J Infect Chemother 25 (2019) 774–779

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Infectious profile in children with ALL during chemotherapy: A report of study group for infections☆

Joanna Zawitkowska [a, *], Katarzyna Drabko [a], Anna Szmydki-Baran [g], Agnieszka Zaucha-Prażmo [a], Monika Lejman [b], Krzysztof Czyżewski [c], Patrycja Zalas-Więcek [d], Olga Gryniewicz–Kwiatkowska [e], Aneta Czajńska-Deptuła [e], Elwira Kulicka [e], Katarzyna Semczuk [f], Łukasz Hutnik [g], Liliana Chełmecka-Wiktorczyk [h], Joanna Klepacka [i], Jowita Frączkiewicz [j], Małgorzata Salamonowicz [j], Renata Tomaszewska [k], Olga Zając-Spychała [l], Ninela Irga-Jaworska [m], Ewa Bień [m], Marcin Płonowski [n], Magdalena Bartnik [o], Tomasz Ociepa [o], Filip Pierlejewski [p], Mariola Woszczyk [q], Zuzanna Gamrot-Pyka [q], Zofia Małas [r], Agnieszka Urbanek-Dądela [s], Weronika Stolpa [t], Jakub Musiał [u], Jan Styczyński [c]

[a] Department of Pediatric Hematology, Oncology and Transplantation, Medical University, Lublin, Poland
[b] Department of Pediatric Hematology, Oncology and Transplantation, University Children's Hospital, Genetic Diagnostic Laboratory, Lublin, Poland
[c] Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University Toruń, Bydgoszcz, Poland
[d] Department of Microbiology, Collegium Medicum, Nicolaus Copernicus University Toruń, Bydgoszcz, Poland
[e] Department of Oncology, Children's Memorial Health Institute, Warszawa, Poland
[f] Department of Microbiology, Children's Memorial Health Institute, Warszawa, Poland
[g] Department of Pediatric Hematology and Oncology, Medical University, Warszawa, Poland
[h] Department of Pediatric Oncology and Hematology, University Children's Hospital, Jagiellonian University Collegium Medicum, Kraków, Poland
[i] Department of Microbiology, University Children's Hospital, Jagiellonian University Collegium Medicum, Kraków, Poland
[j] Department of Pediatric Stem Cell Transplantation, Hematology and Oncology, Medical University, Wrocław, Poland
[k] Department of Pediatric Hematology and Oncology, Silesian Medical University, Zabrze, Poland
[l] Department of Pediatric Oncology, Hematology and Transplantology, University of Medical Sciences, Poznań, Poland
[m] Department of Pediatrics, Hematology and Oncology, Medical University, Gdańsk, Poland
[n] Department of Pediatric Oncology and Hematology, Medical University, Białystok, Poland
[o] Department of Pediatric Hemato-Oncology and Gastroenterology, Pomeranian Medical University, Szczecin, Poland
[p] Department of Pediatric Oncology, Hematology and Diabetology, Medical University, Łódź, Poland
[q] Division of Pediatric Hematology and Oncology, Chorzow Pediatric and Oncology Center, Chorzów, Poland
[r] Division of Pediatric Hematology and Oncology, Children Hospital, Olsztyn, Poland
[s] Division of Pediatric Hematology and Oncology, Children Hospital, Kielce, Poland
[t] Division of Pediatric Oncology, Hematology and Chemotherapy, Department of Pediatric, Silesian Medical University, Katowice, Poland
[u] Division of Pediatric Onoco-hematology, St. Queen Jadwiga's Regional Clinical Hospital No. 2, Rzeszów, Poland

ARTICLE INFO

Article history:
Received 18 February 2019
Received in revised form
20 March 2019
Accepted 6 April 2019
Available online 14 May 2019

