

Diagnostic value of neopterin during neutropenic fever and determination of disease activity in childhood leukemias

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Abstract. Neopterin, a pteridine group compound that is secreted from macrophages is shown to be increased in adult leukemia; however there are few studies in childhood leukemia. This study aimed to investigate neopterin levels during childhood leukemia treatment and neutropenic fever episodes for the possibility of using as a marker for disease activity and differentiation of infections.

A total of 44 children with acute leukemia, 19 children with infection (control group 1) and 21 healthy children (control group 2) were studied. Median serum neopterin level before induction chemotherapy (day 0) in 25 children (patient group 1) was significantly higher (27.7 nmol/L) than those at the beginning of 30 febrile episodes in 19 children in bone marrow remission (2.2 nmol/L) (patient group 2) and in control group 2 (0.4 nmol/L) ($p < 0.05$). It was (27.7 nmol/L) also significantly higher in control group 1 than in patient group 2 and control group 2 ($p < 0.05$). Serum neopterin levels at day 15 (2.1 mmol/L) and day 33 (0.4 mmol/L) of induction were significantly lower than day 0 of ALL subgroup at patient group 1. There were no significant difference in neopterin levels between days 0, 3 and 5 of neutropenic fever as well as between patients with microbiologically and/or clinically documented infections and those with fever of unknown origin in patient group 2 ($p > 0.05$). Serum neopterin did not show significant correlation with absolute neutrophil count and absolute monocyte count ($p > 0.05$). In conclusion, elevated neopterin at diagnosis of leukemia with decrement during induction therapy suggest that it might be an indicator of leukemic process; however larger studies for its role in identifying infections are warranted.

Keywords: Childhood leukemia, neutropenic fever, neopterin

1. Introduction

Neopterin, a pteridine group compound that is secreted from macrophages upon stimulation by interferon- γ regulates the cellular immune response. It is demonstrated that neopterin plays a role at the cytotoxic functioning of the macrophages by increasing the reactive oxygen secretion capacity of macrophage monocyte system. Therefore, neopterin levels can be used as a marker of immunologically induced oxidative stress [1]. Although it is investigated in many viral, autoimmune and malignant diseases as well as adult

leukemias [2–6] little is known about the values in children with hematological malignancies and neutropenic fever [7].

As morbidity and mortality is high and inflammation signs are indistinct early diagnosis of severe infection and prompt initiation of adequate antimicrobial therapy is required during febrile episodes in childhood leukemia [8,9]. Several biomarkers like acute phase proteins, proinflammatory cytokines, soluble adhesion molecules and more recently procalcitonin (PCT) have been investigated in this regard [9,10]. However all of these markers have not been efficacious enough in the etiologic diagnosis of neutropenic fever.

Thus the aim of the present study was to investigate neopterin levels at the time of diagnosis and throughout the induction therapy of childhood leukemias in order to determine if neopterin could be used as a marker both

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in reflecting disease activity and response to therapy and distinguish between etiologically proven infections or fever of unknown origin (FUO). We also aimed to show whether there is relevance between neopterin and other parameters like WBC (white blood cell count), ANC (absolute neutrophil count) and CRP (C-reactive protein) during initial diagnosis and neutropenic fever episodes.

2. Materials and methods

2.1. Study population

This prospective study was planned to evaluate neopterin levels of two patient groups and compare them to that of two control groups. The study was approved by the institutional ethics committee.

2.2. Patients

The study was conducted throughout 2007–2009. During this three years' period 25 patients were diagnosed as leukemia consisting of 20 ALL and 5 AML. Families of all patients were asked for informed consent and it was obtained from all of them. All of the patients are still being followed-up.

2.3. Patient characteristics

Demographic characteristics of patient and control groups are shown in Table 1.

2.3.1. Patient group 1

Twenty five leukemia patients [20 acute lymphoblastic leukemia (ALL), 5 acute myeloblastic leukemia (AML)] at the time of diagnosis were included. Patients were diagnosed to have leukemia when there were > 25% blasts in the Wright stained bone marrow. Chemotherapy was ALL-BFM 95 protocol [11] for ALL and AML MRC-XII protocol [12] for AML subgroups.

