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Analiza zagrażających życiu powikłań we wczesnym okresie po przeszczepieniu komórek hematopoetycznych u dzieci.

Rozprawa doktorska

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SPIS TREŚCI

1. ***Premature cyclosporine cessation and TBI-containing conditioning regimen increase the risk of acute GvHD in children undergoing unrelated donor hematopoietic stem cell transplantation.***

   Autorzy: Szmit Z, Kałwak K, Król A, Mielcarek-Siedziuk M, Salamonowicz M, Frączkiewicz J, Ussowicz M, Owoc-Lempach J, Gorczyńska E.
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2. ***Factors affecting survival in children requiring intensive care after hematopoietic stem cell transplantation. A retrospective single-center***

   Autorzy: Szmit Z, Kośmider-Żurawska M, Król A, Łobos M, Miśkiewicz-Bujna J, Zielińska M, Kałwak K, Mielcarek-Siedziuk M, Salamonowicz-Bodzioch M, Frączkiewicz J, Ussowicz M, Owoc-Lempach J, Gorczyńska E.

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3. ***Veno-occlusive disease in children and adolescents after hematopoietic stem cell transplantation: Did the Modified Seattle Criteria fit the characteristics of pediatric population?***

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4. ***Introduction of new pediatric EBMT criteria for VOD diagnosis: is it time-saving or money-wasting?: Prospective evaluation of pediatric EBMT criteria for VOD***

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ABSTRACT

INTRODUCTION:

Hematopoietic stem cell transplantation (HSCT) is a well-established curative treatment method for a wide range of diseases. It is used for both malignant and non-malignant disorders. Due to advances made over the decades, including individually tailored conditioning regimens, precise HLA-typing, improvements in supportive therapy, and prophylaxis against severe infections, the survival rates of pediatric HSCT recipients are currently rising. However, it remains a high risk procedure, contributing significantly to morbidity and mortality. A thorough understanding of potential post-transplant complications and their risk factors, along with current diagnostic and management strategies, seem to be crucial regarding patients outcomes.

The most common complications occurring in early period post HSCT are severe and opportunistic infections (bacterial, viral, fungal), primary graft rejection and graft failure, severe organ toxicities, and transplant-related endothelial complications including acute graft versus host disease (aGvHD), veno-occlusive disease (VOD) and thrombotic microangiopathy (TMA).

In my doctoral dissertation, I am focusing on the most transplant-specific and potentially fatal complications, which are aGvHD and VOD. Beside those, by analysis of patients requiring intensive care treatment post HSCT, my doctoral thesis embrace the whole population of patients suffering from various but most severe transplant-related complications.

Acute GvHD remains a great obstacle for the broader use of HSCT. It is currently one of the major causes of both early and late transplant-related mortality (TRM).[1] It affects skin, gastrointestinal tract mucosa and liver.[2] Mainly, the diagnosis is set on clinical findings and should be confirmed by a biopsy, if possible. Grading and staging of GvHD should be performed by pediatric-specific criteria published by *Jacobson et al.* [3]

Once the diagnosis of aGvHD is established, it is difficult to treat. It may be prevented by selected methods, but often at the expense of increased risk of relapse or severe infections due to extensive immunosuppression. Despite prophylaxis, the incidence of aGvHD in children receiving stem cells varies from 30% up to 80%.[4–6] The first line treatment is based mainly on steroids but turns out to be abortive in about 50% of cases. Many agents are currently investigated to have a beneficial effect in the treatment of steroid-refractory aGvHD, but for now, none of them have been proven unequivocally effective.[7,8]

Risk factors for aGvHD in the adult population are well known and include HLA-disparity, the intensity of conditioning regimen, recipients' age and donor-recipient sex mismatch.[4,9] However, the differences between adults and children need to be highlighted, particularly regarding indications for transplant, previous treatment, co-morbidities and transplantation regimens. Therefore, in face of lack of effective second-line treatment, precise evaluation of aGvHD risk factors in pediatric population is crucial.

Veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), is an unpredictable and potentially fatal complication primarily associated with HSCT. It may occur in both allogeneic and autologous HSCT recipients.[10] VOD has also been recognized to occur as a result of high-intensity chemotherapy, radiation and immunotoxin-conjugated therapy in nontransplant settings. [11,12] It belongs to a group of conditions increasingly designated as transplant-related endothelial complications. The pathophysiology of VOD involve primary toxic

injury of the sinusoidal endothelial cells that allows extravascular deposition of blood cells and further endothelium dissection leading to central venous occlusion and sinusoidal obstruction resulting in post-sinusoidal portal hypertension. This process results in the clinical syndrome of VOD consisting of weight gain, ascites, hyperbilirubinemia and hepatomegaly, most commonly occurring within the first 20 days post-transplant.[13–15]

The incidence of VOD in the adult population is about 13.7%, but it may be even threefold higher among children, particularly in the youngest group of patients. Contrarily to the adults, pediatric patients more often present with anicteric and late-onset VOD. [16–19] In face of lack of specific biomarkers and diagnostic tools, the diagnosis of VOD is based on clinical and laboratory findings. For years, two sets of diagnostic criteria were used for VOD diagnosis in children- Baltimore criteria and modified Seattle criteria (Table 1). [14,15] They were both quite strict and most importantly they were primarily designed for adult population; therefore, they were not embracing the crucial differences in VOD between adults and children. This gap was fulfilled by newly proposed, more dynamic, pediatric EBMT criteria for VOD diagnosis (Table 2).[20] The major changes included lack of time of onset of VOD, focus rather on bilirubin kinetics than certain cut-off levels and inclusion of consumptive refractory thrombocytopenia (RT) as a diagnostic criterion. However, those criteria were developed solely on experts' opinion. Therefore its careful evaluation in clinical practice seems to be essential. When diagnosed, VOD might be successfully treated with Defibrotide (DF). Its efficacy was proven particularly in the pediatric population what encouraged the need for proper diagnostic criteria for VOD in children.[21]

Table 1. Comparison of Baltimore and Modified Seattle VOD Diagnostic Criteria

Modified Seattle Criteria	**Baltimore Criteria**
Two of following occurring within 21 days of transplantation:	Bilirubin serum level >2mg/dl and at least two of following within 20 days of transplant:
Hepatomegaly or right upper quadrant pain	Hepatomegaly
Bilirubin serum level >2mg/dl	Ascites
Unexplained weight gain >2% from baseline	Weight gain >5% from baseline

Table 2. EBMT diagnostic criteria for hepatic VOD/SOS in children

No limitations for time onset of VOD/SOS
Presence of two or more of the following[a]: • Unexplained, consumptive, transfusion-refractory thrombocytopenia[b] • Otherwise unexplained weight gain in three consecutive days despite use of diuretics or weight gain >5% baseline • [c]Hepatomegaly (best confirmed by imagining) above baseline • [c]Ascites(best confirmed by imagining) above baseline • Rising bilirubin from a baseline value on three consecutive days or bilirubin >=2mg/dl within 72h
[a]With the exclusion of other potential differential diagnosis; [b]>= on weight-adjustet platelet substitution/day to maintain institutional transfusion guidelines; [c]Imagining immediately before HSCT to determine baseline value

Children receiving stem cells are usually heavily pretreated. They are suffering from various conditioning-related toxicities, and due to extensive immunosuppression they are extremely prone to severe and opportunistic infections. Therefore a relevant number of patients post-HSCT would require treatment in the Pediatric Intensive Care Unit (PICU).[22,23] Admission of HSCT recipients to the PICU has been controversial, as many studies are providing extremely high PICU mortality of those patients, reaching the level of 100% if they need mechanical ventilation (MV).[24,25] However, due to major advances made over the decades, in both transplant protocols and intensive care procedures, a significant improvement regarding the general outcome of those patients has been achieved. Pediatric HSCT recipients, while admitted to the PICU, would need an individually tailored approach as they may not fit into a standard prognosis scheme. There are many transplant-related factors, particularly considering immune reconstitution and graft function that might alter their response to treatment.[26]

Major indications for PICU admission post-HSCT are respiratory failure and septic shock, commonly coexisting. In this profoundly immunocompromised population of patients, severe systemic infections contribute significantly to the need for advanced care measures. There are many studies available, investigating the potential risk factors for death in the PICU. Need for MV and renal replacement therapy, underlying malignant disease, presence of aGvHD and intensity of conditioning regimen seem to be major factors decreasing patients' chances for PICU survival.[27–29] However, there is a serious lack of data considering the relation between graft function and PICU mortality. According to the latest multicenter study, there is no difference in long term overall survival (OS) between PICU survivors and those who did not need advanced care measures. Therefore an intensive quest for evaluating and eliminating the risk factors for PICU mortality seems to be crucial.

WORK OVERVIEW

Premature cyclosporine cessation and TBI-containing conditioning regimen increase the risk of acute GvHD in children undergoing unrelated donor hematopoietic stem cell transplantation.

Acute GvHD is one of the most common and severe complications following HSCT in children, and it was thoroughly analysed in the first paper included in my doctoral dissertation. The aim of the study was to evaluate the risk factors for grades II-IV acute GvHD in children undergoing allogeneic HSCT from an unrelated donor. The study enrolled 237 children; every patient received a standard aGvHD prophylaxis consisted of cyclosporine (CsA) and methotrexate. Various clinical and epidemiological features, including graft composition, conditioning regimens, duration and coherence of GvHD prophylaxis, were analyzed as potential risk factors.

Our results confirmed the magnitude of a problem which GvHD is, as almost 60% of patients in the study cohort developed grades II-IV aGvHD. Multivariate statistical analysis revealed that three of the investigated factors significantly increased the risk for aGvHD.

Myeloablative conditioning regimens consisted of TBI were a major risk factor for aGvHD in the study. Treatment-related tissue damage, exacerbated by TBI, particularly in the gastrointestinal tract, may initiate an inflammatory cascade leading to the development of GvHD. What should be emphasized, myeloablative regimens consisted of busulfan did not correlate with higher aGvHD (similar odds ratio to reduced toxicity and reduced intensity regimens). It may suggest that contrary to the TBI, chemo-based regimens do not increase the risk for aGvHD.

Premature discontinuation of CsA relevantly increased the probability of aGvHD. Almost 20% of patients in the study cohort presented with CsA-associated toxicity and required cessation of the drug administration. Those patients were at much higher risk of developing aGvHD. To date, the optimal prophylactic approach to aGvHD has not yet been established. However, presented results suggest that the role of calcineurin inhibitors as a part of aGvHD preventive strategy may be irreplaceable.

Patients undergoing transplantation before the year 2009 (a median year in the study period) have a higher risk of aGvHD. Additionally, patients receiving stem cells in former years had greater TRM and lower OS. This finding is a simple reflection of the advances that were made during the study period in both transplantation proceedings and supportive care. Furthermore, a better general outcome of patients undergoing HSCT after 2009 might be a result of improvements in aGvHD treatment that were achieved during years.

Other analyzed factors, including donor and recipient age, donor-recipients sex mismatch, amount of infused CD34+ cells, stem cell source and underlying disease, failed to be confirmed as risk factors for aGvHD.

Optimal conditioning regimen strategy and proper aGvHD prophylaxis, including continuous administration od CsA might be crucial regarding the risk for aGvHD and patients' general outcome. According to our results, the decision to withdraw CsA should be taken carefully and avoided if possible.

Factors affecting survival in children requiring intensive care after hematopoietic stem cell transplantation. A retrospective single-center study.

The second paper included in my doctoral thesis is a comprehensive analysis of the most severe of post-transplant complications. The study was a retrospective chart analysis of children undergoing first allogeneic HSCT and was mainly focusing on those who required admission to the PICU within one year post transplantation. The detailed objective of the study was to determine potential factors influencing PICU survival. Various clinical and transplant-related data, including conditioning regimens, indications for intensive care, need for mechanical ventilation, the intensity of applied therapies and quality of hematological recovery were assessed.

During the study period, 58 patients required admission to the PICU, what comprises 8.7% of the entire study cohort and places the admission rate on the lower end comparing to literature.[30,31] Among those, 58.6% of patients died within PICU, while 41.5% were successfully discharged. Amidst PICU survivors, 30 days and 6 months survival rates were 91.7% and 66.7%, respectively, what stands in accordance with the latest studies proving that the history of intensive care treatment does not compromise chances for a long term survival.[32] Performed multivariate analysis revealed two of the investigated factors having a significant impact on PICU survival.

Cardiovascular support is a crucial treatment provided in PICU for critically ill patients. Comprehensive cardiac support, including three or more cardiovascular agents, was applied in almost 30% of patients in the study, of whom only two have survived their PICU stay. The need for extensive cardiac support was a major independent factor decreasing chances for PICU survival.

A little is known about the relationship between graft function and PICU mortality. In the study, absolute granulocyte count (ANC) on the last day of treatment differed relevantly between survivors and non-survivors. A significantly lower ANC was observed in those who died in PICU compared to those who were successfully discharged. Contrary to the last day of treatment, ANC and severe neutropenia at the time of PICU admission had no impact on patients' outcomes. Those results suggest that besides advanced care measures, hematologic reconstitution and graft function may be crucial regarding PICU survival. Achieving adequate ANC during PICU stay may significantly improve patients' chances for survival. Therefore it should be taken into account in advance care planning.

The need for MV is one of the most coherent factors correlating with poor PICU prognosis [24,33] However, our study failed to confirm MV as a risk factor for death within PICU. The result may be affected by the fact that the majority of patients in the study received MV (81%), which is a much higher rate than presented in other studies recognizing MV as a risk factor for death. Beyond those requiring MV, five patients in the PICU cohort were treated with non-invasive ventilation (NIV). None of them required subsequent intubation and all were discharged from PICU and survived over 6 months. By preventing potential complications related to MV, early implementation of NIV may be beneficial in this particular group of patients. However, it should not delay intubation if needed. Although very promising, those results are limited by a meager number of patients. Therefore extensive prospective cohort studies evaluating the usefulness of NIV seem to be essential.

To conclude, the study is emphasizing the equal role of both intensity of applied therapies and graft function in the survival of pediatric HSCT recipients admitted to the PICU. A holistic approach to the patient seems to be crucial regarding the general outcome.

Veno-occlusive disease in children and adolescents after hematopoietic stem cell transplantation: Did the Modified Seattle Criteria fit the characteristics of pediatric population?

The third paper that was a part of a publication series is a detailed analysis of another potentially fatal complication following HSCT, which is VOD. Immediate diagnosis of VOD and prompt implementation of proper treatment is crucial regarding patients' general outcomes. Therefore in the presented study, we aimed to assess the usefulness of the modified Seattle criteria, which were widely used for over 25 years for VOD diagnosis in the pediatric population, particularly reflecting recently published first pediatric EBMT VOD diagnostic criteria.

The study enrolled 850 children and adolescents who underwent HSCT during years 2001-2015. In this study cohort, 48 patients with VOD were recognized by Modified Seattle Criteria. This 5.05% incidence of VOD in our center was placed at the lower end compared to other literature reports. One-third of patients diagnosed with VOD, according to modified Seattle criteria, did not meet the obligatory time criterion (<20 days post-transplant); therefore they were diagnosed with "late-onset VOD". Presenting with VOD later than 20 days post-transplant is more common in children than in adults.[16] Thus, considering a relevant number of late-onset cases, our results indicated that the time criterion for the pediatric population may be useless or even interfering.

Another controversial criterion was hyperbilirubinemia. Although it was not mandatory, it remained an important part of the modified Seattle criteria. Similarly to the relatively later onset of the disease, anicteric course of VOD is more commonly observed in children.[18,19] In our cohort, almost 30% of patients never demonstrated bilirubin level above 2mg/dl, as indicated in modified Seattle criteria. Although elevated bilirubin level is a common VOD symptom, lack of hyperbilirubinemia should not lead to a delay in VOD diagnosis and treatment implementation.

The majority of patients that were diagnosed with VOD in our study demonstrated transfusion-refractory consumptive thrombocytopenia. Most commonly, it was observed shortly before VOD diagnosis was established. Although refractory thrombocytopenia was mentioned in the very first reports about VOD, it has never entered the diagnostic criteria. Our results suggest that this symptom, reflecting the pathophysiology of the disease, might be an early hint to suspect VOD.

To conclude, the study indicates that modified Seattle criteria may not be adequate for the pediatric population. They do not embrace the essential differences in VOD among children and adults. Particularly time criterion and strict bilirubin cut-off level may be harmful by causing unnecessary delay in VOD diagnosis. However, refractory thrombocytopenia, as an essential early symptom of VOD, could contribute significantly to the improvement of the diagnostic criteria.

Introduction of new pediatric EBMT criteria for VOD diagnosis: is it time-saving or money-wasting?; Prospective evaluation of pediatric EBMT criteria for VOD.

The concept of the last paper included in my doctoral thesis arises straight from the previous study considering the adequacy of modified Seattle criteria in the pediatric population. Facing the urge to evaluate the new pediatric EBMT criteria for VOD diagnosis in clinical practice, we have started a prospective cohort study to assess its usefulness and impact on patients' general outcomes. Commencing in January 2016, by March 2019 the study enrolled 283 children undergoing either autologous or allogeneic HSCT. It was focusing particularly on those who were diagnosed with VOD according to the new criteria. The results were compared with the above-mentioned study discussing the utility of modified Seattle criteria.

In the analyzed period, the incidence of VOD was 8.9%; it had almost doubled compared to the time when we used modified Seattle criteria, proving that the new, rather dynamic criteria facilitated the diagnosis of VOD. However, this VOD incidence still equates to the lower end of other literature reports;[16,34] therefore, we do believe that the study does not support the concern of over-diagnosing VOD according to new criteria. To evaluate the differences in criteria sensitivity, we retrospectively applied both modified Seattle and Baltimore criteria to the present cohort. The incidence of VOD was 5.7% and 2.83%, respectively. The higher sensitivity of EBMT criteria also reflects in the time of diagnosis - median diagnosis delay when using modified Seattle criteria was three days. According to ongoing trials evaluating the efficacy of Defibrotide, the timepoint of treatment initiation is crucial regarding the duration of treatment and patients' outcomes.

A novel approach to bilirubin levels proposed in the EBMT criteria may reveal that anicteric course of VOD is more common in children than previously expected. In the study, only 20% of patients presented with hyperbilirubinemia (>2mg%) at the moment of diagnosis. Contrarily, 68% of patients met the criterion of rising bilirubin levels on three consecutive days. Emphasis on careful monitoring of bilirubin levels may be beneficial considering the pathophysiology of the disease, as numerous studies confirm that hyperbilirubinemia is a relatively late symptom of VOD and the criterion of poor outcome. According to study results, a more dynamic approach to bilirubin levels proposed in the EBMT criteria allows pre-emptive intervention and may improve patients' outcomes.

