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Araştırma Makalesi / *Research Article*

Investigation of Levels of Periostin, HIF-1α and Phospholipase A2 in COVID19 Patients

COVID-19 Hastalarında Periostin, HIF-1α ve Fosfolipaz A2 Düzeylerinin Araştırılması

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ABSTRACT

Aim: We aimed to reveal the potential roles of periostin, hypoxia-inducible factor-1α (HIF-1α), and phospholipase A2 (PLA2) in inflammation, hypoxia, and tissue damage in patients with coronavirus disease 2019 (COVID-19) and to investigate whether they are useful potential markers in the follow-up and evaluation of disease severity.

Materials and Methods: Serum levels of periostin, HIF-1α, and PLA2 were measured in 32 patients with COVID-19 on days 1, 3, and 7 of hospitalisation. Additionally, samples from 30 healthy individuals were analysed for comparison.

Results: When biomarker levels of the patient and control groups were compared, a statistically significant difference was observed only in periostin levels ($p = 0.023$). Periostin levels were statistically significantly higher on days 3 and 7 compared with the first day of hospitalisation in patients with COVID-19 ($p = 0.018$ and $p = 0.021$, respectively).

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Conclusion: Our study showed that periostin protein is an important biomarker in COVID-19. To the best of our knowledge, our study is one of the first to include the simultaneous analysis of periostin, HIF-1α, and PLA2 in patients with COVID-19. We believe that our study, which provides comprehensive data, is important for the evaluation of the course and severity of COVID-19 infection.

Keywords: Coronavirus disease, Periostin, Hypoxia-inducible factor-1 alpha, Phospholipase A2

ÖZET

Amaç: Bu çalışmada, 2019 Koronavirüs Hastalığı (COVID-19) olan hastalarda periostin, hipoksiyle indüklenen faktör-1 alfa (HIF-1α) ve fosfolipaz A2 (PLA2)'nin inflamasyon, hipoksi ve doku hasarındaki potansiyel rollerini ortaya koymayı ve bu parametrelerin hastalık şiddetinin takibi ve değerlendirilmesinde yararlı ve potansiyel belirteçler olup olmadığını araştırmayı amaçladık.

Gereç ve Yöntem: Otuz iki COVID-19 hastasında, hastaneye yatışın 1., 3. ve 7. günlerinde serum periostin, HIF-1α ve PLA2 düzeyleri ölçüldü. Ayrıca, karşılaştırma amacıyla 30 sağlıklı bireyden alınan örnekler analiz edildi.

Bulgular: Hasta ve kontrol gruplarının biyobelirteç düzeyleri karşılaştırıldığında, yalnızca periostin düzeylerinde istatistiksel olarak anlamlı bir fark gözlendi ($p = 0,023$). Periostin düzeyleri, COVID-19 hastalarında ilk hastaneye yatış gününe kıyasla 3. ve 7. günlerde istatistiksel olarak anlamlı derecede daha yüksekti (sırasıyla $p = 0,018$ ve $p = 0,021$).

Sonuç: Çalışmamız, periostin proteininin COVID-19 hastalığında önemli bir biyobelirteç olduğunu göstermiştir. Bildiğimiz kadarıyla bu çalışma, COVID-19 hastalarında periostin, HIF-1α ve PLA2'nin eş zamanlı analizini içeren ilk çalışmalardan biridir. Kapsamlı veriler sunan çalışmamızın, COVID-19 enfeksiyonunun seyri ve şiddetinin değerlendirilmesine katkı sağlayacağını düşünüyoruz.

Anahtar Sözcükler: Koronavirüs hastalığı, Periostin, Hipoksiyle indüklenen faktör-1 alfa, Fosfolipaz A2

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a pandemic-causing infection caused by the SARS-CoV-2 virus. COVID-19 has a broad clinical spectrum, ranging from asymptomatic infection to severe acute respiratory distress syndrome (ARDS) and multiple organ failure. The immune system, hypoxia and inflammation play a role in the pathophysiology of the disease (1).

Tissue damage caused by the excessive inflammatory response that occurs in COVID-19 is an important determinant of disease severity and mortality (2). Therefore, better elucidation of inflammatory processes and hypoxia mechanisms is crucial in understanding the pathophysiology of the disease and developing treatment strategies.

Periostin is a matrix glycoprotein released in response to cellular stress and inflammation and has been suggested to play an important role in inflammatory diseases and fibrotic processes (3). Considering the intensity of inflammatory processes in COVID-19 patients, it has been suggested that periostin levels may be associated with disease severity (4). In respiratory diseases, periostin stands out as a biomarker that generally reflects inflammation and tissue remodelling processes (5). Considering that COVID-19 may lead to similar inflammatory and fibrotic processes in lung tissue, the potential role of periostin in disease severity is worth investigating.

Hypoxia Inducible Factor-1 alpha (HIF-1α) is an oxygen-sensitive transcription factor that is induced under hypoxic conditions (6,7). Angiotensin converting enzyme 2 (ACE2) expression, which facilitates the entry of SARS-CoV-2 into cells, is reported to be regulated by HIF-1α. Pulmonary damage and hypoxia processes associated with COVID-19 are associated with overexpression of HIF-1α (7).

Phospholipase A2 (PLA2) initiates the production of proinflammatory eicosanoids by catalysing the release of arachidonic acid

from membrane phospholipids and plays a role in various inflammatory diseases (8). It has been reported that PLA2 may affect lung function indirectly by producing lipid mediators or directly by changing the lipid composition of cell membranes or pulmonary surfactant. Increased PLA2 expression has been observed in bronchoalveolar lavage fluid and plasma of ARDS patients, and it has been suggested that PLA2 plays a role as a potential biomarker of ARDS (9). In addition, PLA2 level was found to be significantly higher in the plasma of COVID-19 patients and correlated with the severity of the disease (8,10).

Our study aimed to investigate whether serum levels of periostin, HIF-1α and PLA2 parameters in COVID-19 patients are useful and potential markers in the follow-up and evaluation of the severity of COVID-19 disease.

MATERIALS AND METHODS

Our study was conducted between 20.11. 2021 and 20.09.2022 at the Departments of Emergency Medicine, Intensive Care Unit and Medical Biochemistry, Research Laboratory. Our study was supported by Ege University Scientific Research Projects Coordinatorship with the budget transferred to the project code TGA-2021-23420. Approval for the study was obtained from Ege University Medical Research Ethics Committee dated 23.09.2021 and decision number 21-9.1T/20.

Identification of patients to be included in the study

Thirty-two patients over the age of 18 who presented to the emergency department and were hospitalised with a diagnosis of COVID-19 and who approved the Informed Consent Form were included in the study. Patients who refused treatment, had additional foci of infection, malignancy and acquired or congenital immunodeficiency were excluded. Thirty healthy volunteers were compatible with the average age of the patient group, and who approved the Informed Consent Form were included in the control group.

Collection of Samples

Blood samples of patients diagnosed with COVID-19 and hospitalised were taken by the emergency department and hospitalisation unit nurses on the first day of diagnosis and on the 3rd and 7th days of follow-up and transferred to the Department of Medical Biochemistry. In total, blood samples were taken from 32 patients on the first day of diagnosis, 17 patients on the 3rd day of hospitalisation and 9 patients on the 7th day of hospitalisation. Blood samples were centrifuged at 4000 rpm and +4°C and serum samples were stored at -80°C until analysis. The same procedures were performed for healthy control samples.

Biochemical Analysis

Periostin, HIF-1α and PLA2 biomarker levels in serum samples were analysed spectrophotometrically by sandwich enzyme-linked immunosorbent assay (ELISA) method using FineTest brand ELISA kit (catalogue numbers: EH0255, EH0551, EH3302; Fine Biological Technology Co. Ltd, Wuhan, Hubei, China).

The percentage coefficients of variation (%CV) for the intra-assay variability of the periostin ELISA kit were 4.93, 5.22, and 4.89 for low, medium, and high concentrations, respectively. The inter-assay %CV values were 4.82, 4.68, and 5.12, respectively. For the HIF-1α ELISA kit, the intra-assay %CV values were 5.91, 5.26, and 4.68, while the inter-assay %CV values were 5.22, 5.36, and 4.83 for low, medium, and high concentrations, respectively. For the PLA2 ELISA kit, the intra-assay %CV values were 4.68, 5.22, and 5.36, and the inter-assay %CV values were 4.83, 5.02, and 5.08 for low, medium, and high concentrations, respectively.

In addition, White Blood Cell (WBC), C-Reactive Protein (CRP), D-Dimer, ferritin and procalcitonin values, which are routinely

checked in COVID-19 patients, were obtained from the laboratory information management system. In our laboratory, the WBC test is performed on Sysmex XN3100 (Sysmex Corporation, Kobe, Japan), D-Dimer and fibrinogen on Sysmex CN6000 (Sysmex Corporation, Kobe, Japan), CRP on Roche cobas 702 (Roche Diagnostics GmbH, Mannheim, Germany), ferritin and procalcitonin tests on Roche cobas e801 (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Statistical Package of Social Science (SPSS) Version 20.0 (SPSS, Inc., Chicago, IL, USA) was used to analyse the data. The conformity of the data to the normal distribution was analysed by the Shapiro-Wilk test. Data were expressed as mean ± standard deviation or median (25th-75th percentile). Mann-Whitney U test and Independent samples T test were used to compare the biomarkers of the control-patient group and discharged-died patients. The Friedman test was used to compare biomarkers on the day of diagnosis, days 3 and 7. Paired samples T test and Wilcoxon signed-rank test were used for pairwise comparisons. The relationship between disease severity and biomarkers was analysed by the Spearman correlation test. Statistical significance level was accepted as $p<0.05$.

RESULTS

The mean age of the patients was 70 ± 15 (min 36-max 102) years. Among the 32 COVID-19 patients, 19 were male and 13 were female. During the follow-up, 16 patients were discharged, while 16 patients died. The patient group had WBC levels of 10.51 ± 6.28 x103/μL, CRP levels of 139 ± 110 mg/L, D-Dimer 4360 ± 7129 μg/L, ferritin 878 ± 1312 ng/mL, fibrinogen 472 ± 157 mg/dL, and procalcitonin levels of 3.05 ± 6.64 ng/mL. When the biomarker levels of the patient and control groups were compared, a statistically significant difference was observed only in periostin levels ($p=0.023$) (Table 1).

Periostin levels were statistically significantly higher on days 3 and 7 compared to the first hospitalisation day of COVID-19 patients ($p=0.018$, $p=0.021$, respectively). Although mean HIF-1α and PLA2 levels tended to increase on day 7 compared to day 1, these changes were not statistically significant, and no significant differences were observed between patients and controls for either parameter (Table 2).

With increasing inflammation, a twofold increase in mean periostin levels was observed on day 3 and an approximately fourfold increase on day 7 compared with the first day of hospitalisation. A slight increase in HIF-1α was noted on day 3 compared with day 1 ($p = 0.044$), and on day 7, a twofold and 1.5-fold increase in HIF-1α and PLA2 levels, respectively, was observed compared with day 1. However, no consistent statistically significant differences were found among the follow-up days (Figure 1).

No statistically significant correlation was found between periostin, HIF-1α and PLA2 biomarkers and the number of hospitalisations, mechanical ventilation and intensive care unit days. There was a weak correlation between the number of days of mechanical ventilation and WBC and ferritin levels ($r=0.357$, $p=0.045$; $r=0.417$, $p=0.034$, respectively) and a moderate correlation between procalcitonin ($r=0.562$, $p=0.008$). A weak correlation was observed between the number of intensive care unit days and procalcitonin levels ($r=0.456$, $p=0.038$) (Table 3).

When biomarker levels were compared according to prognosis, a statistically significant difference was observed only in ferritin levels between the died and discharged patients ($p=0.037$) (Table 4).

Table 1. Comparison of periostin, HIF-1α and PLA2 biomarkers between groups
Tablo 1. Gruplar arası periostin, HIF-1α ve PLA2 biyobelirteçlerinin karşılaştırılması

Parameter	Control group N= 30	Patient group N= 32	p value
Periostin (pg/mL)	12.01 (8.18-15.65)	8.95 (1.28-13.20)	**0.023**[a]
HIF-1α (pg/mL)	1.15 ± 0.77	1.31 ± 0.76	0.408[b]
PLA2 (pg/mL)	11.90 ± 9.33	16.81 ± 17.76	0.183[b]

[a]: Mann Whitney U test, [b]: Independent samples T test

Table 2. Changes in periostin, HIF-1α and PLA2 biomarkers over time in patient group
Tablo 2. Hasta grubunda periostin, HIF-1α ve PLA2 biyobelirteçlerinin zaman içindeki değişimi

Parameter	Patient group						
	First day N= 32	3rd day N= 17	7th day N= 9	Friedman test p value	First-3rd day p value	First-7th day p value	3rd-7th day p value
Periostin (pg/mL)	10.46 ± 12.12	23.59 ± 22.55	15.74 (12.19-57.51)	**0.032**	**0.018**[a]	**0.021**[b]	0.110[b]
HIF-1α (pg/mL)	1.31 ± 0.76	1.29 (1.04-2.68)	1.42 (1.02-4.08)	0.459	**0.044**[b]	0.515[b]	0.108[b]
PLA2 (pg/mL)	16.81 ± 17.76	3.96 (1.42-21.41)	24.93 ± 36.56	0.169	0.831[b]	0.392[a]	0.139[b]

[a]: Paired samples T test, [b]: Wilcoxon signed ranks test

Table 3. Relationship between biomarkers and number of hospitalisations, mechanical ventilation and intensive care days in patient group
Tablo 3. Hasta grubunda biyobelirteçler ile hastaneye yatış sayısı, mekanik ventilasyon ve yoğun bakım günü sayısı arasındaki ilişki

Parameter	Number of hospitalisation days		Number of mechanical ventilation days		Number of intensive care days	
	r	p	r	p	r	p
Periostin (First day)	-0.177	0.333	-0.283	0.117	-0.333	0.062
HIF-1α (First day)	0.063	0.733	-0.062	0.735	0.064	0.728
PLA2 (First day)	-0.295	0.101	-0.244	0.178	-0.264	0.144
Periostin (3rd day)	-0.262	0.311	0.005	0.984	0.130	0.619
HIF-1α (3rd day)	-0.424	0.090	0.063	0.809	-0.006	0.981
PLA2 (3rd day)	-0.203	0.434	0.143	0.584	0.043	0.870
Periostin (7th day)	-0.450	0.224	-0.252	0.512	-0.267	0.488
HIF-1α (7th day)	-0.467	0.205	-0.383	0.309	-0.333	0.381
PLA2 (7th day)	-0.433	0.244	0.157	0.687	-0.217	0.576
WBC (First day)	0.041	0.822	**0.357***	**0.045**	0.231	0.204
CRP (First day)	0.155	0.396	0.322	0.072	0.090	0.625
D-Dimer (First day)	0.052	0.780	0.157	0.399	0.085	0.650
Ferritin (First day)	0.000	1.000	**0.417***	**0.034**	0.355	0.075
Fibrinogen (First day)	0.358	0.073	0.065	0.753	0.207	0.309
Procalcitonin (First day)	0.097	0.675	**0.562****	**0.008**	**0.456***	**0.038**

Table 4. Comparison of biomarker levels according to prognosis
Tablo 4. Prognoza göre biyobelirteç düzeylerinin karşılaştırılması

Parameter	Discharged patients N= 16	Died patients N= 16	p value
Periostin (First day)	13.54 ± 15.28	7.38 ± 7.08	0.154[a]
HIF-1α (First day)	1.40 ± 1.02	1.21 ± 0.37	0.476[a]
PLA2 (First day)	22.36 ± 23.03	11.25 ± 7.44	0.083[a]
Periostin (3rd day)	22.70 ± 28.32	24.21 ± 19.18	0.897[a]
HIF-1α (3rd day)	1.21 (0.92-1.32)	1.76 (1.05-3.54)	0.329[b]
PLA2 (3rd day)	1.76 (1.44-6.64)	5.48 (1.40-27.44)	0.525[a]
Periostin (7th day)	18.71 (14.73-75.43)	14.93 (8.06-78.54)	0.462[b]
HIF-1α (7th day)	1.48 (1.25-5.35)	1.17 (0.73-5.22)	0.327[b]
PLA2 (7th day)	23.99 ± 35.74	25.68 ± 41.39	0.950[a]
WBC (First day)	8.36 ± 4.37	12.66 ± 7.25	0.051[a]
CRP (First day)	121.59 ± 122.97	156.40 ± 96.13	0.380[a]
D-Dimer (First day)	2757 ± 2891	6069 ± 9689	0.221[a]
Ferritin (First day)	317 ± 254	1289 ± 1613	**0.037[a]**
Fibrinogen (First day)	492 ± 163	458 ± 157	0.591[a]
Procalcitonin (First day)	0.19 ± 0.10	4.48 ± 7.81	0.061[a]

[a]: Independent samples T test, [b]: Mann Whitney U test

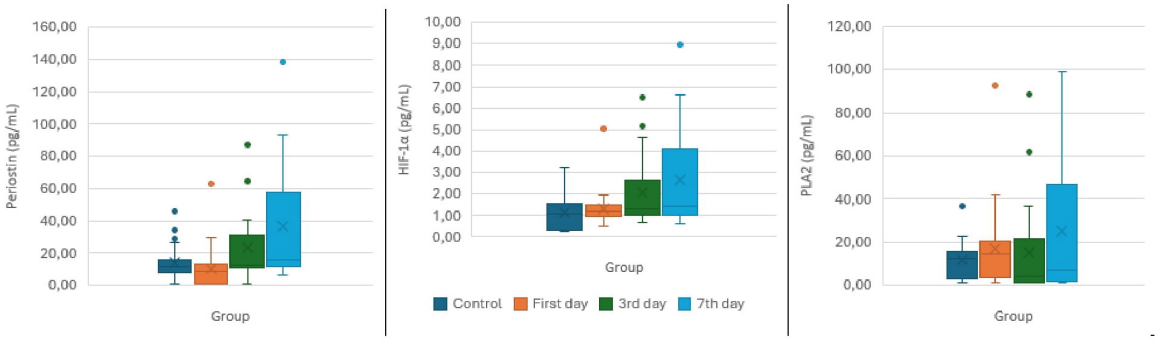

Figure 1. Comparison of periostin, HIF-1α and PLA2 levels
Şekil 1. Periostin, HIF-1α ve PLA2 düzeylerinin karşılaştırılması

DISCUSSION

In our study, periostin, HIF-1α and PLA2 serum levels were evaluated in COVID-19 patients, and a significant difference was observed only in periostin levels between the patient and control groups, and a significant increase was found on days 3 and 7 compared to the first hospitalisation day. A slight increase in HIF-1α was noted on day 3 compared to day 1, and on day 7, a 2- and 1.5-fold increase in HIF-1α and PLA2 parameters, respectively, was observed compared to day 1. However, no consistent statistically significant difference was found between follow-up days.

Periostin is a protein that plays a role in inflammation, fibrosis and tissue repair. It is a part of the extracellular matrix and is reported to be observed at high levels in tissues undergoing fibrotic changes (3). In our study, a statistically significant increase in periostin levels of COVID-19 patients was observed 2-fold on day 3 and approximately 4-fold on day 7 compared to the first

hospitalisation day. The increase in periostin levels in COVID-19 patients supports the role of this parameter in the inflammation process. Cabalak et al. reported that periostin levels were significantly higher in both mild/moderate and severe COVID-19 patients compared to the control group and could be used as a biomarker (4). In the study by Ali et al. investigating the relationship between disease severity and periostin levels in COVID patients, it was found that high serum periostin levels were associated with disease severity and post-COVID lung complications (11). In the study by Tuna et al., periostin levels were found to be significantly higher in COVID-19 patients compared to the control group, and it was suggested that elevated periostin levels observed in the early period may be useful in predicting the development of macrophage activation syndrome (12). In addition to COVID-19, periostin has been shown to play important roles in various non-neoplastic diseases. Yang et al. reviewed the multiple roles of periostin in conditions such as brain injury, allergic diseases, dental diseases, cardiovascular diseases, lung diseases, liver diseases, chronic kidney diseases, inflammatory bowel disease, and osteoarthrosis (13). These findings suggest that periostin levels may be influenced by comorbidities, and therefore, caution should be exercised when interpreting its potential role as a biomarker in COVID-19 patients.

It has been reported that HIF-1α and transcriptionally regulated genes are expressed in the lung cells of COVID-19 patients and may be an important marker of COVID-19-related hypoxia and lung injury (7). In COVID-19 with hypoxia and HIF-1 activity, it has been suggested that suppression of HIF-1 transcription or inhibition of its activity may be effective in reducing inflammation caused by viral infection (6). In a study conducted by Deveci et al., serum HIF-1α levels were found to be higher in the COVID-19 patient group compared to the healthy control group, and levels on the day of hospitalization were higher than those after hospitalization. It has been suggested that low HIF-1α levels in COVID-19 patients, especially in the first week of illness, may contribute to increased clinical severity (14). HIF-1α upregulation observed in a study investigating HIF-1α expression in COVID patients suggests that HIF-1α can be used as a target in molecular therapy as a potential marker for COVID-19 severity (7).

