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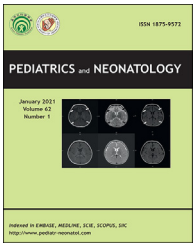

Original Article

The etiologic, microbiologic, clinical and outcome characteristics of immunocompetent young children < 2 years of age hospitalized with acute neutropenia

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Background: To describe the etiologic, microbiologic, clinical and outcome characteristics of acute neutropenia (absolute neutrophil count, ANC, $<1.5 \times 10^9$/L) in hospitalized immunocompetent children.

Methods: Serious bacterial infections (SBI) were defined as culture-positive blood, urine, cerebrospinal fluid, articular fluid or stool infections, alveolar pneumonia, Brucellosis and Rickettsiosis.

Results: 431/671 (64.2%) healthy infants and children hospitalized with acute neutropenia were <2 years of age; 176 (40.8%), 167 (38.8%) and 88 (20.4%) patients were aged 0–3, 4–12 and 13–24 months, respectively. There were 19 (4.4%), 53 (12.3%), 140 (32.5%) and 209 (50.8%) patients with ANC count <200, 200–500, 501–1000 and 1001–1500 $\times 10^9$ cells/L, respectively. Severe neutropenia ($<500 \times 10^9$/L) was recorded in 72 (16.7%) patients. Fever >38 °C was present in 208/431 (48.3%) patients. Blood cultures were positive in 10 (2.3%), with *Brucella melitensis*, *Staphylococcus aureus* and *Enterobacter* spp. identified in 4, 3 and 2 patients, respectively; 5/10 patients with positive blood cultures were <3 months of age. Overall, 55/431 (12.7%) and 65/431 (15.1%) patients were diagnosed with SBIs and bacterial infections, respectively. Nasal washings-PCR for respiratory viruses was positive in 139/293 (47.4%) patients tested. An infectious etiology (bacterial and/or viral) was diagnosed in 190/

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431 (44.1%) patients. Three patients were diagnosed with acute lymphocytic leukemia. Resolution of neutropenia was achieved in 111/208 (53.4%) evaluable patients (63%, 50.6% and 48% of patients aged 0—3, 4—12 and >12 months, respectively and 56.8%, 53.5% and 52% of patients with severe, moderate and mild neutropenia, respectively).
Conclusion: Acute neutropenia is common in immunocompetent children <2 years of age and is frequently associated with viral infections. We showed a substantial involvement of bacterial infections and particularly SBIs in the etiology of acute neutropenia. After a 1-month follow-up, resolution of neutropenia occurred in half of the patients, without association with age subgroups and with neutropenia severity.

1. Introduction

Acute neutropenia occurring as a result of an infectious process is common in immunocompetent infants and children and its differential diagnosis ranges between a severe and even life-threatening disease and a transient and mild condition.[1—5] Viruses and bacteria are well-known agents acting on one or more hematopoietic lines at the bone-marrow level. In patients with hematological malignancies and febrile neutropenia, an infectious etiology is identified in only 30—60 percent of the febrile episodes.[6] Since respiratory viruses are a common cause of fever in the general population, diagnostic screening for respiratory viruses in oncologic patients presenting with febrile neutropenia is considered rational and was used extensively during the last years. Real time PCR for identification of 16 respiratory viruses detected such viruses in 39 (45%) of 87 febrile neutropenia episode in oncological children and Rhinoviruses were the most frequently isolated ones.[7] In Chile,[8] 1044 febrile episodes were recorded in 525 children suffering from malignancies during a five-year period in three hospitals and at least one respiratory virus was identified in 46% of the neutropenic episodes. The most commonly identified viruses (in decreasing order) were Rhinovirus, Respiratory Syncytial Virus (RSV), Parainfluenza, Influenza, Adenovirus and human Metapneumovirus. The authors reported on a benign course of all episodes of neutropenia where a respiratory virus was identified.[8] In India, in an analysis of 81 febrile neutropenia episodes occurring in oncologic children <18 years during 2017, acute respiratory infections were diagnosed in 76.5% of the patients, with rhinoviruses (36.8%) and RSV (13.6%) being the most commonly detected viruses.[9] The median duration of the febrile period and of antibiotic days was longer in cases with detection of respiratory viruses compared with those without a diagnosis of respiratory infection.

Among the age subgroups where neutropenia is recorded, fever in the setting of profound neutropenia diagnosed in neonates and very young infants is a medical emergency requiring, in general, immediate treatment with broad spectrum antibiotics. Patients with ANC of 0.2×10^9/L or less almost invariably require hospital admission for sepsis work-up and intravenous antibiotics, with the choice of drugs depending upon local community and/or hospital flora and antibiotic sensitivities.[10,11]

In a study published in 2018, David et al. described the infectious etiology and disease outcome in 601 immunocompetent children aged 0—18 years hospitalized during 2010—2012 with acute neutropenia.[12] More than 50% of the enrolled patients were <3 months of age and 75.5% were <1 year of age. Severe neutropenia was recorded in 8.5% and SBIs were diagnosed in 17.9% of the patients. An infectious (bacterial and/or viral) etiology was determined in 30.9% of patients. In the whole group of study patients, a direct association was found between duration of neutropenia and patient age, infectious etiology of the condition and severity of neutropenia.[12]

Previous studies did not focus on the sub-group of infants and young children with acute neutropenia, where this diagnosis, particularly during a febrile condition, raises major concerns, and may be a signal of a serious infection. However, the true rates of SBIs associated with acute neutropenia (febrile or non-febrile) in this age subgroup have not yet been studied. Furthermore, the extensive molecular investigations available during the last years for the diagnosis of the viral etiologies associated with acute neutropenia make possible a better understanding of the whole panel of infectious diseases associated with this relatively common pediatric condition.

