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Gamma-glutamyl transpeptidase–platelet ratio, systemic immune inflammation index, and system inflammation response index in invasive *Aspergillosis*

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SUMMARY

OBJECTIVE: Gamma-glutamyl transpeptidase-platelet ratio, system inflammation response index, and systemic immune inflammation index are three systemic immune and inflammation indexes that were investigated for their diagnostic and prognostic proficiencies in cardiovascular diseases and cancers. However, their predictive values for invasive aspergillosis have not yet been studied. The aim of this study was to evaluate Gamma-glutamyl transpeptidase-platelet ratio, system inflammation response index, and systemic immune inflammation index levels and their diagnostic values in invasive aspergillosis.

METHODS: A total of 23 patients with invasive aspergillosis and 23 sex- and age-matched healthy participants were included in this study. Complete blood count parameters and liver function tests were studied. Gamma-glutamyl transpeptidase-platelet ratio, system inflammation response index, and systemic immune inflammation index were calculated.

RESULTS: Leukocyte, neutrophil, lymphocyte, and monocyte levels were statistically significantly higher in IA group (p=0.031, p=0.027, p=0.033, and p=0.001, respectively). In invasive aspergillosis group, platelets were numerically lower; Aspartate transaminase, alanine aminotransferase, and lactic dehydrogenase levels were numerically higher than those in control group but differences between levels were not statistically significant (p>0.05). The γ-glutamyl transpeptidase levels of patients were statistically significantly higher (p=0.007), and in addition, statistically significant differences were found between groups in terms of gamma-glutamyl transpeptidase-platelet ratio, system inflammation response index, and systemic immune inflammation index (p<0.001, p=0.037, p=0.001, respectively). Receiver operating characteristic analysis was performed, and areas under the curves were evaluated. gamma-glutamyl transpeptidase-platelet ratio had the higher area under the curve than systemic immune inflammation index and system inflammation response index (AUC 0.849, 0.798, 0.693, respectively). The results from receiver operating characteristic analysis of the data suggested that the use of a cutoff value of 0.15 for gamma-glutamyl transpeptidase-platelet ratio would be optimum for clinical use to confirm independent predictors of patients with invasive aspergillosis.

CONCLUSIONS: Gamma-glutamyl transpeptidase-platelet ratio is an independent, a useful predictor, and is superior to other evaluated markers in the diagnosis of inflammation in invasive aspergillosis. Gamma-glutamyl transpeptidase-platelet ratio may also be a helpful biomarker for clinicians to follow-up the inflammatory process of these patients.

KEYWORDS: Aspergillosis. Gamma-glutamyl transpeptidase. Platelet. Inflammation.

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INTRODUCTION

Aspergillus species are widely found in soil and rotting plants as saprophytic fungus. In parallel with the increase in susceptible patient groups, there is an increase in *Aspergillus* infections globally. The clinical picture of these infections varies according to the underlying diseases and facilitating factors. For example, invasive aspergillosis (IA) has a high mortality rate in neutropenic patients, whereas fungus balls (aspergilloma) are seen in the presence of cavitary sequelae after diseases such as pulmonary tuberculosis or sarcoidosis. Especially, *Aspergillus fumigatus* is a major cause of IA, severe disseminated fungal infection, and causes high morbidity and mortality in patients with immunodeficiency[1]. IA can be seen not only in patients with long-term neutropenia, allogeneic hematopoietic cell, or solid organ transplantation but also in patients using high doses of corticosteroids or immunosuppressive drugs and in patients with severe genetic immunodeficiency[2].

Platelet-mediated inflammation has been demonstrated in various acute and chronic infections, and it has been stated that platelets contribute to antimicrobial defense mechanisms. In *in vitro* studies, it is stated that platelets may have a role in the immune response against *Aspergillus*[3]. In a recent study by Deshmukh et al., it is suggested that fungal galactosaminogalactans initiate the interaction between complement and platelets, and this situation contributes to excessive inflammation, thrombocytopenia, and thrombosis[4].