Refractory thrombocytopenia was the only criterion fulfilled by 100% of patients in the study. In the majority of cases, it was the very first symptom of VOD. Although it was mention in the primary reports considering VOD and thereafter repeatedly confirmed in further studies in the adult population, until now it has never become a diagnostic criterion. Our results suggest that RT, even if it is unspecific, might be an early hallmark of VOD, encouraging clinicians to become particularly vigilant for other VOD symptoms.

Even if the study did not focus strictly on the financial aspects, it needs to be highlighted that after implementing the new criteria, the time of hospitalization of VOD patients decreased by a median of 12 days. Although the impact of new criteria on cost-effectiveness requires further research, our results suggest that shortening of both Defibrotide administration and length of hospital stay may be economically beneficial, despite the increase in disease incidence.

To conclude, the new EBMT VOD diagnostic criteria acknowledge the differences in various aspects of the disease between children and adults. Earlier VOD diagnosis, facilitated by EBMT criteria, resulting in implementing immediate treatment, significantly improved patients' outcomes.

STRESZCZENIE

WSTĘP:

Transplantacja komórek hematopoetycznych jest uznaną na całym świecie metodą leczenia wielu chorób, zarówno nowotworowych jak i nienowotworowych. Dzięki postępowi jaki przez lata dokonał się w kwestii doboru dawców, protokołów kondycjonowania, profilaktyki przeciw infekcjom oraz intensywnej terapii, wskaźniki przeżycia po HSCT stale rosną. Jednakże transplantacja nadal pozostaje procedurą wyjątkowo wysokiego ryzyka. Dokładne poznanie i zrozumienie komplikacji, które mogą pojawić się po transplantacji, a także aktualnych wytycznych diagnostycznych i terapeutycznych wydaje sie być szczególnie istotnym czynnikiem mogącym wpłynąć na ostateczne wyniki leczenia pacjentów poddawanych HSCT.

Najczęściej pojawiającymi się powikłaniami we wczesnym okresie po transplantacji są ciężkie i oportunistyczne infekcje (bakteryjne, grzybicze, wirusowe), pierwotne odrzucenie przeszczepu lub jego niewydolność, ciężkie toksyczności narządowe, oraz powikłania związane z uszkodzeniem śródbłonka naczyń, które obejmują ostrą chorobę przeszczep przeciwko gospodarzowi (aGvHD), wenookluzyjna chorobę wątroby (VOD) oraz mikroangiopatię zakrzepową (TMA).

W mojej rozprawie doktorskiej szczególny nacisk kładę na komplikacje ściśle związane z transplantacją- aGvHD oraz VOD. Ponadto, poprzez szeroką analizę pacjentów wymagających intensywnej terapii po HSCT, moja rozprawa doktorska obejmuje szczegółowy przegląd licznych, głównie tych najcięższych powikłań po transplantacji.

aGvHD od lat pozostaje główną przeszkodą przeciwko szerszemu zastosowaniu procedury HSCT. Stanowi obecnie jedną z najczęstszych przyczyn zarówno wczesnej jak i późnej śmiertelności związanej z transplantacją (transplant-related mortality, TRM).[1] Narządowa manifestacja aGvHD obejmuje skórę, błonę śluzową przewodu pokarmowego oraz wątrobę.[35] Diagnoza aGvHD stawiana jest głównie na podstawie objawów klinicznych, aczkolwiek o ile to możliwe powinna zostać potwierdzona badaniem histopatologicznym. Ogólne oraz narządowe zaawansowanie choroby powinno być oceniane w oparciu o pediatryczne kryteria opublikowane przez *Jacobson et al.* [3]

Istnieje kilka uznanych strategii profilaktycznych wobec aGvHD, jednakże każda z nich niesie za sobą dodatkowe konsekwencje, najcześciej w postaci zwiększonego ryzyka wznowy oraz większej podatności na ciężkie infekcje. Pomimo profilaktyki, aGvHD rozwija się u około 50-80% dzieci po przeszczepie.[4–6] W leczeniu pierwszego rzutu stosowane są głównie sterydy w dużych dawkach, jednak nawet w 50% przypadków aGvHD pozostaje sterydooporne. Od wielu lat trwają intensywne badania nad ustaleniem skutecznego postępowania wobec sterydoopornego aGvHD, pomimo tego na dzień dzisiejszy żadna ze strategii terapeutycznych nie została jednoznacznie uznana za efektywną.[7,8]

Liczne czynniki ryzyka aGvHD zostały wielokrotnie opisane u dorosłych pacjentów poddawanych transplantacji. Najczęściej wymieniane z nich to kondycjonowanie mieloablacyjne, niezgodność w układzie HLA, wiek biorcy oraz niezgodność płci między dawcą a biorcą (zwłaszcza żeński dawca dla męskiego biorcy).[4,9] Należy jednak podreślić istotne różnice między populacją dorosłych a populacją pediatryczną, głównie pod względem wskazań do transplantacji, wcześniejszego leczenia, chorób współistniejących oraz postępowania okołotransplantacyjnego. Stąd też, wobec braku skutecznego leczenia drugiego rzutu oraz ograniczonej ilości danych na temat czynnikow ryzyka aGvHD u dzieci, dokładna ocena oraz analiza czynników ryzyka aGvHD w populacji pediatrycznej wydaje się być niezbędna.

VOD jest trudną do przewidzenia oraz potencjalnie śmiertelną chorobą pierwotnie związaną z HSCT. Może wystąpić zarówno po transplantacji allogenicznej jak i autologicznej.[10] W bardzo rzadkich przypadkach, VOD może również rozwinąć się niezależnie od tranplantacji, w wyniku intensywnej chemioterapii, radioterapii lub leczenia przeciwciałami monoklonalnymi o działaniu immunotoksycznym.[11,12] Patofizjologia VOD obejmuje pierwotne toksyczne uszkodzenie śródbłonka zatok wątrobowych co pozwala na okołonaczyniowe odkładanie się krwinek, nasilające dalsze rozwarstwianie śródbłonka. W kolejnym etapie prowadzi to do zwężenia światła naczyń, aktywacji procesów zakrzepowych, niedrożności zatok wątrobowych oraz rozwoju nadciśnienia wrotnego. W wyniku tego procesu dochodzi do rozwoju zespołu objawów klinicznych składających się na VOD i obejmujących hepatomegalię, wodobrzusze, wzrost wagi oraz hiperbilirubinemie; objawy standardowo pojawiają się w przeciągu pierwszych 20 dni po transplantacji.[13–15]

Częstośc występowania VOD w populacji dorosłych określana jest na 13.7%, natomiast u dzieci może być trzykrotnie większa osiągając nawet 60% w populacjach wysokiego ryzyka (np. U niemowląt, głównie ze względu na niedojrzałośc wątroby). Ponadto u dzieci znacznie częściej obserwowany jest późniejszy początek objawów VOD (powyżej 20 dni po HSCT) jak i brak znacznego wzrostu bilirubiny w przebiegu choroby.[16–19]

Wobec braku specyficznych dla VOD biomarkerów, diagnoza stawiana jest przede wszystkim na podstawie objawów klinicznych i podstawowych badań laboratoryjnych. Przez ostatnie 25 lat, dwa zestawy kryteriów były wykorzystywane w diagnostyce VOD u dzieci- kryteria z Baltimore oraz zmodyfikowane kryteria z Seattle (Tabele 1 i 2).[14,15] Oby dwa zestawy kryteriów były wyjątkowo rygorystycze, głównie pod względem czasu pojawienia się objawów VOD jak i poziomu bilirubiny. Przede wszystkim jednak, powyższe kryteria zostały pierwotnie dedykowane pacjentom dorosłym toteż nie uwzględniały kluczowych różnic w przebiegu VOD między populacją dorosłych a pacjentami pediatrycznymi. Ta istotna luka została wypełniona przez nowe pediatryczne kryteria VOD zaporponowane przez EBMT (European Society for Bone and Marrow Transplantation).[20] Główne modyfikacje zawarte w kryteriach EBMT obejmują brak ograniczeń czasowych w rozpoznaniu VOD, nacisk na dynamikę zmian w poziomie bilirubiny bez konieczności osiągnięcia konkretnego poziomu oraz włączenie opornej małopłytkowości ze zużycia (RT- refractory thrombocytopenia) jako kryterium diagnostycznego. Powyższe kryteria, jakkolwiek obiecujace, zostały stworzone wyłącznie na podstawie wiedzy i doświadczenia grupy ekspertów w dziedzinie VOD. Ich czułość została później kilkukrotnie potwierdzona w badaniach retrospektywnych, jednakże dokładna, prospektywna ocena ich przydatności w praktyce klinicznej wydaje się być niezbędna.[21]

Pacjenci w momencie przystępowania do procedury HSCT, mają za sobą zazwyczaj dlugie i intensywne leczenie. Po transplantacji, zmagają się z licznymi toksycznościami narządowymi wynikającymi z wcześniejszego leczenia oraz kondycjonowania przed transplantacją. Ze względu na intensywne leczenie immunosupresyjne, są szczególnie podatni na ciężkie i oportunistyczne infekcje. Z tego względu, znaczna liczba dzieci po HSCT wymaga hospitalizacji w Oddziale Intensywnej Terapii Dziecięcej (OITD).[22,23] Wobec dostępnych w literaturze doniesień o bardzo wysokiej, sięgającej nawet 100%, śmiertelności pacjentów po HSCT wymagających hospitalizacji w OIT, wskazania do intensywnej terapii dzieci po transplantacji przez lata pozostawały kontrowersyjne. [24,25] Jednakże, dzięki postępowi jaki dokonał się w ostatnich latach, zarówno w dziedzinie transplantologii jak i intensywnej terapii, obserwuje się znacznie lepsze wyniki leczenia oraz poprawę rokowania dla pacjentów wymagających intensywnej terapii po przeszczepie. Nalezy pamiętać jednak, że dzieci po HSCT, hospitalizowane w OITD mogą wymagać odrębnych, specyficznych protokołów postępowania. Istnieje wiele czynników, zwłaszcza w zakresie

odnowy hematologicznej oraz funkcji szpiku, które mogą znacząco wpłynąć na ich odpowiedź na leczenie.[26]

Głównymi wskazaniami do leczenia w OITD po HSCT są między innymi niewydolność oddechowa oraz wstrząs septyczny, nierzadko ze sobą współistniejące. Wobec konieczności leczenia immunosupresyjnego, ciężkie układowe infekcje pozostają istotym powikłaniem po przeszczepie, w wielu przypadkach wymagające intensywnego, wielokierunkowego leczenia. W literaturze dostępne są liczne badania analizujące wpływ rozmaitych czynników na przeżycie pacjentów po transplantacji wymagających hospitalizacji w OITD. Konieczność wentylacji mechanicznej i terapii nerkozastępczej, choroba nowotworowa, współistnienie aGvHD oraz intensywnośc kondycjonowania wydają się być głównymi czynnikami zmniejszającymi szanse na przeżycie w OITD.[27–29] Jednakże należy zwrócić uwagę na brak doniesień analizujących wpływ odnowy hematologicznej lub wydolności szpiku na śmiertelność dzieci po transplantacji wymagających intensywnej terapii. Wobec najnowszych, wieloośrodkowych badań, dowodzących iż brak jest różnicy w długoterminowym przeżyciu między pacjentami, którzy zostali wypisani z OITD a tymi którzy nie wymagali intensywnej terapii, konieczne jest dokładne określenie czynników wpływających na przeżycie w OITD dzieci po HSCT.

OMÓWIENIE PRAC

Premature cyclosporine cessation and TBI-containing conditioning regimen increase the risk of acute GvHD in children undergoing unrelated donor hematopoietic stem cell transplantation.

W pierwszej publikacji, która wchodzi w skład mojej rozprawy doktorskiej, szczegółowej analizie została poddana jedna z naczęstszych i najcięższych komplikacji po HSCT jaką jest aGvHD. Głównym celem badania było dokładne określenie czynników ryzyka wystąpienia aGvHD u dzieci po transplantacji komórek hematopoetycznych od dawcy niespokrewnionego. Badaniem objęto 237 pacjentów; każdy z nich otrzymał standardową profilaktykę przeciwko aGvHD składającą się z cyklosporyny (CsA) oraz metotreksatu. Liczne czynniki epidemiologiczne i kliniczne, zwłaszcza protokoły kondycjonowania, zawartość materiału przeszczepowego, czas trwania i spójność profilaktyki wobec aGvHD były analizowane jako potencjalnie wpływające na ryzyko pojawienia się aGvHD po HSCT.

Częstość występowania aGvHD (II-IV) w badanej populacji wynosiła prawie 60%. Podkreśla to powszechność i skalę problemu jakim we wczesnym okresie po transplantacji jest aGvHD. Przeprowadzona wieloczynnikowa analiza statystyczna ujawniła trzy czynniki niezależnie wpływające na ryzyko rozwoju aGvHD po HSCT.

Pacjenci poddani mieloablacyjnemu kondycjonowaniu opartemu na naświetlaniu całego ciała (TBI-total body irradiation) znacznie częściej prezentowali objawy aGvHD we wczesnym okresie po HSCT. Istotnym wynikiem był również brak wzrostu ryzyka wystapienia aGvHD u pacjentów otrzymujących kondycjonowanie mieloablacyjne oparte na Busulfanie (podobne ryzyko jak w przypadku protokołów o zmniejszonej intensywności). Powyższe wyniki sugerują, że w przeciwieństwie do TBI, protokoły mieloablacyjne oparte na wysokodawkowej chemioterapii nie wpływają istotnie na ryzyko rozwoju aGvHD po HSCT.

Przedwczesne odstawienie CsA znacząco zwiększało prawdopodobieństwo wystąpienia aGvHD. W grupie badanej, prawie 20% pacjentów zaprezentowało ciężkie objawy niepożądane związane z podażą CsA i wymagało natychmiastowego odstawienia leku. Optymalna strategia profilaktyczna wobec aGvHD, mimo dostępności wielu potencjalnie skutecznych preparatów, nadal nie została jednoznacznie ustalona. Jednakże wyniki powyższego badania sugerują iż, rola inhibitorów kalcyneuryny w profilaktyce aGvHD może być niezastąpiona.

Pacjenci poddani transplantacji przed rokiem 2009 znacznie częściej prezentowali objawy aGvHD niż ci poddani transplantacji w późniejszych latach. Ponadto, wcześniejsze HSCT wiązały się ze znacznie mniejszą przeżywalnością pacjentów i wyższym TRM. Jest to bezpośrednie odzwierciedlenie postępów zarówno w protokołach transplantacyjnych jak i dobrze dawców, jakie dokonały się w czasie, który był objęty badaniem.

Pozostałe analizowane czynniki takie jak wiek dawcy i biorcy, niezgodność płci między dawcą i biorcą, ilość przetoczonych komórek CD34+, źródło materiału przeszczepowego oraz wskazania do transplantacji okazały się nie mieć wpływu na występowanie aGvHD po HSCT.

Dobór optymalnego protokołu kondycjonowania oraz właściwa strategia profilaktyczna wobec aGvHD, zakładająca ciągłą podaż CsA wydają się mieć kluczowy wpływ zarówno na ryzyko wystąpienia aGvHD jak i ostateczne wyniki leczenia pacjentów po HSCT. Powyższe wyniki sugerują iż decyzja o odstawieniu CsA powinna być podejmowana ze szczególną rozwagą.

Factors affecting survival in children requiring intensive care after hematopoietic stem cell transplantation. A retrospective single-center study.

Kolejna publikacja będąca częścią mojej rozprawy doktorskiej obejmuje wielokierunkową analizę pacjentów wymagających leczenia w OITD w przeciągu pierwszego roku po transplantacji komórek hematopoetycznych. Szegółowym celem badania było określenie potencjalnych czynników mogących wpływać na przeżywalność pacjentów hospitalizowanych w OITD. Szereg zmiennych, obejmujący wskazania do leczenia w OITD, protokoły kondycjonowania, intensywność wymaganego leczenia, konieczność wentylacji mechanicznej oraz jakość odnowy hematologicznej analizowano pod kątem potencjalnego wpływu na śmiertelność pacjentów po HSCT. Badaniem objęto wszystkie dzieci poddane transplantacji allogenicznej w latach 2005-2017 (n=668).

Spośród pacjentów włączonych do badania, prawie 9% (n=58) wymagało leczenia w OITD. Jest to niski odsetek w porównaniu do pozostałych badań dostępnych w literaturze.[30,31] Spomiędzy pacjentów hospitalizowanych w OITD, 58.5% zmarło. Wśród tych, którzy zostali wypisani z OITD, 91.7% przeżyło kolejne 30 dni, a 66.7% kolejne 6 miesięcy po wypisie. Otrzymane satysfakcjonujące wyniki dotyczące przezycia po wypisie z OITD, są zgodne z najnowszymi wieloośrodkowymi badaniami dowodzącymi iż wcześniejszy podbyt na OITD nie wpływa znacząco na długoterminowe przeżycie pacjentów po HSCT.

Farmakologiczne wspomaganie układu krążenia jest jedną z głównych form leczenia stosowanych w OITD. Złożona terapia sercowo-naczyniowa, zawierająca co najmniej 3 leki o działaniu intropowym lub wazokonstrykcyjnym była stosowana u 30% pacjentów wymagających leczenia w OITD w grupie badanej. Spośród nich tylko dwoje zostało wypisanych z OITD. W przeprowadzonej wieloczynnikowej analizie, konieczność wdrożenia kompleksowego wspomagania układu krążenia była niezależnym czynnikiem ryzyka śmierci pacjentów po HSCT wymagających intensywnej terapii. Drugim i ostatnim niezależnym czynnikiem ryzyka śmierci w OITD był poziom granulocytów (-(absolute neutrophile count - ANC) w ostatnim dniu leczenia. W literaturze dostępne są jedynie sporadyczne doniesienia na temat zależności między odnową hematologiczną i funkcją szpiku a przezyciem w OITD. W omawianym badaniu znacznie niższe poziomy ANC obserwowane były u pacjentów którzy zmarli w OITD w porównaniu do pacjentów wypisanych z oddziału. W przeciwieństwie do ANC w ostatnim dniu leczenia, poziom granulocytów oraz ciężka neutropenia w dniu przyjęcia do OITD nie miały znaczącego wpływu na śmiertelność pacjentów. Powyższe wyniki podkreślają konieczność indywidualnego podejścia terapeutycznego do dzieci wymagających intensywnej terpii po HSCT, ponieważ osiągnięcie przez nich odnowy hematologicznej i odpowiedniego poziomu ANC może znaczne zmienić ich odpowiedź na leczenie oraz dalsze rokowania.

Najbardziej typowym i najczęściej opisywanym w literaturze czynnikiem pogarszającym przeżycie w OITD pacjentów po HSCT jest konieczność stosowania wentylacji mechanicznej, co nie zostało to potwierdzone w omawianym badaniu.[24,36] W publikacji zaprezentowano za to grupę 5 pacjentów skutecznie leczonych za pomocą wentylacji nieinwazyjnej (NIV), którzy nie wymagali następowej intubacji i zostali wypisani z OITD. Jakkolwiek ograniczone niewielką liczbą przypadków, wyniki te sugerują, iż odpowiednio wcześnie zastosowany NIV może być skuteczną metodą wsparcia układu oddechowego w tej grupie pacjentów.