Keywords:
Acute lymphoblastic leukemia
Children
Chemotherapy
Infection

ABSTRACT

Introduction: The treatment-related mortality in currently published studies of acute lymphoblastic leukemia (ALL) in children is 2–4%, mainly due to infections. The aim of the study was to analyse the incidence, epidemiology, profile of infection and the death rate in children with ALL.
Patients and methods: The retrospective analysis included 1363 patients, aged 1–18 years, with newly diagnosed ALL, who were treated in 17 pediatric hematology centers between 2012 and 2017 in Poland. The patients received therapy according to the ALL IC-BFM 2002 and 2009 (International Berlin-Frankfurt-Munster Study Group) protocols.
Results: In our study, 726 out of 1363 (53.2%) children were reported to have a microbiologically documented bacterial infection during chemotherapy. 1511 episodes of these infection were diagnosed. A total number of 251/1363 (18.4%) children experienced a viral infection. 304 episodes were documented by PCR test (polymerase chain reaction). A fungal infection was reported in 278 (20.4%) children, including 10.1% of probable, 6.0% of proven, 83% of possible diagnosis. A higher frequency of

☆ ICMJE Statement: All authors meet the ICMJE authorship criteria.

* Corresponding author. Department of Pediatric Hematology, Oncology and Transplantology, Medical University of Lublin, 6 Antoni Gębala Street, 20-093 Lublin, Poland.
E-mail address: jzawitkowska1971@gmail.com (J. Zawitkowska).

https://doi.org/10.1016/j.jiac.2019.04.005
1341-321X/

fungal infection was noted in the recent years. In our material, the rate of death was 2.4%, mainly due to fungal infection.

Conclusions: Our results present the epidemiology of infectious disease in the Polish ALL patient population. The most frequent were bacterial infections, followed by fungal and viral ones. Similar to the previously published data, the mortality rate in our material was 2.4%.

1. Introduction

Outcome of acute lymphoblastic leukemia (ALL) in children has been successful in recent decades due to improvement of chemotherapy and supportive care [1]. The rare cause of treatment failure is poor remission in children with ALL. In the study presented by Stary et al. [2] only 31/5060 (0.6%) children died due to resistant disease. However, treatment-related mortality in currently published ALL studies is 2–4%, mainly due to infections [3]. Prolonged and intensive chemotherapy, neutropenia, the use of central catheters was reported to be associated with higher infection-related morbidity and mortality. Therefore, evaluation of frequency and profile of infection-related complications is an important issue to prevent infection-related deaths [3,4].

The aim of the study was to analyse the incidence, epidemiology, profile of infection and the death rate in children with acute lymphoblastic leukemia treated in pediatric hematology centers in Poland.

2. Materials and methods

2.1. Patients

A retrospective analysis included 1363 patients, aged 1–18 years, with newly diagnosed ALL, who were treated in 17 pediatric hematology centers between 2012 and 2017 in Poland. Patients characteristics are presented in each of the analysed subgroups. Children with ALL received therapy according to the ALL IC-BFM 2002 and 2009 protocols, which were randomized trials of the I-BFM-SG (International Berlin-Frankfurt-Munster Study Group). Details of ALL IC-BFM 2002 therapy were published by Stary et al. [2], and the 2009 protocol by Zawitkowska et al. [5].

2.2. Definitions

Bacterial infections were defined in case of the isolation of a pathogen based on the results of blood cultures, stool, urine analyses, or soft tissue swabs. Viral infections were reported when the virus was confirmed by the PCR test (polymerase chain reaction) or the presence of symptomatic varicella or zoster infection. The episodes of invasive fungal infection were qualified as probable, proven and possible, according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) criteria [6].

2.3. Supportive care

Patients in the analysed group received infectious prophylaxis according to institutional standards and recommendations included in the treatment protocols. All patients during chemotherapy received oral co-trimoxazole (25/5 mg/kg/day on 3 consecutive days/week) and oral fluconazole (6 mg/kg/day) in the neutropenic phase. Patients, who were contacted with individuals sustaining varicella or a zoster infection, received oral acyclovir (80 mg/kg/day). Rh-G-CSF (the recombinant human granulocyte colony-stimulating factor; 5 mcg/kg/day subcutaneous injection) was applied during sepsis in neutropenic children or in high-risk patients. All patients received antiemetics and had an assessment of organ function during intensive treatment. A low-bacterial diet and intensified hygiene measures were used in patients in the neutropenic period.

