ORIGINAL ARTICLE

# The utilization of serum thrombopoietin levels as an early biomarker in determining severe acute biliary pancreatitis

**Ahmet Sencer Ergin,[1] Andaç Uzdoğan,[2] Serap Gültekin,[3] Turan Turhan,[2] Özgür Akgül[4]**

[1]Department of General Surgery, Bağcılar Training and Research Hospital, İstanbul-*Türkiye*
[2]Biochemistry Clinic, Ankara Bilkent City Hospital, Ankara-*Türkiye*
[3]Anesthesiology Clinic, Marmaris State Hospital, Muğla-*Türkiye*
[4]Department of General Surgery, Ankara Bilkent City Hospital, Ankara-*Türkiye*

## ABSTRACT

**BACKGROUND:** This study aimed to evaluate the efficacy of thrombopoietin (TPO), a growth factor and acute-phase reactant, as an early prognostic marker for predicting disease severity in patients with acute biliary pancreatitis.

**METHODS:** A total of 72 patients with acute pancreatitis admitted to the Ankara Numune Training and Research Hospital, General Surgery Department, were included in the study. The severity of acute pancreatitis was classified using the 2012 Revised Atlanta Classification, and blood samples were collected from each patient within the first six hours of hospitalization to measure TPO levels. TPO levels were then compared to C-reactive protein (CRP) levels and other prognostic scoring systems.

**RESULTS:** According to the Atlanta Classification, TPO levels were found to be statistically significant in distinguishing severe pancreatitis from moderate and mild cases. When evaluating the sensitivity and specificity ratios of serum TPO levels in predicting the severity of acute pancreatitis, a value of 81.61 pg/dL was identified, with a 86.6% sensitivity and 69% specificity. In our study, the accuracy of TPO levels in detecting severe pancreatitis was compared with other scoring systems. The Balthazar scoring system had the highest precision (area under the curve [AUC]: 0.905) in receiver operating characteristic (ROC) curve analysis for severe pancreatitis (95% confidence interval). Serum TPO levels were identified as the second strongest predictors of severe acute pancreatitis (AUC: 0.831).

**CONCLUSION:** These findings suggest that TPO is a valuable early marker and prognostic indicator for predicting disease severity in patients with acute biliary pancreatitis. However, further randomized studies with larger patient cohorts are still required.

**Keywords:** Acute biliary pancreatitis; thrombopoietin; prognostic marker.

## INTRODUCTION

Acute pancreatitis is one of the most common gastrointestinal diseases, with its incidence increasing in recent years due to advancements in diagnostic methods. This condition can lead to a variety of severe clinical presentations.[1,2] Approximately 80% of patients with acute pancreatitis develop a mild form of the disease, with a 5% mortality rate, while the mortality for severe acute pancreatitis can reach 30%.[1,2] Early determination of acute pancreatitis severity is crucial, as initial management during this period can influence disease progression and the duration of hospitalization.

According to the Atlanta Classification, revised in 2012, acute pancreatitis is categorized into three severity levels: mild, moderate, and severe.[3] In addition to this classification, several other systems are used to assess disease severity, including the Ranson classification, the Acute Physiology and Chronic Health Evaluation (APACHE II) scoring system, the Balthazar classification, and the Bedside Index for Severity in

Cite this article as: Ergin AS, Uzdoğan A, Gültekin S, Turhan T, Akgül Ö. The utilization of serum thrombopoietin levels as an early biomarker in determining severe acute biliary pancreatitis. Ulus Travma Acil Cerrahi Derg 2025;31:214-220.

