North Clin Istanb 2021;8(3):249–254
doi: 10.14744/nci.2020.99896

Original Article PEDIATRICS

Assessment of respiratory tract viruses in febrile neutropenic etiology in children and comparison with healthy children with upper/lower respiratory tract infection

Ayse Bozkurt Turhan,[1] Tercan Us,[2] Ener Cagri Dinleyici,[3] Gonca Kilic Yildirim,[4] Nilgun Kasifoglu,[2] Zeynep Canan Ozdemir,[1] Ozcan Bor[1]

[1]Division of Pediatric Hematology-Oncology, Department of Pediatrics, Eskisehir Osmangazi University Faculty of Medicine, Eskisehir, Turkey
[2]Department of Microbiology, Eskisehir Osmangazi University Faculty of Medicine, Eskisehir, Turkey
[3]Division of Pediatric Intensive Care Unit, Department of Pediatrics, Eskisehir Osmangazi University Faculty of Medicine, Eskisehir, Turkey
[4]Division of Pediatric Nutrition and Metabolism, Department of Pediatrics, Eskisehir Osmangazi University Faculty of Medicine, Eskisehir, Turkey

ABSTRACT

OBJECTIVE: This study aims to compare the frequency of respiratory viruses using real-time and multiplex polymerase chain reaction technology and nasopharyngeal swabs taken during exacerbation of patients aged 0–18 years followed for febrile neutropenia (FN) with non-FN children.

METHODS: This prospective study included a total of 40 patients with FN and malignancies followed at Eskisehir Osmangazi University, Department of Pediatric Hematology and Oncology. The control group (n=76) consisted of age-matched patients with upper respiratory tract infections (URTIs) or lower respiratory tract infections (LRTIs) who were admitted to the emergency service due to fever.

RESULTS: Viral agents were detected in 16 of 53 FN attacks (30.1%). The most commonly isolated viruses were coronavirus (23.7%, n=9), influenza B (18.4%, n=7), and adenovirus (18.4%, n=7). Of 76 children diagnosed with URTI with fever (52.6%) had viral agents, and only 28 of them had a single agent. The most commonly isolated virus was adenovirus (28.6%, n=14). Viral factors were found in 32 of 42 patients (76.1%) patients diagnosed with LRTI, while respiratory syncytial virus was the most common virus in 27 patients (21.7%, n=5).

CONCLUSION: Our study results show that viral agents play an important role in the etiology of FN. This is the first study to show that viral agents play an important role in the etiology of this disease and viral factors in non-neutropenic febrile children at the same time period by detecting respiratory viruses in 30% of FN cases. More similar studies provide antiviral therapy in selected patients, as well as these studies lead to reduce the use of antimicrobial agents or allow more selective use of antibiotics and/or the earlier discontinuation of these antibiotics in febrile neutropenic children who have been shown to have viral cause of respiratory tract infection based on clinical and microbiological/molecular diagnostic criteria.

Keywords: Child; febrile neutropenia; respiratory tract viruses.

Cite this article as: *Bozkurt Turhan A, Us T, Dinleyici EC, Kilic Yildirim G, Kasifoglu N, Ozdemir ZC, et al. Assessment of respiratory tract viruses in febrile neutropenic etiology in children and comparison with healthy children with upper/lower respiratory tract infection. North Clin Istanb 2021;8(3):249–254.*

Received: July 20, 2020 *Accepted:* November 11, 2020 *Online:* January 05, 2021

Correspondence: Ayse BOZKURT TURHAN, MD. Medeniyet University, Goztepe Training and Research Hospital, Istanbul, Turkey.
Phone: +90 532 724 71 80 e-mail: aysebturhan@hotmail.com

© Copyright 2021 by Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

Febrile neutropenia (FN) is one of the life-threatening complications of intensive chemotherapy protocols. The mortality rate is about 2–10% despite early diagnosis and improvement in treatment [1, 2]. In 60–70% of FN cases, the causative microorganism cannot be shown even in the best laboratory conditions [3]. The use of empirical antibiotics for neutropenic fever is a necessity due to the high risk of mortality; however, this also leads to the overuse of broad-spectrum antibiotics, which increases not just the likelihood of undesirable effects in patients but also the cost [4].

