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Original article

Prevalence and outcome of lymphopenia in PICU: a prospective observational study

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ABSTRACT

Aims: Cytopenia (lymphopenia or neutropenia) may be an important marker of altered immunity and risk in children with critical illness. We aimed to evaluate the prevalence, course, and outcome of cytopenia in children admitted to PICU.

Methods: All consecutive children (n=200) admitted to PICU for any reason except malignancy or pre-existing immune deficiency were enrolled during June-August 2018.

Results: Median (IQR) age was 2.2 (0.6-8.5) years. Fifty-two percent (n=103) had undergone a surgical procedure; 34% (n=68) being cardiac surgery. Among medical illnesses, respiratory disorders were the most common (n=39, 20%). Laboratory confirmed infections were present in 63 (31.5%) children. Cytopenia was identified in 135 (67.5%) children in the 1st week; 117 (58.5%) had only lymphopenia, 16 (8%) had both lymphopenia and neutropenia, and 2 (1%) had only neutropenia. In 69 children who had follow-up blood counts, lymphopenia resolved in 33 (48%) within 48 hours and in another 20 (29%) by 4 days, and in a further 10 (14%) by 7 days. Children with lymphopenia had higher frequency of cardiac surgery, longer cardiopulmonary bypass time, greater need for invasive ventilation and vasopressor/inotrope therapy, and a higher probability of organ failure on day 4 and longer hospital stay.

Conclusion: In critically ill children, lymphopenia is very common, often transient, but may be associated with unfavorable outcome. Further studies with follow-up of blood counts in a larger sample are required to determine the course and outcomes of lymphopenia.

Key words: Lymphocytopenia, Cytopenia, Cardiopulmonary bypass, Intensive care unit, Children

INTRODUCTION

Critically ill children in intensive care units may have altered immunity, either an activated or depressed immune response.[1] Impaired adaptive immunity in sepsis may reflect compensatory anti-inflammation. Several mechanisms are responsible for sepsis-induced immuno-suppression, including depletion of immune cells, expression of inhibitory receptors and cellular exhaustion.[2] Similarly, transient systemic immune suppression after cardiopulmonary bypass (CPB) due to Interleukin-10 release and changes in leucocyte subsets have been described.[3]

Lymphopenia may be a simple but overlooked marker of impaired immunity and risk in children with acute illnesses.[4] Circulating cytokine induced dysregulation and Fas-mediated accelerated lymphocyte apoptosis are underlying pathophysiological mechanisms for lymphopenia. Other causes such as corticosteroids, dopamine and hypoprolactinemia may contribute.[5-8] Studies, mainly in adults had shown that low lymphocyte count and decreased lymphocyte-neutrophil ratio were predictive of bacteremia, post-operative infection and adverse intensive care outcome.[9-11] Some reports in children have explored the association between persistent lymphopenia beyond 3 days of admission and adverse outcomes.[6,12] However, most studies were limited by their retrospective study design, differing definitions of cytopenia and a focus on specific cohorts of patients.

Most of the focus for clinicians caring for acutely ill children has been on neutrophil counts. The physiological immune response to acute stress is often characterized by an increase in neutrophil count, a marker utilised in clinical care as a sign of increased risk of bacterial infection. Transient neutropenia is sometimes seen with acute infections and after CPB and if persistent, irrespective of the cause, predisposes children to new infections and may affect overall outcome.[13-15] Studies describing the trends of cytopenia, particularly the trajectory of lymphocyte count and their effect on outcome during acute illness are limited. Hence, we conducted a prospective study to evaluate the prevalence, time-course and outcome of lymphopenia in non-oncology patients admitted to our intensive care unit.

METHODS

Setting and population

We conducted a 3-month prospective study of all admitted patients in the Pediatric Intensive Care Unit (PICU). This is a tertiary referral unit catering to medical, general surgical and cardiac surgical patients. Patients younger than 18 years admitted to PICU for any reason between June and August 2018 were enrolled consecutively. We excluded preterm neonates with gestational age less than 40 weeks, children with malignancy or on chemotherapy, bone marrow failure or immune dysfunction syndromes and documented recent cytopenia in the previous two weeks. The study was approved by the Royal Children's Hospital human research ethics committee.