Wobec porównywalnie istotnego wpływu zarówno funkcji szpiku jak i intensywności leczenia na śmiertelność w OITD, rezultaty powyższego badania podkreślają istotę holistycznego podejścia do pacjentów pediatrycznych wymagających intensywnej terapii po HSCT.

Veno-occlusive disease in children and adolescents after hematopoietic stem cell transplantation: Did the Modified Seattle Criteria fit the characteristics of pediatric population?

Trzecia z publikacji wchodzących w skład cyklu stanowi szczegółowy raport na temat VOD, jednej z najcięższych komplikacji mogących pojawić się we wczesnym okresie po transplantacji. Ponadto, oprócz dogłębnej analizy pacjentów cierpiących na VOD, w badaniu zakwestionowano również obowiązujące ówcześnie kryteria diagnostyczne VOD. Czy rzeczywiście zmodyfikowane kryteria z Seattle były odpowiednim narzędziem do diagnostyki VOD u dzieci?

Retrospektywne badanie objęło 951 procedur HSCT wykonanych u 850 pacjentów pediatrycznych na przestrzeni lat 2001-2015. Spośród wszystkich pacjentów, u 48 zdiagnozowano VOD za pomocą zmodyfikowanych kryteriów diagnostycznych z Seattle. W porównaniu do innych dostępnych w literaturze doniesień na temat VOD, rozpoznawalność tego powikłania na poziomie 5.05% okazała się być wyjątkowo niska. Ponadto, jedna trzecia pacjentów z VOD nie spełniła obowiązkowego kryterium czasu (rozpoznanie poniżej 20 dnia po HSCT), stąd też zostali sklasyfikowani jako „VOD o późnym początku" (late-onset VOD). Późniejszy rozwój objawów VOD jest istotnie częściej obserwowany u dzieci w porównaniu z dorosłymi pacjentami po transplantacji.[16] Stąd też, biorąc pod uwagę znaczną liczbę przypadków zdiagnozowanych ponad 20 po HSCT, wyniki powyższej analizy sugerują, iż kryterium czasu wydaje się być bezużyteczne lub wręcz niekorzystne w rozpoznawaniu VOD w populacji pediatrycznej.

Kolejnym kontrowersyjnym kryterium diagnostycznym jest hiperbilirubinemia. Mimo iż nie jest to kryterium obligatoryjne, hiperbilirubinemia pozostaje istotnym elementem zmodyfikowanych kryteriów z Seattle. Podobnie jak relatywnie późniejszy początek choroby, przebieg VOD bez istotnego wzrostu bilirubiny jest znacznie częściej obserwowany u dzieci niż u dorosłych.[18,19] Ponad 30% pacjentów w badanej grupie nie osiągnęło poziomu bilirubiny sugerowanego w zmodyfikowanych kryteriach z Seattle (>2mg%). Wzrost poziomu bilirubiny jest oczywiście typowym objawem VOD, jednakże brak hiperbilirubinemii nie powinien opóźniać właściwej diagnozy oraz włączenia odpowiedniego leczenia.

Większość pacjentów z VOD w badanej populacji prezentowała nawracającą trombocytopenię ze zużycia (refractory thrombocytopenia-RT), niereagującą na powtarzane trasfuzje koncentratu krwinek płytkowych. RT najczęściej była obserwowana krótko przed ostatecznym postawieniem diagnozy VOD. Mimo, iż omawiana była jako potencjalny objaw już w pierwszych doniesieniach dotyczących VOD, nigdy nie została uznana za kryterium diagnostyczne. Wyniki powyższego badania dowodzą, że RT może być bardzo wczesnym sygnałem sugerującym możliwość rozwoju VOD.

Podsumowując, zmodyfikowane kryteria z Seattle nie wydają się być właściwym narzędziem diagnostycznym VOD w populacji pediatrycznej, głównie ze względu na pominięcie istotnych różnic w przebiegu VOD między dziećmi a pacjentami dorosłymi. Zwłaszcza ograniczenie rozpoznania do 20 po przeszczepie oraz restrykcyjne podejście do poziomu bilirubiny wydają się być szkodliwe, powodując niepotrzebne opóźnienie diagnozy oraz ryzyko pominięcia chorych o łagodnym lub umiarkowanym przebiegu choroby.

Introduction of new pediatric EBMT criteria for VOD diagnosis: is it time-saving or money-wasting? : Prospective evaluation of pediatric EBMT criteria for VOD.

Kolejne badanie będące częścią mojej rozprawy doktorskiej, wynika bezpośrednio z poprzedniej publikacji oceniającej użyteczność zmodyfikowanych kryteriów z Seattle w populacji pediatrycznej. Konieczność pilnej oceny nowych pediatrycznych kryteriów VOD zaproponowanych przez EBMT w praktyce klinicznej poskutkowała rozpoczęciem w styczniu 2016 roku prospektywnego badania analizującego przydatność nowych kryteriów i ich potencjalny wpływ zarówno na ostateczne wyniki leczenia pacjentów, jak i na aspekt ekonomiczny. Do marca 2019 badaniem objęto 282 dzieci, szczegółowo analizując tych pacjentów, u których zdiagnozowano VOD zgodnie z nowymi kryteriami.

W czasie objętym badaniem, częstość występowania VOD wyniosła 8.9%. Jest to prawie dwukrotnie więcej niż w czasie kiedy używane były zmodyfikowane kryteria z Seattle, co dowodzi temu, iż mniej restrykcyjne, a raczej dynamiczne kryteria diagnostyczne ułatwiły rozpoznawanie VOD. Jakkolwiek, częstość występowania tego powikłania na poziomie niecałych 9% jest nadal wyjątkowo niska w porownaniu do innych doniesień dostępnych w literaturze- stąd też, powyższe wyniki raczej nie potwierdzają obaw dotyczących ryzyka nadrozpoznawania VOD dzięki mniej restrykcyjnym kryteriom.[16,34] Aby podkreślić większą czułość nowych kryteriów, przeprowadzono retrospektywną symulację z uzyciem kryteriów z Baltimore oraz zmodyfikowanych kryteriów z Seattle. Nie tylko rozpoznawalnośc VOD przy użyciu starych kryteriów była zdecydowanie niższa, ale także obserwowane było zdecydowane opóźnienie diagnozy w porównaniu do kryteriów EBMT. Zgodnie z najnowszymi badaniami oceniającymi skuteczność Defibrotydu u dzieci, nawet jednodniowe opóźnienie w rozpoczeciu leczenia może znacząco zmniejszych szanse pacjenta na przeżycie.

Odmienne podejście do zmian w poziomie bilirubiny jako symptomu VOD, prezentowane w nowych kryteriach, ujawniło iż przebieg VOD bez hiperilirubinemii może być jeszcze częstszy niż uprzednio oczekiwano. Tylko 20% pacjentów w grupie badanej osiągneło poziom bilirubiny powyżej 2mg/dL w chwili diagnozy VOD, natomiast prawie 70% pacjentów spełniło kryterium dynamicznego wzrostu poziomu bilirubiny przez trzy kolejne dni (bez konieczności osiągnięcia konkretnego poziomu). Dokładne monitorowanie poziomu bilirubiny z szczególnym uwzględnieniem jego kinetyki wydaje się być bardziej przydatne we wczesnym rozpoznawaniu VOD, zwłaszcza wobec licznych badań dowodzących iż hiperbilirubinemia jest raczej późnym objawem VOD oraz złym czynnikiem prognostycznym. RT była najwcześniej obserwowanym kryterium VOD i jedynym które zostało spełnione przez 100% pacjentów w badaniu. Mimo, iż na przestrzeni lat liczne badania dotyczące VOD w populacji dorosłych wskazywały na RT jako istotny objaw choroby, nigdy nie została ona włączona do kryteriów diagnostycznych. Obecnie, RT, będąc prostym odzwierciedleniem procesów patofizjologicznych odpowiedzialnych za VOD, wydaje się być najwcześniejszą i wyjątkowo istotną wskazówką sugerującą rozwój VOD.

Mimo iż w omawianym badaniu nie analizowano szczegółowego wpływu nowych kryteriów na koszty leczenia, wydaje się być istotnym fakt, iż wprowadzenie nowych kryteriów umożliwiło skrócenie czasu hospitalizacji pacjentów z VOD średnio o 12 dni.

Podsumowując, nowe pediatryczne kryteria VOD zaproponowane przez EBMT, dzięki wysokiej czułości pozwalają na wczesną diagnozę oraz natychmiastowe rozpoczęcie terapii, co znacząco poprawiło generalne wyniki leczenia pacjentów. Ponadto, dzięki możliwości skrócenia czasu hospitalizacji, nowe kryteria diagnostyczne wydają się mieć szoroko pojęty, korzystny wpływ na pacjentów cierpiących na VOD po HSCT.

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Premature cyclosporine cessation and TBI-containing conditioning regimen increase the risk of acute GvHD in children undergoing unrelated donor hematopoietic stem cell transplantation

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Abstract

Background. Acute graft-versus-host disease (aGvHD) is a potentially fatal complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Identifying its risk factors would enable the proper prophylaxis and management, which may significantly improve the general outcome of children treated with HSCT.

Objectives. The aim of this single-center, retrospective cohort study was to assess the potential risk factors for grades II–IV of aGvHD in children after the 1st allo-HSCT from an unrelated donor (UD), performed as a result of an underlying malignant disease.

Material and methods. From among patients who received HSCT in our center in the years 2004–2015, 237 were included in the study cohort. All the patients received standard aGvHD prophylaxis consisting of cyclosporine (CsA) and a short course of methotrexate (MTX). Various clinical and epidemiological features, the transplant proceedings, graft composition, conditioning regimens, as well as the duration and coherence of aGvHD prophylaxis were analyzed as potential risk factors for aGvHD.

Results. The incidence of II–IV aGvHD in the study cohort was 58.6%. The median time of the diagnosis of aGvHD was 18 days post-HSCT. In the multivariate analysis, risk factors significantly associated with grades II–IV of aGvHD were: myeloablative conditioning regimen containing total body irradiation (TBI-MAC) (RR (relative risk): 1.69; $p = 0.03$), premature termination of CsA administration due to its toxicity (RR: 1.99; $p = 0.0003$) and HSCT performed before the year 2009 (RR: 1.97; $p = 0.0001$). Donor and recipient age, donor–recipient sex mismatch, stem cell source, risk of disease, and amount of infused CD34+ cells seem to be insignificant as risk factors for aGvHD. The overall survival (OS) of patients with aGvHD was noticeably worse that in those who were aGvHD-free: 60.8% vs 74.1% ($p = 0.08$).

Conclusions. The conditioning regimen and the proper aGvHD prophylaxis, including continuous CsA administration, have a major impact on aGvHD occurrence. According to our results, the termination of CsA therapy should be carefully considered, and avoided if possible.

Key words: risk factors, acute graft-versus-host disease, hematopoietic stem cell transplantation, prophylaxis

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Introduction

Acute graft-versus-host disease (aGvHD) remains one of the most common life-threatening complications after allogeneic hematopoietic stem cells transplantation (allo-HSCT), contributing significantly to morbidity and mortality.[1] Several studies have reported numerous risk factors associated with increased incidence of aGvHD in the adult population. Among them, human leukocyte antigen (HLA) mismatch, type of the conditioning regimen, female donor to male recipient, and higher recipient age have proven to be the most valid.[2–6] However, it needs to be highlighted that pediatric HSCTs differ remarkably from those in the adult population, particularly regarding the indications for transplant, existing comorbidities, previous treatment, and transplantation regimens.

In the pediatric population, the reported incidence of aGvHD varies from 30% to 80%, despite given prophylaxis.[2,7–10] First-line treatment for aGvHD is based mostly on high-dose steroids and turns out to be abortive in about 50% of cases. There are many possible second-line treatment agents and protocols, but, even when providing promising results, none of them have been proven unequivocally effective.[11,12] On that account, not only is an intensive quest for successful second-line treatment for aGvHD needed, but first and foremost, a precise evaluation of its risk factors, especially for a pediatric cohort of patients.

The aim of this single-center, retrospective cohort study was to assess which potential risk factors have a significant influence on the frequency of grades II–IV of aGvHD in children with a malignant disease after the 1st HSCT from an unrelated donor (UD). An analysis of the general outcome, overall survival (OS) and transplant-related mortality (TRM) in the studied cohort was the secondary aim of this research.

Patients and methods

A retrospective analysis of the medical records of the patients who underwent HSCT in the Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation of Wroclaw Medical University (Poland) in the years 2004–2015 was performed. According to the inclusion criteria, all patients under 21 years who were suffering from a malignant disease and underwent the 1st allo-HSCT procedure from an UD were considered in this study. Due to their small number, patients who received cord blood were excluded from the study. The final cohort included a total amount of 237 children and young adults (Table 1).

The grafted cells were obtained from human leukocyte antigen (HLA)-allele matched or mismatched UDs (age: 19–56 years; median age: 31 years). The HLA typing was performed at the high-resolution level (4 digits) in A*, B*, Cw*, DRB1*, and DQB1* alleles. A matched donor was defined as 10/10 or 9/10 HLA-compatible donor. More than 1 mismatched allele was defined as a mismatched unrelated donor (MMUD). According to the ALL-SCT BFM International 2008 criteria, all 10 alleles were high-resolution types.

Table 1. Study cohort characteristics and analyzed risk factors for aGvHD

Patients' characteristics	n	%
Diagnosis		
acute leukemia	162	68.4
chronic myeloblastic leukemia	28	11.8
myelodysplastic syndrome	32	13.5
lymphoma	15	6.3
Patient sex		
male	148	6.3
female	89	37.6
Donor–recipient sex mismatch		
matched	142	59.9
male to female	44	18.6
female to male	51	21.5
Patient age [years]		
≤5	53	22.4
>5 and ≤15	129	54.4
>15	55	23.2
Donor age [years] (median: 31)		
≤median	123	51.9
>median	114	48.1
Amount of CD34+ cells (median: 8.29 × 10^6/kg)		
≤median	119	50.2
>median	118	49.8
Amount of CD34+ cellls (quartile – cut-off value)		
Q1 (5.01 × 10^6/kg)	60	25.3
Q2 (8.29 × 10^6/kg)	59	24.9
Q3 (12.09 × 10^6/kg)	60	25.3
Q4 (51.85 × 10^6/kg)	58	24.5
Stem cells source		
peripheral blood	201	84.8
bone marrow	36	15.2
Conditioning regimen		
TBI-MAC	84	35.4
Bu-MAC	105	44.3
other (RTC/RIC)	48	20.3
Premature cessation of CsA		
yes	47	19.8
no	190	80.2
Risk of disease		
high	115	48.5
standard	122	51.5
Year of transplantation (median: 2009)		
≤2009	126	53.2
>2009	111	46.8
Year of transplantation (quartile)		
Q1 (2004–2006)	72	30.4
Q2 (2007–2009)	54	22.8
Q3 (2010–2012)	58	24.5
Q4 (2013–2015)	53	22.3

TBI-MAC – myeloablative conditioning regimen containing total body irradiation; Bu-MAC – myeloablative conditioning regimen containing busulfan; RTC – reduced-toxicity conditioning; RIC – reduced-intensity conditioning; CsA – cyclosporine.

Graft-versus-host disease prophylaxis

Within the standard prophylaxis for aGvHD, all patients, since the day before HSCT received intravenous cyclosporine (CsA) in a unified initial dose of 1.5 mg/kg twice per day in 2-hour infusions. Further dosage of CsA was adjusted to the CsA trough level (target level: 100–200 µg/L), measured every second day in the majority of patients. Therefore, the dosage was modified to maintain the target level. Cyclosporine was switched into oral formulation when patient was able to tolerate oral intake. According to standard protocols, in patients without signs of GvHD, CsA administration was discontinued on day 120 in the majority of cases, preceded by gradual dose reduction. The 2nd prophylactic agent, methotrexate (MTX), was administered threefold in a standard dose of 10 mg/m^2 on days 1, 3 and 6 after HSCT. All patients were given in vivo T-cell depletion by either rabbit anti-thymocyte globulin (ATG) (n = 229) (ATG-Fresenius/Grafalon® (Neovii Biotech GmbH, Gräfelfing, Germany) – median: 45 mg/kg; or Thymoglobuline® (Genzyme Europe B.V., Naarden, the Netherlands) – median 7.5 mg/kg) or Campath-1H (Genzyme Europe BV) (n = 8) (median: 1 mg/kg).

Acute graft-versus-host disease diagnosis and staging

The diagnosis of aGvHD was based on the clinical findings and/or histopatological findings in the skin, gastrointestinal tract mucosa and liver biopsies.[13–15] Grading and staging of aGvHD was performed using pediatric-specific criteria published by Jacobsohn.[15] In the presented research, only grades II–IV were considered. Patients who presented symptoms of aGvHD after donor lymphocyte infusion were excluded from this study.

Risk factors and definitions

Potential risk factors for developing aGvHD were carefully analyzed and are listed in Table 2. The patients' risk status was defined by our own study-specific modification of the classification proposed by Meisel et al.[16] Acute leukemia (AL) in 1st complete remission (1CR), chronic myeloblastic leukemia (CML) in chronic phase (CP), myelodysplastic syndrome-refractory cytopenia (MDS), and non-Hodgkin lymphoma (NHL) in complete remission (CR) was qualified as a standard risk. Acute leukemia in ≥2CR or non-remission, CML in equal to or greater than accelerating phase, MDS-refractory anemia with an excess of blasts, NHL in non-remission, and juvenile myelomonocytic leukemia (JMML) were defined as a high risk. Early termination of CsA administration was defined as termination before day 60 post-HSCT due to the toxicity of CsA. Cyclosporine-induced nephrotoxicity was diagnosed according to the following criteria: fall in baseline glomerular filtration rate – GFR >25% or doubling the serum creatinine level.[17] Neurotoxicity was defined as seizures, polyneuropathy, ataxy, impaired consciousness, or dizziness.[18] Children with other obvious causes of neuro- and nephrotoxicity were excluded. Only patients who presented first symptoms of aGvHD after the cessation of CsA administration were considered under the influence of this risk factor.

Conditioning regimens

As many as 189 patients received myeloablative conditioning regimen (MAC) based either on myeloablative doses (12.8–19.6 mg/kg) of busulfan (Bu-MAC; n = 105), or on total body irradiation (TBI) at a median dose of 12 Gy (TBI-MAC; n = 84). Thirty-eight patients received reduced-toxicity conditioning (RTC), consisting mainly of fludarabine (160 mg/m^2) and treosulfan (36–42 mg/kg) with thiotepa (10 mg/kg), melphalan (140 mg/m^2) or cyclophosphamide (120 mg/kg). Ten patients received reduced-intensity conditioning (RIC) regimen, consisting of fludarabine with either melphalan or low, non-myeloablative doses of busulfan (2 mg/kg).[19,20] In the statistical analysis, patients receiving RIC and RTC were considered collectively as 1 group.

