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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2021;10(3):946-50

Diagnostic performance of leukocyte count in direct microscopic examination in the diagnosis of septic arthritis

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Received 16 June 2021; Accepted 16 July 2021
Available online 23.08.2021 with doi: 10.5455/medscience.2021.06.209

Abstract

In this study, the value of leukocyte count in the differential diagnosis of culture-negative septic arthritis was investigated. The study was designed retrospectively. Synovial fluid samples sent to Turhal State Hospital Microbiology Laboratory between July 2018 and November 2019 for direct microscopic examination for the differential diagnosis of arthritis were evaluated. In this study, patients who were admitted to Turhal State Hospital between July 2018 and November 2019 and were prediagnosed with septic arthritis and whose synovial fluid samples were sent to the microbiology laboratory for leukocyte count were examined. Synovial fluid samples were counted without centrifugation at 400X magnification under a light microscope in the cell counting chamber. For bacteria isolation, samples were planted on plates of 5% Sheep Blood agar (RDS, Turkey), Chocolate agar (RDS, Turkey) and Eosin Methylene Blue agar (RDS, Turkey) and incubated at 37°C for 24-48 hours. Twenty three (43.3%) of 53 patients included in the study with a pre-diagnosis of septic arthritis were accepted as septic arthritis. Leukocyte was not detected in four (7.5%) and were counted for less than 20,000 in 24 (45.3%), between 20,000-50,000 in nine (17%), between 50,000-100,000 in seven (13.2%), over 100,000 in nine (17%) of the samples. Only three (13%) patients were diagnosed with bacteria isolation. The correlation between high leukocyte count and bacterial isolation was statistically significant (p=0.031). Also, the correlation between the high leukocyte count and the decision to initiate empirical antibiotherapy was statistically significant (p<0.001). Microscopic synovial leukocyte count is a valuable diagnostic parameter for the diagnosis of septic arthritis in secondary healthcare institutions where diagnostic possibilities are limited and where automated culture systems are not available, especially in cases if the pathogen microorganism cannot grown in cultures.

Keywords: Septic arthritis, leukocyte count, synovial fluid

Introduction

Septic arthritis is an infectious inflammation of the joint. Although the pathogen microorganism is generally bacteria, it can also be viruses, fungi and mycobacteria. The incidence of septic arthritis is 2-6 per 100,000 [1]. The incidence is increasing in people with risk factors [2]. It is most common in the knee joint in adults. *Staphylococcus aureus* is the most common agent [3]. It is an infectious disease with high morbidity if early antibiotherapy is not initiated and surgery is not performed [1,4].

The gold standard in the diagnosis of septic arthritis is the isolation of bacteria from synovial fluid [1-3]. Diagnostic difficulties can be experienced in culture negative arthritis. However, a leukocyte count above 50,000 mm^3 in synovial fluid is quite specific for the diagnosis of septic arthritis. Emergency surgery is decided according to this cut-off value [5]. In this study, it was aimed to evaluate the effect of the leukocyte count in the synovial fluid on the clinician's diagnosis and treatment decision in patients who are clinically suspected to be septic arthritis but whose growth in synovial fluid culture is not detected.

Materials and Methods

The study is cross-sectional and descriptive and designed retrospectively. Synovial fluid samples sent to Turhal State Hospital Microbiology Laboratory between July 2018-November 2019 for direct microscopic examination for the differential diagnosis of arthritis were evaluated. Patient data were obtained from the hospital automation system and microbiology records. The demographic data of the patients, involved joints, risk factors, synovial fluid examination results, bacteriological examination results, and the treatments applied were evaluated.

Inclusion criteria were defined as being over 18 years of age and having a clinical diagnosis of septic arthritis. Arthritis was diagnosed by an orthopedist. The presence of at least two of the clinical signs of pain, swelling, temperature increase, limitation

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of movement and immobilization in the joint was accepted as arthritis. The exclusion criteria for the study were determined as being under the age of 18 and not meeting the arthritis criteria.

Joint fluid puncture was performed by an orthopedist under sterile conditions. The sterile synovial fluid samples were quickly delivered to the laboratory. Direct microscopic examination and bacteriological examination of synovial fluid was performed by a microbiologist. Synovial fluid was counted without centrifugation at 400X magnification under a light microscope in the cell counting chamber for leukocyte count. Results are given in mm^3. For bacteria isolation, samples were planted on plates of 5% sheep blood agar (RDS, Turkey), Chocolate agar (RDS, Turkey) and Eosin Methylene Blue agar (RDS, Turkey) and incubated at 37°C for 24-48 hours. Identification of growth detected bacterial strains was performed by biochemical tests. Antibiotic sensitivity was evaluated by Mueller-Hinton Agar disk diffusion method in accordance with the recommendations of The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

The patients were divided into groups according to the number of leukocytes in mm^3. They were divided into groups as those with no leukocytes (group 1), <20,000/mm^3 (group 2); those being between ≥20,000/mm^3, <50,000/mm^3 (group 3); those being between ≥50,000/mm^3, <100,000/mm^3 (group 4); those being between ≥100,000/mm^3 (group 5). Bacterial isolation rates and empirical antibiotherapy initiation rates were evaluated according to the leukocyte count.

The definitive diagnosis of septic arthritis was made in patients meeting one of the 3 criteria.

1. Growth of pathogenic microorganism in culture

2. Detection of leukocyte count above 50,000/mm^3 in synovial fluid analysis

3. Although the leukocyte count is below 50,000/mm^3 in synovial fluid analysis, the history and clinical picture strongly support the diagnosis of septic arthritis.