Address for correspondence: Ahmet Sencer Ergin
General Surgery, Bağcılar Training and Research Hospital, İstanbul, Türkiye
E-mail: sencergin@gmail.com

Ulus Travma Acil Cerrahi Derg 2025;31(3):214-220 DOI: 10.14744/tjtes.2024.23583
Submitted: 08.07.2024 Revised: 05.10.2024 Accepted: 08.12.2024 Published: 03.03.2025
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Acute Pancreatitis (BISAP) score, which are among the most widely utilized methods. Furthermore, numerous studies have examined cytokines, inflammatory molecules, adhesion molecules, hormones, and proteins to evaluate the severity of acute pancreatitis in its early stages.[4-7]

Thrombopoietin (TPO) is the most potent cytokine involved in regulating platelet production in the body. It is synthesized primarily in the liver, kidney, and bone marrow via

interleukin-6 (IL-6) and functions as an acute phase reactant. [8,9] Various pathological conditions lead to increased serum TPO levels, including unstable angina, severe trauma, disseminated intravascular coagulation (DIC), and severe burns. A significant correlation has been observed between the severity of sepsis and elevated TPO levels in sepsis patients, indicating that TPO has strong potential as a disease severity indicator. [10] The present study evaluates the correlation between serum TPO levels at diagnosis and disease prognosis in patients diagnosed with acute biliary pancreatitis.

## MATERIALS AND METHODS

This study received approval from the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (Approval Number: E-17-1366, Date: 26.04.2017). Following ethical committee approval, 72 patients admitted to the hospital's emergency surgery department and diagnosed with acute biliary pancreatitis between July 1, 2017 and June 31, 2018, were included in this prospective study. Written informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki.

The presence of at least two of the three criteria outlined in the updated Atlanta Classification led to the diagnosis of acute biliary pancreatitis: 1) characteristic abdominal pain, 2) serum amylase and/or lipase levels greater than three times the upper limit of normal, and 3) characteristic findings on abdominal imaging, including magnetic resonance imaging, computed tomography, or ultrasonography.

Patients were excluded from the study if they had etiologies other than acute biliary pancreatitis (e.g., nonbiliary pancreatitis, chronic pancreatitis, post-endoscopic retrograde cholangiopancreatography [post-ERCP] pancreatitis), ultrasonography (USG) findings indicative of acute cholecystitis, comorbidities associated with increased TPO levels, age below 18 years, pregnancy, or insufficient data for analysis.

The following data were documented for all included patients: age, gender, laboratory results at 0, 24, and 48 hours after admission, fluid intake and follow-ups, presence of organ failure, pancreatic necrosis, local complications, pleural effusion evaluations, and mortality. Within the first six hours of admission, a blood sample was collected from each patient to assess serum TPO levels. After 30 minutes, the collected samples were centrifuged at 1000 rpm for 15 minutes to separate the serum. The separated serum samples were stored at -80 °C until enzyme-linked immunosorbent assay (ELISA) analysis. TPO levels in the blood were measured using commercial ELISA kits (Quantikine Human TPO Immunoassay, RayBiotech, USA).

Based on these findings, the patients were categorized into three groups: mild, moderate, and severe, according to the Atlanta Classification. Individuals in the mild group exhibited no organ failure or any local or systemic complications. In contrast, those in the moderate group experienced organ failure that resolved within 48 hours and/or had local or systemic complications. Patients classified as severe displayed persistent organ failure, defined as lasting longer than 48 hours.[3] Organ failure classification was conducted using the modified Marshall scoring system for organ dysfunction. The Ranson, Balthazar, and BISAP scores were calculated for all patients.

### Statistical Analysis

SPSS 15.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) and Microsoft Excel 2016 software were used for data analysis. Descriptive statistics for definitive variables were presented as mean and standard deviation (mean ± SD), while categorical variables were expressed as numbers and percentages. The Shapiro-Wilk test was used to assess whether continuous variables followed a normal distribution. The one-way analysis of variance (ANOVA) test was applied to compare the mean values of three customarily distributed continuous variables. Post hoc analysis was conducted to determine the source of any differences between the three groups. When data did not follow a normal distribution, the Wilcoxon test was used for comparisons among the three groups.