2.4. Statistical analysis

Data were analysed using IBM SPSS Statistics 25 and R version 3.5.1. Comparisons of different groups were performed using the chi-square and Fisher's tests. Non-categorical variables were compared with the Mann-Whitney *U* test. A p-value <0.05 was considered to be statistically significant. The cumulative incidence of infection was designed using the Kaplan-Meier method and compared with the log-rank Mantel test.

3. Results

3.1. Bacterial infections

A total number of 726/1363 (53.2%) children were reported to have microbiologically documented bacterial infection during chemotherapy. 1511 episodes of bacterial infection were diagnosed. The number of these episodes was 1–28 per person. Characteristics of patients who experienced 1 and >1 episode are presented in Table 1.

The overall survival of patients who experienced >1 event was lower than survival of those who suffered 1, but no statistically significant difference (Fig. 1). The most common site of bacterial infection was bloodstream (n = 518, 71.3%), followed by gastrointestinal tract (n = 443, 61%) and urinary tract (n = 245, 33.7%). Overall, 298/518 (57.5%) Gram-positive and 199/518 (38.4%) Gram-negative isolates were recovered from the bloodstream. Table 2 shows pathogens in bloodstream infections during study periods. Twenty children (2.75%) died due to a sepsis.

The cumulative incidence of bacterial infections was analysed over two-year periods (Fig. 2a). In our material there was a significant decrease of infection episodes in the years 2014–2015, as compared to 2012–2013. An increase in the frequency of infection was again observed during the 2016–2017 period. Six patients (2.4%) died due to bacterial episodes in the first analysed time period, and 6 (2.8%) and 8 (3.1%) in the consecutive ones.

3.2. Viral infections

During chemotherapy, 251 out of 1363 (18.4%) children experienced a viral infection documented by PCR test. The analysed group included 136 (54.2%) males and 115 (45.8%) females, median age 4.05 years (range 1–18). 304 episodes of viral infections were

Table 1
Characteristics of patients with ALL who experienced 1 and >1 episode of a bacterial infection.

Median of episodes	1	>1	p
Sex			
Male	225 (55.3%)	182 (44.7%)	p = 0.41
Female	166 (52%)	153 (48%)	
Median age (range), year	4.79 (1.07–17.7)	5.30 (1.2–18)	p = 0.43
Death related to infection	5 (1.5%)	15 (4.5%)	p = 0.011 (OR = 3.619)
Median time (range) from diagnosis to death, months	6.2 (0.9–25.6)	10.7 (2.2–16.7)	p = 0.57

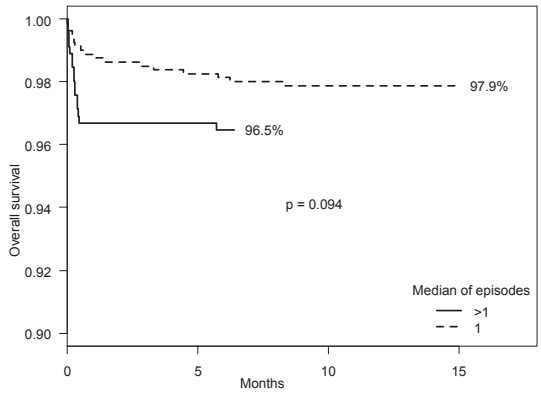

Fig. 1. Survival curves of patients with 1 and >1 episode of a bacterial infection.

identified, ranging 1–4 per person. Five patients (2.0%) in this group died due to these infections. The median time from diagnosis of ALL to death was 9.1 months (4.6–10.8). The distribution of viral infection episodes is presented in Table 3.