2.3.2. Patient group 2

Thirty (71.4%) among 42 neutropenic fever episodes were evaluated in 19 hospitalized leukemic patients in bone marrow remission. Patient group 2 did not consist of all patients of Patient group 1 as all neu-

tropenic episodes could not be included due to unavailability of blood sampling. Neutropenia and neutropenic fever was determined according to 2002 IDSA Guidelines [8]. On the basis of clinical and microbiological findings, patients were grouped in the following categories: microbiologically documented infection (MDI); clinically documented infection (CDI) and fever of unknown origin (FUO). Neutropenic fever attacks were also grouped into three as 'viral infection', 'bacterial infection' and 'FUO' in order to compare markers of infection among different infectious etiologies.

2.3.3. Control groups

Two age and sex matched control groups were set; one consisting of 19 subjects (1–18 years of age) with infectious diseases 31.6% (n: 6) lower respiratory tract disease; 21% (n: 4) upper respiratory tract disease; 21% (n: 4) acute gastroenteritis; 5.4% (n: 1) encephalitis; 10.5% (n: 2) cellulitis and 10.5% (n:2) urinary tract infection without any other known comorbidity as 'control group 1' and 21 healthy subjects (1–18 years of age) as 'control group 2'. Control group 2 consisted of children admitted to pediatrics outpatient clinic for preoperative evaluation before minor surgical procedures like adenoidectomy, tonsillectomy or circumcision. The blood samples for neopterin were collected by a single venipuncture at the time of preoperative laboratory evaluation. This group did not have any sign of infection at the time of admission and laboratory parameters were found to be normal in the whole group.

2.4. Patient sampling methods

In patient group 1, at the time of diagnosis, the subjects were tested for blood biochemistry, viral serologic and polymerase chain reaction (PCR) tests, CRP and microbial/fungal cultures to rule out any comorbidity that may affect initial neopterin levels. Only 9 of the 25 patients had fever at initial diagnosis however remittance of fever very early after initiation of chemotherapy suggested no infectious etiology. None of the patients had fever or any sign of infection at other sampling points. Each patient was evaluated with bone marrow aspirations, complete blood count (CBC) and neopterin levels at 0, 15, 33rd days of induction therapy in ALL and 0, 15th days in AML. In patient group 2, CBC, neopterin levels determined at 0 (first day of neutropenic fever), 3, 5th days of every episode. Microbial and fungal cultures as well as viral and fungal PCR

Table 1
Demographic characteristics of the patient and control groups

Parameter	Patient group 1	Patient group 2	Control group 1	Control group 2
Number of subjects	25	19	19	21
Mean age (years)	7.98 ± 4.61 (0–18)	6.78 ± 3.98	6.78 ± 3.9	6.52 ± 3.34 (1–18)
Diagnosis (ALL/AML)	20/5	15/4		
Sex (F/M)	12/13	10/9	10/9	10/11

ALL: acute lymphoblastic leukemia, AML: acute myeloblastic leukemia, F: female, M: male.