Neutropenic patients are more susceptible to both bacterial infections and viral infections. However, there is not enough literature data on the frequency of viral infection in FN patients, and most centers do not routinely investigate viral infections. Previous studies related to the FN in children have detected only 11–25% of respiratory viruses with classical viral tests [5, 6]. The development of multiplex polymerase chain reaction (PCR) and more sensitive nucleic acid-based viral diagnostic tests have facilitated the identification of viruses.

In this study, we present the results of a one-year prospective study to determine the frequency of respiratory viruses using a nucleic acid amplification test in children followed up with FN and to compare the results of non-FN children concurrently.

Highlight key points

- Neutropenic patients are more susceptible not only to bacterial infections but also to viral infections.
- The most common microorganisms isolated in neutropenic fever are bacteria, but viral infections should also be considered in the etiology of neutropenic fever.
- Viral agents play a role in 1/3 of febrile neutropenia etiology.
- It is coronavirus along with enterovirus and rhinovirus, which are the most commonly isolated viral agents.

MATERIALS AND METHODS

This prospective study included a total of 40 FN patients with hematological malignancies who were followed at Eskişehir Osmangazi University, Faculty of Medicine, Department of Pediatric Hematology and Oncology. The control group (n=76) consisted of only age-matched patients with upper respiratory tract infections (URTIs) or lower respiratory tract infections (LRTIs) who were admitted to the emergency service due to fever without any chronic disease and neutropenia. The patients with an absolute neutrophil count (ANC) of below 500/mm^3 or those with an ANC between 500 and 1500/mm^3 in whom these values were expected to fall below 500/mm^3 within 24–48 h were considered as neutropenic. FN was defined as the presence of fever once >38°C or >37.5°C for at least one hour in the armpits of neutropenic patients [7].

Medical history of each patient and age, sex, diagnosis of primary disease, duration of monitoring, number of neutropenic fever episodes, relapse of disease, duration of fever, prophylactic antibiotic regimen, empirical antibiotic therapy, and complaints suggesting a viral infection (i.e., cough, nasal discharge, respiratory distress, ocular discharge, and rash) were recorded using the patient files. Age, sex, presence of complaints accompanying the fever, physical examination findings, laboratory tests, and fever of children admitted to the emergency service with fever were also noted. Patients with a comorbid chronic disease, neutropenia, or those without an informed consent were excluded from the study.

Virologic Methods

Nasopharyngeal swab specimens were taken from all patients. After entering through both nostrils to the nasopharyngeal region with the swab, specimens were taken by rotating the rods 360° [8]. The swabs were then closed in capped containers containing transport medium (Viral Transport Medium) and samples were stored at +4°C and delivered to the Department of Medical Microbiology, Molecular laboratories within 24–48 h in accordance with the latest cold chain rules. Complete nucleic acid extraction from nasopharyngeal swab specimens was first performed in the relevant laboratory on an automatic extraction device (EasyMAG, Biomeriux, France). The FTD Respiratory Pathogens 33 kit (Fast-track diagnostics Ltd., Luxemburg), working with real-time and multiplex PCR, was applied in accordance with the manufacturer's instructions to detect respiratory tract pathogens on the RotorGene Q platform (Qiagen, Germany). This kit has the ability to distinguish 33 respiratory tract pathogens which are influenza (H3N2 and H1N1), B and C viruses, parainfluenza viruses 1, 2, 3 and 4, NL63, 229E, OC43, and HKU1 types of coronaviruses, human metapneumovirus B, rhinovirus, respiratory syncytial virus (RSV) and B, adenovirus, enterovirus, parechovirus, bocavirus species in a single pass.

