

Comparison of Hematological Inflammatory Indices Between Culture-Positive and Culture-Negative Brucellosis Patients

Bruselloz Hastalarında Hematolojik İnflamasyon İndeks Skorlarının Kültür Pozitifliğini Öngörmedeki Değerleri

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Abstract

Background: Brucellosis, a systemic disease, is a common zoonosis that may be endemic in developing countries. Because the gold-standard diagnostic method, culture, is costly and time-consuming, faster and more practical diagnostic procedures are needed. In this context, we aimed to elucidate the predictive potential of simple and inexpensive parameters.

Materials and Methods: This study included 156 patients diagnosed with brucellosis who had blood cultures obtained prior to treatment. Patients were categorized according to the presence or absence of *Brucella* spp. growth in blood cultures. Biochemical and hematological parameters, as well as hematological inflammatory indices, were compared between the groups.

Results: Blood cultures were positive in 92 patients (59%). No significant differences were observed among groups in hematological parameters (leukocytes, neutrophils, lymphocytes, monocytes, platelets, hemoglobin) or hematological inflammatory indices (NLR: Neutrophil/Lymphocyte ratio, PLR: Platelet/Lymphocyte ratio, NPR: Neutrophil/Platelet ratio, MLR: Monocyte/Lymphocyte ratio, NLPR: Neutrophil/(Lymphocyte × Platelet) ratio, SII: Neutrophil × Platelet/Lymphocyte ratio, SIRI: Neutrophil × Monocyte/Lymphocyte ratio, AISI: Neutrophil × Platelet × Monocyte/Lymphocyte ratio) confirmed by ROC analysis. Biochemical analyses showed significantly higher alanine aminotransferase, aspartate aminotransferase, and C-reactive protein in the culture-positive group compared with culture-negative group.

Conclusions: In our study, we were unable to determine the predictive capacity of hematological parameters or hematological inflammatory indices for culture positivity. However, supporting this finding with prospective, multicenter studies with larger sample sizes that exclude confounding factors is necessary to clarify the issue.

Keywords: Brucellosis, Culture positivity, Biomarkers, Hematological inflammatory indices

Öz

Amaç: Sistemik bir hastalık olan bruselloz, gelişmekte olan ülkelerde endemik olabilen yaygın bir zoonozdur. Altın standart tanı yöntemi olan kültür, maliyetli ve zaman alıcı olduğundan, daha hızlı ve pratik tanı yöntemlerine ihtiyaç vardır. Bu bağlamda, basit ve ucuz parametrelerin öngörü potansiyelini aydınlatmayı amaçladık.

Materyal ve metod: Bu çalışmada, tedavi öncesinde kan kültürü örneği alınan, bruselloz tanısı konmuş 156 hasta değerlendirildi. Hastalar, kan kültüründe *Brucella* spp. üremesi olan ve olmayan gruplar olarak sınıflandırıldı. Biyokimyasal parametreler, hematolojik parametreler ve hematolojik inflamasyon indeksleri gruplar arasında karşılaştırıldı.

Bulgular: Doksan iki hastada (%59) kan kültürleri pozitif. Gruplar arasında hematolojik parametreler (lökosit, nötrofil, lenfosit, monosit, trombosit, hemoglobin) ve hematolojik inflamasyon indeksleri [NLR: Nötrofil/Lenfosit oranı, PLR: Trombosit/Lenfosit oranı, NPR: Nötrofil/Trombosit oranı, MLR: Monosit/Lenfosit oranı, NLPR: Nötrofil/(Lenfosit × Trombosit) oranı, SII: Nötrofil × Trombosit/Lenfosit oranı, SIRI: Nötrofil × Monosit/Lenfosit oranı, AISI: Nötrofil × Trombosit × Monosit/Lenfosit oranı] arasında anlamlı bir fark gözlenmemiştir. Biyokimyasal testler incelendiğinde, kültür pozitif grupta kültür negatif gruba göre anlamlı olarak daha yüksek alanin aminotransferaz, aspartat aminotransferaz ve C-reaktif protein düzeyleri mevcuttu.