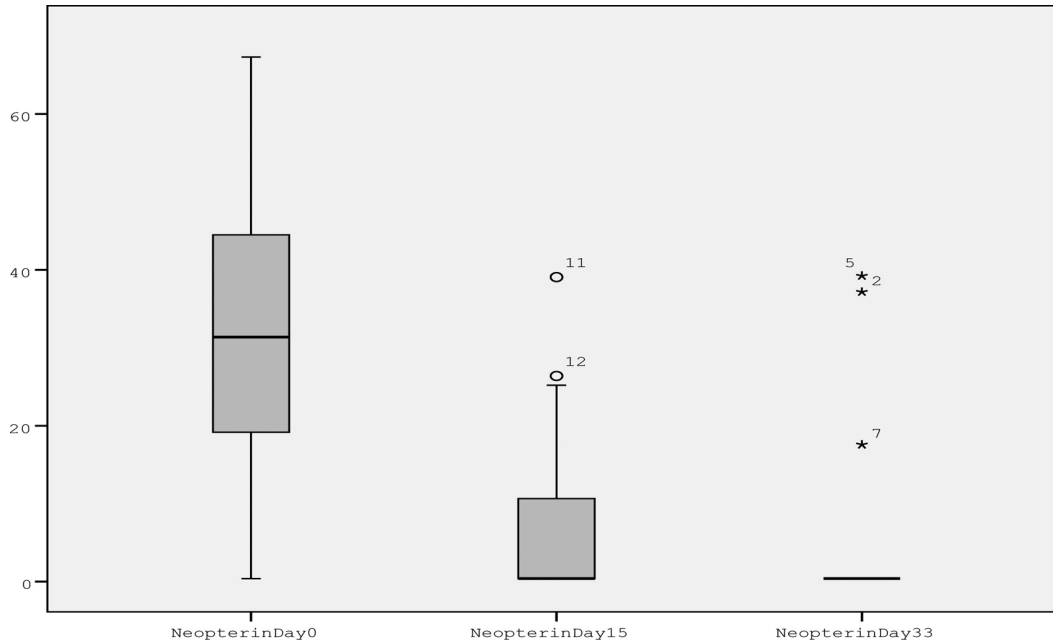


Fig. 1. Neopterin levels of patient group 1 at days 0-15-33. NeopterinDay0: Neopterin level at diagnosis, NeopterinDay15: neopterin level on day 15 of induction, NeopterinDay33: neopterin level on day 33 of induction. Y axis represents neopterin levels as nmol/L.

were performed at the beginning of each febrile episode and were repeated subsequently as needed. Cultures, PCR tests and imaging studies like chest x-ray, high resolution tomography and abdominal ultrasonography (performed according to the severity of neutropenic episode) were used to classify episodes into CDI/MDI and FUO.

Control groups were tested for neopterin levels only once on admission.

2.5. Laboratory methods

Whole blood was obtained for neopterin levels, centrifuged at 3500 rpm for 5 minutes and serum samples were stored at -80°C and were protected from light. Neopterin levels were measured with ELISA method as described by the manufacturer (DRG Diagnostics, Marburg, Germany). Normal values of neopterin in our lab were 1.2–11.8 nmol/L the minimum level of detection

was 0.4 nmol/L and inter-assay variance of neopterin levels is 5.6%. White blood cell counts and blood biochemistry were studied by autoanalysers (Beckman Coulter LH 755-Beckman Coulter Inc. CA 92821-6232, USA). Absolute neutrophil count (ANC) and absolute monocyte count (AMC) were documented by manual methods. CRP was studied with nephelometry quantitative immunoassay and normal levels of CRP at our laboratory was < 6 mg/dl. In patient group 2, blood were drawn at every febrile episode from both peripheral veins and central venous lines (all patients had Hickman Catheters) to be cultured by Bact/ALERT[®] PF (Biomérieux Inc. Durham, NC 27704).

2.6. Statistical analysis

Data were analyzed by SPSS for Windows 11.5 using the nonparametric Mann Whitney U-test and Kruskal Wallis test for comparison between groups. Values