The objectives of the present study were to describe in detail the etiologic, microbiologic, clinical and outcome characteristics of immunocompetent young children <2 years of age hospitalized with acute neutropenia at the Soroka University Medical Center (SUMC) in Southern Israel during 2013—2015.

2. Patients and methods

All healthy immunocompetent infants and young children <2 years of age hospitalized at SUMC with acute neutropenia (febrile or afebrile) during the 3-year period 2013—2015 were enrolled and represented the study population. The pediatric division of the hospital received approximately 40,000 visits per year during the study period.

Patients with previous history of neutropenia and with any primary diagnoses known to cause neutropenia (malignancy, immunosuppressive disorders and therapies or medications causing neutropenia) were excluded from the study.

The study was conducted after obtaining approval from the ethics committee of SUMC.

The indications for hospitalization included: 1) neutropenia <500 WBC $\times$ 10^9/L; 2) the clinical status of the neutropenic patient; 3) the infectious focus diagnosed and the need for initiation of intravenous antibiotic treatment (like pneumonia, urinary tract, dysentery), and 4) diagnosis or suspicion of Brucellosis and Rickettsiosis, need for initiation of empiric or definitive antibiotic treatment for these 2 diseases and need for patient monitoring during hospitalization.

Patients were evaluated in the pediatric emergency department and during hospitalization and followed for a 1-month period, when a WBC measurement was repeated. All patients had the medical records reviewed for demographic and history data, clinical and laboratory findings (presence/absence of fever, risk factors for SBI, total WBC count, ANC and the results of urine, blood, and/or cerebrospinal fluid culture, serology and virology tests), disease management and follow-up.

The analysis of the etiologic, clinical and outcome characteristics compared between 3 age subgroups (0–3, 4–12 and >12 months of age) and in relation to 4 severity grades of neutropenia (0–200, 201–500, 501–1000 and 1001–1500 cells $\times$ 10^9/L). The process of resolution of neutropenia to an ANC >1500 cells $\times$ 10^9/L was analyzed during the 1-month period following the admission with acute neutropenia, as function of age subgroups and neutropenia severity.

2.1. Neutropenia

Neutropenia was defined as an ANC <1.5 $\times$ 10^9/L. According to severity, neutropenia was defined as severe (ANC < 0.5 $\times$ 10^9/L), moderate (ANC between 0.5 and 1.0 $\times$ 10^9/L) or mild (ANC between 1.0 and 1.5 $\times$ 10^9/L).[1,5] Leukopenia was defined as a total WBC count of <5.0 $\times$ 10^9/L. Thrombocytopenia was defined as a platelet count of <150.0 $\times$ 10^9/L. Anemia was defined as hemoglobin value > two standard deviations below the mean for reference population.

2.2. Serious bacterial infections

The following infections were considered SBIs for the purpose of the study: bacteremia, bacterial meningitis, pneumonia, urinary tract infection, bacterial gastroenteritis, osteomyelitis and septic arthritis.[12–14] All cases of pneumonia included were alveolar as this was considered suggestive of a bacterial etiology. Brucellosis and Rickettsiosis, which are extremely common in our geographic area, were also considered SBIs.[15–17]

2.3. Microbiology

Blood cultures were performed using the Bactec Becton Dickinson (Benex Limited, Shannon, County Clare, Ireland) system; the Vitel Bio Merieux (Boston, MA) system was used for bacterial identification. Cerebrospinal fluid was examined by culture for conventional bacterial pathogens and by polymerase chain reaction (PCR, when recommended) for herpes simplex virus and enteroviruses. Serum samples were tested for IgM and IgG antibodies for Epstein–Barr virus (EBV) and cytomegalovirus (CMV). Skin lesions were examined for herpes simplex virus by PCR.

All the respiratory viruses were tested simultaneously with a multiplex real-time polymerase chain reaction (mqRT-PCR) able to identify 12 respiratory viruses.[18] Each sample was tested in parallel, in three test tubes, for the following viruses: influenza A and B, parainfluenza 2 and 3, human respiratory syncytial virus (RSV), human metapneumovirus (hMPV), rhinovirus, adenovirus, and coronaviruses 229E, HKU1, OC43 and NL63. Amplification was carried using the RNA UltraSense One-Step qRT-PCR System (Invitrogen, Carlsbad, CA, USA). The sensitivity rate was identical at 100% for all virus groups except coronaviruses, in which the sensitivity of the pooled samples was 89.3%.