The predictive power of TPO levels, BISAP scores, Balthazar scores, Ranson scores, and C-reactive protein (CRP) levels for determining pancreatitis severity was assessed using receiver operating characteristic (ROC) analysis. The revised Atlanta Classification was used to evaluate severity. For variables with an area under the curve of 0.7 or greater, sensitivity and specificity values, positive likelihood ratio (+LR), negative likelihood ratio (-LR), positive predictive value (PPV), and negative predictive value (NPV) were determined. The highest sensitivity-to-specificity ratio was used to establish the cut-off point following ROC analysis.

## RESULTS

This study included a total of 72 patients, of whom 31.9% (23) were male. The median age of all patients was 60 years (range: 19-87). According to the Atlanta Classification, 58.3% (42) of the patients were classified as having mild acute pancreatitis, 33.3% (24) as moderate, and 8.3% (6) as severe. Two patients succumbed to the disease during follow-up and were

**Table 1.** Clinical features of patients with acute pancreatitis according to the Atlanta Classification

| | Revised Atlanta Classification 2012 | | |
|---|---|---|---|
| | **Mild** | **Moderate*** | **Severe**** |
| Age | 60.1 (21-87) | 59.7 (19-83) | 69.6 (48-82) |
| Sex (M/F) | 14/28 | 5/18 | 4/2 |
| Organ Dysfunction | 0/42 | 10/24 | 6/6 |
| Mortality | 0/42 | 0/24 | 2 (6) |
| CRP mg/L (0th-hour) | 24.4 (1-302) | 40.8 (1-298) | 168.3 (60-374) |
| CRP mg/L (48th-hour) | 74.9 (3-310) | 126.7 (1-293) | 400.6 (249-545) |
| TPO (pg/mL) | 81.47 (67.4-152.2) | 89.12 (80.0-105.1) | 110.4 (83.1-133.2) |

*Transient organ failure (<48 hours). **Persistent organ failure (>48 hours). TPO: Thrombopoietin; CRP: C-reactive protein.

**Table 2.** Distribution of scoring systems according to Atlanta Classification and mean thrombopoietin levels

| **Atlanta Classification** | **Mild** | | **Moderate** | | **Severe** | | **Mean TPO Level (pg/mL)** |
|---|---|---|---|---|---|---|---|
| | **Number** | **Distribution (%)** | **Number** | **Distribution (%)** | **Number** | **Distribution (%)** | |
| Ranson <3 | 35 | 48.61% | 15 | 20.83% | 0 | 0 | 86.40 |
| Ranson ≥3 | 7 | 9.72% | 9 | 12.50% | 6 | 8.33% | 86.41 |
| Balthazar <6 | 42 | 58.33% | 23 | 31.94% | 3 | 4.16% | 84.62 |
| Balthazar ≥6 | 0 | 0 | 1 | 1.38% | 3 | 4.16% | 116.82 |
| BISAP <3 | 42 | 58.33% | 16 | 22.22 | 0 | 0 | 83.98 |
| BISAP ≥3 | 0 | 0 | 8 | 11.11% | 6 | 8.33% | 96.46 |

classified as having severe pancreatitis. The cause of mortality was organ failure and infection that developed within the first few days of hospitalization. The clinical characteristics of patients, categorized according to the Atlanta Classification, are provided in Table 1.

During clinical follow-up, 16 patients experienced temporary or permanent organ failure, as determined by the modified Marshall scoring system. Pancreatic necrosis was detected in seven patients based on computed tomography scans. Table 2 presents a comparison of scoring systems distributions according to the Atlanta Classification, Ranson scores, BISAP scores, Balthazar scores, and mean TPO levels.

