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Research Article

Chitotriosidase and Neutrophil Gelatinase-Associated Lipocalin: New Methods as a Diagnostic Marker of Infection in Patients with Febrile Neutropenia?

Omer Kartal,[1] Orhan Gursel,[2] Ibrahim Eker,[3] Mehmet Emre Tascilar,[4] Serkan Tapan,[5] Ahmet Emin Kurekci[6]

[1]Division of Pediatric Hematology and Oncology, Gulhane Training and Research Hospital, Ankara, Turkey
[2]Division of Pediatric Hematology and Oncology, University of Health Sciences, Ankara, Turkey
[3]Division of Pediatric Hematology and Oncology, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey
[4]Department of Pediatric Endocrinology, Yuksek Ihtisas University, Ankara, Turkey
[5]Department of Biochemistry, Yuksek Ihtisas University, Ankara, Turkey
[6]Department of Pediatric Hematology, Losante Hospital, Ankara, Turkey

Abstract

Objectives: Despite the important improvements in prevention and treatment, febrile neutropenia is still a common challenge in patients with certain hematological disorders. Many biomarkers have been explored to identify high-risk neutropenic patients, which need hospitalization and broad-spectrum antibiotic treatment. In the present study, firstly, we aimed to investigate serum CRP, Chitotriosidase (CtH) and Neutrophil gelatinase-associated lipocalin level (NGAL) in febrile neutropenia, non-febrile neutropenia and control groups. Secondly, to evaluate the usefulness of CRP, CtH and NGAL as a inflammatory biomarker to detect slight inflammatory change associated with infection in these patients.

Methods: This was a single center prospective study that investigated ChT and NGAL as a new biomarkers to detect slight inflammatory change associated with infection in febrile neutropenia, non-febrile neutropenia and control groups. Therefore, 168 serum samples, which were obtained from 26 patients with hematological disorders were divided into three groups; Febrile neutropenia, non-febrile neutropenia and control group.

Results: The mean serum CRP, CtH and NGAL levels of febrile neutropenia group were significantly higher than the other two groups ($p<0.05$). However, there was not any statistically significant difference between non-febrile neutropenia and control groups ($P>0.05$). C-reactive protein had a positive and significant correlation with CtH ($r=0.548$, $p<0.05$) and NGAL ($r=0.311$, $p<0.05$). Moreover, there was a positive and significant correlation between CtH and NGAL ($r=0.247$, $p<0.05$). The area under the curve (AUC) values were found as 0.68 (0.48–0.79), 0.72 (0.56–0.87), 0.72 (0.56–0.87), 0.56 (0.39–0.66) for CRP, CtH and NGAL respectively and there was not any significant difference between any two AUC values ($p>0.05$).

Conclusion: Serum ChT level is the most sensitive and specific biomarker to detect slight inflammatory change associated with infection in febrile neutropenic patients. Therefore, in the future, it may be used instead of or in combination with CRP to discriminate critically ill patients, to prevent unnecessary hospitalization and antibiotic treatment in neutropenia.

Keywords: Chemotherapy, chitotriosidase, C-reactive protein, febrile neutropenia, neutrophil gelatinase-associated lipocalin

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Address for correspondence: Omer Kartal, MD. Gulhane Egitim ve Arastirma Hastanesi, Cocuk Hematoloji ve Onkoloji Klinigi, Ankara, Turkey
Phone: +90 542 461 07 15 **E-mail:** dr.omerkartal@hotmail.com

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Despite the important improvements in prevention and treatment, febrile neutropenia is still a common challenge in patients with certain hematological disorders, especially in those receiving intensive chemotherapy.[1,2] Moreover, these patients are in an immunosuppressive condition, which may cause to life-threatening complications in hours depending on the infectious agent.[3] Because of this reason, rapid administration of empiric antibiotic treatment for these patients is believed vital.[4]

Additionally, low-risk neutropenic patients can follow up as outpatient, therefore, many biomarkers have been explored to identify high-risk neutropenic patients, which need hospitalization and broad-spectrum antibiotic treatment.[5,6]

Chitotriosidase (ChT) is the major active chitinase enzyme, which is produced predominantly by activated macrophages and neutrophils in humans and it participates in host's defense against pathogens.[7,8] Moreover, ChT correlates with leukocyte elastase and it is considered a marker of neutrophil activation.[9]

Neutrophil gelatinase-associated lipocalin (NGAL), which is considered to be a critical component of the first-line host defense is a member of the lipocalins family and it is released from activated neutrophils in case of infection.[10,11]

Up to now, various biomarkers (CRP, PCT, TNF- α, IFN-γ, IL-1, IL-6, IL-8, etc.) have been used to detect slight inflammatory change associated with infection (SICAI), however, they are not yet in the desired sensitivity and specifivity.[12,13] Therefore, first, we aimed to investigate serum CRP, CtH and NGAL level in febrile neutropenia, non-febrile neutropenia and control groups, secondly, to evaluate the usefulness of CRP, CtH and NGAL as a novel biomarker to detect SICAI in these groups.