Statistical analysis

The statistical analysis was performed using R statistical software (https://www.r-project.org/). Variables were analyzed in terms of their prognostic impact on aGvHD, OS and TRM. First, powered by the χ^2 test, we compared the baseline characteristics of patients with II–IV aGvHD and those with either no aGvHD or grade I aGvHD. Thereafter, patient data was entered into a competing risk regression model. We prosecuted 5 variants of regression models, containing either all or only the significant factors. Using the Bayesian Information Criterion (BIC) test, we selected the most adequate model. Acute graft-versus-host disease was defined by the abovementioned criteria, analyzed as time to event with death, relapse or rejection without aGvHD as a competing event. The p-values <0.05 were considered significant. The survival analysis was performed using the Kaplan–Meier estimation and the survival graphs were compared using the log-rank test. Since aGvHD is a time-dependent variable, we applied the landmark analysis at day 100 to the poor-prognosis group in order to avoid bias connected with autoselection. All patients who died or did not undergo a follow-up before day 100 were deleted from the survival analysis.

Results

A total of 237 patients underwent the 1st allo-HSCT from an UD. Two hundred thirty patients received stem cells from matched donor and 7 patients received stem cells

from MMUD (8/10). One hundred thirty-nine patients (58.6%) developed aGvHD stage II–IV within 100 days post-HSCT. The time of aGvHD diagnosis varied from 5 days post-HSCT up to 92 days post-HSCT (median: 18 days). Seventy-eight patients (32.9%) did not develop aGvHD or developed only stage I with no need for systemic treatment. Twenty (8.5%) of those who did not develop aGvHD encountered a competing event, such as graft rejection, relapse or death before day 100.

Forty-seven patients (19.8%) presented CsA-associated toxicity and required cessation of the therapy. The most common CsA toxicity observed in the study population was nephrotoxicity (n = 22) and neurotoxicity (n = 19). Six patients presented other, more unspecific symptoms, such as allergic reactions (n = 5) and microangiopathy (n = 1). The beginning of CsA treatment discontinuation varied from 1 day post-HSCT up to 52 days post-HSCT (median: 15 days). Instead of CsA, the majority of patients (n = 45; 96%) received Mycofenolate Mofetil (MMF) intravenously at a standard prophylactic dose (20 mg/kg/day). Four of those patients received additional steroids at a standard dose of 1 mg/kg/day. Two (4%) patients received steroids alone.

Initially, we performed the univariate statistical analysis. In this analysis, 3 factors significantly increased the probability of aGvHD ($p < 0.05$): TBI-MAC ($p = 0.0272$),

Table 2. Univariate analysis of risk factors for acute graft-versus-host disease (aGvHD)

Risk factors	aGvHD			p-value
	yes n [%]	no (without a competing event) n [%]	no (with a competing event) n [%]	
Patient sex				
male	87 (62.6)	53 (67.9)	8 (40)	0.0705
female	52 (37.4)	25 (32.1)	12 (60)	
Sex mismatch				
matched	85 (61.2)	48 (61.6)	9 (45)	0.1221
male to female	27 (19.4)	15 (19.2)	2 (10)	
female to male	27 (19.4)	15 (19.2)	9 (45)	
Patient age [years]				
≤5	33 (23.7)	13 (16.7)	7 (35)	0.4084
>5 and <15	76 (54.7)	45 (57.7)	8 (40)	
≥15	30 (21.6)	20 (25.6)	5 (25)	
Donor age [years] (median: 31)				
≤median	74 (53.2)	41 (52.6)	8 (40)	0.5358
>median	65 (46.8)	37 (47.4)	12 (60)	
Amount of CD34+ cells (median: 8.29 × 10^6/kg)				
≤median	65 (46.8)	42 (53.8)	12 (60)	0.3985
>median	74 (53.2)	36 (46.2)	8 (40)	
Amount of CD34+ cells (quartile – cut-off value)				
Q1 (5.01 × 10^6/kg)	32 (23)	22 (28.2)	6 (30)	0.7970
Q2 (8.29 × 10^6/kg)	33 (23.7)	20 (25.6)	6 (30)	
Q3 (12.09 × 10^6/kg)	35 (25.2)	21 (26.9)	4 (20)	
Q4 (51.85 × 10^6/kg)	39 (28.1)	15 (19.3)	4 (20)	
Stem cells source				
peripheral blood	115 (82.7)	68 (87.2)	18 (90)	0.5425
bone marrow	24 (17.3)	10 (12.8)	2 (10)	
Conditioning regimen				
TBI-MAC	59 (42.4)	39 (50)	7 (35)	0.0272
Bu-MAC	59 (42.4)	19 (24.4)	6 (30)	
other (RTC/RIC)	21 (15.2)	20 (25.6)	7 (35)	
Premature discontinuation of CsA				
yes	36 (25.9)	7 (9)	2 (10)	0.0201
no	103 (74.1)	71 (91)	18 (90)	
Risk of disease				
high	71 (51.1)	36 (46.2)	8 (40)	0.5711
standard	68 (48.9)	42 (53.8)	12 (60)	
Year of transplantation (median: 2009)				
≤2009	88 (63.3)	33 (42.3)	5 (25)	0.0004
>2009	51 (36.7)	45 (57.7)	15 (75)	
Year of transplantation (quartile)				
Q1 (2004–2006)	54 (38.8)	17 (21.8)	1 (5)	0.0003
Q2 (2007–2009)	34 (24.5)	16 (20.5)	4 (20)	
Q3 (2010–2012)	32 (23)	17 (21.8)	9 (45)	
Q4 (2013–2015)	19 (13.7)	28 (35.9)	6 (30)	

preemptive discontinuation of CsA (p = 0.0201) and transplantation performed before year 2009 (p = 0.0004). Donor and recipient age, patient sex, donor–recipient sex mismatch, amount of infused CD34+ cells, stem cell source, and underlying disease stage seemed to be insignificant as risk factors for aGvHD (Table 2).

The same factors as in the univariate analysis proved to be significant in the multivariate analysis. The myeloablative conditioning regimen containing total body irradiation puts patients in a greater risk of aGvHD (RR (relative risk): 1.69; 95% confidence interval (95% CI) = 1.047–2.74; p = 0.0320). Premature discontinuation of CsA significantly increased the risk of aGvHD (RR = 1.99; 95% CI = 1.369–2.89; p = 0.0003). Transplantation performed before 2009 enhanced the possibility of aGvHD (RR = 1.97; 95% CI = 1.400–2.78; p = 0.0001). The relative risk regression model containing all significant factors is presented in Table 3.

Table 3. Results of multivariate analysis, competing risk regression model

Risk factors	RR (95% CI)	p-value
Conditioning regimen		
other (RTC/RIC)	1	–
Bu-MAC	1.11 (0.673–1.85)	0.67
TBI-MAC	1.69 (1.047–2.74)	0.0320
Early cessation of CsA		
no	1	–
yes	1.99 (1.369–2.89)	0.0003
Year of transplantation (median: 2009)		
>median	1	–
≤median	1.97 (1.400–2.78)	0.0001

RR – relative risk; CI – confidence interval.

The median follow-up in the study cohort, which was 2.3 years after HSCT, revealed that 138 patients remained alive. The general outcome in the aGvHD cohort was noticeably worse (TRM = 22.5%; OS = 60.8%) compared to aGvHD-free patients (TRM = 12%; OS = 74.1%). The difference between OS (p = 0.08) and TRM in both groups was at the limit of statistical significance (p = 0.08). Transplantation performed before 2009 contributed significantly to a worse survival (p = 0.019) and increased TRM (p = 0.049) (Fig. 1,2). Other factors, including those which statistically impact the incidence of aGvHD, did not influence OS and TRM (data not shown). Complete OS and TRM statistics are presented in Table 4.

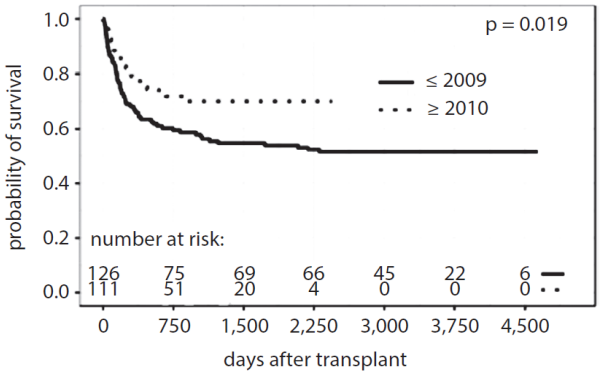

Fig. 1. Comparison of overall survival (OS) for patients receiving hematopoietic stem cell transplantation (HSCT) before and after 2009

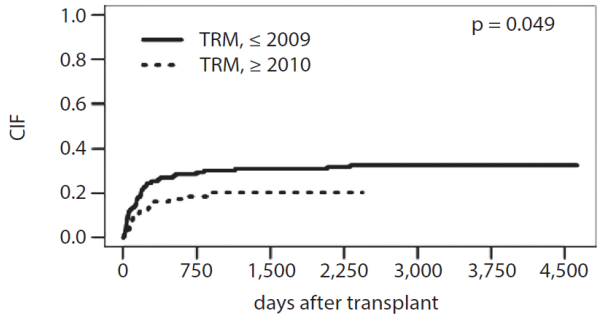

Fig. 2. Comparison of transplant-related mortality (TRM) for patients receiving hematopoietic stem cell transplantation (HSCT) before and after 2009

CIF – cumulative incidence function.

Discussion

Acute graft-versus-host disease as a major factor affecting morbidity and mortality in the early period post-HSCT still remains a challenge for clinicians and researchers worldwide. There is a great number of published studies attributing both the incidence of aGvHD and the general outcome to the influence of potential risk factors. Repeatedly, those studies demonstrate different or even contradictory results. However, it should be emphasized that most of the studies were performed on adult patients and lack of great cohort research conducted on the pediatric population is unquestionable.

Table 4. Comparison of overall survival (OS) and transplant-related mortality (TRM) in patients with or without acute graft-versus-host disease (aGvHD)

Time post HSCT	OS [%]		p-value	TRM [%]		p-value
	aGvHD	no aGvHD		aGvHD	no aGvHD	
1 year	87.0	90.3		13.8	8.7	
3 years	72.2	85.3		18.6	12.2	
5 years	60.4	74.1	0.084	25.9	12.8	0.145

The incidence of aGvHD in our study (58.6%) is very similar to that presented in other studies, but there are also some reports referring to a much higher frequency of this complication, reaching the level of 80%.[1,2,9,10,23,24] Such discrepancies may be due to meaningful differences in the studied cohorts. In our study, patient selection was defined by strict criteria; therefore, the analyzed cohort was relatively homogenous.

A comparison of conditioning regimens Bu-MAC vs TBI-MAC vs other (RTC & RIC) shows TBI as a major risk factor for aGvHD in the pediatric population.[21,22] Tissue damage, particularly in the gastrointestinal tract, undoubtedly exacerbated by TBI, is thought to be the 1st stage of inflammatory reaction through the activation of host antigen-presenting cells (APCs) and cytokine production. This may initiate an inflammatory cascade, leading to the development of aGvHD.[28] Lack of significant differences between the Bu-MAC patients and the group receiving RTC and RIC may suggest that chemo-based myeloablative regimens would not influence aGvHD development.[29,30] This finding only highlights the contribution of TBI to aGvHD. There are also some studies suggesting that in younger recipients, a low dose of TBI may have a beneficial effect without increasing the risk for aGvHD. This indicates that reduced-toxicity regimens, containing low doses of TBI, could be further investigated also in pediatric HSCT recipients. However, in our study, the best survival rate was noted in the group of patients with Bu-MAC (data not shown). This finding may suggest that chemo-based MAC could be a golden standard for pediatric patients.[31]

Premature discontinuation of CsA increased the probability of aGvHD. There are a lot of reports in the literature about the essential role of calcineurin inhibitors in aGvHD prophylaxis. A large part of these reports focus on the superiority of one of them (CsA or tacrolimus) over the other. Another great part of the literature proves that a low plasma concentration level of CsA, especially in the early period post-HSCT (i.e., 2–3 weeks post-HSCT) is associated with a much higher risk of aGvHD.[26,32–35] To our knowledge, this is the only study providing results that refer to the influence of early cessation of CsA on the occurrence of aGvHD. There is only one reference regarding premature discontinuation of CsA – the research conducted by Cadenas et al.[32] Although this study was performed on a completely different population of patients, it contains a brief suggestion that there is no connection between discontinuation of CsA and aGvHD. The quoted study primarily focused on low CsA plasma concentration levels as a risk factor for aGvHD. To date, the optimal therapeutic approach for CsA therapy as aGvHD prophylaxis after HSCT remains unclear in terms of doses as well as the monitoring strategy.[36,37] However, our results indicate that calcineurin inhibitors as part of aGvHD prophylaxis may be irreplaceable.

Hematopoietic stem cells transplantation performed before 2009 proved to be a crucial risk factor for the development of aGvHD and contributed to a worse general outcome. This finding is a reflection of all the improvements in donor selection, transplantation regimens and supportive care that were made during the analyzed period of time. Furthermore, improved OS and reduced TRM after 2009 result from relevant advances in aGvHD therapy that were made during these years. Among all implemented GvHD treatment strategies, extracorporeal photopheresis seems to be promising both in our center (data not shown) and worldwide.

The CD34+ cell dose is one of the most ambiguous factors that have an impact on aGvHD and survival after HSCT. The results of our study showed no correlation between a greater amount of transplanted CD34+ cells and a higher incidence of aGvHD. In parallel, we found no interdependence between the CD34+ cells dose and either OS or TRM. Several studies, performed in both the adult and pediatric population of patients, confirm our findings. Previous research conducted in our center by Kałwak et al. also proves that there is no correlation between the CD34+ cell dose and aGvHD; notwithstanding, a greater amount of transplanted CD34+ cells contributed to better OS and general outcome.[9] Similar results were reported by Pulsipher at al. in a multi-center study performed on a great cohort of adult and pediatric patients.[38] Another study demonstrating no correlation between CD34+ and aGvHD was carried out by Tsirigotis et al.[39] It also reports that the number of infused CD34+ cells has no influence on the general outcome. On the contrary, there are some reports suggesting that a higher number of infused CD34 cells may be a risk factor for aGvHD.[5,40] The extremely discrepant findings concerning the impact of the CD34+ cell dose on aGvHD show that this variable should be analyzed individually, following the patient requirements. This knowledge can be beneficial, allowing clinicians to tailor the composition of the graft in accordance with the patient status and other peri-transplantation variables.

Other analyzed variables, such as donor and recipient age as well as donor–recipient sex mismatch, are well-known risk factors for aGvHD in the adult population. In children, however, the data is sparse. In our study, female donor to male recipient did not prove to be a risk factor for aGVHD, which was, however, noted as a risk factor in our population of male matched sibling donor recipients, when no ATG was given (data not shown).

Conclusions

To conclude, conditioning regimen and adequate immunosuppressive prophylaxis, including continuous CsA use, remain major factors affecting the incidence of aGvHD in children with malignant disorders undergoing UD-HSCT. However, to date, it is not possible to provide strict guidelines for minimizing the risk of aGvHD. According to our results,

the decision to stop CsA administration should be carefully considered, and avoided if possible. Choosing an approach may be discussed if CsA needs to be discontinued for some reason. There is no clear answer. Both MMF and steroids could be useful, but in our belief, MMF seems to be the most appropriate option, with less toxicity.

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ORIGINAL ARTICLE

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Factors affecting survival in children requiring intensive care after hematopoietic stem cell transplantation. A retrospective single-center study

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Abstract

Allo-HSCT is associated with life-threatening complications. Therefore, a considerable number of patients require admission to a PICU. We evaluated the incidence and outcome of PICU admissions after allo-HSCT in children, along with the potential factors influencing PICU survival. A retrospective chart review of 668 children who underwent first allo-HSCT in the Department of Pediatric Hematology/Oncology and BMT in Wrocław during years 2005-2017, particularly focusing on patients admitted to the PICU within 1-year post-HSCT. Fifty-eight (8.7%) patients required 64 admissions to the PICU. Twenty-four (41.5%) were discharged, and 34 (58.6%) patients died. Among the discharged patients, 6-month survival was 66.7%. Compared with survivors, death cases were more likely to have required MV (31/34; 91.2% vs. 16/24; 66.7% P = .049), received more aggressive cardiac support (17/34; 50% vs. 2/24; 8.3% P = .002), and had a lower ANC on the last day of their PICU stay (P = .004). Five patients were successfully treated with NIV and survived longer than 6 months post-discharge. The intensity of cardiac support and ANC on the last day of PICU treatment was independent factors influencing PICU survival. Children admitted to the PICU after allo-HSCT have a high mortality rate. Mainly those who needed a more aggressive approach and had a lower ANC on the last day of treatment had a greater risk of death. While requiring MV is associated with decreased PICU survival, early implementation of NIV might be considered.

KEYWORDS

complications, critical care, granulocytes, hematopoietic stem cell transplantation, mechanical ventilation, non-invasive ventilation

Abbreviations: aGvHD, acute graft-versus-host disease; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ANC, absolute neutrophil count; ARDS, acute respiratory distress syndrome; CPAP, continuous positive airway pressure; CsA, cyclosporine; CVVHDF, continuous venous-venous hemodiafiltration; G-CSF, granulocyte colony-stimulating factor; MAC, myeloablative conditioning; MMRD, mismatched related donor; MOF, multiorgan failure; MSD, matched sibling donor; MTX, methotrexate; MUD, matched unrelated donor; MV, mechanical ventilation; NIV, non-invasive ventilation; NMA, non-myeloablative conditioning; Non-MAC, including non-myeloablative, reduced intensity and reduced toxicity conditioning regimens; OS, overall survival; PICU, pediatric intensive care unit; RIC, reduced intensity conditioning; RRT, renal replacement therapy; SCA, sudden cardiac arrest; SIRS, systemic inflammatory response syndrome; VOD, veno-occlusive disease.

1 | INTRODUCTION

Allo-HSCT is a curative therapy for children with a wide range of malignant and non-malignant diseases. Despite advances made over the decades, HSCT remains a high-risk procedure, contributing significantly to morbidity and mortality.[1] HSCT is associated with life-threatening toxicities leading directly to organ and tissue damage, while long-lasting immunosuppression and slow recovery of the immune system make the children susceptible to infections.[2,3] Therefore, transplant children often require admission to a PICU.

The increasing number of children undergoing HSCT has escalated the demand for adequate pediatric intensive care services capable of managing a full spectrum of transplant-related complications.[1] According to the literature, the mortality of children admitted to the PICU post-HSCT remains high.[4-7] Over the years, a few studies have reported improved outcomes for adult post-HSCT ICU patients.[8,9] However, in the pediatric population, the data are sparse.

