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Semi-quantitative reagent strip analysis of leukocyte esterase and glucose in synovial fluid as bioindicators for rapid diagnosing bacterial arthritis

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ABSTRACT

Aim: To evaluate the diagnostic performance of synovial leukocyte esterase (LE) and glucose reagent strip tests in differentiating septic arthritis from inflammatory arthritis.

Methods: All aspirates were evaluated by culturing, gram staining, and analyzing the cell count and the polymorphonuclear cell percentage. We interpreted all LE and glucose results by semi-quantitative visual assessments. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated.

Results: While LE positivity was more common in the septic arthritis group, glucose levels were found to be significantly lower. As bioindicators; levels of synovial LE, synovial glucose and combined levels of LE and glucose were found to have significantly different ROC areas in discriminating septic arthritis patients from aseptic arthritis patients.

Conclusions: The test can be used as a valuable and inexpensive assessment tool for patients to quickly perform the differential diagnosis of septic arthritis in primary care or emergency settings. However, LE and glucose strip test results should not be viewed as an independent test. Rather, the results should be assessed as auxiliary test results that facilitate the diagnosis of septic changes in body fluids.

Keywords: Bacterial arthritis, septic arthritis, leukocyte esterase, glucose, reagent strip test.

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1. Introduction

Septic arthritis is a severe joint infection that constitutes a medical emergency, often leading to substantial acute and chronic morbidity. Prompt diagnosis and treatment are crucial to prevent irreversible damage [1]. When diagnosis and treatment are delayed, the disease can cause permanent damage, leading to chondral injury and joint destruction. Diagnosis of acute bacterial arthritis relies on the clinical presentation, laboratory results, imaging studies, and arthrocentesis findings. However, none of these parameters are sufficiently specific or sensitive for the diagnosis of acute bacterial arthritis [1,2]. Diagnosis is based on the examination of aspirated synovial fluid specimens by Gram

staining, bacterial cultures, and differential cell counts [3]. Synovial fluid cultures are time consuming. Gram staining of synovial fluid is readily available but has limited sensitivity. Of all tests that provide results in real-time, a cell count in the synovial fluid is the most reliable one. However, this procedure has limited availability [3,4,5]. Despite these diagnostic tools, a rapid and highly reliable test for diagnosing septic arthritis remains elusive. Therefore, there is an ongoing need for accessible, efficient, and practical tests to aid in the timely diagnosis of bacterial arthritis [2]. Recent studies have shown that the diagnostic ability of reagent strip tests for glucose and leukocyte esterase (LE) in the synovial fluid may be feasible [1,3,6]. These tests are performed using colorimetric strips. The test method is fast, easy, simple, and inexpensive, and provides immediate results [1,4,5]. LE, an enzyme released by neutrophils, has been found at elevated levels during inflammatory processes. Initially used as a diagnostic tool for urinary tract infections, LE strips can semi-quantitatively detect leukocytes within minutes [4,5]. Similarly, a reduction in glucose concentration within synovial fluid, compared to plasma levels, is a strong indicator of infection, as glucose is metabolized by bacteria [3,5].

The aim of our study was to evaluate the feasibility of using leukocyte esterase and glucose strip tests as rapid diagnostic parameters for bacterial septic arthritis.

2. Materials and methods

2.1. Study design: This study adhered to the Declaration of Helsinki and was approved by the local ethics committee of the University of Health Sciences Yıldırım Beyazıt Training and Research Hospital (28/06/2021-114/20). Synovial fluid samples were collected between June 2019 and September 2019. Samples meeting the inclusion criteria were included in the study. Cell counts were performed on the synovial fluid samples, which were then inoculated onto culture media. Both fresh and Gram-stained specimens underwent microscopic examination. After routine microbiological examinations, the specimens were centrifuged. A 250 µl aliquot of the supernatant from each specimen was collected and stored at -80 °C until testing.

2.2. Study population: Eligible individuals meeting the inclusion criteria were included in the study. The inclusion criteria were as follows: to be ≥ 18 years old, to have acute arthritis of the knee joint, the presence of clinical symptoms and signs of acute arthritis, and the availability of adequate synovial fluid volume of appropriate morphology.

2.3. Exclusion criteria: The criteria were as follows: to be younger than 18 years old, to have mechanic and infectious conditions developing secondary to bone fractures or implants, potentially leading to poor skin condition, sinus, blood in the aspirate, or to making a diagnosis of hemophilia or any other bleeding disorder.