Methods

This was a single center (Gülhane Training and Research Hospital) prospective study that investigated ChT and NGAL as a new biomarkers to detect SICAI in febrile neutropenia, non-febrile neutropenia and control groups between January 1, 2016 and December 31, 2019. Therefore, 168 serum samples, which were obtained from 26 patients with hematological disorders were divided into three groups; Febrile neutropenia, non-febrile neutropenia and control group. Detailed characteristics of these groups were summarized in Table 1.

Febrile neutropenia was defined as an absolute neutrophil count <0.5x109/l and either with a axillary temperature of ≥38.3 °C in a single measurement, or with a temperature

Table 1. Clinical and laboratory characteristics of the study participants

Parameters, n (%)	Febrile neutropenia group (n=17)	Non-febrile neutropenia group (n=19)	Control group (n=20)	p
Age, yr (Mean±SD)	8.9±4.3	8.7±3.7	9.2±3.9	0.950
Gender (M/F), (n, %)	10 (58.8)/7 (41.2)	10 (52.6)/9 (47.4)	11 (55)/9 (45)	0.932
Disease, (n, %)				0.720
AA	0 (0)	1 (5.3)	0 (0)	
ALL	9 (52.9)	13 (68.4)	13 (65)	
AML	7(41.2)	2 (10.5)	5 (25)	
CML	0 (0)	3 (15.8)	1 (5)	
MDS	1 (5.9)	0 (0)	1 (5)	
Treatment, (n, %)				0.432
ALL-BFM 95	8 (47)	13 (57.7)	12 (60)	
AML-BFM 2004	4 (23.5)	3 (10.5)	3 (15)	
ALL-REZ-BFM 2002	2 (11.7)	1 (5.3)	3 (15)	
HSCT	3 (17.6)	2 (26.3)	2 (10)	
WBC	600±536	1121±944	5305±3650	<0.001
ANC	111±103	259±274	3103±1099	<0.001
CRP	38.3±10.3	7.8±4.1	4±2.1	<0.001
ChT	77.3±17.2	16.1±5.5	14.8±3.3	<0.001
NGAL	12.6±1.8	6.1±1.2	4.5±0.7	0.010

AA: Aplastic Anemia; ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; CML: Chronic myeloid leukemia; MDS: Myelodysplastic syndrome; ALL-BFM 95: ALL Berlin-Frankfurt-Münster Study Group 95; AML-BFM 2004; AML Berlin-Frankfurt-Münster Study Group 2004; ALL-REZ-BFM 2002; BFM 2002 protocol for Relapsed ALL; HSCT: Hematopoietic stem cell transplantation; WBC: White blood cell count: (/μL); ANC: Absolute Neutrophil Count, (/μL); CRP: C-reactive protein: (mg/l); ChT: Chitotriosidase; (nmol/mL substrate/hr); NGAL: Neutrophil gelatinase associated lipocalin, (ng/mL).

of between 37.6 ºC and 38 ºC in two consecutive measurements sustained over one hour period.

Non-febrile neutropenia was defined as an absolute neutrophil count <0.5x109/l and with an axillary temperature <37.6 °C.

Control group was defined as an absolute neutrophil count ≥1500x109/l and with an axillary temperature <37.6 °C.

The study protocol was approved by the local medical ethics committee and carried out in accordance with the ethical principles described by the Declaration of Helsinki. An informed written consent was obtained from each patient and their parents.

All of the venous blood samples were obtained on the first day of neutropenia. Complete blood count parameters were measured by an automatic blood counter (BECKMAN Coulter GEN-S, Florida, USA). C-reactive protein was measured using the turbidimetric method (Boehringer, Mannheim, Germany). Chitotriosidase activity was measured with using a Microfluor fluorometer (BIO-TEK SynergyHT; Biotek Instruments Inc., Winooski, VT, USA) based on the method described by Hollak et al. and were expressed as nanomoles of substrate hydrolyzed per milliliter per hour (nmol/mL/h). Serum NGAL level was measured with using Human Neutrophil Gelatinase Associated Lipocalin ELISA Kit (Hycult Biotech, Wayne, PA, United States).