In the study by Kuypers et al., it was suggested that PLA2 level was associated with COVID-19 severity in children and played a role in disease pathogenesis. It has been reported that PLA2 may be a useful biomarker to classify risk and guide patient management in children with acute COVID-19 (15). In the study by Snider et al., it was reported that PLA2 levels were significantly higher in the plasma of COVID-19 patients and correlated with the severity of the disease (10). In Urazov et al.'s study, PLA2 levels increased statistically significantly in patients who died and were transferred to the intensive care unit (as the severity of COVID-19 infection increased), and it was suggested that PLA2 could be considered as an early marker of worsening of COVID-19 infection (9). The results from these studies support the possible benefit of PLA2 inhibitors in the treatment of COVID-19.

An important negative finding of our study is that no statistically significant differences were observed in HIF-1α and PLA2 levels, either between COVID-19 patients and healthy controls or across the follow-up days. Although a trend toward increased values was noted on day 7, these changes did not reach statistical significance. Reporting such negative results is scientifically valuable, as it indicates that, within the limitations of our cohort, HIF-1α and PLA2 may not serve as reliable biomarkers for monitoring disease severity.

In addition, we observed a moderate correlation between procalcitonin levels and the number of mechanical ventilation days. This finding suggests that elevated

procalcitonin may reflect the severity of systemic inflammation and the presence of bacterial co-infections, which could contribute to prolonged respiratory support needs in COVID-19 patients. Previous studies have similarly reported that higher procalcitonin levels are associated with worse outcomes. For example, Lippi and Plebani showed that elevated procalcitonin is linked to increased risk of severe disease in COVID-19 patients (16). Hu et al. demonstrated that procalcitonin was an independent predictor of disease severity and prognosis of COVID-19 (17). Likewise, elevated procalcitonin levels have been reported to be associated with higher mortality in hospitalized COVID-19 patients (18). Our results are consistent with these findings and highlight the potential of procalcitonin as a prognostic biomarker, not only for identifying severe disease but also for predicting the duration of mechanical ventilation in critically ill patients.

To our knowledge, our study is one of the first to include the simultaneous analysis of periostin, HIF-1α, and PLA2 in COVID-19 patients, providing preliminary data on their potential roles. We think that our study, which provides comprehensive data by measuring these parameters together in COVID-19 patients, is important in terms of public health and public interest in the follow-up and evaluation of the severity of COVID-19 infection.

Limitations of our study include the relatively small number of patients and the further reduction in sample size at day 3 and day 7 follow-up due to discharge or death. This significantly reduces the statistical power of the analysis and may have caused the observed differences in HIF-1α and PLA2 levels to be statistically insignificant. Furthermore, the short follow-up period limits the generalizability of our findings. Therefore, our results should be interpreted with caution and validated in larger, prospective studies with longer follow-up periods. Another limitation is that routine laboratory tests, including WBC, CRP, D-dimer, fibrinogen, ferritin, and procalcitonin, were systematically requested from all patients only on admission (the first day). Because these tests were performed selectively on days 3 and 7, depending on the patient's clinical condition and treatment needs, statistical comparisons could not be made due to missing data. Another limitation of our study is the relatively high mean age of the patient population (70 ± 15 years). Therefore, the generalizability of our findings to younger COVID-19 patients is limited, and further studies involving broader age groups are warranted.

In conclusion, periostin levels were significantly increased in COVID-19 patients, suggesting that periostin may play a role in the inflammatory response associated with the disease. No significant changes were observed in HIF-1α and PLA2 levels, which may be related to the limited sample size and clinical heterogeneity of the study population. These findings provide preliminary insight into the potential involvement of periostin in COVID-19 pathophysiology.

Conflicts of interest: The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Trends in Ethanol Positivity by Age and Gender: A Laboratory-Based Study

Yaş ve Cinsiyete Göre Etanol Pozitifliğindeki Eğilim: Laboratuvar Tabanlı Bir Çalışma

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ABSTRACT

Aim: This study aimed to evaluate the distribution and trends of ethanol analysis results according to age, gender, and ethanol concentration levels over a four-year period.

Materials and Methods: In this retrospective study, a total of 11,684 ethanol analysis results performed between January 1, 2021, and December 31, 2024, at our hospital laboratory were extracted from the laboratory information system. Ethanol test results were categorized by age groups (≤19 years, 20–34 years, 35–50 years, and >50 years), gender, and ethanol concentration levels (<10 mg/dL, 10–50 mg/dL, and >50 mg/dL). Ethanol levels ≥10 mg/dL were considered positive.

Results: Ethanol positivity was detected in 1,355 of 10,868 male cases (12.5%) and 94 of 816 female cases (11.5%). The highest number of tests and the highest positivity rate (13.7%) were observed in the 20–34 age group, which accounted for 56.3% of all positive cases. Of the 1,449 ethanol-positive cases identified over the four-year period, 93.5% were male and 6.5% were female. The number of cases with ethanol concentrations <10 mg/dL, 10–50 mg/dL, and >50 mg/dL were 10,235 (87.6%), 365 (3.1%), and 1,084 (9.3%), respectively.

Conclusion: Ethanol positivity was most frequently observed among young adults aged 20–34 years. Over the four-year period, the ≤19, 35–50, and >50 age groups demonstrated a consistent decline in ethanol positivity rates.

Keywords: Ethanol, Alcohol screening, Laboratory analysis

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ÖZET

Amaç: Bu çalışmada, dört yıl boyunca analiz edilen etanol sonuçlarının yaş, cinsiyet ve etanol konsantrasyon düzeylerine göre dağılımlarını ve pozitiflik eğilimlerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Bu retrospektif çalışmada, 1 Ocak 2021 ile 31 Aralık 2024 tarihleri arasında hastanemiz laboratuvarında gerçekleştirilen toplam 11.684 etanol analiz sonucu laboratuvar bilgi sisteminden elde edildi. Etanol sonuçları yaş gruplarına (≤19 yıl, 20-34 yıl, 35-50 yıl ve >50 yıl), cinsiyete ve etanol konsantrasyon düzeylerine (<10 mg/dL, 10-50 mg/dL ve >50 mg/dL) göre kategorize edildi. ≥ 10 mg/dL etanol düzeyleri pozitif olarak kabul edildi.

Bulgular: 10.868 erkek vakanın 1.355'inde (%12,5) ve 816 kadın vakanın 94'ünde (%11,5) etanol pozitifliği saptandı. En fazla test sayısı ve en yüksek pozitiflik oranı (%13,7) tüm pozitif vakaların %56,3'ünü oluşturan 20-34 yaş grubunda görüldü. Dört yıllık süreçte saptanan 1.449 etanol pozitif vakanın %93,5'i erkek, %6,5'i kadındı. Etanol konsantrasyonu <10 mg/dL olan vakaların sayısı 10.235 (%87,6), 10-50 mg/dL arasında olan vakaların sayısı 365 (%3,1) ve >50 mg/dL olan vakaların sayısı ise 1.084 (%9,3) olarak bulundu.

Sonuç: Etanol pozitifliği en sık 20-34 yaş aralığındaki genç yetişkinlerde gözlendi. Dört yıllık süre boyunca, ≤19, 35-50 ve >50 yaş gruplarında etanol pozitifliği oranlarında istikrarlı bir düşüş görüldü.

Anahtar Kelimeler: Etanol, Alkol taraması, Laboratuvar analizi

INTRODUCTION

Alcohol poses significant public health risks due to its psychoactive properties, toxicity, and potential for addiction. According to the World Health Organization (WHO), alcohol consumption accounted for 4.7% of all global deaths in 2019, corresponding to approximately 2.6 million fatalities (1). Alcohol use is a risk factor for the development of cardiovascular diseases, diabetes, liver disorders, and cancers (2). Beyond its detrimental effects on the individual, alcohol consumption also contributes to a range of physical, psychological, and social harms, including violence, traffic accidents, family breakdown, reduced economic and emotional well-being of families, increased crime rates, and both direct and indirect economic burdens on societies (3).

Despite ongoing public health efforts, the health and social burden related to alcohol consumption remains unacceptably high worldwide (1). To develop effective preventive and protective strategies that reduce alcohol-related harm, reliable and objective data are essential (4). In this study, we aimed to retrospectively evaluate changes in laboratory ethanol test results over a four-year period according to age, gender, and different ethanol levels.

MATERIAL AND METHODS

Data Collection and Evaluation

In this retrospective study, all ethanol test results performed in the emergency laboratory of Mehmet Akif İnan Training and Research Hospital between January 2021 and December 2024 were retrieved from the hospital information system. No exclusion criteria were applied, and all available results were included in the analysis. The data were categorized by age group [children and adolescents (≤19 years), young adults (20–34 years), middle-aged adults (35–50 years), and older adults (>50 years)], gender, and ethanol concentration ranges (<10 mg/dL, 10–50 mg/dL, and >50 mg/dL). Ethanol levels ≥10 mg/dL were considered positive.

In Türkiye, the Road Traffic Act sets the maximum allowable blood alcohol concentration for private vehicle drivers at 50 mg/dL (5). Therefore, ethanol-positive samples were evaluated separately as 10–50 mg/dL and >50 mg/dL.

Ethanol levels were determined using an enzymatic method. From January 2021 to December 2021, analyses were performed using the Indiko Plus analyzer (Thermo Fisher Scientific, Vantaa, Finland), and from January 2022 to December 2024 using the Cobas c501 analyzer (Roche Diagnostics,

Mannheim, Germany), all operated with the respective reagent kits. According to the manufacturers' datasheets, the analytical measurement ranges were 10–600 mg/dL for the Indiko Plus analyzer and 10.1–498 mg/dL for the Cobas c501 analyzer.

In our laboratory, internal quality control was performed daily, and external quality assessment was conducted monthly through the Bio-Rad External Quality Assurance Services program.

Statistical Analysis

All analyses were performed using IBM SPSS Statistic software. Continuous variables were expressed as median [interquartile range], and categorical variables as number (percentage). Comparisons between categorical variables (ethanol positivity by gender) were performed using chi-square test. All statistical tests were two-tailed, with a significance level set at $p<0.05$.

Ethical Considerations

This study was approved by the Clinical Research Ethics Committee of Harran University (Decision Number: HRÜ/24.21.08, Date: 30.12.2024).

RESULTS

A total of 11,684 ethanol test results were included in the study, comprising 10,868 results from 10,295 male individuals and 816 results from 799 female individuals. The ≤19 years, 20–34 years, 35–50 years, and >50 years age groups accounted for 1,998, 5,947, 2,593, and 1,146 results, respectively. The median (IQR) ages for these groups were 18 (16–19), 26 (22–30), 41 (37–45), and 58 (54–64) years, respectively. The distribution of results by year was as follows: 2,007 in 2021; 2,116 in 2022; 3,897 in 2023; and 3,664 in 2024.

Table 1 and Figures 1 and 2 summarize the ethanol analysis results by year across different age and gender groups. Among the male cases, 1,355 (12.5%) tested positive for ethanol, whereas 94 (11.5%) of the female cases showed ethanol positivity. The highest number of ethanol tests was performed in the 20–34 age group, which also had the highest positivity rate at 13.7%.

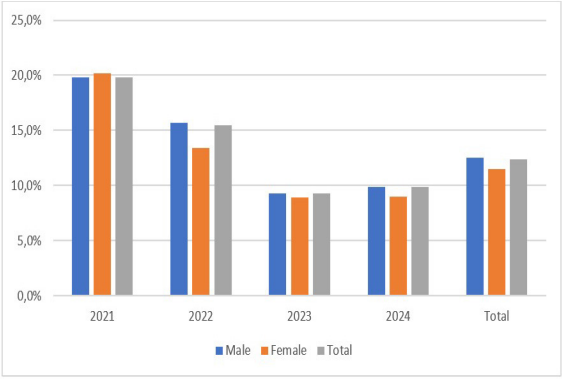

Figure 1. Change in Alcohol Positivity Rates by Gender Over The Years
Şekil 1. Cinsiyete Göre Alkol Pozitiflik Oranlarındaki Değişim

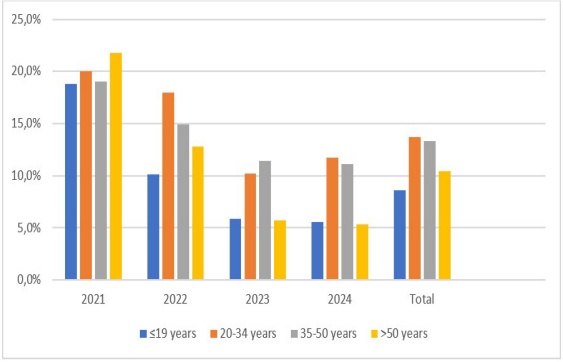

Figure 2. Change in Alcohol Positivity Rates by Age Groups Over The Years
Şekil 2. Yaş Gruplarına Göre Alkol Pozitiflik Oranlarındaki Değişim

Table 2 presents the distribution of ethanol levels across different concentration ranges by year. The number of cases with ethanol levels below 10 mg/dL, between 10 and 50 mg/dL, and above 50 mg/dL were 10,235 (87.6%), 365 (3.1%), and 1,084 (9.3%), respectively.

Table 3 shows ethanol positivity rates by gender across different age groups. In the 35–50 age group, ethanol positivity was significantly higher among males compared to females ($p<0.05$). In the other age groups (≤19, 20–34, and >50 years), no statistically significant gender differences were observed in ethanol positivity rates ($p>0.05$).

Table 1. Ethanol Analysis Results of Different Age and Gender Groups by Year
Tablo 1. Farklı Yaş ve Cinsiyet Gruplarının Yıllara Göre Etanol Analiz Sonuçları

	2021		2022		2023		2024		Total	
	Negative	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Age Groups, n (%)										
≤19 years	260 (81.2)	60 (18.8)	303 (89.9)	34 (10.1)	621 (94.1)	39 (5.9)	643 (94.4)	38 (5.6)	1827 (91.4)	171 (8.6)
20-34 years	789 (80)	197 (20)	891 (82)	196 (18)	1786 (89.8)	202 (10.2)	1666 (88.3)	220 (11.7)	5132 (86.3)	815 (13.7)
35-50 years	375 (81)	88 (19)	389 (85.1)	68 (14.9)	795 (88.6)	102 (11.4)	690 (88.9)	86 (11.1)	2249 (86.7)	344 (13.3)
>50 years	186 (78.2)	52 (21.8)	205 (87.2)	30 (12.8)	332 (94.3)	20 (5.7)	304 (94.7)	17 (5.3)	1027 (89.6)	119 (10.4)
Gender, n (%)										
Male	1515 (80.2)	373 (19.8)	1639 (84.3)	305 (15.7)	3309 (90.7)	341 (9.3)	3050 (90.1)	336 (9.9)	9513 (87.5)	1355 (12.5)
Female	95 (79.8)	24 (20.2)	149 (86.6)	23 (13.4)	225 (91.1)	22 (8.9)	253 (91)	25 (9)	722 (88.5)	94 (11.5)
Total	1610 (80.2)	397 (19.8)	1788 (84.5)	328 (15.5)	3534 (90.7)	363 (9.3)	3303 (90.1)	361 (9.9)	10235(87.6)	1449 (12.4)

Table 2. Distribution of Ethanol Analysis Results by Concentration Ranges and Year
Tablo 2. Konsantrasyon Aralıkları ve Yıla Göre Etanol Analiz Sonuçlarının Dağılımı

Ethanol Level	**2021 (n/%)**	**2022 (n/%)**	**2023 (n/%)**	**2024 (n/%)**	**Total (n/%)**
<10 mg/dL	1,610 (80.2%)	1,788 (84.5%)	3,534 (90.7%)	3,303 (90.1%)	10,235 (87.6%)
10–50 mg/dL	178 (8.9%)	48 (2.3%)	60 (1.5%)	79 (2.2%)	365 (3.1%)
>50 mg/dL	219 (10.9%)	280 (13.2%)	303 (7.8%)	282 (7.7%)	1,084 (9.3%)

Table 3. Ethanol Positivity by Gender Across Different Age Groups
Tablo 3. Farklı Yaş Gruplarında Cinsiyete Göre Etanol Pozitifliği

Age Group (years)	*Ethanol Level*	*Female, n (%)*	*Male, n (%)*	*p-value*
≤19 years	Negative	80 (88.9%)	1,747 (91.6%)	0.376
	Positive	10 (11.1%)	161 (8.4%)	
20–34 years	Negative	402 (86.1%)	4,730 (86.3%)	0.888
	Positive	65 (13.9%)	750 (13.7%)	
35–50 years	Negative	194 (92.4%)	2,055 (86.2%)	**0.012***
	Positive	16 (7.6%)	328 (13.8%)	
>50years	Negative	46 (93.9%)	981 (89.4%)	0.318
	Positive	3 (6.1%)	116 (10.6%)	

*Chi-square test was used, *indicates statistically significant difference.*

DISCUSSION

In 2019, the WHO European Region had the highest per capita alcohol consumption worldwide. However, recent reports have indicated a decline in per capita alcohol intake among adults in the European Region (1). A study evaluating alcohol consumption trends in Germany between 1995 and 2018 demonstrated a gradual decrease in prevalence among both males and females (6). Similarly, a study assessing alcohol consumption patterns in Spain from 1990 to 2019 reported reductions during specific periods (7).

According to data from the Turkish Statistical Institute, the past-year alcohol use prevalence among individuals aged 15 years and older in Türkiye was 12.1% in 2022 (18.4% in males and 5.9% in females), compared with 14.9% in 2019 (23.3% in males and 6.6% in females), indicating a decline in alcohol consumption over this period (8). In a survey conducted by İlhan et al. in the province of Şanlıurfa, the lifetime prevalence of alcohol use among the local population was 3.5%, while the past-year alcohol use prevalence was 1.9% (9).

Our study, based on the analysis of laboratory data, revealed that between 2021 and 2024, ethanol positivity rates ranged from 8.9% to 20.2% in females and from 9.3% to 19.8% in males, with overall positivity rates ranging from 9.3% to 19.8%. Over the four-year period, the overall ethanol positivity rate was 11.5% in females, 12.5% in males, and 12.4% in total. Notably, during the final two years of the study period, ethanol positivity rates declined to their lowest levels in both sexes and overall. In addition, 93.5% of all positive results were observed in male individuals, whereas only 6.5% occurred in females.

A similar study conducted by Gök et al., based on laboratory ethanol data from 2021 and 2022, reported positivity rates (≥10 mg/dL) of 15.0% in females, 18.82% in males, and 18.26% overall. In that study, 71.4% of the analyzed samples were obtained from males and 28.6% from females (10). Many countries implement public health policies aimed at minimizing alcohol-related harms, and strong alcohol control policies represent the first line of defense against such harms (1). The decrease in ethanol positivity observed in recent years in our study may therefore be associated with the implementation of these alcohol control policies.

Alcohol consumption is highly prevalent among adolescents, with rates varying considerably across countries. Using data from the Global School-based Student Health Survey (2003–2018), one study reported a prevalence of alcohol consumption of 25.2% among adolescents aged 11–16 years (11). In Thailand, 31.01% of adolescents aged 10–14 years were reported to have experienced alcohol use (12). Similarly, a study from

Ethiopia found that 29% of adolescents aged 13–19 years had consumed alcohol at least once (13).

In Türkiye, a 2012 survey conducted by Evren et al. reported that 34.2% of 10th-grade students had consumed alcohol at least once in their lifetime (females: 33.8%, males: 35.6%) (14). Likewise, a survey by Ünlü et al. conducted in 2010 found the lifetime prevalence of alcohol use among high school students to be 34.8% in males and 30.6% in females (15).

According to our study results, 8.6% of ethanol analyses in the ≤19 age group were positive, accounting for 11.8% of all ethanol-positive results. No statistically significant difference in ethanol positivity was observed between males and females in this age group ($p > 0.05$). Consistent with our findings, a study by Gök et al. based on laboratory data reported alcohol positivity rates of 6.12% in the 1–10 age group (females: 0%, males: 10%) and 17.12% in the 11–20 age group (females: 16.86%, males: 17.23%), with no significant gender differences observed in these age categories (10).

Among young adults, a study conducted in Türkiye between 2019 and 2020 among university students aged 18–35 years found that 34.7% reported never having consumed alcohol, while the lifetime prevalence of alcohol use was 65.3% (16). Similarly, a 2020 survey by Bahar et al. reported that 17.6% of university students were active alcohol users, whereas 69.1% reported never having consumed alcohol (17).

In line with these findings, our data showed that the highest number of ethanol analyses was performed in individuals aged 20–34 years, followed by those aged 35–50 years. These age groups also exhibited the highest ethanol positivity rates (13.7% and 13.3%, respectively). Specifically, 56.3% of all ethanol-positive cases occurred in individuals aged 20–34 years, while 23.7% were observed in the 35–50 age group, indicating that approximately 80% of all positive cases were among individuals aged 20–50 years.