2.4. Study groups: Patients were categorized into two groups: the septic arthritis group (n=34) and the noninfectious arthritis group (n=33). Septic arthritis was diagnosed if a bacterial pathogen was isolated from the synovial fluid, purulent material was observed in the joint space, or Gram staining was positive for any bacterial morphology. Patients with lupus arthritis, rheumatoid arthritis, Behçet's disease, gout, or those not meeting the criteria for septic arthritis were classified as having noninfectious arthritis.

2.5. Microbiological assessment and cell count of synovial fluid samples: All aspirates were evaluated through culturing, Gram staining, and cell count analysis, including the percentage of polymorphonuclear (PMN) cells. Microbiological analysis was conducted in the

microbiology laboratory of our hospital using standard culture methods. Standard microbiological methods were used for Gram staining, leukocyte counting, and calculating the PMN percentage. Inflammatory arthritis was defined by a leukocyte count of ≥ 2000/mm³, while counts < 2000/mm³ indicated mechanical joint conditions. A septic condition was diagnosed if a positive bacterial culture was obtained after 72 hours.

2.6. Reagent Strip Test for Leukocyte Esterase and Glucose: We interpreted leukocyte esterase (LE) and glucose results using semi-quantitative visual assessments with the LabStrip U11 Plus GL (Elektronika, Hungary). One drop of synovial fluid was directly applied to the reagent strip. After two minutes, the color change on the strip was compared to the color scale provided in the strip box. For LE, possible test results were negative, positive (+), positive (++), or positive (+++). Leukocyte values of ≥ 1+ were considered indicative of infection. For glucose, possible results included negative, positive (+), positive (++), positive (+++), or positive (++++). A negative (–) result indicated a reduction in glucose concentration in the joint fluid, while scores of (+) or (++++) were considered positive. Semi-quantitative values specified by the manufacturer for LE and glucose are illustrated in Fig 1 and Fig 2, respectively.

2.7. Statistical Analyses: Statistical analyses were performed using SPSS 20.0 (IBM, Chicago, IL, USA). Descriptive statistics were presented as mean ± SD or median (Q1-Q3) for numerical data and as frequencies (percentages) for categorical variables. Data normality was assessed using the Shapiro-Wilk test, revealing that none of the continuous variables followed a normal distribution. Thus, the Mann-Whitney U test was used to compare septic and noninfectious arthritis groups. Categorical variables were analyzed using the exact Chi-square test. Receiver operating characteristic (ROC) analysis was conducted to evaluate the diagnostic sensitivity of LE and glucose, and the area under the ROC curve (AUC) was used to determine cut-off values. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for LE, glucose, and their combination. A p-value <0.05 was considered statistically significant.

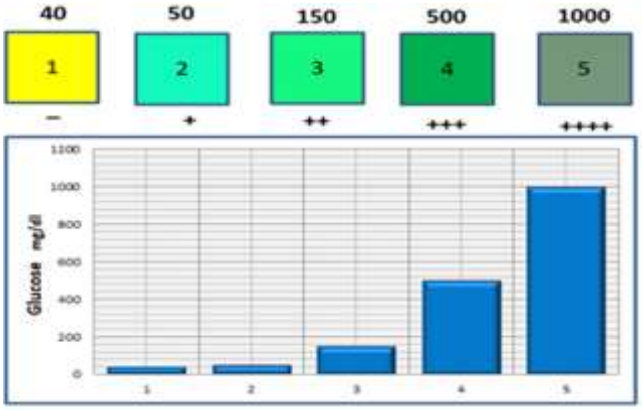

Figure 1. A color change that indicates a value of less than 50 mg/dl (2.8 mmol/L) is considered negative. The color fields correspond to the following results in the glucose concentration range as negative, 50(+), 150(++), 500(+++), and 1000(++++) mg/dl.