Statistical Analysis

Data were analyzed with SPSS (Statistical Package for Social Sciences) 21.0 program for Windows. Continuous variables were calculated as mean+standard deviation. Mann–Whitney U test and Kruskal–Wallis test were used to compare non-parametric data. Correlations between variables were assessed using Spearman's correlations. Receiver operating characteristic curve was used to determine the optimum cut off levels of CRP, CtH and NGAL. A p-value <0.05 was evaluated significant.

Results

A total of 168 serum samples from 26 patients with hematological disorders were studied. Male/female ratio of febrile neutropenia and non-febrile neutropenia groups was 10/7 and 10/9, respectively, while it was 11/9 for control group (p >0.05). Mean age of febrile neutropenia, non-febrile neutropenia and control groups were 8.9±4.3; 8.7±3.7; 9.2±3.9 years, respectively (p>0.05). The characteristics of all participants are shown in Table 1.

The mean serum CRP levels of febrile neutropenia, non-febrile neutropenia and control groups were 38.3±10.3, 7.8±4.1 and 4±2.1, respectively (p<0.001). The mean serum CtH levels of febrile neutropenia, non-febrile neutropenia and control groups were 77.3±17.2, 16.1±5.5 and 14.8±3.3, respectively (p<0.001). The mean serum NGAL levels of febrile neutropenia, non-febrile neutropenia and control groups were 12.6±1.8, 6.1±1.2 and 4.5±0.7, respectively (p<0.001). The mean serum CRP, CtH and NGAL levels of febrile neutropenia group were significantly higher than the other two groups (p<0.05). However, there was not any statistically significant difference between non-febrile neutropenia and control groups (p>0.05).

C-reactive protein had a positive and significant correlation with CtH (r=0.548, p<0. 05) and NGAL (r=0.311, p<0.05). Moreover, there was a positive and significant correlation between CtH and NGAL (r=0.247, p<0. 05), (Fig. 1).

Figure 2 shows the sensitivity and specificity for CRP, CtH and NGAL to detect SICAI in febrile neutropenia patients at cut-off values of 18 mg/l for CRP (sensitivity 77%; specificity 57%), 30 nmol/mL substrate/hr for CtH (sensitivity 90%; specificity 76%) and 10 ng/mL for NGAL (sensitivity 66%; specificity 49%). The area under the curve (AUC) values were found as 0.68 (0.48–0.79), 0.72 (0.56–0.87), 0.72 (0.56–0.87), 0.56 (0.39–0.66) for CRP, CtH and NGAL respectively and there was not any significant difference between any two AUC values (p>0.05), (Fig. 2).

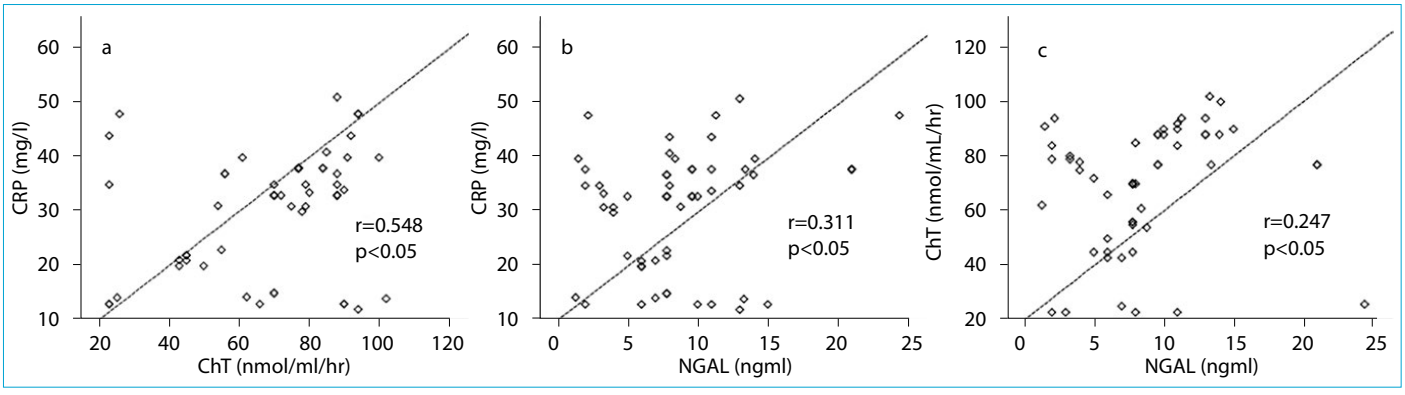

Figure 1. Correlation analysis showing statistically significant positive correlation between **(a)** CRP and ChT; **(b)** CRP and NGAL; **(c)** ChT and NGAL.