Aspartate transaminase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT) are transferase enzymes with high clinical relevance, and they are primary biomarkers of liver functions[5]. GGT-platelet (PLT) ratio (GPR) is an inflammatory marker used in the early determination of the prognosis of liver diseases[6]. Recently, two new inflammatory markers comprising platelet and leukocyte subgroups, the systemic immune inflammation index (SII) and the system inflammation response index (SIRI), have been associated with poor prognosis in various diseases and cancers[7,8]. However, there is no study in the literature regarding the diagnostic and prognostic value of these three markers in IA patients. This study aims to determine the diagnostic values of GPR, SII, and SIRI in IA patients for the first time in the literature.

METHODS

Study groups

The study group consisted of 23 patients who were treated for IA infections between June 2011 and May 2012 at the University Hospital. All patients had underlying medical histories including tuberculosis, sarcoidosis, acute lymphoblastic leukemia, lung cancer, colon cancer, breast cancer, and chronic renal failure with renal transplantation. The control group consisted of 23 age- and gender-matched participants who applied to our hospital outpatient clinics and whose laboratory test results were not in favor of infection and/or inflammation.

Laboratory analysis

Clinical samples of 23 patients diagnosed with IA or possible IA in the University hospitals in line with the criteria of the European Cancer Research and Treatment Organization Mycosis Study Group (EORTC-MSG)[9] were included. Complete blood count parameters such as leukocyte (WBC), platelet (PLT), neutrophil (N), lymphocyte (L), and monocyte (M) in both groups were analyzed using the Siemens ADVIA 2120i autoanalyzer, biochemical test parameters such as lactic dehydrogenase (LDH), aspartate transaminase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT) were analyzed using the Abbot Architect C8000 autoanalyzer, and all these results were evaluated retrospectively. GPR, SII (using the PLT×N/L formula), and SIRI (using the N×M/L formula) were calculated.

In studies for the identification of *Aspergillus* species, microscopic and macroscopic features of clinical samples were defined first[10,11]. Colonies growing on Sabouraud dextrose agar were inoculated into potato dextrose agar to increase their conidia and to produce pigments and then inoculated to Czapek–Dox agar for the identification of the species. Colony color, morphology, and growth characteristics at 25, 35, and 44°C were recorded for each growing species. Lactophenol cotton blue was prepared for microscopic examination, and conidia formation and its color, number of sterigmata, shape of vesicles, structure of conidiophores, and shapes of Hulle cells were examined. More detailed studies using the molecular methodology of DNA sequencing analysis confirmed this preliminary identification[12,13]. Sequencing of the Internal Transcribed Spacer (ITS)-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') regions flanking 5.8S ribosomal DNA were used as a reference method for all isolates studied in the determination of species. Subsequently, thermal environments for 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min 30 s at 72°C, followed by the final thermal environment for 10 min at 72°C were used. Purification of amplicons was done by using the MinElute PCR kit (QIAGEN, Germany). Sequencing of both chains with primer ITS-1 or ITS-4 and BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) were performed using the ABI Prism 3100 genetic analyzer (Applied Biosystems)[14]. The elongation of PCR products was sequenced, and the species were identified using the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST/)

database. The obtained isolates were matched with the species showing 99% sequence identity in the searched database.

This study was approved by the University Medical Sciences Research Ethics Committee (Resolution number: TSA-11-3244).

Statistical analysis

Data were analyzed using the Windows SPSS version 23.0 (SPSS Inc., USA) program. The Mann–Whitney U test was used to evaluate the differences between groups, and the results were shown as median and interquartile range (25–75%). The receiver operating characteristic (ROC) curves were drawn to show the diagnostic performance of inflammation markers in IA, and the best cutoff point was determined. Values of $p<0.05$ were considered statistically significant.

RESULTS

The demographic data and laboratory results of 9 female and 14 male patients included in the IA group and 9 female and 14 male participants included in the control group are presented in Table 1.

A. fumigatus in 12 samples, *Aspergillus flavus* in seven samples, *Aspergillus niger* in three samples, and *Aspergillus terreus* in 1 sample were isolated as a result of DNA sequence analysis of 23 samples including lung tissue, eye tissue, and bronchoalveolar lavage fluid. The mean ages of the patients and control group participants were 43±20 years and 42.71±17.98 years, respectively. There was no statistically significant difference in terms of age between both groups ($p>0.05$).

