL, the incidence of events in the blinatumomab versus chemotherapy groups was 31% versus 57% (p <0.001) [6]. The most severe adverse effects include infections, anemia, thrombocytopenia, febrile neutropenia and CRS [5].

Another approach to immunotherapy in ALL is represented by drug-antibody conjugates such as inotuzumab ozogamicin, which is a CD22-directed monoclonal antibody conjugated to the cytotoxin calicheamicin. In the Children Oncology Group study, inotuzumab proved to be effective in children with relapsed ALL, with a response rate of 58% [CR or CR with incomplete bone marrow recovery (CRi)] [7]. Similar promising effects were shown in a study in an infant and young child population, where 50% of patients achieved CR [8]. The most severe and life-threatening complication of inotuzumab treatment is sinusoidal obstruction syndrome which can occur in as many as 50% of patients who have received allo-HSCT as part of their ALL treatment [8].

CAR-T has recently emerged as a breakthrough therapy for patients with refractory or relapsed B-ALL. CAR-T are T-cells engineered from the patient to express a chimeric antigen receptor for targeted receptor on B-ALL cells. Currently, only CD-19 targeted CAR-T are approved for childhood B-ALL treatment [4, 9]. CR after CAR-T infusion has reached 70–90% in clinical trials, depending on the CAR-T type and CR definition [9]. However, it is still unclear whether CAR-T should be considered as a final treatment, or whether it should be followed by allo-HSCT [9, 10]. On the other hand, CAR-T manufacturing can be challenging and the treatment is related to manageable, albeit life-threatening, complications such as CRS or neurotoxicity [9, 11, 12].

In this report we have described two cases of primary refractory childhood ALL. In both patients, a lack of remission at the end of induction was combined with other risk factors such as MLL rearrangements and high hyperleukocytosis at diagnosis [1]. Because of the failure of standard therapy, both children were qualified to second line therapy with immunotherapy as one of the available options. In both cases, monoclonal antibodies as well as intensification of classical chemotherapy have been shown to be only partially effective. Unfortunately, CAR-T therapy was unavailable in the first case, but it proved highly effective in the second patient, being a curative option. Since September 2021, CAR-T therapy has been reimbursed in Poland for children and adults up to the age of 25 with refractory or relapsed B-ALL.

We hope that innovative therapies will improve outcomes in patients with primary refractory disease, as until now the prognosis in these patients has been highly unfavorable.

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Authors' contributions

JS — data collection and interpretation, description of results, manuscript preparation. NB, ED, AK, MRP — data collection and interpretation. JS — thesis draft, critical review and important intellectual content, acceptance of final version for publication.

Conflict of interest

The authors declare no conflict of interest.

Ethics

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

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ORIGINAL RESEARCH ARTICLE

VIA MEDICA

Immunological response to chimeric antigen receptor T-cell therapy in pediatric relapsed/refractory acute lymphoblastic leukemia: peak of cytokine levels on day 4 post-infusion

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Abstract

Chimeric antigen receptor (CAR) T-cell therapy is associated with specific toxicities related to immunological response to the therapy. The aim of this study was to analyze the kinetics of cytokine responses after CAR T-cell infusion and its correlation with observed toxicities. Data from patients treated with CAR T-cell therapy at a single children's oncology center in 2023–2024 was analyzed. The data included CAR T-cell count in peripheral blood, hematology laboratory data and proinflammatory biomarkers (IL-2, IL-4, IL-6, IL-10, IL-17A, TNFα, IFNγ, CRP, and ferritin), and observed toxicities. Six patients were treated with CD19 CAR-T cell therapy for relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL). The expansion of CAR T-cells was observed during the first 14 days following infusion, with the peak occurring between days 6 and 12. Cytokine release syndrome was observed in 5/6 analyzed cases, mostly during the first week after the infusion. Increased plasma concentrations of IL2, IL10, and IFNγ were observed on the fourth day after CAR T-cell infusion. Biphasic peaks were detected in the plasma concentrations of I-4 (on days 1 and 4), and IL-6 and IL-17a (on the day after the infusion and on days 8 and 9). Day 4 post-infusion was characterized by elevated plasma levels of most of the analyzed cytokines and this correlated in terms of time with CRS and ICANS. We also observed an immunological response to the infusion itself, along with CAR-T cell expansion.

Keywords: chimeric antigen receptor T-cell, CAR T-cell, relapsed/refractory acute lymphoblastic leukemia, children, cytokines

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Introduction

Chimeric antigen receptor (CAR) T-cells engineered to target CD19 have shown significant efficacy in treating relapsed or refractory (R/R) B-cell malignancies, with particularly high complete remission (CR) rates in R/R acute lymphoblastic leukemia (ALL) [1]. In pediatric patients with R/R ALL, CD19 CAR T-cell therapy has been associated with a CR >80% in a highly pretreated group [1–3].

Yet despite its high efficacy, CAR T-cell therapy is associated with severe toxicities that differ significantly from those observed with conventional cytotoxic treatment [4]. The two main potentially life-threatening adverse effects of CAR T-cells are cytokine release syndrome (CRS),

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and immune effector cell-associated neurotoxicity syndrome (ICANS). CRS is characterized by systemic inflammation leading to fever, chills, fatigue, anorexia, and, in severe cases, cardiovascular instability, whereas ICANS, which is related to cytokine dysregulation in the central nervous system (CNS), typically manifests as toxic encephalopathy with confusion, somnolence, cranial palsy and seizures [2, 4, 5].

As CAR T-cell therapy becomes more widely used in clinical practice, predicting the onset and severity of toxicity and optimizing its management have become essential [6]. Analyses of immunological response after CAR T infusion have shown that certain proinflammatory cytokines can be used to identify patients at risk of severe adverse effects [2, 6, 7]. The incorporation of CAR T-cells and cytokine monitoring can guide therapeutic approaches for identifying possible toxicities and side effects.

The aim of this analysis was to study the kinetics of cytokine responses after CAR T-cell infusion, and to determine their correlation with observed toxicities. The study was approved (KB577/2021) by the Ethics Committee of Collegium Medicum, Nicolas Copernicus University, Bydgoszcz, and was conducted in accordance with the Declaration of Helsinki.

Material and methods

Patients and study design

Data from patients treated with CAR T-cell therapy at the Department of Pediatrics, Hematology and Oncology, University Hospital No. 1 in Bydgoszcz, Poland from May 2023 to August 2024 inclusive was analyzed. The indication for CAR T-cell therapy was R/R ALL. Patients received a single dose of CAR T-cell therapy with tisagenlecleucel (Kymriah, Novartis Europharm, Basel, Switzerland). All patients were observed for the occurrence of CRS and ICANS for 14 days, or longer if clinically indicated. The entire follow-up period was determined based on the infusion time and the study design.

Data analyzed in this study included serial measurements of CAR T-cell count in peripheral blood, hematology laboratory data such as white blood cell count (WBC), absolute neutrophil count (ANC) and percentage, absolute lymphocyte count (ALC) and percentage, with monitoring of CD3 and CD19 number/percentage and proinflammatory biomarkers (C-reactive protein (CRP), ferritin and specific cytokines).

Disease burden was assessed in bone marrow from biopsies performed before CAR T-cell infusion, 30 days after the infusion, and, in cases of clinical indications (*e.g.* prolonged cytopenias, suspicion of relapse), during the follow-up.

Owing to the small sample size and deviation from a normal distribution, most quantitative variables are presented as medians with trend lines or ranges. The language was corrected using the TRINKA grammar checker on the website: www.trinka.ai website.

Flow cytometric detection of CAR T-cells

A four-color flow cytometry panel was used based on a commercial CD19 CAR detection reagent kit (CD19 CAR detection reagent human, biotin; CD3 antibody, anti-human, PE, REAfinity; CD45 antibody anti-human, VioBlue, REAfinity; Biotin antibody, APC, REAfinity, Miltenyi Biotec, Bergisch Gladbach, Germany). This reagent consisted of a biotinylated CD19 antigen that binds CD19-targeted CAR-Ts. In a second incubation step, the biotin-labeled CAR T-cells were then stained with a fluorochrome-conjugated anti-biotin antibody.

Flow cytometric detection of cytokines

Cytokines were detected using the flow cytometry method (CBA and BD Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit, Becton Dickinson, Franklin Lakes, NJ, USA). The following cytokines were analyzed: IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α, and IFN-γ. Serial samples for serum cytokine levels were obtained within the two days before CAR T cell infusion and during the 14 days after it.

Definitions

Cytokine release syndrome and ICANS severity was graded according to the stage system proposed in the ASTCT Consensus Grading for CRS and ICANS [8]. Disease burden was assessed using standard morphological examination and polymerase chain reaction (PCR) analysis of bone marrow and peripheral blood samples. Minimal residual disease (MRD) was measured by detecting clone-specific immunoglobulin and T-cell receptor gene rearrangements using PCR amplification in bone marrow [9]. CR was defined as MRD negativity with at least one, and if possible two, markers with a sensitivity and quantitative range of at least 10e-4.

Results

During the analysis period, six patients were treated with CD19 CAR-T cell therapy with tisagenlecleucel for R/R ALL and were included in the analysis. The median follow-up period was 11 months (range 1–15) after CAR T-cell infusion. Specific data regarding indication for CAR T-cell therapy, transduced CAR T-cell dosage, and treatment complications is set out in Table 1. Bridging therapy was based on the FRALLPOST 2004 and IntReALL 2010 protocols [9, 10]. Three patients had previously undergone allogeneic hematopoietic stem cell transplantation (HCT), with the times from HCT to CAR T-cell infusion being 11 years (Patient 2), two years (Patient 3), and 18 months (Patient 4). All patients received CAR T-cells at one infusion, with an average dose of 3.28×10^6 cells (range $2.4–5.12 \times 10^6$ cells).

Table I. Clinical data of patient characteristics with indication for CAR T-cell therapy, bringing therapy, transduced CAR T-cell dosage, treatment complications and outcomes

No.	Age (years)	Indication for CAR T-cell[1]	Bridging therapy	CAR-T/kg	CRS day	CRS grade	ICANS day	ICANS grade	MRD before CAR T-cell infusion	MRD 30 days after CAR T-cell infusion	Last MRD
1	5	1st line refractory disease	FRALL-POST[4] 2004	2.4×10^6 cells	4 + 8	1	4 + 8	2 + 2	5.5×10^{-1}	$<5 \times 10^{-4}$	Negative
2	17	Relapse after HCT[2]	FRALL-POST[4] 2004	2.6×10^6 cells	3	1	NA[7]	0	Negative	Negative	Negative
3	6	Relapse after HCT[2]	IntReALL[5]: SIA[6]	3.2×10^6 cells	2	1	NA[7]	0	5×10^{-3}	Negative	Negative
4	7	Relapse after HCT[2]	IntReALL[5]: SIA[6]	3.9×10^6 cells	4	1	5	1	$<1 \times 10^{-4}$	Negative	Negative
5	10	2nd ALL[3] relapse	FRALL -POST[4] 2004	2.46×10^6 cells	2	2	2	2	Negative	Negative	Negative
6	11	2nd ALL relapse	IntReALL[5]: SIA[6]	5.12×10^6 cells	NA[7]	0	NA[7]	0	No molecular markers		

[1]CAR T-cell – chimeric antigen receptor T-cell; [2]HCT – hematopoietic stem cell transplantation; [3]ALL – acute lymphoblastic leukemia; [4]FRALLPOST – Frankfurt relapse acute lymphoblastic leukemia post stem cell transplantation protocol; [5]IntReALL – an international randomised phase III study for treatment of standard risk childhood relapsed acute lymphoblastic leukemia; [6]SIA – dexamethasone, vincristine, methotrexate, PEG-asparaginase, cytarabine, prednisolone; [7]NA – not applicable

CRS and neurotoxicity

Cytokine release syndrome was observed in 5/6 analyzed cases. Three children experienced ICANS. None of the analyzed patients had grade 3–4 CRS or ICANS. The incidence and severity of CRS are set out in Table I. The majority of CRS and ICANS cases were observed during the first week after CAR-T infusion, with the exception of the first patient who developed CRS and ICANS on days 4 and 8 after the infusion.

Cytokine release syndrome and ICANS were managed according to the 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Hematology Association (EHA) [8]. All patients with CRS received tocilizumab 1–4 doses every eight hours, depending on the clinical course and response to therapy. Two patients received steroids. None of the patients required admission to the intensive care unit (ICU) for CRS or ICANS management.

CAR T and cytokines kinetics

An expansion of CAR T-cells was observed during the first 14 days following infusion, with the peak in CAR T-cells number and percentage occurring between days 6 and 12 (Fig. 1). The expansion of CAR T-cells was associated with a rapid decrease in CD19-positive cells. The percentages of CD3, CD19 and CAR T-cells over the follow-up period for respective patients are set out in Figure 2.

C-reactive protein and ferritin levels, but not procalcitonin levels, increased in a time-dependent correlation with the observed toxicities (see Fig. 3). Increased plasma concentrations of IL-2, IL-10, and interferon gamma (IFN-γ) were observed on the fourth day after CAR T-cell infusion, as shown in Figure 4. Two peaks in IL-4 concentrations were observed on the first and fourth days after CAR T-cell infusion. Biphasic peaks were also detected in the plasma concentrations of IL-6 and IL-17a. Individual patients' cytokines levels, along with median results and trend lines for each cytokine, are set out in Figure 4.

Patients who developed grade 2 CRS exhibited higher concentrations of IL-4, IL-17a, and IFN-γ compared to the median results of patients with grade 1 CRS, while no significant differences were observed in the levels of IL-2, IL-6, IL-10, or TNF-α (see Fig. 5).

Hematological toxicities

All patients developed neutropenia following CAR T-cell infusion, with the majority experiencing severe neutropenia, defined as an absolute neutrophil count (ANC) of below $0.5 \times 10^3/\mu L$. The dynamics of ANC over the three-month follow-up period after CAR T-cell infusion are illustrated in Figure 6.

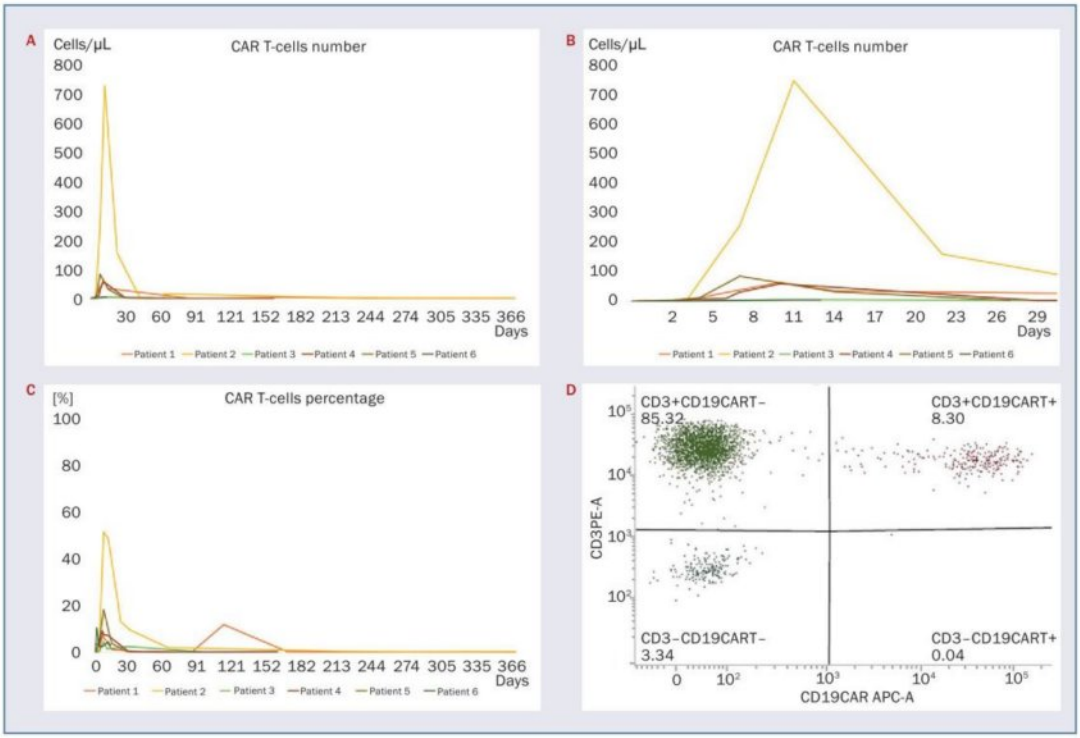

Figure 1. Chimeric antigen receptor T-cells expansion. **A.** Chimeric antigen receptor T (CAR T) cells number for six analyzed patients during follow-up period; **B.** CAR T-cells number for six analyzed patients during first month after infusion; **C.** CAR T-cells percentage during follow-up period; **D.** Representative flow cytometry plot for CAR-positive CD3+ lymphocytes detection

Outcomes

On day 30 after the infusion, 4/5 patients with detectable molecular markers had negative MRD, and the final MRD before this article's submission for publication was negative for all patients with detectable molecular markers. All patients remain in remission up to the present day.

Discussion

The introduction of CAR T-cell therapy has radically altered the treatment paradigm in R/R B-cell malignancies. Despite better characterization of CAR T-cell kinetics and its immunological effects, monitoring for adverse events remains a challenge [11]. Both CRS and ICANS are associated with the significant production of proinflammatory cytokines, triggered by the interaction between the CAR T-cell receptor and its target [11]. This process initiates an inflammatory cascade, wherein activated CAR T-cells and lyzed cancer cells induce massive production of cytokines such as IL-6, TNF-α, and IL-10. While this process can enhance the antileukemic efficacy of CAR T-cells, it can also progress into an uncontrolled and damaging condition [2, 4, 11].

Routine cytokine assessment has not yet been integrated into clinical practice. Moreover, there was limited reporting of cytokine measurements during the clinical trials of CAR T-cell therapy [7]. On the other hand, most toxicities are strongly correlated with elevated plasma concentrations of specific cytokines [2, 7, 11]. In the analyzed cohort, day 4 post-CAR T-cell infusion showed a marked increase in IL-2, IL-10, and IFNγ. Interleukin 4 plasma levels exhibited two peaks: on day 1, likely as an immune response to the infusion, and on day 4. The dynamics of IL-6 and IL-17a were characterized by a rise in the first few days post-infusion, with the peak on days 8 and 9, which correlated with the peak of CAR T-cells expansion.

The majority of CRS cases occurred on days 2 and 3, with plasma cytokine peaks temporally correlated with the onset of toxicities. This was consistent with findings from previous studies [12]. The association between proinflammatory cytokine peaks and CRS resulted in the introduction of tocilizumab, an anti-IL6 monoclonal antibody, to CRS treatment. In the analyzed cohort, all patients who experienced CRS were successfully treated with tocilizumab, leading to a rapid resolution of symptoms. Targeting this cytokine effectively disrupted the

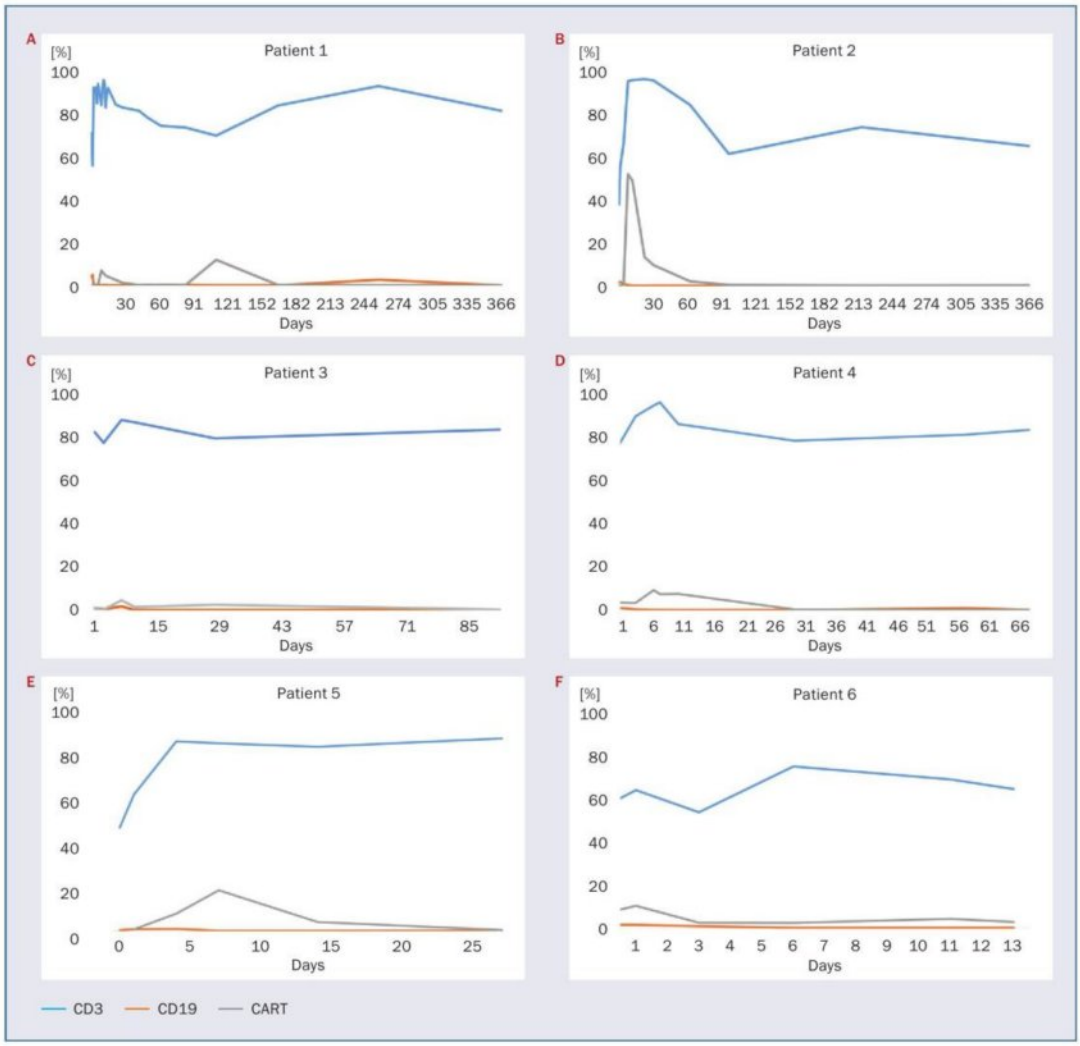

Figure 2. CD3, CD19 and chimeric antigen receptor T-cell percentages for six analyzed patients during follow-up

immune feedback loop, and reduced the harmful effects of cytokine activity.