The results of the ROC curve analysis results for thrombopoietin levels and scoring systems predicting severe acute pancreatitis are summarized in Table 3. When comparing the predictive power of TPO levels with various scoring systems for diagnosing severe pancreatitis, the Balthazar scoring system demonstrated the highest accuracy, with an area under the curve (AUC) of 0.905 in predicting severe pancreatitis (95% confidence interval) (Fig. 1). Serum TPO levels emerged as the second-best predictor of severe acute pancreatitis, with an AUC of 0.831 (Fig. 2). The ROC curves for BISAP, Ranson, and CRP levels are presented in Figures 3, 4, and 5, respectively.

Table 4 presents the sensitivity and specificity ratios calculated using the cut-off values obtained by combining the highest sensitivity and specificity of the scoring systems. Although the AUC for the Balthazar scoring system was high, sensitivity, specificity, -LR, and NPV calculations based on the commonly used cut-off value of 6 yielded rates of 13%, 100%, 0.86%, and 53.48%, respectively. PPV and +LR could not be determined, indicating that this scoring method is unable to accurately identify severe pancreatitis patients based on this cut-off value. When calculations were performed using a cut-off value of 2, the threshold at which the maximum sensitivity and specificity were obtained in this study, the sensitivity, specificity, +LR, -LR, PPV, and NPV were 90%, 76.1%, 3.78%, 0.13%, 79%, and 88.3%, respectively (Table 4).

The serum TPO level at the time of admission for acute pancreatitis diagnosis was statistically significant in distinguishing severe pancreatitis from mild and moderate cases. However, no significant difference was observed between mild and moderate pancreatitis cases.