Data collection

This study was observational and did not involve any intervention or influence in the clinical management or frequency of blood sampling. Data of all enrolled children were collected from the hospital electronic medical record (EMR) system on a predesigned case record form. Demographic data, clinical features, onset of cytopenia and daily trends, details of infections and organisms, surgical procedures, organ dysfunctions, therapeutic interventions and outcome were recorded. In children with more than one full blood count in 24 hours period, the lowest cell count value was taken for analysing daily trends. All enrolled children were monitored for occurrence of cytopenia in the first 7 days and followed until PICU discharge, death or 28 days from enrolment for outcome measurements.

Definition of cytopenia

Cytopenia was considered to be present if blood count examination showed lymphopenia and/or neutropenia. Lymphopenia was defined according to age; absolute lymphocyte count <3.0 X 10^9/L in children younger than 2 years, <2.0 X 10^9/L in 2-5 years and <1.5 X 10^9/L in 6-18 years age group were defined as lymphopenia.[16,17] Neutropenia was defined as an absolute neutrophil count below 1.5 X 10^9/L, irrespective of age.

Classification of severity of illness and organ dysfunction

Severity of illness was classified according to Pediatric Index of Mortality 3 (PIM 3).[18] Standard definitions were used to diagnose organ dysfunction.[19] New onset of multiorgan dysfunction syndrome (MODS) was diagnosed if a patient with no or one organ dysfunction at admission developed two or more organ dysfunctions after enrolment. Progressive MODS was considered present if a patient who already had MODS at study enrolment developed dysfunction of at least one other organ after study enrolment. We report the level of respiratory support, inotrope and vasopressor use, length of PICU stay and outcome.

Sample size

As there were no previous data, we planned to include a sample of at least 200 children and about 400 blood count measurements to obtain a reasonable estimate of prevalence of cytopenia.

Statistical analysis

Descriptive statistics such as mean (SD) and median (IQR) for continuous variables and frequencies (proportions) for categorical variables were used. Groups with and without lymphopenia and with or without known persistent lymphopenia (>4 days) were compared using unpaired Student's t-test for normally distributed continuous variables and by chi-square and Fisher's exact tests for binary categorical variables. Non-parametric continuous variables were analysed using Mann-Whitney U test.

Based on previous literature, variables that could potentially be associated with development of lymphopenia such as age, severity of illness, presence of infection, MODS, cardiac surgery and use of steroids were subjected to multivariable logistic regression analysis to determine the independent predictors of lymphopenia.[5,6,8,12] For all tests, a two-sided *p*-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS software v.22.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient population

Between June and August 2018, 247 consecutive PICU admissions were screened for eligibility and 47 were excluded. Twenty-two children were excluded because blood count examination was not performed during their PICU admission. Twenty-five children were excluded due to reasons decided apriori (premature infants <40 weeks gestation =10; malignancy and/or on chemotherapy =12; previous neutropenia in last 2 weeks =3).

Of 200 children enrolled into the study, infants (<1 year), young children (1-5 years) and older age group (>5 years) constituted approximately a third each; median age was 2.2 (0.6-8.5) years with a slight predominance of males. About two thirds (n=123, 61.5%) were admitted to the general PICU and the remainder (n=77, 38.5%) in the cardiac PICU. Nearly half (n=103, 51.5%) underwent a surgical procedure, 34% (n=68) being cardiothoracic surgery. Among medical illnesses, acute lower respiratory infections were the most common; pneumonia and bronchiolitis accounting for 20% (n=39). Laboratory confirmed infections were identified in 63 (31.5%) children; the majority (n=50, 25%) were respiratory pathogens with respiratory syncytial virus predominating (n=30, 15%). Six children (3%) had bacteremia.

Severity of illness and organ dysfunction

The overall severity of illness predicted by median (IQR) PIM 3 probability of death was 1.22 (0.7-1.8). The majority had no (n=99, 50%) or one (n=79, 40%) organ dysfunction at PICU admission. Two or more organ dysfunctions were observed in 22 (10%) children at admission and in 16 (8%) at day 4 of PICU stay. Two of these patients had new MODS and 6 had progressive MODS. Respiratory support was required by 161 (80%); 117 (58%) required invasive ventilation for a median (IQR) duration of 17.7 (8-45) hours. Inotrope and vasopressor therapy was used in 44 (22%) children. Six children underwent extracorporeal therapies. The median (IQR) length of PICU stay was 45 (23-91) hours and length of hospital stay was 7 (4-15) days (Table 1).