Both CRS and ICANS treatments were divided into subsequent steps according to their complications severity (grades 1–4) [8]. While tocilizumab has become the standard treatment for CRS, a subset of patients remains refractory to this therapy. Moreover, the anti-IL6 monoclonal antibody is not effective in treating ICANS. Corticosteroids are the next line of CRS treatment; however, the prolonged administration of high-dose steroids can compromise the antitumor efficacy of CAR T-cells and should be reserved for severe cases of CRS [8].

To address the therapeutic gap in CRS management, additional cytokines are being investigated as potential targets. In the analyzed cohort, plasma concentrations of IL-2, IL-10, and IFNγ were characterized by similar dynamic profiles, a correlation also noted in previous studies [13, 14]. Moreover, elevated levels of IFNγ have been associated with increased mortality during follow-up [12]. IFNγ is currently under investigation as a novel therapeutic target for CRS. Emapalumab, a human monoclonal antibody directed against IFNγ, has demonstrated efficacy in preclinical murine *in vivo* models, mitigating severe CAR T--cell-associated toxicities without impairing CAR T-cell activation [15]. It also has been successfully applied in the therapy of a patient with grade 4 CRS refractory to both tocilizumab and methylprednisolone [16].

Other common adverse effects of CAR T-cell therapy are hematological toxicities, with neutropenia and

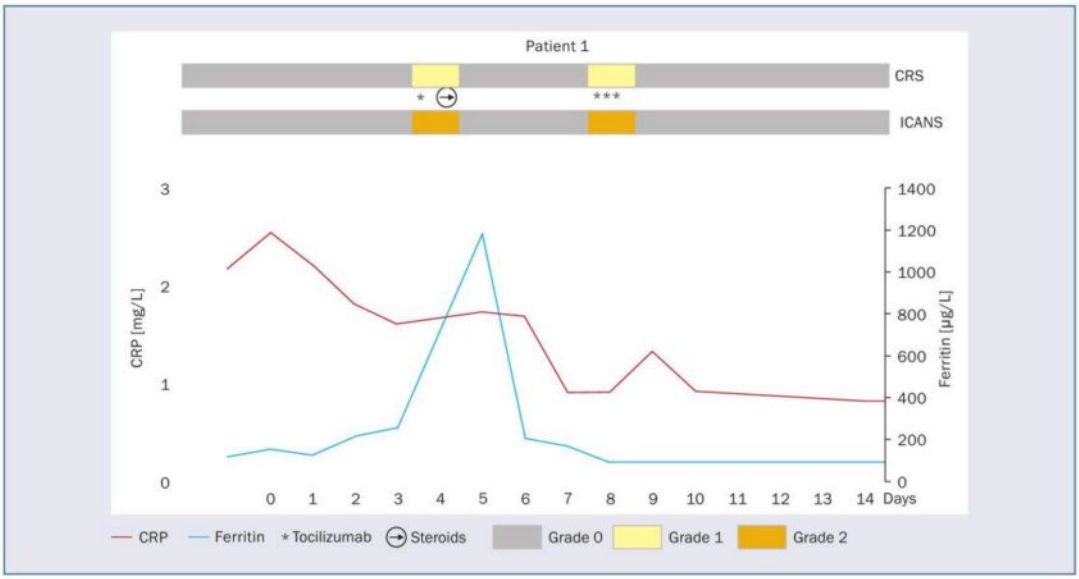

Figure 3. Kinetics of C-reactive protein (CRP) and ferritin with correlation of cytokine-release syndrome, immune effector cell-associated neurotoxicity syndrome grades, and used therapy over 14 days following infusion in Patient 1. Kinetics of C-reactive protein (CRP) and ferritin with correlation of cytokine-release syndrome, immune effector cell-associated neurotoxicity syndrome grades, and used therapy over 14 days following infusion in Patient 1

thrombocytopenia being the most frequently observed [17]. Cytopenias usually present with a biphasic pattern, with neutropenia recovery by the second week after the infusion in c.50% of patients [17]. A similar pattern was observed in our analyzed patients. The dynamics of ANC reflect the mechanism of therapy, with severe neutropenia following initially post-lymphodepleting therapy. Although lymphodepletion chemotherapy leads to early cytopenia, prolonged neutropenia may have multiple contributory factors, and appears to be strongly linked to inflammation [18]. A second nadir of neutropenia is associated with elevations of the serum levels of IFN-γ, IL-6 and IL-8, a state similar to those observed in acquired bone marrow failure such as aplastic anemia and hypocellular myelodysplastic syndrome [18, 19]. This mechanism is especially active in children because of increased thymic activity and sustained immune dysregulation, leading directly to hematopoietic suppression or immune-mediated mature blood cell destruction [18]. Moreover, as shown in Figure 2, most patients exhibited prolonged B-cell aplasia, which correlated with the presence of CAR T-cells. B-cell aplasia is a well-documented phenomenon associated with disease remission [8, 19, 20].

Our study has some obvious limitations. The methodology follows conventional approaches. However, the uniformity in cytokine assay methods, data reporting, and correlation with clinical outcomes enables meaningful cross-study comparisons. While parallel studies have become more common, many studies focusing on CAR T-cell toxicities have not reported cytokine data at all. Furthermore, not all articles have explored correlations between a broad panel of cytokines and the presence or severity of CRS. The analyzed group was small, but CAR T-cell therapy has only become available in Poland in recent years, and there is limited data on cytokine kinetics following CAR T-cell infusion in Polish populations. Given the relatively homogeneous genetic profile within this population, reporting such results is crucial in order to identify potential differences in immunological responses.

Conclusions

Incorporating cytokine profiling into clinical practice could improve our understanding of the correlations between specific cytokines levels and distinct toxicities, potentially guiding new therapeutic approaches. In our study, day 4 post-infusion was characterized by elevated plasma levels of most of the analyzed cytokines and there was a correlation in terms of time with CRS and ICANS. We also observed an immunological response to the infusion itself, along with CAR T-cell expansion. Hematological toxicities were common, with a biphasic neutropenia pattern.

Cytokine profiling provides valuable insights into the *in vivo* functionality of CAR T-cells, and can facilitate the early identification of patients at risk of severe adverse effects.

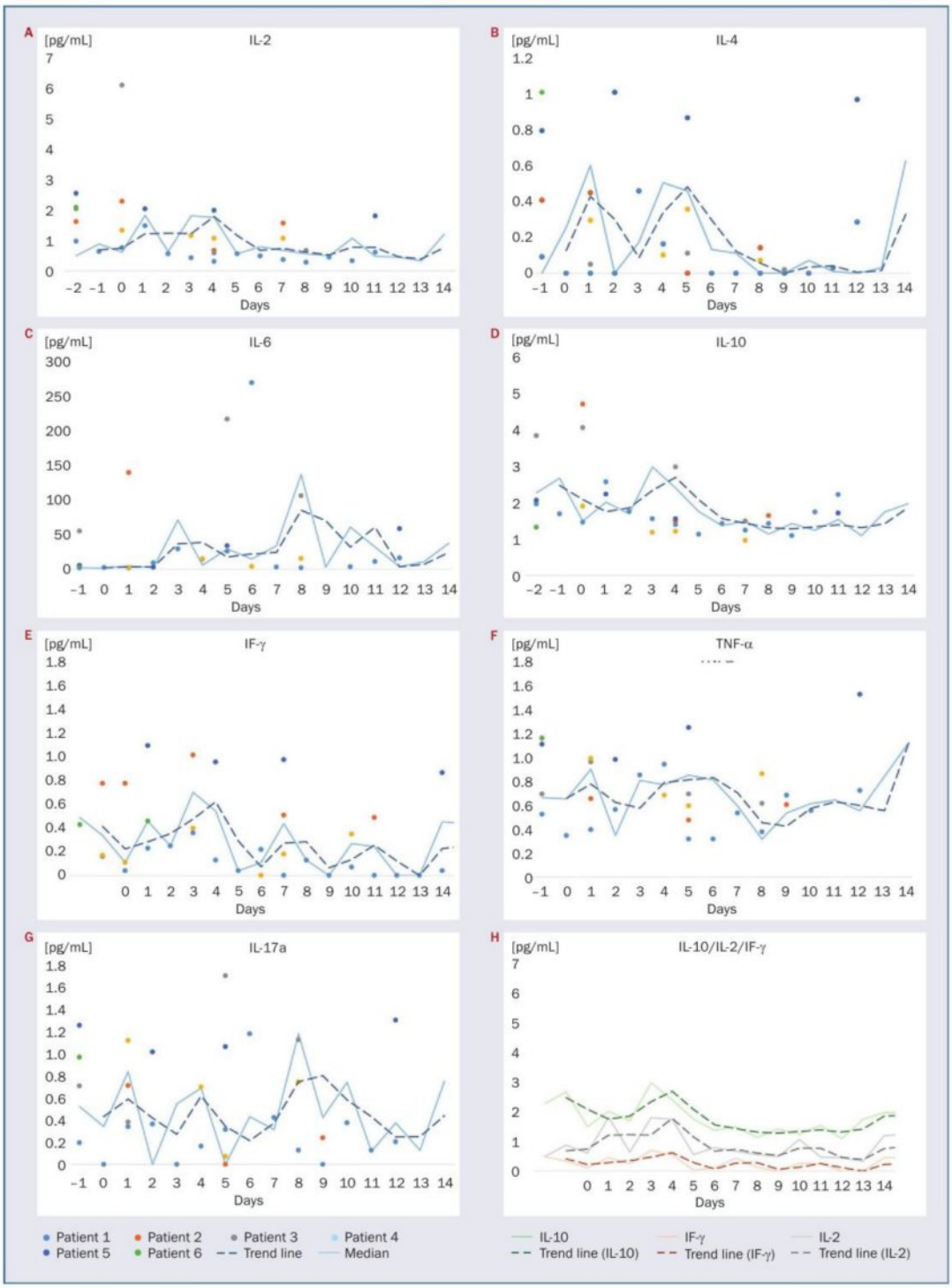

Figure 4. A.–G. Kinetics of analyzed cytokines day before and during 14 days following chimeric antigen receptor T-cells infusion, along with median results and trend lines. Dots represent respective measurements of individual patients; **H.** Median results and trend lines for interleukin-10, interleukin-2 and IFN-γ

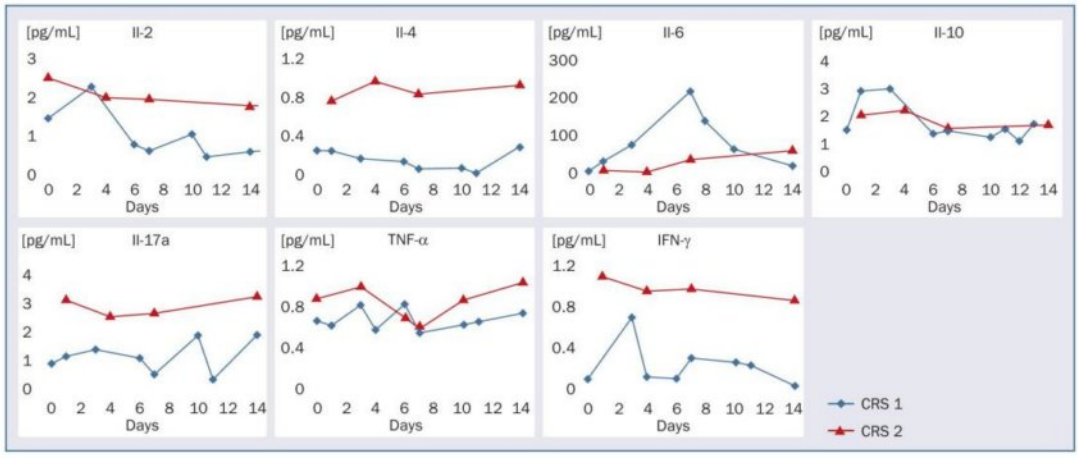

Figure 5. Kinetics of analyzed cytokines in patient with grade 2 cytokine-release syndrome (CRS) compared to median result of patients who developed grade 1 CRP over 14 days following infusion

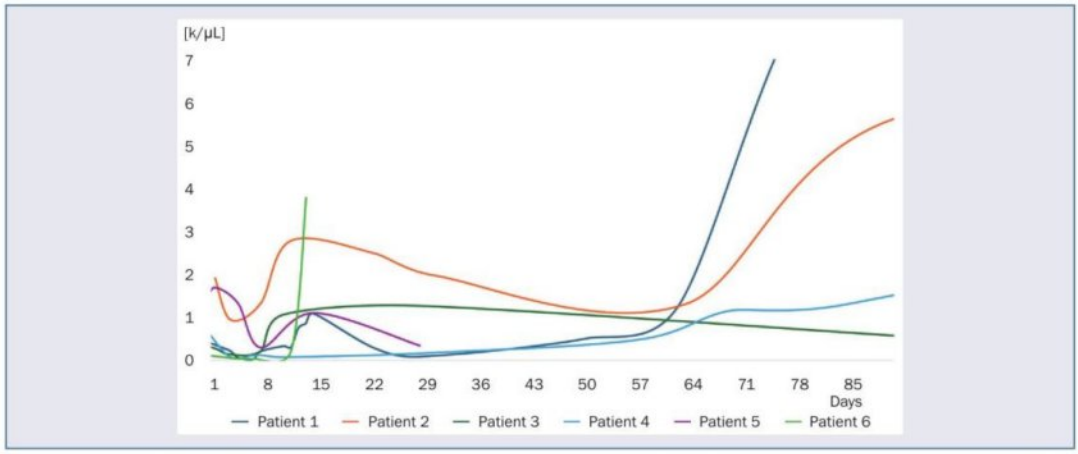

Figure 6. Absolute neutrophil count over period of three months following chimeric antigen receptor T-cells infusion

Article information and declarations

Data availability statement

Data is available upon reasonable request from the corresponding author.

Ethics statement

The study was approved (KB577/2021) by the Ethics Committee of Collegium Medicum, Nicolas Copernicus University, Bydgoszcz, and was conducted in accordance with the Declaration of Helsinki.

Authors' contributions

JoannaS — design of work, data interpretation, statistical analysis, description of results, drafting work, final approval of version to be published; EP — conception of work, acquisition and analysis of data for work, drafting work, final approval of version to be published; KC, RD, JC — conception and design of work, acquisition of data for work, critical review and important intellectual content, final approval of version to be published; MK, BKR — conception of work, acquisition and analysis of data for work, reviewing it critically for important intellectual content, final approval of version to be published; JanS, MRP — conception and design of work, drafting work, reviewing it critically for important intellectual content, final approval of version to be published. All authors agree to be accountable for all aspects of work in ensuring that questions related to accuracy or integrity of any part of work are appropriately investigated and resolved.

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Conflict of interest

None.

Supplementary material

None.

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Improved Outcome in Pediatric Acute Myeloid Leukemia: Progress With Hematopoietic Cell Transplantation

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Abstract. *Background/Aim: Pediatric acute myeloid leukemia (AML) is a heterogenous disease; however, development of diagnostic methods has led to a better understanding of its nature and improvement in therapy outcomes. In this study, we evaluated treatment results in children with AML treated in a single oncology center in comparison with international results. Patients and Methods: Data from 77 children with AML treated in a single oncology center between 1994 and 2020 were analyzed. Patients had been treated according to five consecutive therapy protocols: AML NOPHO 88; ANLL 98; BFM AML 2004; BFM AML 2012, and BFM AML 2019. Five-year overall survival (OS), 5-year event-free survival (EFS) and 5-year relapse-free survival (RFS) were evaluated for each therapy protocol to determine prognostic factors and clarify differences between international and individual center results. Results: During the observational period, 5-year OS increased from 55.6% to 85.7%, 5-year EFS from 45.7% to 87.5% and 5-year RFS from 51.4% to 85.7%. Hematopoietic cells transplantation (HCT) introduction in early 2000' improved treatment outcomes in the high-risk group, what has been mostly seen in the results of 5-year EFS. Treatment-related mortality was the most frequent cause of death in the analyzed group. Conclusion: Despite the significant improvement in therapy of pediatric AML, treatment outcomes remain unsatisfying. Introduction of HCT relevantly improved therapy results, especially in the high-risk group. International cooperation is crucial because of the small patient numbers in individual oncology centers.*

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Key Words: Acute myeloid leukemia, children, therapeutic era, survival.

During the last 30 years, significant improvement in pediatric acute myeloid leukemia (AML) treatment has been made. International collaboration is necessary in AML trials because of the small number of patients in regional oncology centers, the need of standardized risk group stratification and implementing unified protocols (1, 2). In 1983, The Polish Pediatric Leukemia/Lymphoma Study Group introduced international treatment protocols in AML therapy in Poland. Multi-aspects national transformation enabled better implementation of the treatment guidelines, which led to improvement in treatment outcomes in children with AML (3, 4). The progress can be seen nationwide as well as in individual oncology centers. The aim of the study was to determine specific prognostic factors in each therapeutic era and compare a single center performance with international results. We analyzed data from 77 patients treated in the Department of Pediatrics, Hematology and Oncology in Bydgoszcz between 1994 and 2020 with five consecutive AML protocols.

Patients and Methods

Design of the study. In this study, we retrospectively analyzed outcomes in all patients diagnosed with AML at a single children oncology center in Poland between 1994 and 2020. Treatment results were divided into specific therapeutic groups, defined by several therapy protocols.

Patients. Data of patients aged under 18 years with AML treated between 1994 and 2020 in the Department of Pediatrics, Hematology and Oncology in Bydgoszcz were analyzed. The inclusion criteria included patients with AML, including AML as myelodysplastic syndrome (MDS) transformations, patients with comorbidities or congenital malformations (including Down syndrome) and also biphenotypic or bilineage leukemia with AML component.

Diagnosis. In all cases, diagnosis was established based on bone marrow biopsy including cell morphology with the French-American-British (FAB) classification and immunophenotyping. Since 2003, cytogenetic analyses have been performed. Central verification of cell morphology, immunophenotyping, and cytogenetics have been performed since 2005. Complete blood count, ultrasonography of the liver and spleen, lumbar puncture,

Table I. *Risk group definitions*.