The study protocol was approved by the institutional Eskisehir Osmangazi University Faculty of Medicine, Clinical Research Ethics Committee (805587721/11). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Table 1. Characteristics of episodes of febrile neutropenia

Characteristics	n/%
Age (year), median	6
Gender (male), %	40
Diagnosis, %	
Acute lymphoblastic leukemia	65
Acute myeloblastic leukemia	17.5
Solid tumors	12.5
Aplastic anemia	5
Presence of catheter, % (n)	50
Duration of fever (day), median	4
Duration of neutropenia (day), median	6
Presence of respiratory symptoms, %	28.3

Statistical Analysis

Statistical analysis was performed using the SPSS version 18 for Windows software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed in mean ± standard deviation, and range (min-max) for normally distributed intermittent and continuous numerical variables and in median (25–75%) for abnormally distributed variables. One-way analysis of variance was used to compare mean values. The Mann Whitney U test was used to compare the differences between the groups. The Chi-square (χ^2) test was used to analyze categorical variables. P=0.05 was considered statistically significant.

RESULTS

A total of 53 FN episodes involving forty patients (24 females and 16 males) who were followed for malignancy were included in the study. The median age of the patients was six years (range: 3–10 years). According to the disease distribution, 26 of them (65%) had acute lymphoblastic leukemia (two of them in relapse), seven (17.5%) had acute myeloblastic leukemia, five (12.5%) had solid tumors (one patient with NHL, three patients with Wilms, one patient with Ewing's sarcoma), and two (5%) had aplastic anemia.

The use of the catheter was 50%. The median value for duration of neutropenia was 6 days (range: 5–9 days), while the median value for duration of fever was four days (range: 3–5 days). A total of 28.3% of the patients had respiratory symptoms (Table 1). Prophylactic antibiotic therapy was initiated in all patients diagnosed with FN. Combined antibiotic therapy and monotherapy were applied in 92% and 8% of cases, respectively.

During the study period, 76 of the patients (38 females and 38 males) with URTIs and 42 patients with LRTIs (15 females and 27 males) who were admitted to the emergency service were included as the control group. The median age of patients with URTIs was 6 years (range: 4–10 years) and the median age of the patients with LRTIs was four years (range: 3–6 years). There was no significant difference in the age and sex of the patients between the groups.

Of 53 FN episodes, viral factors were shown in 16 cases (30.1%): 11 patients had a viral agent (two patients with influenza A, three patients with influenza B, one patient with parainfluenza type 3, one patient with coronavirus NL63, three patient with adenovirus), and four of them with concurrent two viral agents (one patient with coronavirus 229E and adenovirus, one patient with rhinovirus and adenovirus, one patient with coronavirus 229E and coronavirus NL63, one patient with coronavirus OC43 and adenovirus), and one of them with concurrent three viral agents (rhinovirus, influenza B, and

Table 2. Underlying etiology of neutropenia for patients, FN episodes, and viruses detected

Diagnose	Number of patients (n)	Episodes of FN's (n)	Single respiratory viral infection (% of FN episodes, n)	Multiple respiratory viral infection (% of FN episodes, n)
ALL	26	32	25 (8)	9.4 (3)
AML	7	7	14.3 (1)	14.3 (1)
Solid tumors	5	9	11.1 (1)	11.1 (1)
Aplastic anemia	2	5	20 (1)	0

ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; FN: Febrile neutropenia.

Table 3. Demographic information and respiratory virus diagnostic results for children with febrile neutropenic episode

Characteristics	
Number of patients (n)	40
Number of episodes of febrile neutropenia (n)	53
Positive viral tests among all episodes (%)	30.2
Single virus detected (%)	20.8
Multiple viruses detected (%)	9.4
Single respiratory viral infections	11
Influenza (%)	36.3
Adenovirus (%)	27.2
Coronavirus (%)	18.1
Respiratory syncytial virus (%)	9.1
Multiple respiratory viral infections (n)	5
Adenovirus, coronavirus (%)	20
Rhinovirus, adenovirus (%)	20
Coronavirus 229 E, coronavirus NL63 (%)	20
Coronavirus, adenovirus (%)	20
Rhinovirus, influenza B and adenovirus (%)	20

adenovirus). The most commonly isolated viruses were coronavirus (23.7%, n=9), influenza B (18.4%, n=7), and adenovirus (18.4%, n=7), respectively. A total of 9.4% of the patients had multiple viral infections (Table 2, 3). The most common respiratory symptom was cough (45.3%).