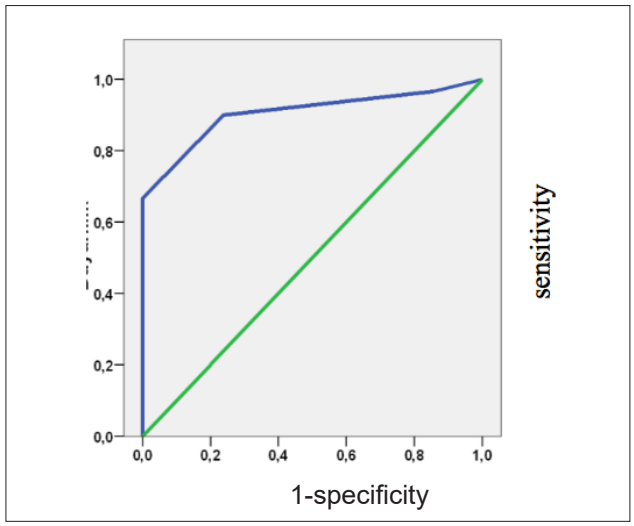


**Figure 1.** Receiver operating characteristic (ROC) curve of the Balthazar scoring system.

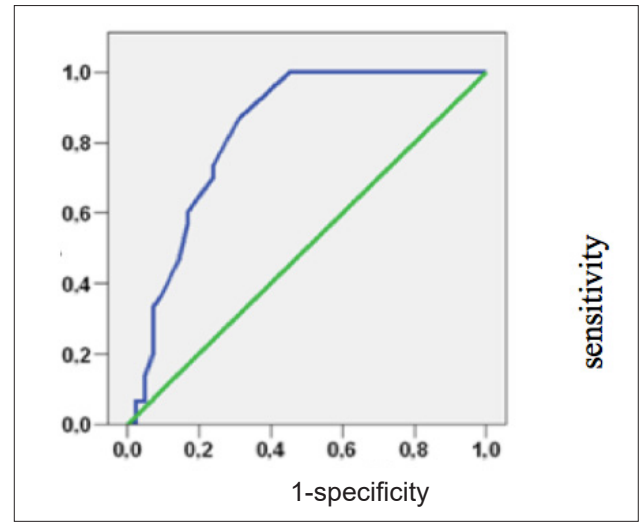


**Figure 2.** Receiver operating characteristic (ROC) curve of thrombopoietin.

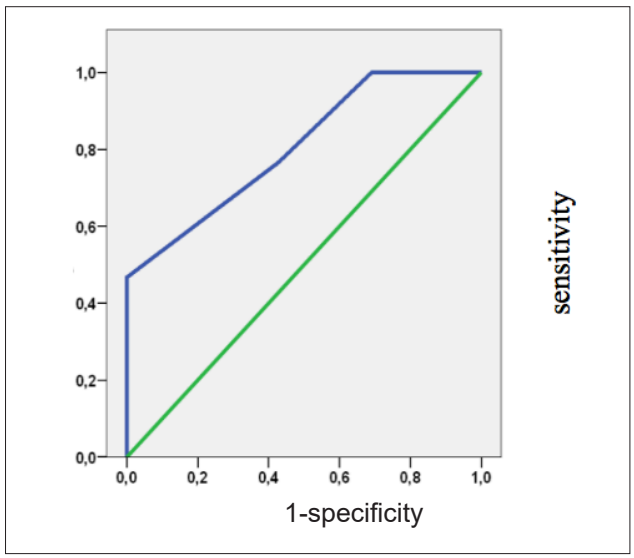


**Figure 3.** Receiver operating characteristic (ROC) curve of the Bedside Index for Severity in Acute Pancreatitis (BISAP).

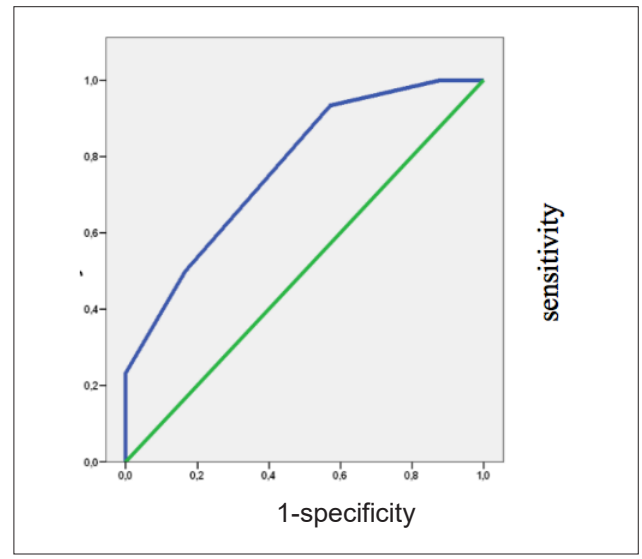


**Figure 4.** Receiver operating characteristic (ROC) curve of the Ranson score.

**Table 3.** Receiver operating characteristic (ROC) curve analysis of thrombopoietin and scoring systems in predicting severe acute pancreatitis

| | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | **AUC** | **Standard Error** | **P-value** | **Threshold** | **Upper Limit** |
| Thrombopoietin | 0.831 | 0.048 | <0.001 | 0.736 | 0.925 |
| BISAP | 0.805 | 0.052 | <0.001 | 0.704 | 0.906 |
| Balthazar | 0.905 | 0.041 | <0.01 | 0.824 | 0.986 |
| Ranson | 0.769 | 0.055 | <0.001 | 0.662 | 0.877 |
| CRP (0th-hour) | 0.636 | 0.069 | 0.050 | 0.500 | 0.772 |
| CRP (48th-hour) | 0.755 | 0.060 | <0.01 | 0.639 | 0.872 |

AUC: Area under the curve.