Protocol	Risk group	Definition
AML NOPHO 88	No risk group stratification	
ANLL 98	SR	FAB other than M5, ≤5% blasts in BM on day 15, no increase in blast count after day 15
	HR	Patients not qualified as SR group
AML BFM 2004	SR	M1/M2 with Auer rods[1,2], AML with t(8;21)[1,2], M4Eo with inv16a[1,2], M3 AML in Down's syndrome
	HR	M0 M1/M2 without Auer rods M4, M5, M6, M7
AML BFM 2012	SR	t(8;21), inv(16), t(1;11),NPM1, CEBPαdm
	IR	Patients not qualified as SR group or HR group
	HR	t(4;11), t(5;11), t(6;11), t(10;11), t(6;9), t(7;12), der12p, isolated monosomy 7, t(9;22), FLT3-ITD-WT1mut, complex karyotype
AML BFM 2019	SR[3,4]	Inv(16)(p13.1q22), t(16;16)(p13;q22), t(8;21)(q22;q22), t(1;11) (q21;q23), normal karyotype and NPM1-mutation, normal karyotype and CEBPA (double mutation)
	IR[4]	Patients not qualified as SR group or HR group
	HR	Abnormalities in chromosome 12p/ t(2;12), monosomy 5/5q-, WT1mut and FLT-ITD, monosomy 7 (not in combination with favorable/MLL- aberrations), t(4;11)(q21;q23); MLL/AF4, t(5;11)(q35.3;p15); NUP98/NSD1, t(6;11)(q27;q23)); MLL/AF6, t(10;11)(p12;q23); MLL/AF10, t(6;9)(p23;q34), t(7;12)(q36;p13), t(9;22)(q34;q11), complex karyotype, inv(3)(q21q26.2)/t(3;3)(q21;q26.2), t(16;21)(p11;q22); FUS/ERG, Inv(16)(p13.3q24.3) CBFA2T3-GLIS2

AML NOPHO: Acute myeloid leukemia Nordic Society of Pediatric Hematology and Oncology; AML-BFM: acute myeloid leukemia-Berlin–Frankfurt–Munster; BM: bone marrow; SR: standard risk; IR: intermediate risk; HR: high risk; FAB: French–American–British. [1]FLT3-ITD/TKD positive patients were re-stratified to HR group. [2]patients with ≥5% blasts in BM on day 15 or blastic reconstitution between day 15 and 28 were re-stratified to HR group. [3]patients with nonresponse after first induction or FLT3-ITD/TKD positive were re-stratified into IR group. [4]patients SR group or IR group with nonresponse after second induction were re-stratified into HR group.

chest X-ray and echocardiography (ECG) were performed in all cases. Most children were also examined by an ophthalmologist and children's neurologist for central nervous system (CNS) assessment.

Treatment protocols and risk group stratification. Patients were treated according to five therapeutic protocols: AML Nordic Society of Pediatric Hematology and Oncology (NOPHO) 88 in the period from March 1994 to March 1998 (5); ANLL 98 from March 1998 to November 2005 (3); Berlin–Frankfurt–Munster (BFM) AML 2004 from November 2005 to August 2013 (6); BFM AML 2012 from August 2013 to January 2020 (7); BFM AML 2019 from January 2020. Stratification to each risk group was performed since ANLL 98 protocol and the risk group definition for each protocol is shown in Table I. Hematopoietic cell transplantation (HCT) accessibility was limited before 2000 and in the first years, transplantation was performed in all forms (autologous, matched related, and unrelated donors), after individual assessment of the advantages and risk related to the procedure.

Definitions. Complete remission was defined as ≤5% of blasts in bone marrow (BM) of normal or only slightly decreased cellularity, with signs of regeneration of normal hematopoiesis, regeneration of normal cell production in peripheral blood (PB), lack of blasts in PB and the disappearance of any extramedullary sites. Relapse was defined as reappearance of leukemic blasts in the peripheral blood, re-infiltration of BM with ≥5% blasts, or leukemic infiltration elsewhere following complete remission (CR), lasting at least 4 weeks. Second complete remission (CR2) was defined as achievement of CR after relapse. An event was defined as failure to achieve complete remission during the first 42 days of treatment, relapse, death of any causes, or secondary malignancies after AML treatment. Overall survival (OS) was defined as the time from AML diagnosis until death of any cause, relapse-free survival (RFS) was defined as the time from AML diagnosis until relapse occurrence and event-free survival (EFS) was defined as the time from diagnosis to event occurrence.

Statistical methods. Data were given as median, range and proportion. Differences between groups were calculated using Ch-squared test and in specific subgroups relative odds ratio (OR) and relative risk (RR) were determined, with 95%CI (confidence interval). The endpoints of the study were 5-year OS, 5-year EFS and 5-year RFS. Survival rates were calculated with the Kaplan-Meier methods and compared with the log-rank test. MedCalc 20.100 (MedCalc Software, Mariakerke, Belgium) statistical software was used. Data were considered statistically significant when $p<0.05$. The study was approved (KB 577/2021) by the Ethics Committee of Collegium Medicum, Nicolas Copernicus University, Bydgoszcz.

Results

Demographics. In the period between 1994 and 2022, overall, 77 patients were diagnosed with AML. Patient characteristics in specific therapeutic protocols are shown in Table II. The median age at diagnosis was 10.0 years (range=0.1-18.2 years). Risk group stratification was introduced in ANLL 98 protocol. Since then, out of 72 children, 32 (44.4%) have been stratified into a high-risk group (HR). Relapse occurred in 21 children. Fifteen children had isolated bone marrow (BM) relapse, two isolated CNS relapse, two combined BM and CNS relapse, and two patients had BM and localized relapse (in both cases

Table II. *Patient characteristics at diagnosis in five acute myeloid leukemia (AML) protocols.*

	AML NOPHO 88	ANLL 98	AML BFM 2004	AML BFM 2012	AML BFM 2019
Number of patients	7	16	32	14	8
Sex					
Male	4	8	16	6	3
Female	3	8	16	8	5
Age (years) median range	7.9	10.1	11.2	8.0	10.2
	3.6-13.8	0.8-16.4	0.0-19.7	0.4-17.8	1.4-17.7
WBC ($\times 10^3$) median range	69.3	44.9	37.9	45.5	31.1
	2.2-454.4	0.4-168.0	1.0-219.0	1.1-207.4	0.8-188.2
PLT ($\times 10^3$) median range	59.1	63.4	65.4	88.4	92.0
	19-105	5-180	4-341	7-351	12-318
HGB (g/l) median range	8.5	8.2	8.5	7.8	7.5
	7.0-11.8	4.4-12.6	4.4-19.1	2.7-12.0	2.7-11.4
CNS involvement	2	2	6	9	0
Extramedullary organs involvement	7	11	24	11	4
FAB types					
M0	0	1	3	0	1
M1	2	4	9	3	2
M2	2	7	11	5	4
M3	0	2	0	1	1
M4	0	1	5	0	0
M5	2	1	2	4	0
M6	0	0	1	0	0
M7	0	0	0	1	0
NA	1	0	1	0	0
Risk group					
SR	NA	11	11	4	2
IR	NA	NA	NA	4	2
HR	NA	5	21	6	4

AML NOPHO: Acute myeloid leukemia Nordic Society of Pediatric Hematology and Oncology; AML-BFM: acute myeloid leukemia-Berlin–Frankfurt–Munster; WBC: white blood cells; PLT: platelet count; HGB-hemoglobin; CNS: central nervous system; FAB: French–American–British; SR: standard risk; IR: intermediate risk; HR: high risk.

nasopharyngeal mass). High-risk group patients had fourfold higher risk of relapse (OR=4.35, 95%CI=1.22-15.44, p=0.023) compared to other patients, but no higher risk of death was observed (OR=1.04, 95%CI=0.36-2.99, p=0.934).

Achievement of remission. CR was achieved in 67 patients (87.0%). Failure to achieve CR after two cycles of induction chemotherapy was one of the risk factors of death (OR=60.0, 95%CI=3.37-1088.9, p=0.005).

Overall survival. Five-year OS was 62.1% for the entire group. For each protocol, 5-year OS was respectively 55.6% for AML-NOPHO88 protocol, 68.2% for ANLL98 protocol, 48.0% for BFM AML 2004 protocol, 85.7% for BFM AML 2012 protocol and 100.0% for BFM AML 2019 protocol (3 years of median follow up for BFM AML 2019 protocol). The results are shown in Figure 1A. There were no statistically significant differences between groups (p=0.416). Among 32 patients stratified into the HR group, 5-year OS rate in patients treated with HCT was 76.0% compared with 57.1% in patients treated without HCT. There was a significant difference between patients with primary AML and MDS transformation or other comorbidities in 5-year OS (65.5% vs. 42.4%, p=0.048). This was mainly due to the higher rate of death after disease progression in the second group (10.0% vs. 27.3%).

Event-free survival. Five-year EFS was 51.4% for the entire group. For each protocol, 5-year EFS was 45.7% for AML-NOPHO88 protocol, 62.5% for ANLL98 protocol, 28.7% for BFM AML 2004 protocol, 85.7% for BFM AML 2012 protocol, and 87.5% for BFM AML 2019 protocol. The results are shown in Figure 1B. The differences were statistically significant (p=0.007). Among patients in the HR group, 5-year EFS was 58.0% in those who underwent HCT compared with 16.7% in those treated without HCT (p=0.007).

Relapse-free survival. In patients who relapsed, the average time to relapse was 2.45 years (range=0.1-15.3 years). Incidence of relapse was an independent risk factor of death

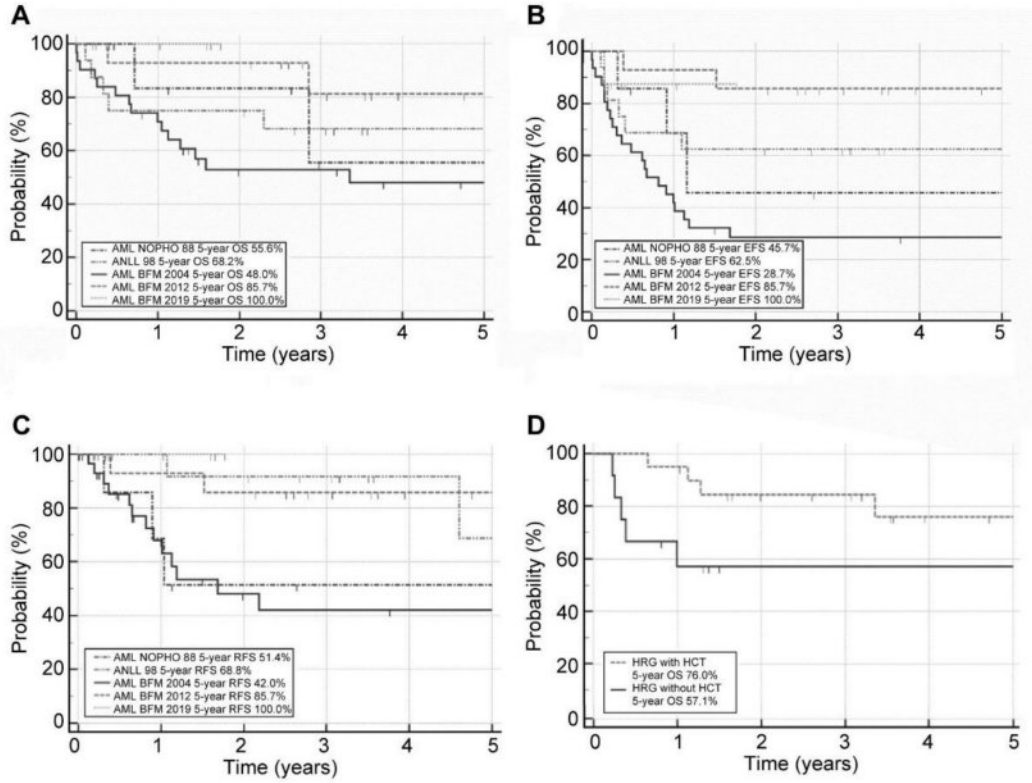

Figure 1. *Therapy outcomes for acute myeloid leukemia children treated between 1994-2022, according to 5 consecutive protocols: (A) Overall survival; (B) Event-free survival; (C) Relapse-free survival; (D) Overall survival of patients treated with chemotherapy+ hematopoietic cell transplantation (HCT) vs. chemotherapy without HCT.*

(OR=5.66, 95%CI=1.48-21.56, p=0.01) (Table III). Five-year RFS was 62.7% for the entire group. Regarding each protocol, 5-year RFS was 51.4% for AML-NOPHO88 protocol, 68.8% for ANLL98 protocol, 42.0% for BFM AML 2004 protocol, 85.7% for BFM AML 2012 protocol, and 100.0% for BFM AML 2019 protocol. The results are shown in Figure 1C. The differences were also statistically significant (p=0.01). CR2 was achieved in 14 patients (66.7%) and HCT in CR2 was performed in 13 relapsed patients. Five-year OS in patients who underwent HCT was 48.9% compared with 25.0% in children treated without HCT. The difference was of borderline significance (p=0.078).

Table III. *Independent risk factors of death.*

Risk factor	OR	95%CI	p-Value
Age at diagnosis	0.97	0.86-1.10	0.79
Sex	1.22	0.24-5.78	0.74
HR	0.71	0.14-3.55	0.67
HCT	0.94	0.17-5.22	0.95
Relapse	5.66	1.48-21.56	0.01

CI: Confidence interval; HR: high risk; HCT: hematopoietic cell transplantation.

Causes of death. In the entire observation period, 27 patients died (35.0%). Treatment-related mortality (TRM) was the most frequent cause of death (11 patients, 40.7%), among which 6 patients died from infections, 2 from treatment toxicity, 1 from graft *versus* host disease (GvHD) and 2 from other treatment-related complications. Nine patients died from disease progression and other 7 from relapse (3 in second relapse and 4 in third and further relapses). There was no impact of white blood cells (WBC), platelets (PLT) or hemoglobin (HGB) levels at diagnosis on the risk of death.

Discussion

Therapy results in children with AML have improved significantly over the last 3 decades, mostly due to the development of cytogenetics and molecular diagnostics, better supportive care, and introduction of HCT. The progress can be seen in international and nationwide reports (8). This study shows the results of a single oncology center experience in pediatric AML treatment and provides the opportunity to compare individual center results with international reports.

During the described period, OS improved from 55.6% between 1994 and 1998 to 87.5% from 2012 to early 2020. OS in patients treated since January 2020 remains 100.0%. In this group, observational period is short; however, it is worth emphasizing that there was no early death in patients treated by BFM AML 2019 protocol. OS in the majority of therapeutic protocols was slightly superior in our analysis compared to international results (55. 6% vs. 46.0% for AML-NOPHO 88, 68.2% vs. 50.0% for ANLL 98 and 85.7% vs. 82.0% for BFM AML 2012) (3, 5, 7). The only exception were the results of BFM AML 2004 protocol (48.0% vs. 74.0%), but also, in the nationwide analysis in Poland, OS was inferior in comparison to international results (3, 6). This was mostly due to the high rate of relapse and treatment-related mortality, including deaths after HCT. Because HCT accessibility in Poland was limited before 2000, poor OS in 2004-2012 could be the result of the unsatisfying results of transplantations during the first years of their performance.

AML is a heterogenous disease and the diagnosis relies predominantly on the identification of mutations in myeloid cells. Immunophenotyping and cytogenetics became available in Poland after 2005, and since then, most patients have been stratified according to immunophenotype and characteristic mutations in blast cells, which allows the adjustment of therapy in some patient group. It is worth noting, that the biggest progress in childhood AML treatment has been made in specific subgroups of patients, who received targeted therapy (AML M3, patients with Down syndrome). Unfortunately, therapeutic outcomes in some subgroups remain highly unsatisfying (secondary AML, patients with FTL3 mutation) (9, 10).

AML remains the most frequent cause of HCT performance (11). Despite the controversial role of HCT in first remission in non-HR patients, undoubtedly it has improved outcomes in this particular group (1, 12). In our analysis, HCT had relevant impact on therapy results in the HR group and almost doubled chances of survival in relapsed patients.

The obvious limitation of the study was its small group size. Childhood AML is a rare disease with an incidence of 5-8/1,000000, which leads to insufficient patient numbers treated in separated centers; therefore, international cooperation is necessary (13). Other limitations include its retrospective nature and lack of complete data (*e.g.*, uniformed genetic diagnosis before 2005) in some patients. However, the data and therapy results reflect the gradual progress in the diagnostic and therapeutic capability of oncology centers in Poland.

In conclusion, despite the fact that therapeutic results in pediatric patients with AML remain unsatisfying, we can observe significant progress, especially in some patients with well-defined mutations in blast cells and targeted therapy. Further research and international collaborations are needed to obtain a better understanding of the disease and improve outcomes.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Joanna Stankiewicz– data collection and interpretation, statistical analysis, writing the manuscript, Jan Styczyński - critical review, acceptance of final version for publication.

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ORIGINAL ARTICLE

Therapy results in pediatric Hodgkin lymphoma — does less mean better? Experience from a single children's oncology center

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Abstract
Therapy results in pediatric Hodgkin lymphoma reflect remarkable progress in pediatric oncology. In the last decade, relevant development of new therapeutic options for children with refractory or relapsed disease has been made. In this study, we retrospectively analyzed therapy results and risk factors in children treated in a single oncology center according to five therapeutic protocols. Data from 114 children treated by a single institution between 1997 and 2022 were analyzed. Classic Hodgkin lymphoma therapy results were divided into four therapeutic periods: 1997–2009, 2009–2014, 2014–2019, and 2019–2022. For nodular lymphocyte-predominant Hodgkin lymphoma, data from one therapeutic protocol was analyzed. For the entire group, the 5-year probability of overall survival was 93.5%. There were no statistically significant differences between therapeutic periods. The occurrence of B symptoms at diagnosis and incidence of relapse were risk factors for death ($p=0.018$ and $p<0.001$). Relapse occurred in 5 cases. The 5-year probability of relapse-free survival for the entire group was 95.2%, without significant differences between groups. Patients treated between 1997 and 2009 had over a sixfold higher risk for events, defined as primary progression, relapse, death, or incidence of secondary malignancies (OR = 6.25, $p=0.086$). The 5-year probability of event-free survival for all patients was 91.3%. Five patients died, and the most common cause of death was relapse. Modern therapeutic protocols in pediatric Hodgkin lymphoma are marked by excellent outcomes. Patients with disease relapses have a notably high risk of death, and the development of new therapeutic options for this group remains one of the main goals of current trials.

Keywords Hodgkin lymphoma · Children · Therapeutic era · Survival · Radiotherapy

Introduction

Hodgkin lymphoma (HL) represents about 5–7% of all malignancies in children, with an incidence of 5:100,000 per year in children up to 15 years old [1]. The etiology of the disease is not well understood, but there is growing evidence of an Epstein-Barr infection in the pathogenesis of HL. Moreover, patients with immunodeficiencies or on immunosuppressive therapy have an increased risk of HL [1, 2]. The pathomorphological classification divides HL into classical HL with four subtypes (nodular sclerosis, mixed cellularity, lymphocyte rich, and lymphocyte depleted) and nodular lymphocyte-predominant HL (nLPHL) type, which represent about 10% of all new cases. Treatment of HL consists of chemotherapy combined with radiotherapy in most cases. During the last few years, new therapeutic options for children with targeted agents and immunotherapy have been under investigation in clinical trials [2–4].

Therapy results in pediatric HL have made remarkable progress in the treatment of childhood malignancies. Whereas the 5-year probability of overall survival (pOS) exceeded 90% over 20 years ago, further efforts have focused on reducing side effects, especially the late ones [5, 6]. In

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1997, the Polish Pediatric Leukemia and Lymphoma Study Group introduced the first unitized therapy protocol for newly diagnosed children with HL in Poland. The aim of the protocol was not only to improve therapy results but also to minimize long-term complications [7]. Since 2007, pediatric oncology centers in Poland have been part of the international EuroNet-PHL trials that have focused on therapy individualization according to risk group stratification and treatment response [8].

In this study, we analyzed therapy outcomes in children with HL treated in a single oncology center in Poland over a period of 25 years.

Materials and methods

Design of the study

Retrospective analysis of therapy results and risk factors in children treated for HL in a single oncology center according to different therapy protocols.

Patients

In the study, data from children aged 3.5–20.8 years, treated by the Department of Pediatrics, Hematology and Oncology in Bydgoszcz during 1997–2022, were analyzed. The inclusion criteria involved children with a confirmed diagnosis of HL, both classical HL (nodular sclerosis, mixed cellularity, lymphocyte rich, and lymphocyte depletion subtypes) as well as nLPHL. Patients with incomplete data, as well as patients treated according to the EuroNet-PHL-C2 clinical trial (ClinicalTrials.gov identifier: NCT02797717), were excluded, as the results of that study have not been published yet.

Diagnosis

The diagnosis of HL was established according to pathology results. A detailed medical interview focusing on the B symptoms (unexplained fever, weight loss, and night sweats) was performed in each case. Complete blood count, lactate dehydrogenase (LDH) level, erythrocyte sedimentation rate (ESR), ultrasonography (USG) of the lymph nodes, liver, and spleen, and echocardiography (ECG) were performed in all cases. Bone marrow trephine biopsy was performed up to 2009. All patients also had computer tomography (CT) scans performed on the neck, chest, abdomen, and pelvis. Since 2009, whole-body positron emission tomography-CT (PET-CT) scans were performed.

Lymph nodes were considered involved in cases of enlargement above 2 cm between 1997 and 2009. Starting in 2009, all patients had a whole body PET-CT scan, and lymph nodes were defined as involved in cases with a diameter of 1–2 cm with a positive PET-CT or a diameter exceeding 2 cm independent of PET-CT results.