Sonuç: Çalışmamızda, hematolojik parametrelerin ve hematolojik inflamasyon indekslerinin kültür pozitifliği için öngörücü potansiyelini belirleyemedik. Ancak, bu bulguyu, karıştırıcı faktörlerin dışlanabileceği, geniş hasta örneklemleri, prospektif, çok merkezli çalışmalarla desteklemek, konuyu açıklığa kavuşturmak için gereklidir.

Anahtar Kelimeler: Bruselloz, Kültür pozitifliği, Biyobelirteçler, Hematolojik inflamasyon indeksleri

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Introduction

Brucellosis, a systemic zoonosis, is widespread and remains endemic in developing countries. The most common agents of the disease are *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, and other *Brucella species*; *Brucella melitensis* is the most virulent. These bacteria are facultative intracellular microorganisms that reside within macrophages (1). It is reported that the primary routes of transmission are close contact with infected animals, such as cows, sheep, dogs, goats, and pigs, and the consumption of contaminated animal products, including uncooked meat and dairy products. The disease, which can present as acute, subacute, chronic, or asymptomatic brucellosis, can mimic a wide range of clinical presentations because it is characterized by nonspecific symptoms such as weakness, sweating, arthralgia, and fever (1,2).

Various laboratory methods are used to diagnose brucellosis. The Rose Bengal card test, a rapid screening test, is recommended for screening because it has high sensitivity and low specificity. While a titer of $\geq 1:160$ is significant in the standard tube agglutination test (SAT), which is accepted as one of the reference methods for diagnosis, isolating the causative agent's deoxyribonucleic acid (DNA) with the polymerase chain reaction (PCR), which is more costly, may also be used in diagnosis (2–4). However, as in many infection clinics, isolating the agent is essential for definitive diagnosis in brucellosis. For this reason, culture is considered the gold standard for diagnosing brucellosis (4,5). It has been reported that blood culture positivity rates in acute brucellosis cases (15–90%) can reach an average of 70%. Positivity rates in cultures obtained by bone marrow aspiration exceed 90%, however, the procedural difficulty, invasive nature, and lower acceptance by both clinicians and patients limit its routine use (2). However, the fact that the success rate in growing the agent in blood culture varies in a wide range from 15% to 90% has been attributed to the method being affected by many factors, such as age, disease form (acute, chronic, focal, or relapsing), antibiotic history, sample volume, culture system used, and incubation period. Because it is influenced by numerous variables, blood culture examination is widely used as a diagnostic method with low sensitivity but high specificity (1,5,6). This situation highlights the need for more practical, cheaper,

and faster tests that can predict the agent's isolation in culture, which is essential for definitive diagnosis.

C-reactive protein (CRP) and white blood cell count are inflammatory markers frequently used in the diagnosis and follow-up of brucellosis treatment (7). However, it is known that these values vary widely depending on factors such as age, gender, non-infectious inflammatory diseases, and malignancies (8). For these and similar reasons, the levels of many parameters that may guide the diagnosis and treatment of brucellosis have long attracted researchers' attention. Alterations in circulating hematological parameters occur in response to various stress factors, including bacterial infections. The low specificity of the leukocyte count alone has prompted researchers to calculate various ratios of hematological parameters to derive multiple hematological inflammatory indices and investigate the impact of these scores on various diseases (9,10). The first hematological inflammatory index used for this purpose is the neutrophil-to-lymphocyte ratio (NLR) (11). Subsequently, many hematological inflammatory indices have been developed, and in recent years, studies investigating their effects in brucellosis have increased (7,12–15).

In our research, we aimed to address the gap in the predictive of parameters potentially relevant to culture positivity and designed our study accordingly. In this context, our study evaluated the potential of hematological parameters and hematological inflammatory indices to predict culture positivity.

Materials and Methods

The study included patients admitted to the Infectious Diseases and Clinical Microbiology outpatient clinics of Batman Education and Research Hospital between 01.05.2022 and 01.05.2024 and diagnosed with brucellosis. Patients who were younger than 18 years old, older than 90 years old, in intensive care, with severe renal dysfunction, or receiving chemotherapy were not included. Among the patients who applied to the outpatient clinics, those who were between the ages of 18 and 90, those who had a clinical presentation compatible with brucellosis and whose SAT and/or Coombs result was $\geq 1:160$, or those who had *Brucella spp.* growth in blood culture and whose routine biochemical and hematological parameters were studied within the same week were included in the study.