**Table 4.** Sensitivity and specificity analyses of scoring systems

| | Cut-off Value | Sensitivity | Specificity | +LR | -LR | PPV | NPV |
|---|---|---|---|---|---|---|---|
| BISAP[1] | ≥3 | 46% | 100.0% | - | 0.53 | - | 64.9% |
| BISAP[2] | ≥2 | 76.7% | 57.1% | 1.78 | 0.40 | 64.1% | 71.0% |
| BALTHAZAR[1] | ≥6 | 13% | 100.0% | - | 0.86 | - | 53.4% |
| BALTHAZAR[2] | ≥2 | 90.0% | 76.1% | 3.78 | 0.13 | 79.0% | 88.3% |
| RANSON | ≥3 | 50.0% | 83.3% | 3.00 | 0.60 | 74.6% | 62.4% |
| 48th-hour CRP | ≥200 | 36.6% | 92.8% | 5.13 | 0.68 | 81.8% | 58.9% |
| Thrombopoietin (pg/mL) | >81.61 | 86.7% | 69% | 2.79 | 0.19 | 73.6% | 83.8% |

[1]Cut-off values from the literature. [2]Cut-off values from this study with the highest sensitivity and specificity levels. LR: Likelihood ratio; PPV: Positive predictive value; NPV: Negative predictive value.

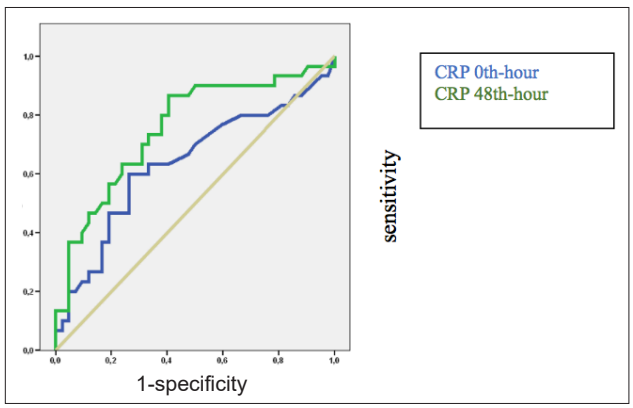


**Figure 5.** Receiver operating characteristic (ROC) curve of C-reactive protein (CRP).

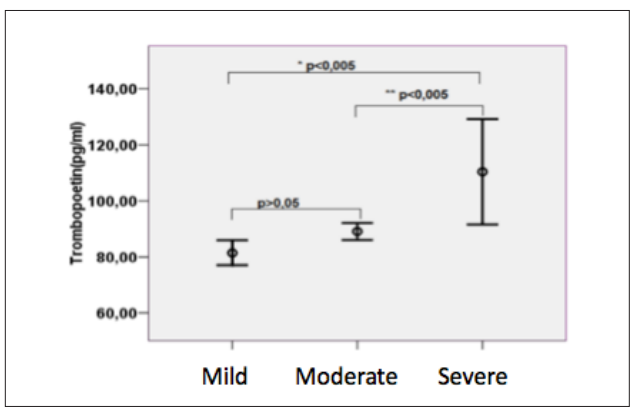


**Figure 6.** Box plot of thrombopoietin (TPO) levels and severity of pancreatitis.

When the sensitivity and specificity of serum TPO levels were analyzed to determine the severity of acute pancreatitis, and the highest sum of sensitivity and specificity was considered the cut-off value, 81.61 pg/dL was identified as the threshold. At this cut-off value, the sensitivity of serum TPO was 86.6%, specificity was 69%, +LR was 2.79, -LR was 0.19, PPV was 73.6%, and NPV was 83.8%. A box plot illustrating the relationship between TPO levels and pancreatitis severity is provided in Figure 6.

## DISCUSSION

Multifactorial scoring systems, radiological scores, and biochemical markers can assist in the early prediction of disease severity, pancreatic necrosis, and mortality in patients with acute pancreatitis. In this study, we compared several commonly used predictive tools with TPO to evaluate their effectiveness in predicting morbidity and mortality in acute pancreatitis. Our findings suggest that TPO can reliably indicate the severity of this condition in its early stages. To the best of our knowledge, this is the second prospective study in the literature to investigate the relationship between TPO levels and acute pancreatitis severity.