When trends in ethanol positivity over the four-year study period were evaluated, the lowest rates were observed during the final two years, particularly among individuals aged 35–50 years, in whom a consistent annual decline was noted. No statistically significant gender difference was observed in ethanol positivity rates within the 20–34 age group ($p > 0.05$). However, in the 35–50 age group, ethanol positivity rates were significantly higher in males than in females ($p < 0.05$).

Similarly, in the study by Gök et al., the majority of ethanol tests were conducted in the 21–30 and 31–40 age groups, which also exhibited the highest positivity rates. The overall positivity rates in these groups were 19.33% (females: 20.66%, males: 18.79%) and 20.77% (females: 15.6%, males: 22.89%), respectively. While no statistically significant gender difference was reported in the 21–30 age group, males demonstrated significantly higher ethanol positivity rates than females in the 31–40 age group (10).

In our study, the overall ethanol positivity rate among older adults (>50 years) was 10.4% (females: 6.1%, males: 10.6%), and this age group accounted for 8.2% of all ethanol-positive cases. No statistically significant difference in ethanol positivity was observed between males and females in this age group ($p > 0.05$). The lowest positivity rate in this group was recorded in the final year of the study period, with a consistent annual decline observed.

According to the findings of Gök et al., ethanol positivity rates were 16.06% among individuals aged 51–60 years (females: 6.81%, males: 19.52%) and 12.06% among those aged over 60 years (females: 4.1%, males: 15.1%). In both older age groups, ethanol positivity rates were significantly higher in males compared to females (10).

One of the main limitations of our study is its single-center design, which restricts the

generalizability of the findings. Another limitation is that the dataset is result-based rather than person-based, which may partially introduce bias. An additional methodological limitation is the use of two different analyzers during the study period without evaluating potential differences in their analytical performance. Previous studies on measurement uncertainty (MU) in ethanol testing indicate that MU can vary between different analyzer brands due to factors such as calibrator uncertainty, reagent stability, and analytical repeatability (18–20). We did not assess the potential impact of MU on results near the defined threshold values. Ethanol levels ≥10 mg/dL were considered positive, and positive samples were evaluated separately as 10–50 mg/dL and >50 mg/dL. Accounting for MU, particularly for results close to 10 mg/dL and 50 mg/dL, would have provided a more robust assessment.

Nevertheless, the strengths of our study include its large sample size and the analysis of a continuous four-year dataset, which enabled the evaluation of temporal trends.

CONCLUSIONS

In Türkiye, many studies on alcohol use prevalence are based on self-reported survey data, which have inherent limitations in accurately assessing alcohol consumption. In this context, laboratory-based ethanol measurements obtained from biological samples may provide more objective and reliable insights (4). According to our findings, the highest number of ethanol analyses and the highest positivity rate were observed in the 20–34 age group. Overall, 93.5% of all ethanol-positive cases were male, while only 6.5% were female. A statistically significant gender difference in ethanol positivity was observed only in the 35–50 age group, with higher rates among males, whereas no significant gender differences were detected in other age groups. Over the four-year period, the ≤19, 35–50, and >50 age groups demonstrated a consistent decline in ethanol positivity rates, with the lowest rates recorded in the final two years of the study. This decline may be associated with alcohol control policies; however, further up-to-date and multi-center studies are required to elucidate the underlying causes.

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Conflict of Interest

The authors declare no conflicts of interest.

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Unnecessary Repeated Tests in The Biochemistry Laboratory

Biyokimya Labroratwarında Gereksiz Tekrarlanan Testler

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ABSTRACT

Aim: This study aims to evaluate repeated tests and unnecessary repeated laboratory tests based on minimum retest intervals.

Materials and Methods: Data regarding thyroid-stimulating hormone (TSH), ferritin, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) tests analysed in the İzmir Kemalpaşa State Hospital laboratory during 2023 were retrospectively obtained from the laboratory information system. Minimum retest intervals for these tests were determined according to the National Minimum Re-testing Intervals in Pathology guidelines developed by The Royal College of Pathologists, The Association for Clinical Biochemistry and Laboratory Medicine, and The Institute of Biomedical Science. Accordingly, the minimum retest intervals were defined as 28 days for TSH, 30 days for ferritin, 24 hours for CRP, and 7 days for ESR. Tests performed more than once per patient during the study period were categorized as repeated tests, whereas tests repeated within a period shorter than the minimum retest interval were classified as unnecessary repeated tests.

Results: The total numbers of TSH, ferritin, CRP, and ESR tests performed in the laboratory were 27,516, 19,836, 32,100, and 7,200, respectively. The number (percentage) of repeated tests was 1,402 (5.10%) for TSH, 1,726 (8.70%) for ferritin, 1,827 (5.69%) for CRP, and 1,020 (14.2%) for ESR. Among these repeated tests, the numbers (percentages) classified as unnecessary repeated tests were 148 (10.6%), 134 (7.76%), 118 (6.46%), and 107 (10.49%), respectively.

Conclusion: Unnecessary repeated laboratory tests contribute significantly to increased healthcare costs and workload inefficiencies. Increasing awareness among healthcare administrators and clinicians regarding the prevalence of unnecessary testing is critical for improving resource utilization and reducing avoidable expenditures.

Keywords: Unnecessary repeated tests, Retest interval, Laboratory costs.

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ÖZET

Amaç: Bu çalışmanın amacı, minimum yeniden test aralıklarına dayanarak "tekrarlanan testler" ve "gereksiz tekrarlanan laboratuvar testleri"ni değerlendirmektir.

Gereç ve Yöntem: 2023 yılı boyunca İzmir Kemalpaşa Devlet Hastanesi laboratuvarında çalışılan tiroid uyarıcı hormon (TSH), ferritin, C-reaktif protein (CRP) ve eritrosit sedimantasyon hızı (ESH) testlerine ait veriler laboratuvar bilgi yönetim sisteminden retrospektif olarak elde edildi. Bu testler için minimum yeniden test aralıkları; The Royal College of Pathologists, The Association for Clinical Biochemistry and Laboratory Medicine ve The Institute of Biomedical Science tarafından geliştirilen Ulusal Patolojide Minimum Yeniden Test Aralıkları kılavuzuna göre belirlendi. Buna göre minimum yeniden test aralıkları; TSH için 28 gün, ferritin için 30 gün, CRP için 24 saat ve ESH için 7 gün olarak kabul edildi. Çalışma süresi boyunca bir hastada birden fazla kez yapılan testler "tekrarlanan testler" olarak, minimum yeniden test aralığından daha kısa sürede tekrar edilen testler ise "gereksiz tekrarlanan testler" olarak sınıflandırıldı.

Bulgular: Laboratuvarda yapılan toplam TSH, ferritin, CRP ve ESH test sayıları sırasıyla 27.516, 19.836, 32.100 ve 7.200 olarak belirlendi. Tekrarlanan testlerin sayısı (yüzdesi) TSH için 1.402 (%5,10), ferritin için 1.726 (%8,70), CRP için 1.827 (%5,69) ve ESH için 1.020 (%14,2) idi. Bu tekrarlanan testler içerisinde gereksiz tekrarlanan test olarak sınıflandırılanların sayısı (yüzdesi) sırasıyla 148 (%10,6), 134 (%7,76), 118 (%6,46) ve 107 (%10,49) olarak hesaplandı.

Sonuç: Gereksiz tekrarlanan laboratuvar testleri, sağlık hizmetlerinde maliyet artışına ve iş yükü verimsizliğine önemli ölçüde katkıda bulunmaktadır. Gereksiz testlerin yaygınlığı konusunda sağlık yöneticileri ve klinisyenlerin farkındalığının artırılması, kaynak kullanımının iyileştirilmesi ve önlenebilir harcamaların azaltılması açısından kritik öneme sahiptir.

Anahtar kelimeler: Gereksiz tekrarlanan testler, Tekrar test aralığı, Laboratuvar maliyetleri

INTRODUCTION

Unnecessary repeat laboratory testing is a significant concern in modern healthcare, as it can lead to patient discomfort, increased healthcare costs, and potential diagnostic inaccuracies. Over-testing often results from a lack of adherence to established guidelines for re-testing intervals, leading to redundant data that may complicate clinical decision-making. Implementing standardized re-testing intervals, based on evidence-based guidelines, is crucial to reducing these redundant tests. This approach not only conserves resources but also enhances the accuracy of patient diagnoses and the overall quality of care. By minimizing unnecessary tests, the efficiency of healthcare systems can be increased, patient burden reduced, and a higher standard of clinical practice achieved (1-4).

Despite the widespread occurrence of laboratory test overuse, healthcare providers often remain ambivalent about its significance. While the overutilization of these tests is acknowledged as a serious concern in terms of patient safety and financial burden, it is frequently regarded as less critical compared to other areas of healthcare. Physicians recognize the importance of addressing this issue but note that time constraints in daily practice prevent them from exercising sufficient care when ordering tests (5, 6).

The "National Minimum Re-testing Intervals in Pathology" report, collaboratively produced by The Royal College of Pathologists, The Association for Clinical Biochemistry and Laboratory Medicine, and The Institute of Biomedical Science, serves as a comprehensive guideline for clinicians and laboratory professionals. It provides evidence-based recommendations on the minimum intervals required to avoid unnecessary repeat testing. This helps to optimize patient care by reducing the risk of over-testing, minimizing patient discomfort, and improving the efficiency of laboratory services. The report emphasizes the importance of adhering to these intervals to ensure accurate and clinically relevant results, ultimately contributing to more effective healthcare management (7).

One of the critical strategies to reduce excessive and unnecessary laboratory test utilization is for healthcare institutions to

identify and address unnecessary test requests. In this context, our study aims to evaluate "repeat testing" and "unnecessary repeat laboratory tests" in biochemistry laboratories based on the recommendations outlined in the "National Minimum Re-testing Intervals in Pathology" report.

MATERIALS AND METHODS

Data regarding thyroid stimulating hormone (TSH), ferritin, human C-reactive protein (CRP), and sedimentation tests analyzed in the İzmir Kemalpaşa State Hospital laboratory during 2023 were retrospectively obtained from the laboratory information system. Minimum retest intervals for these tests were determined according to the National Minimum Re-testing Intervals in Pathology guidelines, developed by The Royal College of Pathologists, The Association for Clinical Biochemistry and Laboratory Medicine, and The Institute of Biomedical Science. Accordingly, the minimum retest intervals were established as 28 days for TSH, 30 days for ferritin, 24 hours for CRP, and 7 days for sedimentation.

The selection of these four tests was based on their clearly defined minimum re-testing intervals and the low incidence of clinical scenarios that would justify earlier repeat testing. In addition, these tests represent distinct laboratory categories—hormonal (TSH), iron metabolism (ferritin), inflammatory (CRP), and general screening (erythrocyte sedimentation rate)—and are frequently used in routine clinical practice. This approach allowed a broader assessment of unnecessary repeat testing across different types of laboratory investigations.

Tests performed more than once per patient during the study period were categorized as "repeated tests," while tests repeated within a period shorter than the minimum retest interval were classified as "unnecessary repeated tests." This classification was based on clear, evidence-supported criteria rather than subjective clinical interpretation.

Descriptive statistics were used for data analysis, and no comparative statistical tests were performed, as the primary aim of the study was to determine the prevalence of repeated and unnecessary repeated laboratory tests rather than to compare test groups statistically.

Ethical approval for the study was obtained from the Harran University Faculty of Medicine Ethics Committee (HRÜ/24.17.07).

RESULTS

Total numbers of TSH, ferritin, CRP, and sedimentation tests performed in the laboratory were 27516, 19836, 32100, and 7200, respectively. The number (percentage) of repeated tests were 1402 (5.10%), 1726 (8.70%), 1827 (5.69%), and 1020 (14.2%) for TSH, ferritin, CRP, and sedimentation, respectively. From these repeated tests, the percentages classified as unnecessary repeated tests were calculated as 148 (10.6%), 134 (7.76%), 118 (6.46%), and 107 (10.49%), respectively (Table 1). The numbers and percentages of total, repeated, and unnecessary tests for TSH, ferritin, CRP, and sedimentation according to services were presented in Tables 2 – 5.

Table 1. The Numbers and Percentages of Total, Repeated, and Unnecessary Tests for TSH, Ferritin, CRP, and Sedimentation

Tablo 1. TSH, Ferritin, CRP ve Sedimentasyon için Toplam, Tekrarlanan ve Gereksiz Testlerin Sayıları ve Yüzdeleri

Tests	Number of Tests	Number of Repeated Tests (%)	Number of Unnecessary Tests (%)
TSH	27516	1402 (5.10)	148 (10.6)
Ferritin	19836	1726 (8.70)	134 (7.76)
CRP	32100	1827 (5.69)	118 (6.46)
Sedimentation	7200	1020 (14.2)	107 (10.49)

Table 2. The Numbers and Percentages of Total, Repeated, and Unnecessary Tests for TSH According to Services
Tablo 2. Servislere Göre TSH İçin Toplam, Tekrarlanan ve Gereksiz Testlerin Sayıları ve Yüzdeleri

Services	Total TSH Tests	Number of Repeated Tests (%)	Number of Unnecessary Tests (%)
Neurology	1100	78 (7.09)	13 (16.67)
Family Medicine	1650	135 (8.18)	15 (11.11)
Internal Medicine	17610	602 (3.42)	65 (10.80)
Obstetrics & Gynecology	3301	315 (9.54)	31 (9.84)
Cardiology	1128	81 (7.18)	8 (9.88)
General Surgery	1155	90 (7.79)	5 (5.56)
Other Clinics	1572	101 (6.42)	11 (10.89)

Table 3. The Numbers and Percentages of Total, Repeated, and Unnecessary Tests for Ferritin According to Services
Tablo 3. Servislere Göre Ferritin İçin Toplam, Tekrarlanan ve Gereksiz Testlerin Sayıları ve Yüzdeleri

Services	Total Ferritin Tests	Number of Repeated Tests (%)	Number of Unnecessary Tests (%)
Internal Medicine	12201	924 (7.57)	87 (9.42)
General Surgery	508	65 (12.8)	4 (6.15)
Obstetrics & Gynecology	4506	396 (8.79)	24 (6.06)
Cardiology	102	14 (13.73)	1 (7.14)
Family Medicine	1387	213 (15.36)	12 (5.63)
Neurology	901	95 (10.54)	5 (5.26)
Other Clinics	231	19 (8.23)	1 (5.26)

Table 4. The Numbers and Percentages of Total, Repeated, and Unnecessary Tests for CRP According to Services
Tablo 4. Servislere Göre CRP İçin Toplam, Tekrarlanan ve Gereksiz Testlerin Sayıları ve Yüzdeleri

Services	Total CRP Tests	Number of Repeated Tests (%)	Number of Unnecessary Tests (%)
Emergency	11306	46 (0.41)	6 (13.04)
Neurology	1001	115 (11.49)	9 (7.83)
Obstetrics & Gynecology	1031	157 (15.23)	11 (7.01)
Internal Medicine	14201	824 (5.80)	65 (7.89)
Urology	992	104 (10.48)	6,0 (5.77)
Family Medicine	1465	110 (7.51)	4 (3.64)
Chest Diseases	1723	412 (23.91)	10,0 (2.43)
Other Clinics	381	59 (15.49)	7 (11.86)

Table 5. The Numbers and Percentages of Total, Repeated, and Unnecessary Tests for Sedimentation According to Services
Tablo 5. Servislere Göre Sedimentasyon İçin Toplam, Tekrarlanan ve Gereksiz Testlerin Sayıları ve Yüzdeleri

Services	Number of Sedimentation Tests	Number of Repeated Tests (%)	Number of Unnecessary Tests (%)
General Surgery	203	42 (20.69)	5 (11.9)
Internal Medicine	4391	725 (16.51)	80 (11.03)
Orthopedics and Traumatology	601	91 (15.14)	9 (9.89)
Physical Therapy and Rehabilitation	1566	82 (5.24)	8 (9.76)
Infectious Diseases	58	21 (36.21)	1 (4.76)
Other Clinics	381	59 (15.49)	4 (6.78)

DISCUSSION

The findings of this study highlight the significant prevalence of both repeated and unnecessary laboratory testing, particularly in the context of TSH, ferritin, CRP, and sedimentation tests. In line with previous studies, the results indicate that a substantial proportion of repeated tests were unnecessary, thus underscoring the need for stricter adherence to established guidelines for re-testing intervals (8). The unnecessary repeat test rates of 10.6% for TSH, 7.76% for ferritin, 6.46 % for CRP, and 10.49 % for sedimentation demonstrate the potential for improvement in laboratory test utilization.

This study contributes to the current literature by presenting quantitative data on the prevalence of repeated and unnecessary repeat testing for frequently ordered biochemical parameters in a secondary hospital in Turkey. To our knowledge, there are few national data addressing this issue in accordance with international re-testing interval guidelines.

The analysis of repeated and unnecessary tests across clinical departments highlights significant inefficiencies in test utilization. High repetition rates, such as for ferritin in family medicine service (15.36%) and CRP in chest diseases service (23.91%), suggest potential overuse. Similarly, neurology service exhibited the highest unnecessary TSH test rate (16.67%), while emergency service had the highest unnecessary CRP rate (13.04%).

One of the contributors to this issue is the failure to follow the minimum re-testing intervals recommendations, which are designed to prevent overuse of tests. This not only leads to resource wastage but also increases the likelihood of patient discomfort and confusion due to excessive or irrelevant test results. Previous studies have shown that unnecessary repeat testing can increase the complexity of clinical decision-making and may even result in inappropriate treatment or delays in care (2-4). Our findings align with those of Kwok and Jones (2005) and Bai et al. (2020), who similarly reported high rates of redundant sedimentation tests and thyroid function testing in routine clinical settings.

The variation in unnecessary repeat test rates across different tests suggests that some laboratory parameters are more prone to overuse than others (8). The relatively high percentage of unnecessary sedimentation tests (10.49%) could be attributed to clinicians' reliance on this test as a routine inflammatory marker, despite the availability of more specific tests such as CRP. Similarly, the elevated rate of unnecessary TSH tests (10.6%) may be related to the chronic management of thyroid disorders, where frequent monitoring is often performed without consideration of recommended intervals. The persistence of high rates of repeat sedimentation tests and TSH testing despite guideline recommendations may also reflect systemic, institutional, and cognitive factors. In many healthcare settings, outdated laboratory protocols continue to permit early reordering of common tests, even when newer guidelines advise against it. In addition, limited feedback mechanisms mean that clinicians are often unaware of redundant orders. Diagnostic uncertainty and a desire for confirmatory evidence — a form of 'defensive medicine' — also contribute to repeated testing. Previous research has highlighted that such behavioral and organizational drivers play a central role in laboratory test overuse (2, 3, 5).

To address these issues, it is essential that healthcare providers are made more aware of re-testing interval guidelines and are encouraged to incorporate them into their daily practice. Educational interventions targeting both clinicians and laboratory personnel could be beneficial in reducing unnecessary testing. In this context, the implementation of automated systems—which integrate minimum retesting intervals into the laboratory information system to flag premature requests while ultimately preserving clinician discretion, as recommended by authorities such as the Canadian Agency for

Drugs and Technologies in Health (CADTH)—emerges as a balanced and effective solution to reduce redundant testing. Studies have shown that such system-based interventions can significantly reduce the frequency of unnecessary testing and improve the overall efficiency of healthcare services. However, the success of these interventions often depends on clinician engagement and system usability; poorly designed alerts may lead to alert fatigue, limiting their effectiveness (8-14).

The results of this study also point to the potential for significant cost savings and better resource allocation in healthcare. Reducing unnecessary laboratory tests can lower overall healthcare costs, conserve laboratory resources, and reduce the burden on both patients and healthcare providers. In a time when healthcare systems worldwide are striving for efficiency and cost-effectiveness, optimizing laboratory test utilization by adhering to evidence-based guidelines can make a meaningful impact (15). Beyond economic implications, minimizing redundant testing can reduce patient anxiety, unnecessary follow-up investigations, and iatrogenic harm from misinterpreted results. Systematic reviews have shown that diagnostic test overuse is common and may expose patients to unnecessary harm, including anxiety, unnecessary follow-up procedures, and potential iatrogenic effects (16).

While this study provides important insights into the prevalence of repeated and unnecessary testing, it has limitations that should be considered. The analysis was confined to a single hospital and focused on a limited set of tests. Further research, including multi-center studies with a broader range of tests, would provide a more comprehensive understanding of the scope of unnecessary testing across different settings. Additionally, future studies could explore the underlying reasons for repeated testing, such as clinician decision-making processes, patient expectations, or institutional policies, to develop targeted interventions for reducing overuse. Displaying information such as the test half-lives or the recommended minimum retest intervals on patient result forms may help increase clinician awareness and reduce unnecessary repeat testing. Such strategies could serve as simple yet effective educational tools to promote adherence to evidence-based re-testing intervals.