Blood examination and presence of cytopenia

Most (n=198, 99%) had their first blood count examination within 24 hours of PICU admission. The median number of blood count measurement was 2; 53 (26.5%) had one, 50 (25%) had two and 56

(28%) had three blood count measurements during PICU stay. One hundred and thirty-five (67.5%) children developed some form of cytopenia during the first week of the PICU admission; 117 (58.5%) had only lymphopenia, 2 (1%) had only neutropenia and 16 (8%) had both lymphopenia and neutropenia.

Of the 133 children who developed lymphopenia with or without neutropenia, the time of detection was day 1 of PICU admission for 95 (71%); and day 2 of admission for 27 (20%). Figure 1 shows the trend of lymphocyte count during initial days. The duration of lymphopenia was studied in 69 children who had one or more follow up blood counts available during PICU stay. The lymphocyte count returned to normal in 33 (48%) children within 48 hours and in another 20 (29%) the lymphopenia resolved by 4 days. In the remaining 16 children, 10 (14%) had resolution of lymphopenia between 5 and 7 days, while in 6 (9%) lymphopenia persisted beyond 7 days.

Of 18 children with neutropenia, 16 had associated lymphopenia. Most children were neutropenic (n=16, 88%) within 48 hours of PICU admission and in about half (n=10, 55%) the onset of neutropenia coincided with the onset of lymphopenia. Thirteen children had follow-up blood counts; neutrophil count returned to normal in 11 children between 1 and 5 days while 2 had persistent neutropenia beyond 7 days. Children with lymphopenia also had lower total leucocyte count, however, their neutrophil counts were comparable to children who did not develop lymphopenia. (Figure 2)

Comparison of children with and without lymphopenia

Table 2 shows the comparative analysis of children with and without lymphopenia. Children with lymphopenia group were more represented among children with Indigenous Australian or Pacific island ethnicity, children undergoing surgical procedures particularly cardiac surgery, increased CPB time, higher serum procalcitonin, greater need for invasive ventilation and vasopressor/inotrope therapy. Children without lymphopenia had a higher frequency of laboratory confirmed viral infection. The proportions with organ failure on day 4 and length of hospital stay were significantly greater among children with lymphopenia. The incidence of health care associated infections and mortality were also higher in children with lymphopenia, but the difference was not statistically significant. On multivariable analysis using logistic regression model, only cardiac surgery was independently associated with development of lymphopenia. (Table 3)

Of 69 children whom duration of lymphopenia was assessed, 16 children had persistent lymphopenia beyond 4 days. Table 4 shows the characteristics and outcomes of children with known prolonged lymphopenia compared to those with known transient lymphopenia.

DISCUSSION

Our study showed that cytopenia, particularly lymphopenia is very common in children receiving intensive care. Two third of all children admitted to PICU developed lymphopenia in the first 7 days of PICU admission, in most the onset was within 48 hours of admission. Undergoing cardiac surgery was a common aetiology with nearly a four-fold increase in likelihood for the development of lymphopenia, and the duration of CPB in children who developed lymphopenia was twice as for long as those who did not. Studies have explored the influence of open-heart surgery and CPB on lymphocyte number and function in children. Both surgical stress and CPB are known to induce peripheral blood lymphocyte apoptosis through the Fas mediated pathway however the degree of apoptosis was greater in surgeries involving CPB than in those without CPB.[5] CD4+ T-helper cells are more profoundly affected and their sustained decrease in the first 24 hours after CPB resulted in lower CD4+/CD8+ ratios.[20] Additionally, hypothermia, thymectomy and chylous effusions may contribute to the altered T-cell subpopulations. CPB induced alterations in cell count are often comparable in children and adults, however unlike in adults, the prognostic significance has not been clearly demonstrated in children. In adult patients, perioperative lymphopenia, and elevated neutrophil / lymphocyte ratio were shown to predict postoperative outcome in cardiac surgery (however the latter ratio risk may be more to do with the height of the neutrophil count as how low the lymphocyte count is).[21,22] Studies in children do not allow conclusions due to heterogeneity in age, maturity of immune system and biological difference in different lesions and surgical interventions.[3] Effect of sepsis and other injuries on lymphocyte subsets are comparable to CPB-related changes but are relatively better understood. Apoptosis has been identified as the pathophysiological process for an acute decrease in lymphocyte count in sepsis however the causes for prolonged lymphopenia are likely multifactorial. Alterations in adrenocorticotrophic hormone-cortisol axis, steroid use, hypoprolactinemia due to various reasons including dopamine infusion have been proposed as contributory to lymphopenia in ICU.[6-8]