Diagnosis of Brucellosis was established according to a clinical presentation compatible with the disease, a positive blood culture and/or a standard tube agglutination test titer >1/160 obtained at admission in all patients.[15,16] Diagnosis of Rickettsiosis was established by determining the presence of IgG and IgM antibodies to murine typhus and spotted fever group rickettsia, by using a micro immunofluorescence assay.[17]

2.4. Management

During the study period, the management of patients with neutropenia was dictated by patients' condition and neutropenia severity. Ill-appearing patients, regardless of neutropenia severity, and those with severe neutropenia <0.5 cells $\times$ 10^9/L were considered at risk of SBI and were hospitalized.

Diagnosis of UTI was made on the basis of the presence of at least 50,000 colonies/ml of one or two uropathogenic organisms in a specimen of urine obtained by suprapubic aspiration or bladder catheterization.

All hospitalized febrile and afebrile patients <1 months of age with acute neutropenia underwent a complete sepsis work-up and were treated with antibiotics according to the infectious focus diagnosed. If no infectious focus was present, the departmental protocol for the initial empiric treatment included intravenous ampicillin (50 mg/kg/day three times/day for infants > 2 kg who are $\leq$ 7 days old while for infants > 7 days old the dose was 50 mg/kg/dose every 6 h) plus gentamicin (5 mg/kg/day once/day) till blood cultures results were available and afterwards was continued (or discontinued) according to patients' general condition and resolution (or lack of resolution) of neutropenia.

For infants aged 1–2 months without a source of infection, the protocol for the initial empiric treatment (following a complete or partial sepsis work-up) consisted of intravenous ampicillin (50 mg/kg/day three times/day) plus gentamicin (5 mg/kg/day once/day).

Patients >2 months of age with an ANC <0.5 $\times$ 10^9/L and without any source of infection were treated empirically with intravenous ceftriaxone (50 mg/kg/day once/day) till blood cultures results were available and afterwards the treatment was continued (or discontinued) according to

patients' general condition and resolution (or lack of resolution) of neutropenia.

Every hospitalized patient was followed with at least one WBC count during the one-month period after discharge.

2.5. Data analysis

Data analysis was conducted using the SPSS 22.0 package. Analysis of variance and *t* tests were used to compare continuous variables. The $\chi 2$ or Fisher exact tests were used for comparison of categorical variables. Kaplan–Meier survival curves were built for the three neutropenia severity groups, and these groups were compared with use of the log-rank test. *P* values less than 0.05 were considered significant.

3. Results

A total of 671 healthy immunocompetent infants and children were hospitalized with acute neutropenia during the study period. Of them, 431 (64.2%) were <2 years of age and they represent the study population; there were 176 (40.8%), 167 (38.8%) and 88 (20.4%) patients aged 0–3, 4–12 and 13–24 months, respectively. Overall, 343/431 (79.6%) patients were <1 year of age. There were 248 (56.6%) males and 183 (42.4%) females.

Overall, 398/431 (92.3%) of the enrolled patients were in good general health prior to admission, with no previous hospitalizations or any background medical conditions at admission. Thirty-three (7.7%) were previously diagnosed with various diseases/conditions, including failure to thrive (11, 2.6% of study patients), gastrointestinal diseases (10, 2.3%, 5 of them diagnosed with Hirschprung's disease), metabolic diseases (6, 1.4%), renal/urologic diseases (4, 0.92%), cardiovascular diseases (4, 0.9%), neurologic diseases (2, 0.5%) and others, respectively.

Fever >38 °C was present in 208/431 (48.3%) patients. The clinical picture associated with acute neutropenia at admission included respiratory, gastrointestinal, urinary, skin, central nervous system and otorhinolaryngology conditions in 85 (19.7%), 54 (12.5%), 23 (5.3%), 14 (3.2%), 13 (3.0%) and 10 (2.3%) patients, respectively. The most commonly diagnosed respiratory tract diseases associated with neutropenia were bronchiolitis (7.2% of all enrolled patients), upper respiratory infection (4.6%) and pneumonia (1.6%). Urinary tract infections were diagnosed in 18 (4.2%) patients. Six (1.4%) patients suffered from febrile convulsions and 6 additional ones from afebrile convulsions. Fourteen (3.2%) had skin rashes and 7 (1.6%) had acute otitis media at admission. A bone marrow examination was performed in 3 patients (all 3 subsequently diagnosed with leukemia).

There were 19 (4.4%), 53 (12.3%), 140 (32.5%) and 209 (50.8%) patients with an ANC count <200, 200–500, 501–1000 and 1001–1500 $\times$ 10^9 cells/L, respectively. Overall, severe neutropenia (<500 $\times$ 10^9/L) was recorded in 72 (16.7%) patients. There were more patients with mild and moderate neutropenia among all 3 age sub-groups (<3 months, 3–12 months and >12 months) – $P = 0.02$. More patients with ANC <200 and <500 $\times$ 10^9/L were found in the group of patients aged 3–12 months compared with the group of patients aged <3 months ($P = 0.03$ and $P = 0.02$, respectively). Leukopenia <5000 $\times$ 10^9/L was recorded in 118 (27.1%) neutropenic patients, with an increase in the percentages of leukopenic patients with increase in patient age ($P = 0.006$). Hemoglobin values <10 mg/dL were found in 112 (25.5%) and thrombocytopenia <150,000 cells/mm^3 in 68 (14.5%) patients.