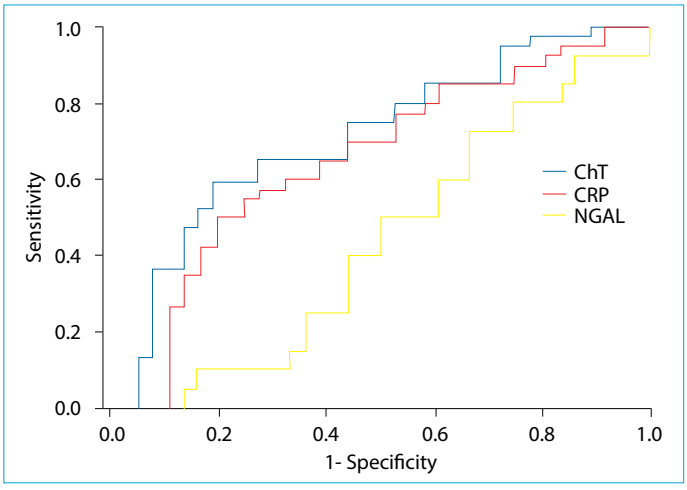

Figure 2. A plot for comparison of ROC curves among ChT, CRP, NGAL variables.

Discussion

Patients with neutropenia can follow up as outpatient, however, in the presence of fever, they need hospitalization and broad-spectrum antibiotic treatment.[14] For that reason, to prevent unnecessary hospitalization and antibiotic treatment and to detect SICAI, we need early, reliable and sensitive biomarkers. Moreover, new markers are also necessary to precisely differentiate infectious and noninfectious etiologies.

C-reactive protein is the most common biomarker used to detect inflammation associated with infection, however, it may not detect SICAI early and precisely.[15,16] Recently, the study, which was conducted in Germany by Michel et al., shown that CRP was a weak biomarker for identifying critically ill patients.[13] Similar observations were reported by Massaro et al.[17] We speculated that c-reactive protein may increase with not only to inflammation, but also to blastic cells. Consistent with these findings, in the present study, CRP has a significant association, but, lower specificity than ChT to show SICAI.

Chitinases are enzymes that degrade ubiquitous chitin and protect against pathogens containing chitin.[18] In humans, ChT is released by phagocytic cells of the immune system, predominantly macrophages and neutrophils.[9,19] The study, which was reported by Labadaridis et al. evaluated eight neonates with fungal and 15 neonates with bacterial infection and they showed that both groups had an increased plasma chitotriosidase activity regardless of infectious etiology.[20] That has been confirmed in another study conducted by Chiarla et al.[8] Similar to these consequences, in the present study, ChT was found the most sensitive and specific biomarkers to detect SICAI in febrile neutropenic patients.

Neutrophil gelatinase–associated lipocalin is a protein, which is synthesized by many types of cells, including neutrophils, kidney tubule, liver, etc.[11,21] In the present study, we tested whether or not NGAL has the potential biomarker to detect SICAI in neutropenic patients. In the present study, there was a statistically significant elevation of serum ngal level in febrile neutropenic patients. However, it had the lowest sensitivity and specificity to identify SICAI compared to other biomarker. Consistent with the present study, a prospective study which was carried out in Seoul by Kim et al., found that serum NGAL level is a valuable biomarker to assess the inflammation.[22] Similar result was also reported by Saleh et al.[23]

Another important result in this study is that there is a positive and significant correlation between CRP, ChT and NGAL. The study, which was conducted by Suchojad et al. showed also a positive correlation between serum NGAL and CRP in 57 preterm infants admitted to the Neonatal Intensive Care Unit.[24] Similar observation was reported by Basok et al. They found a positive and significant correlation between CRP and ChT in 27 patients with rheumatoid arthritis.[25]

The most strength of the present study is that it was managed prospectively with a control group. However, the most obvious limitations of its are a relatively small sample size and participants which belonged to a single center.

Conclusion

Serum ChT level is the most sensitive and specific biomarker to SICAI in febrile neutropenic patients. Therefore, in the future, it may be used instead of or in combination with CRP to discriminate critically ill patients, to prevent unnecessary hospitalization and antibiotic treatment in neutropenia.

Disclosures

Ethics Committee Approval: The Ethics Committee of Gülhane Training and Research Hospital provided the ethics committee approval for this study (09/1539).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – O.K., O.G., M.E.T., A.E.K.; Design – O.K., O.G.; Supervision – O.K., O.G., M.E.T., A.E.K.; Data collection and/or processing – O.K.; Data Analysis and/or interpretation – O.K., O.G., I.E., M.E.T., S.T., A.E.K.; Writing – O.K.; Critical review – O.K., O.G., M.E.T., A.E.K.

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