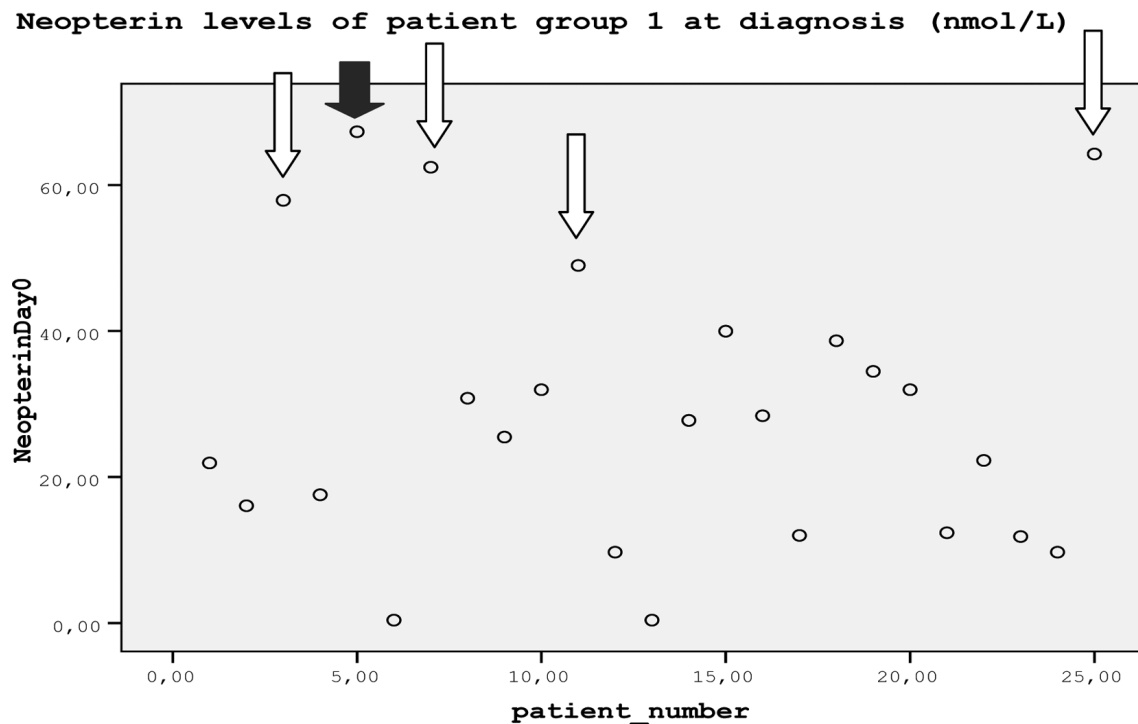


Fig. 2. Neopterin levels of patient group 1 at diagnosis. The tall arrows represent four patients (patients 3, 7, 11 and 25) who have high initial neopterin levels. Two of these patients had an isolated CNS relapse later (patients 11, 25). One of these, patient 11 also suffered from MTX encephalopathy during second induction therapy after relapse. Patients 3 and 7 are in remission receiving maintenance therapy however; during consolidation therapy patient 7 had also suffered from MTX encephalopathy. The neopterin level shown with a grey arrow represents the highest initial neopterin level in patient 5 who is also in remission. Y axis represents neopterin levels as nmol/L.

were expressed as median, range and 25–75 percentiles due to inhomogeneous distribution of the values. Nominal variables were expressed as number of observations and percentages. Associations were considered statistically significant with a p value < 0.05 .

3. Results

3.1. Patient group 1

All patients in patient group 1 had bone marrow diagnosis of M3 (with 25% blasts in bone marrow) at the time of diagnosis. Bone marrow aspirations revealed M1 (with $< 5\%$ blasts in bone marrow) in 96% (n: 24) and M2 (with 5–25% blasts in bone marrow) in 4% (n: 1) of the patients at 15th day.

On 33rd day of induction therapy, bone marrow aspiration was performed in 20 ALL patients, among them 95% (n: 19) was M1 and 5% (n: 1) M2. Changes in the laboratory parameters of both ALL and AML subgroups throughout the induction period are shown in Table 2. In ALL subgroup, a significant decrease in

WBC levels was found throughout the induction period, especially passing through 0th to 15th days ($p = 0.000$). Absolute neutrophil count and AMC did not change significantly at days 0 and 15 ($p > 0.05$). The median neopterin level at the time of diagnosis was 29.5 nmol/L (25–75 percentiles: 12.9–39.6 nmol/L) decreasing to 2.1 nmol/L (0.4–12.4 nmol/L) at the 15th day and to 0.4 nmol/L (0–0.4 nmol/L) at the 33rd day (Fig. 1).

In the AML subgroup there was a significant difference between WBC, ANC, bone marrow blast percentage and neopterin ($p < 0.05$) but not for AMC and CRP ($p > 0.05$). Neopterin levels of ALL and AML subgroups were not compared as AML subgroup consisted of only five subjects making it inappropriate to draw any conclusions between two subgroups. The patients in patient group 1 were ranked from 1–25. The highest initial neopterin levels were detected in five patients (patients 3, 5, 7, 11 and 25) with ALL two of whom were later diagnosed with isolated CNS relapse (patients 11 and 25). One of the patients without (patient 7) and one with CNS relapse (patient 11) had al-