3.1. Etiology

3.1.1. Bacterial etiology

Blood cultures were obtained in all study patients and returned positive in 10 (2.3%), with *Brucella melitensis* identified in 4 (0.9%) patients (1 with positive blood culture plus positive serology and 3 with positive serology only); *Staphylococcus aureus* and *Enterobacter* spp. identified in 3 and 2 patients, respectively (Table 1). Five of the 10 patients with positive blood cultures were <3 months of age (2 *Enterobacter* spp., 1 *Candida albicans*, 1 *Salmonella* spp. and 1 *Escherichia coli*).

Urine cultures were obtained in 152 (35.2%) patients and returned positive in 30 (6.9%).

The most commonly isolated pathogen was *E. coli* (20 positive urine cultures, 4.6%).

All 55 lumbar punctures performed were negative. Alveolar pneumonia was diagnosed in 7 (1.6%) patients. Overall, 55/431 (12.7%) of the study patients were diagnosed with SBI. In addition, culture positive-acute otitis media, acute tonsillitis and impetigo were diagnosed in 3, 6 and 1 patient, respectively.

More SBIs than non-invasive diseases were recorded in the study patients (55/431, 12.7% *vs.* 10/431, 2.3%, $P < 0.01$). No differences were recorded in the number of SBI cases between febrile *vs.* nonfebrile patients (26//208, 12.5% vs. 29/223, 13%, $P = 0.9$).

3.1.2. Viral etiology (Table 2)

Investigations for respiratory viruses (PCR from nasal washings) were performed in 293/431 (68.0%) patients and returned positive on 139/293 (47.4%). Of them, 45 (15.3%), 37 (12.6%), 31 (10.5%) and 25 (8.5%) were positive for Adenovirus, Respiratory Syncytial Virus, Parainfluenza virus 1, 2, 3 and Influenza A, respectively. EBV was detected in 5/39 (12.8%) and CMV in 11/40 (27.5%) of the evaluated patients. Overall, a definitive viral diagnosis was made in 158/394 (33.9%) patients.

The number of cases with a viral etiology increased with increase in patient age ($P = 0.012$).

In summary, an infectious etiology (bacterial and/or viral) was made in 190/431 (44.1%) of the patients with acute neutropenia enrolled in this study.

3.1.3. Malignancy

Three patients were diagnosed with acute lymphocytic leukemia. Two of them were febrile at admission, all three were leukopenic and none of them suffered from anemia or thrombocytopenia. All three were examined at the emergency room due to an acute disease/condition (vomiting, acute otitis media and upper respiratory infection, respectively). One of these patients had RSV recovered from the nasopharyngeal wash, the second one had

Table 1 Bacterial etiology: 431 <2 years of age patients with newly diagnosed neutropenia.

	Number	% patients
Invasive infection		
Blood cultures	**431**	100
Positive	10	2.3
Staphylococcus aureus	3	0.7
Enterobacter spp.	2	0.4
Brucella spp.	1	0.2
Salmonella spp.	1	0.2
E. coli	1	0.2
Candida spp.	1	0.2
Bacillus spp.	1	0.2
Brucellosis (positive serology IgM and/or IgG)	**4**	**0.9**
Urine cultures	**152**	**35.2**
Positive	30	6.9
Escherichia coli	20	4.6
Klebsiella spp.	4	0.9
Other	6	1.3
CSF cultures	**55**	**12.7**
Positive	0	0
Stool cultures	**20**	**4.6**
Positive	4	0.9
Campylobacter spp.	2	0.4
Shigella sonnei	1	0.2
Salmonella spp.	1	0.2
Pneumonia	**7**	**1.6**
Total invasive	**55/431**	**12.7**
Non-invasive infection		
Ear cultures	**8**	**1.8**
Positive	3	0.7
Haemophilus influenzae nontypeable	2	0.4
Streptococcus pneumoniae	1	0.2
Pharyngeal cultures	**19**	**4.4**
Positive	6	1.3
Group A *Streptococcus*	4	0.9
Group C *Streptococcus*	2	0.4
Impetigo	**1**	**0.2**
Total non-invasive	**10/431**	**2.3**
Total bacterial	**65/431**	**15.1**

nontypeable *Haemophilus influenzae*-acute otitis media and the third had adenovirus recovered from the nasopharyngeal wash. Two suffered from mild neutropenia and the third from moderate neutropenia (ANC 560 $\times$ 10^9/L). The diagnosis of leukemia was made during hospitalization following a bone marrow examination.

3.1.4. Relationship between patient age and severity of neutropenia in various etiologic groups (Table 3)

No differences were recorded on the distribution of cases with an infectious etiology among patients with severe neutropenia versus those with mild/moderate neutropenia ($P = 0.9$).

Among patients with a non-infectious etiology, the number of patients with severe neutropenia as well as the number of patients with moderate and mild neutropenia decreased with increase in patient age ($P = 0.01$).

Among patients with a viral etiology, the number of patients with severe neutropenia as well as the number of patients with moderate and mild neutropenia decreased with increase in patient age (P 0.06).

Among patients with bacterial and mixed etiology and also in patients with SBI, the number of patients with severe neutropenia as well as patients with moderate and mild neutropenia did not change with increase in patient age ($P = 0.364$, $P = 0.487$ and $P = 0.168$, respectively).