In addition, 40 of 76 children (52.6%) with an URTI and fever had viral agents and only 28 patients with a viral URTI had a single viral factor. The most common virus was adenovirus (28.6%, n=14) in patients with URTIs. However, of 42 patients with LRTI, 32 (76.1%) were detected to have viral agents and 27 of them were with a single agent. The most commonly isolated virus was RSV in five patients (21.7%) with a LRTI.

None of the FN patients needed to be followed in the intensive care unit and no mortality was seen in either group during the study.

DISCUSSION

This study documented the role of viral agents in one-third of FN etiology based on the detection of respiratory tract viruses in 30% of pediatric FN cases and reported the viral factors in non-FN children to determine the frequency of viral infection in society at the same time period.

Although the most frequently isolated factors in FN cases are bacteria, the risk of fungal infection increases as the duration of fever is prolonged. Viral infections are often overlooked in the etiology of neutropenic fever. Since the infection can be fatal in immunocompromised FN children, it is of utmost importance to define that the agent whether it is viral [9, 10], bacterial [11, 12], or fungal to tailor the treatment.

In the present study, viral agents were found in 30.1% of all neutropenic attacks. Previous studies reported various rates. In the studies of Lindblom et al. [5], Torres et al. [13], Suryadevara et al. [14], Hakim et al. [15], Castagnola et al. [16], Lehrnbecher et al. [17] and Koskenvuo et al. [18], the rate of viral agents was reported as 46%, 52%, 57%, 33%, 14%, 8%, and 59%, respectively. This can be, in part, attributed to the differences in the patient population and methods used in the aforementioned studies. In parallel with the advance in PCR technology, the frequency of detection of viruses has also increased.

The most frequently isolated viral agents have been reported as rhinovirus/enterovirus [5, 14, 18] and rhinovirus [19]. It was also shown that coronaviruses were relatively rare, accounting for <5% of isolated respiratory viruses [13, 20]. In our study, the incidence of coronavirus was 23.7%, unlike the literature data. In previous studies, viruses such as coronavirus and metapneumovirus were less emphasized, as the methods used for virus detection were not highly sensitive [21, 22]. As a more sensitive method, real-time PCR (RT-PCR) was used in our study which provided a higher frequency of coronavirus.

In addition, it is noteworthy that coronavirus is usually found in multiple virus breeding rather than as the sole determinant. Although human coronavirus is considered as a common cause of infectious diseases of the upper respiratory tract and LRTIs in immunocompromised patients, the effect on immunocompromised children is not fully known, and more studies are required.

Unlike other studies in the literature, the frequency of viral agents as a parallel group was investigated in patients with urgent fever and non-neutropenic URTI/LRTI to determine the types and frequency of respiratory viruses in the same seasonal period. In these groups, the rate of viral agents was higher than other factors. Strikingly, the rate was much higher (76.1%), particularly in patients with LRTI. When the diversity of viral agents was examined, we determined that the active viruses (adenovirus and RSV) were different and observed more frequently. It can be assumed that the reason for the viral factors being different is that they do not exhibit a direct parallel-

ism with the community-based factors because the FN patients are followed up in isolated services, more sterile conditions, and different immunological systems.

Respiratory viral infection is a rare cause of mild illness in immunocompromised children whereas this infection can be a common cause of mild illness. None of the patients with FN required intensive care during the study and none were lost. Complications are more common in younger patients and those with underlying pulmonary disease.