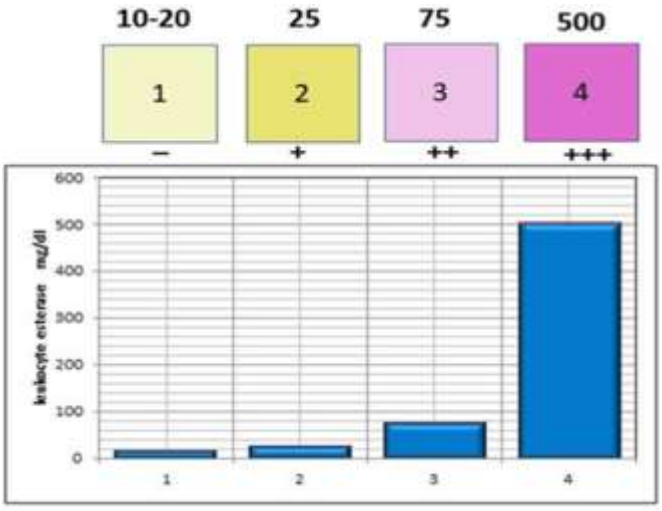

Figure 2. A color change that indicates a value of less than 25 mg/dl is considered negative. The color fields correspond to the following results in the leukocyte esterase concentration range as negative, 25(+), 75(++), 500(+++) mg/dl.

3. Results

A total of 67 adults were included in the study, comprising 34 patients with septic arthritis and 33 patients with inflammatory arthritis. Among the participants, 32 (52.2%) were women and 35 (47.8%) were men. There was no statistically significant difference in gender distribution between the groups (p=0.547). The mean age was 58.79 ± 20.88 years for the septic arthritis group and 53.06 ± 24.98 years for the inflammatory arthritis group, with no significant difference in age between the groups (p=0.350). There was not a statistically significant difference in age between the groups. *Staphylococcus aureus* was the most frequently isolated microorganism, identified in 53.3% of the culture tests. Other isolated microorganisms included *Staphylococcus epidermidis* (17.6%), *Staphylococcus haemolyticus* (11.7%), *Staphylococcus hominis* (5.8%), *Streptococcus pneumoniae* (5.8%), and *Streptococcus pyogenes* (5.8%). LE-negativity was more common in the inflammatory arthritis group, while LE-positivity was more prevalent in the septic arthritis group (Figure 3).

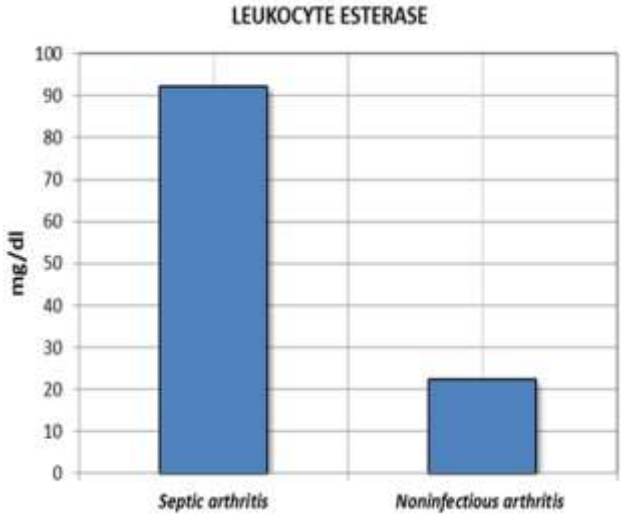

Figure 3. Synovial fluid leukocyte esterase concentration according to the semiquantitative analysis by the reagent strip test. The values are shown as mean and the standard deviation.

Glucose levels were significantly lower in the septic arthritis group compared to the inflammatory arthritis group (Figure 4).

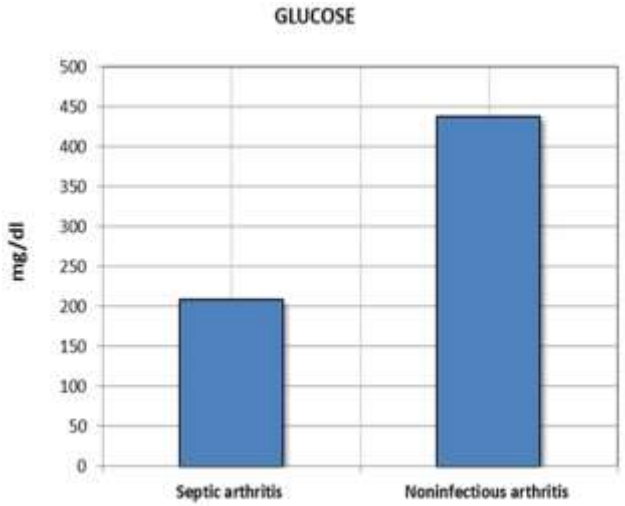

Figure 4. Synovial fluid glucose concentration according to the semiquantitative analysis of the reagent strip test. The values are shown as the mean and the standard deviation.