Studies on demographic and clinical profile of lymphopenia in hospitalised children are limited by restricted age groups and small sample sizes. Adamski et al reported transient lymphopenia in 229 infants under 3 months of age presenting to emergency department with acute illness.[12] The incidence of lymphopenia in that study was 18% (n=42). A greater proportion of infants with lymphopenia had infection as their underlying diagnosis and a greater probability of needing resuscitation and PICU admission. The duration of lymphopenia was studied in 26 (62%) children; lymphocyte counts returned to normal in 73% and 96% children within 2 and 7 days respectively. Another study of 14 children with a median age of two years reported a significant increase in total lymphocyte count and CD4 and CD8 subpopulations between the first and seventh days of PICU stay.[23] Our findings are consistent with these

observations that lymphopenia in hospitalised children is mostly transient. We observed that in only 9% of children, did lymphopenia persist beyond 7 days.

There is a renewed interest in the significance of lymphopenia in ICU patients as a marker reflecting sepsis-related compensatory anti-inflammation. Several observational studies linking severity and duration of lymphopenia and clinical outcomes are available in adults.[7,9-11,24] In patients with severe sepsis and septic shock, severe lymphopenia defined as count less than $0.5x10^9$/L was independently associated with 28-day mortality [adjusted hazard ratio (95%CI) =3.5 (1.5-8.4); P=0.004].[7] Another study showed that persistence of lymphopenia at day 4 of diagnosis of sepsis predicted mortality at 28 days and 1 year.[11] Similarly, failure to normalize lymphocyte count by day 4 was predictive of mortality in severely injured trauma patients.[24] A recent large study of 753 adults with shock evaluated the relation between baseline lymphocyte count and its evolution in day 3 and the risk of ICU acquired infection and death.[9] Nearly two-thirds (61%) of all patients had lymphopenia and about 20% had persistently low lymphocyte count or a non-significant increase on day 3. Both these variables predicted the risk of ICU acquired infection in addition to persistent lymphopenia being associated with 28-day mortality. Studies in children investigating the association between lymphopenia and hospital outcome are very limited. In a cohort of 113 critically ill children with diverse etiologies including sepsis, surgery, trauma and transplant, Felmet et al observed that an absolute lymphocyte count $<1x10^9$/L identified children at risk of health care associated infection.[6] In that study, persistent lymphopenia beyond 7 days was predictive of death from uneradicated infection. We too observed that significantly higher proportion of children with lymphopenia had health care associated infections and progressed to multiorgan failure on day 4. The mortality was also higher (7 vs 1) although the difference was not statistically significant.

Our study has several strengths. It is one of the largest prospective reports in children examining the prevalence, pattern and course of lymphopenia during PICU admission. Our cohort was a combined general and cardiac intensive care population including children with acute infections, other medical and surgical illnesses and injuries, and cardiac surgical patients. The definition of lymphopenia was based on studies of normal range age specific thresholds and centiles. A notable limitation was that only half of children with lymphopenia had follow up blood counts to determine the duration of lymphopenia. Unbeknown to the authors at the time of this study, lymphopenia would become an identified marker of COVID-19 infection and related inflammation in 2020 and 2020. Our study establishes a baseline incidence of lymphopenia in PICU, and gives some perspective on these recent observations.

CONCLUSIONS

In critically ill children in a PICU, lymphopenia is common, often transient, but may be associated with unfavorable outcome or more prolonged recovery. Further studies with follow up of full blood counts at regular intervals until resolution are required to draw conclusions on the significance of lymphopenia in acutely ill children.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Children with critical illness often display altered adaptive immunity during PICU admission
- Lymphocyte count is commonly measured in PICU but is frequently overlooked as a marker of immune response.
- Studies, mainly in adults had shown that lymphopenia and decreased lymphocyte-neutrophil ratio were predictive of bacteremia, post-operative infection and intensive care outcome

WHAT THIS STUDY ADDS

- Lymphopenia is very common during critically illness, seen in up to two-thirds of children admitted to PICU. Cardiac surgery was the independent risk factor for development of lymphopenia.
- The majority of PICU patients had transient lymphopenia in the initial 48 hours that resolved by 4 days, and only 9% of lymphopenia persisted at 7 days.
- Persistent lymphopenia may be associated with unfavourable outcomes or prolonged recovery, and this requires confirmation in further studies.