Various scoring systems have been developed to assess the severity of acute pancreatitis. These systems use clinical, laboratory, and radiological data to determine disease severity at an early stage. Some scoring systems require data at the time of admission, while others rely on data collected 48 hours later. Additionally, the large number of parameters in some of these scoring systems makes them difficult to recall and creates computational difficulties in practical application. Despite these limitations, they are still widely used due to the absence of more effective predictive parameters.

Despite its widespread use, the Ranson scoring system is not always considered sufficient for assessing the severity of acute pancreatitis, as demonstrated by numerous studies evaluating its effectiveness. In a prospective cohort study examining the effectiveness of multifactorial scoring systems in predicting hospital mortality, the sensitivity, specificity, and accuracy of the Ranson score for predicting severe acute pancreatitis were reported as 84.2%, 89.8%, and 94%, respectively. In another meta-analysis involving 1,300 patients, the Ranson score demonstrated a sensitivity of 75% and a specificity of 77%.[11,12] In a retrospective study involving 289 patients, the

BISAP score threshold for determining severe pancreatitis was found to be 3, with a sensitivity of 45.5%, specificity of 98.9%, positive predictive value of 76.9%, and negative predictive value of 95.8%. However, in a different study, the optimal BISAP score threshold was determined to be 2, with corresponding sensitivity and specificity of 61.4% and 83.1%, respectively.[13,14]

In 2002, Balthazar introduced a severity stratification method based on the extent of necrosis and pancreatic morphological changes observed on intravenous (IV) contrast-enhanced computed tomography (CT). This scoring system has a maximum of 10 points, with patients scoring above 6 experiencing higher rates of complications and mortality. Studies have shown that mortality rates begin to increase at a cut-off value of 2 and above in the Balthazar classification system when assessing the severity of acute pancreatitis.[15-18] The results for the Balthazar score were consistent with the evidence presented in the literature. However, in our analysis, the Balthazar score results may have been influenced by certain limitations, including the small sample size and the limited number of patients in one of the subgroups. Consequently, we cannot draw definitive conclusions regarding the prognostic value of this scoring system.

Several studies in recent years have investigated the use of various serum markers to predict the prognosis of acute pancreatitis. These serum markers include CRP, procalcitonin, TPO, lipoproteins, cytokeratin 18, serum amyloid A, urinary trypsinogen-activation peptide, and methemalbumin.[4-7] In a study comparing the effectiveness of inflammatory markers, CRP demonstrated a sensitivity of 86.2% and a specificity of 100% for detecting severe acute pancreatitis at a cut-off value of 150 mg/L.[18,19] When comparing our results, we found that the Ranson scoring system had lower specificity and sensitivity in our patient population. Similarly, the BISAP scoring system also exhibited lower sensitivity in our study. Our findings regarding the CRP score were consistent with the literature; however, the low sensitivity of the scoring systems may be attributed to the insufficient sample size for multi-parameter scoring analysis in this study.

In another study, it was found that measuring serum TPO levels was effective in identifying temporary organ failure and intensive care requirements. It involved calculations using different cut-off values for serum TPO levels, and the findings indicated that serum TPO levels could effectively differentiate between severe, moderate, and mild acute pancreatitis.[9]

A 2012 study conducted in China on animal models demonstrated that serum TPO levels were associated with pancreatitis severity in a mouse model induced by L-arginine and acinar cell necrosis. Additionally, the study found that mice treated with anti-TPO antibodies experienced reduced organ damage from pancreatitis.[20] In a more recent study, researchers examined the relationship between serum TPO levels and disease severity in 53 patients admitted to the emergency department with conditions such as systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, or septic shock. The results demonstrated a significant association between disease severity and serum TPO levels.[21]

In our study, we found that TPO effectively detected the severity of acute pancreatitis with high sensitivity and specificity, comparable to multifactorial scoring systems. Our findings were consistent with those of a previous study, despite using different cut-off values for TPO.