The lymph regions involved were scored based on the Ann Arbor classification in Cotswold modification [9].

Bulk was defined as present if the volume of the largest contiguous lymph node mass was ≥ 200 mL. Spleen involvement occurred when the spleen was PET-CT positive or multiple small focal changes were detected on USG. Lung involvement was diagnosed in cases of 2 or more lesions with a diameter of 2–10 mm or at least one lesion ≥ 10 mm in diameter. Liver involvement was defined as PET-CT positive changes or a minimum of one focal change detected with other imaging methods. Bone marrow involvement was defined as the presence of lymphoma cells in one marrow biopsy before 2009 and more than two PET-CT positive bone lesions after 2009. Bone involvement was detected based on CT scans of the bones. Extra-lymphatic structures or organs infiltrated per continuum were termed E-lesions.

Between 1997 and 2009, the total volume of involved lymph nodes was calculated, followed by the formula that lymph nodes of a diameter below 2 cm were equal to 1 unit, between 2 and 5 cm — 2 units, and above 5 cm — 3 units. In cases of mediastinal involvement, if the largest diameter of lymphoma mass was lower than 1/4 diameter of the chest, it was equal to 1 unit, 1/4–1/3 of the chest was equal to 2 units, and more than 1/3 of the chest — 3 units. In cases of bilateral mediastinal involvement, the unit numbers doubled [7].

Therapy protocols and risk group stratification

Children have been treated according to consecutive therapeutic protocols: PGP-HD-97 from January 1997 to June 2009 [7]; EuroNet-PHL-C1 from July 2009 to February 2014 [8]; EuroNet-PHL-Interim Phase from March 2014 to September 2019, and EuroNet-PHL-C2 from October 2019 [10]. Patients diagnosed with nLPHL were treated according to the Euro-Net-PHL-LP1 protocol (ClinicalTrials.gov identifier: NCT01088750).

Protocol schemes are shown in Fig. 1.

During the PGP-HD-97 therapeutic period, patients were stratified into 4 risk groups, according to lymph node regions involved and the presence of B symptoms, defined as below:

- Group 1 — Stages IA and IB (with the exception of the lymphocyte depletion subtype).
- Group 2 — Stage IIA in children ≤ 10 years old and a total lymph node mass below 8 units.
- Group 3 — Stage IIA in children > 10 years old and total lymph node mass 8 units and above or stages IIB and IIIA (both with the exception of the lymphocyte depletion subtype).

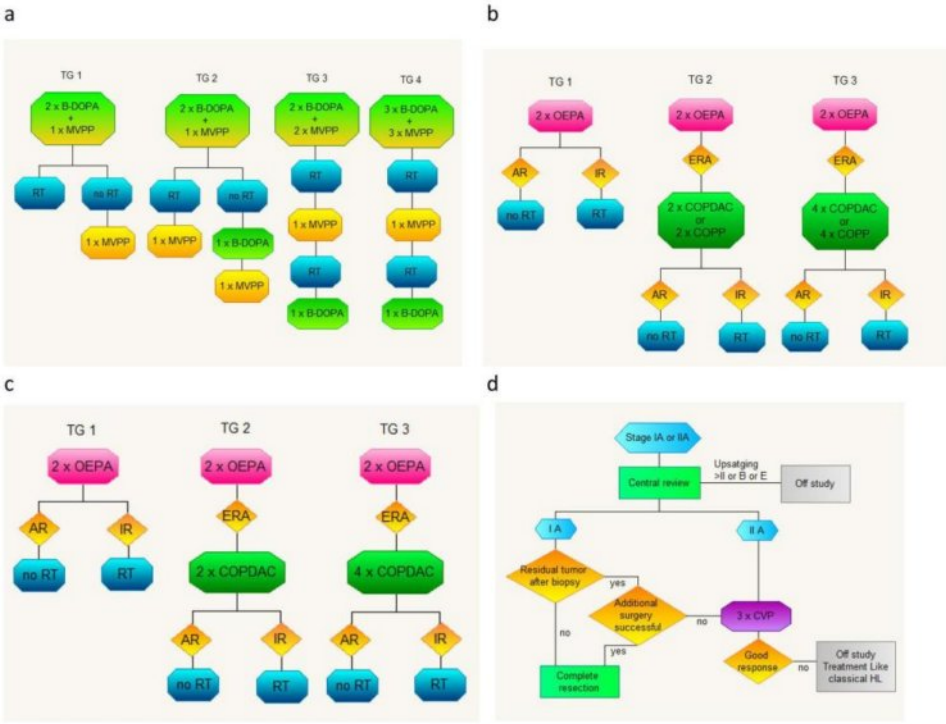

Fig. 1 Schemas of four consecutive protocols analyzed in the study. **a** PGP-HD-97 protocol; **b** EuroNet-PHL-C1 protocol; **c** EuroNet-PHL-Interim protocol; **d** Euro-Net-PHL-LP1 protocol; TG 1 — therapeutic group 1; TG 2 — therapeutic group 2; TG 3 — therapeutic group 3; TG 4 — therapeutic group 4; B-DOPA — bleomycin, dacarbazine, vincristine, doxorubicin, prednisone; MVPP – nitrogranulogen, vinblastine, procarbazine, prednisone; RT — radiotherapy; OEPA — vincristine, etoposide, prednisone, doxorubicin; AR — adequate response; IR — inadequate response; ERA — early response assessment; COPP — cyclophosphamide, vincristine, procarbazine, prednisone; COPDAC — cyclophosphamide, vincristine, prednisone, dacarbazine; DECOPDAC — dacarbazine, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone; B — B symptoms, E — E-lesions; CVP — cyclophosphamide, vinblastine, prednisone

– Group 4 — Stages IIIB and IV, and all stages of lymphocyte depletion subtype.

According to EuroNet-PHL-C1, EuroNet-PHL-interim phase, and EuroNet-PHL-C2 patients were stratified into 3 therapeutic groups (TG):

– TG1 — Stages IA and IIA without risk factors defined as ESR ≥ 30, missing ESR, or occurrence of bulk.
– TG2 — Stages IA and IIA with at least one risk factor or stage IIB and IIIA without E-lesions.
– TG3 — Stages IIB and IIIA with E-lesions, or stages IIIB or IV.

Euro-Net-PHL-LP1 was dedicated to children with nLPHL in stages IA and IIA, and it did not define specific therapeutic groups. The protocol offered two paths of treatment, one for stage IA and a second one for stage IIA.

Response criteria

Response to initial therapy was evaluated after 3 cycles of chemotherapy during the PGP-HD-97 protocol and was defined as adequate in cases of at least 70% reduction of the primary lymphoma mass on CT.

In EuroNet PHL-C1, EuroNet-PHL-Interim Phase, and EuroNet PHL-C2 trials, the response was evaluated after 2 cycles of chemotherapy using CT or MRI and fluorodeoxyglucose (FDG)-PET scan results. The response was defined as adequate, if the response assessment PET was negative or the residual tumor volume was less than or equal to 5% of the reference volume and less than or equal to 2 ml, or all disease symptoms disappeared and the response assessment PET was unclear.

During Euro-Net-PHL-LP1, the response was assessed after 3 cycles of chemotherapy in stage IIA or just after initial surgery in stage IA according to CT or MRI and

FDG-PET scan results. Complete remission was defined as the disappearance of all disease symptoms, non-measurable tumor volumes in all initially involved regions, and negative results on the response assessment PET scans.

Definitions

Progression was defined as the recurrence or occurrence of new disease symptoms which could not be explained otherwise, the occurrence of new lymphatic or extra-lymphatic lesions, or at least one initially involved extra-nodal site or region with non-measurable tumor volume was locally progressive during therapy or within 3 months after the end of therapy. Relapse was defined as the occurrence of new lymphatic or extra-lymphatic lesions or if at least one initially involved an extra-nodal site or involved a region with a non-measurable tumor volume that progressed 3 months after the end of therapy or further. An event was defined as primary progression, relapse, death of any causes, or incidence of secondary malignancies. Overall survival was defined as the time from HL diagnosis until death of any cause or end of follow-up. Relapse-free survival (RFS) was defined as the time from HL diagnosis until a relapse occurred. Event-free survival (EFS) was defined as the time from diagnosis to event occurrence.

Statistical analysis

The endpoints of the study included a 5-year pOS, a 5-year probability of EFS (pEFS), and a 5-year probability of RFS (pRFS). Survival curves of pOS, pEFS, and pRFS were calculated using the Kaplan–Meier method and compared by log-rank tests. All clinical, laboratory, and imaging data were analyzed in terms of their impact on outcomes. Univariate analysis was used to assess prognostic factors. The significant factors from univariate analysis were used in the multivariate regression model. Data were considered statistically significant when the p value was < 0.05. All calculations were performed using the statistical software MedCalc 20.100 (MedCalc Software, Mariakerke, Belgium). The study was approved (KB 577/2021) by the Ethics Committee of Collegium Medicum, Nicolas Copernicus University, Bydgoszcz.

Results

A total of 123 patients were treated for HL between April 1997 and March 2022. Five patients were excluded because of insufficient or missing data and another four were excluded because they were part of the EuroNet-PHL-C2 clinical trial. Data from 114 children were analyzed. Fifty-four of them (47.4%) were male and 60 (52.6%) were female (M/F 1:1.1). The mean age at diagnosis was 14.1 years and the median age was 15.3 years. Over 90% of patients had more than 10 years at diagnosis. The most common manifestation of the disease was a mediastinal mass (89 patients, 78.1%), followed by cervical lymphadenopathy (87 patients, 76.3%) and supraclavicular lymph node enlargement (84 patients, 73.7%). All three sites were involved in over half of the cases ($n = 61$, 53.4%). B symptoms occurred in 52 children (45.6%). E-lesions were observed in 7 cases (6.1%), bulky disease in 24 (21.1%), 18 patients (15.8%) had lung involvement, and another 12 (10.5%) had skeletal involvement.

One hundred and eleven patients were diagnosed with classical HL and three (2.6%) with nLPHL. In the group of classical HL, the most frequent histological subtype was nodular sclerosis ($n = 69$, 62.1%), followed by mixed cellularity ($n = 18$, 16.2%). Two patients had the nodular predominant subtype and another two lymphocyte rich (1.8% in each case). The characteristics of patients from consecutive therapeutic protocols are shown in Table 1.

Therapy results

Adequate response after initial therapy was observed in 62.9% of patients and for the consecutive protocols a total of 67.7% for PGP-HD-97, 53.3% for EuroNet-PHL-C1, 48.1% for EuroNet-PHL-Interim, and 60.0% for EuroNet-PHL-C2. Patients with inadequate responses to initial therapy had almost a fivefold higher risk of death, but the differences were not statistically significant (OR = 4.68, 95% CI 0.50 to 43.12, $p = 0.172$). Fifty-eight patients (50.8%) were treated with radiotherapy.

The 5-year pOS for the entire group was 93.5%. For each protocol, the 5-year pOS was 87.0% for PGP-HD-97, 100.0% for EuroNet-PHL-C1, 96.3% for EuroNet-PHL-Interim, 100.0% for EuroNet-PHL-C2, and 100.0% for EuroNet-PHL-LP1. The results are shown in Fig. 2. The differences between protocols were not statistically significant ($p = 0.602$). Risk factors related to poor outcomes was the occurrence of B symptoms (5-year pOS 86.5% vs. 93.5%, $p = 0.018$). Patients treated according to PGP-HD-97 had an 11-fold higher risk of death compared to the others (OR 11.18, 95% CI 0.62–200.31, $p = 0.080$). Outcomes for the consecutive therapeutic protocols are shown in Table 2.

Relapse occurred in 5 cases with a median time to relapse of 0.86 years. In five patients with relapse, second-line therapy consisted of chemotherapy with autologous hematopoietic cell transplantation (auto-HCT) in two patients, chemotherapy only in one patient, and chemotherapy and radiotherapy in one patient. One patient was treated according to the EuroNet-PHL-C2 protocol after a 2020 relapse due to early therapy termination because of

Table 1 The characteristics of patients from consecutive therapeutic protocols

	PGP-HD-97	EuroNet-PHL-C1	EuroNet-PHL-Interim Phase	EuroNet-PHL-C2	Euro-Net-PHL-LP1
Number of patients	51	17	27	14	3
Gender					
Male	25	8	10	5	3
Female	26	9	17	9	0
Age (years) mean range	14.1 4.1–18.8	14.4 3.9–20.8	13.7 3.5–17.7	14.2 5.7–18.2	12.6 6.8–15.7
WBC (× 10^3) mean range	10.2 2.4–33.0	11.5 6.5–21.7	10.5 4.2–18.8	11.2 4.9–27.1	8.7 7.4–9.8
PLT (× 10^3) mean range	377 153–972	349 67–646	354 178–541	424 163–792	304 222–366
HGB (g/l) mean range	11.8 8.7–16.3	11.9 8.8–16.0	11.8 8.2–14.1	11.7 7.6–15.3	13.9 13.2–14.9
ESR mean range	49 2–135	38 1–83	46 11–140	46 4–105	7 2–16
B symptoms					
Fever	7	3	8	3	0
Weight loss	17	7	3	4	0
Night sweats	10	4	10	3	0
Lymph nodes regions involvement – median range	4 1–9	5 2–7	3 1–9	5 2–7	2 1–2
Liver involvement	6	2	2	2	0
Spleen involvement	11	2	9	4	0
Skeletal involvement	3	3	5	3	0
Lung involvement	5	4	5	5	0
Bulky disease	5	2	9	3	0
E-lesions	1	6	7	0	0
Stage at diagnosis					
I	3	0	0	0	1
II	26	10	11	6	2
III	12	1	8	2	0
IV	10	6	8	6	0

WBC White blood cells, *PLT* platelet count, *HGB* hemoglobin

treatment toxicity. In this case, the first chemotherapy cycle was complicated with *Clostridium perfringens* sepsis that required treatment in the intensive care department. After he recovered from sepsis, the treatment was continued, but after a few days, he presented severe bleeding from the gastrointestinal tract, which was complicated by sudden cardiac arrest with successful resuscitation. Because of the severe adverse effects of chemotherapy, he was switched to second-line therapy with brentuximab vedotin (BV) in combination with nivolumab. He is still completing therapy with good clinical and radiological responses. Three patients died after relapse. The 5-year pRFS for the entire group was 95.2%. For each protocol, the 5-year pRFS was 92.1% for PGP-HD-97, 100.0% for EuroNet-PHL-C1, 100.0% for EuroNet-PHL-Interim, 87.5% for EuroNet-PHL-C2, and 100.0% for Euro-Net-PHL-LP1. The results are shown in Fig. 3. There were no statistically significant differences between protocols and risk groups ($p=0.277$, $p=0.763$). Platelets count above $450\times10^{\wedge}9$/L at diagnosis was an independent risk factor for relapse ($p=0.013$). The incidence of relapse was the most important risk factor for death ($p<0.001$).

The 5-year pEFS for all patients was 91.3%. For each protocol, the 5-year pEFS was 88.2% for PGP-HD-97, 94.4% for EuroNet-PHL-C1, 96.3% for EuroNet-PHL-Interim, 87.5% for EuroNet-PHL-C2, and 100.0% for Euro-Net-PHL-LP1, without statistically significant differences between protocols ($p=0.721$). The results are shown in Fig. 4. There were no differences in event occurrences between risk groups ($p=0.907$). However, therapy with the PGP-HD-97 protocol was related to considerably poorer outcomes with an over sixfold higher risk of events (OR = 6.25, 95% CI 0.76–50.84, $p=0.086$). Primary progression occurred in two patients,

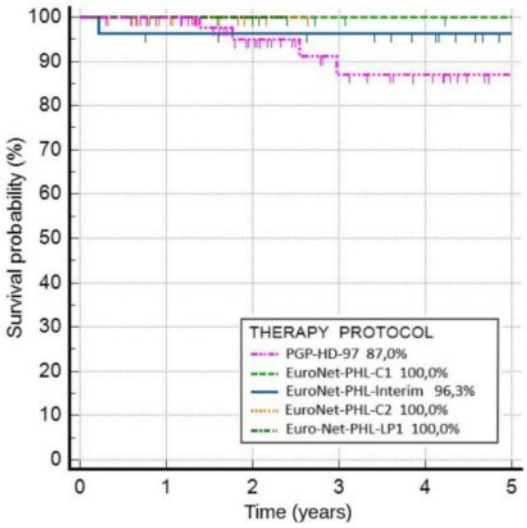

Fig. 2 Five-year pOS for children treated because of HL between 1997 and 2022 according to 5 consecutive protocols

and both of them were successfully treated with second-line therapy (chemotherapy only in one case, chemotherapy with auto-HCT in the other). Secondary malignancy occurred in one girl treated primarily with chemotherapy and radiotherapy, who was diagnosed with acute myelocytic leukemia 0.6 years after she had finished HL treatment.

Death

During the entire analysis period, 5 patients died. The mean time from diagnosis to death was 1.54 years (median 1.57 years, range 0.22 to 2.97 years). Two patients died of relapse and one of a secondary malignancy. Among patients who died of a relapse, the second-line therapy differed significantly: the first patient was treated with etoposide, ifosfamide, and prednisone (IEP) and vindesine, lomustine, and melphalan (CAD) chemotherapy combined with radiotherapy and the second one with IEP and ifosfamide, vinorelbine, gemcitabine, and prednisone (IGEV) chemotherapy followed by auto-HCT, and the third one with IEP and doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) chemotherapy followed by auto-HCT. There was also one case of treatment-related mortality because of an infection during chemotherapy. The most important risk factor for death was the incidence of relapse ($p < 0.001$).

Discussion

During the last 30 years, treatment results in pediatric HL have improved significantly [1]. Despite great progress in cure rates, side effects of therapy remain a relevant problem. Long-term observations have proved patients treated in childhood for HL are at risk of long-term complications, including gonadotoxicity, cardiovascular complications, thyroid dysfunctions, and secondary malignancies [11]. One of the milestones of the current therapeutic approach is to reduce radiotherapy in patients who can be treated satisfactorily with chemotherapy alone.

In Poland, the PGP-HD-97 protocol was the first protocol to omit radiotherapy in specific groups of patients [7]. The aim of radiotherapy exclusion was to protect children from the late side effects, especially cardiotoxicity, as well as thyroid and lung damage [7]. In a nationwide analysis, the 5-year pOS, pEFS, and pRFS were 92%, 96%, and 97%, respectively [7]. Results from our center were slightly inferior to the national rates, mostly due to a high rate of death due to relapse, progression, and treatment-related toxicities. Although there were no statistically significant differences in the outcomes between individual protocols, patients treated according to the PGP-HD-97 protocol had a higher risk of events compared to patients treated according to the EuroNet-PHL trials. Moreover, the number of patients with late therapy complications remained high, despite the decreased number of children who received radiotherapy during treatment [12].

Table 2 Outcomes of five consecutive therapeutic protocols in the analyzed group

Outcome	PGP-HD-97	EuroNet-PHL-C1	EuroNet-PHL-interim phase	EuroNet-PHL-C2	Euro-Net-PHL-LP1
Primary progression	1	1	0	0	0
Relapse	4	0	0	1	0
Secondary malignancy	1	0	0	0	0
Death	4	0	1	0	0
Reason of death					
Relapse	3	0	0	0	0
Treatment-related mortality	0	0	1	0	0
Secondary malignancy	1	0	0	0	0

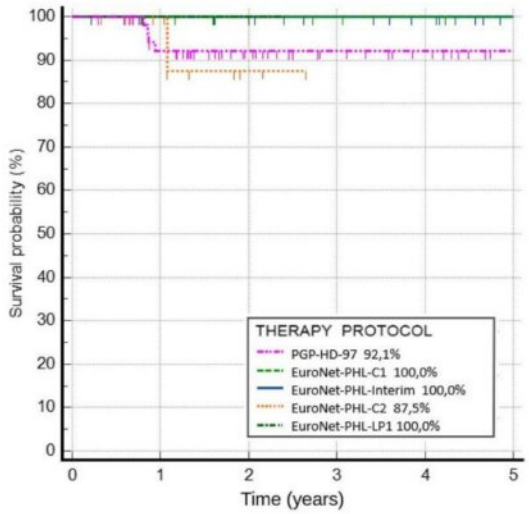

Fig. 3 Five-year pRFS for children treated because of HL between 1997 and 2022 according to 5 consecutive protocols

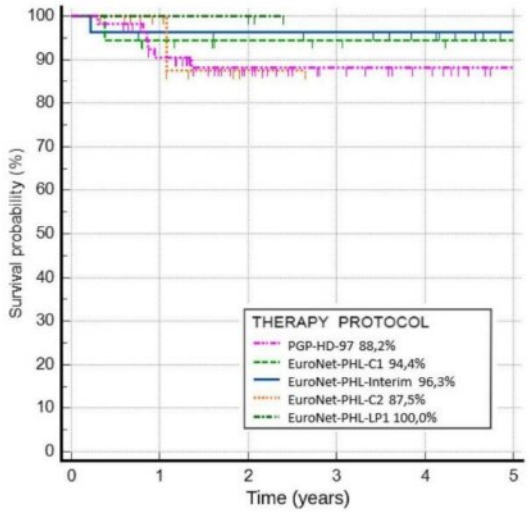

Fig. 4 Five-year pEFS for children treated because of HL between 1997 and 2022 according to 5 consecutive protocols

Encouraging results from the Multicenter Trial GPOH-HD 95 and GPOH-HD-2002 Study resulted in the multinational EuroNet-PHL-C1 randomized trial [8, 13, 14]. This study started in January 2007 and was introduced in our department in July 2009. The treatment was based on response-adapted therapy, measured with PET with radiotherapy omission in patients who had an adequate response to the induction. The second objective of the trial was to replace procarbazine with dacarbazine during consolidation to reduce gonadotoxicity. Results of the EuroNet-PHL-C1 protocol in the analyzed group were excellent, with a 5-year pOS of 100.0% and a 5-year pEFS of 96.3%. During this therapeutic period, there was only one case of primary progressive disease, successfully treated with second-line therapy. The followed therapeutic EuroNet protocols have shown similar satisfying results in our cohort.