Pediatric transplant recipients, when admitted to the PICU, may require a different approach compared to the general patient population, as they do not fit into standard prognosis schemes. Indeed, many transplant-specific factors influence the outcome of children requiring treatment within the PICU after HSCT. Therefore, advanced care planning and end-of-life care issues remain an enduring challenge for physicians. Lack of disease- and HSCT-specific intensive care guidelines has led to major discrepancies in proceedings worldwide.[10]

The aim of this single-center retrospective study was to assess the incidence and outcome of pediatric patients admitted to the PICU within 1-year post-HSCT. We provide detailed characteristics of the children requiring intensive care after HSCT, as well as an analysis of the potential factors that likely affect the overall outcome of PICU treatment.

2 | MATERIALS AND METHODS

The study was based on retrospective data analysis of 668 children and adolescents (under 21 years of age) who underwent allogeneic HSCT in the Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation in Wrocław during the years 2005-2017. The baseline characteristics of the patients are presented in Table 1. In particular, we analyzed patients requiring transfer to the PICU within 1 year of transplantation. Epidemiological and demographic data, as well as the underlying disease, type of stem cell donor, and peri-transplantation procedures were assessed. Patient charts were reviewed to obtain the time-lapse between HSCT and PICU admission, the presence of aGvHD prior to admission, and indications for intensive care, including the need for MV, the use of vasopressors, and RRT. Hematological reconstitution, particularly granulocyte count, was ascertained in patients at the time of admission and either on the day of discharge or death. All listed features were investigated as potential factors influencing the general outcome of patients requiring intensive care post-HSCT. The study was reviewed and approved by the Wrocław Medical University Ethics Committee.

TABLE 1 Characteristics of study cohort

Patients/HSCT analyzed	n = 668
MSD	153
MUD	460
MMRD	55
Male/Female	391/277
Age	2 mo-21 y (median: 8.5 y)
Stem cells source	
Bone marrow	143
Peripheral blood	504
Cord blood	21
Conditioning regimen	
Treosulfan-based	192
Busulfan-based	239
TBI-based	126
NMA	82
Other	29
Diagnosis	
Acute leukemia	322
Solid tumor	20
Immunodeficiency	89
Myelodysplastic syndrome	63
Anemia	90
Lymphoma	29
Metabolic disorders	30
Chronic leukemia	25

2.1 | Study endpoints

Primary endpoints of this study were discharge from the PICU or death within the PICU. The 30-day and 6-month survival post-discharge were assessed as secondary endpoints.

2.2 | Definitions

Indications for PICU admission were defined as followed:

Respiratory failure—compromise of the respiratory system resulting in either desaturation <90%, hypoxemia (PO2 < 60 mm Hg), or hypercapnia (PaCO2 > 45 mm Hg) on room air combined with severe dyspnea or other clinical symptoms of respiratory distress;

Sepsis—documented SIRS[11] with concurrent documented or assumed infection;

Acute renal impairment—severe renal injury with elevated creatinine plasma levels above 1.5 times baseline, or anuria (<0.5 mL/kg), leading to the need for RRT, such as CVVHDF, despite adequate conservative treatment, regardless of etiology;
Cardiac failure—hemodynamic instability requiring the use of cardiac supportive drugs (including adrenaline, noradrenaline, dopamine, or dobutamine in continuous infusions) despite adequate fluid balance;
Neurological disorder—major deterioration in consciousness with or without seizures, paralysis, and neuroinfection;
MOF—clinical or laboratory signs of altered organ function, including two or more organs when hemostasis cannot be maintained without intervention.
The *MV* group included all patients requiring endotracheal intubation and mechanical ventilatory support at some point during PICU admission.
The *NIV* group included patients treated with non-invasive positive pressure ventilation—either continuous (CPAP) or Bilevel (BiPAP).

Donor type and stem cell source: Stem cells were obtained from three types of donors—MSDs, MUDs, and mismatched (haploidentical) related donors (MMRD). HLA typing was performed at high resolution (four digits) in A* B* Cw* DRB1* and DQB1* alleles. Matched donors were defined as 10/10 HLA-compatible donors. According to ALL international BFMSCT 2008 criteria, all ten alleles were high resolution typed.[12]

Diagnosis of aGvHD was set based on clinical and/or histopathological findings in skin, gastrointestinal tract mucosa, and liver biopsies. Grading and staging of aGvHD were performed using pediatric-specific criteria published by Jacobson et al,[13] and only grades II-IV were considered for our research. As standard prophylaxis for aGvHD, all patients received intravenous CsA at an initial dose of 1.5 mg/kg twice per day, starting one day prior to HSCT, which was switched to an oral formulation when the patient was able to tolerate oral intake. Further dosage of CsA was adjusted to the CsA trough level (target 100-200 mcg/L), measured every second day in the majority of patients. Three subsequent doses of MTX at the standard dosing of 10 mg/m^2 were given on days one, three, and six after HSCT.

Patients discharged from the PICU and transferred back to the transplant unit were considered PICU survivors, regardless of the subsequent outcome. For those who required multiple PICU admissions, the last admission was taken into account. Patients admitted to the PICU for central venous catheter replacement, plasmapheresis, or routine post-operative care were excluded from this study.

2.2.1 | Statistical methods

The data were described as a percentage for discrete variables and a median for continuous variables. They were analyzed for prognostic impact on discharge from PICU. Utilizing either chi-squared or Fisher's exact tests for discrete variables and the Wilcoxon nonparametric rank sum test for continuous data, we first compared the baseline characteristics of patients who survived or died within the PICU. Thereafter, patient data were entered into the logistic regression model. *P*-values lower than .05 were considered significant. Survival analysis was performed using Kaplan-Meier estimation. Statistical analysis was performed using R statistical software.

TABLE 2 Baseline characteristics of PICU cohort

Epidemiological and transplant-specific features	n = 58
Age	0.14-17.9 y median: 5.57 y
Gender	
Male	32 (55.2%)
Female	26 (44.8%)
Underlying disease	
Acute leukemia	36 (62%)
Immunodeficiency	8 (15%)
Metabolic disorder	5 (8.6%)
Anemia	4 (6.9%)
Solid tumor	3 (5.2%)
Lymphoma	1 (1.7%)
Myelodysplastic syndrome	1 (1.7%)
Type of donor	
MUD	41 (70.7%)
MSD	9 (15.5%)
MMRD	8 (13.8%)
Conditioning regimen	
MAC	43 (74.1%)
Non-MAC	15 (25.9%)
Stem cells source	
Peripheral blood	41 (70.7%)
Bone marrow	11 (19.0%)
Cord blood	6 (10.3%)

3 | RESULTS

Among the 668 patients who underwent allo-HSCT in the study period, 58 (8.7%) required a total of 64 admissions to PICU—32 males (55.2%) and 26 females (44.8%). Fifty-two (89.6%) patients were admitted once, while six patients required a second admission to the PICU. The median age at the time of HSCT was 5.5yrs (2.5 months-19.7 years). In the PICU cohort, malignancy was an underlying disease in the majority of patients (40/58; 69%). Demographic and transplant-related data are shown in Table 2.

The median time between transplantation and PICU admission was 61 days (1-297 days). The median length of PICU hospitalization was 7 days (1-104 days). Major indications for PICU admission were respiratory failure (24 of 64 admissions; 36.9%) and septic shock

(16/64; 24.6%). The most common causes of respiratory failure were pneumonia (7/24; 29.2%) and ARDS (7/24; 29.2%), while in five patients, respiratory failure was attributed aGvHD. A summary of the causes for all 64 admissions is reported in Table 3.

Among the 58 PICU patients, 24 (41.3%) were successfully discharged, while 34 (58.6%) died in the PICU. Among those discharged from the PICU, the 30-day survival rate post-PICU discharge was 91.7% (22/24), and the 6-month survival rate was 66.7% (16/24). Non-relapse mortality was a major cause of death in those who died within 6 months post-discharge (6/8; 75%). The 5-year OS in the whole PICU cohort was 17.9% (Figure 1.) and differed significantly from the OS in the general allo-HSCT cohort (17.9% vs. 69%; $P < .001$). All factors potentially influencing the outcome for pediatric HSCT recipients admitted to the PICU are listed in Table 4.

A total of 52 patients required respiratory support during their PICU stay. Forty-seven patients (47/58; 81%) received MV; six of them underwent emergency intubation within the transplant unit and were then transferred to the PICU. Among those treated with MV, 31 (67%) patients died. The need for MV was a significant factor influencing PICU survival. The number of patients requiring MV differed relevantly between non-survivor and survivor group (31/34; 91.2% vs. 16/24; 66.7% $P = .049$).

In our study cohort, apart from those requiring MV, five patients (8.6%) were treated with NIV with no prior or subsequent need for intubation. NIV was applied to three patients with severe pneumonia, one patient with septic shock, and one patient with VOD-associated respiratory failure that required simultaneous RRT. In one case, NIV treatment was attempted in the transplant unit; however, due to further deterioration of the patient's condition, PICU transfer was unavoidable. Nevertheless, the patient did not require intubation post-admission and recovered on NIV alone. In two patients, NIV therapy was intermittent and combined with short periods of spontaneous breathing (ie, 3 hours NIV/30 minutes unassisted breathing). The median time for NIV was 2.5 days. All patients treated with NIV were successfully discharged from the PICU and survived over 6 months post-discharge. The complete characteristics of the NIV group are presented in Table 5.

The use of previously defined vasopressors or inotropes also significantly influenced patient outcomes. A total of 43 patients were treated with cardiac supportive drugs. Among the non-survivors, 29 patients required cardiac support compared to 14 patients in the survivors' group (29/34; 85.3% vs. 14/24; 58.3% $P = .021$). Nineteen patients received extensive cardiac support defined as three or more cardiovascular agents. Majority of those patients (12/19; 63%) were suffering from severe sepsis and concomitant septic shock. The more aggressive the approach needed, the lower the chance of survival observed—only two patients among those receiving extensive cardiac support were discharged from the PICU (17/34; 50% vs. 2/24; 8.3% $P = .001$).

Nine patients (15%) experienced SCA within PICU. The most common cause of SCA was sepsis-associated profound hypotension and hemodynamic shock (n = 4). Three of those who experienced SCA were successfully discharged and survived over 6 months after PICU discharge. The causes of SCA in survivors were as follows: anaphylactic shock post-drug administration, arrhythmia due to hypertrophic cardiomyopathy, and hypovolemic shock due to severe gastrointestinal bleeding.

The ANC at the end day of PICU treatment (either discharge or death) differed significantly in patients who survived (median 2.7×10^{6}/mL) and those who died in the PICU (median 0.9×10^{6}/mL; $P = .004$). Nineteen patients died in severe neutropenia within the PICU; nine of them were admitted to the PICU in severe neutropenia and never engrafted, while ten patients had lost their graft during their PICU stay. Four dead cases presented a substantial drop in ANC within the PICU.

The ANC on the day of PICU admission did not diverge significantly between survivors and non-survivors. Among the patients admitted to the PICU in severe neutropenia (26, 44.8%), 12 achieved neutrophil recovery; eight of them were discharged from the PICU, and six survived more than 6 months. Four patients died despite achieving engraftment.

Five patients received granulocyte concentrate for severe neutropenia, all of whom were discharged from the PICU and four survived over 6 months. Contrary to the ANC at the end of PICU treatment, profound neutropenia on the day of PICU admission did not diminish patients' chance of survival.

Among the nine patients (15%) who required RRT (CVVHDF), three were successfully discharged from the PICU. Two of them survived more than 6 months while one died within 30 days. The complete results from our univariate analyses are presented in Table 4.

TABLE 3 Analysis of indications for PICU admission post-HSCT

Indications for admission	Number of admissions = 64
Respiratory failure	24 (37.5%)
Septic shock	16 (25%)
Cardiovascular failure	5 (7.8%)
Neurological disorders	6 (9.4%)
Multiorgan failure	7 (10.9%)
Acute renal failure	5 (7.8%)
Anaphylactic shock	1 (1.6%)

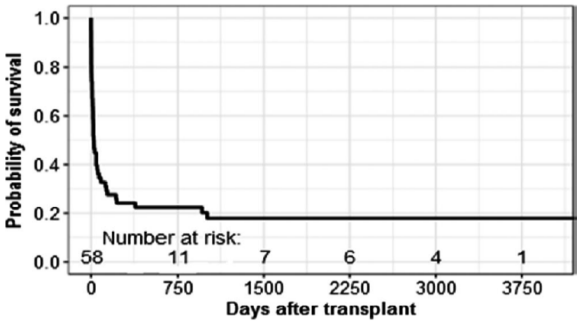

FIGURE 1 Kaplan-Meier survival plot of 58 patients admitted to the PICU post-HSCT

TABLE 4 Results of univariate analysis of factors influencing PICU survival

Investigated factors	Non-survivors n (%)	Survivors n (%)	*P*-value
Total	34 (58.6)	24 (41.4)	
Sex			.23
Female	13 (38.2)	13 (54.2)	
Male	21 (61.8)	11 (45.8)	
Age (median = 5.6 y)			.11
≤5.6	20 (58.8)	9 (37.5)	
>5.6	14 (41.2)	15 (62.5)	
Underlying disease			.404
Malignant	22 (64.7)	18 (75)	
Non-malignant	12 (35.3)	6 (25)	
Type of donor			.167
MMRD	7 (20.6)	1 (4.2)	
MUD	23 (67.6)	18 (75)	
MSD	4 (11.8)	5 (20.8)	
Stem cells source			.688
Bone marrow	5 (14.7)	6 (25)	
Cord blood	4 (11.8)	2 (8.3)	
Peripheral blood	25 (73.5)	16 (66.7)	
aGvHD			.469
No	16 (47.1)	9 (37.5)	
Yes	18 (52.9)	15 (62.5)	
Severe neutropenia			.142
No	17 (51.5)	16 (66.7)	
Yes	16 (48.5)	8 (33.3)	
Ventilatory support			**.049**
No	3 (8.8)	3 (12.5)	
NIV	0	5 (20.8)	
MV	31 (91.2)	16 (66.7)	
Cardiac supportive drugs			**.021**
No	5 (14.7)	10 (41.7)	
Yes	29 (85.3)	14 (58.3)	
Extensive cardiac support (≥3 drugs)			**<.001**
No	17 (50)	22 (91.7)	
Yes	17 (50)	2 (8.3)	
RRT			.449
Yes	6 (17.6)	3 (8.3)	
No	28 (82.4)	21 (91.7)	
Disease status at admission			~1
Remission	32 (94.1)	22 (91.7)	
Relapse	2 (5.9)	2 (8.3)	
Conditioning regimen			.95
Myeloablative	24 (70.6)	19 (79.2)	
Non-myeloablative	2 (5.9)	1 (4.2)	
Reduced intensity	5 (14.7)	2 (8.3)	
Reduced toxicity	3 (8.8)	2 (8.3)	
Day post-HSCT at admission (median; d)	44	96.5	.097
Length of stay (median; d)	7	6.5	.93
ANC at admission (median $\times 10^6$/mL)	0.56	2.43	.143
ANC at the end of PICU stay (median $\times 10^6$/mL)	0.9	2.7	**.004**
Age (median y)	3.38	6.89	.43

The investigated factors were put into the logistic regression model. The analysis revealed that two of them were significant and independent factors influencing PICU survival post-HSCT. Extensive cardiac support (ie, three or more cardiac supportive drugs) relevantly diminished PICU survival (OR 0.02, 95%CI 0-0.33, *P* = .005). A lower ANC on the last day of PICU treatment considerably correlated with worse survival (OR 1.63 95% CI 1.1-2.41 *P* = .014).

4 | DISCUSSION

Pediatric recipients of HSCT are at high risk for life-threatening complications. Transplant-related toxicity, as well as extensive immunosuppression, leads to increased vulnerability to severe opportunistic infections and the risk of organ failure. In addition, most patients are suffering from previous organ damage due to heavy pretreatment, including conditioning and chemotherapy for an underlying disease. Therefore, a relevant number of patients will require advanced supportive measures provided within the PICU.

During the analyzed period of time, the PICU admission rate was 9.3% (58/668). In comparison with reports from the literature, which describe admission rates reaching up to 35% of HSCT recipients, our admission rate was relatively low.[14-19] Such a discrepancy may be attributed to different indications for PICU care, as every center adopts its own criteria for PICU admission.[10,20-23] Similarly to other researches, most children in our study required intensive care due to respiratory failure of multifarious origin. According to reports, respiratory failure, as an indication for PICU admission, may be a major factor influencing poor survival. However, this finding was not confirmed by our results.[15,16,24-26]

PICU survival rate in our study was 41.3%, which correlates with those reported in many previous studies.[4,5,15,17,19,24-27] However, the latest multicenter study by Zinter et al[28] reported

TABLE 5 Characteristics of patients treated with NIV

No.	Sex	Age (y)	Underlying disease	Indication for PICU Admission	Day post HSTC at PICU admission	Duration of PICU stay (d)	Indications for NIV	Duration of NIV (d)
1	M	6.3	ALL	Respiratory failure, Pneumonia	288	5	Hypoxemia Increased oxygen demand Dyspnea	2.5
2	M	14	MDS	Respiratory failure, Pneumonia	129	11	Hypoxemia Increased oxygen demand Dyspnea	4
3	M	8	ALL	VOD-associated MOF	21	11	Exhaustion Increased work of breathing	2.5
4	F	17	AML	Respiratory failure, Pneumonia	72	3	Hypoxemia Increased oxygen demand Exhaustion	2
5	M	7	ALL	Septic shock	2	5	Hypercapnia Increased work of breathing Neurological deterioration	1.5

a very low mortality rate of children requiring critical care post-HSCT. It may suggest that the timing and PICU admission criteria in our center should be reconsidered. Nonetheless, we report promising 30-day and 6-month survival rates in patients who were discharged from the PICU, placing our results at the higher end of the published data.[17,24,26] Comparable findings were recently reported by Duncan et al[29] in a large multicenter study proving no difference in the long-term outcome of those who did and did not require intensive cardiopulmonary support and survived until hospital discharge.

Many factors are being investigated for having a possible influence on the outcome of HSCT patients requiring PICU care. The need for MV and its duration were recognized as major factors related to poor PICU prognosis.[6,8,9,22,23,30,31] In our study, the significant influence of MV in univariate analysis failed to be confirmed by the relative risk regression model. Therefore, contrary to many other studies, MV was not an independent factor affecting PICU survival in our cohort. This statistical result may be influenced by the fact that the majority of patients in our study received MV (81%). This rate is considerably higher than in studies revealing MV as a risk factor for death within the PICU.[23,24] Differences in PICU admission criteria, indications for MV, or patient selection bias may also influence the results. Nonetheless, due to a considerable lack of data, we were not able to analyze independent indicators of respiratory function (ie, Oxygenation Index or PaO2/Fi02 ratio), which is a noticeable limitation of the study.