Table 2
WBC, ANC, AMC, CRP, bone marrow blast percentage and neopterin levels during induction therapy in patient group 1

Parameter	All subgroup (n = 20)			AML subgroup (n = 5)		
	Median	25p-75p	Min-max	Median	25p-75p	Min-max
WBC (/mm³)						
Days 0	6910*	2700-19200	590-150000	13800**	7710-26500	3810-35000
15	1030*	840-3320	204-13600	499**	328.50-520.50	238-539
33	1690*	981-3840	219-6110			
ANC (/mm³)						
Days 0	625	252-2324	0-21000	1404**	698.50-3122	340-3864
15	488	104-976	310-9520	17**	2.50-39	50
33	743	117-1911	5-3480			
AMC (/mm³)						
Days 0	178	0-768	0-6246	0	0-3123	0-6246
15	21	0-118	0-1960	8	1.5-20	0-30
33	109	0-272	0-1302			
CRP(mg/dl)						
Days 0	26**	0-48	0-180	6	0-107.5	0-180
15	0**	0-12	0-152	48	6-62	0-83.5
33	0**	0-0	0-80			
BM blast percentage						
Days 0	100*	90-100	20-100	47**	32-95	25-100
15	1*	1-3	0-10	1**	0-1.5	0-2
33	1*	0-2	0-20			
Neopterin (nmol/L)						
Days 0	29.5*	12.9-39.6	0.4-68.7	12.3**	6.1-30.3	0.4-38.6
15	2.1*	0.4-12.4	0.4-39.1	0.4**	0.4-10.1	0.4-11.6
33	0.4*	0.0-0.4	0.4-39.2			

WBC: white blood cell, ANC: absolute neutrophil count, AMC: absolute monocyte count, CRP: c-reactive protein, ALL: acute lymphoblastic leukemia, BM: bone marrow, AML: acute myeloblastic leukemia 25p-75p: 25th-75th percentile, Min: minimum, max: maximum.

p* < 0.001 *p* < 0.05.

The values of different variables at days 0, 15, 33 are compared in subgroups ALL and AML. As AML group is too small, the p values written for variables of AML subgroup reflects the comparison of the values among different days of AML subgroup not comparison of AML and ALL. There was significant difference between WBC levels on days 0-15; 0-33 and 15-33 (**p* < 0.001). Difference between CRP levels of days 0-15 and 0-33 was found in ALL subgroup. Significant difference in neopterin levels was found between days 0-15 and 0-33 (**p* < 0.001) but not between days 15-33 (**p* < 0.001).

so suffered from methotrexate (MTX) encephalopathy (Fig. 2).

3.2. Patient group 2

Median ANC levels were 9/mm³ (0-1200/mm³) at the beginning of fever; 12/mm³ (0-7780/mm³) at day 3 and 90/mm³ (0-11360/mm³) at day 5 of the fever (Table 3). No significant difference between WBC levels of days 0, 3, 5 of fever episode was found however median ANC and AMC showed an increase especially towards day 5 (*p* < 0.005). There was no difference in both CRP and neopterin levels along the days of fever (*p* > 0.05). The febrile episodes were retrospectively classified into three outcomes: CDI (50%, n: 15), MDI (23.3%, n: 7) and FUO (26.7%, n: 8). No difference in WBC, AMC, ANC, CRP and neopterin levels were found among these three groups at 0, 3, 5th days of fever episodes (*p* > 0.05) (Data not shown). White blood cell

count, ANC, AMC, neopterin and CRP levels at days 0, 3, 5 of fever were also compared among viral infection, bacterial infection and FUO groups. No difference in these parameters were found among the groups along the days of fever as well (*p* > 0.05) (Table 4).

3.3. Control groups

In control group 1 median magnitude of fever was 38.7°C, median WBC was 13000/mm³ (4500-24000/mm³) and none of the patients had bacteriemia. The median neopterin level of control group 1 was 27.7 nmol/L (0.4-107.5 nmol/L) with the 107.5 nmol/L value belonging to an 8 year old patient with herpetic encephalitis. Median neopterin level of control group 2 was 0.4 nmol/L (0.4-16.9 nmol/L). No correlation was found between WBC, ANC and AMC counts with respect to neopterin levels any of the groups (*p* > 0.05).