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Table 1: Clinical characteristics and outcome of study population

Variables	n=200
Age in years	2.2 (0.6-8.5)
• Neonates	6 (3)
• Infants (>28 days to 1 year)	65 (32.5)
• 1-5 years	62 (31)
• >5 years	67 (33.5)
Male sex	119 (59.5)
Weight in Kg	12.9 (6.8-28.2)
Ethnicity or country of descent	
• Caucasian / Europe	159 (79.5)
• Aboriginal or Pacific Island	6 (3)
• Middle east / Arabic	17 (8.5)
• Asia	10 (5)
• Indian sub-continent / Sri Lanka	7 (3.5)
• Africa	1 (0.5)
System of primary involvement	
• Cardiovascular	78 (39)
• Respiratory	60 (30)
• Gastrointestinal	13 (6.5)
• Central nervous system	17 (8.5)
• Neuromuscular	8 (4)
• Trauma	7 (3.5)
• Systemic disorders	9 (4.5)
• Others	8 (4)
Common diagnoses	
• Congenital heart disease	66
• Pneumonia	20
• Bronchiolitis	19
• Sepsis	9
• Spinal instrumentation for scoliosis	7
• Meningitis	5
• Seizures / status epilepticus	5
• Out of hospital cardiac arrest	5
Comorbidities	31
• Cerebral palsy	17
• Syndromes and malformations	5
• Inborn errors of metabolism	3
• Rheumatic heart disease	2
• Neurodegenerative disease	1
• Chronic liver disease	1
• Chronic kidney disease	1
• Type 1 Diabetes Mellitus	1

Children with hospitalization in previous 4 weeks	19 (9.5)
Children with pre-PICU stay >24 hours	24 (12)
Duration of illness in days	2 (1-5)
Children who underwent surgical procedure	103 (51.5)
Children who underwent a cardiac surgery	68 (34)
• RACHS percent risk of death	4.7 (2.0-8.4)
• Cardiopulmonary bypass (CPB) duration in minutes	90 (55-152)
• Aortic cross clamp time (XCT) duration in minutes	43 (14-75)
• Thymectomy	7
• Chylothorax	3
Children with microbiologically confirmed infections	63 (31.5)
Site	
• Blood	6
• Nasopharyngeal aspirate	35
• Lower respiratory specimens (Sputum/tracheal aspirate/BAL)	15
• Cerebrospinal fluid (CSF)	3
• Urine	2
Common organisms	
• Pseudomonas aeruginosa	7
• Streptococcus species	6
• N. Meningitidis	3
• E. Coli	2
• Klebsiella	2
• Respiratory syncytial virus	30
• Human metapneumovirus	3
• Parainfluenza virus	3
• Influenza virus	1
Organ supportive care	
• Respiratory support	161 (80.5)
• Invasive ventilation	117 (58.5)
• Duration of invasive ventilation in hours	17.7 (7.8-45.6)
• Vasoactive inotrope therapy	44 (22)
• Renal replacement therapy	2 (1)
• Plasma exchange	1 (0.5)
• ECMO	3 (1.5)
• No. of children prescribed antibiotics	77 (38.5)
• No. of children given steroids	27 (13)
• Duration of steroids in hours	72 (24-102)
PIM 3 predicted mortality	1.22 (0.7-1.8)
ICU length of stay in hours	45.1 (23.7-91.5)
Hospital length of stay in days	7 (4-15)
Mortality	8 (4)

Values are expressed in numbers (%) or Median (Interquartile range)

(RACHS – Risk Adjustment for Congenital Heart Surgery, BAL – Bronchoalveolar lavage, PCR – Polymerase Chain Reaction, CRP – C-reactive protein, ECMO – Extracorporeal membrane oxygenation, PIM 3 – Pediatric Index of Mortality 3)