In synovial fluid samples, leukocyte count, percentage of PMNs, and CRP levels were significantly higher in septic arthritis compared to inflammatory arthritis (Table 1).

Table 1. Distribution of the reagent strip test results between septic arthritis and noninfectious arthritis groups.

Synovial fluid analysis	**Septic arthritis (n=34) mean ±SD**	**Noninfectious arthritis (n=33) mean ±SD**	***p***
Glucose (*mg/dl*)	208.8±287	437.87±340	*<0.001**
Leukocyte esterase (*mg/dl*)	92.35±149	22.35±12.21	*<0.001**
WBC (*cell/mm^3*)	11,255±3301	10,012±3508	*0.089*
PMN (%)	84.70±14.61	70.60±31.68	*0.024**
CRP (*mg/l*)	58.45±50.06	24.03±20.45	*0.043**

*PMN: (polymorphonuclear neutrophil) cells; CRP: C-reactive protein; WBC: White Blood Cell count; *: significant at 0.05 level according to Mann-Whitney U test.*

Cross-tabulation of the semi-quantitative LE and glucose test results showed statistically significant differences between septic and non-

septic samples. Specifically, a positive LE strip test result, a negative glucose strip test result, and a combination of positive LE and negative glucose test results were indicative of bacterial arthritis (Table 2).

Table 2. Comparison of leukocyte esterase and glucose reagent strip test results by the diagnosis.

Tests	**Septic arthritis n (%)**	**Noninfectious arthritis n (%)**	***p***
LE Strip test *(>25 mg/dl)*			
Positive	22 (66.6)	8 (24.2)	*0.031**
Negative	12 (36.4)	25 (75.8)	
GLC strip test *(<50 mg/dl)*			
Negative	18 (52.9)	3 (9.1)	*<0.001**
Positive	16 (47.1)	30 (90.9)	
LE & GLC			
Negative (others)	23 (67.6)	31 (93.9)	*0.007**
Positive (LE>*25 mg/dl* & GLC<*50 mg/dl)*	11 (32.4)	2 (6.1)	

LE: Leukocyte esterase strip test; GLC: glucose strip test.

As bioindicators, levels of synovial LE (Figure 5), synovial glucose (Figure 6), and the combination of LE and glucose were found to have significantly different ROC areas in distinguishing septic arthritis from inflammatory arthritis.

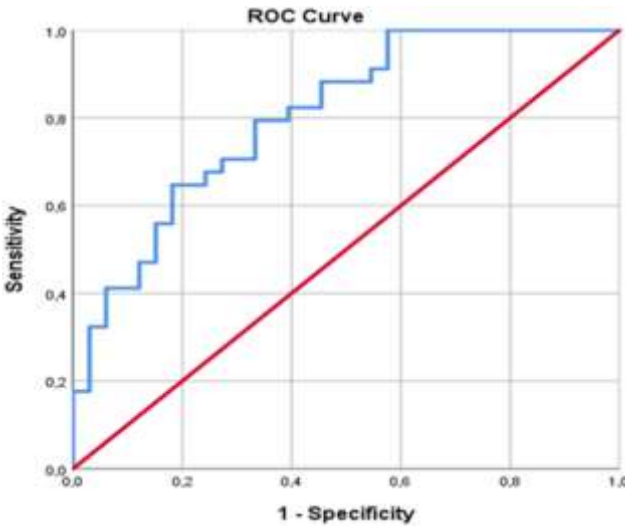

Figure 5. ROC curve for semi-quantitatively tested leukocyte esterase values.

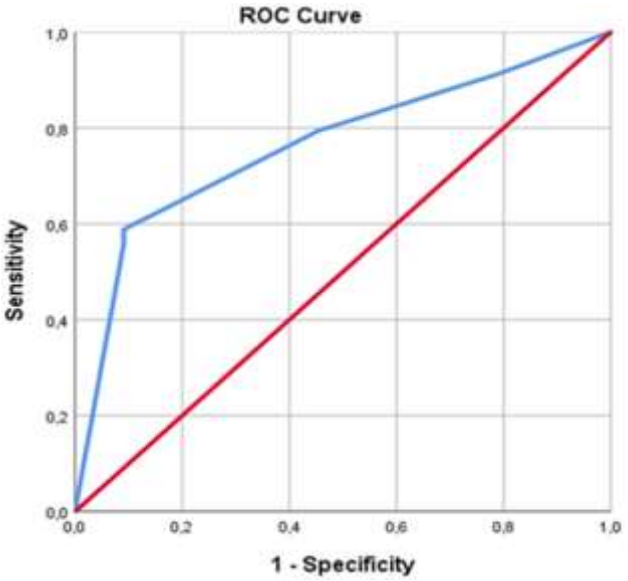

Figure 6. ROC curve for semi-quantitatively tested glucose values.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy values for synovial LE, synovial glucose, and the combined LE-positive and glucose-negative reagent strip test results are shown in Table 3.