3.4. Neopterin levels among patient and control groups

Levels of neopterin of patient group 1, control group 1, patient group 2 and control group 2 were found to be 27.7 nmol/L, 27.7 nmol/L, 2.2 nmol/L and 0.4 nmol/L respectively (Fig. 3).

4. Discussion

Studies in adult patients revealed that neopterin is elevated in hematological malignancies including Hodgkin's disease, non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and adult T-cell lymphoblastic leukemia at diagnosis and during progression of disease burden [5,6,13,14]. Neopterin levels have also been shown to be increased in hematopoietic stem cell transplant recipients during Graft versus host disease and viral infections [15]. In studies including adult patients with tumors of genitourinary tract or hematopoietic neoplasms, urinary neopterin levels were correlated with tumor burden and became normal when remission was achieved [16]. However, there is only one pediatric study showing higher cerebrospinal fluid (CSF) neopterin levels with meningeal relapse of hematologic malignancies [7].

The first objective of our study was to establish the neopterin levels in childhood leukemia at the time of diagnosis and levels throughout the induction therapy. We hypothesized that neopterin levels would be higher than healthy controls at the time of diagnosis and would gradually decrease parallel to the decrement of immunological stimulation by the leukemic blasts. A gradual decrease was found in neopterin levels through the induction therapy days in concordance with the abrupt decrease in the bone marrow blast percentage ($p < 0.001$). Our study was consistent with the previous adult studies by observing a decrease of neopterin levels throughout leukemia treatment [5,6,13]. However, one limitation of our study is the low median neopterin levels especially in the lower range of the results as the lowest neopterin levels are found to be higher than 1 nmol/L in most studies [6,7]. This may be attributed to a problem of initial calibration of microsta software programme that we used to analyse the sample standard curve. Median neopterin levels of patient group 1 and control group 1 were close to each other being highest among four groups 27.7 nmol/L and 27.7 nmol/L respectively. Median neopterin levels of patient group 1 were also significantly higher com-

pared to that of control group 2 consisting of healthy subjects. This result suggested that neopterin levels in patients with active leukemic disease were much higher than those of healthy subjects and as high as those in otherwise healthy patients with infection. All subjects in our study group were newly diagnosed without relapses. Soon after the first remission, two of the patients had an early isolated CNS relapse. We found out that these two patients (patients 11 and 25 in Fig. 2) had substantially high initial neopterin levels. One of these (patient 11) also suffered from MTX encephalopathy during second induction therapy (1 gr/m² MTX infused 6 times according to Rez-BFM 2002 protocol) after relapse. There were three other patients with significant high neopterin levels in patient group 1 (patients 3, 5 and 7) without relapses who are under maintenance therapy and in remission. Interestingly, one of them (patient 7) also had MTX encephalopathy and seizures during previous consolidation therapy. These individual results may suggest that there may be a probable relation between initial high levels of neopterin in serum or CSF with early isolated CNS relapses. Our observation that neopterin levels are identified in patients who later experienced CNS relapses and the possible interaction between MTX toxicity is interesting, however as we did not expect any CNS relapses from the patients with initially increased neopterin levels we did not collect CSF samples for neopterin as determination of the relationship between initial neopterin levels which is a limitation of our study. On the other hand, we found this observation is worth to be shared that may give rise to new questions. Whether increased neopterin originates from CNS leukemic cells, leukemic burden induced monocytes or glial cells are a further question that needs to be solved. Short time period of the study to observe relapses is another limitation of this study. Further prospective studies including newly diagnosed and relapsed patients with larger subject groups should be planned to estimate the predictive value of initial neopterin levels on CNS relapses or CNS toxicity of MTX, which arises from our study. We found that the patients at diagnosis had significantly higher levels than normal subjects and levels decreased in concert with decreasing WBC and blast percentage.