The cumulative incidence of viral infections is illustrated in Fig. 2b. The highest incidence of viral events was observed in the years 2014–2015 ($p < 0.001$). In the remaining periods, the frequency of viral episodes was similar. Two children (2.9%) died from *CMV* and *AH1N1* infection during the first period, 1 (0.8%) due to *AH1N1*, 2 (3.2%) due to CMV and RSV infection in the following ones, respectively.

3.3. Fungal infections

A total number of 278/1363 (20.4%%) children were reported as having a fungal infection during therapy of ALL. The analysed group

Table 2
Pathogens in bloodstream infections, presented by the number of infection episodes.

The type of infection	Number of bacterial infection episodes (n = 497)	Number of death
Gram-positive organism	**298 (60%)**	
Staphylococci species	228	1 (0.2%)
S. aureus	30	1 (0.2%)
MR-CNS	2	–
Streptococci species	21	–
Enterococcus species	11	–
Bacillus species	4	–
Listeria monocytogenes	2	1 (0.2%)
Gram-negative organism	**199 (40%)**	
Escherichia coli	85	3 (0.6%)
Klebsiella species	38	1 (0.2%)
Pseudomonas species		
P. aeruginosa	36	6 (1.2%)
Acinetobacter baumani	3	1 (0.2%)
Stenotrophomonas maltophilia	1	1 (0.2%)
Enterobacter species	35	4 (0.8%)
Morganella morgani	2	1 (0.2%)
Serratia marcescens	1	–
Others	21	–

MR-CNS - methicillin-resistant coagulase-negative Staphylococci.

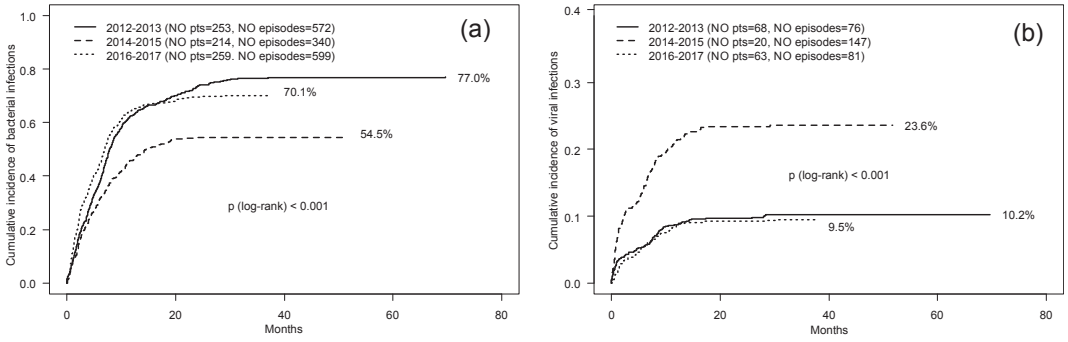

Fig. 2. The cumulative incidence of bacterial (a) and viral (b) infections in patients with ALL.

Table 3
The distribution of viral infection episodes.

Type of viral infection	Number of viral infection episodes (n = 304)	Number of death
Human herpes virus (HHV)		
Cytomegalovirus (CMV, HHV5)	34 (11.1%)	2 (0.8%)
Varicella- Zoster virus (VZV, HHV 3)	26 (8.6%)	–
Epstein – Barr (EBV, HHV4)	7 (2.3%)	–
Herpes simplex virus (HSV-1, HHV1)	4 (1.3%)	–
Human herpes virus 6 (HHV6)	3 (1.0%)	–
Human parainfluenza virus (HPIV)	23 (7.6%)	–
Influenza		
Type A	14 (4.6%)	–
AH1N1	7 (2.3%)	2 (0.8%)
Type B	4 (1.3%)	–
Adenovirus (ADV)	25 (8.2%)	–
Respiratory syncytial virus (RSV)	12 (3.9%)	1 (0.4%)
Enteroviruses		
Rhinovirus (HRV)	10 (3.3%)	–
Coxsackie virus	1 (0.3%)	–
Rotavirus	111 (36.5%)	–
Norovirus	19 (6.3%)	–
Rubella virus (RuV)	1 (0.3%)	–
Hepatitis C virus (HCV)	1 (0.3%)	–
Human metapneumovirus (MPV)	1 (0.3%)	–
BK virus (BKV)	1 (0.3%)	–