Nevertheless, we report promising results for NIV in our cohort. Among the patients treated with NIV, none required subsequent intubation and all survived more than 6 months post-PICU discharge. Recently published studies have suggested the limited usefulness of NIV in adult transplant patients, as it often leads to delayed intubation.[6,32,33] Nevertheless, in children the data are sparse. Early NIV implementation seems to be effective in pediatric intensive care recipients, particularly by decreasing the risk of life-threatening complications related to MV[34,35]; however, several studies investigating pediatric respiratory failure of multifarious origin became inconclusive about the usefulness of NIV.[36-38] Our results suggest that the use of NIV in pediatric HSCT recipients should be strongly considered, as it may prevent further deterioration of pulmonary function; however, our findings are limited by a low number of patients receiving NIV. Nevertheless, the use of NIV should not delay MV implementation, if needed. Considering the differences in institutional indications for NIV implementation, leading to major discrepancies in the outcomes of patients treated with NIV, further large prospective cohort studies are essential.

Numerous studies identify aGvHD as an independent factor worsening the prognosis of HSCT recipients treated within the PICU. Indeed, some researchers suggest that severe aGvHD may be an indication for restraining intensive therapy and introducing palliative care.[39] In our cohort, aGvHD had no impact on survival within the PICU, suggesting that those patients should be treated aggressively, despite the presence of aGvHD.

It is worth emphasizing that among the nine patients in our cohort treated with RRT, three survived their PICU stay, and two survived more than 6 months after PICU discharge. In the face of reports suggesting extremely high mortality rates in patients requiring RRT, our result may encourage clinicians, supporting the idea that RRT therapy is not necessarily fatal in HSCT recipients. Furthermore, as reported by DiCarlo et al,[40] early implementation of RRT might improve the outcome in this group of patients.[9,10,14,16,19,20,31,39,41]

The intensity and comprehensiveness of therapies applied within the PICU are also well-known factors influencing survival. Beside MV, pharmacologic cardiovascular support is an essential

treatment provided within PICU. In our cohort, patients receiving cardiac support had a significantly lower chance of surviving their PICU stay.[9,24,25] Particularly, extensive cardiac support (ie, three or more cardiovascular agents) was an independent factor correlating with poor PICU prognosis. Considering the fact that extensive cardiac support was most commonly applied in patients with septic shock, vigilance for signs of infection in this vulnerable population of patients might be crucial. A relatively high number of patients receiving cardiac support in our study (74%; 43/58) illustrate the critical condition of majority of patients admitted to the PICU. Nevertheless, we must highlight the fact that 33% (3/9) of patients who experienced a SCA within the PICU survived more than 6 months post-discharge. The outcome of HSCT patients requiring resuscitation in our study is quite satisfying compared to those reported in other contributing studies of general PICU cohorts; nonetheless, it is limited by the meager number of patients.[42]

Beyond the intensity of cardiac support, in multivariate analysis the ANC appeared to be another significant factor influencing PICU survival. The ANC on the last day of a PICU stay differed significantly between survivors and non-survivors. Patients who died within the PICU had a lower ANC compared to those successfully discharged. On the contrary, the ANC and severe neutropenia on the day of PICU admission did not affect patient outcomes. A comparable result about the impact of initial neutropenia and neutrophil recovery on the outcome of HSCT patients treated with MV was recently published by Moffet et al[43] Other recent research by Garcia et al,[44] proved the substantial role of primary engraftment within the PICU. However, to our knowledge, this is the only study investigating the broad impact of ANC on PICU survival. Our findings suggest that besides numerous intensive care measures applied during PICU treatment, hematological recovery and proper graft function may have a major impact on patient outcomes. The influence of implemented therapies on graft function should be taken into account and not neglected by clinicians providing intensive care. Hematological recovery and graft function also ought to be considered in advanced care planning, while achieving an adequate ANC may relevantly improve patient prognosis. Therefore, the use of G-CSF and/or granulocyte transfusions should be considered as potentially effective.

5 | CONCLUSIONS

To conclude, we would like to highlight the equal roles of comprehensive intensive care and graft function on the survival of HSCT in children requiring hospitalization within the PICU. In our hands, almost half of the patients requiring intensive care survived until PICU discharge, which confirms the promising trend of increased PICU survival post-HSCT. Comprehensive supportive care including the prompt implementation of NIV, the use of granulocyte concentrates and G-CSF, a holistic approach to the patient and close cooperation of intensive care providers and pediatric transplant experts could relevantly improve the general outcomes of children admitted to the PICU following HSCT.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest. All authors have nothing to disclose.

AUTHORS' CONTRIBUTIONS

ZS and M.Ł.: Designed the study. JM-B., M.Ł., JO-L., and AK: Reviewed patients' charts and collected the data. ZS, MK-Ż., MZ, JF, MS, and MM-S.: Analyzed collected data. AK: Was responsible for statistical analysis. ZS: Wrote the manuscript with substantial help from MU, KK, EG, and MZ The paper was critically revised by KK and EG who were also supervising the study.

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Veno-occlusive disease in children and adolescents after hematopoietic stem cell transplantation: Did the Modified Seattle Criteria fit the characteristics of pediatric population?

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Abstract

Background. Hepatic veno-occlusive disease (VOD) is a life-threatening complication following hematopoietic stem cell transplantation (HSCT) and associated with a high mortality rate. Therefore, accurate and immediate diagnosis is crucial for implementing appropriate treatment.

Objectives. In our single-center retrospective study, we assessed the accuracy of the Modified Seattle Criteria in children and adolescents undergoing HSCT, and compared them to the diagnostic criteria recently established by the European Society for Blood and Marrow Transplantation (EBMT).

Material and methods. Retrospective analysis of medical records of 951 HSCT procedures performed in 850 children and young adults in the years 2001–2015 in the Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation of Wroclaw Medical University Supraregional Center of Pediatric Oncology "Cape of Hope" in Wrocław, Poland.

Results. Among the 850 children, 48 were diagnosed with VOD according to the Modified Seattle Criteria (5.05%). Thirteen patients (27%) developed VOD later than within 20 days after transplantation, as required in the diagnostic criteria. Five of the 6 patients who died from VOD were diagnosed with late-onset VOD. Using the categories of symptoms described in the Modified Seattle Criteria, hepatomegaly and weight gain were the most common symptoms in the analyzed cohort (81.25% and 68.75%). Fourteen patients (29%) never demonstrated elevated plasma bilirubin level (>2 mg/dL), as suggested in the Modified Seattle Criteria. Twenty-nine patients (64%) had increased platelet consumption requiring daily transfusions. Only 5 patients with decreased plasma antithrombin III (ATIII) activity level (<80%) on the day of HSCT developed VOD despite supplementation of ATIII.

Conclusions. The Modified Seattle Criteria seemed to not meet the special needs of the pediatric population. The new diagnostic criteria proposed by the EBMT appear to be more adequately tailored to the pediatric population and may significantly change the conception of VOD in the future. The surprisingly low incidence of VOD in our cohort may suggest a beneficial role of monitoring and early supplementation of ATIII.

Key words: hematopoietic stem cell transplantation, pediatric, veno-occlusive disease

Introduction

Hematopoietic stem cell transplantation (HSCT) has been used as a curative therapy for various kinds of disorders, both malignant and nonmalignant. Despite the increasing rate of successful transplantation procedures, the widespread use of the treatment is limited by concerns of life-threatening complications.

Hepatic veno-occlusive disease (VOD), also known as sinusoidal obstructive syndrome (SOS), is frequent and may be one of the most severe complications in the early post-HSCT period. This syndrome is characterized by clinical features like rapid weight gain, ascites, painful hepatomegaly, and jaundice.[1] Clinical suspicion of VOD may be supplemented by noninvasive imaging, such as ultrasonography (USG), particularly to identify attenuated or reversed hepatic venous flow, a typical USG finding in VOD.[2,3] However, none of the diagnostic criteria, laboratory tests or USG findings are specific to VOD. Its incidence in the adult population historically has been reported in up to 60%[1,4–6] of patients undergoing HSCT, but nowadays it appears to be 13.7%, with values ranging from 0% to 60.2% in different studies.[7] Such great range of results depends on the diagnostic criteria used – the Baltimore or the Seattle Criteria (Table 1).[6,8] In the face of the newly presented European Society for Blood and Marrow Transplantation (EBMT) diagnostic criteria for VOD in both the adult and pediatric population, the prevalence of this complication may change significantly in upcoming years (Table 2).[9,10]

Several risk factors for the development of VOD have been identified and inclusion of patients with each of these risk factors has differed between studies. The most widely accepted risk factors are allogeneic HSCT, previous liver disease, history of abdominal radiation, advanced disease (beyond the 2nd complete remission or relapse) and busulfan- and cyclophosphamide-based regimens.[4,7,11] In the pediatric population, low body weight, low albumin level, autologous HSCT for neuroblastoma, younger age, some underlying diseases (i.e., hemophagocytic lymphohistiocytosis (HLH) and osteopetrosis) and previous treatment (i.e., eculizumab, gemtuzumab ozogamicin) were identified as specific risk factors for VOD.[12–15] The severity of VOD could be retrospectively classified as mild, moderate or severe based on the dysfunction of the liver and associated organs.

Table 1. Comparison of Baltimore and Modified Seattle VOD Diagnostic Criteria

Modified Seattle Criteria	Baltimore Criteria
Two of following occurring within 21 days of transplantation:	Bilirubin serum level >2 mg/dL and at least 2 of following within 20 days of transplant:
hepatomegaly or right upper quadrant pain	hepatomegaly
bilirubin serum level >2 mg%	ascites
unexplained weight gain >2% from baseline	weight gain >5% from baseline

Table 2. European Society for Blood and Marrow Transplantation (EBMT) diagnostic criteria for hepatic VOD/SOS in children

No limitations for time of the onset of VOD/SOS
Presence of 2 or more of the following[a]: – unexplained, consumptive, transfusion-refractory thrombocytopenia[b]; – otherwise unexplained weight gain in 3 consecutive days despite use of diuretics or weight gain >5% baseline; – hepatomegaly (best confirmed by imagining) above baseline[c]; – ascites (best confirmed by imagining) above baseline[c]; – rising bilirubin from a baseline value on 3 consecutive days or bilirubin ≥2 mg/dL within 72 h.

[a] with the exclusion of other potential differential diagnosis;
[b] ≥ on weight-adjusted platelet substitution/day to maintain institutional transfusion guidelines;
[c] imaging immediately before HSCT to determine baseline value.
VOD – veno-occlusive disease; SOS – sinusoidal obstructive syndrome.

In addition to the recently proposed diagnostic criteria, the EBMT has established a new severity classification that would affect the structure of VOD incidence in transplant populations.[9] Therapy options for VOD are still very limited and defibrotide is the only drug approved by both the European Union and the USA for VOD treatment. Many previous and ongoing trials provide promising results about the safety and efficacy of defibrotide as a crucial agent in both prophylaxis and treatment regimens for VOD.[16–20] Additional treatment and prophylactic strategies using other agents such as heparin, antithrombin III, prednisone, or ursodiol have been studied, but none of them have been proven unequivocally effective.[21–25]

Given the limited number of large cohort studies on VOD in the pediatric population, in our single-center retrospective study, we have reported detailed VOD characteristics in children. In our research, we have evaluated the usefulness of the Modified Seattle Criteria, which have been widely used for over 25 years, particularly compared to the recently published EBMT VOD criteria in the pediatric population. We have also assessed the influence of antithrombin III (ATIII) supplementation on the prevalence of VOD in our center.

Material and methods

We retrospectively analyzed the medical records of 951 HSCT procedures performed on 850 children and young adults in the years 2001–2015 in the Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation of Wroclaw Medical University Supraregional Center of Pediatric Oncology "Cape of Hope" in Wrocław, Poland. Seven hundred sixty-two patients underwent a single HSCT procedure, 76 patients required 2 HSCTs, 3 HSCTs were performed in 10 patients, and 4 HSCTs in 2 patients. Among the 951 transplantations performed, 246 were autologous and 705 were allogeneic. Among the allo-HSCTs, the stem cells were derived from bone marrow in 236 cases and from peripheral blood in 451 cases. Eighteen patients

Table 3. Characteristic of study cohort

Parameter	Value
HSCT analyzed autologous allogeneic	951 246 705
Patients after 1 transplantation after 2 transplantations after 3 transplantations after 4 transplantations	850 762 76 10 2
Male/female	534/316
Age	3 months–21 years (median: 8.87 years)
Stem cells source bone marrow peripheral blood cord blood unit	 237 696 18
Donor characteristic autologous MSD MUD MMUD	 246 184 420 101

MSD – matched sibling donor; MUD – matched unrelated donor; MMUD – mismatched unrelated donor.

Table 4. Indications for HSCT in study cohort

Diagnosis		Number of patients	
Hematological malignancies	ALL AML CML MDS LYM	227 125 36 65 53	total n = 506
Anemias	SAA BDA FA	62 10 13	total n = 85
Solid tumors	neuroblastoma Ewing sarcoma other	100 40 35	total n = 175
Immuno-deficiencies	SCID X-CGD Omenn syndrome other	26 11 8 21	total n = 66
Metabolic disorders	ALD MLD other	7 2 9	total n = 18

ALL – acute lymphoblastic leukemia; AML – acute myeloblastic leukemia; CML – chronic myeloid leukemia; MDS – myelodysplastic syndrome; LYM – lymphoma; SAA – severe anaplastic anemia; BDA – Blackfan–Diamond anemia; FA – Fanconi anemia; SCID – severe congenital immunodeficiency; X-CGD – chronic granulomatous disease; ALD – adrenoleukodystrophy; MLD – metachromatic leukodystrophy.

received stem cells from a cord blood unit. The complete clinical and demographic analysis of the patient cohort is presented in Tables 3 and 4.

Definitions

Each case of VOD was diagnosed using the Modified Seattle Criteria, which requires at least 2 of the following clinical findings within 20 days of transplantation: painful hepatomegaly, weight gain >2% above baseline and plasma bilirubin level >2 mg/dL (34 mcmol/L). The severity was evaluated on the basis of criteria proposed by McDonald et al.[1]: mild for cases that resolved spontaneously, moderate when treatment was required but the symptoms were resolved completely and severe when there was associated multiorgan failure (MOF) or the symptoms were not resolved by day 100 post-HSCT.

Prophylaxis and treatment

Every patient was scheduled for careful ATIII activity monitoring, starting from the first day of conditioning. Measurements of ATIII were repeated twice a week and continued till day 100 post-HSCT. If a patient developed low levels of ATIII, its activity was measured more often, i.e., every day.

Every patient who demonstrated decreased plasma ATIII activity in the period between the beginning of the conditioning up to 100 days post-HSCT received an additional supplementation of ATIII to maintain its activity over 80%. The treatment strategies for patients with confirmed VOD depended on the severity of the symptoms and any coexisting multiorgan failure. They varied from conservative measures such as fluid restriction and ursodiol in mild cases up to combined treatment with defibrotide (daily dose 25 mg/kg b.w.) and additional diuretics, anticoagulant agents and multiple platelets transfusions.

Results

Veno-occlusive disease was diagnosed in 48 patients – 20 females and 28 males. Forty-seven patients developed VOD after the 1st HSCT. There was only 1 case of VOD following consecutive transplantation. Patients were from 7 months to 19.5 years of age at the time of HSCT. Veno-occlusive disease developed in 26 of the 246 autologous HSCT recipients (10.6%) and in 22 of the 705 allogeneic HSCT recipients (3.1%). Mild VOD occurred in 7 patients (14.6% of VOD), and severe occurred in 39 patients (81.25% of VOD). Two patients developed moderate VOD. The overall incidence of VOD in the study cohort was 5.05%. There was a significantly higher difference in VOD incidence when the analyzed autologous HSCT was compared to allogeneic HSCT ($p < 0.05$). The indications for transplant in the VOD cohort was most commonly solid tumors (26 patients), mainly neuroblastoma. Conditioning regimens, as a well-known risk factor for VOD, were also assessed. All patients, except for one, who were diagnosed with VOD, received a myeloablative regimen prior to HSCT. The majority of patients (38) received busulfan/melphalan, while 9 received a TBI-based regimen (Table 5).

Time from HSCT to VOD onset varied between 3 days to 67 days post-transplantation (median: 14.5 days). Cases of VOD diagnosed after 20 days post-transplantation (13 patients, 27%) were confirmed by the presence of specific

Table 5. Detailed characteristic of VOD patients

Parameters		Values
Sex	male	28
	female	20
Type of transplant and donor	AUTO	26
	ALLO: MSD MUD MMUD	22 6 13 3
Stem cells source	bone marrow	7
	peripheral blood	41
Conditioning regimen	MAC: TBI BU-MEL MEC	47 9 36 2
	RIC	1
Diagnosis	AL	16
	CML	1
	IE	1
	ID	1
	LYM	3
	solid tumor: NBL Ewing Yolk sac Wilms	26 20 4 1 1
Staging at the moment of HSCT	advanced	40
	non-advanced	8
Age [years]	range: 0.6–19.5, median: 6.75	

AUTO – autologous transplantation; ALLO – allogeneic transplantation; MSD – matched sibling donor; MUD – matched unrelated donor; MMUD – mismatched unrelated donor; MAC – myeloablative conditioning; TBI – total body irradiation 12 Gy; BU-MEL – busulfan (16–19 mg/kg)+melphalan (140 mg/m^2)-based regimen; RIC – reduced intensity regimen; RTC – reduced toxicity regimen (Busulfan (9–12 mg/kg)+Fludarabine 150 mg/m^2); MEC – Melphalan (140 mg/m^2)+Etoposide (60 mg/kg) conditioning regimen; AL – acute leukemia; IE – metabolic disorders; ID – immunodeficiency; LYM – lymphoma; NBL – neuroblastoma. Non-advanced disease – 1CR (1st complete remission); CML – chronic phase, non-malignant disease; advanced – anything below non-advanced.

findings in an abdominal USG such as attenuated or even reversed blood flow in the portal vein. Nine cases of late-onset of VOD were confirmed with liver biopsy.

Among the diagnostic criteria used in the Modified Seattle Criteria, hepatomegaly was most commonly observed in our patients (n = 39; 81.25%). Ascites and weight gain occurred accordingly in 32 (66.7%) and 33 (68.75%) patients. Plasma bilirubin level range at diagnosis was 0.7–32.5 mg/dL (median 2.83 mg/dL). Fourteen patients (29%) never demonstrated a bilirubin level higher than 2 mg/dL, as indicated in the Modified Seattle Criteria, neither on the day of diagnosis nor during the course of treatment for VOD.