In a study comparing pneumonia with viral or bacterial origin, it was found out that the levels of CRP > 10 mg/dl and PCT > 0.1 μ g/L may be used to rule out a bacterial infection in 60% to 95% of cases whereas neopterin > 10 nmol/L may be used to rule out viral infection in 97% of patients [17]. Prat et al. measured levels of several biomarkers like PCT, CRP, IL-6, IL-8

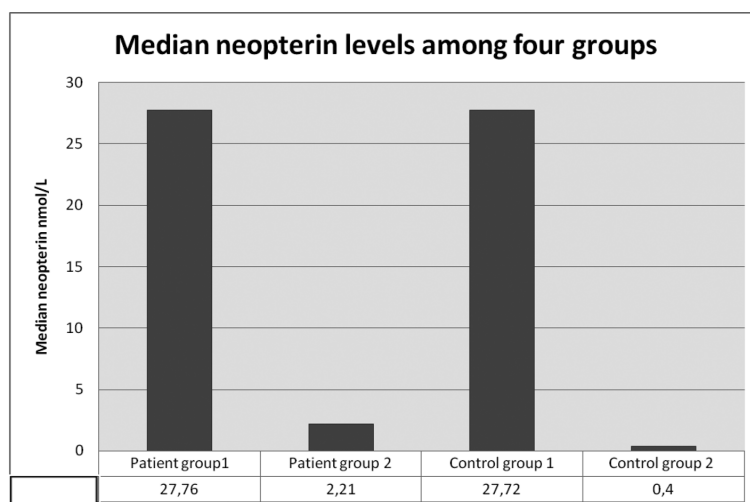


Fig. 3. Comparison of neopterin median levels among four groups * Significant difference was found between the median neopterin levels when groups 1–2; 2–3; 2–4 and 3–4 are compared ($p < 0.05$). There was no difference between the median neopterin levels of groups 1–3 and 1–4.

and neopterin in patients with hematological malignancy [18]. Neopterin levels were not elevated at diagnosis of neutropenia besides no significant difference was found according to the origin or etiology of fever. In our study, although a gradual increase in ANC and AMC towards day 5 was shown there was no correlation with neopterin ($p > 0.05$). There was also no significant difference regarding CRP and neopterin levels ($p > 0.05$). Median neopterin level of patient group 2 was found to be 2.2 nmol/L which was lower than that of patient group 1 and control group 1 but significantly higher than the level of 0.4 nmol/L of control group 2 ($p < 0.05$).

Our results for neopterin during neutropenic fever were similar to Prat et al that it could not differentiate between different etiologies, as a first among pediatric population. This made us think that patients with neutropenic fever were not able to increase neopterin levels as high as in infections of immune competent subjects due to lack of enough cellular response. We postulated that neopterin would be a marker for viral or fungal infections as it shows the cellular immune response, compared to increased CRP mainly in bacterial infections. Nevertheless, both markers were unable to differentiate different etiologies of fever episodes (CDI and MDI versus FUO). This may be due to the inability of immune compromised leukemic children to produce a sufficient cellular immune response reflected by the lack of estimated increase in neopterin levels. Furthermore, in most of the episodes, symptoms of viral and bacterial infections were together and indistinct making the classification more difficult. However, as the

highest neopterin level in all groups was found in a patient with herpetic encephalitis in control group 1, the postulation that neopterin may be a candidate to specify viral intracellular infections is still worth to be further investigated in larger studies.

In conclusion, neopterin levels are high at the time of diagnosis, decrease to normal levels during induction therapy, and so probably could be used in the determination of the activity of childhood leukemia. Whether neopterin can differentiate between etiologically proven infections or FUO should be considered.

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References

- [1] Weiss G, Fuchs D, Hausen A, Reibnegger G, Werner ER, Werner-Felmayer G, Semenitz E et al. Neopterin modulates toxicity mediated by reactive oxygen and chlorine species. *FEBS Lett.* 1993; 321: 89-92.
- [2] Murr C, Bergant A, Widschwendter M, Heim K, Schröcksnadel H, Fuchs D. Neopterin is an independent prognostic variable in females with breast cancer, *Clin Chem* 1999; 45: 1998-2004.
- [3] Melichar B, Solichová D, Freedman RS. Neopterin as an indicator of immune activation and prognosis in patients with gynecological malignancies. *Int J Gynecol Cancer* 2006; 16: 240-52.
- [4] Weiss G, Kronberger P, Conrad F, Bodner E, Wachter H, Reibnegger G. Neopterin and prognosis in patient with adenocarcinoma of the colon. *Cancer Res* 1993; 53: 260-5.