In adult patients, the role of FDG-PET in the response assessment is well established [15]. It has also been proved that the use of FDG-PET in an initial evaluation and response assessment has better specificity than CT alone for baseline measurements and relapse predictions [16]. The introduction of PET-based response-adapted therapy in children can help in the identification of patients with inadequate responses to initial therapy, who may benefit from additional cycles of chemotherapy or radiotherapy. It can also identify patients with a good initial response and low risk of relapse, who can be treated without radiotherapy and avoid potentially life-limiting late effects [8].

The stage at diagnosis is an important prognostic factor in HL. It was used for stratification in the EuroNet trials as well as the previous HD-97 therapeutic protocol. Additionally, in EuroNet protocols, the occurrence of B symptoms, ESR equal to or above 30 mm/h, and bulky disease were considered risk factors and stratified patients into a higher risk group [8]. In our analysis, the occurrence of B symptoms was related to a significantly lower pOS, despite being used in therapeutic group stratification. Platelet counts above $450 \times 10^{\wedge}3/\mu l$ at diagnosis were another risk factor related to relapse. Similar observations were published by other authors [17].

The incidence of relapse was the strongest risk factor for death. Because of the long period of analysis, the therapy for relapsed disease differed widely between individual patients. From the year 2002, auto-HCT became available for relapse treatment in our patients. Although auto-HCT is the standard treatment for relapsed adult patients, there is a lack of randomized trials in children that proves the superiority of high-dose chemotherapy (HDC) with auto-HCT versus standard-dose chemotherapy (SDC) [18, 19]. Furthermore, the evidence for a survival benefit in children treated with HDC with auto-HCT is limited [19–21]. According to the EuroNet Pediatric Hodgkin Lymphoma Group's recommendations, HDC with auto-HCT should be limited to standard and high-risk groups, while children in the low-risk group at relapse should be treated with SDC and radiotherapy [19]. Targeted agents and immunotherapy play an emerging role in relapse treatment, but their place in the treatment of relapsed/refractory HL in the pediatric population is not well specified [4, 18, 19, 22]. In our analysis, one patient was treated with BV combined with nivolumab, as his parents did not consent

to chemotherapy and auto-HCT for second-line treatment. He had a complete metabolic response after 4 cycles of BV and nivolumab infusions. Although the results from trials with BV and nivolumab as second-line therapy in adults are promising, we need more information about its efficacy in children [3, 23].nLPHL represents a small subset of all HL cases in children [2]. Children with nLPHL are usually diagnosed in the early stages of the disease (IA or IIA) and have excellent outcomes with a pOS of approximately 100% [24]. Because of the indolent character of nLPHL, the therapeutic approach differs from the one used in classical HL. First-line therapy for patients with stage IA is surgery only, with further observation for patients with complete resection [2, 24]. According to the Euro-Net-PHL-LP1 protocol, 3 cycles of chemotherapy with cyclophosphamide, vincristine, and prednisone (CVP) is recommended for patients with stage IIA. In the analyzed group, there were 3 cases of nLPHL, two with stage IIA, and one with stage IA. All of them were treated with CVP chemotherapy (the patient with stage IA was because of incomplete initial resection), with a 5-year pOS, pEFS, and pRFS of 100%. These results were superior to the national analysis of the Polish Pediatric Leukemia/Lymphoma Study Group, where patients with IA and IIA stages were characterized with a 5-year pOS of 100% and a 5-year pRFS of 86.7% [25]. The prognosis for patients with stages III and IV is considerably worse than for the early stages of nLPHL, with particular concern for transformation to an aggressive, diffuse large B cell lymphoma [2].

An obvious limitation of our study is a very heterogeneous group of patients who were treated during a period of over 20 years. Due to the retrospective design, the long period of time considered during which the therapeutic protocols for pediatric HL evolved, the evaluation of disease extension at diagnosis was made in different manners, and the evaluation of response was made with different timing, methods, and criteria. Despite the effect of different therapeutic protocols, progress in supportive care as well as the development of new diagnostic tools has undoubtedly significantly impacted the outcomes. However, there were a relatively large number of cases, and despite the long period of analysis, most of the important information about clinical, laboratory, and radiological results were available. A large multicenter analysis is needed for further conclusions. We eagerly await the results of the EuroNet-PHL-C2 clinical trial.

Conclusion

In conclusion, progress in the treatment of childhood HL is visible in both international as well as single-institution analyses. Despite some obvious limitations of our study, our analysis shows that modern therapeutic protocols are marked by excellent outcomes. Currently, the greatest emphasis is put on toxicity reduction, with radiotherapy omission as one of the core goals of new trials. Furthermore, targeted therapy and immunotherapy remain emerging therapeutic options, especially for children with relapsed or progressive disease as a group with unfavorable prognosis. Further investigation with multicenter studies is needed to confirm the initially satisfying results.

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Data Availability Data used in the article is available from the corresponding author in case of reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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Toward Inclusive Oncology: Challenges in the Therapy of Pediatric Non-B Non-Hodgkin Lymphomas

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Abstract. *Background/Aim: Non-B non-Hodgkin lymphomas (NHL) represent over 30 T/NK lymphoma types. The majority of them are T-cell lymphoblastic lymphomas (TLL) and anaplastic large cell lymphomas (ALCL). Other rare non-B NHLs represent a diverse group of neoplasms, usually excluded from clinical trials. This study analyzed outcomes in pediatric patients with non-B NHL in a single oncology center with particular emphasis on patients with rare NHLs. Patients and Methods: We retrospectively analyzed data from patients <18 years with newly diagnosed non-B NHL treated at the Department of Pediatric Hematology and Oncology in Bydgoszcz between 2002 and 2022. The probability of 5-year overall survival (pOS) and event-free survival (pEFS) were calculated for the entire cohort and patients with TLL and ALCL. The clinical course for patients with rare non-B NHL was described in detail. Results: Twenty-six children were eligible for analysis. Fourteen patients were diagnosed with ALCL, nine with TLL, and three with rare NHL types (subcutaneous panniculitis-like T-cell lymphoma, extranodal NK/T-cell lymphoma and hydroa vacciniforme-like lymphoproliferative disease associated lymphoma). For the entire group, the 5-year pOS was 83.7% and the 5-year pEFS was 72.4%. For TLL and ALCL, the outcomes were comparable with those achieved in clinical trials. Patients with rare NHL were treated according to individualized therapy recommendations based on physicians' expertise and available case report descriptions. Conclusion: There is a lack of knowledge on optimal therapeutic strategies for rare NHLs. It is crucial to create trials dedicated to uncommon NHLs and establish therapy guidelines for these patients.*

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Non-Hodgkin lymphomas (NHL) represent a heterogeneous group of lymphoid malignancies. According to the fifth edition of the World Health Organization Classification, non-B NHL comprises over 30 T/NK lymphoid proliferation and lymphoma types, many with subtypes (1). However, in the pediatric population, approximately 90% of patients with non-B NHL are diagnosed with T-cell lymphoblastic lymphomas (TLL) and anaplastic large cell lymphomas (ALCL) (2, 3). The remaining 10% represent a diverse group of neoplasms with very low incidences, characterized by limited understanding of their pathogenesis and biology. This has resulted in a lack of standardized diagnostic and therapeutic approaches, as well as insufficient knowledge of long-term outcomes (4, 5).

In the Children's Oncology Group's (COG) 2023 research blueprint, the primary goal of the COG Committee was to provide optimal cures for all children with NHL (6). Currently, for rare peripheral T-cell NHL, the 5-year event-free survival (EFS) ranges from 47±7% to 61±11% and the 5-year overall survival (OS) ranges from 56±7% to 65±11% (7, 8). Priorities in NHL therapy include developing clinical trials for rare NHL types and expanding studies on their biology to facilitate targeted and personalized therapies (5, 6, 9). Although further studies are necessary, adhering to specific clinical protocols can be challenging, particularly for oncology centers with limited funding or those located in developing countries.

In this study we retrospectively analyze outcomes in pediatric patients diagnosed with non-B NHL in a single

oncology center with particular emphasis on the challenges and opportunities, that pediatric oncologists face in therapy of children with rare tumors.

Patients and Methods

Design of the study. In this study, we retrospectively analyzed outcomes for pediatric non-B NHL patients, who received treatment at a single oncology center in Poland between 2002 and 2022, with particular emphasis on patients with rare types of lymphomas. The study was approved by Ethics Committee of Collegium Medicum, Nicolas Copernicus University, Bydgoszcz (KB 577/2021).

Patients. The medical records of patients diagnosed with non-B NHL at the Department of Pediatric Hematology and Oncology in Bydgoszcz were analyzed. Patients <18 years with newly diagnosed non-B NHL were eligible for the study. Children with missing or incomplete data were excluded from the analysis.

Diagnosis. The diagnosis was established on the basis of histological analysis from a tissue sample and confirmed by the national reference laboratory. Staging workup included medical interview information, laboratory test results (complete blood count, lactate dehydrogenase (LDH) activity, cerebrospinal fluid (CSF) analysis) and imaging test results (ultrasonography of the abdomen and peripheral lymph nodes, computed tomography (CT) of the neck, thorax, abdomen and pelvis).

Definitions. Progression was defined as reappearance or increase in size of residual lesions or appearance of new manifestations during treatment or up to three months after completion of the treatment. Relapse was defined as reappearance or appearance of new manifestations more than three months after therapy completion. Bone marrow progression/relapse was diagnosed in case of >25% lymphoma cells in the bone marrow. Central nervous system (CNS) progression/relapse was diagnosed, if lymphoma cells were present in the CNS with a cell count of ≥5/μl or in case of appearance of an intra-cerebral tumor. The diagnosis of relapse and progression were proved by biopsy and histology.

An event was defined as death due to any cause, progression, relapse, or secondary malignancy. Overall survival was calculated as the time in years from the first day of treatment to death of any cause or last follow-up contact for alive patients. Event free survival was calculated as the time in years from the first day of treatment to the event.

Statistical analysis and language corrections. The probability of OS (pOS) and the probability of EFS (pEFS) were calculated using the Kaplan–Meier method and compared using the log-rank test. Statistical calculations were performed using MedCalc 20.100 software (MedCalc Software, Mariakerke, Belgium). The language was corrected using the TRINKA grammar checker on the www.trinka.ai website.

Results

Demographics. During the analyzed period, 32 pediatric patients were treated for non-B NHL. Six children were excluded from the analysis because of incomplete or missing data. The median age at diagnosis was 11.0 years (range=1.3-17.8 years). One patient was diagnosed with immunodeficiency before NHL diagnosis, and none was diagnosed with cancer predisposition syndrome. Patient characteristic is shown in Table I.

Table I. *Patients characteristics at diagnosis.*

	Whole group	ALCL	TLL
Number of patients	26	9	14
Sex			
Male	15	5	8
Female	11	4	6
Age (years), median	11.4	11.0	10.6
Range	1.3-17.6	8.3-17.6	1.3-16.7
WBC (1,000/μl), median	6.67	5.95	6.67
Range	2.03-21.75	3.80-12.91	2.40-21.75
HGB (g/dl) median	11.8	10.5	12.1
Range	6.5-15.3	9.9-15.3	9.8-13.6
PLT (1,000/μl), median	297	342	266
Range	3-781	3-721	86-781
LDH (IU/l), median	325	270	325
Range	155-3,313	155-1,013	171-3,313
B symptoms			
Fever	4	2	2
Weight loss	2	1	1
Night sweats	3	2	1
Primary sites involvement			
Lymph nodes	24	9	14
Mediastinal	17	5	12
Lung	2	1	1
Skin	3	1	0
Skeletal	2	1	1
BM	4	1	3
CNS	0	0	0

WBC: White blood cells; HGB: hemoglobin; PLT: platelets; LDH: lactate dehydrogenase; BM: bone marrow; CNS: central nervous system; TLL: T-cell lymphoblastic lymphoma; ALCL: anaplastic large cell lymphoma.

Diagnosis and therapy. In the analyzed cohort, the following histological types were diagnosed: TLL (n=14), ALCL (n=9), subcutaneous panniculitis-like T-cell lymphoma (SPTCL) (n=1), extranodal NK/T-cell lymphoma (ENKL) (n=1), and hydroa vacciniforme-like lymphoproliferative disease associated lymphoma (HV-LPDL) (n=1).

Patients treated for TLL and ALCL received therapy according to standardized therapy protocols. Thirteen patients diagnosed with TLL were treated according to the EURO-LB02 protocol and one with the LBL 2018 protocol (10). All patients diagnosed with ALCL were treated according to the ALCL99 protocol (11). Among the analyzed group three patients were diagnosed with rare types of NHL (SPTCL, ENKL and HV-LPDL).

A girl diagnosed with SPTCL was treated with six cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)

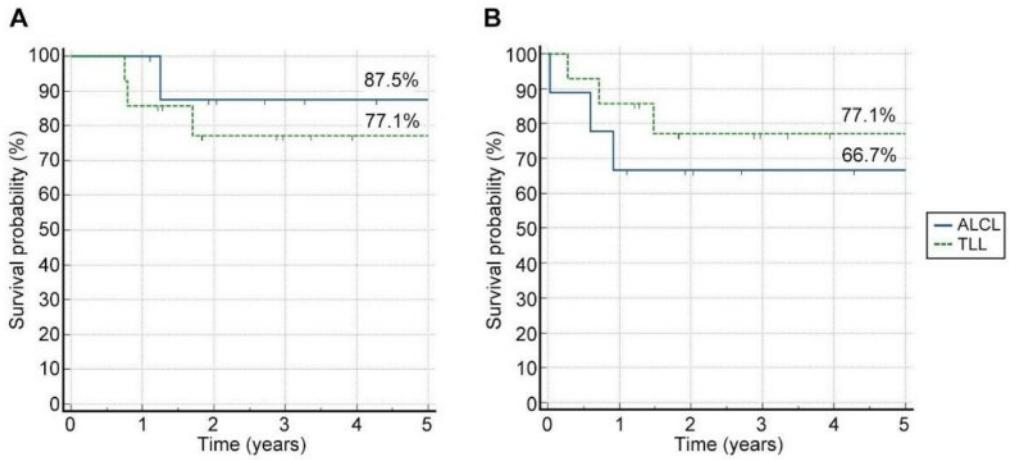

Figure 1. *Five-year probability of overall survival and probability of event-free survival for non-Hodgkin lymphoma subtypes. A) The 5-year probability of overall survival for T-cell lymphoblastic lymphoma (TLL) and anaplastic large cell lymphoma (ALCL); B) The 5-year probability of event-free survival for TLL and ALCL.*

chemotherapy. She presented with laboratory and clinical symptoms of hemophagocytic lymphohistiocytosis at diagnosis, which resolved after the first treatment course. She presented also a significant decrease of subcutaneous lymphoma lesions after the two first cycles of chemotherapy and a complete remission after the fourth cycle. She received two additional consolidation cycles of CHOP regimens and has remained free of disease during the 16-year follow-up.

A boy diagnosed with ENKL presented with lymphoma lesions in the nasal structures, skin of the cheeks, and upper lip at diagnosis. He underwent four cycles of CHOP therapy, resulting in a significant reduction in tumor lesions. The boy did not agree to local radiotherapy as a consolidation therapy; therefore, he received bexarotene as a maintenance therapy. After the completion of maintenance treatment, magnetic resonance imaging (MRI) results showed enhancement at the primary involved sites. He received an additional three cycles of CHOP chemotherapy, achieving complete remission as confirmed by positron emission tomography scan results. However, 12 months post-therapy completion, he was diagnosed with relapse and died nine months later due to disease progression.

A case of a boy with common variable immunodeficiency complicated by systemic lymphoma, developed from hydroa vacciniforme-like lymphoproliferative disorder was described previously (12). In this case, systemic chemotherapy combined with antiviral drugs was unsuccessful. Finally, he received treatment with third-party donor Epstein Barr virus-specific cytotoxic T-cells (tabelecleucel), followed by allogenic hematopoietic stem cell transplantation (HSCT). He remains in lymphoma remission up to the date of the article publication.

Outcomes. For the entire group, the 5-year pOS was 83.7% and the 5-year pEFS was 72.4%. For TLL and ALCL, the 5-year pOS and pEFS were comparable to those achieved in clinical trials (Figure 1) (10, 11).

Three patients presented with progression during the frontline therapy. Among them one was diagnosed with ALCL and two with TLL. The patient with progressed ALCL was successfully treated with a second cycle of induction therapy according to the ALCL99 protocol. Two patients with progressed TLL were treated with salvage chemotherapy, but both subsequently died due to disease progression.

Three patients were diagnosed with relapse. The patient with relapsed ENKL was described above. Another two patients with relapsed disease were primarily diagnosed with ALCL. Both presented with relapse in peripheral lymph nodes within the first year after completion of frontline therapy. The first patient, diagnosed in 2005, received salvage chemotherapy. However, the treatment was unsuccessful, and he died from disease progression. The second patient with relapsed ALCL, treated in 2020, received salvage chemotherapy combined with an anti-CD30 antibody-drug conjugate brentuximab vedotin (BV), followed by allogenic hematopoietic stem cell transplantation (allo-HSCT). He has remained free of disease up to the date of the article publication. Five patients died, and the most common reason for death was disease progression (n=4, 80.0%). One patient died because of treatment toxicity (infection).

Discussion

This study is a comprehensive, single-center analysis of therapeutic outcomes in pediatric patients with non-B-cell non-Hodgkin lymphomas. Although the analysis includes relatively small number of patients, it reflects the challenges in the treatment of children with non-B NHL. According to the Polish Central Statistical Office's annual report from 2021, there are approximately 1100 new cases of pediatric cancer diagnosed in Poland each year (13). Non-Hodgkin lymphomas represent 7% of them, with an annual incidence 0.9 per 100,000 children, what makes the whole group of NHL an ultrarare disease (13, 14). In our department, over a period of 20 years, only 32 patients were diagnosed with non-B NHL, which gives a number of little higher than 1.5 patient per year in a region inhabited by 2.09 million people (15).

TLL and ALCL constitute the vast majority of pediatric non-B NHL cases (2, 3). Despite extensive research on prognostic factors in TLL, only disease stage has proven to be applicable in risk group stratification. There is also a lack of new drugs or other therapeutic options for pediatric patients with TLL (16). The opposite situation is observed in the therapy of ALCL, with multiple therapeutic options emerging in recent years. Although the 5-year OS in pediatric patients with ALCL has reached over 90%, the 5-year EFS remains at an unacceptable level of 70%, despite several modifications in frontline treatment (17). Recent advances in understanding ALCL biology resulted in the introduction of BV to standard chemotherapy, primarily in refractory or relapsed ALCL, and subsequently as a part of frontline therapy (18-20). Another therapeutic option for patients with relapsed or refractory ALK-positive ALCL are ALK inhibitors. Although their role in children's therapy is still under investigation, they show promising results in adult patients, particularly in refractory ALCL (17, 20). In relapsed disease, allo-HSCT remains the treatment of choice (21, 22). However, the high incidence of treatment related mortality raises questions about the potential role of alternative treatments (21).