Table 3. Diagnostic performance of the reagent strip test in predicting septic arthritis according to ROC analysis.

	GLC	**LE**	**LE+GLC**
AUC	0.764	0.668	
P	*0.001*	*0.018*	
Cut-off	125	237.50	
Sensitivity	52.94 (35.13-70.22)	50.00 (32.43-67.57)	32.35 (17.39-50.53)
Specificity	90.91 (75.67-98.08)	75.76 (57.74-88.91)	93.94 (79.77-99.26)
PPV	85.71 (66.09-94.86)	68.00 (51.58-80.91)	84.62 (56.86-95.82)
NPV	65.22 (56.37-73.13)	59.52 (49.95-68.42)	57.41 (51.26-63.33)
Accuracy	71.64 (59.31-81.99)	62.69 (50.01-74.20)	62.69 (50.01-74.20)

The values are given as estimates within a 95% CI specified in parentheses. AUC: Area under the curve; LE: leukocyte esterase strip test; GLC: glucose strip test.

4. Discussion

In patients with joint effusion and functional joint limitations, it is essential to promptly exclude infective etiology. Delayed diagnosis is closely associated with rapid joint destruction and increased morbidity and mortality. Current diagnostic methods often provide delayed results and demonstrate insufficient performance [4,5]. In our study, we evaluated whether biochemical parameters such as glucose and leukocyte esterase could serve as rapid and cost-effective additional diagnostic criteria for septic arthritis.

Septic arthritis is a serious clinical condition associated with morbidity and mortality that can cause permanent joint cartilage damage. Early diagnosis and appropriate treatment are essential to prevent disease progression. Diagnosis can be difficult because of numerous potential diagnoses to be ruled out in the differential diagnoses including osteoarthritis and other inflammatory types of arthritis. Delayed diagnosis has been associated with high mortality rates between 15% and 56% [6,7]. Various diagnostic tools such as synovial fluid cultures, Gram stains, synovial fluid analysis, and blood analysis are used to differentiate septic arthritis from other arthritis types. All these procedures have strengths and limitations. The gold standard for the diagnosis of septic arthritis is the synovial culture having a sensitivity range from 75% to 90%. However, the culturing process is time consuming and takes 24-96 hours. Gram staining usually results in an hour,but the sensitivity range is 29-50% [7,8]. A synovial white blood cell counts and the percentage of polymorphonuclear cells are diagnostic tests that provide results readily. However, their limited availability in primary or secondary care settings limits their feasibility for emergencies. Therefore, there is a need for new approaches that aid to make a fast and reliable diagnosis of septic arthritis [8,9].

The successful application of the leukocyte esterase (LE) strip test in diagnosing urinary tract infections, peritonitis, meningitis, and periprosthetic joint infections underscores its value as a reliable infection marker [4,6,10]. LE is specifically released by neutrophils in infected fluids. Synovial LE can be measured using a rapid, straightforward, and cost-effective colorimetric strip test that provides immediate results [4,5]. Gautam et al. reported a sensitivity of 79.2% and specificity of 80.8% for LE in acute bacterial arthritis in natural joints [11]. A meta-analysis indicated even higher values, with sensitivity and specificity of the synovial fluid LE test at 90% and 97%, respectively. Additionally, synovial LE has been identified as a specific biomarker for differentiating prosthetic joint infections [1]. Colvin et al. found lower sensitivity and specificity values, but a 100% negative predictive value (NPV) for synovial LE tests in natural joints [12]. Coiffier et al. demonstrated that synovial LE tests can effectively distinguish inflammatory from non-inflammatory arthritis. That study demonstrated that synovial LE tests could discriminate inflammatory arthritis from non-inflammatory arthritis correctly [13]. Another study found the sensitivity, specificity, positive predictive value (PPV), and NPV of the synovial LE test to be 80.8%, 78.6%, 70.0%, and 86.8%, respectively, compared to positive culture results. This study concluded that the LE test is a quick and accurate method for differentiating between juvenile idiopathic arthritis and septic arthritis [14].A recent study confirmed that synovial fluid LE levels can diagnose septic arthritis with 82% specificity, 95% sensitivity, 47% PPV, and 99% NPV. In our study, the sensitivity, specificity, PPV, NPV, and accuracy of the synovial LE test were 50.0%, 75.7%, 68.0%, 59.5%, and 62.6%,

respectively, in distinguishing septic arthritis from noninfectious arthritis [15].