Our study did not implement an intervention regarding retesting. The results of our study can form the basis for planned institutional measures to prevent inappropriate retesting, including LIS-based alerts and clinician education programs to increase awareness of retest intervals.

In conclusion, the unnecessary repetition of laboratory tests is a persistent issue that not only affects healthcare efficiency but also patient care. By adhering to established guidelines for minimum re-testing intervals and implementing system-based interventions, healthcare providers can substantially reduce the rates of redundant testing. Displaying information such as the test half-lives or the recommended minimum retest intervals on patient result forms may help increase clinician awareness and reduce unnecessary repeat testing. Such strategies could serve as simple yet effective educational tools to promote adherence to evidence-based re-testing intervals. Future efforts should focus on integrating laboratory stewardship principles into medical education and hospital policy, ensuring sustainable adherence to evidence-based retesting intervals.

Ethics Committee Approval: Ethical approval for the study was obtained (Harran University Faculty of Medicine, HRÜ/24.17.07).

Conflict of Interest

The authors declare no conflict of interest.

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Performance Assessment of Sysmex CS-5100 Versus ACL Top 700 in Routine Coagulation Testing

Rutin Koagülasyon Testlerinde Sysmex CS-5100 ve ACL Top 700'ün Performans Değerlendirmesi

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ABSTRACT

Aim: This study aimed to evaluate the performance characteristics of preliminary coagulation tests on the Sysmex CS-5100 coagulation autoanalyzer and compare it with the ACL Top 700 autoanalyzer. Both analyzers were assessed for their ability to measure prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer levels, using optical clot detection and immunological techniques.

Materials and Methods: Blood samples were analyzed on both platforms, and key performance metrics such as within-day and between-day imprecision, bias, and total error were evaluated following CLSI guidelines. Method comparison was conducted using Bland-Altman plots, Passing-Bablok regression, and correlation analyses.

Results: Our results demonstrated that both analyzers provided precise and reliable results for most parameters. However, significant differences were observed in D-dimer measurements, where the Sysmex CS-5100 consistently reported lower values compared to the ACL Top 700, particularly at higher concentrations. Despite these differences, no diagnostic discrepancies were found among patient samples, and strong correlations were observed for all other parameters.

Conclusion: The findings suggest that the Sysmex CS-5100 is a reliable alternative to the ACL Top 700, although further standardization, particularly for D-dimer measurements, may be needed to ensure consistency across platforms.

Keywords: Blood Coagulation tests, Precision, Method comparison.

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ÖZET

Amaç: Bu çalışmanın amacı, Sysmex CS-5100 koagülasyon otoanalizöründe yapılan temel koagülasyon testlerinin performans özelliklerini değerlendirmek ve bu cihazı ACL Top 700 otoanalizörü ile karşılaştırmaktır. Her iki analizör, protrombin zamanı (PT), aktive parsiyel tromboplastin zamanı (aPTT), fibrinojen ve D-dimer düzeylerini ölçme performansları açısından, optik pıhtı tespiti ve immünolojik teknikler kullanılarak değerlendirilmiştir.

Gereç ve Yöntem: Kan örnekleri her iki cihazda analiz edilmiştir ve gün içi ile günler arası imprecison, bias ve toplam hata gibi temel performans ölçütleri CLSI kılavuzları doğrultusunda değerlendirilmiştir. Yöntem karşılaştırması Bland-Altman grafikleri, Passing-Bablok regresyonu ve korelasyon analizleri kullanılarak gerçekleştirilmiştir.

Bulgular: Sonuçlarımız, her iki analizörün de çoğu parametre için kesin ve güvenilir sonuçlar verdiğini göstermiştir. Ancak, D-dimer ölçümlerinde anlamlı farklılıklar gözlenmiş, Sysmex CS-5100 özellikle yüksek konsantrasyonlarda ACL Top 700'e kıyasla sistematik olarak daha düşük değerler bildirmiştir. Bu farklara rağmen, hasta örneklerinde herhangi bir tanısal uyumsuzluk saptanmamış ve diğer tüm parametreler için güçlü korelasyonlar gözlenmiştir.

Sonuç: Bulgular, Sysmex CS-5100'ün ACL Top 700'e güvenilir bir alternatif olduğunu göstermektedir. Ancak, özellikle D-dimer ölçümleri için cihazlar arası tutarlılığın sağlanabilmesi adına ilave standardizasyon gerekebilir.

Anahtar Kelimeler: Kan koagülasyon testleri, Presizyon, Metod karşılaştırma

INTRODUCTION

Today, preliminary coagulation tests are among the basic tests conducted in the biochemistry laboratories of large-scale hospitals (1). In Turkey, the number of prothrombin time tests performed in the biochemistry laboratories of these hospitals can approach up to 1000 test/day. Coagulation autoanalyzers play a crucial role in diagnosing and managing bleeding and clotting disorders, delivering precise and reliable results quickly and efficiently.

There are numerous coagulation autoanalyzers and coagulation test kits marketed by various manufacturers. These kits can exhibit differences that may be reflected in test results, primarily due to the components they contain—particularly thromboplastin—being sourced from different origins (2). However, coagulation test results must be comparable and standardized for patient safety and medical advancement. For these reasons, it is necessary to evaluate the performance of coagulation test methods and conduct comparison studies.

Recent work has continued to evaluate high-throughput hemostasis systems and to characterize inter-assay variability that directly affects clinical interpretation. Since 2022, studies using the Sysmex CS-5100 coagulation autoanalyzer (Siemens Healthcare Diagnostics, Erlangen, Germany) have reported solid analytical performance in routine settings (e.g., Six-Sigma and analytical-phase evaluations) and explored preanalytical effects such as hemolysis on common coagulation tests measured on the Sysmex CS-5100. These reports collectively reinforce that platform-specific detection principles, reagent formulations, and calibration strategies can yield systematic differences that matter in practice—especially for D-dimer, where assay heterogeneity is well-documented and continues to influence diagnostic pathways and imaging yields (3-5).

Against this backdrop, our study compares two high-capacity analyzers [Sysmex CS-5100 as a candidate measurement procedure (MP) and ACL Top 700 (Instrumentation Laboratory, Milan, Italy) as a comparative MP] that are widely used in tertiary-care laboratories. By quantifying precision, bias, and agreement across PT, INR, aPTT, fibrinogen, and D-dimer—and by interpreting differences considering current evidence on inter-assay variability—we aim to provide actionable guidance for result interpretation, analyzer harmonization, and reflex testing policies in busy core labs.

MATERIALS and METHODS

2.1. Analyzers and choice of reagents

This study was conducted to evaluate the analytical performance of two fully automated coagulation analyzers: the Sysmex CS-5100 (Siemens Healthcare Diagnostics, Germany) and the ACL Top 700 (Instrumentation Laboratory, Werfen Group, Germany). Both systems can perform routine coagulation tests including PT, aPTT, fibrinogen, and D-dimer, utilizing optical clot detection methods.

The Sysmex CS-5100 is equipped with multi-wavelength optical detection technology (340, 405, 575, 660, and 800 nm) and provides additional capabilities such as pre-analytical sample integrity checks, including automatic detection of hemolysis, icterus, and lipemia (HIL indices). The analyzer also verifies sample volume and performs automatic cuvette loading and reagent monitoring, enhancing its suitability for high-throughput laboratories.

The ACL Top 700 analyzer also utilizes optical clot detection but lacks integrated pre-analytical HIL checks. Both analyzers use different reagents for the same test parameters, which is an important source of variability in result comparison. The reagents and reference intervals are summarized in Table 1. All reagents were used according to the manufacturers' instructions, and calibration and quality control procedures were performed using manufacturer-recommended calibrators and controls.

2.2. Collection of blood samples

Sterile vacutainer tubes (Vacusera, Disera, İzmir, Türkiye) containing 3,2% sodium citrate as an anticoagulant were used to draw venous blood samples from patients. The blood was collected by a trained phlebotomist following aseptic techniques. After collection, the samples were centrifuged at 1500 g for 15 minutes at 20°C to obtain platelet-poor plasma, which was then analyzed within 4 hours. The samples were directly tested on both the ACL Top 700 and Sysmex CS-5100 instruments.

Only excess material from patient samples, previously collected during standard clinical care, was used in the study. Additionally, all samples used in the study were anonymized to protect patient privacy. Our study was approved by the Ethics Committee of Health Science University Antalya Research and Training Hospital (2024-329) and conducted in accordance with the ethical principles of the Declaration of Helsinki (1964).

2.3. Performance Characteristics

Within-day and between-day imprecision, bias, and total error were assessed following the CLSI EP15-A3 guideline (6). This study was conducted at three levels using lyophilized control materials (Siemens Healthcare Diagnostics). In addition to normal and pathological levels, a third level was created by mixing these two controls in equal proportions. Each control material was tested five times a day for five consecutive days. On each testing day, a new control material from the same lot was reconstituted. Desirable imprecision (CVA), bias and total allowable error (TAE) goals was determined according to the following formulae (7):

$CV_A < 0.5CV_I$

$$\text{Bias} < 0.25\sqrt{CV_I^2 + CV_G^2}$$

$TAE < 1.65CV_A + \text{Bias}$

(CV_I: within-subject biological variation, CV_G: between-subject biological variation).

Updated CVI and CVG values of PT, INR, aPTT, fibrinogen and D-dimer were obtained from European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Database (8). Additionally, between-day imprecision values were compared with the manufacturer's stated imprecision values. The precision design and targets are summarized in Table 2.

Table 1. Charactheristics of the assays on the ACL Top 700 and SYSMEX Cs-5100.
Tablo 1. ACL Top 700 ve SYSMEX CS-5100 koagülasyon testlerinin özellikleri

	ACL Top 700			SYSMEX CS-5100		
	Reagent[a]	Method	Ref. Int.[b]	Reagent[a]	Method	Ref. Int.[b]
aPTT(s)	aPTT-SP silica dispersion with synthetic phospholipid activator (liq)	Opticalclotdetection	23.4–36.9	Actin FS with ellagic acid activator(liq)	Opticalclot detection	21.6–28.7
PT(s)	RecombiPlasTin 2G withrecombinanthumantissue factor (lyo)	Optical clotdetection	9.4–12.3	Thromborel® S withhuman placental thromboplastin (lyo)	Optical clotdetection	9.9–12.3
D-dimer (µg/L)	D-Dimer HS 500 (liq)	Particle-enhancedimmuno turbidimetry	<230	INNOVANCE® D-Dimer(lyo)	Particle-enhancedimmuno turbidimetry	<550
Fibrinogen (mg/dL)	HemosIL Q.P.A. with bovine thrombin (lyo)	Claussclottingassay withopticaldetection	200–393	Dade® Thrombinwithbovinethrombin(lyo)	Claussclottingassaywith optical detection	170-420

aPTT: activated partial thromboplastin time. PT: prothrombin time.
[a]liq: liquid reagent; lyo: lyophilized reagent.
[b]Ref. Int.: reference interval (as stated by the manufacturer).

Table 2. Precision and accuracy indicators of the coagulation tests on the SYSMEX CS-5100 coagulation autoanalyzer.
Tablo 2. SYSMEX CS-5100 koagulasyon otoanalizöründe koagulasyon testlerinin doğruluk ve kesinlik göstergeleri.

Control Material	Assay	Target Value	Within-day Imprecision (%)	Criteria Sysmex (%)	Between-day Imprecision (%)	Criteria Sysmex (%)	Minimum Criteria Imprecision Ricos (%)	Desirable Criteria Imprecision Ricos (%)	Bias (%)	Minimum Criteria bias Ricos (%)	Desirable Criteria bias Ricos (%)	Total Error (%)	Minimum Criteria total error Ricos (%)	Desirable Criteria total error Ricos (%)
Control N	PT (s)	12.6	0.9	≤2	0.1	≤5	2	1.3	-2	2.1	1.4	3.73	5.4	3.6
	INR	1.09	0.9	≤2	0.2	≤5	1.9	1.3	**3.3**	2	1.3	5.1	5.1	3.4
	aPTT (s)	24.3	0.6	≤2	0.4	≤5	2.1	1.4	0.8	2.9	1.9	1.3	6.4	4.2
	Fibrinogen (mg/dL)	256	3.5	≤4	0.1	≤10	7.7	5.1	0.5	7.5	5	7.6	20.1	13.4
Control P	PT (s)	20	1.1	≤2	0.8	≤5	2	1.3	**-4.6**	2.1	1.4	**7.2**	5.4	3.6
	INR	1.77	1	≤2	0.6	≤5	1.9	1.3	1.6	2	1.3	3.9	5.1	3.4
	Fibrinogen (mg/dL)	98	1.2	≤2	2.5	≤10	7.7	5.1	**-8.8**	7.5	5	13.9	20.1	13.4
Citrol	aPTT (s)	43.9	0.4	≤2	0.5	≤5	2.1	1.4	2.3	2.9	1.9	3.5	6.4	4.2
D-Dimer Control 1	D-Dimer (µg/L)	380	1.9	≤10	0.5	≤15	18.9	12.6	7	16.3	10.9	11.6	47.5	31.7
D-Dimer Control 2	D- Dimer (µg/L)	2530	1.2	≤10	0.1	≤15	18.9	12.6	3.5	16.3	10.9	5.9	47.5	31.7

Bolded values exceed the minimum criteria defined. Ricos criteria have been established considering biological variation (7).

2.4. Method comparison

The method comparison study was carried out in accordance with the CLSI EP09-A3 guideline (9). We identified Sysmex CS-5100 as the candidate measurement procedure and ACL Top 700 as the comparative measurement procedure. Table III presents data on the number of samples used and the range of values compared for each test. The Comparison results between the two analyzers were visualized and assessed by Bland–Altman difference plots, Passing–Bablok regression analysis, and a Spearman's Rank correlation coefficient. Acceptable method comparison performance criteria were as follows: The 95% confidence interval of the intercept and slope included point zero and point one respectively in Passing -Bablok regression analysis, the magnitude of the percentual difference between the two analytical methods was below the desirable criteria for total error of Ricos, and the Spearman's Rank correlation coefficient was greater than 0.95.

2.5. Statistical Analysis

All statistical analysis were performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK).

RESULTS

3.1. Performance Characteristics

Precision, bias, and total error were assessed using lyophilized control materials across three concentration levels—normal (Control N), pathological (Control P), and an intermediate level created by mixing equal parts of both controls. Measurements followed CLSI EP15-A3 guidelines, and analytical performance was evaluated using biological variation-based targets (Ricos et al.), including both minimum and desirable criteria.

As shown in Table 2, within-day and between-day imprecision results for all parameters, including D-dimer, were well within both the manufacturer's specifications and the desirable biological variation limits. This indicates acceptable analytical precision of the Sysmex CS-5100 for PT, INR, aPTT, fibrinogen, and D-dimer measurements.

Bias analysis revealed that most parameters demonstrated satisfactory agreement with biological variation-based criteria. However, notable deviations were observed in PT (Control P), INR (Control N), and fibrinogen (Control P), where bias values exceeded both the minimum and desirable thresholds defined by Ricos et al. These values suggest a tendency toward systematic deviation, particularly at pathological levels. In contrast, the remaining parameters—including aPTT and both D-dimer controls—showed bias values well within acceptable limits, indicating strong analytical consistency across a broad measurement range.

Total error (TE%) analysis further supported the analytical performance of the Sysmex CS-5100. Most test results fell within the minimum and desirable total allowable error limits defined by biological variation data. Exception was observed in PT (Control P), which exceeded both minimum and desirable criteria. Despite these deviations, all other parameters—including aPTT, fibrinogen (Control N), and D-dimer at both concentration levels—remained well within acceptable boundaries. Notably, D-dimer total error values (Control 1: 11.6%, Control 2: 5.9%) were significantly lower than both Ricos thresholds, underscoring the robustness of the method in measuring fibrin degradation products.

3.2. Method Comparison

Method comparison was conducted to evaluate the agreement between the Sysmex CS-5100 and ACL Top 700 analyzers for PT, INR, aPTT, fibrinogen, and D-dimer. Statistical assessments included Bland-Altman difference plots and Passing-Bablok regression analysis.

Figure 1 illustrates the Bland-Altman difference plots for all parameters. The mean differences for PT, INR, and fibrinogen were within acceptable bias limits, indicating good agreement. For aPTT, the 95% confidence

interval of the estimated bias was close to the lower limit of acceptability. However, for D-dimer, the bias was outside the acceptable range and exhibited increasing divergence at higher concentrations, suggesting a proportional bias between the two methods.

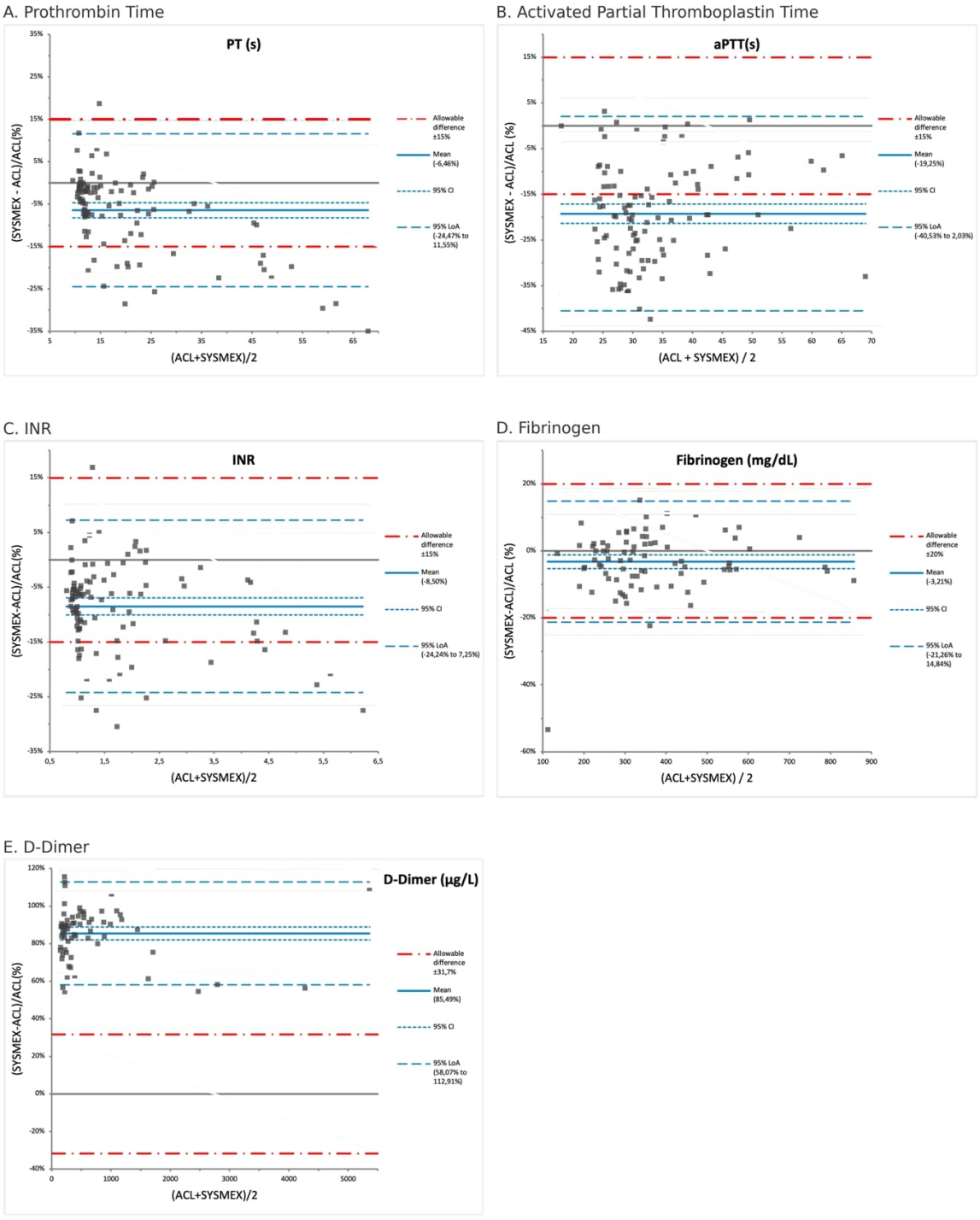

Figure 1. Method comparison analysis: Bland-Altman difference plots.
Şekil 1. Metod karşılaştırma analizi: Bland-Altman fark grafiği.

Table 3 presents the results of the Passing-Bablok regression analysis and Figure 2 displays the Passing-Bablok regression plots, showing the degree of agreement between the Sysmex CS-5100 and ACL Top 700 analyzers. Excellent correlations were observed for all parameters, with the highest coefficients seen in PT and INR followed closely by fibrinogen and aPTT. D-dimer also demonstrated strong correlation, despite a markedly different slope. Specifically, the slope for D-dimer, indicating a significant proportional bias. PT also showed both constant and proportional bias, as reflected in its intercept, which deviated from the ideal values. Conversely, INR, aPTT, and fibrinogen showed intercepts including zero and slopes approaching one, suggesting minimal systematic error. These findings confirm strong agreement between platforms for most parameters, while reinforcing the need for cautious interpretation of PT and D-dimer due to significant proportional deviations.