In the entire study cohort, 19% of patients (n = 181) demonstrated decreased ATIII activity level on day 0 and received immediate ATIII supplementation. Antithrombin III activity range on day 0 was 44–152% (median: 101%). Only 5 patients, among those who demonstrated decreased ATIII levels on the day of HSCT, developed VOD, despite being given prophylaxis. In the VOD cohort, 10 patients (21%) had normal ATIII activity levels since the beginning of pre-HSCT conditioning through the resolution of symptoms of VOD (9 patients) or death (1 patient). The remainder of patients that were diagnosed with VOD (n = 33, 68.8%) developed decreased ATIII plasma activity between 19 days prior, up to the day of diagnosis of VOD (median: 3 days before diagnosis of VOD).

Twenty-nine patients (64%) demonstrated increased platelet consumption followed by refractory thrombocytopenia and required daily platelet transfusions to maintain transfusion criteria (platelet count below 20,000/mcL).

Resolution of VOD symptoms was defined as platelet level sustainability and platelet transfusion independence, resolution of painful hepatomegaly, resolution of jaundice, and reduction of ascites (which was measured by a decrease of waist circumference to the patient's baseline as well as weight reduction). Using this definition, duration of VOD symptoms ranged from 3 to 47 days (median: 19 days). Eight patients (16.6%) died before the resolution of symptoms.

During therapy, 29 patients (60.4%) with VOD received defibrotide. The response rate for defibrotide was 82.76%. The time of treatment ranged from 4 days to 26 days (median: 12 days). Each patient was given a standard dose of 25 mg/kg b.w. daily.

Day 100 mortality rate in the VOD cohort was 21% (n = 10), compared to the 15.52% (n = 132) day 100 mortality rate in the entire cohort of patients. In the VOD cohort, 10 more patients died after day 100 but their causes of death were not directly related to the HSCT.

Day 100 mortality rate among patients treated with defibrotide was 17% (n = 5). Veno-occlusive disease was recognized as the cause of death in 6 patients (12.5%). Two more patients died from septic shock and gastrointestinal hemorrhage with coexisting active VOD. Importantly, only 1 patient among those who died of VOD was diagnosed within 20 days post-HSCT. Detailed cause-of-death information is presented in Table 6.

Table 6. Causes of day +100 transplant-related mortality in veno-occlusive disease (VOD) cohort

Cause of death	n = 10
VOD	6
Infection	3
Hemorrhage	1

Discussion

The incidence of VOD in our study was 5.05%. Other publications that have also focused strictly on VOD in children have reported incidence ranges from 13% to 28%.[12–15,26–30] This high variability in rates of VOD might be caused by the selected study cohort, different risk factors and, of course, ambiguous diagnostic criteria.

However, in light of the recently implemented diagnostic criteria, the incidence of VOD in the pediatric population may shift drastically. The most recent research conducted by Corbacioglu et al.[9] demonstrates major differences in VOD prevalence depending on the criteria used, suggesting that VOD may be more common than previously thought. Given the criteria proposed by EBMT, there is also a possibility that VOD was underdiagnosed in our center, even taking into account 13 late-onset cases which did not meet the Modified Seattle Criteria.

Our study revealed that patients undergoing autologous-HSCT more likely develop VOD than patients receiving allogeneic graft – 10.6% compared to 3.12%. This result is quite opposite to those presented in the literature concerning adult patients, as allogeneic stem cell transplantation is a well-known risk factor for VOD in the adult population. There are only a few studies performed on very limited study cohorts (86 and 116 patients) analyzing VOD after autotransplantations in children. Those studies report an extremely high incidence of VOD, reaching 39%.[31,32] The difference in frequency of VOD after autologous and allogeneic transplantations presented in our analysis is most likely caused by the fact that almost all of the patients treated with autotransplantation were suffering from neuroblastoma, a known risk factor for VOD.[32] The exact mechanism for this increased risk in neuroblastoma is not known, but it likely relates to the association with other known VOD risk factors such as previous abdominal radiation, specific chemotherapy agents including tandem transplantations and lower albumin level prior to HSCT.[32]

The majority of patients in our VOD cohort were recipients of a 1st HSCT. Only 1 out of 88 patients receiving multiple transplants developed VOD. This observation may indicate that it is not the cumulative doses of chemotherapeutic agents that are a risk factor for VOD, but rather the intensity of treatment in a limited period of time that has a significant impact, given that several other studies have found a correlation with increased risk following subsequent transplantation.[13]

The prevalence of late-onset VOD has never been properly assessed in the pediatric population.[33] Several studies report a minority of VOD occurrences diagnosed later than 20 days post-transplantation. In our cohort, almost 1/3 (27%) of patients did not meet the time criterion of VOD required in the Modified Seattle Criteria. Our results are in compliance with the absence of time limitation in the newly proposed diagnostic criteria, where VOD may be diagnosed regardless of the time when the first symptoms occurred. This may be particularly important given the high mortality rate associated with late-onset VOD in our cohort. Five of the 6 patients who died of VOD in our cohort were diagnosed with late-onset VOD. New EBMT VOD criteria for adults (Mohty et al.[10]) have highlighted for the first time the presence of anicteric VOD. This phenomenon seems to be even more frequent in children, which has been emphasized by Corbacioglu et al.[9]

Fourteen patients (27%) never demonstrated a bilirubin level over 2 mg/dL, as suggested in the Modified Seattle or Baltimore VOD diagnostic norms. A similar percentage of anicteric patients were reported in other studies[26,27]; nonetheless, bilirubin level, even if not mandatory, still remained a part of the VOD diagnostic criteria. The novel approach to VOD emphasizes the importance of bilirubin kinetics, instead of bilirubin plasma levels alone. Although elevated bilirubin level is a common VOD symptom, lack of hyperbilirubinemia should not lead to a delay in diagnosis and treatment. As reported by Corbacioglu,[16] the response to treatment with defibrotide is significantly lower when implemented more than 2 days from diagnosis. Therefore, a proper and immediate diagnosis, based on adequate criteria, is crucial for general patient outcome.

Bilirubin levels and its kinetics are suggested to be more a prognostic factor than a strict diagnostic criterion.[9]

Increased platelet consumption was a prominent feature of our VOD cohort. Sixty-four percent of patients required daily platelet transfusions to maintain adequate hematologic parameters. The median time of transfusion dependence related to VOD was 14 days. Even if mentioned in the very first studies about VOD, refractory thrombocytopenia (RT) has been overlooked as a VOD symptom for years. Despite not being a part of the diagnostic criteria, in our center, RT has remained an important symptom and diagnostic hint for VOD. Correlation of ATIII activity level and development of VOD is still unclear. Only half of patients demonstrated a sudden drop in ATIII activity, as indicated in previous studies. Even if such an evident drop was observed, it mostly happened only 3 days before a diagnosis was made. Haussmann et al. demonstrated an even shorter time lapse between ATIII activity decline and diagnosis of VOD.[24] This makes plasma ATIII activity drop a poor prognostic factor for VOD. Although both Haussman and Peres emphasize a beneficial effect of early intervention with ATIII,[24,25] it has never been highlighted in the major expert opinions. Looking at the relevantly lower incidence of VOD, this beneficial effect of ATIII supplementation seems to be confirmed by our study.

Conclusions

Veno-occlusive disease is a quite frequent, partially unexpected and potentially fatal complication following HSCT. New VOD diagnostic criteria provided by EBMT seem to be more adequate for the pediatric population than the recently used Modified Seattle Criteria. The newly proposed criteria correlate better with actual clinical findings in children diagnosed with VOD after HSCT. Removal of the time factor and hyperbilirubinemia, and incorporation of RT into the diagnostic criteria were crucial. The lower incidence of VOD in our center might suggest that early supplementation of ATIII to maintain its activity over 80% could be an efficacious prophylaxis against VOD. This novel finding requires further studies.

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ARTICLE

Introduction of new pediatric EBMT criteria for VOD diagnosis: is it time-saving or money-wasting?

Prospective evaluation of pediatric EBMT criteria for VOD

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Abstract

Hepatic veno-occlusive disease (VOD) is a potentially fatal complication following hematopoietic stem cell transplantation (HSCT). We evaluated in prospective analysis the usefulness of the pediatric EBMT criteria for VOD diagnosis and their presumable impact on cost effectiveness and patients' outcome. Study included all 282 HSCT procedures performed in Department of Pediatric Hematology/Oncology and BMT in Wrocław between January 2016 and March 2019. Data were compared with previous VOD research conducted in our center before year 2016. Twenty-five (8.9%) patients (median age 3.5 years) were diagnosed with VOD. Duration of defibrotide (DF) administration varied from 4 to 34 days (median: 16.5), with 96% response rate. Overall survival was 88%. If applying Baltimore and modified Seattle criteria, VOD incidence was 2.13% and 5.7%, respectively. Median diagnosis delay based on modified Seattle criteria was 3 days. Before 2016, VOD incidence was 4.9%, with 74% DF response rate ($p = 0.033$) and 56.2% OS ($p = 0.008$). After implementing new criteria length of hospitalization for VOD patients decreased by median of 12 days ($p = 0.009$). Earlier VOD diagnosis, facilitated by EBMT criteria, resulting in implementing immediate treatment significantly improved patients' outcome. Furthermore, it allows shortening of DF administration and minimizes length of hospital stay.

Veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), is an unpredictable and potentially fatal complication following haematopoietic stem cell transplantation (HSCT) [1]. It belongs to a group of conditions increasingly designated as transplant-related endothelial complications. VOD may also occur as a result of chemotherapy, radiation, and immunotoxin-conjugated therapy [2, 3]. A toxic injury of the sinusoidal endothelial cells, allows extravascular deposition of blood cells and further endothelium dissection leading to central venous occlusion and sinusoidal obstruction resulting in post-sinusoidal portal hypertension. This pathophysiology process leads to the clinical syndrome of VOD consisting of painful hepatomegaly, ascites, weight gain and hyperbilirubinemia [4–7]. Persistence of reduced blood flow leads to hepatocyte damage and cell death.

The mean incidence of VOD is 13.7% in adult patients, but it may be threefold higher among children, reaching a level of 60% in certain high-risk populations [8–12]. The reported prevalence of this complication is variable due to the different diagnostic criteria applied [9, 13, 14].

Many transplant-related factors such as high-dose busulfan or TBI-based myeloablative conditioning, a second transplant and prior hepatic injury have been demonstrated to increase the risk of VOD [8, 13, 15–17]. Beyond those, there are a number of pediatric conditions, in particular younger age (i.e., infants due to their liver immaturity) and underlying pediatric disorders such as HLH, neuroblastoma, osteopetrosis, or thalassemia, which may enhance the likelihood of VOD [9, 18–21].

Apart from risk factors, pediatric VOD differs significantly from adults in terms of their clinical manifestation.

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Table 1 Comparison of Baltimore and modified Seattle VOD diagnostic criteria.

Modified seattle criteria	Baltimore criteria
Two of following occurring within 21 days of transplantation:	Bilirubin serum level >2 mg/dL and at least two of following within 20 days of transplant:
Hepatomegaly or right upper quadrant pain	Hepatomegaly
Bilirubin serum level >2 mg%	Ascites
Unexplained weight gain >2% from baseline	Weight gain >5% from baseline

Table 2 EBMT diagnostic criteria for hepatic VOD/SOS in children.

No limitations for time onset of VOD/SOS

Presence of two or more of the following[a]:

- Unexplained, consumptive, transfusion-refractory thrombocytopenia[b]
- Otherwise unexplained weight gain in three consecutive days despite use of diuretics or weight gain >5% baseline
- [c]Hepatomegaly (best confirmed by imagining) above baseline
- [c]Ascites (best confirmed by imagining) above baseline
- Rising bilirubin from a baseline value on three consecutive days or bilirubin ≥2 mg/dl within 72 h

[a]With the exclusion of other potential differential diagnosis.

[b]≥on weight-adjusted platelet substitution/day to maintain institutional transfusion guidelines.

[c]Imagining immediately before HSCT to determine baseline value.

The anicteric course of VOD is more commonly observed in children (about 30% of cases), similarly to relatively later onset of the disease [22, 23]. In almost 20% of pediatric cases, VOD develops beyond day +30 post-HSCT which is rare in the adult population [9, 24]. Children are also more prone to rapid weight changes, partially due to iatrogenic fluid overload, making the weight gain less objective as a diagnostic hint.

In the absence of specific biomarkers or imagining tools, a VOD diagnosis is based on clinical findings [25, 26]. For the last three decades, two sets of criteria were used to diagnose VOD—Baltimore or Seattle criteria (Table 1) [5, 6]. Neither of those was designated for the pediatric population. Therefore, EBMT recently proposed VOD diagnostic criteria for children, along with a new severity classification (Table 2). Major changes include no limitation of the time of onset of VOD, no specific bilirubin cut-off level, a more objective assessment of ascites and hepatomegaly as well as the inclusion of refractory thrombocytopenia (RT) as a diagnostic criterion [14].

The primary aim of this single-center prospective cohort study was to evaluate the usefulness of the new EBMT VOD diagnostic criteria in comparison with the historically used Seattle and Baltimore criteria. Furthermore, the presumable impact of the new criteria on patients' outcome was assessed.

Study design

All HSCT procedures performed in the Department of Pediatric Hematology, Oncology, and Bone Marrow Transplantation in Wroclaw between January 2016 and March 2019 were eligible for the study. Therefore, the study cohort incorporated 282 children and adolescents, aged from 2 months up to 21 years (median: 7.6 years) who underwent either allogeneic ($n = 209$) or autologous ($n = 73$) HSCT. The indications for transplant comprised both malignant ($n = 207$) and non-malignant ($n = 75$) disorders. The median follow-up was 22.3 months. The patient characteristics are presented in Table 3. In this study, further detailed analysis was focused on 25 patients diagnosed with VOD post-HSCT during the above-mentioned period. The new pediatric EBMT VOD criteria were officially published in June 2017 but were discussed within the Pediatric Diseases Working Party of the EBMT (personal communication Selim Corbacioglu) so that we became familiar with the preliminary drafts of the published criteria as early as the beginning of 2016. Since then, the approach to the diagnosis of VOD in our center changed accordingly and we were able to start our prospective pilot study using the new EBMT criteria.

VOD diagnosis and treatment

Each case of VOD was diagnosed using pediatric EBMT diagnostic criteria, along with a severity assessment. Weight was measured once daily; the serum bilirubin level was evaluated at least every 2 days or more frequently if needed. Antithrombin III, AST, and ALT were measured twice a week. An abdominal ultrasound was performed prior to HSCT and repeated in the case of any abdominal symptoms. Refractory thrombocytopenia, manifesting as consumptive thrombocytopenia with refractoriness for platelet transfusion, was defined as the need for an otherwise unexplained daily weight-adjusted platelet transfusion to maintain the transfusion criteria (PLT above 20,000/mL). For the severity assessment, respiratory and renal failure was defined according to CTCEA 4 (Common Terminology Criteria for Adverse Events) VOD severity classification [14]. Neurological impairment was defined as a newly observed cerebral deterioration, i.e., a drop in the level of

Table 3 Characteristics of study cohort.

Patients/HSCT analyzed	$n = 282$
Autologous	73 (25.9%)
Allogeneic	209 (74.1%)
MSD	*46*
MUD	*151*
MMD	*12*
Male/Female	180 (63.8%)/102 (36.2%)
Age	2 months to 21 years (median: 7.6 years)
Less than 2 years	56 (19.9%)
Stem cells source	
Bone marrow	35 (12.4%)
Peripheral blood	242 (85.8%)
Cord blood	5 (1.8%)
Conditioning regimen	
Treosulfan-based	131 (46.5%)
Busulfan-based	64 (22.7%)
TBI-based	11 (3.9%)
RIC	27 (9.6%)
NMA	45 (16%)
Other	4 (1.4%)
Diagnosis	
Acute leukemia	95 (33.7%)
Solid tumor	62 (22%)
Neuroblastoma	*41 (66.13%)*
Other	*21 (33.87%)*
Immunodeficiency	34 (12.1%)
HLH	*4 (11.8%)*
Myelodysplastic syndrome	27 (9.6%)
Anemia	29 (10.3%)
Lymphoma	21 (7.4%)
Metabolic disorders	10 (3.5%)
Chronic leukemia	4 (1.4%)

MSD matched sibling donor, *MUD* matched unrelated dono, *MMUD* mismatched unrelated donor, *NMA* non-myeloablative conditioning, *RIC* reduced intensity conditioning, *HLH* hemophagocytic lymphohistiocytosis.

consciousness, seizures. If a patient required repeated fresh frozen plasma (FFP) transfusions to maintain an INR < 1.5, it was classified as coagulation impairment [14]. Complete remission (CR) of VOD was considered as a resolution of RT and ascites along with normalization of the serum bilirubin level [27].

According to institutional guidelines, ATIII supplementation was provided to every patient who presented with a drop in ATIII level below 80%. In our center, the treatment of VOD was conducted according to the expanded-access protocol; therefore Defibrotide (DF) was administered at 25 mg/kg/day [28] to all VOD patients, starting from the day of diagnosis until CR was achieved. In our cohort, no patients received DF as a prophylaxis.

Comparison cohort

To compare modified Seattle criteria, formerly used in our institution with the EBMT criteria in clinical practice, the collected data were compared with the results of previous VOD research, conducted in our center during the years 2001–2015 [29]. This former cohort included 48 cases of VOD diagnosed with modified Seattle criteria among the 951 HSCT that were performed in the analyzed period of time. The baseline characteristics of the former VOD cohort are presented in Table 4.

Statistical methods

The data were described as percentage for discrete variables and median for continuous variables. Utilizing either chi-squared or Fisher exact tests for discrete variables and Wilcoxon nonparametric rank sum test for continuous data, we perform simple comparison of outcomes of VOD patients in both cohorts. *P* values lower than 0.05 were considered significant. Survival analysis was performed using Kaplan–Meier estimation. Statistical analysis was performed using R statistical software.

Results

Among the 282 HSCTs analyzed, 25 patients (14 males, 11 females) were diagnosed with VOD according to the EBMT criteria; therefore, the VOD incidence in our center was 8.9% (25/282). The median age in the VOD cohort was 3.5 years (range 9 months to 17 years) which is significantly lower compared with the general population of patients (3.5 years vs 7.4 years, $p = 0.002$). In 11 patients VOD occurred after an autologous HSCT, while in 14 it was post allogeneic HSCT. According to the new severity classification, over half of the VOD cases met the criteria of severe and very severe VOD (13/25, 53%) (Table 5).

The median time post-HSCT at the moment of VOD diagnosis was 16 days (8–25 days). Five cases (20%) were diagnosed over 21 days post-transplant and would be historically classified as late-onset VOD.

The earliest and most consistent symptom of VOD in our patients was RT. It was observed in 100% of children, commonly as the first sign of VOD. Nineteen (76%) patients presented US-confirmed ascites, while both hepatomegaly and weight gain were observed in 23 (92%) patients respectively. None developed reversed blood flow in the portal vein (Table 6).

Table 4 Characteristics of patients diagnosed with VOD using Modified Seattle Criteria in years 2001–2015.