As TLL and ALCL represent the majority of pediatric non-B NHL cases, it is not surprising that most efforts are directed toward the therapy development for these patients (2, 16, 18). Moreover, pediatric patients with rare types of non-B NHL are generally excluded from clinical trials (4, 23). In a single oncology center, treatment decisions are usually made by individual physicians or therapeutic teams, often relying on limited available data on outcomes derived from case reports. Many patients receive CHOP-based regimens as a potentially efficient therapeutic option, but the results vary according to the NHL type (4, 23, 24). The challenges in treatment of rare types NHL have been addressed by COG by initiating the Rare and Cutaneous NHL registry (COG protocol ANHL 04B1) (5). The first report from the registry published in 2016 highlighted the lack of treatment guidelines and variety of regimens are administered in therapy of patients with rare non-B NHL types, starting from observation or surgery alone to HSCT (5).

Other limitations of therapeutic options for NHL patients are funding issues, particularly for newly discovered or experimental therapies (25). Our analysis demonstrated how the oncology center's financial status influenced changes in therapeutic approaches. The first two patients with rare types of NHL (SPTCL and ENKL) were treated in the early 2000s, a period marked by limited funding in our country. Therefore, they received only CHOP-based regimens, resulting in varied outcomes. The last patient with HV-LPDL was treated in the early 2020s, when Poland become a developed country that directly impacted funding opportunities for oncology centers, including our Department. Therefore, the patient was able to receive innovative treatment, which proved to be successful in his case. A similar situation can be observed in the therapy of relapsed ALCL patients. The first patient, treated in 2005, received only standard salvage chemotherapy and subsequently died due to disease progression. In contrast, the second patient, diagnosed with relapse in 2020, was treated with chemotherapy combined with BV and followed by allo-HSCT, which proved to be successful in his case.

Despite funding concerns, we must also consider, that smaller institutions may be discouraged from participating in large studies due to the disease's rarity (25). Therefore, we need to provide therapy recommendations adapted to the local capabilities. Addressing those needs in the United States and Canada, Priya Mayahan et al. described the multidisciplinary monthly Rare Tumor Tele Tumor Boards created in 2018 (26). During these meetings, pediatric rare solid tumor cases were discussed to determine the best diagnostic and therapeutic options (26). The multidisciplinary approach facilitates therapeutic approaches including not only systemic chemotherapy or targeted treatment but also surgery, radiation, and the consideration of other non-medical aspects (25-28). A similar solution could be applied to pediatric patients with rare NHL types, on a local, national, or international level. Besides providing access to multidisciplinary consultation, this approach offers patients and their families the chance to receive treatment close to home, thereby reducing the financial and personal costs associated with treatment (29).

In conclusion, non-B NHL represent a heterogeneous group of rare diseases with varied therapeutic approaches and outcomes. Although we observe a significant progress in therapy for ALCL, results for TLL treatment remain unsatisfactory. Moreover, many rare non-B NHL types are excluded from clinical trials and further research. In these cases, treatment decisions often rely on individual center expertise and available case reports. However, there is a lack of comprehensive literature and knowledge on optimal therapeutic strategies.

Many individual oncology centers feel the pressure to demonstrate outcomes comparable to those achieved in international trials. However, their unique value lies in the

deep understanding of challenges they face every day. While celebrating successes is important, it is equally crucial to identify institutional limitations, to create new directions for therapy development.

Conflicts of Interest

The Authors have no competing interests to declare that are relevant to the content of this article.

Authors' Contributions

Joanna S. and Jan S. contributed to the study conception and design. Material preparation, data collection and analysis were performed by Joanna S., Anna J. and Paweł T. The first draft of the manuscript was written by Joanna S. and all authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

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From Local to International Approach: Prognostic Factors and Treatment Outcomes in Neuroblastoma—A 30-Year Single-Center Retrospective Analysis

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Abstract: Background/Objectives: Over the past three decades, significant progress has been made in understanding the biology of neuroblastoma. The integration of prognostic factors has facilitated risk stratification and the development of targeted treatment approaches. This study aims to analyze the outcomes of pediatric patients with neuroblastoma treated at a single oncology center over a 30-year period. **Methods**: This retrospective study analyzed data from patients aged 0–18 years with neuroblastoma, treated at the Department of Pediatric Hematology and Oncology in Bydgoszcz, Poland, between 1993 and 2023. The study endpoints included the 5-year probability of overall survival (pOS), event-free survival (pEFS), and relapse-free survival (pRFS), analyzed separately for low/intermediate- and high-risk groups. **Results**: Seventy-five patients met the inclusion criteria. Thirty-two children were categorized as high-risk patients and forty-three as low/intermediate risk. During the study period, outcomes in the low/intermediate-risk group improved significantly (the 5-year pOS 85.7% vs. 100.0%, $p = 0.019$; the 5-year pRFS 85.7% vs. 100.0%, $p = 0.662$; the 5-year pEFS 83.3% vs. 100.0%, $p = 0.038$). In the high-risk group, outcomes improved but did not reach statistical significance (the 5-year pOS 0.0% vs. 41.1%, $p = 0.342$; the 5-year pRFS 0.0% vs. 32.5%, $p = 0.180$; and the 5-year pEFS 0.0% vs. 21.5%, $p = 0.537$). Sixteen patients experienced relapse, of whom only three survived; stem cell transplantation at relapse significantly improved survival (OS 0.0% vs. 50.0%, $p = 0.001$). In the multivariable analysis, stage at diagnosis was a prognostic factor for pOS (HR 6.0; 95%CI 0.7–49.6, $p = 0.096$), while pelvic localization was a risk factor for pRFS (HR 3.0; 95%CI 0.8–10.5; $p = 0.084$). **Conclusions**: This analysis highlights significant advancements in the diagnosis and treatment of neuroblastoma. Nevertheless, outcomes for high-risk patients and those who experience relapse remain poor, underscoring the need for further therapeutic improvements.

Keywords: neuroblastoma; chemotherapy; therapeutic era; survival

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1. Introduction

Neuroblastoma (NBL) is the most common extracranial solid tumor of childhood, characterized by heterogeneous biology and a variable clinical course. The primary tumor arises from primordial neural crest cells and can develop anywhere where sympathetic tissue is present. However, the majority of cases are diagnosed in the adrenal glands or paravertebral ganglia [1,2]. Clinical presentation ranges from an incidentally detected adrenal mass to advanced metastatic disease with systemic manifestations. Moreover, the natural history of neuroblastoma is highly heterogeneous, with some cases exhibiting

spontaneous regression or differentiation, while others follow a highly aggressive course with rapid tumor progression and widespread metastasis [1–3].

For more than three decades, neuroblastoma treatment has served as a paradigm for tailored, personalized therapy in pediatric oncology [1,4–10]. Using known biological and clinical factors, patients with neuroblastoma can be divided into three therapeutic groups: low-risk, intermediate-risk, and high-risk. As knowledge about prognostic factors and therapy responses continues to expand, each risk group is further subdivided into smaller, more specific therapeutic subgroups. Although the overall survival rate in the low- and intermediate-risk groups exceeds 90%, high-risk patients have a long-term survival rate of less than 50% [2,5,11–14]. The implementation of multimodal, intensive chemotherapy, followed by megatherapy with autologous hematopoietic stem cell transplantation (auto-SCT) and maintenance therapy with 13-cis-retinoic acid (13-cisRA), as well as the introduction of immunotherapy with anti-GD2 monoclonal antibodies, has significantly improved outcomes in the HR group [1,6,9–11,14,15]. However, children with refractory or relapsed disease have a particularly unfavorable prognosis, and currently, no established effective therapy exists for these patients [16–20]. The gap in our understanding of effective therapies for refractory and relapsed disease remains an important clinical problem.

This study aims to analyze the risk factors, treatment strategies, and outcomes of the entire cohort of pediatric patients with NBL treated at a single oncology center in Poland over a 30-year period. The analysis reflects the expanding knowledge about neuroblastoma biology and the improved therapy results related to the introduction of novel therapeutic modalities in NBL treatment.

2. Materials and Methods

This retrospective study analyzed the prognostic factors and outcomes in pediatric patients diagnosed with neuroblastoma and treated in a single tertiary oncology center in Poland during the respective therapeutic periods.

Data from patients aged 0–18 years treated at the Department of Pediatric Hematology and Oncology, University Hospital No. 1 in Bydgoszcz between January 1993 and December 2023 were analyzed. The observations were completed in November 2024. The study included newly diagnosed tumors of neuronal origin that were confirmed as neuroblastomas by pathologists with experience in pediatric malignancies. Clinical data for patients treated between 1994 and 2003 were obtained from traditional medical records, whereas data from 2004 onward were stored and reviewed electronically. Patients were excluded if they had incomplete medical data, were diagnosed with malignancies other than neuroblastoma (e.g., ganglioneuroma, paraganglioma), were lost in follow-up, or were treated at other centers and referred to the Department solely for stem cell transplantation (SCT) as part of therapy.

Patients' medical histories were reviewed with a particular focus on the diagnosis of cancer-predisposition syndromes before NBL incidence and family history of malignancies. The symptoms presenting at diagnosis included hypertension (blood pressure above the 95 percentile for age and height), tachycardia, pain, constipation, diarrhea, Horner syndrome (unilateral ptosis, anhidrosis, and miosis), signs of spinal cord compression (muscle weakness, sensory deficits, urinary and rectal sphincter dysfunction), and opsoclonus–myoclonus–ataxia (OMA) syndrome.

The histological diagnosis of neuroblastoma was based on conventional tissue-staining histology with additional immunohistochemistry, if indicated. The following NBL subtypes were diagnosed in the analyzed cohort: differentiating NBL, poorly differentiated NBL, undifferentiated NBL, and ganglioneuroblastoma. If the histologic

subtype was not indicated in the medical records, neuroblastoma not otherwise specified (NBL NOS) was diagnosed.

At diagnosis, blood tests for hematologic and biochemistry (complete blood count, ferritin, and lactate dehydrogenase levels) and urinary dopamine and catecholamine metabolites, including homovanillic acid (HVA) and vanillylmandelic acid (VMA), were performed. The radiologic work-up included chest X-rays, abdominal ultrasonography (USG), computed tomography (CT), or magnetic resonance imaging (MRI) of the primary tumor with tumor volume calculation and iodine-123-labeled metaiodobenzylguanidine (MIBG) scan (technetium bone scan if primary tumor MIBG negative). Bone marrow involvement was evaluated using bone marrow aspirations and bone marrow trephines (both from two separate evaluable sites). Since 2001, tumor studies have been conducted for the MYC gene copy number status, and since 2015, for genomic copy number profiles with high-resolution array comparative genomic hybridization (aCGH) and ALK gene mutations.

All patients were staged according to the revised International Neuroblastoma Staging System (INNS) [21]. Since 2009, an additional staging system according to the International Neuroblastoma Risk Group Staging System (INRGSS) has been used [22].

The definition of risk groups varies between different protocols and therapeutic periods, as successive research has introduced new prognostic factors for risk group stratifications [5–8,12–14,21–23]. Currently, risk group stratification is based on various features related to symptoms, diagnosis, and tumor biology, including tumor genetic profiles. However, many of these factors, measurements, and results were not available during earlier analysis periods, particularly before the 2000s. To enable reliable comparisons between specific therapeutic approaches in this study, patients were divided into two risk groups based on age and disease stage at diagnosis. Patients were classified as low/intermediate risk if they were diagnosed with stage 1–3 neuroblastoma according to INNSS or with stage 4 disease within the first 12 months of life (including stage 4S). Patients older than 12 months with stage 4 disease were classified as high risk.

Patients were treated according to therapeutic protocols dedicated to the relevant risk groups.

The high-risk group was treated according to the Study Group of Japan for Treatment of Advanced Neuroblastoma Tokyo (TOKYO) protocol from January 1993 to January 2002 and the High Risk Neuroblastoma Study 1 (HR-NBL1) of SIOPEN from February 2002 onwards [6,7,9,14,15,24,25].

Patients from the low/intermediate-risk group were treated according to the French Society of Pediatric Oncology Neuroblastoma 90 and 94 therapeutic protocols (SFOP NBL) from January 1993 to October 2002, and a subgroup of patients at stage 1–2 was treated according to the SFOP NBL study to June 2011 [4,5,11,23]. From November 2002 to June 2011, stage 3 patients and patients at stage 4 younger than 12 months were treated according to a Multicenter Study for Infants designed by the International Society of Pediatric Oncology European Neuroblastoma Study Group (SIOPEN INES) [12,13]. From July 2011 onwards, all patients from the low/intermediate-risk group were treated according to the SIOPEN European Low and Intermediate Risk Neuroblastoma study Version 3.0 (LINES 3.0) [8]. One patient from the low/intermediate-risk group was treated according to the TOKYO protocol, and one according to HR-NBL1, based on the individual decision of the therapeutic team.

Surgery of primary tumors was performed according to the respective therapeutic protocols and guidelines. The main recommendations are summarized in Table 1.

Table 1. The main recommendations for surgery according to the analyzed therapeutic protocols.

Therapeutic Protocols	Surgery Guidelines
TOKYO [1]	The timing and method of resection (gross complete resection, partial resection, or biopsy) were determined by each individual institution. Surgery of the primary tumor and lymph node metastases was performed between chemotherapy courses [6,14,15].
HR-NBL1 [2]	CME [3] of the primary tumor and involved lymph nodes was encouraged, ideally before SCR [4]. The operation was to be postponed if nephrectomy was necessary. Tumor resection was permitted at one of three defined time points: within 60 days after the end of induction therapy, after 60 days of induction completion, followed by a topotecan, vincristine, and doxorubicin chemotherapy course, or after SCR [7].
SFOP NBL 90 [5] **and SFOP NBL 94** [6]	The final decision regarding the extent of surgery (primary or delayed excision attempt versus partial resection or biopsy) was made by an interdisciplinary team comprising a pediatric oncologist, radiologist, and surgeon. A localized tumor was considered unresectable if it crossed the midline, infiltrated major vessels, or posed a high risk of major surgical complications or macroscopically incomplete resection. Surgery with the risk of major organ removal (e.g., kidney, bladder, ureter) was not recommended unless initial chemotherapy had been administered before [4,5,23].
SIOPEN INES [7]	Primary surgery could be performed as complete, near-complete, or incomplete excision of the tumor mass, or it may be limited to an open or needle-core biopsy, depending on the objective and subjective SRFs [8] defined based on imaging characteristics. Objective SRFs include infiltration of or close relation to major blood vessels, infiltration of intervertebral foramina, or crossing the midline. The protocol also defines SRFs related to the specific localization of the primary tumor. Subjective SRFs include the ratio between tumor and child size as well as tumor fragility. In the presence of SRFs, primary biopsy was encouraged [12,13].
LINES 3.0 [9]	Primary resection was indicated for patients with localized tumors without IDRFs [10] as listed in the study (e.g., tumor encasing major arteries and vessels, tumor encasing vital neural structures such as brachial plexus roots, invasion of more than one-third of the spinal canal, tumor encasing the trachea or principal bronchi, and infiltration of adjacent organs and structures). Biopsy at presentation was indicated for localized tumors with IDRFs and metastatic tumors. Excision of the primary tumor may serve as an alternative diagnostic procedure to biopsy in metastatic tumors, provided the primary tumor is IDRF-negative. The decision to perform delayed resection was made individually based on the child's age and the presence of IDRFs, as defined in the protocol [8].

[1] TOKYO—The Study Group of Japan for Treatment of Advanced Neuroblastoma Tokyo; [2] HR-NBL1—the High Risk Neuroblastoma Study 1 of The International Society of Paediatric Oncology European Neuroblastoma (SIOPEN); [3] complete macroscopic excision; [4] stem cell rescue; [5] SFOP NBL 90—The French Society of Pediatric Oncology Neuroblastoma 90 therapeutic protocol; [6] SFOP NBL 94—The French Society of Pediatric Oncology Neuroblastoma 94 therapeutic protocol; [7] SIOPEN INES—a Multicenter Study for Infants designed by the International Society of Pediatric Oncology European Neuroblastoma Study Group; [8] SRF—surgical risk factor; [9] LINES 3.0—The International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) European Low and Intermediate Risk Neuroblastoma study Version 3.0; [10] IDRF—image-defined risk factor.

Complete resection was defined as the macroscopically complete removal of the tumor, permitting the presence of microscopic residuals. Complete remission (CR) was defined as the absence of tumor in any site. Progression was defined as the appearance of a new site of disease, an increase of any measurable lesion by >25%, or a previous negative marrow positive for tumor during first-line treatment [21]. Relapse was defined as the reappearance of the tumor after achieving CR. Event was defined as relapse, progression, secondary malignancy, or death from any cause. Overall survival (OS) was the time calculated from diagnosis to death or last observation, event-free survival (EFS) included the time from diagnosis to an event, and relapse-free survival (RFS) was calculated as the time from diagnosis to relapse.

The endpoints of the study were a 5-year probability of overall survival (pOS), a 5-year probability of event-free survival (pEFS), and a 5-year probability of relapse-free survival (pRFS).

Survival curves of pOS, pEFS, and pRFS were analyzed according to the Kaplan–Meier method and compared by the log-rank test. All features measured at diagnosis were included in the univariate analysis to determine their impact on outcomes. Factors significant in the univariate analysis were used in the multivariate Cox proportional hazards regression model. A value of $p < 0.05$ was considered statistically significant. Statistical

analysis was performed using MedCalc® statistical software Version 23.1.3 (MedCalc Software, Mariakerke, Belgium).

3. Results

3.1. Patient Characteristics

During the period from January 1993 to December 2023, a total of 113 patients aged 0–18 years were hospitalized in the Department of Pediatric Hematology and Oncology with the diagnosis of tumors of neuronal origin. Among them, 107 were finally diagnosed with neuroblastoma, 4 with ganglioneuroma, and 2 with paraganglioma. Within the 107 neuroblastoma patients, 20 have been treated in different oncology centers and referred to our department for stem cell rescue procedures. Another one was hospitalized solely for the stem cell harvesting procedure without SCT. Eight patients were excluded because of insufficient or incomplete data available, and three patients were lost in follow-up (they were transferred to other oncology centers by their parents' decision). In total, 75 patients were included in the analysis.

Median age at diagnosis was 1.8 years (range 0.0–12.4 years), and 28 patients (37.3%) were younger than 12 months at diagnosis. None of the analyzed children had a family history of neuroblastoma or other malignancies, and one was earlier diagnosed with cancer predisposition syndrome (neurofibromatosis type 1). However, two patients (a 6-month-old boy and a 4.6-year-old girl) had café au lait spots in clinical examinations at diagnosis. Stage 1 disease was diagnosed in 11 patients (14.7%), stage 2 in 6 (8.0%), and stage 3 in 15 cases (20.0%). Most patients presented with metastatic disease (n = 43, 57.3%), of whom six presented with stage 4S. Thirty-two children were categorized as high-risk patients and forty-three as low/intermediate risk. Patients' detailed characteristics are shown in Table 2.

Table 2. Patients' characteristics considering respective therapeutic protocols.

	TOKYO [11]	HR-NBL1 [12]	SFOP NBL [13]	SIOPEN INES [14]	LINES 3.0 [15]
Number of patients	8	27	9	12	19
Male	4	14	5	6	14
Female	4	13	4	6	5
Age (years) mean range	5.5 1.0–12.4	3.8 0.4–10.5	2.7 0.0–12.4	1.3 0.1–7.4	1.5 0.0–11.5
WBC [1] ($\times 10^3$) mean range	9.21 2.60–17.00	7.49 4.41–12.7	9.21 4.80–18.00	10.73 8.60–12.40	10.18 2.96–15.40
PLT [2] ($\times 10^3$) mean range	322 107–653	301 48–536	502 259–780	379 161–647	488 254–849
HGB [3] (g/L) mean range	9.9 7.0–13.5	8.9 4.8–12.4	11.7 9.3–13.4	10.7 9.6–13.2	11.3 7.4–15.9
LDH [4] (IU/L) mean range	1313 403–4258	1613 168–7029	548 410–740	627 282–1360	344 223–874
Ferritin(ng/mL) mean range	488 271–706	538 25–2480	85 37–181	119 20–292	97 6–298
Histology					
Differentiating NBL [5]	2	2	3	1	1
Poorly differentiated NBL	0	5	1	6	12
Undifferentiated NBL	1	4	0	0	2
Ganglioneuroblastoma	0	6	2	2	2

Table 2. *Cont.*

	TOKYO [11]	HR-NBL1 [12]	SFOP NBL [13]	SIOPEN INES [14]	LINES 3.0 [15]
NBL NOS [6]	5	10	3	3	2
Catecholamine metabolites	4	20	3	7	11
MIBG [7] positive tumors	5	18	4	9	10
N-MYC amplification	NA [10]	13	NA	3	1
SCA [8]	NA	7	NA	NA	5
NCA [9]	NA	3	NA	NA	7
ALK gene mutation	NA	0	NA	NA	0
Symptoms					
Hypertension	2	6	1	2	1
Tachycardia	2	3	0	0	1
Pain	7	14	1	4	2
Constipation	3	3	1	3	1
Diarrhea	1	0	0	2	3
Horner syndrome	0	0	0	1	0
Spinal cord compression	2	5	0	3	1
Opsoclonus–myoclonus–ataxia	0	0	0	0	0
Primary tumor localization					
Adrenal gland	6	22	8	7	16
Retroperitoneal	6	22	1	7	5
Mediastinal	2	12	1	5	1
Pelvis	2	12	0	4	1
Neck	1	3	0	0	0

[1] WBC—white blood cells; [2] PLT—platelet count; [3] HGB—hemoglobin; [4] LDH—lactate dehydrogenase; [5] NBL—neuroblastoma; [6] NOS—not otherwise specified; [7] MIBG—iodine-123-labeled metaiodobenzylguanidine; [8] SCA—structural chromosomal alterations, [9] NCA—numerical chromosomal alterations; [10] NA—not applicable; [11] TOKYO—The Study Group of Japan for Treatment of Advanced Neuroblastoma Tokyo; [12] HR-NBL1—the High Risk Neuroblastoma Study 1 of The International Society of Paediatric Oncology European Neuroblastoma (SIOPEN); [13] SFOP NLB—The French Society of Pediatric Oncology Neuroblastoma 90 and 94 therapeutic protocols; [14] SIOPEN INES—A Multicenter Study for Infants designed by the International Society of Pediatric Oncology European Neuroblastoma Study Group; [15] LINES 3.0—The International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) European Low and Intermediate Risk Neuroblastoma study Version 3.0.