Table 2. Comparison of children with lymphopenia and without lymphopenia

	Lymphopenia (n=133)	No lymphopenia (n=67)	P value
Age in years	2.5 (0.6- 8.5)	1.6 (0.4-10.4)	0.42
Infants < 1 year	45 (34)	26 (39)	0.49
Male sex	77 (58)	42 (62.7)	0.52
Weight in Kg	14 (7.5- 29.5)	11.8 (6.6-24)	0.49
Ethnicity or country of origin			0.04
Caucasian / Europe	110 (82.7)	48 (73)	
Indigenous Australia or Pacific islands	6 (4.5)	0	
Middle East / Arabic	8 (6)	9 (13.4)	
Asia or India	8 (6)	9 (13.4)	
Africa	1 (0.8)	0	
Children with hospitalization in previous 4 weeks	16 (12)	3 (3.5)	0.08
Duration of illness in days	2 (1-5)	2.5 (1.3-5)	0.59
Lymphocyte count ($x10^9$ cells/L)			
Day 1 (n= 198)	1.73 (1.2-2.5)	3.86 (2.8-5.3)	<0.001
Day 2 (n=129)	1.54 (1-2.4)	3.11 (2.0-3.6)	<0.001
Day 4 (n=71)	2.0 (1.4-3.7)	3.7 (2.2-5.5)	0.007
No. of children who underwent surgical procedure	80 (60)	23 (34.3)	0.001
No. of children who underwent cardiac surgery	58 (43.6)	10 (15)	<0.001
• RACHS percent risk of death	4.7 (3-11.6)	2.9 (0.5-6.6)	0.12
• CPB duration in hours	118 (75-177)	60.5 (55-85)	0.04
• XCT duration in hours	59.5 (25-90)	37 (29-57)	0.45
• Thymectomy	5	2	0.27
• Chylothorax	3	0	1.0
No. of children with microbiologically confirmed infection	36 (27)	28 (42)	0.03
• Bacterial infection	24 (18)	7 (10)	0.16
• Viral infection	15 (11)	21 (31)	<0.001
• Procalcitonin ng/ml (n=75)	1.76 (0.3-8.7)	0.34 (0.1-1.4)	0.01
• CRP mg/dL (n=40)	45 (18-175)	21 (11-50)	0.2
No. of children with organ failure			
• Day 1	69 (52)	32 (47)	0.45
• Day 4	35 (26)	8 (12)	0.02
No. of children with MODS			
• Day 1	16 (12)	6 (9)	0.51
• Day 4	14 (10.5)	2 (3)	0.06
No. of children with new or progressive MODS	8 (6)	1 (1.5)	0.27
No. of children received respiratory support	110 (83)	52 (78)	0.38
No. of children received Invasive ventilation	86 (65)	31 (46)	0.01

• Duration of invasive ventilation in hours	20.2 (7.5-65.5)	17 (10.1-39.5)	0.67
No. of children who received vasoactive drugs	36 (27)	8 (12)	0.02
Health care associated infections	7 (5.3)	0	0.12
No. of children prescribed antibiotics	48 (36)	29 (43)	0.32
No. of children who received steroids	18 (13.5)	9 (13.4)	0.98
• Duration of steroid therapy in hours	60 (24-84)	84 (21-114)	0.61
PIM 3 predicted mortality	1.2 (0.7-2.0)	1.2 (0.4-1.7)	0.15
PICU length of stay in hours	47.8 (23.8-99.3)	41.5 (23.3-75.4)	0.29
Hospital length of stay in days	7 (5-15)	5 (3.7-14)	0.01
Mortality	7 (5.3)	1 (1.5)	0.27

Values expressed are in numbers (%) or median (interquartile range)

(*RACHS – Risk Adjustment for Congenital Heart Surgery, CPB – Cardiopulmonary bypass, XCT – Aortic cross clamp time, CRP – C-reactive protein, MODS – Multiorgan dysfunction syndrome, PIM 3 – Pediatric Index of Mortality 3)*

Table 3. Multivariable analysis evaluating characteristics of children with lymphopenia

No.		Lymphopenia (n=133)	No lymphopenia (n=67)	OR (95% CI)	p-value
1.	Age in years	2.5 (0.6-8.5)	1.6 (0.4-10.4)	1.0 (0.9-1.0)	0.53
2.	PIM 3 predicted mortality	1.2 (0.7-2.0)	1.2 (0.4-1.7)	1.0 (0.9-1.1)	0.38
3.	Cardiac surgery	58 (43.6)	10 (15)	3.9 (1.75-8.7)	0.001
4.	Bacterial infection	24 (18)	7 (10)	2.2 (0.8-5.6)	0.10
5.	Viral infection	15 (11)	21 (31)	0.47 (0.2-1.0)	0.06
6.	Steroid therapy	18 (13.5)	9 (13.4)	0.69 (0.3-1.7)	0.43
7.	MODS at admission	16 (12)	6 (9)	1.3 (0.4-3.9)	0.69