VOD patients	$n = 48$
Male/Female	28/20
Age	7 months to 19.5 years (median 6.7 years)
Type of transplant	
Autologous	54.2% (26/48)
Allogeneic	45.8% (22/48)
MSD	6
MUD	13
MMD	3
Underlying disease	
Neuroblastoma	20
Acute leukemia	16
Ewing tumor	4
Lymphoma	3
Chronic leukemia	1
Wiskott–Aldrich syndrome	1
SCID	1
Yolk sac tumor	1
Wilms tumor	1
Stem cells source	
Bone marrow	14.6% (7/48)
Peripheral blood	12.2% (41/48)
Conditioning regimen	
MAC	97.9% (47/48)
RIC	2.1% (1/48)
Severity of VOD	
Mild	14.6% (7/48)
Moderate	4.2% (2/48)
Severe	81.3% (39/48)
Time of VOD diagnosis	3–67 days (median 16.5 days)
Late-onset cases of VOD	27% (13/48)
Serum bilirubin level	0.7–32.5 mg/dl (median 2.83 mg/dl)
DF response rate	74% (22/30)
OS	56.3% (27/48)
Day 100 TRM	20.3% (11/48)

MSD matched sibling donor, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *MAC* myeloablative conditioning, *RIC* reduced intensity conditioning.

Table 5 Characteristics of patients diagnosed with VOD according to EBMT VOD diagnostic criteria.

Number of VOD patients	$n = 25$
Age	9 months to 17 years (3.5 years)
Sex	
Male	14
Female	11
Type of transplant	
Autologous	11
Allogeneic	14
MUD	9
MSD	3
MMD	2
Underlying disease	
Neuroblastoma	11
Acute leukemia	4
JMML	2
Lymphoma	2
Osteopetrosis	1
Wiskott-Aldrich syndrome	1
HLH	1
Krabbe disease	1
Mucopolisacharydosis	1
Wilms Tumor	1
Severity assessment	
Mild	6
Moderate	6
Severe	6
Very severe	7
Days post HSCT at diagnosis	8–25 (median 16 days)
VOD symptoms	
RT	100% (25/25)
Ascites	76% (19/25)
Hepatomegaly	92% (23/25)
Weight gain	92% (23/25)
Rising bilirubin on 3 days	68% (17/25)
Hyperbilirubinemia at diagnosis	20% (5/25)
Serum bilirubin level at diagnosis	0.4–4.5 mg/dl (1.2 mg/dl)
Peak serum bilirubin level	0.4–20.3 mg/dl (1.6 mg/dl)
AST at diagnosis	7–3118 U/l (median: 59 U/l)
ALT at diagnosis	4 U/l-1686 U/l (median: 34 U/l)
Peak AST	13–4502 U/l (median: 115 U/l)
Peak ALT	22–2336 U/l (median: 80 U/l)
VOD acquired organ dysfunction	
Coagulation impairment	28% (7/25)
Oxygen supplementation	24% (6/25)
Mechanical ventilation	4% (1/25)
Renal impairment	12% (3/25)
Cerebral deterioration	4% (1/25)
DF response rate	96% (24/25)
OS	88% (22/25)
Day100 TRM	4% (1/25)

JMML juvenile myelomonocytic leukemia, *HLH* hemophagocytic lymphohistiocytosis.

Only five (20%) patients presented with hyperbilirubinemia >2 mg/dl at diagnosis, while 17 (68%) met the criterion of a rising serum bilirubin level on three consecutive days, not necessarily reaching any certain threshold. Median bilirubin level at the diagnosis was 1.2 mg/dl (range 0.4 mg/dl–4.5 mg/dl). The peak bilirubin level in the VOD cohort varied from 0.4–20.3 mg/dl (median 1.6 mg/dl). Primarily obligatory hyperbilirubinemia (>2 mg/dl) was achieved by seven (28%) patients during the whole course of VOD. A rapid increase in the bilirubin level (doubling within 48 h) was observed in six cases. None of the patients had a prior history of cirrhosis.

Table 6 Details of the VOD diagnostic process.

No	Type of transplant	Day of VOD diagnosis (post-HSCT)	Symptoms at diagnosis	Fever	Rash	aGvHD at VOD diagnosis day	Other latter VOD symptoms according to diagnostic criteria
1	Auto-HSCT	22	RT, rising bilirubin, ascites, weight gain	No	No	No	
2	MUD-HSCT	12	RT, hepatomegaly, rising bilirubin on 3 days	No	Yes	Skin grade II	Bb doubling 48 h, ascites, weight gain
3	MMUD-HSCT	14	RT, weight gain, hepatomegaly	No	No	No	Ascites, Hyperbilirubinemia
4	Auto-HSCT	16	RT, hepatomegaly,	No	No	No	Ascites, weight gain
5	MSD-HSCT	25	RT, ascites, rising bilirubin	No	yes	Skin\gut grade III	Weight gain
6	Auto-HSCT	16	RT, weight gain, rising bilirubin	No	no	No	hepatomegaly
7	Auto-HSCT	9	RT, hepatomegaly, hyperbilirubinemia	No	No	No	Bilirubin doubling within 48 h, ascites, weight gain,
8	Auto-HSCT	20	RT, hepatomegaly, rising bilirubin on 3 days	No	No	No	Weight gain
9	MSD-HSCT	7	RT, hyperbilirubinemia, hepatomegaly	Yes	No	No	Ascites, weight gain
10	MUD-HSCT	21	RT, ascites, hepatomegaly	No	No	No	rising bilirubin, Hyperbilirubinemia, weight gain
11	Auto-HSCT	19	RT, rising bilirubin, hepatomegaly	no	No	No	weight gain, ascites
12	MUD-HSCT	18	RT, ascites, weight gain,	no	No	No	Rising bilirubin hepatomegaly
13	Auto-HSCT	15	RT, rising bilirubin, hepatomegaly	no	no	No	ascites, weight gain
14	Auto-HSCT	18	RT, hepatomegaly,	No	No	No	Ascites, weight gain
15	MUD-HSCT	12	RT, rising bilirubin, hepatomegaly,	No	No	No	weight gain
16	MUD-HSCT	12	RT, rising bilirubin, hepatomegaly	No	No	No	Bilirubin doubling within 48 h, weight gain
17	MSD-HSCT	20	RT, rising bilirubin, hepatomegaly	No	No	No	weight gain, ascites
18	MUD-HSCT	21	RT, hyperbilirubinemia, hepatomegaly, weight gain, ascites	No	Yes	Skin\ gut grade IV	
19	MUD-HSCT	16	RT, rising bilirubin, hepatomegaly, ascites, weight gain	No	yes	Skin grade III	Bilirubin doubling 48 h,
20	MUD-HSCT	10	RT, rising bilirubin, hepatomegaly	No	No	No	Hyperbilirubinemia
21	Auto-HSCT	22	RT, rising bilirubin, ascites, weight gain	No	No	No	Bilirubin doubling within 48 h, hepatomegaly
22	Auto-HSCT	20	RT, ascites, rising bilirubin, hepatomegaly	No	No	No	Weight gain
23	Auto-HSCT	20	RT, ascites, hepatomegaly, weight gain, rising bilirubin	No	No	No	Bilirubin doubling within 48 h
24	MUD-HSCT	12	RT, hyperbilirubinemia,	No	No	No	hepatomegaly, weight gain, ascites
25	MSD-HSCT	8	RT, hyperbilirubinemia, ascites, hepatomegaly	Yes	No	No	Bilirubin doubling within 48 h

Auto-HSCT autologus hematopoietic stem cell transplantation, *MUD-HSCT* matched unrelated donor hematopoietic stem cell transplantation, *MSD-HSCT* matched sibbling donor hematopoietic stem cell transplantation, *RT* refractory thrombocytopenia.

Table 7 Comparison of cohorts diagnosed by EBMT VOD criteria and modified Seattle criteria.

	2016–2019 EBMT diagnostic criteria Total $n = 282$	2001–2015 modified Seattle criteria Total $n = 951$	p value
VOD incidence	8.9% (25/282)	4.9% (48/951)	0.021
"Late-onset" VOD incidence	20% (5/25)	27% (13/48)	0.06
Median serum bilirubin level	1.2 mg/dl	2.83 mg/dl	0.0001
DF response rate	96% (24/25)	74% (22/30)	0.033
OS	88% (22/25)	56.2% (27/48)	0.008
TRM	4% (1/25)	20% (11/48)	0.12
Median length of hospitalization	42 days	54 days	0.009

With applying the Baltimore criteria to the analyzed cohort, only 6 (24%) patients with VOD would have been identified, while 16 (65%) would be diagnosed by the modified Seattle criteria. The median delay to diagnosis of VOD using the modified Seattle criteria compared with the EBMT criteria was 3 days (0–11 days).

With the diagnosis of VOD, DF was started in every patient. The decision to withdraw DF was based on clinical evaluation. Therefore, the duration of treatment was determined by individual presentation of 'dynamic' and 'static' criteria according to recommendations of Corbacioglu et al. [27] and was made if patients achieved VOD CR. The median length of DF treatment was 16 days (4–34 days). Two patients needed treatment beyond 28 days. The DF response rate was 96% (24/25 patients). In our study, we did not observe any significant adverse effects of DF.

Seven patients developed severely impaired coagulation and required repeated FFP transfusions. Four patients with VOD presented with mild respiratory distress, resulting in increased oxygen demand (>2 l/min). One patient developed VOD-associated acute respiratory and renal failure requiring admission to the pediatric intensive care unit (PICU). In the analyzed cohort, three patients died; two due to relapse and one with septic shock and fulminant VOD. The overall survival (OS) and day 100 transplant-related mortality (TRM) were 88% and 4%, respectively.

The above-mentioned results considering the incidence and clinical manifestation of VOD were compared with patients who were diagnosed with VOD in our center before 2015 using the modified Seattle criteria. There were significant differences between these two groups (Table 7). With the implementation of the pediatric EBMT criteria, the incidence of VOD in our center doubled (8.9% vs 4.9%; $p = 0.024$). Even if there was no relevant difference in the median time of diagnosis, the rate of "late-onset" cases has changed (5/25—20% vs 13/48—27%). The median serum bilirubin levels at the time of VOD diagnosis were significantly higher in the modified Seattle cohort (2.83 mg/dl vs 1.2 mg/dl; $p < 0.0001$), so was the duration of hospitalization significantly longer compared with the period when patients were diagnosed with the pediatric EBMT criteria (54 days vs 42 days; $p = 0.0021$). However, the major difference in both cohorts consisted of the DF response rate (74% vs 96%; $p = 0.033$) and the OS (56,3% vs 88%; $p = 0.008$). It is worth mentioning that among patients diagnosed according to Seattle criteria, six patients died due to VOD, while in the present cohort only one patient died due to VOD-acquired multiorgan failure (MOF).

Discussion

For decades, similar criteria were used for VOD diagnosis in children and adults, despite ample evidence that the disease differs significantly between adults and children in terms of clinical manifestation and response to treatment [14]. The pediatric EBMT criteria were developed to provide the first pediatric-specific, and a more dynamic diagnostic tool for VOD in children. The need for new criteria was encouraged by the evident therapeutic activity of DF, particularly in the pediatric population [30]. However, the new criteria were developed solely on expert opinion. Despite several retrospective analyses confirmed the sensitivity of the EBMT criteria, its prospective evaluation is essential [13, 31].

The incidence of VOD in our cohort was 8.9%. Although this equates to the lower end of the other studies, after implementing the new criteria, it has doubled compared with our previous reports (4.9%) [9, 10, 12, 29, 32, 33]. These findings suggest that less strict but more dynamic EBMT criteria facilitate VOD diagnosis, preventing mild and moderate cases which may benefit from immediate treatment being overlooked. On the other hand, our relatively low incidence does not support the concern of overdiagnosing VOD due to the new criteria [13, 33].

A relatively high percentage of anicteric VOD, particularly in children, has been reported in the last few years [22, 23]. Nevertheless, a specific serum bilirubin level remained an important part of diagnosis, especially in the Baltimore criteria. An emphasis on bilirubin kinetics as proposed in the new criteria, rather than the cut-off level, reveals that the anicteric course of VOD in children may be even more common than previously expected. In our study cohort, only 20% of patients presented with hyperbilirubinemia (>2 mg/dl) on the day of VOD diagnosis. There was also a significant difference in serum bilirubin levels between the present and historical VOD cohorts. Numerous reports demonstrated that hyperbilirubinemia >2 mg/dl is rather a criterion for poor outcome, so that the conservative criteria with mandatory

hyperbilirubinemia seem to cause an unnecessary delay in VOD diagnosis [9, 22, 33, 34]. A more dynamic approach and careful monitoring of the serum bilirubin level as proposed in the EBMT criteria allows pre-emptive intervention, preventing progression to severe VOD/MOF and may improve patients' outcome [23, 35].

RT, however, is not a novel observation but was mentioned already in one of the very first reports about VOD [7] and was repeatedly described thereafter, all in adult patients [5, 36–39]. Unfortunately, RT never entered diagnostic criteria and is therefore the most novel criterion of the pediatric EBMT criteria. Although unspecific, it was the only and earliest criterion fulfilled by 100% of our patients, confirming recent publications [31, 33]. Even if it is uneasy to establish [40], RT may be the very first hallmark of VOD, encouraging clinicians to be particularly vigilant about other VOD symptoms consecutively, especially in high-risk patients [5, 30, 38, 41].

To illustrate the changes provided by the new criteria, we retrospectively applied both the Baltimore and modified Seattle criteria to our EBMT VOD cohort. The biggest discrepancy between the EBMT criteria and the old criteria was noted in its sensitivity. If Baltimore or modified Seattle criteria were still in use in our center, VOD incidence would be 2.13% (6/282) and 5.7% (16/282) respectively. A considerable number of patients with VOD would not be recognized by those criteria and therefore would not have the chance to receive the proper treatment. Another crucial difference between EBMT criteria and modified Seattle criteria is time of diagnosis. In those 16 patients retrospectively diagnosed by the modified Seattle criteria, the median diagnostic delay compared with the EBMT criteria was 3 days [13, 27]. According to published evidence and ongoing trials, the timepoint of initiation of VOD treatment is critical with regard to outcome. Despite the unequivocal curative potential of DF, any delay of diagnosis, even beyond 1 day, results in longer and less effective treatment with a decrease in OS. [30, 34, 42–44].

Prompt diagnosis along with immediate treatment supported by the new VOD criteria led to a promising general outcome of our study group (OS = 88% and TRM = 4%). These results need to be highlighted as they are outstanding compared with the latest other reports considering DF efficacy in children [34, 43, 45]. Twenty-four patients achieved VOD CR, therefore the treatment response in our cohort was 96% [30, 33]. Only one (4%) patient died due to refractory VOD. Nevertheless, we do believe that this patient's other serious conditions such as respiratory failure, sepsis and the need for PICU treatment, resulted in a delayed VOD diagnosis which may have influenced his fatal outcome. In comparison to our previous VOD cohort, the new EBMT criteria led to great improvements both in terms of OS and DF response rate [29]. Although our results are promising, they are limited by the relatively small number of patients and the inclusion of patients with mild/moderate VOD; further prospective studies are essential.

Despite being registered solely for severe VOD in the European Union, due to the new diagnostic criteria, the use of DF was moved to almost first suspicion of VOD [28, 34, 44, 46]. According to Corbacioglu et al, the duration of treatment may correlate directly with the initiation of treatment based on the criteria used. While labeling for DF recommends its administration for at least 21 days, the duration of treatment should be tailored individually to reflect the dynamics of the disease [27, 28, 47]. The withdrawal of DF should be based on the resolution of dynamic symptoms (i.e., RT, active ascites, and coagulopathy) as they represent active process within the endothelium [27]. In our cohort, the median DF treatment was 16 days. Only three patients required the full 21 days of DF. As we observed rapid resolution of symptoms in the majority of patients, without any rebound effect post treatment cessation, we believe that the shortening of DF administration in our cohort was enabled by its pre-emptive implementation [41].

Although our study was not strictly focused on financial aspects, it is worth emphasizing that VOD patients diagnosed by the EBMT criteria required a significantly shorter time of hospitalization compared with the previous cohort diagnosed using the modified Seattle criteria. In the new cohort, only one patient needed PICU admission compared with six patients in the historical cohort. However, the direct impact of the new VOD criteria on cost effectiveness requires separate research. Shorter treatment and length of hospitalization along with a decreased need for advanced supportive care may be accompanied by an economical benefit despite the increase in the incidence of the disease [48].

To conclude, the new EBMT VOD diagnostic criteria justify the differences in various aspects of the disease between children and adults and offer the potential for early diagnosis. According to up-to-date studies, pre-emptive intervention in VOD is crucial for patients' outcome. The superiority of immediate diagnosis, especially in correlation with the expanded-access DF treatment protocol was confirmed by our study. Due to the possibility of shortening the treatment and length of hospital stay, the new EBMT VOD criteria seem to have improved the outcomes of patients suffering from VOD post-HSCT.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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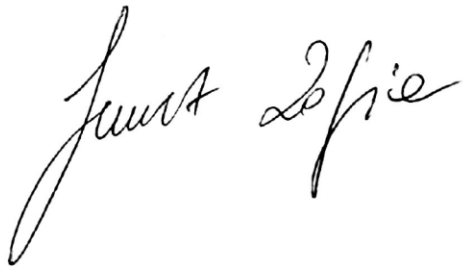
Mój udział polegał na przygotowaniu koncepcji i projektu badania, zebraniu, zestawieniu i interpretacji danych oraz napisaniu artykułu.



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Wrocław, 27.07.2020

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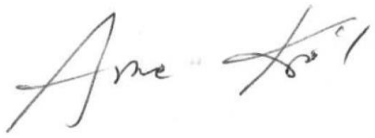

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Opublikowanej w Advances of Clinical and Experimental Medicine 2020 Mar;29(3):339-344.

Mój udział polegał na stworzeniu koncepcji badania, zebraniu i interpretacji danych klinicznych i laboratoryjnych oraz stworzeniu manuskryptu.

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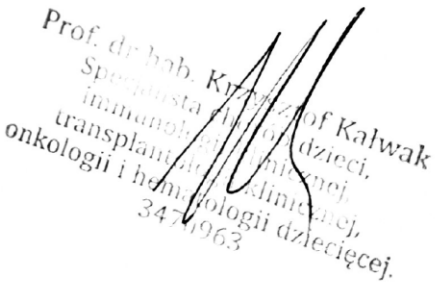

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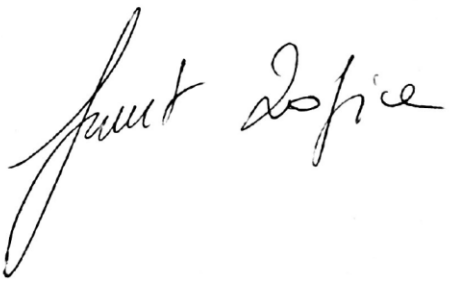

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