Glucose concentrations in serum and normal joint fluid are generally found to be in similar proportions. However, bacterial metabolism causes a reduction in glucose levels within body fluids. Measuring synovial glucose using a colorimetric strip offers a quick, simple, and cost-effective method for obtaining results [10,14-16]. This approach seems practical for distinguishing bacterial arthritis from other conditions. Kinugasa et al. demonstrated that synovial glucose levels serve as a highly specific indicator for differentiating between septic and reactive arthritis [17]. Similarly, Berthoud et al. found that median synovial glucose levels were lower in patients with septic arthritis compared to those with acute non-septic arthritis, aligning with our study findings [18]. Another study revealed that the lowest synovial fluid (SF) glucose levels, as measured by colorimetric strips, were linked to joint damage caused by disease [19]. De Vecchi et al. reported that the synovial glucose reagent strip test had a sensitivity of 73.7%, specificity of 70%, PPV of 77.8%, and NPV of 89.6% [20]. Similarly, another study reported sensitivity and specificity of 71.4% for the synovial glucose strip test in diagnosing septic arthritis [21]. More recently, a study confirmed that semiquantitative glucose strip tests could detect septic arthritis with 90% sensitivity, 98% specificity, 90% PPV, and 98% NPV [15]. In our study, the synovial glucose strip test showed a sensitivity of 52.9%, specificity of 90.9%, PPV of 85.7%, NPV of 65.2%, and an accuracy of 71.6% in distinguishing septic arthritis from noninfectious arthritis.

In the literature, combinations of leukocyte esterase positivity and glucose negativity were used in the evaluation of septic and aseptic samples. A positive LE result in the presence of a negative glucose result detects septic arthritis with 100% specificity, 85% sensitivity, 100% PPV, and 98% NPV [15]. In another study, the combination of leukocyte esterase positivity and synovial glucose negativity was reported to be associated with 71.4% sensitivity and 92.9% specificity with an AUC of 0.82 in the ROC analysis [17]. Similarly, Omar et al. demonstrated a sensitivity of 89.5%, specificity of 99.2%, a positive predictive value of 94.4%, and a negative predictive value of 98.4%. [5]. The studies indicated that semi-quantitative analysis of glucose and LE using strip tests could detect septic arthritis. Both the sensitivity and specificity of the individual LE and glucose tests were found to be high. On the contrary no significant difference was observed when glucose and LE test results were evaluated in combination [20].In our study; the sensitivity, specificity, PPV, NPV, and accuracy of the LE positivity combined with a negative glucose strip test were found as 32.3%, 93.9%, 84.6%, 57.4%, and 62.6%, respectively, in discriminating septic arthritis patients from noninfectious arthritis patients.

4.1. Conclusion

This study aimed to assess the diagnostic performance of synovial leukocyte esterase and glucose reagent strip tests for differentiating septic arthritis from inflammatory arthritis. Our results, in conjunction with existing literature, demonstrate that these reagent strip tests are effective for distinguishing between septic and inflammatory arthritis. The combined evaluation of LE and glucose tests in synovial fluid emerged as a valuable biomarker for diagnosing septic arthritis. The rapid exclusion of septic arthritis is crucial, and the major advantage of these reagent strip tests is their ability to provide results within minutes. This makes the tests a valuable, cost-effective tool for quickly diagnosing septic arthritis in primary care or emergency settings where other diagnostic options may be limited.

However, the combined LE and glucose test results should be considered as supplementary rather than standalone diagnostic tools. Further large-scale studies are needed to confirm the specificity and sensitivity of the combined LE and glucose test results. We believe this study provides a foundational basis for future research into the efficacy of strip tests in diagnosing septic arthritis.

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Conflict of Interest: *The other authors declare that they have no conflicts of interest to report.*

Ethical Statement: *The local ethics committee of the University of Health Sciences Yıldırım Beyazit Training and Research Hospital approved the study (28/06/2021-114/20).*

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