Table 1. Demographical data and laboratory findings of patients with invasive aspergillosis and control participants.

	IA group (n=22)	Control group (n=22)	p-valor
Age	43±20[b]	42.71±17.98[b]	p>0.05
Sex (female/male)	9/13	9/13	–
Underlying diseases			
Tuberculosis	1	–	–
Sarcoidosis	1	–	–
Acute lymphoblastic leukemia	4	–	–
Lung cancer	10	–	–
Colon cancer	2	–	–
Breast cancer	1	–	–
Renal transplantation	3	–	–
Fungal infection sites			
Lung	20	–	–
Eye	2	–	–
WBC	11.74 (8.14–16.20)	6.70 (5.44–9.31)	p=0.031[a]
Neutrophil (%)	64.70 (55.80–83.70)	58.30 (52.40–60.70)	p=0.027[a]
Lymphocyte (%)	19.50 (10.30–32.30)	31.70 (29.50–36.90)	p=0.033[a]
Monocyte (%)	5.80 (4.70–6.70)	7.90 (6.50–8.40)	p=0.001[a]
Platelet	256 (180–410)	270 (215–291)	p=0.303
SII	1104.91 (521.39–2315.15)	477.76 (321.54–581.92)	p=0.001[a]
SIRI	19.14 (10.72–67.50)	13.29 (10.32–16.53)	p=0.037[a]
LDH	357.23 (246.0–414.62)	278.0 (204.50–333.50)	p=0.097
AST	25 (17–36)	19 (13.75–31.25)	p=0.191
ALT	22 (16.25–44.82)	17.50 (13.50–40.0)	p=0.364
GGT	79.0 (32.0–91.64)	32.50 (19.0–46.75)	p=0.007[a]
GPR	0.25 (0.12–0.63)	0.08 (0.06–0.14)	p<0.001[a]

WBC: leukocyte; LDH: lactic dehydrogenase; AST: aspartate transaminase; ALT: alanine aminotransferase; GGT: γ-glutamyl transpeptidase; SII: systemic immune inflammation index; SIRI: system inflammatory response index; GPR: γ-glutamyl transpeptidase-platelet ratio. The Mann–Whitney U test was used; data were summarized as median and interquartile range (25–75%). [a]Statistically significant, $p<0.05$. [b]Data were summarized as mean±SD.

Complete blood count parameters, such as WBC, neutrophil, lymphocyte, and monocyte levels, were evaluated in patients with IA and found to be statistically significantly higher than the values of the controls (p=0.031, p=0.027, p=0.033, and p=0.001, respectively). There was no statistically significant difference between the patients and the controls in terms of platelet values (p>0.05).

Among the biochemical parameters, AST, ALT, and LDH values were found to be numerically higher in the patient group, whereas no statistically significant differences were found between these three enzyme levels in both groups (p>0.05). However, GGT values were statistically significantly higher in the patient group compared with the control (p=0.007). SII, SIRI, and GPR indices, which were the inflammation markers calculated by their formulae, were also shown to be statistically significantly higher in the patient group compared with the controls (p=0.001, p=0.037, and p<0.001, respectively; (Table 1). The ROC analysis was performed, and the curves were drawn to determine the diagnostic performances of inflammatory markers in IA. Areas under the curves were compared (Table 2). It was stated that GPR values were statistically significantly higher than SII and SIRI values in determining IA patients. Then, the optimal cutoff point for GPR was determined using the maximum value of Youden's index (sensitivity+specificity–1). It was predicted that the values above 0.15 for GPR can be used in the diagnosis of IA with 70% of sensitivity and 85% of specificity (Table 2, Figure 1).