Cases in which a blood culture sample was taken before treatment initiation were included in the study. Patients diagnosed with brucellosis based on positive SAT and/or Coombs test results, but for whom no blood culture sample was taken at the start of treatment, were excluded from the study.

This study was approved by the Batman Training and Research Hospital Clinical Research Ethics Committee (approval no: 389, date: April 26, 2024).

After excluding cases without a culture, biochemical parameters, hematological parameters, and hematological inflammatory indices were compared between the culture-positive and culture-negative groups. The biochemical parameters included in the study were alanine aminotransferase (ALT), aspartate aminotransferase (AST), CRP, and erythrocyte sedimentation rate (ESR). Hematological parameters were based on hemogram components (leukocytes, neutrophils, lymphocytes, monocytes, platelets, and hemoglobin). The hematological inflammatory indices used, along with their formulas, are listed below.

- NLR: Neutrophil/Lymphocyte ratio,
- PLR: Platelet/Lymphocyte ratio,
- NPR: Neutrophil/ Platelet ratio,
- MLR: Monocyte/Lymphocyte ratio,
- NLPR: Neutrophil/ (Lymphocyte × Platelet) ratio,
- SII (Systemic immune/inflammation index): Neutrophil × Platelet/Lymphocyte ratio,
- SIRI (Systemic inflammatory response index): Neutrophil × Monocyte/Lymphocyte ratio,
- AISI (Aggregate index of systemic inflammation): Neutrophil × Platelet × Monocyte/Lymphocyte ratio.

Biochemical parameters for all patients with laboratory requests were measured using Beckman Coulter AU5800 and AU680 (Beckman Coulter, Brea, USA), and hematologic parameters were measured using Mindray BC6800 (Mindray, Shenzhen, China). Blood culture samples from patients were analyzed using the fully automated Render BC128 (Render Biotech Co., Ltd., China).

All data were analyzed using IBM SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as counts (n) and percentages (%) for categorical variables, and as medians and interquartile ranges (IQRs) for continuous variables. Normality of numerical variables was assessed using the Shapiro-Wilk normality test and Q-Q plots. Since the variables did not show normal distribution, all comparisons between groups were made using the Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was used to evaluate the ability of hematological inflammatory index scores and biochemical examinations to predict culture positivity. Statistical significance was determined as $p < 0.05$.

Results

A total of 156 patients diagnosed with brucellosis who applied to our hospital between 01.05.2022 and 01.05.2024 were included in the study. The mean age of the patients was 39.0 ± 15.2 years; 91 (58.3%) were female. According to blood culture results, growth was observed in 92 (59.0%) cases, and cultures were negative in 64 (41.0%) cases. There were no differences between the groups in terms of age and gender ($p > 0.05$). No significant difference was found between these groups in terms of hematological parameters (leukocytes, neutrophils, lymphocytes, monocytes, platelets, hemoglobin) and hematological inflammatory indices (NLR, PLR, NPR, MLR, NLPR, SII, SIRI, AISI) ($p > 0.05$). ALT, AST, and CRP levels were significantly higher in the culture-positive group ($p < 0.05$).

Table 1. Hematological parameters, hematological inflammatory index scores and biochemical examination results of the groups

Parameters	All patients N:158 (%100) Median ± IQR	Culture positive N:92 (%52) Median ± IQR	Culture negative N:64 (%36) Median ± IQR	p*
Age [mean (SD)]	39.0 (15.2)	37.4 (15.5)	41.4 (14.4)	0.055*
Gender				
Female [n (%)]	91 (58.3)	56 (60.9)	35 (54.7)	0.566**
Male [n (%)]	65 (41.7)	36 (39.1)	29 (45.3)	