None of the patients requested admission to the intensive care unit and no fatalities were recorded among the study patients.

3.1.5. One-month follow-up on WBC counts by age, etiology and severity of neutropenia

Data on the one-month follow-up after the resolution of neutropenia in the study patients were available in 208 (48.2%) patients (Table 4). Resolution of neutropenia to values >1500 $\times$ 10^9/L was achieved in 111 (53.4%) evaluable patients. Resolution of neutropenia was recorded in 63%, 50.6% and 48% of the patients aged 0—3, 4—12 and >12 months, respectively ($P = 0.326$).

Follow-up data in patients with bacterial etiology was available in 18/65 (27.6%) patients and resolution of neutropenia was achieved in 10/18 (55.6%), with a significant decrease in resolution percentages with increase in patient age ($P = 0.01$).

Follow-up data in patients with SBI was available in 26/55 (47.2%) and resolution on neutropenia was achieved in 15/26 (57.7%), with no differences in the percentages of resolution between the 3 age subgroups.

Follow-up data in patients with viral etiology was available in 91/155 (58.7%) and resolution of neutropenia was achieved in 38/91 (41.8%), with no differences in the percentages of resolution between the 3 age subgroups.

Resolution of neutropenia was achieved in 56.8%, 53.5% and 52% evaluable patients with severe, moderate and mild neutropenia, respectively, with no differences between the 3 severity subgroups.

Figs. 1—3 present the resolution of neutropenia (after a 1-month period of follow-up) by Kaplan Meier survival curves for the 3 age subgroups and the 3 neutropenia severity subgroups, respectively. No differences were recorded in the resolution of neutropenia between the age subgroups and between the neutropenia severities groups studied.

4. Discussion

In the 10 studies published in the pediatric medical literature during 2005—2020 and dealing with the topic of acute neutropenia diagnosed in immunocompetent children, 2 were prospective, 7 enrolled only febrile patients and the other 3 enrolled both non-febrile and febrile neutropenic patients.[12,19—27] The number of patients enrolled varied from study to study (range 32—1888) and all included a considerable number of young infants, although the inclusion criteria, in respect to patient age, differed between studies. The percentages of patients with severe neutropenia (ANC < 0.5 cells $\times$ 10^9/L) ranged from 8.5% to 100% (two studies enrolled only patients with severe

Table 2 Viral etiology.

Etiology	Positive (%)	Positive (%) (0–3 m)	Positive (%) (4–12 m)	Positive (%) (>12 m)
Respiratory (nasal washings)	**139 (47.4)**	**49/130 (37.7)**	**56/104 (53.8)**	**34/59 (57.6)**
N = 293 (67%)				
RSV	37 (12.2)	19 (14.5)	14 (12.8)	4 (6.3)
Adenovirus	45 (14.8)	10 (7.6)	21 (19.3)	14 (21.9)
Influenza A	25 (8.2)	6 (4.6)	10 (9.2)	9 (14.1)
Influenza B	10 (3.3)	3 (2.3)	5 (4.6)	2 (3.1)
Parainfluenza 1, 2 3	31 (10.2)	9 (10.7)	10 (9.3)	12 (11)
Metapneumovirus	13 (4.3)	5 (3.8)	5 (4.6)	3 (4.7)
EBV	5 (12.8)	1/8	0/12	4/19
N = 39				
CMV	11 (27.5)	2/9	6/14	3/17
N = 40				
Herpes simplex 1	0	0/9	0/1	0/1
N = 11				
Herpes simplex 2	0	0/9	0/1	0/1
N = 11				

Table 3 Relationship between patient age and severity of neutropenia in various etiologic groups.

Etiology	ANC[a] < 500 ($\times 10^9$/L) *N* = 72	ANC > 500 ($\times 10^9$/L) *N* = 359	*P* value
Bacterial only[b]			0.364
0–3 m (15)	1 (1.3)	14 (3.8)	
3–12 m (13)	2 (2.7)	11 (3.0)	
>12 m (11)	0	11 (3.0)	
Viral only[c]			0.059
0–3 m (46)	4 (5.5)	42 (11.6)	
3–12 m (52)	12 (16.6)	40 (11.1)	
>12 m (35)	10 (13.8)	25 (6.9)	
Mixed infection (bacterial and viral agent in the same patient)			0.487
0–3 m (5)	1 (1.3)	4 (1.1)	
3–12 m (8)	2 (2.7)	6 (1.6)	
>12 m (5)	0	5 (1.3)	
SBI[d]			0.168
0–3 m (18)	2 (2.7)	16 (4.4)	
3–12 m (17)	4 (5.5)	13 (3.6)	
>12 m (12)	0	15 (3.3)	
Infectious etiology	32/72 (44.4)	158/359 (42.3)	0.9
No infectious etiology			0.010
0–3 m (110)	13 (18.0)	98 (27.3)	
3–12 m (91)	24 (33.3)	68 (18.9)	
>12 m (34)	3 (4.2)	31 (8.6)	
	P[e] = 0.03	P[e] < 0.001	

[a] ANC = absolute neutrophil count.
[b] Bacterial only group include: positive blood culture (not including *Brucellosis*), urine culture, CSF culture, joint culture, stool culture, pharyngeal culture and ear culture + diagnosis of pneumonia.
[c] Viral only: positive nasal wash, positive EBV and CMV serology and positive HSV PCR.
[d] SBI: positive blood culture, positive urine culture, positive CSF culture, positive joint culture, dysentery, pneumonia and Brucellosis by culture and/or serology.
[e] Chi-square for linear trends in proportion.