included 141 (50.7%) males and 137 (49.3%) females, median age 4.94 years (range 1–18). 406 episodes of a fungal infection were observed (probable - 41, proven– 28, possible- 337), range 1–7 per person. Eight children (2.9%) died due to the fungal infection. Three of those patients had proven and five had possible episodes. The median time from diagnosis of ALL to death was 4.4 months (range: 1–16.6). The localizations of proven infections were the lungs (10 pts), the gastrointestinal tract (7 pts), the blood (7 pts), the central nervous system (CNS) (3 pts) and an eye socket (1 pts). The types of pathogens of the proven infections are presented in Table 4.

There was a significant predominance of possible fungal episodes. A higher incidence of fungal infections was noted in the recent years ($p < 0.001$) (Fig. 3a, b). Three patients (3.2%) died from fungal infection in the first analysed time period, 2 (1.5%) and 3 (1.7%) in the consecutive ones.

4. Discussion

The main causes of treatment failure and the life-threatening complications are fungal infection and sepsis in children with ALL who are undergoing intensive chemotherapy. The infection-related mortality during treatment for childhood acute lymphoblastic leukemia was 1.7–2.4% in published trials [3].

Infection reports by the Polish Society of Pediatric Hematology and Oncology in hematologic centers started from 2012 [4]. In the present study, we focus on the profile of documented infections in children with ALL during a six-year period. In our report most patients experienced a bacterial bloodstream infection. Gram-positive pathogens were the most common microbiologically documented etiology of those infections. The increase in the frequency of bacterial infections in the recent period may be related to the fact that our patients did not use prophylactic antibacterial treatment eg. ciprofloxacin. Several studies presented a potential reduction in infection with quinolone prophylaxis without an increase in the incidence of fungal infections [7,8]. On the other hand, there is an increasing risk of selection of antibiotic-resistant bacteria. Further study is essential in order to find the optimal regimens for this group of children [9]. Analysing viral infections over the two-year period, the cumulative incidence was significantly lower during the years 2012–2013. This was probably caused by the low detection of viral infection. Since 2014, access to this diagnostic test such as the PCR methods has been improved. This may result in increased detection of viral infections and, consequently, earlier diagnosis and treatment implementation. Similarly, the improvement of supportive care measures was observed. After the introduction of antiviral treatment with acyclovir and varicella-zoster immune globulin (VZIG), the mortality rate of varicella-infections in children with immune suppression decreased significantly (<1%), compared to that before the introduction of antiviral therapy (7%) [10].

An increased number of possible invasive fungal infections in our study was also observed in other publications and could be related to improved diagnostic procedures (e.g. high-resolution computed tomography imaging) [11]. On the other hand, there was no increase in the frequency of probable and proven infections. This may be explained by the fact that antifungal prophylaxis was applied. In our study, the fungal infection-related mortality decreased over time. The EORTC/MSG consensus group revised the

Table 4
Pathogens of proven fungal infection episodes.

The type of pathogen	Number of fungal infection episodes (n = 41)	Number of death
Candida species		
C. glabrata	8 (28.6%)	–
C. crusei	1 (3.6%)	1 (2.4%)
C. albicans	5 (12.2%)	1 (2.4%)
C. parapsilosis	4 (14.3%)	–
C. metapsilosis	1 (3.6%)	–
C. famata	1 (3.6%)	–
Aspergillus fumigatus	6 (14.6%)	1 (2.4%)
Mucor	2 (7.1%)	–