While this study has several strengths, it is important to acknowledge its limitations. The relatively small sample size in each group restricted our ability to detect clinically significant differences and reduced the generalizability of our findings. Additionally, the study was conducted at a single center over a limited timeframe. Therefore, caution should be exercised when applying these findings to other groups.

## CONCLUSION

Determining disease prognosis using a single serum parameter, rather than costly, time-consuming, and labor-intensive examinations, could minimize patient expenses and reduce workload. However, randomized controlled trials with a larger patient population are needed to support the conclusions of this research.

**Ethics Committee Approval:** This study received approval from the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (Date: 26.04.2017, Decision No: E-17-1366).

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions:** Concept: A.S.E.; Design: A.S.E., A.U., Ö.A.; Supervision: T.T., Ö.A.; Resource: A.U., S.G., T.T.; Materials: A.U., T.T.; Data Collection and/or Processing: A.S.E., A.U., S.G.; Analysis and/or Interpretation: A.S.E., Ö.A.; Literature Review: A.S.E., A.U.; Writing: A.S.E., S.G., T.T..; Critical Review: T.T., Ö.A.

**Conflict of Interest:** None declared.

**Financial Disclosure:** The author declared that this study has received no financial support.

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ORİJİNAL ÇALIŞMA - *ÖZ*

## Şiddetli akut biliyer pankreatitin belirlenmesinde serum trombopoietin düzeylerinin erken biyobelirteç olarak kullanımı

AMAÇ: Bu çalışmada, akut biliyer pankreatitli hastalarda erken prognostik bir belirteç olarak, bir büyüme faktörü ve aynı zamanda bir akut faz reaktanı olan trombopoietinin (TPO) hastalığın ciddiyetini öngörmedeki etkinliğinin belirlenmesi amaçlandı.
GEREÇ VE YÖNTEM: Çalışmaya Ankara Numune Eğitim ve Araştırma Hastanesi Genel Cerrahi Bölümü'ne başvuran 72 akut pankreatit hastası dahil edildi. Hastaların akut pankreatitinin ciddiyeti, 2012'de revize edilmiş Atlanta Sınıflandırması kullanılarak belirlendi ve TPO seviyelerini belirlemek için her hastadan hastaneye yatıştan sonraki ilk 6 saat içinde kan alındı. TPO seviyeleri, C-reaktif protein (CRP) seviyeleri ve diğer prognostik skorlama sistemleriyle karşılaştırıldı.
BULGULAR: Atlanta Sınıflamasına göre TPO, şiddetli pankreatit grubu ile orta ve hafif pankreatit grupları arasındaki farkı belirlemede istatistiksel olarak anlamlıydı. Akut pankreatit şiddetini değerlendirmede serum TPO düzeyinin duyarlılık ve seçicilik oranları incelendiğinde, 81.61 pg/dl değeri, duyarlılığı %86.6, seçiciliği ise %69 olarak belirlendi. Çalışmamızda TPO düzeylerinin şiddetli pankreatit tespitindeki doğruluğu diğer skorlama sistemleriyle karşılaştırıldığında, Balthazar skorlama sisteminin ROC eğrisi analizlerinde ağır pankreatiti öngörmede en yüksek duyarlılığa (AUC: 0.905) sahip olduğu tespit edildi (%95 güven aralığı). Öte yandan serum TPO düzeyi şiddetli akut pankreatitin ikinci en güçlü göstergesiydi (AUC: 0.831).
SONUÇ: Bulgulara göre TPO, akut biliyer pankreatitli hastalarda hastalığın ciddiyetini tahmin etmede erken bir belirteç ve prognostik belirteç olarak faydalıdır. Ancak daha geniş hasta grupları ile randomize çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Akut biliyor pankreatit; trombopoetin; prognostik faktör.

Ulus Travma Acil Cerrahi Derg 2025;31(3):214-220 DOI: 10.14744/tjtes.2024.23583