Figure 2 displays the Passing-Bablok regression plots. The correlation coefficients (Spearman's rho) for PT, INR, and fibrinogen, indicating strong agreement. The coefficients for aPTT and D-dimer were lower, reflecting the variability noted in Bland-Altman plots. The 95% confidence intervals for the intercept included zero for all parameters, while the slope confidence intervals included one for aPTT and fibrinogen only. Notably, the slope for D-dimer was substantially greater than one, confirming the presence of a proportional bias.

Taken together, these results indicate that while PT, INR, aPTT, and fibrinogen measurements are comparable between analyzers, D-dimer results differ significantly, likely due to differences in reagent kits and analytical methodologies. No diagnostic discrepancies were observed based on clinical cutoffs, but the proportional differences should be considered when interpreting D-dimer values across platforms.

Table 3. Passing-Bablok regression analysis.
Tablo 3. Passing-Bablok regresyon analizi.

	n	Range	Median (2,5–97,5 percentile)	Intercept (95% CI)	Slope (95% CI)	r
PT (s)	106	9,5-79,8	13,8 (10,4 - 61,6)	**1,76 (1,08 - 2,34)**	**0,83 (0,78 - 0,89)**	0,987
INR	104	0,83-7,08	1,2 (0,88 - 5,47)	0,06 (-0,011 - 0,1)	**0,88 (0,84 - 0,95)**	0,989
aPTT (s)	105	18-80,4	34,43 (25,1 – 63,7)	-2,52 (-6,1 - 1,3)	0,9 (0,78 - 1,01)	0,926
Fibrinogen (mg/dL)	80	136-895	331 (183,9 – 806,3)	-5,06 (-23,4 - 10,8)	0,98 (0,94 - 1,06)	0,982
D-Dimer (µg/L)	71	88-3070	198 (91,8 – 2144,5)	-17,32 (-46,12 - 16,59)	**2,68 (2,42 - 2,87)**	0,940

PT: prothrombin time, INR: international normalized ratio, aPTT: activated partial thromboplastin time, n: number of samples, r: correlation coefficient. Range of results are shown according to ACL TOP 500. Bold characters indicate cases where the confidence intervals for the intercept and slope do not include zero and one, respectively.

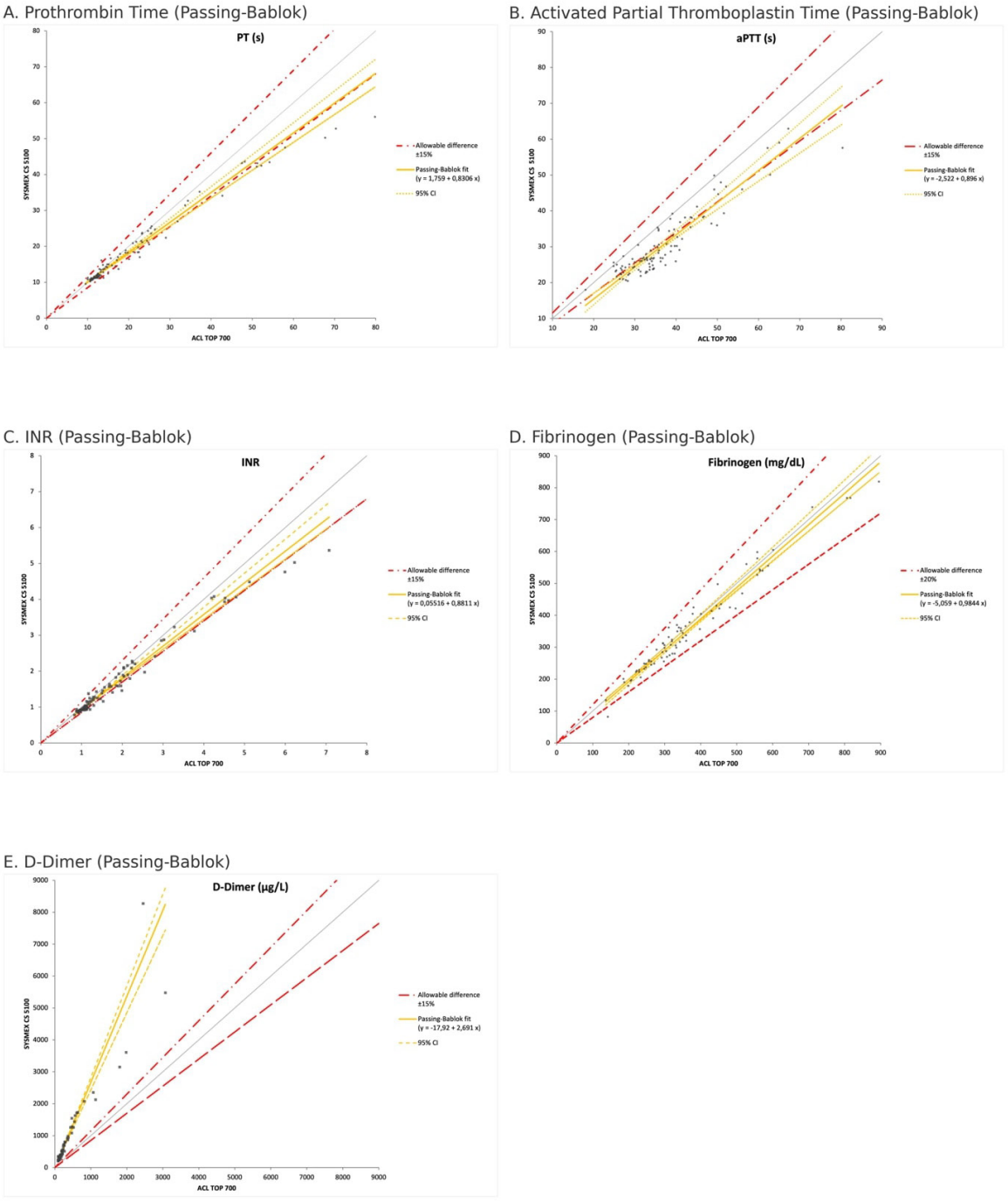

Figure 2. Method Comparison analysis: Passing-Bablok regression analysis.
Şekil 2. Metod karşılaştırma analizi: Passing-Bablok regresyon analizi.

DISCUSSION

This study aimed to assess the analytical performance of routine coagulation parameters—including PT, INR, aPTT, fibrinogen, and D-dimer—using the Sysmex CS-5100 analyzer and to compare these results with those from the ACL Top 700. Precision, accuracy, and method comparison analyses were conducted in accordance with

CLSI guidelines and evaluated using biological variation criteria.

When interpreting the overall analytical performance of the Sysmex CS-5100, it is important to consider the combined outcomes of precision, bias, and total error analyses. The analyzer demonstrated excellent within- and between-day imprecision for all test parameters, consistently meeting both minimum and desirable biological variation thresholds. Most bias and total error values also complied with Ricos criteria (7). But some deviations were observed in the bias and total error values for PT, fibrinogen, and INR. Specifically, the bias values for INR at the normal control level and fibrinogen at the pathological level did not meet the desirable Ricos thresholds; however, their total error values remained within acceptable limits, indicating no significant impact on clinical interpretation. The PT results, at the pathological control level, failed to meet the minimum Ricos specifications for both bias and total error. This contrasts with the findings of Geens et al., who demonstrated that both bias and total error satisfied the Ricos criteria when using the Dade Innovin (PT) kit on the same autoanalyzer (10). Further analysis using the Bland–Altman plot for PT shows that dispersion widens in the negative direction as PT increases (Figure 1). This suggests that the elevated absolute values of bias and total error observed with pathological control materials are not random but rather reflect a systematic source of error. This interpretation is supported by the fact that precision met the acceptance thresholds. In a related observation, a review of the external quality control results (RIQAS Cycle 15, Coagulation Program) determined that, while the overall results were distributed within the ± 1standard deviation range, the specific PT results for external quality control samples (ranging between 10.9-15.2 s) remained within the normal reference range, and no prolonged results were obtained.

We also note that converting PT seconds to INR substantially attenuates the proportional deviations seen at prolonged clotting times, thereby improving comparability between different measurement methods (11). In routine clinical care, particularly for monitoring vitamin K antagonist therapy, clinical decisions are primarily based on the INR value rather than the raw PT in seconds. Nevertheless, markedly prolonged PT values may still be encountered (e.g., at the high end of the range or in the presence of analytical interferences/flags), and these deviations may not be fully captured by a near-therapeutic INR. Consequently, clinicians should be explicitly alerted when the PT result is unusually high, allowing the results to be interpreted within the appropriate clinical and preanalytical context.

The method comparison analysis revealed strong agreement between the two analyzers for PT, INR, aPTT, and fibrinogen. This was supported by high correlation coefficients, regression slopes close to 1, and Bland-Altman plots showing minimal bias within acceptable limits. Notably, the INR results were particularly robust, with minimal total error and near-perfect alignment between devices. These results affirm the suitability of the Sysmex CS-5100 for clinical use in measuring these parameters.

However, significant discrepancies were identified in the D-dimer results. The Sysmex CS-5100 consistently reported lower values than the ACL Top 700, particularly at higher concentrations. The Bland-Altman plot (Figure 1) showed a proportional bias that increased with D-dimer concentration. This finding was corroborated by the Passing-Bablok regression analysis (Figure 2), which showed a slope significantly deviating from unity (2.69), indicative of a proportional systematic difference.

Consistent with our findings, D-dimer values tended to be higher on the Sysmex platform than on the comparator system. In the CN-6000 vs. STA-R Max study, the regression slope for D-dimer was >1 (1.17), indicating higher readings on CN-6000 across the range (12). Similarly, another CN-6000 vs. STA-R comparison reported a D-dimer slope

above 1 (1.10–1.21), again pointing to slightly higher Sysmex results despite good overall agreement (13).

We attribute this variation primarily to the different D-dimer reagents used by each analyzer: the Sysmex CS-5100 utilizes the INNOVANCE® D-Dimer assay (cutoff <550 μg/L), while the ACL Top 700 uses the D-Dimer HS 500 assay (cutoff <230 μg/L). External quality control (EQC) data (RIQAS Cycle 15 & 16, Coagulation Program) over one year showed consistent performance of the INNOVANCE® assay on the CS-5100, with results remaining within ±0.5 SD of the peer group mean. Conversely, EQC participants using the HS-500 reagent reported generally lower values, aligning with our observations. These findings emphasize the need for caution when comparing D-dimer results between platforms using different assays, even when both fall within clinically accepted ranges.

Importantly, when we applied the manufacturer-recommended cutoffs to the D-dimer results from both analyzers, no diagnostic discrepancies were observed in any of the 71 patient samples. This suggests that despite numerical differences, clinical interpretation remained consistent between the platforms.

Beyond our head-to-head comparison, current literature supports three themes. First, CS-series analyzers (including CS-5100) continue to demonstrate imprecision comfortably within biological-variation targets in routine use, aligning with our precision estimates (10,14). Second, high throughput and robust analytical concordance between the CS-5100 and ACL Top systems, supporting the platform's clinical applicability (14). Third, inter-assay variability for D-dimer remains clinically relevant: contemporary studies comparing multiple D-dimer assays (including HemosIL HS/HS-500 and Innovance families) show different specificity profiles and demonstrate that harmonization or unified calibration can improve cross-system consistency (12,13,15). These points collectively support our interpretation that the PT and especially D-dimer differences we observed are primarily assay-driven and should be managed with analyzer-specific cutoffs and, where feasible, local verification or calibration alignment.

A major strength of this study is the inclusion of a relatively high number of patient samples for method comparison, which enhances the generalizability of the findings, particularly in a real-world clinical laboratory setting. In addition, the study also has limitations. This was a single-center evaluation and done without subgroup analyses across clinically distinct populations (e.g., oncology, pregnancy, renal impairment). We did not assess turnaround time, reagent consumption, or cost-effectiveness, nor did we perform external validation across multiple sites. Future work should include multi-center cohorts, analyzer-specific reference interval verification in special populations, and participation in harmonization initiatives for D-dimer calibration. In this study, the analytical performance of the Sysmex CS-5100 coagulation analyzer was evaluated and compared with the ACL Top 700 across key preliminary coagulation tests, including PT, INR, aPTT, fibrinogen, and D-dimer. The Sysmex CS-5100 demonstrated excellent precision and strong agreement with the ACL Top 700 for most parameters, supporting its reliability and suitability for routine clinical use.

While measurements of PT, INR, aPTT, and fibrinogen were consistent between the two analyzers, significant proportional differences were observed in D-dimer results, with the Sysmex CS-5100 consistently yielding lower values. These discrepancies were attributed to differences in assay design and calibration standards between reagent kits. Nonetheless, no diagnostic misclassifications occurred when analyzer-specific reference ranges were applied, reaffirming the clinical acceptability of both systems.

Overall, the Sysmex CS-5100 offers a robust and efficient alternative to the ACL Top 700 for coagulation testing in high-volume laboratory settings. However, assay-specific standardization—particularly for D-dimer—is essential to ensure cross-platform harmonization and accurate interpretation of patient results.

Conflict of Interest

The authors declare no conflict of interest.

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Measurement Uncertainty of Cardiac Markers: High-Sensitivity Troponin T and Myoglobin

Kardiyak Belirteçlerin Ölçüm Belirsizliği: Yüksek Hassasiyetli Troponin T ve Miyoglobin

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ABSTRACT

Aim: The measurement uncertainty (MU) of high-sensitivity troponin T (hs-TnT) and myoglobin assays was calculated in accordance with ISO/TS 20914:2019. The calculated MU values were subsequently compared with the relevant analytical performance specifications (APS).

Materials and Methods: Internal quality control (IQC) and external quality control (EQC) data collected between January and June 2024 were analyzed. Level 1 and Level 2 control materials for hs-TnT and myoglobin were measured daily. Expanded combined relative measurement uncertainty (Ucrel) were calculated with calibrator uncertainties included, and level-specific estimates were combined to derive the total device-level expanded measurement uncertainty, t(Ucrel).

Results: For the hs-TnT assay, Ucrel values at Level 1 exceeded the allowable APS on both the Cobas 8000 and Cobas Pro analyzers, whereas Level 2 results remained within acceptable limits. t(Ucrel) values on both analyzers (19.63% and 14.24%) exceeded the minimum APS of 13%. For myoglobin, Ucrel values were below the APS limit of 13% at both control levels on the Cobas 8000 analyzer, whereas they slightly exceeded the APS at both levels on the Cobas Pro analyzer. Accordingly, the t(Ucrel) value exceeded the APS on the Cobas Pro analyzer (13.45%) but remained acceptable on the Cobas 8000 analyzer (11.49%).

Conclusion: Routine monitoring and reporting of MU can facilitate more reliable interpretation of laboratory results, particularly those close to clinical decision thresholds. Improved communication of MU between laboratories and clinicians may enhance the clinical utility of cardiac biomarkers and support more informed diagnostic decision-making.

Keywords: Troponin T, Myoglobin, Uncertainty, Quality Control

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ÖZET

Amaç: Yüksek duyarlılıklı troponin T (hs-TnT) ve miyoglobin testlerinin ölçüm belirsizliği (MU), ISO/TS 20914:2019 standardına uygun olarak hesaplandı. Hesaplanan MU değerleri daha sonra ilgili analitik performans spesifikasyonları (APS) ile karşılaştırıldı.

Gereç ve Yöntemler: Ocak–Haziran 2024 döneminde toplanan iç kalite kontrol (IQC) ve dış kalite kontrol (EQC) verileri analiz edildi. hs-TnT ve miyoglobin için Seviye 1 ve Seviye 2 kontrol materyalleri günlük olarak ölçüldü. Kalibratör belirsizlikleri dâhil edilerek seviye-özel genişletilmiş birleşik bağıl ölçüm belirsizlikleri (Ucrel) hesaplandı ve bu seviye-özel tahminler birleştirilerek cihaz düzeyindeki toplam genişletilmiş ölçüm belirsizliği, t(Ucrel), elde edildi.

Bulgular: hs-TnT testi için Seviye 1'deki Ucrel değerleri hem Cobas 8000 hem de Cobas Pro analizörlerinde izin verilen APS sınırlarını aşarken, Seviye 2 sonuçları kabul edilebilir sınırlar içinde kaldı. Her iki analizörde elde edilen t(Ucrel) değerleri (%19,63 ve %14,24), %13 olan minimum APS değerini aştı. Miyoglobin için ise Cobas 8000 analizöründe her iki seviyedeki Ucrel değerleri %13'lük APS sınırının altında kalırken, Cobas Pro analizöründe her iki seviyede de APS'yi hafifçe aştı; buna bağlı olarak Cobas Pro analizöründe t(Ucrel) değeri (%13,45) APS'yi aşarken, Cobas 8000 analizöründe (%11,49) kabul edilebilir düzeyde kaldı.

Sonuç: Ölçüm belirsizliğinin rutin olarak izlenmesi ve raporlanması, özellikle klinik karar eşiklerine yakın sonuçların daha güvenilir şekilde yorumlanmasını kolaylaştırabilir. Laboratuvarlar ile klinisyenler arasında ölçüm belirsizliğinin daha iyi iletişimi, kardiyak biyobelirteçlerin klinik faydasını artırabilir ve daha bilinçli tanısal kararların verilmesini destekleyebilir.

Anahtar kelimeler: Troponin T, Miyoglobin, Belirsizlik, Kalite kontrol

INTRODUCTION

Clinical laboratories play a crucial role in supporting a wide range of clinical decisions, including early and accurate diagnosis, guiding treatment selection, preventing delays in therapy, and reducing the need for palliative care (1,2). A survey conducted among clinicians in Germany and the United States reported that laboratory test results influence approximately 60–70% of clinical decisions (3). Therefore, clinical laboratories focus on improving and maintaining quality through the use of quality assurance tools. To enhance the clinical value of laboratory tests, results must be reported in a manner that supports effective clinical decision-making. In addition, clinical laboratories should provide consultative services that understand clinicians' needs and collaborate with them regarding test selection and interpretation. To this end, it is essential for laboratory professionals to share clinically relevant information with clinicians through face-to-face meetings, telephone consultations, and written reports in order to enhance the clinical value of laboratory tests (4). Laboratory testing involves numerous potential sources of 'uncertainty' that may significantly influence results, including preanalytical errors in sample collection or transport, biological variation, medication use, and recording or reporting errors (5). The evaluation of measurement uncertainty (MU) is essential for interpreting laboratory results, diagnosing diseases, and monitoring treatment. MU is defined as a parameter that describes the dispersion of values reasonably attributable to a measurand, and reporting MU alongside test results can influence clinical decision-making (6). Determining MU in medical laboratories provides several important benefits. These include offering objective information about the quality of individual laboratory performance, supporting appropriate clinical decisions, identifying tests that require analytical improvement before clinical application, encouraging IVD manufacturers to enhance the quality of their analytical performance, and enabling the discontinuation of analytical methods that demonstrate inadequate quality (7,8). According to the ISO 15189 accreditation standard, if requested, the MU of laboratory tests can be calculated and reported alongside the test results. However, despite these recommendations and guidelines, most calibrator insert documents do not include MU values.

There are two fundamental approaches to calculating MU: the bottom-up and top-down approaches. In the bottom-up approach, all individual factors contributing to uncertainty and their influence ratios are included separately in the calculation. In contrast, the top-down approach relies on existing analytical performance data obtained from quality control materials (9,10). For the calculation of MU, the International Vocabulary of Metrology (VIM2), the Guide to the Expression of Uncertainty in Measurement (GUM1), and the International Organization for Standardization / International Electrotechnical Commission (ISO/IEC) guidelines provide metrological methodologies (11). The ISO/TS 20914:2019 guideline specifically recommends the calculation of MU (9). The Nordtest guide, which is widely used as a standard reference for estimating MU in environmental laboratories across Europe, employs a top-down approach and aims to provide a clear and practical framework for MU calculation (12).

Myocardial infarction (MI), the most severe form of coronary artery disease, is a life-threatening condition and a major cause of global mortality (13). Measurement of cardiac troponins is the cornerstone of MI diagnosis (14). High-sensitivity troponin assays (hs-troponin) enable the rapid exclusion of MI and help prevent unnecessary hospitalizations (15).

Suboptimal analytical performance in troponin testing—including device-to-device variability and differences in reagents and calibrator lots—can significantly affect measurements at low troponin concentrations and lead to patient misclassification within MI diagnostic algorithms used in emergency departments (16). Therefore, medical laboratories should calculate the MU of troponin assays and report it alongside test results to assist clinicians. Clinicians should interpret hs-troponin results near clinical decision cutoffs by taking MU into account (17).