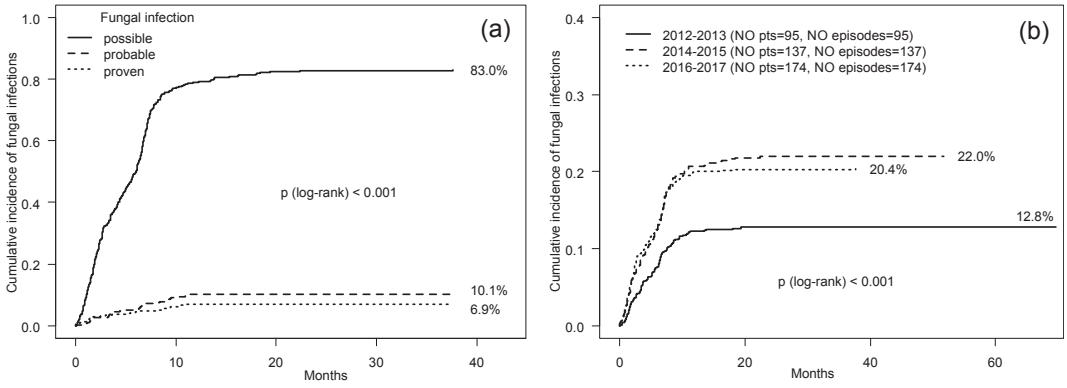

Fig. 3. The cumulative incidence of fungal infections (probable, proven and possible) (a) and overall infections in children with ALL (b).

definition of the current state of knowledge on infectious fungal diseases in pediatric patients with cancer in order to improve diagnosis, prevention and management [12].

A lower mortality was presented by Inaba et al. [3] in the study in which 4409 (0.98%) patients with ALL died of bacterial infection: 2 patients died due to *Bacillus cereus*, the postmortem culture in another patient showed *Clostridium* species and in still another one the causative organism was not identified. The authors did not observe fungal infection–related mortality. These differences could be explained by different prophylactic measures and a smaller group size. Some studies reported that fungal infections accounted for about 20% of cases of infection-related mortality [13].

Christensen et al. [14] analysed 1652 children with ALL who were treated according to the NOPHO-ALL92 protocol between 1991 and 2001. Out of 1652 patients, 56 (3.4%) died due to complications of therapy. Two-thirds (n- = 38, 68%) of all TRDs (treatment-related deaths) were infections: 17 monobacterial (14 Gram negative, three Gram positive), 2 mixed (Candida + *Staphylococcus aureus*; CMV + *Staphylococcus aureus*), 6 fungal (two Candida, one Pneumocystis jiroveci pneumonia, three other fungi), 3 viral (one CMV, one adenovirus, one influenza B virus), 8 FUO (fever of unknown origin).

Wiegering et al. [15] analysed 119 patients with VZV-infection treated between 2004 and 2010. A total number of 9 children (16.7%) had an oncologic disorder and among those 7 were diagnosed with acute lymphoblastic leukemia (ALL). All received intravenous acyclovir as antiviral treatment immediately. The authors recorded one VZV-related death in a child with ALL: a 4-year old boy who showed a rapidly fatal outcome of his simultaneous varicella-infection. There are studies showing that routine vaccination of populations against varicella reduces the number of infections and infection-related complications also in immunocompromised patients [15,16].

In conclusion, our results demonstrate that microbiologically documented infections and infection-related mortality remain a problem in childhood ALL and they present the epidemiology of infectious disease in the Polish ALL patient population. Similar to the previously published data, the mortality rate in our material was 2–2.9%. The profile of infections in ALL children undergoing chemotherapy in Poland may be useful for the development of future therapeutic interventions, such as early initiation of empiric broad-spectrum antifungal therapy, or prophylaxis with oral antibiotic agents in children receiving intensive chemotherapy.

Declarations of interest

None.