3.2. Treatment in the Low/Intermediate-Risk Group

In the low/intermediate-risk group, most patients were treated according to the LINES 3.0 therapeutic protocol (n = 20, 46.2%). Complete resection during either primary or delayed surgery was achieved in 28 patients (65.1%). Among these, 18 patients underwent complete macroscopic removal of the primary tumor during initial surgery. Primary surgery served as the definitive therapy in 15 cases, including 12 stage 1 patients, 2 stage 2 patients, and 1 stage 4S patient. Three stage 4S patients had only a biopsy of the primary tumor without further treatment. Radiotherapy was administered as a complementary therapy in eight cases (18.6%).

3.3. Treatment in the High-Risk Group

The majority of patients in the high-risk group were treated according to the HR-NBL1 therapeutic protocol (n = 25, 78.1%). All patients were diagnosed based on a biopsy of either the primary tumor or a metastatic site and received primary chemotherapy. Delayed complete resection was achieved in three cases (9.4%). Twenty patients underwent autologous-stem cell transplantation (auto-SCT). In 15 cases, local radiotherapy (RTX) was performed following induction chemotherapy, surgery, and auto-SCT. Sixteen children received maintenance treatment with 13-cis-retinoic acid (13-cisRA), and six patients were eligible to receive dinutuximab beta (anti-GD2 monoclonal antibodies) during maintenance therapy.

3.4. Outcomes

Complete remission was achieved by 82.7% of patients (n = 62). For the entire cohort, the 5-year pOS was 66.7%. Patients in the low/intermediate-risk group had a significantly better 5-year pOS compared to those in the high-risk group (91.9% vs. 35.9%, $p < 0.001$). Relapse occurred in 16 cases, with a median time to relapse of 0.8 years (range 0.0–3.2 years). In seven cases, relapse was observed at the primary tumor site, while in five cases, the patients presented with disseminated disease at the time of relapse diagnosis. Characteristics of patients with relapsed disease and details of subsequent therapies are presented in Table 3. Among patients who experienced relapse, only three survived. Stem cell transplantation (SCT) in relapse was the only therapeutic modality that significantly improved outcomes in this group (OS 0.0% vs. 50.0% for patients who underwent SCT in relapse, $p = 0.001$).

The 5-year pRFS for the whole cohort was 68.3%. Patients in the high-risk group had a significantly higher risk of relapse compared to those in the low/intermediate-risk group (the 5-year pRFS 28.3% vs. 94.9%, $p < 0.001$).

The 5-year pEFS for all analyzed patients was 57.3%, with a 5-year pEFS of 94.9% for the low/intermediate-risk group and 28.3% for the high-risk group ($p < 0.001$). Disease progression was observed in five cases. Four patients with progression were treated with chemotherapy, of whom two underwent SCT; one child received palliative care only. None of the patients with disease progression survived. No cases of secondary malignancies were observed.

Patients treated according to modern therapeutic protocols, utilizing genetic-based stratification, had better outcomes. Patients treated during the second half of the analyzed period have significantly better pOS, pEFS, and pRFS compared to the cohort treated before the year 2008 (Figure 1).

Twenty-four patients died, with the most common causes of death being disease relapse (n = 9, 37.5%) and treatment-related complications (n = 9, 37.5%). Four patients died due to primary disease progression, and in two cases, the cause of death was unknown (both patients died outside our hospital). Among the treatment-related deaths, four were attributed to infectious complications, four to treatment toxicity, and one to complications following primary tumor resection. The median time from diagnosis to death was 1.3 years.

Table 3. Detailed characteristics of patients with relapse.

No.	Sex	Age at Diagnosis (yrs)	Therapy Protocol in 1st Line	Time from Start of Treatment to Relapse (yrs)	2nd Line Chemotherapy	RTX [1]	Auto-SCT [2]	13-cisRA [3]	Anti-GD2 [4]	MIBG [5]	Outcome	Time from Relapse to Death (yrs)
1.	M	7.6	TOKYO [6]	0.9	2nd therapy with TOKYO protocol	0	0	0	0	0	Death (disease progression)	0.1
2.	M	12.4	TOKYO	0.2	SPIC [7]	0	0	0	0	0	Death (disease progression)	0.2
3.	F	0.1	SIOPEN INES [8]	0.1	HR-NBL1 [9]	1	1	0	0	0	Alive	NA
4.	M	0.5	HR-NBL1	0.1	Palliative care	0	0	0	0	0	Death (disease progression)	0.0
5.	F	2.1	TOKYO	0.0	Palliative care	0	0	0	0	0	Death (disease progression)	0.2
6.	F	8.3	SFOP NBL [10]	1.0	TOKYO	1	0	0	0	0	Death (disease progression)	1.7
7.	M	3.2	HR-NBL1	2.3	TVD [11], Cisplatin, Etoposide + ZVAC [12] + Temozolomide	0	0	0	0	0	Death (disease progression)	1.7
8.	F	4.6	HR-NBL1	0.3	SIOPEN INES	0	0	0	0	0	Death (disease progression)	0.3
9.	M	1.7	HR-NBL1	3.1	CIT [13], VOIT [14], ICE [15] + Topotecan	0	1	0	0	0	Death (disease progression)	2.5
10.	M	0.1	HR-NBL1	2.0	ICE + Topotecan, TOTEM [16]	0	1 *	1	0 **	1	Death (TRM [17]—infection)	5.9
11.	M	0.9	SIOPEN INES	1.1	CIT, ICE+ Topotecan	1	1	1	0	0	Alive	NA
12.	F	2.5	HR-NBL1	2.7	Cyclophosphamide + Vincristine	0	0	0	0	0	Death (TRM—treatment toxicity)	0.1

Table 3. *Cont.*

No.	Sex	Age at Diagnosis (yrs)	Therapy Protocol in 1st Line	Time from Start of Treatment to Relapse (yrs)	2nd Line Chemotherapy	RTX [1]	Auto-SCT [2]	13-cisRA [3]	Anti-GD2 [4]	MIBG [5]	Outcome	Time from Relapse to Death (yrs)
13.	F	3.1	HR-NBL1	3.2	Cyclophosphamide + Topotecan, Temozolomide + Irinotecan	1	1 *	1	1	1	Alive	NA
14.	M	3.1	HR-NBL1	0.6	Bevacizumab + Irinotecan + Temozolomide	0	0	0	0	0	Death (disease progression)	0.7
15.	F	0.1	HR-NBL1	0.1	Etoposide + Carboplatin + Doxorubicin	1	0	0	0	0	Death (disease progression)	0.1
16.	M	0.3	HR-NBL1	0.1	TOTEM	0	0	0	0	0	Death (disease progression)	0.2

[1] RTX—radiotherapy; [2] Auto-SCT—autologous stem cell transplantation; [3] 13-cisRA—13-cis-retinoic acid; [4] Anti-GD2—anti-GD2 monoclonal antibodies; [5] MIBG—I-131-labeled metaiodobenzylguanidine therapy; [6] TOKYO—The Study Group of Japan for Treatment of Advanced Neuroblastoma Tokyo; [7] SPIC—sequential postoperative intraperitoneal chemotherapy; [8] SIOPEN INES—a Multicenter Study for Infants designed by the International Society of Pediatric Oncology European Neuroblastoma Study Group; [9] HR-NBL1—the High Risk Neuroblastoma Study 1 of The International Society of Paediatric Oncology European Neuroblastoma (SIOPEN); [10] SFOP NBL—The French Society of Pediatric Oncology Neuroblastoma 90 and 94 therapeutic protocols; [11] TVD—topotecan, vincristine, doxorubicin; [12] ZVAC—dexrazoxane, vincristine, doxorubicin, cyclophosphamide; [13] CIT—carboplatin, irinotecan, temozolomide; [14] VOIT—vincristine, oral irinotecan, temozolomide; [15] ICE—ifosfamide, carboplatin, etoposide; [16] TOTEM—temozolomide and topotecan; [17] TRM—treatment-related mortality; * patients received two auto-SCTs, ** patient was disqualified from a therapy with anti-GD2 monoclonal antibodies due to restrictive lung disease as a complication of first-line treatment.

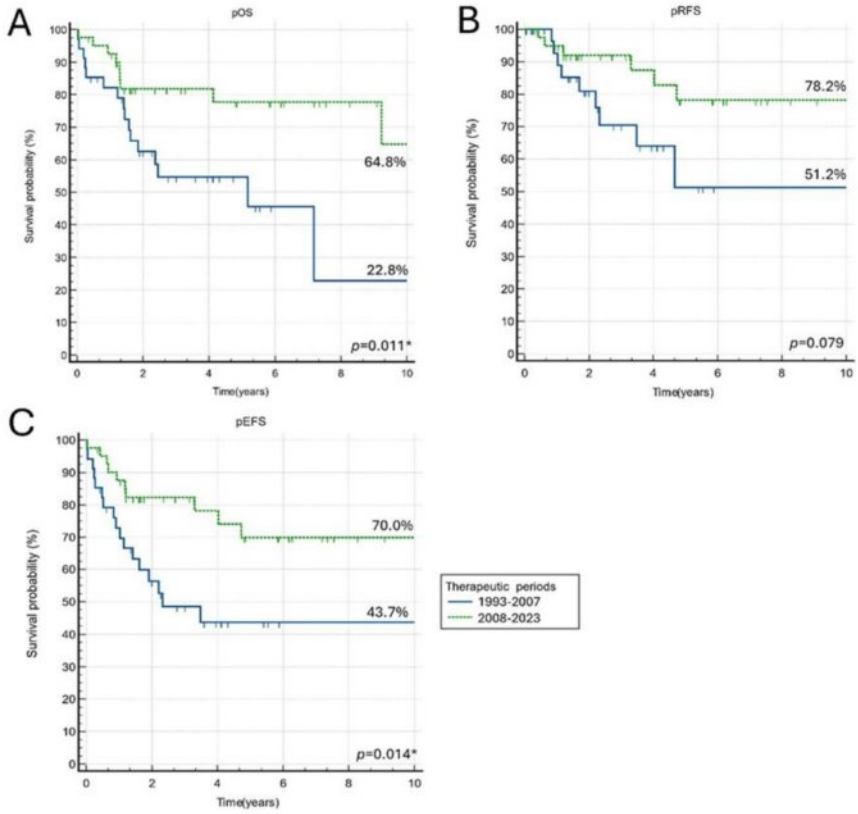

Figure 1. The long-term probability of overall survival (pOS), event-free survival (pEFS), and relapse-free survival (pRFS) divided by therapeutic periods. (**A**) The 10-year pOS for the whole cohort divided by therapeutic periods; (**B**) The 10-year pRFS for the whole cohort divided by therapeutic periods; (**C**) The 10-year pEFS for the whole cohort divided by therapeutic periods. * $p < 0.05$.

3.5. Therapy Results in the Low/Intermediate Group

The differences between therapeutic protocols in 5-year pOS and pEFS were statistically significant, with the best results observed in patients treated according to LINES 3.0 (Figure 2). No progression was observed in this group. One patient treated with the TOKYO protocol died due to treatment-related complications; one patient treated with the HR-NBL1 protocol is alive in remission. Relapse occurred in three patients. After relapse, one patient received chemotherapy only, one received chemotherapy followed by auto-SCT and additional RTX, and one received chemotherapy followed by auto-SCT, RTX, and maintenance with 13-cisRA. Three patients died: one in relapse (patient primary treated with SFOP NBL 94 protocol and with TOKYO after relapse), one due to treatment-related toxicity (infection), and in one case, the cause of death was unknown (the patient died in a different hospital after completion of therapy).

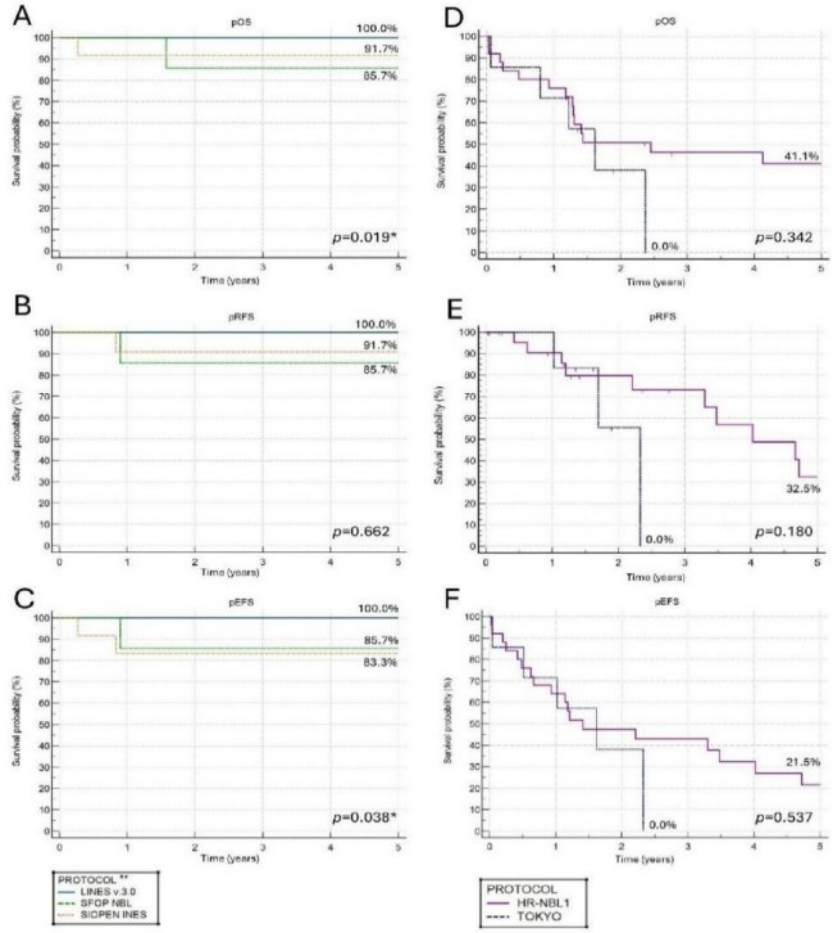

Figure 2. The 5-year probability of overall survival (pOS), event-free survival (pEFS), and relapse-free survival (pRFS) for consecutive therapeutic protocols divided by risk groups. (**A**) The 5-year pOS for low/intermediate-risk group patients according to therapy protocols; (**B**) The 5-year pRFS for low/intermediate-risk group patients according to therapy protocols; (**C**) The 5-year pEFS for low/intermediate-risk group patients according to therapy protocols; (**D**) The 5-year pOS for high-risk group patients according to therapy protocols; (**E**) The 5-year pRFS for high-risk group patients according to therapy protocols; (**F**) The 5-year pEFS for high-risk group patients according to therapy protocols; * $p < 0.05$; ** One patient treated with TOKYO and one patient treated with HR-NBL1 protocol were excluded from the (**A**–**C**).

3.6. Therapy Results in the High-Risk Group

Figure 2 shows that 5-year pOS was superior in patients treated according to the HR-NBL1 protocol. However, the results did not reach statistical significance. Patients treated with auto-SCT and maintenance with 13-cisRA had a better prognosis (the 5-year pOS 50.3% vs. 24.0%, $p = 0.001$). In the subgroup treated in maintenance with an addition of anti-GD2 monoclonal antibodies, the 5-year pOS was better, but the difference did not reach statistical significance (the 5-year pOS 50.0% vs. 38.8%, $p = 0.449$). However, prolonged observations indicated prominent improvement in long-term outcomes among patients treated with anti-GD2 monoclonal antibodies (the 10-year pOS 50.0% vs. 12.1%).

Thirteen patients experienced relapse, with a median time to relapse of 0.9 years. The differences in 5-year pRFS between protocols were visible but not statistically significant

(0.0% vs. 32.5%, $p = 0.180$, Figure 2). None of the therapy modalities used in first-line treatment (auto-SCT procedure, maintenance with 13-cisRA, or anti-GD2 monoclonal antibodies) significantly reduced the probability of relapse.

The 5-year pEFS was 0.0% for TOKYO and 21.5% for the HR-NBL1 protocol (Figure 2). Although therapy results with the auto-SCT procedure were better (the 5-year pEFS 26.8% vs. 20.0%, $p = 0.001$), the outcomes remained poor. The addition of maintenance with 13-cisRA significantly improved the 5-year pEFS (34.8% vs. 0.0%, $p = 0.001$ for 13-cisRA). Patients treated with anti-GD2 monoclonal antibodies had a higher 5-year pEFS, but the differences were not statistically significant (50.0% vs. 13.8%, $p = 0.308$).

3.7. Prognostic Factors

Stage at diagnosis was a significant prognostic factor. Patients with stage 1 and 4S disease had an excellent 5-year pOS and pEFS of 100.0%. In patients with stage 2 and 3 neuroblastoma, the 5-year pOS and pEFS reached 80.0% or above, while patients with stage 4 neuroblastoma had the 5-year pOS of 46.3%, the 5-year pRFS of 37.9%, and the 5-year pEFS of 28.5% (Figure 3).

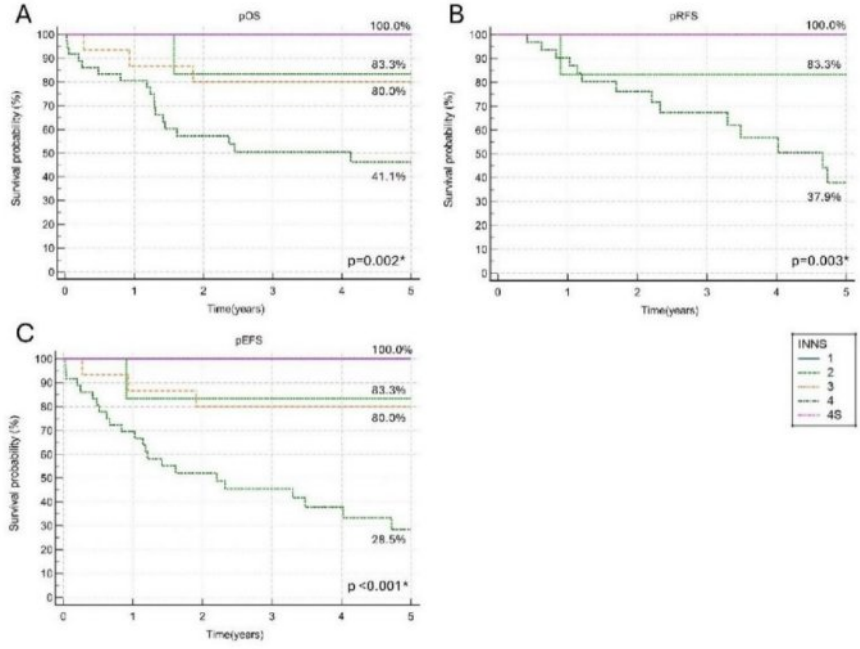

Figure 3. The outcomes according to five consecutive International Neuroblastoma Staging System (INNS) disease stages. (**A**) The 5-year probability of overall survival (pOS) for five consecutive INNS disease stages; (**B**) The 5-year probability of relapse-free survival (pRFS) for five consecutive INNS disease stages; (**C**) The 5-year probability of event-free survival (pEFS) for five consecutive INNS disease stages. * $p < 0.05$.