In this study, we aimed to determine the MU of hs-TnT and myoglobin assays performed in our laboratory in accordance with ISO/TS 20914:2019, and to compare the calculated MU values with the analytical performance specifications (APS) for MU.

MATERIALS AND METHODS

Study Setting

Six months of internal quality control (IQC) data collected between January and June 2024 were used to determine the MU of hs-TnT and myoglobin assays. MU values were calculated separately for the Roche cobas® pro and Roche cobas® 8000 analyzers (Roche Diagnostics, Mannheim, Germany) used in our laboratory.

The IQC materials consisted of Roche PreciControl Troponin (Lot No. 79059301) and Roche PreciControl Cardiac II (Lot No. 79446901). Level 1 and Level 2 IQC samples for the hs-TnT and myoglobin assays were analyzed in duplicate on a daily basis. For hs-TnT, a total of 904 Level 1 and 928 Level 2 IQC results were evaluated. For the myoglobin assay, 450 IQC results were obtained for each control level.

External quality control (EQC) was performed monthly using Cardiac RQ9186 and Cardiac Plus RQ9190 samples (RIQAS, Randox Laboratories Ltd., Crumlin, UK). Although EQC results were reviewed to assess long-term analytical stability, they were not included in the MU calculation, as ISO/TS 20914:2019 recommends the use of internally generated control data for estimating standard MU (18).

Calculating Measurement Uncertainty

For each control level, the relative standard MU due to imprecision (uRw%) was calculated using IQC data. The relative calibrator uncertainty (ucal%) was obtained from the manufacturer-provided uncertainty information given in Table 1. Each calibrator had an assigned concentration value and an expanded uncertainty (U) with a coverage

factor of k = 2. The corresponding standard uncertainty was calculated by dividing U by k, and ucal% was expressed as a percentage of the assigned calibrator concentration.

Table 1. Calibrator uncertaintiy values
Tablo 1. Kalibratör belirsizlik değerleri

Parameter	ucal% (k=1)
Troponin T Calibrator (C1)	0.64
Troponin T Calibrator (C2)	0.97
Myoglobin Calibrator (C1)	0.74
Myoglobin Calibrator (C2)	2.17

For each control level, the combined relative standard measurement uncertainty (ucrel) was calculated by combining uRw% and ucal%. These level-specific combined uncertainties were then expanded using a coverage factor of k = 2 and reported as the expanded combined relative measurement uncertainty (Ucrel) (Table 2).

To estimate the total device-level measurement uncertainty, the ucrel values obtained from Level 1 and Level 2 were combined using the pooled variance approach. The resulting total combined relative standard uncertainty t(ucrel) was then multiplied by a coverage factor of k = 2 to obtain the total device-level expanded combined relative measurement uncertainty, denoted as t(Ucrel) (Table 3).

The ucrel was calculated separately for each control level using the following formula:

$$u_{crel} = \sqrt{u_{Rw}^2 + u_{cal}^2}$$

The expanded ucrel for each control level was then calculated as:

$$U_{crel} = u_{crel} \times k$$

The t(ucrel) was calculated at the device level by combining the ucrel values from both control levels and multiplying the resulting value by the coverage factor (k = 2) to obtain t(Ucrel).

$$u_{crel,\,combined}(\%) = \sqrt{\frac{(n_{level1} - 1)\,u_{crel,level1}^2 + (n_{level2} - 1)\,u_{crel,level2}^2}{n_{level1} + n_{level2} - 2}}$$

Table 2. Measurement uncertainty values for hs-troponin T and myoglobin
Tablo 2. hs-troponin T ve miyoglobin için ölçüm belirsizliği değerleri

Test	Analyzer	Level	uRw (%)	ucal (%)	u_{crel} (%)	U_{crel} (k=2, %)
hs-troponin T	Cobas 8000	Level 1	13.37	0.64	13.39	26.78
hs-troponin T	Cobas 8000	Level 2	3.82	0.97	3.94	7.89
hs-troponin T	Cobas Pro	Level 1	8.23	0.64	8.26	16.52
hs-troponin T	Cobas Pro	Level 2	5.72	0.97	5.80	11.61
Myoglobin	Cobas 8000	Level 1	5.53	0.74	5.58	11.16
Myoglobin	Cobas 8000	Level 2	5.48	2.17	5.90	11.81
Myoglobin	Cobas Pro	Level 1	6.65	0.74	6.69	13.39
Myoglobin	Cobas Pro	Level 2	6.40	2.17	6.76	13.52

Table 3. Total Expanded Combined Relative Measurement Uncertainty t(Ucrel)
Tablo 3. Toplam Genişletilmiş Birleşik Bağıl Ölçüm Belirsizliği t(Ucrel)

Test	Analyzer	Level	t (U_{crel}) (k=2, %)	Target (%)	Status
hs-Troponin T	Cobas 8000	Total	19.63	Min 13 / Desirable 9.4	Not acceptable
hs-Troponin T	Cobas Pro	Total	14.24	Min 13 / Desirable 9.4	Not acceptable
Myoglobin	Cobas 8000	Total	11.49	13	Acceptable
Myoglobin	Cobas Pro	Total	13.45	13	Not acceptable

RESULTS

Six-month EQC data for both tests were within acceptable limits for all results, confirming the absence of clinically significant bias. The calibrator uncertainty values (ucal%) for hs-TnT and myoglobin are listed in Table 1. The MU values for hs-TnT and myoglobin at both control levels, including uRw, ucal, ucrel, and Ucrel, are summarized in Table 2. For hs-TnT, the APS values for standard MU were defined as 13% at the minimum level and 9.4% at the desirable level. Because the APS for the standard MU of myoglobin is not clearly defined in the literature, the maximum allowable standard MU (MAu) was estimated using a biological variation–based approach (20). Within-subject biological variation (CVI) values were obtained from the referenced study, and MAu was defined according to the criterion MAu < 2 × 0.5 × CVI (i.e., MAu < CVI). Accordingly, myoglobin results were evaluated using an APS threshold of 13%. For hs-TnT, Level 2 results remained within acceptable limits on both analyzers, whereas Level 1 and t(Ucrel) values exceeded the threshold of 13% on both analyzers. For myoglobin, Ucrel values on the Cobas 8000 analyzer were below 13% at both control levels. In contrast, Ucrel values on the Cobas Pro analyzer slightly exceeded the MAu at both Level 1 (13.39%) and Level 2 (13.52%). The t(Ucrel) value for myoglobin was acceptable on the Cobas 8000 analyzer but exceeded the MAu limit of 13% on the Cobas Pro analyzer (Table 3).

DISCUSSION

In this study, MU of hs-TnT and myoglobin was evaluated across two analyzer platforms and two control levels using an extensive IQC dataset. For myoglobin, the t(Ucrel) value exceeded the current APS limit of 13% only on the Cobas Pro analyzer. The t(Ucrel) value for hs-TnT exceeded the minimum allowable APS threshold on both analyzers, indicating unacceptable MU according to the predefined criteria. Notably, the Cobas 8000 analyzer exhibited a higher t(Ucrel) value (19.63%) than the Cobas Pro analyzer (14.24%), suggesting greater overall analytical variability for hs-TnT on this platform. In addition, Level 1 results for hs-TnT on the Cobas 8000 exceeded the acceptable APS threshold, which may partly reflect the increasingly stringent clinical performance requirements applied to cardiac troponin assays at low concentration levels over the past two decades (21).

Previous studies have reported that MU values for cardiac biomarkers vary depending on the analyte and analytical methodology. For instance, studies on troponin I assays have shown that high sensitivity methods generally remain within acceptable MU limits across clinically relevant concentration ranges (17). In contrast, myoglobin has frequently been reported to exhibit higher analytical variability, which has been attributed to its wide physiological distribution and limited cardiac specificity (22). These findings indicate that MU performance characteristics can vary markedly across biomarkers and analytical platforms, especially near clinical decision thresholds, consistent with the observations of the present study. Laboratory information plays an increasingly central role in diagnosis and treatment; however, as with all clinical data, the inherent limitations of diagnostic tests may influence clinical interpretation. Effective communication of MU can strengthen collaboration between clinicians, patients, and medical laboratories (21). Previous studies have emphasized the critical role of MU reporting in clinical decision-making, and MU has also been incorporated into quality assessment criteria and international standards. Nevertheless, unless MU calculations are readily applicable in routine laboratory practice, their widespread implementation remains limited. Therefore, practical MU models that can be calculated using existing data without requiring additional resources or budget allocation are both important and valuable (22). The CCLM guidelines also emphasize the importance of MU assessment in supporting compliance

with ISO 15189 and in interpreting results close to clinical decision thresholds (23–24). Unlike bias, MU cannot be reduced to zero; therefore, the objective is to keep uncertainty within predefined targets to prevent excessive uncertainty that could compromise the clinical utility of test results. (25). Taken together, these findings underscore that MU is not merely a statistical parameter but a clinically relevant factor that may affect result interpretation, particularly in borderline cases. Differences in t(Ucrel) values between analyzers underscore the need to account for uncertainty when evaluating cardiac biomarkers, as such differences may influence clinical judgment in distinguishing acute myocardial injury from non-cardiac causes of biomarker elevation in emergency settings.

This study has several limitations. First, only two analyzers and two levels of IQC materials were evaluated, and variability related to patient samples was not assessed. Second, long-term trends, lot-to lot variability, and the potential impact of reagent or calibrator changes were not investigated. Despite these limitations, the study has notable strengths. It is based on a large IQC dataset collected over a six-month period, applies the most recent ISO/TS 20914:2019 framework, and provides a detailed comparison of MU across two widely used analyzers. Collectively, these features enhance the practical relevance of the findings and support their applicability in routine laboratory practice.

CONCLUSION

Routine monitoring and reporting of MU can facilitate more reliable interpretation of laboratory results, particularly those close to clinical decision thresholds. Furthermore, improved communication of MU between laboratories and clinicians may enhance the clinical utility of cardiac biomarkers and support more informed diagnostic decision making.

Conflict of Interests

The authors declare no conflicts of interest in this study.

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Çocukluk çağı epilepsilerinde PIV ve SII oranlarının valproik asit düzeyleri ile ilişkisi

Relationship PIV and SII with Valproic acide levels in childhood epilepsy

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ÖZET

Amaç: Valproik asit çocukluk çağı epilepsilerinde sık kullanılan bir terapötik ajandır. Valproik asit toksik dozlarının takibi hepatotoksisite ve ensefalopati gibi ciddi yan etkilerinden ötürü önemlidir. Çalışmamızda inflamatuvar belirteçler olan panimmün inflamasyon (PIV) ve sistemik immün inflamasyon (SII) ile valproik asit düzeylerinin ilişkileri ortaya konulmaya çalışılmıştır.

Gereç ve Yöntem: Retrospektif veri değerlendirmesi olarak tasarladığımız çalışmamızda, Ocak 2023-Aralık 2024 arasında hastane bilgi sisteminden alınan valproik asit sonucu etkin olmayan (<50 mg/L), etkin düzeyde (50-100 mg/L) olan ve toksik düzeyde (>100 mg/L) olmak üzere üç gruba ayrılmıştır. Hemogram sonuçları ve bu sonuçlardan hesaplayarak elde ettiğimiz SII ve PIV ile NLO, platelet/lenfosit oranı (PLO), Nötrofil sayısıx Lenfosit sayısı/Platelet sayısı oranı (NLPO) ve sistemik inflamatuvar cevap indeksi (SIRI) karşılaştırılmıştır**.** SPSS 25.0 paket programıyla analiz edilmiştir.

Bulgular: 154 hasta bireye ait toplam 4596 veri değerlendirildi. Gruplar birbiri ile karşılaştırıldığında 1-2. gruplar arası platelet sayısı (p=0,0001), 1-3. gruplar karşılaştırıldığında bazofil (p=0,037), platelet sayısı (p= 0,0001), PLO (p= 0,001), PIV oranı (p=0,027) ve SII oranı (p=0,003), 2-3. grup karşılaştırıldığında ise platelet sayısı (p= 0,0001), PLO (p= 0,001), PIV (p=0,027) ve SII oranı (p=0,003) istatistiksel olarak anlamlı bulundu.

Sonuç: Çalışmamız, SII ve PIV oranlarının valproik asit toksik düzeylerini tahmin ettirmede etkin olduğunu göstermiştir. 3. Basamağa ulaşımı zor olan epilepsili çocukların kolay ulaşılabilen birinci basamak testleri ile toksik doz takibi literatüre katkı olarak sunulmaya çalışıldı.

Anahtar Sözcükler: Valproik asit, İnflamatuvar oranlar, Çocukluk çağı epilepsi, Veri analizi, Toksik doz

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ABSTRACT

Aim: Valproic acid is a commonly used therapeutic agent in childhood epilepsies. Monitoring toxic doses of valproic acid is important due to serious adverse effects such as hepatotoxicity and encephalopathy. In this study, we aimed to investigate the relationship between valproic acid levels and inflammatory markers, including the pan-immune inflammation value (PIV) and the systemic immune-inflammation index (SII).

Materials and Methods: This retrospective study evaluated data obtained from the hospital information system between January 2023 and December 2024. Valproic acid results were classified into three groups: subtherapeutic (<50 mg/L), therapeutic (50–100 mg/L), and toxic (>100 mg/L). Hemogram parameters and derived inflammatory indices—SII, PIV, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), neutrophil × lymphocyte / platelet ratio (NLPR), and systemic inflammatory response index (SIRI)—were compared among groups. Statistical analyses were performed using SPSS version 25.0.

Results: A total of 4,596 data points from 154 patients were evaluated. When groups were compared, platelet count was significantly different between Groups 1 and 2 (p=0.0001). In comparisons between Groups 1 and 3, basophil count (p=0.037), platelet count (p=0.0001), PLR (p=0.001), PIV (p=0.027), and SII (p=0.003) were statistically significant. Between Groups 2 and 3, platelet count (p=0.0001), PLR (p=0.001), PIV (p=0.027), and SII (p=0.003) showed statistically significant differences.

Conclusion: Our study demonstrated that SII and PIV ratios are effective in predicting valproic acid toxic levels. This study aimed to contribute to the literature by presenting easily accessible primary care tests for epileptic children who have difficulty accessing tertiary care, thus monitoring toxic doses.

Keywords: Valproic acid, Inflammatory indices, Childhood epilepsy, Data analysis, Toxic dose

GİRİŞ

Çocukluk çağında en sık görülen nörolojik bozukluklardan biri olan epilepsi, klinik olarak 24 saatten uzun aralıklarla ortaya çıkan iki veya daha fazla provoke edilmemiş nöbet ile tanımlanmaktadır (1). En yüksek insidans yaşamın ilk yıllarında görülmektedir (2). Pediatrik başlangıçlı epilepsiler, erişkin epilepsilerinden farklı olarak gelişimsel, psikiyatrik ve davranışsal komorbiditeler açısından daha yüksek risk taşımaktadır (3). Epilepsi, uzun süreli tedavi gerektiren kronik bir hastalıktır (4). Genel olarak epilepsi; fokal, jeneralize, başlangıcı bilinmeyen ve sınıflandırılamayan olmak üzere dört ana gruba ayrılmaktadır (5). Valproik asit (VPA), pediatrik epilepsi sendromlarında ve özellikle idiyopatik jeneralize epilepside, diğer antiepileptik ilaçlara kıyasla nispeten daha etkili bulunması nedeniyle sık kullanılan geniş spektrumlu bir antiepileptik ajandır (6). Oral yolla alınan VPA, gastrointestinal emilimin ardından karaciğerde hepatik enzimler aracılığıyla primer metabolizmaya uğramakta, daha sonra glukuronik asit ile konjuge edilerek renal klerens yoluyla vücuttan uzaklaştırılmaktadır. Yeni tanı konmuş epilepsili çocuklarda en sık reçete edilen antiepileptik ilaçlardan biridir. Terapötik aralıkta nadir görülen yan etkilerine rağmen, VPA toksik dozlarda trombositopeni, çeşitli metabolik bozukluklar, hepatotoksisite ve hiperamonyemik ensefalopati gibi ciddi yan etkilere neden olabilmektedir (7). VPA'nın toksik düzeylerde inflamasyonla ilişkisi hücresel düzeyde tam olarak açıklanamamış olmakla birlikte, reaktif oksijen türleri ve serbest radikallerin başlattığı hücresel hasarın bu ciddi yan etkilere yol açtığı önceki çalışmalarda vurgulanmıştır (8). VPA'ya bağlı ensefalopatide, beyin toksisitesi ve buna bağlı olarak ensefalopatinin gelişmesinde öne sürülen mekanizma; amonyağın glutamin formunda hücre içine girmesi ve ardından mitokondride yeniden amonyağa ve glutamata dönüşerek oksidatif strese neden olmasıdır (9).

Her ne kadar randomize klinik çalışmalar terapötik ilaç izleminin klinik sonuçlar üzerine olumlu etkisini net olarak ortaya koyamamış olsa da, serum antiepileptik ilaç düzeylerinin klinik durumla birlikte

değerlendirilmesinin hasta yönetiminde değerli olduğu bildirilmektedir. Serum ilaç düzeylerine ek olarak biyokimyasal ve hemogram parametrelerinin izlenmesinin, yan etkilerin anlaşılması ve prognostik süreçte klinik müdahalelere yön vermesi açısından faydalı olabileceği belirtilmektedir (10,11).

Histon deasetilaz inhibitörü olarak VPA, histon H3 ve H4 asetilasyonunu artırarak lenfoid ilişkili gen ekspresyonunu desteklemekte, miyeloid seri farklılaşmasını ise baskılamaktadır. Bu durum lenfosit sayısında artış ve nötrofil sayısında azalma ile sonuçlanmaktadır (12). Ayrıca VPA'nın düzenleyici T hücrelerinin sayı ve fonksiyonlarını artırdığı, monosit–makrofaj ve dendritik hücre olgunlaşmasını modüle ettiği ve bağışıklık hücre dengesi üzerinde lenfosit lehine bir etki oluşturduğu bildirilmiştir (13).

Son yıllarda literatürde, hemogramdan elde edilen hücre sayıları ve bu hücrelerden hesaplanan inflamatuvar oranların kronik hastalıkların izleminde kullanılabilirliği giderek daha fazla tartışılmaktadır. Bu kapsamda, epilepsi tanısı almış ve antiepileptik tedavi kullanan hastalarda nötrofil-lenfosit oranı (NLO) ve platelet-lenfosit oranı (PLO) gibi birçok parametre değerlendirilmiştir (14–16).

Patsalos ve Hiemke, en iyi terapötik etkinliğin VPA için 50–100 mg/L serum düzeyleri arasında sağlandığını bildirmiştir (10,17). Bu doğrultuda çalışmamızda; serum VPA düzeyi <50 mg/L olan hastalar Grup 1, 50–100 mg/L arasında olanlar Grup 2, >100 mg/L olanlar ise toksik doz grubu (Grup 3) olarak sınıflandırılmıştır.

Çalışmamızda, 2–18 yaş arası VPA kullanan epilepsili çocuk hastalarda hemogram verilerinden elde edilen hücre sayıları ile bu hücrelerden hesaplanan PLO, nötrofil sayısı × lenfosit sayısı / platelet sayısı oranı (NLPO), sistemik inflamatuvar cevap indeksi (SIRI), sistemik immün inflamasyon indeksi (SII) ve pan-immün inflamatuvar indeks (PIV) değerlendirilmiştir. Bu oranların VPA'nın terapötik ve toksik dozlarındaki farklılıklarını ortaya koymak ve özellikle toksik doz grubundaki tahmin ettirme gücünü inceleyerek birinci basamakta kullanılan kolay erişilebilir testlerin klinik faydasını değerlendirmek amaçlanmıştır.

GEREÇ VE YÖNTEMLER

Retrospektif olarak tasarlanan bu çalışmada, Ocak 2023–Aralık 2024 tarihleri arasında Pamukkale Üniversitesi Hastanesi Çocuk Nöroloji Servisi ve Polikliniği'ne başvuran, eş zamanlı VPA ve hemogram sonucu bulunan, 2–18 yaş arası çocuk hastalara ait veriler Laboratuvar Bilgi Sistemi (LBS) üzerinden elde edilmiştir. Eş zamanlı VPA ve hemogram sonucu bulunmayan hastaların verileri çalışmaya dahil edilmemiştir.

Serum VPA düzeyleri Cobas 801 analizöründe (Roche Cobas Sistemleri, Mannheim, Almanya) homojen enzim immünoassay yöntemi kullanılarak ölçülmüştür. Tam kan sayımı analizleri Mindray BC-6800 otoanalizöründe (Mindray CAL8000 sistemleri, Şanghay, Çin) optik dansite ve empedans yöntemleri ile gerçekleştirilmiştir. VPA ölçümleri için varyasyon katsayısı (CV) değerleri genel olarak <%10 iken, hemogram parametrelerine ait CV değerleri %5–6 arasında değişiklik göstermiştir. Dış kalite değerlendirme sonuçları incelendiğinde, ±2 Z-skoru dışında kalan herhangi bir sonuç saptanmamıştır.