DISCUSSION

IA is an infectious disease that causes deterioration in liver functions. Liver dysfunction and liver fibrosis are common pathological processes in various liver diseases. Early detection of liver failure or fibrosis in these patients is an important factor in planning treatment and predicting prognosis. Also, the presence of significant liver fibrosis is a strong indication to start antiviral therapy[15]. The gold standard in liver fibrosis is liver biopsy, but it cannot be performed in every hospital because of its invasive procedure, sample errors, or complications. Therefore, the use of combinations of biochemical markers in the determination of liver fibrosis or liver function damage has become an important research subject in recent years[16]. In vitro studies state that platelets may play a role in the immune response against *Aspergillus*[3]. Therefore, by investigating the combination of two important parameters, such as GGT and PLT, in this study, it was thought that these two pathological conditions could be evaluated together and the diagnostic values could be compared in IA patients with inflammation and liver dysfunction. Wang et al. reported that the diagnostic performance of GPR is higher than ALT, AST, and GGT in patients with chronic hepatitis B and especially in the presence of HBeAg positivity. They also suggested that GPR could be a new and easy-to-use index for determining significant liver inflammation in chronic hepatitis B[17]. In this study, although the AST, ALT, and LDH levels of IA patients were statistically higher than the control group values, the differences were statistically insignificant, but GGT values of the IA patients were statistically significantly higher than the controls (p=0.007). In recent studies, it has been stated that the predictive values of SII and SIRI in cancers are higher than the N–L ratio (NLR) and P–L ratio (PLR)[18,19]. However, the relationship of these parameters with fungal infections such as IA has not yet been evaluated. Therefore, for the first time in the literature, GPR, SII, and SIRI levels in IA patients were evaluated in this study, and the diagnostic performances of these parameters in IA were compared. In addition, the diagnostic

Table 2. Receiver operating characteristic analysis of γ-glutamyl transpeptidase-platelet ratio, systemic immune inflammation index, and system inflammatory response index in patients with *Aspergillus* infection.

	Area under the curve	standard deviation error	p-valor	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
GPR	0.849	0.059	<0.001	0.732	0.965
SII	0.798	0.080	0.001	0.641	0.954
SIRI	0.693	0.089	0.037	0.518	0.867

GPR: γ-glutamyl transpeptidase-platelet ratio; SII: systemic immune inflammation index; SIRI: system inflammatory response index.

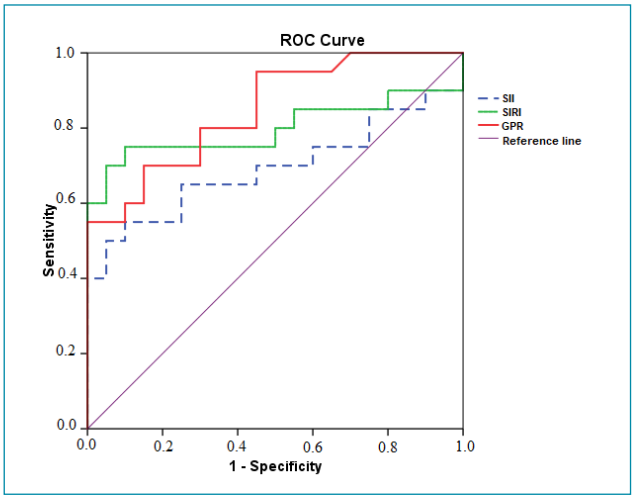

Figure 1. Receiver operating characteristic curves of GPR, SII, and SIRI in patients with *Aspergillus* infection.

performance of GPR in IA patients has been shown to be more significant and higher than ALT, AST, and GGT, in addition to SII and SIRI values (Table 2).

CONCLUSION

It is anticipated in this study that SII, SIRI, and especially GPR values, which are easy to achieve and easy to use in assessing the inflammation and liver dysfunction, may also be useful in evaluating inflammation and liver function in IA. Since this was a single-centered retrospective study with relatively small study groups, these results should be supported with further prospective studies with a larger number of participants.

AUTHORS' CONTRIBUTIONS

NC: Data Curation, Investigation, Methodology, Resources, Supervision, Validation, Writing – Review & Editing. **ANK:** Data Curation, Investigation, Methodology, Resources, Supervision, Validation, Writing – Review & Editing.

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