Age above 12 months at diagnosis was a risk factor for death (OR 7.9, 95% CI 2.1 to 29.9, $p = 0.002$), event (OR 6.8, 95% CI 2.1 to 22.7, $p = 0.014$), and relapse (OR 13.6, 95% CI 1.7 to 109.3, $p = 0.014$). Other significant risk factors included symptoms such as pain, hypertension, and tachycardia at diagnosis, increased LDH and ferritin levels, poorly differentiated histology, primary tumor localization in the pelvis or retroperitoneum, MIBG-positive tumors, N-MYC gene amplification, and structural chromosomal alterations (SCA) in tumor tissue (Table 4). In the multivariable analysis, INNS stage was a risk

factor of borderline significance for pOS and pEFS, while pelvis localization was a factor of borderline significance for pRFS (Table 5).

Table 4. Univariate analysis of risk factors.

	5-Year pOS [1]	pOS; *p*=	5-Year pEFS [2]	pEFS; *p*=	5-Year pRFS [3]	pRFS; *p*=
Male Female	75.0% 57.0%	0.693	62.0% 52.1%	0.893	70.6% 65.1%	0.854
Age < 12 months Age > 12 months	88.2% 54.1%	0.003 *	84.4% 42.4%	0.002 *	96.2% 52.5%	0.003 *
LDH [4] high level LDH normal level	54.8% 82.0%	0.019 *	38.5% 82.3%	0.005 *	51.9% 88.1%	0.066
Ferritin high level Ferritin normal level	53.1% 88.7%	0.005 *	38.0% 88.9%	0.001 *	49.7% 96.0%	0.004 *
Histology						
Differentiating NBL [5] Histology other than differentiating NBL	64.8% 66.9%	0.955	64.4% 56.3%	0.807	80.0% 66.3%	0.504
Poorly differentiated NBL Histology other than poorly differentiated NBL	87.1% 58.5%	0.042 *	87.1% 45.9%	0.011 *	100.0% 56.4%	0.006 *
Undifferentiated NBL Histology other than undifferentiated NBL	68.2% 57.1%	0.692	57.1% 57.6%	0.813	71.4% 68.1%	0.581
Ganglioneuroblastoma Histology other than ganglioneuroblastoma	63.7% 81.5%	0.897	61.7% 56.5%	0.737	68.6% 68.7%	0.521
Catecholamine metabolites positive Catecholamine metabolites negative	64.8% 84.4%	0.129	49.4% 85.7%	0.057	59.7% 83.6%	0.232
N-MYC [6] positive N-MYC negative	55.7% 87.6%	0.047 *	57.9% 71.9%	0.199	65.3% 82.1%	0.090
SCA [7] SCA negative	59.3% 100.0%	0.026 *	49.4% 100.0%	0.016 *	70.3% 100.0%	0.125
NCA negative [8] NCA positive	42.9% 84.6%	0.351	42.9% 84.6%	0.085	0.0% 92.3%	0.425
Symptoms at diagnosis						
Hypertension present Hypertension absence	32.1% 73.3%	0.002 *	18.8% 63.5%	0.003 *	31.5% 70.0%	0.030 *
Tachycardia present Tachycardia absent	44.1% 68.8%	0.163	22.2% 60.0%	0.148	22.2% 71.1%	0.003 *
Pain present Pain absent	44.2% 81.9%	0.001 *	26.2% 76.8%	<0.001 *	37.1% 81.3%	0.001 *
Constipation present Constipation absent	48.2% 69.1%	0.497	50.0% 56.9%	0.833	60.0% 66.6%	0.533
Diarrhea present Diarrhea absent	66.7% 66.6%	0.979	66.7% 55.4%	0.940	100.0% 63.5%	0.223
Spinal cord compression present Spinal cord compression absent	72.2% 65.2%	0.847	58.3% 55.7%	0.931	72.7% 64.3%	0.924
Primary tumor localization						
Adrenal gland Other than adrenal gland	67.0% 68.5%	0.514	54.2% 69.5%	0.515	66.3% 72.7%	0.909
Retroperitoneal Other than retroperitoneal	53.1% 86.8%	0.007 *	41.2% 81.0%	0.004 *	56.8% 82.6%	0.140
Pelvis Other than pelvis	44.9% 73.2%	0.021 *	29.8% 65.7%	0.003 *	44.5% 74.1%	0.023 *
Neck Other than neck	25.0% 70.6%	0.133	25.0% 60.3%	0.193	33.3% 71.4%	0.182

[1] pOS—probability of overall survival; [2] pEFS—probability of event-free survival; [3] pRFS—probability of relapse-free survival; [4] LDH—lactate dehydrogenase; [5] NBL—neuroblastoma; [6] N-MYC gene amplification; [7] SCA—structural chromosomal alterations; [8] NCA—numerical chromosomal alterations; * $p < 0.05$.

Table 5. Multivariable analysis of risk factors.

Independent Variable	pOS [4] HR [5]	pOS HR 95% CI [7]	pOS *p*=	pEFS [8] HR	pEFS HR 95% CI	pEFS *p*=	pRFS [9] HR	pRFS HR 95% CI	pRFS *p*=
Hypertension	3.7	0.7 to 19.6	0.124	2.9	0.7 to 12.9	0.142	1.3	0.2 to 7.7	0.741
Pain	1.0	0.2 to 6.3	0.992	1.2	0.2 to 5.4	0.816	1.7	0.5 to 5.8	0.415
Tachycardia	NA [6]	NA	NA	NA	NA	NA	2.0	0.3 to 11.5	0.447
Ferritin high level	3.1	0.3 to 30.0	0.334	3.5	0.4 to 32.5	0.266	1.4	0.1 to 14.2	0.771
LDH [1] high level	0.4	0.5 to 3.2	0.381	0.6	0.1 to 4.5	0.640	NA	NA	NA
N-MYC [2] positive	1.2	0.3 to 4.6	0.780	NA	NA	NA	NA	NA	NA
Pelvis	1.9	0.4 to 10.7	0.447	2.0	0.5 to 7.9	0.319	3.0	0.8 to 10.5	0.084 *
Retroperitoneal	1.5	0.2 to 15.3	0.714	0.9	0.1 to 7.9	0.973	NA	NA	NA
INNS [3]	6.0	0.7 to 49.6	0.096 *	7.4	0.9 to 57.5	0.056 *	2.9	0.7 to 12.5	0.134
Age < 12 months	0.2	0.1 to 3.9	0.301	0.2	0.1 to 3.7	0.306	3.4	0.3 to 34.8	0.670
Poorly differentiated histology	0.4	0.1 to 3.1	0.377	0.2	0.1 to 1.6	0.149	0.8	0.1 to 5.8	0.860

[1] LDH—lactate dehydrogenase; [2] N-MYC gene amplification; [3] INNS—International Neuroblastoma Staging System; [4] pOS—probability of overall survival; [5] HR—hazard ratio; [6] NA—not applicable; [7] CI—confidence interval; [8] pEFS—probability of event-free survival; [9] pRFS—probability of relapse-free survival. Structural chromosomal alterations were excluded from the multivariable analysis due to a low number of patients with aCGH test results; * p = 0.05–0.10.

4. Discussion

This 30-year, single-center analysis reflects significant improvements in the diagnostic and therapeutic approaches for NBL. As Poland has never established a national therapeutic program for neuroblastoma, our department has used therapeutic protocols from other countries [4–9,11–15,23,26]. In the 1990s, patients were treated using Japanese and French protocols. The results obtained in our cohort were inferior to those reported by the Neuroblastoma Study Group of the Société Francaise d'Oncologie Pédiatrique and the Study Group of Japan [4,6,14,15]. Poorer outcomes could be attributed to limited access to detailed therapy guidelines and high rates of treatment-related mortality. Since the early 2000s, patients have been treated according to international therapy protocols, leading to significant improvements in treatment outcomes.

During the study period, diagnostic and therapeutic recommendations have evolved into more specific and comprehensive guidelines, enabling precise execution of all therapeutic stages. An excellent example of the development is surgical guidelines. In the 1980s and 1990s, the surgery range and acceptable extension of the surgical site, as well as surgery timing, were assessed individually by surgeons [6]. Currently, the surgical guidelines are detailedly described in therapeutic protocols, with well-recognized image-defined risk factors and surgery time [1,2,7,8,27]. Moreover, the final decision regarding the surgical range is made by an interdisciplinary team comprising a pediatric oncologist, radiologist, and surgeon. Multidisciplinary collaboration plays a crucial role in the preparation of comprehensive treatment plans while minimizing postoperative complications.

In the last three decades, significant progress has been made in our understanding of neuroblastoma biology, particularly regarding the genetic and molecular background of the tumor and its distinct natural course in infants [2,3,12,22]. The stage at diagnosis is one of the most important prognostic factors. Prognostic factors other than age and stage include elevated LDH and ferritin levels, segmental chromosomal aberrations, MYC oncogene amplification, and unfavorable histology [1,2,8,13,16,23]. Although most risk factors were also identified in this study, in our cohort, clinical manifestations, such as hypertension and pain, were associated with decreased pOS, pRFS, and pEFS, whereas tachycardia was specifically linked to lower pEFS in a univariate analysis.

The integration of prognostic factors into clinical practice has facilitated effective risk stratification, allowing for adjusted treatment approaches. In the analyzed cohort, patients in the low/intermediate-risk group had a favorable prognosis, with a 5-year pOS and pEFS above 80.0%. A subgroup of patients treated with the LINES 3.0 protocol achieved excellent outcomes, with a 5-year pOS and pEFS of 100.0%. Significant progress in therapeutic outcomes for the low- and intermediate-risk groups was achieved by minimizing invasive treatment in subgroups with highly favorable prognoses while intensifying therapy for patients with identified risk factors. This risk-adapted strategy has optimized treatment intensity while reducing toxicity and improving overall patient outcomes.

Intensive treatment regimens also resulted in gradual improvements in survival among children in the high-risk group. Although overall outcomes in this group remained unfavorable, the 5-year pOS of the analyzed cohort increased from 0.0% to 41.1% during the study period. The TOKYO protocol was one of the first to incorporate multimodal, intensive chemotherapy for high-risk patients [6,14,15]. Although the results of the protocol were unsatisfactory in our department, it paved the way for further intensive treatment according to the HR-NBL1 protocol, which yielded significantly better outcomes. Therapy with anti-GD2 monoclonal antibodies did not significantly improve pOS and pEFS in our cohort. However, prolonged observation suggests that the addition of anti-GD2 monoclonal antibodies improves long-term outcomes.

More than half of children with high-risk neuroblastoma either fail to respond to standard therapies or experience relapse [16,19,20,28]. Patients with relapse have particularly unfavorable outcomes, with long-term pOS ranging from 14.1% to 20.0% [17–20]. Although no standard second-line therapy has been established, limited evidence indicates improved survival following myeloablative chemotherapy with auto-SCT [20,29]. This trend was also observed in our study, with SCT in relapse being the only therapeutic modality that significantly improved outcomes. Nevertheless, the choice of bridging therapy remains a topic of discussion, as reflected by the variety of salvage chemotherapy used during the study period. Regimens containing temozolomide and topotecan are currently the standard backbone of bridging chemotherapy [17,18,30]. However, most second-line therapy trials for neuroblastoma have been single-arm studies, lacking a valid comparator and primarily assessing short-term outcomes, such as response rate [17,18,30]. Given the heterogeneity of the disease and second-line therapy, there is limited knowledge of which patients are more likely to respond to treatment and achieve long-term survival after primary therapy failure [16,17,29].

Due to the retrospective nature of the analysis, this study has some obvious limitations. Some patients were excluded from the analysis due to missing medical data. The group comprised heterogeneous patients treated within 30 years, and the number of patients treated according to consecutive protocols was relatively small. Although some genetic diagnostic tests were not available in the early period of the analysis, the most important information about the clinical and laboratory results was included. Moreover, the lack of genetic testing reflects the limited knowledge about neuroblastoma biology in the 1990s and early 2000s. Furthermore, the endpoints were achieved in all analyzed cases, which enabled a comparison between therapeutic approaches. The data from this study may serve as a background for further research, particularly those focusing on relapsed disease.

5. Conclusions

This study highlights the significant progress that has been made in the diagnosis and treatment of neuroblastoma. The development of modern diagnostic methods, including genetic testing of tumor tissues, has facilitated precise risk stratification. Tailored treatment approaches, focused on minimizing invasive interventions for subgroups with highly favor-

able prognoses while intensifying therapy for high-risk patients, have optimized treatment strategies and improved overall patient outcomes. In our department, treatment guidelines have evolved from individualized therapeutic decisions based on scientific reports to standardized protocols within international trials. However, prognosis remains poor for high-risk patients and those who experience relapse, emphasizing the need for further therapeutic improvements. The lack of standardized second-line treatment guidelines for patients with relapse underscores the urgency for additional clinical trials. With the upcoming SIOPEN HR-NBL-2 trial on the horizon, we expect further improvements in outcomes for patients with high-risk neuroblastoma.

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Informed Consent Statement: Patient consent was waived due to the retrospective nature of the study and anonymization of patients' data.

Data Availability Statement: Data are available on reasonable request from the corresponding author.

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Rozdział 14.

Wykaz publikacji stanowiących rozprawę doktorską

1. Stankiewicz, J., Demidowicz, E., Jatczak-Gaca, A., Bartoszewicz, N., Kołtan, A., Kołtan, S., Czyżewski, K., Richert-Przygońska, M., Dębski, R., Pogorzała, M., Tejza, B., Księżniakiewicz, P., Cisek, J., Ewertowska, M., Marjańska, A., Dąbrowska, A., Urbańczyk, A., Grześk, E., Jaremek, K., Winogrodzka, E., Kołuda, D., Łęcka, M., Adamkiewicz, M., Wałach, S., Grochowska, O., Tarasenko, S., Mazalon, M., Dziedzic, M., Kubicka, M., Kuryło-Rafińska, B., Dembna, E., Majk, A., Wysocki, M. & Styczyński, J. (2023). Changing risk factors in childhood acute lymphoblastic leukemia: experience from Kujawsko-Pomorski region 1976–2018. *Acta Haematologica Polonica*, 54(1), 11-17. DOI: 10.5603/AHP.a2022.0003; IF(-); MNiSW 100 pkt.
2. Stankiewicz, J., Demidowicz, E., Bartoszewicz, N., Kołtan, A., Richert-Przygońska, M. & Styczyński, J. (2023). The impact of CAR-T therapy for outcomes in primary refractory acute lymphoblastic leukemia. *Acta Haematologica Polonica*, *54*(4), 248-251. DOI:10.5603/AHP.a2023.0041; IF(-); MNiSW 100 pkt.
3. Stankiewicz, J., Pukownik, E., Czyżewski, K., Dębski, R., Cisek, J., Kubicka, M., Kuryło-Rafińska B., Styczyński J. & Richert-Przygońska, M. (2025). Immunological response to chimeric antigen receptor T-cell therapy in pediatric relapsed/refractory acute lymphoblastic leukemia: peak of cytokine levels on day 4 post-infusion. *Acta Haematologica Polonica*, *56*(2), 108-116. DOI: 10.5603/ahp.103404; IF(-); MNiSW 100 pkt.
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5. Stankiewicz, J., Kołtan, A., Demidowicz, E., Bartoszewicz, N., Kołtan, S., Czyżewski, K. Richert-Przygońska M.,Dębski R., Pogorzała M., Tejza B., Cisek

J., Księżniakiewicz P., Jatczak-Gaca A., Marjańska A., Salamon M., Dąbrowska A., Urbańczyk A., Grześk E., Jaremek K., Łęcka M., Grochowska O. & Styczyński, J. (2023). Therapy results in pediatric Hodgkin lymphoma—does less mean better? Experience from a single children's oncology center. *Annals of Hematology*, *102*(8), 2109-2117. DOI:10.1007/s00277-023-05268-5; IF 3,0; MNiSW 70 pkt.

6. Stankiewicz, J., Jabłońska, A., Treichel, P. & Styczyński, J. (2024). Toward Inclusive Oncology: Challenges in the Therapy of Pediatric Non-B Non-Hodgkin Lymphomas. *In Vivo*, *38*(5), 2404-2409. DOI: 10.21873/invivo.13708; IF 1,8; MNiSW 40 pkt.
7. Stankiewicz, J., Pogorzała, M., Księżniakiewicz, P. & Styczyński, J. (2025). From Local to International Approach: Prognostic Factors and Treatment Outcomes in Neuroblastoma—A 30-Year Single-Center Retrospective Analysis. *Children*, *12*(4), 525. DOI: 10.3390/children12040525; IF 2,1; MNiSW 40 pkt.

Uniwersytet Mikołaja Kopernika w Toruniu

Collegium Medicum im L. Rydygiera w Bydgoszczy

KOMISJA BIOETYCZNA

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

KB 577/2021 Bydgoszcz, 16.11.2021 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 **16.11.2021 r.** przeanalizowała wniosek, który złożył kierownik badania:

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

z zespołem w składzie

lek. med. Joanna Konieczek, prof. dr hab. med. Mariusz Wysocki, Katarzyna Peszyńska-Białczyk, dr n. med. Ewa Demidowicz, dr n. med. Krzysztof Czyżewski, dr n. med. Robert Dębski, dr n. med. Monika Pogorzała, dr n. med. Andrzej Kołtan, dr n. med. Natalia Bartoszewicz, dr n. med. Monika Richter-Przygońska, dr n. med. Barbara Tejza, lek. med. Piotr Księżniakiewicz, lek. med. Joanna Cisek, dr n. med. Agnieszka Jatczak-Gaca, dr hab. n. med. Sylwia Kołtan, prof. UMK, dr n. med. Anna Dąbrowska, dr n. med. Elżbieta Grześk, lek. med. Anna Urbańczyk, lek. med. Kamila Jaremek, lek. med. Eugenia Winogrodzka, lek. med. Dominika Koluda, lek. med. Monika Łęcka, lek. med. Sandra Wałach, lek. med. Agata Marjańska, lek. med. Magdalena Dziedzic, lek. med. Katarzyna Balci, lek. med. Sonia Tarasenko, lek. med. Oliwia Grochowska, lek. med. Hanna Żołnowska, lek. med. Marlena Ewertowska, lek. med. Marta Mazalon, lek. med. Monika Adamkiewicz, dr n. med. Małgorzata Kubicka, dr n. med. Beata Kuryło-Rafińska

w sprawie badania:

„Skuteczność terapii innowacyjnych w onkologii dziecięcej w świetle wyników postępowania przeciwnowotworowego w najczęstszych nowotworach wieku dziecięcego".

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:

- w odniesieniu do części retrospektywnej badania:

- posiadania 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).

- w odniesieniu do części prospektywnej badania:

- poinformowania na piśmie uczestników badania o celu oraz zakresie badań i uzyskania od nich (obojga 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 pozytywnej opinii o badaniu;
- 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 świadomej zgody od nich;
- zachowania tajemnicy wszystkich danych, w tym danych osobowych uczestników badania, 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 w ramach jednego dokumentu.

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 (rodziców/opiekunów prawnych), a także klauzule, że uczestnik badania wyraża zgodę na przetwarzanie danych osobowych dotyczących realizacji tematu badawczego, zgodnie z obowiązującym prawem (RODO).

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

Zgoda obowiązuje od daty podjęcia uchwały (16.11.2021 r.) do końca 2026 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
Collegium Medicum w Bydgoszczy UMK w Toruniu

Lista obecności

na posiedzeniu Komisji Bioetycznej

w dniu 16.11.2021 r.

Lp.	Imię i nazwisko	Funkcja/ Specjalizacja	Podpis
1.	Prof. dr hab. n. med. Karol Śliwka	*medycyna sądowa* przewodniczący	
2.	Mgr prawa Joanna Połetek-Żygas	*prawniczka* zastępca przewodniczącego	
3.	Prof. dr hab. n. med. Mieczysława Czerwionka-Szaflarska	*pediatra, alergologia i gastroenterologia dziecięca*	
4.	Prof. dr hab. n. med. Marek Grabiec	*położnictwo, ginekologia onkologiczna*	
5.	Prof. dr hab. n. med. Maria Kłopocka	*choroby wewnętrzne, gastroenterologia*	
6.	Prof. dr hab. n. med. Zbigniew Włodarczyk	*chirurgia ogólna, transplantologia kliniczna*	
7.	Dr hab. n. med. Maciej Słupski, prof. UMK	*chirurgia ogólna, transplantologia kliniczna*	
8.	Dr hab. n. med. Katarzyna Sierakowska, prof. UMK	*anestezjologia i intensywna terapia*	
9.	Ks. dr hab. Wojciech Szukalski, prof. UAM	*duchowny*	
10.	Dr n. med. Radosława Staszak-Kowalska	*pediatria, choroby płuc*	
11.	Mgr prawa Patrycja Brzezicka	*prawniczka*	
12.	Mgr farm. Aleksandra Adamczyk	*farmaceutka*	
13.	Mgr Lidia Iwińska-Tarczykowska	*pielęgniarka*	