Table 4 1-month follow-up on neutropenia by age, etiology and severity of neutropenia.

	Number of patients investigated	Neutropenia Resolved	Neutropenia not resolved	*P* value
Patients with follow-up CBC	208/431 (48.2%)	111/208 (53.4%)	97/208 (46.6%)	
Age				
0–3 m	73 (35.1%)	44/73 (60.3%)	29/73 (29.9%)	0.326
3–12 m	85 (40.9%)	43/85 (50.6%)	42/85 (49.4%)	
>12 m	50 (24.0%)	24/50 (48.0%)	26/50 (52.0%)	
Etiology				
Bacterial[a]	18/65 (27.6%)	10/18 (55.6%)	8/18 (44.4%)	0.01
0–3 m	7/18 (38.9%)	6/7 (85.7%)	1/7 (14.3%)	
3–12 m	6/18 (33.3%)	4/6 (66.7%)	2/6 (33.3%)	
>12 m	5/18 (27.8%)	0/5 (0%)	5/5 (100%)	
SBI[b]	26/55 (47.2%)	15/26 (57.7%)	11/26 (42.3%)	0.623
0–3 m	9/26 (34.6%)	7/9 (77.8%)	2/9 (22.2%)	
3–12 m	9/26 (34.6%)	6/9 (66.7%)	3/9 (33.3%)	
>12 m	8/26 (30.8%)	2/8 (25.0%)	6/8 (75.0%)	
Viral only[c]	91/155 (58.7%)	38/91 (41.8%)	53/91 (58.2%)	0.417
0–3 m	30/91 (33%)	14/30 (46.7%)	16/30 (53.3%)	
3–12 m	36/91 (39.6%)	12/36 (33.3%)	24/36 (66.7%)	
>12 m	25/91 (27.5%)	12/25 (48.0%)	13/25 (52.0%)	
Severity of neutropenia				
ANC < 500 cells/mm^3	37/72 (51.4%)	21/37 (56.8%)	16/37 (43.2%)	0.311
0–3 m	9/37 (24.3%)	7/9 (77.8%)	2/9 (22.2%)	
3–12 m	21/37 (56.8%)	11/21 (52.4%)	10/21 (47.6%)	
>12 m	7/37 (18.9%)	3/7 (42.9%)	4/7 (57.1%)	
500 < ANC < 1000 cells/mm^3	71/140 (50.7%)	38/71 (53.5%)	33/71 (46.5%)	0.55
0–3 m	21/71 (29.6%)	13/21 (61.9%)	8/21 (38.1%)	
3–12 m	32/71 (45.1%)	15/32 (46.9%)	17/32 (53.1%)	
>12 m	18/71 (25.4%)	10/18 (55.6%)	8/18 (44.4%)	
ANC ≥ 1000 cells/mm^3	100/219 (45.6%)	52/100 (52%)	48/100 (48%)	0.65
0–3 m	43/100 (43%)	24/43 (55.8%)	19/43 (44.2%)	
3–12 m	32/100 (32%)	17/32 (53.1%)	15/32 (46.9%)	
>12 m	25/100 (25%)	11/25 (44.0%)	14/25 (56.0%)	

[a] Bacterial only group include: positive blood culture, urine culture, CSF culture, joint culture, stool culture, pharyngeal culture, ear culture + diagnosis of pneumonia and diagnosis of empyema.
[b] SBI: positive blood culture, positive urine culture, positive CSF culture, positive joint culture, dysentery, pneumonia and Brucellosis by culture and/or serology.
[c] Viral only: positive nasal wash, positive EBV and CMV serology and positive HSV PCR.

neutropenia).[20,27] The rates of SBIs among the enrolled patients were reported in 6 studies and ranged from 1.9 to 23.6%.[12,20,21,25–27] No SBIs were reported in 4 studies.[19,22–24] Infectious diseases were associated with acute neutropenia in 12.1%–63.8% patients.[12,20,22–24,26] Bacterial infections associated with neutropenia were reported in 5.3%–48.9% patients.[12,19,21–24,26] Viral investigations were completed in six studies[12,20,22–24,26] and the isolation ranges were between 5.1% and 55% of the enrolled patients. In the two studies where a detailed definitive viral diagnosis was reported, human herpes virus 6, enteroviruses and influenza A virus[22] and RSV, influenza A and parainfluenza 1 viruses[12] were the most commonly isolated pathogens. In one of the 3 studies completed in Greece, neutropenia following a Coxsackie, mumps, EBV or RSV infection lasted for more than one month.[23] Vlacha et al.[19] reported a 91.7% recovery of neutropenia rate at discharge while two additional studies reported a mean time for recovery of the ANC ranging from 6 to 16.7 days.[22,26]

The purpose of this study was to continue and broaden our previously mentioned study[18] and describe the epidemiologic, etiologic, laboratory and short-time outcome characteristics of acute neutropenia in the youngest age group (0–2 years) most commonly affected by this condition. During 2013–2015 we enrolled a considerable number of immunocompetent young children <2 years of age hospitalized with acute neutropenia in southern Israel and investigated its infectious and non-infectious etiology, the patient distribution according to 3 age subgroups (0–3, 4–12 and 13–24 months of age) and to severity of neutropenia and followed after the resolution of neutropenia for a short time period after discharge. We considered that the broad use of modern molecular technologies for the detection of viral pathogens during the last years will enable us to reach more definitive etiologic diagnoses and determine in detail the relevant responsible etiology of this condition in young children.