Hemogram parametreleri; hemoglobin (g/dL), eritrosit sayısı (RBC, M/μL), eritrosit dağılım genişliği (RDW-SD, fL), platelet sayısı (PLT, K/μL), beyaz küre (WBC), nötrofil, lenfosit, monosit, eozinofil ve bazofil sayıları (K/μL) olarak kaydedilmiştir. Bu veriler kullanılarak platelet/lenfosit oranı (PLO), nötrofil × lenfosit / platelet oranı (NLPO), sistemik inflamatuvar cevap indeksi (SIRI = nötrofil × monosit / lenfosit), pan-immün inflamatuvar indeks (PIV = nötrofil × platelet × monosit / lenfosit) ve sistemik immün inflamasyon indeksi (SII = platelet × nötrofil / lenfosit) belirtilen formüller kullanılarak hesaplanmıştır.

Serum VPA düzeyleri <50 mg/L etkin olmayan doz, 50–100 mg/L terapötik doz ve >100 mg/L toksik doz olarak sınıflandırılmıştır (10,17). Belirlenen tarihler arasında başvuran ve çalışma kriterlerini karşılayan tüm tanılı çocuk hastalara ait mevcut veriler analize dahil edilmiştir.

İstatistiksel analizler SPSS 25.0 paket programı (IBM SPSS Statistics 25, Armonk, NY, ABD) kullanılarak yapılmıştır. Sürekli değişkenler ortalama ± standart sapma veya medyan (IQR: 25.–75. persentil) olarak, kategorik değişkenler sayı ve yüzde şeklinde ifade edilmiştir. Gruplar arası karşılaştırmalarda parametrik test varsayımlarının sağlandığı durumlarda tek yönlü varyans analizi, sağlanmadığı durumlarda ise Kruskal–Wallis testi kullanılmıştır. Sürekli değişkenler arasındaki ilişkiler Spearman korelasyon analizi ile değerlendirilmiştir. Değişkenlerin ayırt edicilik gücünü ve tanısal performanslarını belirlemek amacıyla ROC analizi uygulanmış, en uygun kesim noktası Youden indeksi kullanılarak belirlenmiştir. Tüm istatistiksel analizlerde $p<0.05$ değeri istatistiksel olarak anlamlı kabul edilmiştir. Bu çalışma için gerekli etik onay, Pamukkale Üniversitesi Tıp Fakültesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu'ndan alınmıştır (Tarih: 03.06.2025, Karar No: E-60116787-020-703297).

BULGULAR

LBS'den Ocak 2023–Aralık 2024 tarihleri arasında belirlenen kriterlere uygun toplam 4596 veri elde edilmiştir. Verileri değerlendirilen çocuk hastaların 64'ü kız, 90'ı erkek olup yaşları 2–18 yıl arasında değişmekteydi. LBS'den 154 çocuk hastaya ait toplam 309 eş zamanlı VPA ve hemogram sonucu alınmıştır. Bazı hastaların birden fazla başvurusu bulunması nedeniyle, aynı hastaya ait birden fazla eş zamanlı VPA ve hemogram sonucu mevcuttu. Serum VPA düzeylerine göre yapılan sınıflandırmada, <50 mg/L olanlar 1. grup, 50–100 mg/L arasında olanlar 2. grup ve >100 mg/L olanlar toksik doz grubu (3. grup) olarak tanımlanmıştır. Buna göre 1. grupta 98, 2. grupta 184 ve 3. grupta 27 ölçüm yer almıştır. Gruplar arası karşılaştırmalara ait ayrıntılı veriler Tablo 1'de sunulmuştur. Gruplar birbiriyle karşılaştırıldığında, 1. ve 2. gruplar arasında platelet sayısı açısından istatistiksel olarak anlamlı fark saptanmıştır ($p=0,0001$). 1. ve 3. grupların karşılaştırılmasında bazofil sayısı ($p=0,037$), platelet sayısı ($p=0,0001$), PLO ($p=0,001$), PIV ($p=0,027$) ve SII ($p=0,003$) değerleri istatistiksel olarak anlamlı bulunmuştur. 2. ve 3. gruplar karşılaştırıldığında ise platelet sayısı ($p=0,0001$), PLO ($p=0,001$), PIV ($p=0,027$) ve SII ($p=0,003$) açısından istatistiksel olarak anlamlı fark gözlenmiştir. SIRI ve NLPO indeksleri üç grup arasında istatistiksel olarak anlamlı farklılık göstermemiştir (Tablo 1).

Hematolojik parametreler ve bu parametrelerden elde edilen inflamatuvar oranlar için yapılan ROC analizinde, eğri altında kalan alan (AUC) değerleri Tablo 2'de sunulmuştur. Buna göre en yüksek AUC değeri platelet sayısına ait olup 0,72 olarak saptanmıştır. Bunu sırasıyla PLO (0,695), SII (0,689) ve PIV (0,658) izlemiştir. PIV ve SII için ROC eğrileri Şekil 1'de gösterilmiştir.

Tablo 1. Hemogram testleri ve testlerden elde edilen oranların valproik asit düzeyine göre ayrılmış üç grup içinde karşılaştırılması

Table 1. Comparison of hemogram parameters and derived ratios among three groups stratified by Valproic acid level

Hemogram testleri ve oranlar		Valproik Asit Düzeyi			p
		<50 mg/L (1)	50- 100 mg/L (2)	>100 mg/L (3)	
	n	98	184	27	
Bazofil sayısı (K/uL)	A.O. ± S.S.	0,034 ± 0,0169	0,0334± 0,0216	0,0248 ± 0,0126	0,037* (kw=6,598) (1-3)
	Med (IQR)	0,03 (0,02 - 0,04)	0,03 (0,02 - 0,04)	0,02 (0,015 - 0,03)	
Eozinofil sayısı (K/uL)	A.O. ± S.S.	0,25 ± 0,22	0,22 ± 0,2	0,22 ± 0,26	0,161 (kw=3,655)
	Med (IQR)	0,19 (0,11 - 0,33)	0,15 (0,09 - 0,31)	0,12 (0,07 - 0,32)	
Hemoglobin(g/dL)	A.O. ± S.S.	12,71 ± 1,54	12,75 ± 1,29	12,71 ± 1,19	0,977 (F=0,023)
	Med (IQR)	12,8 (11,55 - 13,75)	12,7 (12 - 13,6)	12,6 (11,7 - 13,6)	
Lenfosit sayısı (K/uL)	A.O. ± S.S.	3,1 ± 1,06	2,94 ± 1,13	3,13 ± 1,01	0,43 (kw=1,689)
	Med (IQR)	2,91 (2,33 - 3,71)	2,75 (2,18 - 3,49)	2,77 (2,48 - 3,85)	
Monosit(K/uL)	A.O. ± S.S.	0,57 ± 0,21	0,61 ± 0,27	0,58 ± 0,19	0,515 (kw=1,325)
	Med (IQR)	0,54 (0,44 - 0,66)	0,55 (0,45 - 0,69)	0,56 (0,47 - 0,7)	
Nötrofil Sayısı (K/uL)	A.O. ± S.S.	3,68 ± 1,94	3,63 ± 2,01	3,02 ± 1,33	0,443 (kw=1,627)
	Med (IQR)	3,17 (2,47 - 4,05)	3,15 (2,43 - 4,21)	2,87 (2,36 - 3,95)	
Platelet sayısı (K/uL)	A.O. ± S.S.	285,4 ± 75,21	247,38 ± 82,85	199,64 ± 63,54	0,0001* (kw=29,644) (1-2, 1-3, 2-3)
	Med (IQR)	273 (224,5 - 341,5)	240 (186,5 - 296,5)	205 (156,5 - 227,5)	
Eritrosit (RBC) sayısı (M/uL)	A.O. ± S.S.	4,56 ± 0,5	4,48 ± 0,5	4,33 ± 0,5	0,103 (F=2,295)
	Med (IQR)	4,54 (4,23 - 4,87)	4,45 (4,17 - 4,82)	4,32 (4,04 - 4,61)	
Eritrosit Dağılım Genişliği (RDW-SD) (fL)	A.O. ± S.S.	13,98 ± 1,5	13,83 ± 1,54	13,85 ± 1,41	0,553 (kw=1,186)
	Med (IQR)	13,7 (12,9 - 14,65)	13,5 (12,9 - 14,2)	13,5 (12,8 - 14,9)	
Beyaz küre (WBC) sayısı (K/uL)	A.O. ± S.S.	7,64 ± 2,41	7,43 ± 2,54	6,98 ± 1,75	0,596 (kw=1,035)
	Med (IQR)	7,08 (6,2 - 8,7)	6,93 (5,9 - 8,29)	6,79 (5,55 - 8,05)	
Nötrofil /Lenfosit oranı (NLO)	A.O. ± S.S.	1,32 ± 0,81	1,46 ± 1,13	1,08 ± 0,66	0,234 (kw=2,907)
	Med (IQR)	1,18 (0,71 – 1,61)	1,15 (0,79 – 1,63)	1,05 (0,58 – 1,41)	
Platelet/Lenfosit oranı (PLO)	A.O. ± S.S.	101,15 ± 37,59	94,9 ± 44,9	70,46 ± 32,72	0,001* (kw=14,361) (1-3, 2-3)
	Med (IQR)	98,25 (73,9 - 121,57)	87,38 (66,46 - 110,36)	67,79 (43,7 - 90)	
Nötrofil*Lenfosit/Platelet oranı (NLPO)	A.O. ± S.S.	0,49 ± 0,31	0,67 ± 0,7	0,6 ± 0,47	0,096 (kw=4,683)
	Med (IQR)	0,44 (0,24 - 0,64)	0,47 (0,3 - 0,79)	0,52 (0,28 - 0,7)	
SIRI (Nötrofil*Monosit/Lenfosit oranı)	A.O. ± S.S.	0,82 ± 0,84	0,99 ± 1,26	0,68 ± 0,61	0,322 (kw=2,266)
	Med (IQR)	0,57 (0,4 - 0,93)	0,64 (0,4 - 0,97)	0,55 (0,35 - 0,8)	
PIV (Nötrofil*Monosit*Platelet/ Lenfosit Oranı)	A.O. ± S.S.	242,25 ± 267,36	258,83 ± 376,12	150,13 ± 189,8	0,027* (kw=7,216) (1-3, 2-3)
	Med (IQR)	152,01 (94,35 - 262,93)	140,35 (93,37 - 254,43)	93,07 (49,66 - 181,44)	
SII (Platelet*Nötrofil/Lenfosit Oranı)	A.O. ± S.S.	382,01 ± 289,51	363,9 ± 324,75	228,19 ± 198,7	0,003* (kw=11,478) (1-3, 2-3)
	Med (IQR)	314,33 (196,15 - 457,08)	264,28 (191,91 - 392,97)	155,6 (102,44 - 301,13)	

*p<0,05 istatistiksel olarak anlamlı farklılık; A.O: Aritmetik ortalama; S.S: Standart sapma; Med (IQR): Ortanca (25. – 75. yüzdelikler); F: Tek Yönlü varyans analizi; kw: Kruskal Wallis Varyans analizi; 1-2: 50 altı ve 50-100 arası gruplar arası anlamlı farklılık; 1-3: 50 altı ve 100 üzeri arası gruplar arası anlamlı farklılık; 2-3: 50-100 ve 10 üzeri arası gruplar arası anlamlı farklılık

Tablo 2. Hemogram sonuçları ve bu sonuçlardan elde edilen oranların 95% güven aralığında eğri altında kalan alanları

Table 2. Areas under the curve with 95% confidence intervals for hemogram parameters and derived ratios

Analitler	EAA	Std, Hata	p	%95 G,A, Alt - Üst
Bazofil sayısı (K/uL)	0,646	0,054	0,015*	0,54 - 0,753
Eozinofil sayısı (K/uL)	0,569	0,064	0,255	0,443 - 0,694
Hemoglobin(g/dL)	0,509	0,057	0,887	0,397 - 0,62
Lenfosit sayısı (K/uL)	0,461	0,055	0,52	0,352 - 0,57
Monosit(K/uL)	0,476	0,06	0,691	0,359 - 0,593
Nötrofil Sayısı (K/uL)	0,576	0,056	0,21	0,466 - 0,686
Platelet sayısı (K/uL)	0,72	0,049	0,0001*	0,623 - 0,817
Eritrosit (RBC) sayısı (M/uL)	0,606	0,06	0,08	0,488 - 0,723
Eritrosit Dağılım Genişliği (RDW-SD) (fL)	0,5	0,066	0,998	0,371 - 0,629
Beyaz küre (WBC) sayısı	0,538	0,058	0,532	0,425 - 0,651
Nötrofil / Lenfosit oranı (NLO)	0,598	0,058	0,106	0,484 - 0,712
Platelet / Lenfosit oranı (PLO)	0,695	0,057	0,001*	0,583 - 0,808
Nötrofil x Lenfosit/Platelet oranı (NLPO)	0,478	0,061	0,721	0,36 - 0,597
SIRI (Nötrofil x Monosit / Lenfosit oranı)	0,57	0,058	0,247	0,457 - 0,683
PIV (Nötrofil x Monosit x Platelet / Lenfosit Oranı)	0,658	0,061	0,009*	0,538 - 0,777
SII (Platelet x Nötrofil / Lenfosit Oranı)	0,689	0,06	0,002*	0,573 - 0,806

*p<0,05 istatistiksel olarak anlamlı ayırt edicilik; EAA: eğri altında kalan alan (AUC); %95 G.A: %95 Güven Aralığı

TARTIŞMA

Retrospektif olarak kurgulanan bu çalışmada, hemogram parametrelerinden elde edilen SII ve PIV, VPA düzeylerinin 50–100 mg/L ile >100 mg/L olma durumunu ayırt etmede etkili olduğu gösterilmiştir. Kumar ve arkadaşlarının yayımladığı bir derlemede, VPA tedavisinin çeşitli koagülopatik yan etkilere yol açtığı ve bunlar arasında en sık görülenin trombositopeni olduğu bildirilmiştir (18). Buoli ve arkadaşları, "VPA" ve "trombositopeni" anahtar kelimeleri ile en az dört veri tabanında yaptıkları taramada 159 özet metni incelemiş ve dokuz çalışma dışında kalan tüm araştırmalarda, uzun süreli VPA tedavisinin (>9 ay) trombosit sayısında 130.000/μL ve altına düşüşe neden olduğunu ortaya koymuştur (19). Delgado ve arkadaşları, 306 çocuk hasta ile gerçekleştirdikleri çalışmada %21 oranında trombositopeni geliştiğini, 64 hastanın 32'sinde trombosit sayısının mm³'te 100.000'in altında olduğunu ve sekiz hastada kanama bulgularının görüldüğünü bildirmiştir. Araştırmacılar, ilaç dozundaki azalma ile trombosit sayısındaki artışın doğru orantılı olduğunu belirtmişlerdir (20).

Bu çalışmada da VPA düzeylerine göre oluşturulan üç grup karşılaştırıldığında platelet sayıları arasında istatistiksel olarak anlamlı fark saptanmış ve >100 mg/L VPA düzeyine sahip grupta ortalama platelet sayısı mm³'te 199,64 ± 63,54 olarak bulunmuştur. Bu bulgu, VPA tedavisinin trombosit sayısı üzerindeki etkilerine ilişkin mevcut literatürle uyumludur. VPA tedavisinin trombosit sayısı ve fonksiyonu üzerinde çeşitli etkileri olduğu bilinmektedir. Tedavi alan hastalarda yapılan kemik iliği incelemelerinde megakaryosit sayısının normal veya artmış bulunması, trombositopeninin kemik iliği baskılanmasından ziyade periferik trombosit yıkımının artmasına bağlı olabileceğini düşündürmektedir. Ayrıca VPA'nın trombosit membranı üzerine doğrudan toksik etki göstererek malondialdehit üretimini azaltması, trombosit bütünlüğünü ve yaşam süresini olumsuz etkileyebileceği bildirilmiştir. Bununla birlikte VPA'nın, fibrinojen bağlanmasının azalması ve P-selektin ekspresyonunun artması yoluyla trombosit agregasyonunu bozarak trombosit disfonksiyonuna neden olduğu, bu etkinin araşidonik asit yolu ve siklooksijenaz

aktivitesinin inhibisyonu sonucu tromboksan A sentezinin azalması ve ATP salınımının bozulması ile ilişkili olduğu belirtilmiştir (21).

Veri analizi ile gerçekleştirilen bu çalışmada, platelet ve lenfosit sayılarının oranlanmasıyla hesaplanan PLO, VPA düzeyi >100 mg/L olan grupta diğer iki gruba kıyasla anlamlı olarak düşük bulunmuştur. Literatürde antiepileptik ilaç kullanan bireylerde PLO oranını inceleyen tek çalışma olan Çağ ve arkadaşlarının çalışmasında, 17 karbamazepin, 21 VPA ve 22 levetirasetam kullanan hasta grubu 40 sağlıklı kontrol ile karşılaştırılmış; bu çalışmanın aksine PLO açısından anlamlı fark saptanmamıştır (14). VPA kullanan hastalarda PLO'yu özel olarak değerlendiren başka bir çalışmaya literatürde rastlanmamıştır. Epilepsi hastaları ile sağlıklı kontrollerin karşılaştırıldığı ve PLO oranlarının değerlendirildiği bir başka çalışmada ise PLO'nun epilepsi hastalarında sağlıklı bireylere göre daha yüksek olduğu bildirilmiştir; ancak bu çalışmada hastaların yalnızca VPA değil farklı tedavi modaliteleri de kullandığı belirtilmiştir (22). Hosseini ve arkadaşları, PubMed, Scopus ve Web of Science veri tabanlarında yaptıkları taramada, NLO epilepsi hastaları ile sağlıklı bireyleri ayırt etmede tanısal bir değere sahip olmadığını öne sürmüşlerdir (23). Bu çalışmada da VPA düzeylerine göre üç gruba ayrılarak yapılan NLO analizinde gruplar arasında anlamlı bir fark saptanmamıştır.

Bu çalışmada nötrofil, monosit ve lenfosit sayıları kullanılarak hesaplanan SIRI açısından gruplar arasında istatistiksel olarak anlamlı bir farklılık izlenmemiştir. Literatürde SIRI indeksinin epilepsi hastalarında değerlendirildiği başka bir çalışmaya rastlanmamış olup, bu açıdan elde edilen bulguların karşılaştırılabilmesi için ileri çalışmalara ihtiyaç bulunmaktadır. Mevcut literatürde SIRI'nin daha çok kanser hastalıklarında prognoz ve mortaliteyi öngörmede, ayrıca vasküler endotelyal hasar ile seyreden romatolojik hastalıklar ve koroner arter hastalıkları gibi klinik durumlarla ilişkili olduğu bildirilmektedir (24–26).

Bu çalışmada SII ve PIV değerleri üç farklı VPA doz grubunda karşılaştırıldığında, toksik doz grubu ile diğer gruplar arasında istatistiksel olarak anlamlı farklar saptanmıştır. Literatürde SII ve PIV oranlarını VPA düzeyleri ile birlikte değerlendiren bir çalışmaya rastlanmamıştır. Bu yönüyle, VPA düzeylerine göre gruplandırılarak kurgulanan bu çalışma, SII ve PIV oranlarının toksik VPA düzeyleri ile ilişkisini ilk kez ortaya koymaktadır. Bu oranların laboratuvar bilgi sistemlerine entegre edilmesi, özellikle VPA'nın toksik dozlarının öngörülmesinde laboratuvar uzmanları ve klinisyenler için yol gösterici olabilir.

Çalışmanın bazı kısıtlılıkları bulunmaktadır. Retrospektif tasarım nedeniyle preanalitik evreye ait olası hatalar değerlendirilememiştir. Hastaların ayrıntılı nörolojik muayene kayıtlarına ulaşılamadığından eşlik eden ilaç kullanımları ve olası ilaç-düzey etkileşimleri değerlendirilememiştir. Ayrıca C-reaktif protein (CRP) düzeyleri rutin olarak istenmediğinden inflamasyonun katkısı doğrudan analiz edilememiştir.

Özellikle kırsal bölgelerde yaşayan, ikinci ve üçüncü basamak sağlık kuruluşlarına erişimde ekonomik ve fiziksel güçlükler yaşayan ancak birinci basamak sağlık hizmetlerine ulaşabilen VPA kullanan hastalarda, basit hemogram testleri aracılığıyla ilaç düzey takibinin kolaylaştırılması mümkün olabilir. Büyük veri analizleri ile elde edilen anlamlı ilişkilerin, sağlık sistemlerinde giderek artan veri yükünün etkin şekilde işlenmesi ve hasta ile sağlık çalışanlarının yararına kullanılması açısından kaçınılmaz olduğu düşünülmektedir (27).