It is well-established that reverse transcription PCR (RT-PCR) is more sensitive to virus detection than viral culture and antigen tests, as is the case with previous studies using nucleic acid-based methods. However, although viruses such as rhinovirus/enterovirus, RSV, and influenza have been detected, particularly in immunosuppressed patients, these viruses may not be the etiologic cause for FN. A positive PCR may result from subclinical infection, as well as this result may also be indicative of post-infectious viral spillage or only intracellular non-replicated viral nucleic acid residues. However, it has been shown that only 5% of the nasopharyngeal aspirate samples taken 2 weeks apart have the same virus and rarely have the same virus genotype [23]. Clinical signs of the study patients were compatible with infections caused by specific viruses detected by the PCR-based method, and none of these patients had the same virus in the recurrent episode.

The lack of power analysis was the main limitation of the study.

Conclusion

The use of anti-viral drugs in FN patients is not a routine practice and the data on antiviral therapy is very limited. Non-specific viral symptoms, difficulty and long time in the isolation of viral agents, and the lack of efficacy in treatment have major roles in routine practice. However, chemoprophylaxis or treatment is possible for specific viral agents. If bacteria are isolated from only about one-third of neutropenic patients and many patients are still considered to have unexplained fever, with the increase in similar studies, it is possible that administering antiviral treatment to selected patients or reduction in the use of antimicrobial agents for FN children who have been shown to have viral cause of respiratory tract infection based on strict clinical and microbiological/molecular diagnostic criteria [24] as well as more selective use and/or earlier discontinuation of antibiotics can be achieved.

Ethics Committee Approval: The Eskisehir Osmangazi University Faculty of Medicine, Clinical Research Ethics Committee granted approval for this study (date: 02.01.2014, number: 805587721/11).

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by the Scientific Research Project Unit in Eskisehir Osmangazi University (BAP 2014/11036).

Authorship Contributions: Concept – ABT, TU, ECD; Design – ABT, ECD, GKY; Supervision – NK, ZCO, OB; Materials – TU, GKY; Data collection and/or processing – ABT, ZCO; Analysis and/or interpretation – ABT, ECD, OB; Literature review – ABT, NKOB; Writing – ABT, ECD; Critical review – ABT, TU, ECD, OB.

REFERENCES

1. Paul M, Yahav D, Fraser A, Leibovici L. Empirical antibiotic monotherapy for febrile neutropenia: systematic review and meta-analysis of randomized controlled trials. J Antimicrob Chemother 2006;57:176–89.
2. Laoprasopwattana K, Pruekprasert P, Laosombat V, Wongchanchailert M. Clinical outcome of febrile neutropenia in children with cancer using ceftazidime and aminoglycosides. Pediatr Hematol Oncol 2007;24:595–606. [CrossRef]
3. Pizzo PA. Management of fever in patients with cancer and treatment-induced neutropenia. N Engl J Med 1993;328:1323–32. [CrossRef]
4. Sipsas NV, Bodey GP, Kontoyiannis DP. Perspectives for the management of febrile neutropenic patients with cancer in the 21st century. Cancer 2005;103:1103–13. [CrossRef]
5. Lindblom A, Bhadri V, Söderhäll S, Ohrmalm L, Wong M, Norbeck O, et al. Respiratory viruses, a common microbiological finding in neutropenic children with fever. J Clin Virol 2010;47:234–7. [CrossRef]
6. Täger F M, Zolezzi R P, Folatre B I, Navarrete C M, Rojas P J. Respiratory virus infections in children with acute lymphoblastic leukemia and febrile neutropenia: a prospective study. [Article in Spanish]. Rev Chilena Infectol 2006;23:118–23. [CrossRef]
7. Kebudi R, Devecioğlu Ö, Gürler N. Pediatric febrile neuropathy guide: definitions and diagnostic methods. Flora 2004;2:73–105.
8. Robinson JL, Lee BE, Kothapalli S, Craig WR, Fox JD. Use of throat swab or saliva specimens for detection of respiratory viruses in children. Clin Infect Dis 2008;46:e61–4. [CrossRef]
9. Chemaly RF, Shah DP, Boeckh MJ. Management of respiratory viral infections in hematopoietic cell transplant recipients and patients with hematologic malignancies. Clin Infect Dis 2014;59 Suppl 5:S344–51.
10. Dotan A, Ben-Shimol S, Fruchtman Y, Avni-Shemer Y, Kapelushnik J, Ben-Harush M, et al. Influenza A/H1N1 in pediatric oncology patients. J Pediatr Hematol Oncol 2014;36:e271–4. [CrossRef]
11. Lv H, Ning B. Pathogenesis of bloodstream infection in children with blood cancer. Exp Ther Med 2013;5:201–4. [CrossRef]
12. Paulus SC, van Saene HK, Hemsworth S, Hughes J, Ng A, Pizer BL. A prospective study of septicaemia on a paediatric oncology unit: a three-year experience at The Royal Liverpool Children's Hospital, Alder Hey, UK. Eur J Cancer 2005;41:2132–40. [CrossRef]
13. Torres JP, Labraña Y, Ibañez C, Kasaneva P, Farfán MJ, De la Maza V, et al. Frequency and clinical outcome of respiratory viral infections and mixed viral-bacterial infections in children with cancer, fever and neutropenia. Pediatr Infect Dis J 2012;31:889–93. [CrossRef]
14. Suryadevara M, Tabarani CM, Bartholoma N, Rosenberg HF, Doma-