Values expressed are in numbers (%) or median (interquartile range)

(PIM 3 – Pediatric index of mortality 3, MODS – Multi-organ dysfunction syndrome)

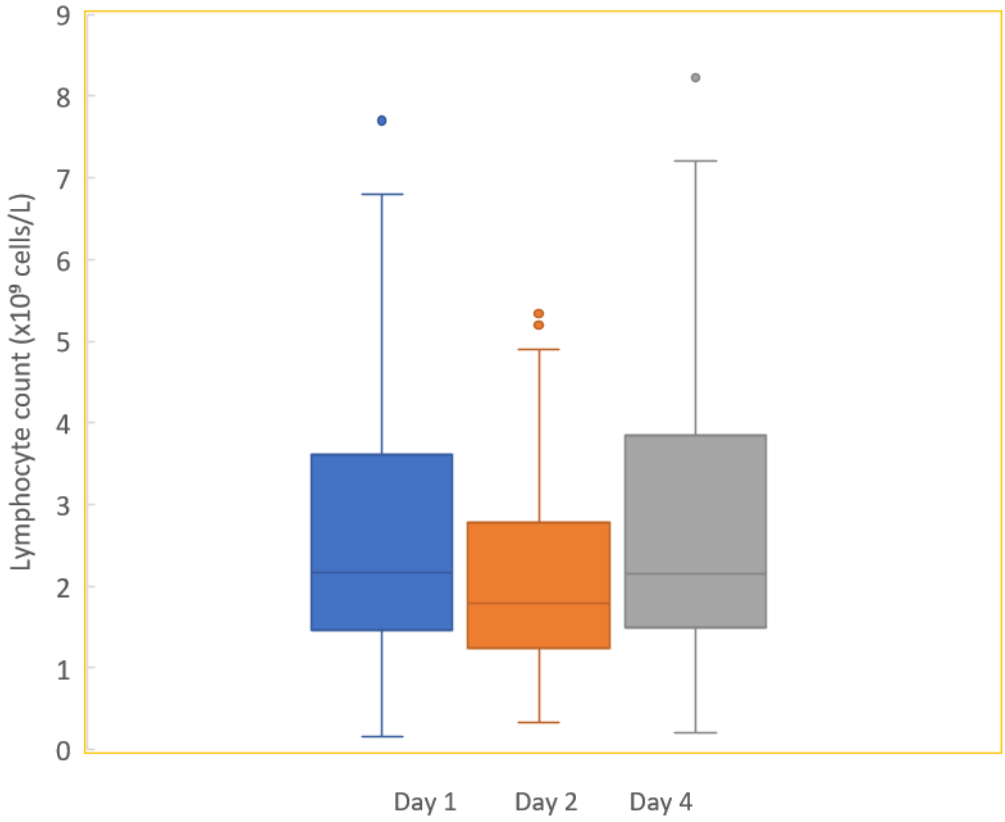
Table 4. Characteristics and outcome of those children with known lymphopenia beyond 4 days

Characteristics and outcome	**No lymphopenia N=67**	**Transient lymphopenia (< 4 days) N=53**	**Persistent lymphopenia (> 4 days) N=16**	**P value (Transient vs Persistent lymphopenia)**
PIM 3 predicted mortality	1.2 (0.4-1.7)	1.2 (0.7-2.0)	1.3 (1.0-2.9)	0.71
Cardiac surgical procedure n (%)	10 (15)	52 (98)	6 (38)	0.88
Confirmed infections	28 (42)	15 (28)	10 (63)	0.02
Bacterial infection	7 (10)	12 (23)	9 (56)	0.01
Viral infection	21 (31)	6 (11)	1 (6)	1.00
Any organ failure				
Day 1	32 (47)	29 (55)	12 (75)	0.24
Day 4	8 (12)	9 (17)	14 (88)	0.03
MODS				
Day 1	6 (9)	5 (9)	5 (31)	0.05
Day 4	2 (3)	2 (4)	6 (38)	0.001
Healthcare associated infections n (%)	0	3 (6)	4 (25)	0.02
PICU length of stay (hours)	41.5 (23-75)	44.4 (23 – 92)	164.8 (71 – 278)	0.004
Hospital length of stay (days)	5 (3.7-14)	7 (4.3 – 12.8)	22 (8-26)	0.02
Mortality n (%)	1 (1.5)	2 (4)	1 (6)	0.55