Leukocyte (10 ³)	7.02 (2.78)	6.90 (2.37)	7.14 (3.0)	0.712*
Neutrophil (10 ³)	3.72 (1.95)	3.65 (1.67)	3.93 (2.38)	0.174*
Lymphocyte (10 ³)	2.57 (1.08)	2.60 (1.20)	2.37 (1.03)	0.115*
Monocyte (10 ³)	0.50 (0.26)	0.51 (0.26)	0.50 (0.26)	0.927*
Platelet (10 ³)	235 (99)	234 (103)	240 (90.5)	0.880*
HGB (g/dL)	13.1 (2.35)	12.9 (2.2)	13.2 (2.5)	0.274*
NLR	1.46 (1.01)	1.39 (0.95)	1.66 (1.17)	0.077*
PLR	94.02 (45.48)	90.4(47.1)	96.9 (41.4)	0.387*
NPR	0.002 (0.001)	0.015 (0.009)	0.016 (0.008)	0.158*
MLR	0.19 (0.10)	0.19 (0.11)	0.20 (0.12)	0.221*
NLPR	0.01 (0.01)	0.005 (0.01)	0.007 (0.004)	0.113*
SII	348.35 (266.47)	316 (249)	334 (207)	0.138*
SIRI	0.79 (0.66)	0.703 (0.63)	0.755 (0.53)	0.108*
AISI	177.46 (158.71)	161 (154)	179 (122)	0.147*
ALT	24.0 (22.0)	28.0 (30.0)	20.5 (16.0)	<0.001*
AST	24.0 (15.0)	27.0 (18.0)	20.0 (7.0)	<0.001*
CRP	10.3 (20.7)	12.8 (23.6)	4.9 (12.9)	<0.001*
ESR	32.0 (24.0)	32.0 (23.0)	31.0 (26.0)	0.430*

* The Mann-Whitney U test was used in the pairwise comparisons between the cultur-positive and culture negative groups. ** The Chi-squared test was used in the pairwise comparisons between the cultur-positive and culture negative groups.
 IQR: Interquartile range, SD: Standard deviation, HGB:Hemoglobin, NLR: Neutrophil/Lymphocyte rate, PLR: Platelet/Lymphocyte rate, NPR: Neutrophil/ Platelet rate, MLR: Monocyte/Lymphocyte rate, NLPR: Neutrophil/(Lymphocyte × Platelet) rate, SII (Systemic immune/inflammation index): Neutrophil × Platelet/Lymphocyte rate, SIRI (Systemic inflammatory response index): Neutrophil × Monocyte/Lymphocyte rate, AISI (Aggregate index of systemic inflammation): Neutrophil × Platelet × Monocyte/Lymphocyte rate, ALT: Alanine aminotransferase, AST: Aspartate transferase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

When ROC analysis was performed, similar results were obtained, and no statistically significant differences were found between the groups for hematological parameters and hematological inflammatory indices. In ROC analysis, ALT, AST,

and CRP levels were significantly higher in the culture-positive group. ESR levels did not differ significantly between the groups (Tables 2 and Figure 1).

Variables	AUC (%95 CI)	Cutt-off	Sensitivity (%) (%95, CI)	Specitivity (%) (%95, CI)
NLR	0.583	1.490	0.565	0.563
PLR	0.541	94.02	0.511	0.516
NPR	0.567	0.016	0.576	0.563
MLR	0.558	0.196	0.533	0.531
NLPR	0.575	0.006	0.543	0.547
SII	0.568	343.8	0.533	0.535
SIRI	0.570	0.785	0.543	0.547
AISI	0.563	181.3	0.576	0.570
ALT	0.653	24.50	0.573	0.613
AST	0.688	23.50	0.648	0.677
CRP	0.663	9.150	0.644	0.645
ESR	0.510	32.50	0.508	0.519

NLR: Neutrophil/Lymphocyte rate, PLR: Platelet/Lymphocyte rate, NPR: Neutrophil/ Platelet rate, MLR: Monocyte/Lymphocyte rate, NLPR: Neutrophil/(Lymphocyte × Platelet) rate, SII (Systemic immune/inflammation index): Neutrophil × Platelet/Lymphocyte rate, SIRI (Systemic inflammatory response index): Neutrophil × Monocyte/Lymphocyte rate, AISI (Aggregate index of systemic inflammation): Neutrophil × Platelet × Monocyte/Lymphocyte rate, ALT: Alanine aminotransferase, AST: Aspartate transferase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