chowske JB. Nasopharyngeal detection of respiratory viruses in febrile neutropenic children. Clin Pediatr (Phila) 2012;51:1164–7. [CrossRef]
15. Hakim H, Flynn PM, Knapp KM, Srivastava DK, Gaur AH. Etiology and clinical course of febrile neutropenia in children with cancer. J Pediatr Hematol Oncol 2009;31:623–9. [CrossRef]
16. Castagnola E, Fontana V, Caviglia I, Caruso S, Faraci M, Fioredda F, et al. A prospective study on the epidemiology of febrile episodes during chemotherapy-induced neutropenia in children with cancer or after hemopoietic stem cell transplantation. Clin Infect Dis 2007;45:1296–304. [CrossRef]
17. Lehrnbecher T, Varwig D, Kaiser J, Reinhardt D, Klingebiel T, Creutzig U. Infectious complications in pediatric acute myeloid leukemia: analysis of the prospective multi-institutional clinical trial AML-BFM 93. Leukemia 2004;18:72–7. [CrossRef]
18. Koskenvuo M, Möttönen M, Rahiala J, Saarinen-Pihkala UM, Rikonen P, Waris M, et al. Respiratory viral infections in children with leukemia. Pediatr Infect Dis J 2008;27:974–80. [CrossRef]
19. Söderman M, Rhedin S, Tolfvenstam T, Rotzén-Östlund M, Albert J, Broliden K, et al. Frequent Respiratory viral infections in children with febrile neutropenia - a prospective follow-up study. PLoS One 2016;11:e0157398. [CrossRef]
20. Bredius RG, Templeton KE, Scheltinga SA, Claas EC, Kroes AC, Vossen JM. Prospective study of respiratory viral infections in pediatric hemopoietic stem cell transplantation patients. Pediatr Infect Dis J 2004;23:518–22. [CrossRef]
21. Gerna G, Campanini G, Rovida F, Percivalle E, Sarasini A, Marchi A, et al. Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and immunocompromised patients. J Med Virol 2006;78:938–49. [CrossRef]
22. Esposito S, Bosis S, Niesters HG, Tremolati E, Begliatti E, Rognoni A, et al. Impact of human coronavirus infections in otherwise healthy children who attended an emergency department. J Med Virol 2006;78:1609–15. [CrossRef]
23. Jartti T, Lee WM, Pappas T, Evans M, Lemanske RF Jr, Gern JE. Serial viral infections in infants with recurrent respiratory illnesses. Eur Respir J 2008;32:314–20. [CrossRef]
24. Santolaya ME, Alvarez AM, Acuña M, Avilés CL, Salgado C, Tordecilla J, et al. Efficacy and safety of withholding antimicrobial treatment in children with cancer, fever and neutropenia, with a demonstrated viral respiratory infection: a randomized clinical trial. Clin Microbiol Infect 2017;23:173–8. [CrossRef]