FIGURE LEGENDS

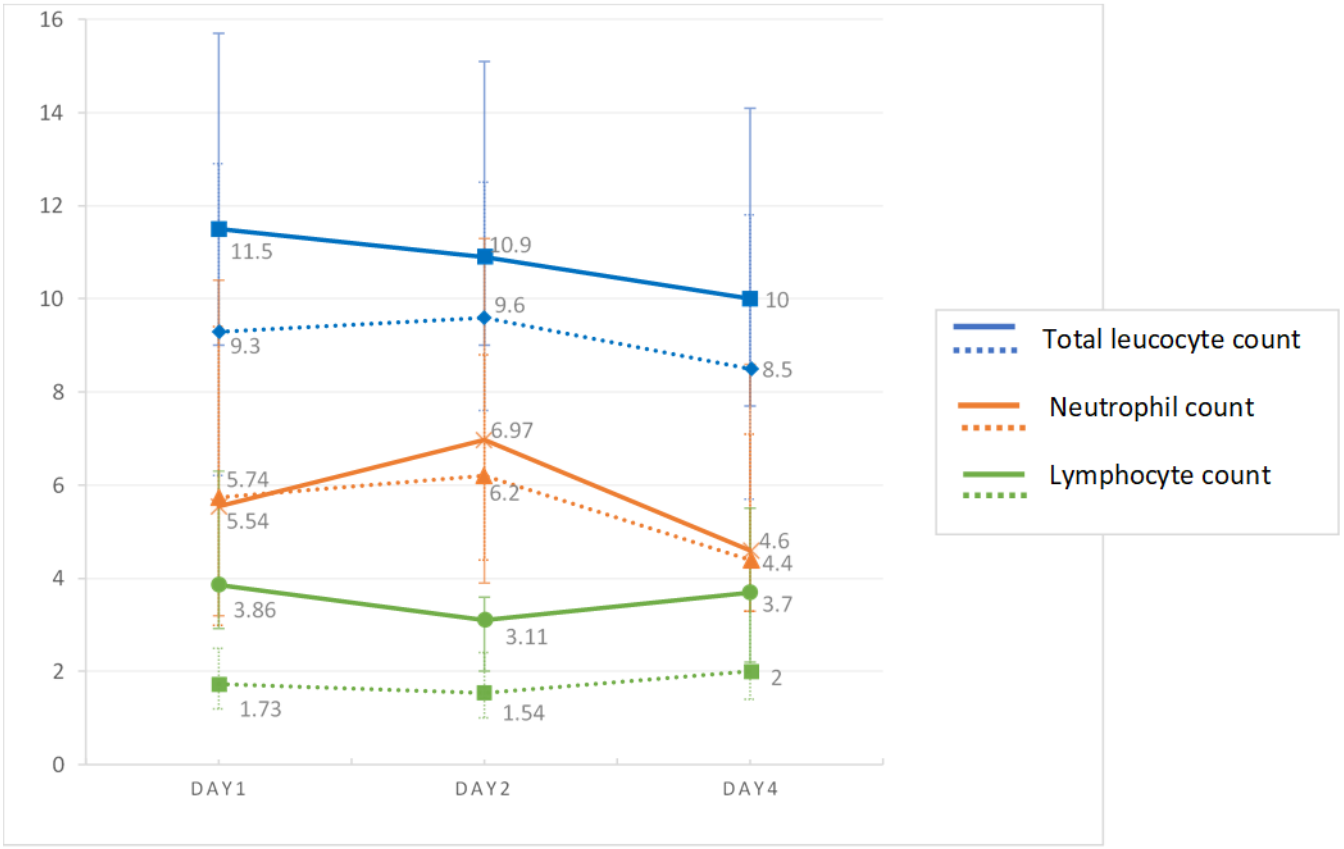
Figure 1. Box Whisker plot depicting serial lymphocyte count of the study population

Figure 2: Comparison of serial blood counts in children with lymphopenia and without lymphopenia



The number of children at each time points: Day 1 = 198, Day 2 = 129, Day 4 =71

JPC_16183_Figure1.tif



Solid lines represent non-lymphopenia group and dotted lines represent lymphopenia group. The number of children at each time points: Day 1 = 198, Day 2 – 129, Day 4- 71. Median cell counts are given in the figure against each time points. Error bars indicate interquartile ranges.

JPC_16183_Figure2.tif