Formatting of funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet 2013;381:1943–55. https://doi.org/10.1016/S0140-6736(12)62187-4.
[2] Stary J, Zimmermann M, Campbell M, Castillo L, Dibar E, Donska S, et al. Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL IC-BFM 2002. J Clin Oncol 2014;32(3):174–84. https://doi.org/10.1200/JCO.2013.48.6522.
[3] Inaba H, Pei D, Wolf J, Howard SC, Hayden RT, Go M, et al. Infection-related complications during treatment for childhood acute lymphoblastic leukemia. Ann Oncol 2017;28(2):386–92. https://doi.org/10.1093/annonc/mdw557.
[4] Styczyński J, Czyżewski K, Zalas-Więcek P, Gryniewicz-Kwiatkowska O. Profile of infections in Polish pediatric hematology, oncology and stem cell transplantation centers in 2014–2015. Post N Med 2016;8:555–60. https://doi.org/10.5604/08606196.1215446.
[5] Zawitkowska J, Lejman M, Zaucha-Prazmo A, Drabko K, Plonowski M, Bulsa J, et al. Clinical characteristics and analysis of treatment result in children with Ph-positive acute lymphoblastic leukaemia in Poland between 2005 and 2017. Eur J Haematol 2018;101(4):542–8. https://doi.org/10.1111/ejh.13142.
[6] De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the National Institute of Allergy and infectious diseases Mycoses study group (EORTC/MSG) consensus group. Clin Infect Dis 2008;46(12):1813–21. https://doi.org/10.1086/588660.
[7] Wolf J, Tang L, Flynn PM, Pui CH, Gaur AH, Sun Y, et al. Levofloxacin prophylaxis during induction therapy for pediatric acute lymphoblastic leukemia. Clin Infect Dis 2017;65(11):1790–8. https://doi.org/10.1093/cid/cix644.
[8] Yeh TC, Liu HC, Hou JY, Chen KH, Huang TH, Chang CY, et al. Severe infections in children with acute leukemia undergoing intensive chemotherapy can successfully be prevented by ciprofloxacin, voriconazole, or micafungin prophylaxis. Cancer 2014;120(8):1255–62. https://doi.org/10.1002/cncr.28524.
[9] Calitri C, Ruberto E, Castagnola E. Antibiotic prophylaxis in neutropenic children with acute leukemia: do the presently available data really support this practice? Eur J Haematol 2018;101(6):721–7. https://doi.org/10.1111/ejh.13162.
[10] Matsuzaki A, Suminoe A, Koga Y, Kusuhara K, Hara T, Ogata R, et al. Fatal visceral varicella-zoster virus infection without skin involvement in a child with acute lymphoblastic leukemia. Pediatr Hematol Oncol 2008;25(3):237–42. https://doi.org/10.1080/08880010801938215.
[11] Bochennek K, Hassler A, Perner C, Gilfert J, Schoning S, Klingebiel T, et al. Infectious complications in children with acute myeloid leukemia: decreased mortality in multicenter trial AML-BFM 2004. Blood Cancer J 2016;6:e382. https://doi.org/10.1038/bcj.2015.110.
[12] Groll AH, Castagnola E, Cesaro S, Dalle JH, Engelhard D, Hope W, et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. Lancet Oncol 2014;15(8):e327–40. https://doi.org/10.1016/S1470-2045(14)70017-8.
[13] O'Connor D, Bate J, Wade R, Clack R, Dhir S, Hough R, et al. Infection-related mortality in children with acute lymphoblastic leukemia: an analysis of infectious deaths on UKALL2003. Blood 2014;124(7):1056–61. https://doi.org/10.1182/blood-2014-03-560847.

[14] Christensen MS, Heyman M, Mottonen M, Zeller B, Jonmundsson G, Hasle H, et al. Treatment-related death in childhood acute lymphoblastic leukaemia in the Nordic countries: 1992–2001. Br J Haematol 2005;131(1):50–8. https://doi.org/10.1111/j.1365-2141.2005.05736.x.

[15] Wiegering V, Schick J, Beer M, Weissbrich B, Gattenlohner S, Girschick HJ, et al. Varicella-zoster virus infections in immunocompromised patients - a single centre 6-years analysis. BMC Pediatr 2011;11:31. https://doi.org/10.1186/1471-2431-11-31.

[16] Siedler A, Arndt U. Impact of the routine varicella vaccination programme on varicella epidemiology in Germany. Euro Surveill 2010;15(13). https://doi.org/10.25646/546.