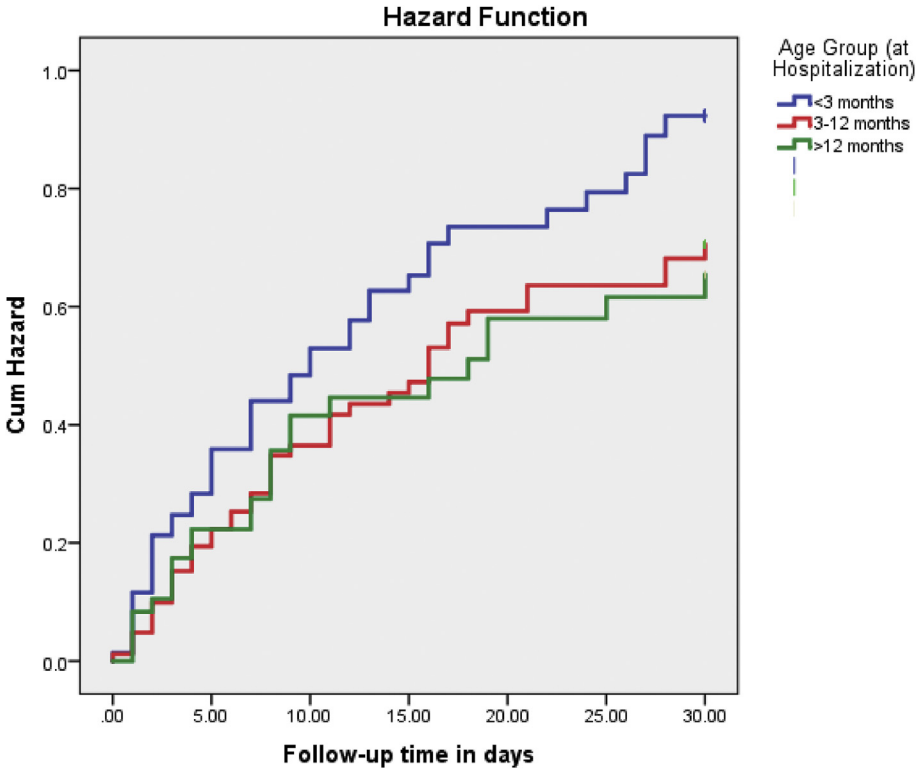

Figure 1 One month follow-up of correction of neutropenia according to age groups. Kaplan Meier survival curves for the 3 age subgroups ($P = 0.138$).

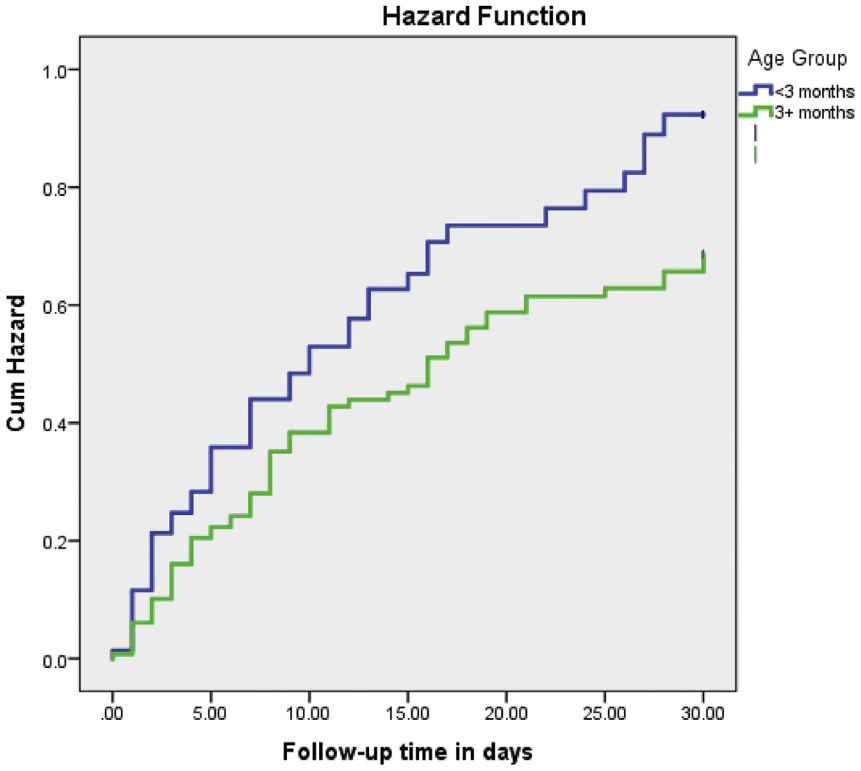

Figure 2 One month follow-up of correction of neutropenia according to age groups. Kaplan Meier survival curves for 2 age subgroups (<3 months versus 3—24 months) — $P = 0.106$.