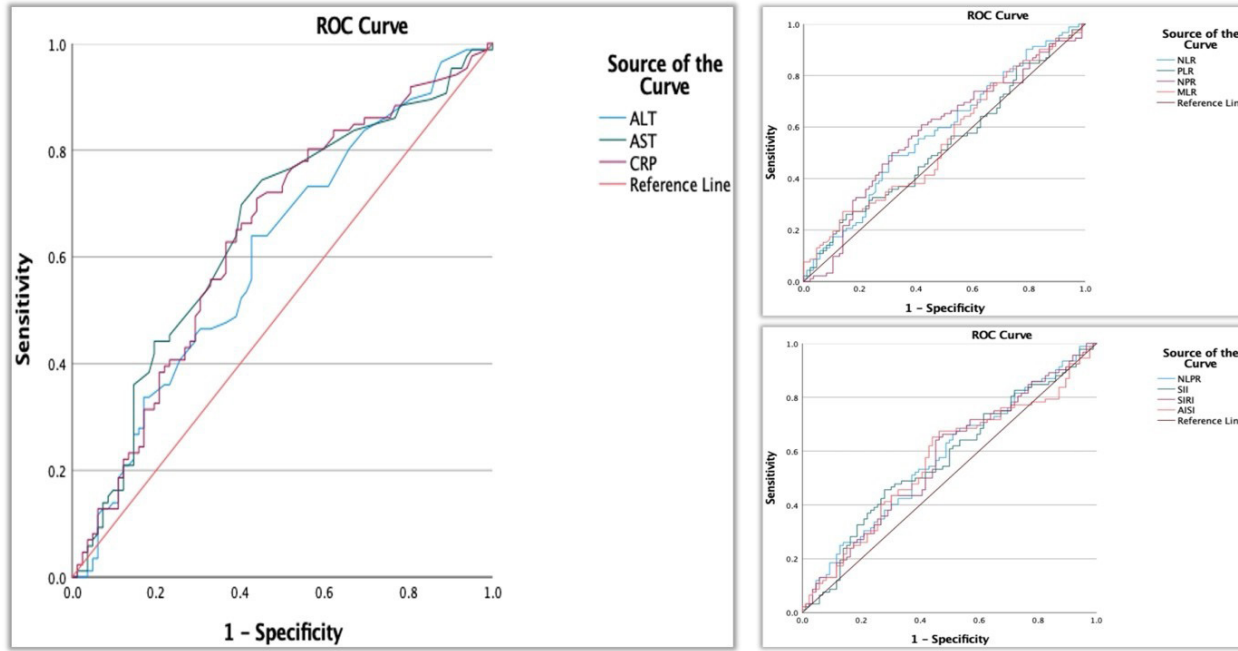


Figure 1. ROC analysis graphs of hematological inflammatory index scores and biochemical examinations between culture positive and negative groups

NPR: Neutrophil/ Platelet rate, NLR: Neutrophil/Lymphocyte rate, MLR: Monocyte/Lymphocyte rate, PLR: Platelet/Lymphocyte rate, NLPR: Neutrophil/(Lymphocyte × Platelet) rate, SII: Systemic immune/inflammation index, Neutrophil × Platelet/Lymphocyte rate, SIRI (Systemic inflammatory response index): Neutrophil × Monocyte/Lymphocyte rate, AISI (Aggregate index of systemic inflammation): Neutrophil × Platelet × Monocyte/Lymphocyte rate

Discussion

In our study, the predictive value of hematological parameters and indices for culture positivity was investigated. Although culture is a costly and time-consuming method for brucellosis, it remains the gold standard diagnostic method (4,5). Faster and more practical parameters that can predict culture positivity are needed. In this context, in the study we conducted, no predictive potential for culture positivity was found in the hematological parameters (leukocyte, neutrophil, lymphocyte, monocyte, platelet, hemoglobin) and hematological inflammatory indices that were compared.