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Olgu Sunumu / *Case Report*

A Case of Unexpected Elevated Bilirubin Levels in Urine

İdrarda Beklenmeyen Bilirubin Yüksekliği Vakası

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ABSTRACT

Complete urinalysis is commonly requested by clinicians, because it provides important information about diabetes, liver or urinary system diseases, hydration, urinary tract infections and many other clinical conditions. The results of complete urinalysis can be influenced by various factors, such as diet, dietary supplements and medications. In this case report, a false-positive urine bilirubin result caused by taking an anti-inflammatory drug containing etodolac was presented.

Keywords: Bilirubin, Etodolac, Interference, Complete urinalysis.

ÖZET

Tam idrar analizi diyabet, karaciğer veya üriner sistem hastalıkları, hidrasyon, idrar yolu enfeksiyonları ve birçok klinik durum hakkında önemli bilgiler sağladığı için klinisyenler tarafından sıklıkla istenir. Tam idrar analizi sonuçları diyet, gıda takviyeleri ve ilaç tedavileri dahil olmak üzere birçok faktörden etkilenebilir. Bu vaka sunumunda idrarda etodolak etken maddeli antienflamatuvar bir ilaç alımı kaynaklı yanlış pozitif idrar bilirubin vakası sunuldu.

Anahtar sözcükler: Bilirubin, Etodolak, İnterferans, Tam idrar analizi.

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Etik onay: Hastadan bilgilendirilmiş onam formu alınmıştır

INTRODUCTION

Complete urinalysis is a non-invasive, rapid, and cost-effective diagnostic tool in clinical practice (1). It is widely used for the diagnosis and clinical monitoring of various systemic and urinary disorders. Under normal physiological conditions, bilirubin is absent in urine. Its presence is always considered pathological and may indicate an underlying hepatobiliary disorder.

Dipstick tests used for urinalysis may yield false-positive or false-negative results in urinary bilirubin detection (2). Inaccurate urinalysis results are often caused by pre-analytical errors such as improper storage conditions, delayed transport, or interference from certain medications and their metabolites (3). Several drugs that interfere with urinary bilirubin testing have been reported in the literature (4). In this case report, we present a patient with a false-positive urinary bilirubin test result associated with the use of a nonsteroidal anti-inflammatory drug containing etodolac.

CASE PRESENTATION

A 60-year-old male patient was referred to our laboratory during routine check-ups because of +3 urinary bilirubin positivity, despite a serum total bilirubin level of 0.43 mg/dL and a direct bilirubin level of 0.20 mg/dL. The patient had no known comorbidities or abnormal physical examination findings apart from hypothyroidism, hyperlipidemia, and benign prostatic hypertrophy. The patient's clinical chemistry and complete urinalysis test results are presented in Table 1.

Complete urinalysis was performed using both the DIRUI FUS-200 and DIRUI FUS-2000 urine analyzers (DIRUI Industrial Co., China). No pathological findings were detected other than +3 bilirubin positivity, and further investigation was initially deemed unnecessary based on the remaining medical laboratory test results. In addition, the physical characteristics of the urine did not support the presence of bilirubinuria (Figure 1).

To rule out pre-analytical and analytical errors, a fresh spot urine sample was requested from the patient on the same day. In this sample, bilirubin was detected as +2 positive using both the DIRUI FUS-200 and DIRUI FUS-2000 urine analyzers, as well as by manual urine dipstick testing. Due to suspicion of a false-positive urinary bilirubin result, the patient was asked whether he was using any herbal products, dietary supplements, or medications. The patient reported the use of a nonsteroidal anti-inflammatory drug containing etodolac. Furthermore, review of the assay kit package insert revealed that etodolac metabolites may interfere with urinary bilirubin testing and potentially lead to false-positive results.

Figure 1. The patient's urine specimen collected in the etodolac treatment period

Şekil 1. Etodolak tedavisi döneminde toplanan hastaya ait idrar örneği

Table 1. Clinical chemistry and complete urinalysis test results of the patient
Tablo 1. Hastanın klinik kimya ve tam idrar tahlili test sonuçları

Parameters	Results	Reference Values
Glucose, mg/dL	101	70-110
Urea, mg/dL	15.50	6-20
Creatinine, mg/dL	0.85	0.7-1.2
AST, U/L	15	0-37
ALT, U/L	13	0-41
ALP, U/L	109	0-129
GGT, U/L	19	10-71
Total bilirubin, mg/dL	0.43	0-1.1
Direct bilirubin, mg/dL	0.20	0-0.3
Indirect bilirubin, mg/dL	0.23	0-0.8
Na^+, mEq/L	140	135-145
K^+, mEq/L	4.90	3.5-5.1
Cl^-, mEq/L	101	98-107
Complete urinalysis		
Color	Light yellow	Light yellow
Clarity	Clear	Clear
Specific gravity	1018	1015-1025
pH	6	5.0-8.5
Glucose	Negative	Negative
Protein	Negative	Negative
Ketone	Negative	Negative
Bilirubin	+3	Negative
Urobilinogen	Normal	Normal
Nitrite	Negative	Negative
Ascorbic acid	Negative	Negative
Blood	Negative	Negative
Leukocyte esterase	Negative	Negative
Red blood cell/HPF	1	0-3
White blood cell/HPF	1	0-5

DISCUSSION

Etodolac is a nonsteroidal anti-inflammatory drug with relatively high selectivity for cyclooxygenase-2 (COX-2) (5). It is primarily metabolized in the liver through hydroxylation and glucuronidation and is excreted mainly via the kidneys in urine, with a smaller proportion eliminated through the bile.

The reaction of bilirubin with diazotized sulfanilic acid, known as the diazo (Van den Bergh) reaction, was introduced by Van den Bergh in 1918 and is widely used for the quantitative measurement of bilirubin in serum and urine. In this reaction, two isomeric colored azo pigments are formed, exhibiting maximum absorbance at 530 nm. The intensity of the resulting color is directly proportional to the bilirubin concentration (6).

Numerous conditions that interfere with urinary bilirubin measurement have been described. For example, ascorbic acid, prolonged storage time, and light exposure may cause false-negative results, whereas the presence of urine-coloring pigments, elevated urobilinogen levels, and various medical therapies may lead to false-positive results. One of the medications known to

cause false-positive urinary bilirubin results is etodolac (7). In such cases, electron-rich regions of the phenolic ring may be attacked by the electrophilic diazonium salt, resulting in the formation of a colored product.

Sho et al. analyzed urine samples from patients with normal serum bilirubin levels but positive urinary bilirubin results using high-performance liquid chromatography (HPLC). Three positive fractions were obtained following extraction of etodolac metabolites with ethyl acetate. The HPLC retention times of two of these fractions corresponded to the 6-hydroxy and 7-hydroxy derivatives of etodolac metabolites (8). In another case report, a 14-year-old girl exhibited a positive urine dipstick bilirubin result during etodolac treatment (9).

Medical therapies can affect certain clinical chemistry test results, such as complete urinalysis. In cases of unexpected or clinically inconsistent urinalysis findings, medication use should be carefully reviewed, and clinicians should be informed about the possibility of false-positive or false-negative results. In patients with unexplained urinary bilirubin positivity and normal liver function test results, etodolac therapy should be considered as a potential interfering factor. This approach may help prevent unnecessary additional testing and invasive procedures. Furthermore, the duration of false-positive urinary bilirubin results in patients using etodolac can be determined through follow-up evaluation.

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Conflict of Interest

The authors declare no conflicts of interest.

Informed consent was obtained from the participant.

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Olgu Sunumu / *Case Report*

From Laboratory Clue to Diagnosis: Multiple Myeloma

Laboratuvar İpucundan Tanıya: Multipl Miyelom

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ABSTRACT

Preanalytical irregularities in serum separator tubes are frequently regarded as technical artifacts. In this case, repeated failure of serum separation led to the diagnosis of multiple myeloma. A 77-year-old woman presented with chest pain and fatigue, and successive serum samples failed to form an adequate gel barrier, yielding only minimal serum despite prolonged centrifugation. Biochemical analyses revealed hypoalbuminemia, elevated total protein, increased IgA with suppressed IgM and IgG levels, and a markedly decreased kappa/lambda ratio. Serum and urine immunofixation identified an IgA–lambda monoclonal band. PET-CT demonstrated multiple lytic bone lesions. Bone marrow biopsy revealed a hypercellular marrow with 80% plasma cell infiltration, confirming the diagnosis of multiple myeloma. These findings underscore that abnormal serum separation, often dismissed as a technical issue, may serve as an early indicator of underlying hematologic malignancy.

Keywords: Multiple myeloma, Serum separator tube, Preanalytical error, Immunofixation, Hyperproteinemia.

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ÖZET

Serum ayırıcı tüplerde görülen preanalitik düzensizlikler sıklıkla teknik artefaktlar olarak değerlendirilir. Bu olguda, tekrarlayan serum ayrışma başarısızlığı multipl miyelom tanısına yol açmıştır. Yetmiş yedi yaşındaki bir kadın hasta göğüs ağrısı ve halsizlik şikâyetleriyle başvurmuş, ardışık serum örneklerinde yeterli jel bariyer oluşmamış ve uzun süreli santrifügasyona rağmen yalnızca minimal miktarda serum elde edilmiştir. Biyokimyasal analizlerde hipoalbuminemi, artmış total protein, IgM ve IgG baskılanması ile birlikte artmış IgA düzeyi ve belirgin derecede azalmış kappa/lambda oranı saptanmıştır. Serum ve idrar immünfiksasyonunda IgA–lambda monoklonal bant tespit edilmiştir. PET-BT incelemesinde çok sayıda litik kemik lezyonu gösterilmiştir. Kemik iliği biyopsisinde %80 plazma hücresi infiltrasyonu ile hipersellüler bir kemik iliği saptanmış ve multipl miyelom tanısı doğrulanmıştır. Bu bulgular, sıklıkla teknik bir sorun olarak göz ardı edilen anormal serum ayrışmasının, altta yatan hematolojik malignitenin erken bir göstergesi olabileceğini vurgulamaktadır.

Anahtar Kelimeler: Multipl miyelom, Serum ayırıcı tüp, Preanalitik hata, İmmünfiksasyon, Hiperproteinemi.

INTRODUCTION

Gel barrier displacement or failure to form in serum separator tubes is predominantly considered a pre-analytical artifact (1). Nevertheless, such anomalies may occasionally serve as harbingers of underlying severe pathologies (2). The localization of the gel barrier is influenced by multiple factors, including sample viscosity, specific gravity, tube composition, and laboratory handling conditions. Additionally, patient-specific variables such as heparin therapy, anemia, elevated plasma protein concentrations, or sample contamination may precipitate gel barrier failure (3). This case report delineates the diagnosis of multiple myeloma in a 77-year-old female patient, subsequent to recurrent gel barrier failure in serum separator tubes.

Case Presentation

A 77-year-old female patient initially presented to an external medical center with complaints of chest pain, fatigue, generalized weakness, and musculoskeletal pain, mainly affecting both legs. She reported increased sleep duration, loss of appetite, and approximately 7 kg of weight loss over the past three months. In addition, she described recurrent episodes of nasal bleeding and severe leg pain during the last three days.

On physical examination, no jaundice, cyanosis, or clubbing was observed. Vital signs were stable (heart rate: 84/min, blood pressure: 130/80 mmHg). Cardiovascular and abdominal examinations were within normal limits. Based on the initial evaluation at the external center, a need for blood transfusion was identified, and the patient was referred to our hospital for further investigation and management.

The initial blood sample was collected in a Greiner Bio-One VACUETTE Serum Sep Clot Activator tube (Lot A240839S) and centrifuged at 2000 × g for 10 minutes. Post-centrifugation, gel barrier formation was absent. Despite prolonging the centrifugation duration, the anomaly persisted. (Figure 1) A subsequent blood draw yielded minimal serum from the upper fraction. Biochemical analyses, including albumin, total protein, immunoglobulins, and free light chains, were performed using the Beckman Coulter AU5800 analyzer (Beckman Coulter Inc., Brea, CA, USA). Serum and urine immunofixation electrophoresis were performed using the Helena SAS-1 Plus system (Helena Biosciences, Sunderland, UK).

Oncologic PET using F-18 fluorodeoxyglucose (FDG), performed at diagnosis, revealed a mildly hypermetabolic lytic lesion measuring 30 × 17 mm located at the anterior aspect of the right iliac bone. Additionally, multiple lytic hypodense lesions exhibiting mild to minimal intramedullary hypermetabolism were observed throughout the skeletal system, with more prominent

involvement of the calvarium, bilateral humeri, pelvic bones, and femora. Subsequently, a bone marrow biopsy was performed.

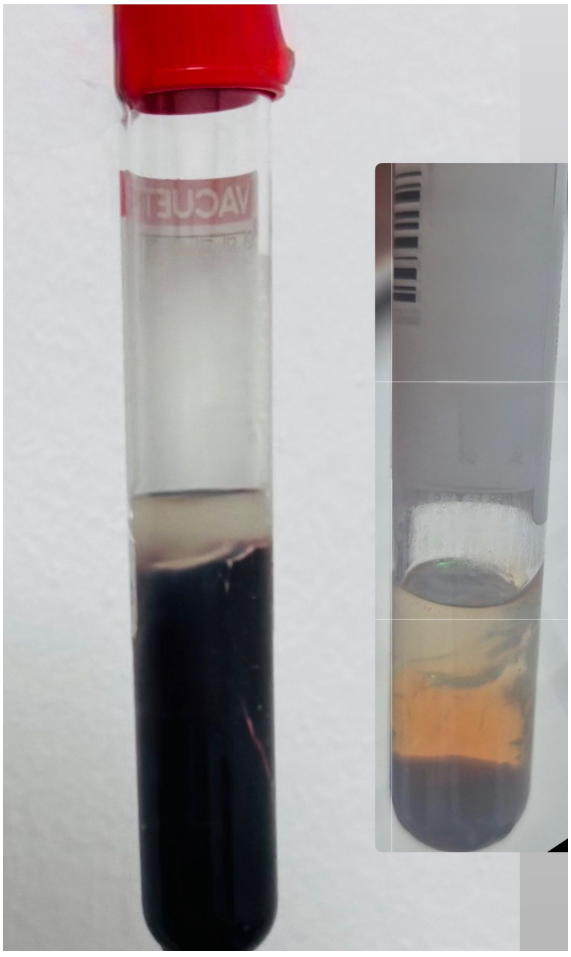

Figure 1. Abnormal floating separator gel.
Şekil 1. Ayırıcı Jelde Anormal Konumlanma

Laboratory Findings

Serum biochemistry revealed hypoalbuminemia (23 g/L; reference range: 35–52 g/L) concomitant with hyperproteinemia (119 g/L; reference range: 66–83 g/L). Immunoglobulin profiling demonstrated markedly elevated IgA (16.68 g/L; reference range: 0.7–4 g/L), with depressed IgM (0.20 g/L; reference range: 0.4–2.3 g/L) and IgG (2.07 g/L; reference range: 7–16 g/L) concentrations. Free light chain assays showed reduced kappa chains (1.48 mg/L; reference range: 2.37–20.73 mg/L) and normal lambda chains (26.42 mg/L; reference range: 4.23–27.69 mg/L), resulting in a markedly decreased kappa/lambda ratio of 0.056 (reference range: 0.22–1.74). Serum and urine immunofixation electrophoresis revealed an IgA lambda monoclonal band, representing a laboratory finding compatible with a monoclonal gammopathy (Figure 2). A differential diagnosis of multiple myeloma was considered, and to confirm it, a bone marrow biopsy was performed, which revealed plasma cell dyscrasia consistent with multiple myeloma. Additionally, the bone marrow biopsy showed a hypercellular marrow with 80% plasma cells and 3% blasts. Based on these findings, a definitive diagnosis of multiple myeloma was established.

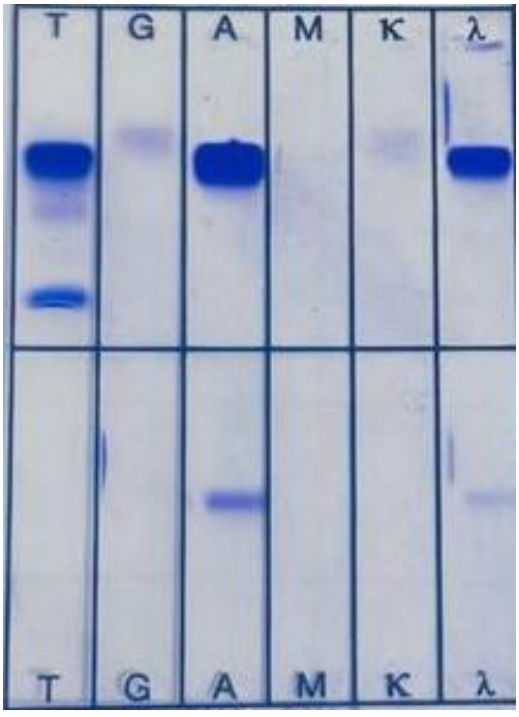

Figure 2. IgA lambda monoclonal band on serum and urine immunofixation electrophoresis.
Şekil 2. Serum ve idrar immünofiksasyon elektroforezinde IgA lambda monoklonal band.

DISCUSSION

Failure or aberrant positioning of the gel barrier in serum separator tubes is commonly attributed to preanalytical technical errors. However, accumulating evidence suggests that such abnormalities may serve as early indicators of hematologic

malignancies and other serious clinical conditions (1,2,4). In particular, gel barrier failure has been repeatedly reported in patients with multiple myeloma, a disorder characterized by excessive paraprotein production and increased plasma viscosity (3,5,6).

In multiple myeloma, overproduction of monoclonal immunoglobulins and free light chains alters the physicochemical properties of serum, including density and viscosity, thereby interfering with normal gel barrier migration during centrifugation. Tan et al. described serum separator tube gel failures associated with elevated IgA concentrations, findings that closely parallel the present case and emphasize the importance of heightened awareness in clinical laboratory practice (1).

Similarly, Kim et al demonstrated that discrepancies between serum separator and plasma tubes in immunoglobulin measurement are influenced by both tube composition and serum viscosity, highlighting the critical role of appropriate tube selection in laboratory diagnostics (2). Demir et al. reported abnormal gel flotation in hemodialysis patients with hyperproteinemia, further supporting the notion that elevated protein concentrations disrupt proper gel positioning (3).

Increased serum viscosity, a hallmark of hyperproteinemic states, has been strongly implicated in serum separator tube failures. Kisselev et al. identified hyperviscosity syndrome as a key contributor to separation errors, adversely affecting assay reliability (5). Likewise, Smith et al. demonstrated that elevated viscosity promotes gel barrier displacement during centrifugation, underscoring its relevance to preanalytical quality control (7). Collectively, these findings indicate that excessive serum protein levels disturb the physicochemical equilibrium within serum separator tubes.

In addition to patient-related factors, variability in tube manufacturing and gel composition may influence gel barrier performance. Jones et al. showed that differences in gel chemistry and density among manufacturers affect gel stability and protein electrophoresis outcomes (8). Lee et al. further reported reduced reliability of certain tube brands in samples with high protein content, often necessitating repeat testing (9). These observations underscore the importance of judicious tube selection in clinical laboratories.

In the present case, all samples were collected using the same type of serum separator tube, ensuring consistency in tube composition. Therefore, the observed gel barrier failure cannot be attributed to inter-tube variability. Nevertheless, samples were obtained at different time points during the patient's clinical evaluation. Minor temporal changes in serum viscosity—potentially related to fluctuations in paraprotein concentrations or slight delays between sample collection and centrifugation—may have contributed to variations in gel migration. Despite these potential factors, the predominant cause of the abnormal separation was most likely the markedly elevated serum protein concentration associated with multiple myeloma.

Brown et al. emphasized the diagnostic value of preanalytical abnormalities in serum separator tubes as early warning signs of hematologic malignancies. Prompt recognition of such anomalies may facilitate earlier diagnosis and improve clinical outcomes (10).

Additional patient-related factors, including anemia, anticoagulant therapy, and contamination with contrast agents, may also contribute to gel barrier abnormalities (11,12). Accordingly, preanalytical protocols should be adapted to account for individual patient characteristics.

In conclusion, heightened awareness of serum separator gel anomalies during the pre-analytical phase is essential for laboratory personnel. Such vigilance facilitates the early detection of life-threatening conditions like multiple myeloma.

Limitations and Contributions

A limitation of this study is that it is based on a single patient case, which constrains the assessment of variability in pre-analytical and analytical conditions. Factors such as serum viscosity, sample handling, and centrifugation parameters were observed qualitatively rather than systematically quantified. Despite this, the study underscores the importance of monitoring gel barrier behavior as apotential indicator of altered serum physicochemical properties, such as those seen in multiple myeloma. By linking repeated gel barrier failure to a specific hematologic malignancy, this report contributes methodologically by highlighting a practical pre-analytical clue that can inform laboratory quality control and early diagnostic vigilance.

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Conflict of Interest

The authors declare no conflicts of interest.

Informed consent was obtained from the participant.

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