- [5] Motyl T, Traczyk Z, Holska W, Daniewska-Michalska D, Cieśluk S, Kukulska W et al. Comparison of urinary neopterin and pseudouridine in patients with malignant proliferative diseases. *Eur J Clin Chem Clin Biochem* 1993; 31: 205-9.
- [6] Caenazzo A, Pietrogrande F, Sgarabotto D, Dazzi F, Sartori D, Vattamattathil KP et al. Serum neopterin levels in haematological malignancies. *Haematologica* 1993; 78: 225-9.
- [7] Millot F, Dhondt JL, Mazingue F, Mechinaud F, Auget JL, Bauters F et al. Cerebrospinal fluid neopterin levels in children with central nervous system leukemia. *Leuk Lymphoma* 1993; 9: 351-6.
- [8] Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T et al. Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 2002; 34: 730-51.
- [9] Klastersky J, Paesmans M, Rubinstein EB, Boyer M, Elting L, Feld R et al. The multinational association for supportive care in cancer risk index: a multinational scoring system for identify low-risk febrile neutropenic cancer patients. *J Clin Oncol* 2000; 18: 3038-51.
- [10] Kallio R, Surcel H-M, Bloigu A, Syrjälä H. C-reactive protein, procalcitonin and interleukin-8 in the primary diagnosis of cancer patients. *Eur J Cancer* 2000; 36: 889-94.
- [11] Kaya Z, Gursel T, Bakkaloglu SA, Kocak U, Atasever T, Oktar SO. Evaluation of renal function in Turkish children receiving BFM-95 therapy for acute lymphoblastic leukemia. *Pediatr Hematol Oncol* 2007; 24: 257-67.
- [12] Burnett AK, Hills RK, Milligan DW, Goldstone AH, Prentice AG, McMullin MF, Duncombe A, Gibson B, Wheatley K. Attempts to optimize induction and consolidation treatment in acute myeloid leukemia: results of the MRC AML12 trial. *J Clin Oncol* 2010; 28: 586-95.
- [13] Davies SV, Carter C, Willims MD, Pollock A, Leeming RJ. The significance of neopterin and biopterin concentrations at presentation in hematological malignancies. *Clin Lab Hematol* 1992; 14: 41-6.
- [14] Giusti RM, Maloney EM, Hanchard B, Morgan OS, Steinberg SM, Wachter H, Williams E, Cranston B, Fuchs D, Manns A. Differential patterns of serum patterns of serum biomarkers of immune activation in human T-cell lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis, and adult T-cell leukemia/lymphoma. *Cancer Epidemiol Biomarkers Prev* 1996; 5: 699-704.
- [15] Niederwieser D, Huber C, Gratwahl A, Bannert P, Fuchs D, Hausen A, Reibnegger G, Speck B, Wachter H. Neopterin as a new biochemical marker in the clinical monitoring of bone marrow transplant recipients. *Transplantation* 1984, 38(5): 497-500.
- [16] Fuchs D, Hausen A, Huber C, Reibnegger G, Wachter H. Urinary neopterin in the diagnosis and follow-up of neoplasia: a biochemical parameter to detect cell-mediated immune response. *Tumor Biol* 1984; 5(3-4): 199-209.
- [17] Ip M, Rainer TH, Lee N, Chan C, Chau SS, Leung W, Leung MF, Tam TK, Antonio GE, Lui G, Lau TK, Hui DS, Fuchs D, Renneberg R, Chan PK. Value of serum procalcitonin, neopterin, and C-reactive protein in differentiating bacterial from viral etiologies in patients presenting with lower respiratory tract infections. *Diagn Microbiol Infect Dis* 2007; 59: 131-6.
- [18] Prat C, Snacho JM, Dominguez V, Xicoy B, Gimenez M, Ferra C et al. Evaluation of procalcitonin, neopterin, C-reactive protein, IL6 and IL8 as a diagnostic marker of infection in patients with febrile neutropenia. *Leuk Lymphoma* 2008; 49: 1752-61.