In diagnostic methodology, various practical and widely used tests for brucellosis are employed, with culture positivity accepted as the gold standard. While these tests, which may have variable diagnostic advantages, are frequently used, the culture method, the gold standard, is used in fewer centers because of factors such as limited infrastructure and its lack of speed and practicality (2,3,5). Therefore, the development of faster and more practical tests that yield results comparable to the gold-standard diagnostic method remains essential. While planning our study, which we designed and conducted within

this scope, we found that the literature contained few studies on the subject. However, the parameters of brucellosis cases and healthy controls are often compared in these reported studies (13–18). While designing our study, we classified brucellosis patients according to culture requests, accounting for this factor. We compared the parameters of culture-positive and culture-negative groups, both of which consisted of patients. In addition, because no study in the literature covers all hematological inflammatory indices, such as NLR, PLR, NPR, MLR, NLPR, SII, SIRI, and AISI, we aimed to make an original contribution by providing a comprehensive comparison of these indices.

The first study to examine NLR levels in brucellosis (16) reported that NLR levels decreased in brucellosis patients compared with healthy controls. In the study, hematological parameters, including NLR, PLR, white blood cells (WBC), and hemoglobin, were compared between the groups. Only NLR and hemoglobin differed significantly across groups; the others were not correlated. Although the literature reports elevated NLR in inflammatory processes, this study found that NLR levels in brucellosis patients were significantly lower than those in healthy individuals. Although ROC was found to be significant in the study, no statistical significance was observed in the

analysis using Fisher's Exact Test. Another study (17), conducted in 2020, was a retrospective case-control study compared with healthy controls. WBC, CRP, and neutrophil values were significantly higher in patients. In contrast, no statistically significant differences were observed between the groups in platelet (PLT), mean platelet volume (MPV), and platelet distribution width (PDW). In a retrospective study published in 2025 (13), the PMI (Platelet Mass Index), NLR, and SII indices were compared between healthy pediatric controls and patient groups to assess their potential roles in predicting diagnosis and focal involvement. No difference was observed between patient groups with and without focal participation with respect to these parameters. In the comparison between patients and healthy controls, hemoglobin and platelet counts were significantly lower, whereas CRP and ferritin were significantly higher in patients. While there was no difference in NLR, the PMI and SII indices were considerably lower. Gulten et al. (7) evaluated the treatment response of NLR, PLR, and red blood cell distribution width (RDW), only NLR correlated with changes in CRP, suggesting that it may be valuable for assessing treatment response.

Sen et al. (19) compared patients by grouping them according to their complications and organ involvement, rather than by patient status versus healthy controls. This study was reported as the first to evaluate PLR, NLR, and lymphocyte-to-monocyte ratio (LMR) parameters in predicting specific organ involvement and complications. In this study, ESR and PLR elevations were the most predictive parameters for both specific organ involvement and complications. NLR elevation and decreases in LMR and MPV were reported to be significant only in predicting particular organ involvement. However, the study made no comparison of blood culture positivity across the parameters and indices examined. Because contradictory findings across these studies may be attributable to confounding factors such as disease stage and treatment process, new studies are needed to address these variables.

In a study comparing bacteremic and non-bacteremic patient groups, Çelik et al. (20) reported that ferritin, eosinophil, and ESR parameters had acceptable discriminatory power in the bacteremic group. In a study comparing indices from patient and healthy groups (18), the indices were significantly higher in the patient group, suggesting an increased inflammatory response in the disease. A study conducted on male brucellosis patients admitted from China found that ALT, AST, PLT, and ESR levels showed a statistically significant relationship with culture positivity (21). Since this finding, which also supports the starting point of our study, was obtained by comparing patient

and healthy groups, we compared culture-positive and culture-negative patient groups in our study. When the results from our study were examined, significant differences were observed in AST, ALT, and CRP, consistent with the studies mentioned above. However, no difference was found between the groups in the hematological inflammatory indices we compared.

We would like to state that it has some limitations, such as the small sample size, the retrospective nature of the study, and the possibility of confounding factors such as age, gender, disease stage (acute, chronic), and antibiotic history. Similarly, another significant limitation of the study is its single-center design, which limits the sample to patients from the same demographic region and the parameters studied to the same devices. Since our study is not a prospective study, causality cannot be established, and only the existence of a relationship has been investigated.

Conclusion

The findings obtained in our study showed that hematological parameters and hematological inflammatory indices do not have the potential to predict culture positivity, which is the gold standard diagnostic method for brucellosis. However, supporting this finding with prospective, multicenter studies with large patient samples, that exclude confounding factors, is necessary to clarify the issue.

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