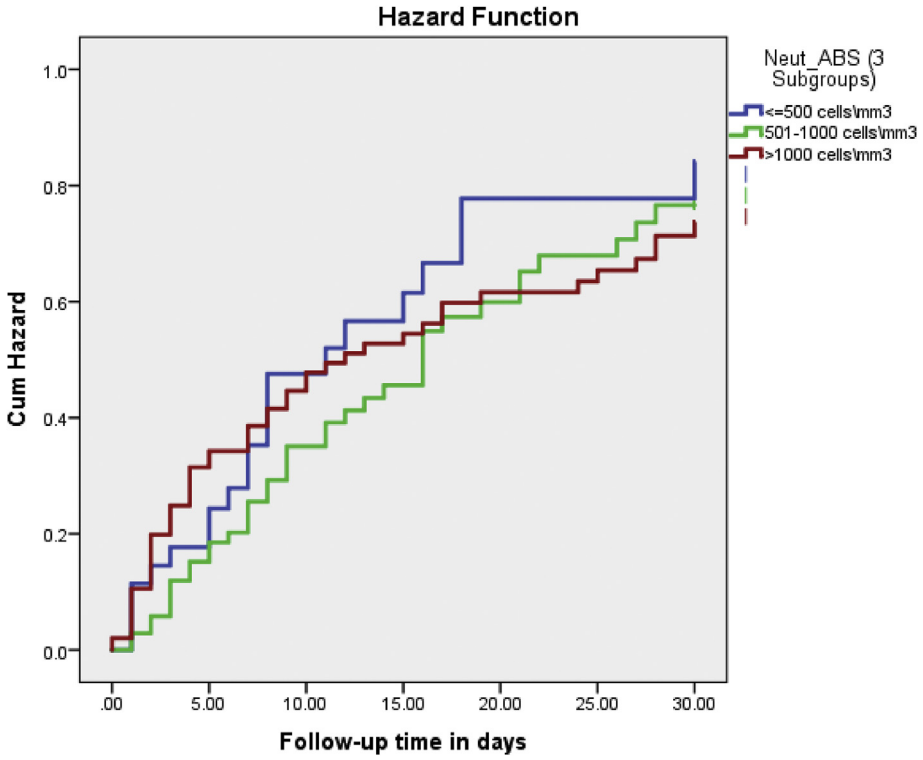

Figure 3 One month follow-up of correction of neutropenia according to severity of neutropenia. Kaplan Meier survival curves for the 3 neutropenia severity groups ($P = 0.814$).

We found in the present study that infants and young children <2 years of age represented the majority of patients diagnosed with acute neutropenia hospitalized during the study period. The number of cases of severe neutropenia recorded among the study patients was considerable (16.7%). SBIs were recorded in 12.7% of children <2 years of age, a percentage similar to that previously mentioned in the medical literature. The number of cases diagnosed with a viral infection was high (39.3%), making this etiology the main risk factor associated with neutropenia in the study group. A final infectious etiology (bacterial and/or viral) was made in 44% of the patients with acute neutropenia. Resolution of neutropenia was reported (after a short one month follow-up) in almost half of the study patients, without differences between the 3 age subgroups studied and without association with the severity of neutropenia. However, a significant decrease in resolution percentages was recorded in association with increase in age in patients with bacterial etiology of neutropenia.

We found in our study that, similarly to the study of David et al. published in our medical center and covering the whole pediatric population <16 years of age with acute neutropenia,[12] infants and young children <2 years of age represent the majority of cases diagnosed with acute neutropenia among immunocompetent children. We found that the rates of SBIs were lower in the group of patients <2 years of age compared with the previously SBIs rates in children <16 years of age, most probably due to lower rates of Brucellosis and Rickettsiosis in the younger age group. During the study period, we were also able to perform a considerable number of molecular and serologic tests for the diagnosis of viral infections associated with acute neutropenia and reported a 47.4% positivity in the patients tested. We found that RSV, Adenovirus, Parainfluenza and Influenza A were the main viruses associated with acute neutropenia.

The limitations of our study are mainly related to its retrospective nature, and it is possible that some information on the enrolled patients was missed or incorrectly presented.

In addition, we cannot rule out the possibility that some of the patients enrolled in our study might have suffered from congenital or from ethnical neutropenia which had not been previously diagnosed.[2–4,28,29] Another limitation is related to lack of follow-up data for a considerable number of patients enrolled in the study and, as mentioned, the lack of a longer follow-up period with repeated WBC counts after discharge from hospital.

In conclusion, in this study we described the infectious and non-infectious etiology of acute neutropenia in immunocompetent infants and young children <2 years of age and reported that this condition is common in this age group. We established that viral etiology is frequently associated with this condition and characterized the main viruses associated with acute neutropenia. We reported on the involvement of bacterial infections in the etiology of acute neutropenia in this age group with emphasis on the major role played by SBIs. In addition, we determined that, during the short follow-up period following the diagnosis,

resolution of neutropenia occurred in around half of the study patients without any association with age subgroups and severity of neutropenia.

Declaration of competing interest

There is no conflict of interest.

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