Statistical analysis

SPSS 22 (Inc. Chicago, Illinois, USA) statistics package program was used to calculate the data. The conformity of the variables to normal distribution was examined by visual (histo-gram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics were made as numbers and percentages for categorical variables, mean ± standard deviation for normally distributed continuous variables, and median (minimum-maximum) for non-normally distributed continuous variables. Chi-square and Fisher's exact test were used to compare categorical variables between groups. Statistical significance value was accepted as $p<0.05$ in all analyzes.

Results

Fifty three patients with a pre-diagnosis of septic arthritis were included in the study. Thirty (56.6%) of the patients were male and the mean age was 60.96±16.63. Three (5.6%) patients had more than one joint involvement. The most involved joint was knee joint (n=35, 62.5%). The most important risk factors; presence of implant/prosthesis in the joint and/or its neighborhood (n=7, 13.2%); diabetes mellitus (DM) (n=6, 11.3%) and degenerative joint disease (n=4, 7.5%) (Table 1). In direct microscopic examination of synovial fluids, leukocytes were not detected in four (7.5%), less than 20,000/mm^3 detected in 24 (45.3%), between 20,000-50,000/mm^3 detected in nine (17%), and between 50,000-100,000/mm^3 detected in seven (13.2%), and over 100,000/mm^3 were detected in nine (17%) of them (Table 2).

Table 1. Demographic data, joint involvement and risk factors of the patients

		Patients with pre-diagnosis of septic arthritis (n=53)	Patients with definitive diagnosis of septic arthritis (n=23)
Age		60.96±16.63	61.7±18.96
Gender	**n(%)**		
-female		23 (43.4)	11 (47.8)
-male		30 (56.6)	12 (52.2)
Joint involved	**n(%)**		
-knee		35 (62.5)	18 (75)
-ankle		7 (12.5)	1 (4.1)
-hip		7 (12.5)	4 (16.6)
-shoulder		3 (5.3)	-
-elbow		2 (5.3)	1 (4.1)
-wrist		2 (3.5)	-
Risk factors	**n(%)**		
- implant/prosthesis in the joint and/or its neighborhood		7 (13.2)	7 (30.4)
-diabetes mellitus		6 (11.3)	3 (13)
-degenerative joint disease		4 (7.5)	2 (8.6)
-arthroscopic intervention		3 (5.6)	1 (4.3)
-trauma		3 (5.6)	1 (4.3)
-Immunosuppressive drug usage		2 (3.7)	1 (4.3)

Table 2. Comparison of bacterial isolation and empirical antibiotherapy initiation rates according to synovial leukocyte count

	group 1 (leukocyte absent) n=4	group 2 (leukocyte <20,000/mm³) n=24	group 3 (leukocyte ≥20,000/mm³, <50,000/mm³) n=9	group 4 (leukocyte ≥50,000/mm³, <100,000/mm³) n=7	group 5 (leukocyte ≥100,000/mm³) n=9	P
Bacteria isolation	-	1	-	-	3	0.031
Empirical antibiotherapy initiation	-	2	5	7	9	<0.001

Twenty three (43.3%) of 53 patients with a pre-diagnosis of septic arthritis were accepted as septic arthritis. Twelve (52.2%) of the patients were male and the mean age was 61.7±18.96. One (4.3%) patient had more than one joint involvement. The most involved joint was knee joint (n=18, 75%). The most important risk factors were determined as presence of implant/ prosthesis in the joint and/or its neighborhood (n=7, 30.4%); DM (n=3, 13%) and degenerative joint disease (n=2, 8.6%) (Table 1).

Bacteria isolation could be achieved in only four patients (7.5%). *Coagulase negative staphylococcus* (CNS) was isolated in one patient, and *S. aureus* was isolated in three patients. In the patient with CNS growth, 1200/mm^3 leukocytes were counted on direct examination. In all patients with growth of *S. aureus*, leukocyte counts were >100,000/mm^3. The difference between high leukocyte count and bacterial isolation was found to be statistically significant (p=0.031).

Septic arthritis was diagnosed in three (13%) patients by isolating the causative microorganism from the synovial fluid, in 13 (56.5%) patients by the leukocyte count above 50,000/mm^3 in the synovial fluid, and in seven (30.4%) patients with the presence of joint prosthesis and clinical findings, although the leukocyte count was below 50,000/mm^3. In the patient with CNS growth in culture, since CNS is not always a pathogenic bacteria, found in the skin flora, and laboratory findings do not support septic arthritis, growth was evaluated as contamination. Septic arthritis was not diagnosed.

Empirical antibiotics were not initiated in any of the patients in whom leukocytes were not found in the synovial fluid analysis. Empirical antibiotics were initiated to two (8.6%) of those with cell counts below 20,000/mm^3, to five (55.5%) of those with cell counts between 20,000-50,000/mm^3, and to all those with cell counts > 50,000. The difference between the leukocyte count and the decision to initiate empirical antibiotherapy was statistically significant (p<0.001) (Table 2).

Discussion

Septic arthritis is an infectious disease with high morbidity. Early initiation of antibiotherapy and surgical intervention are very important. Laboratory support is very important when there is clinical doubt. Microscopic examination of the leukocyte count is a valuable diagnostic pa-rameter for the diagnosis of septic arthritis in secondary healthcare institutions where diagnostic possibilities are limited and where automated culture systems are not available, especially in cases when the agent cannot be isolated [2,3]. In the presented study, we were able to diagnose septic arthritis in only three patients with bacterial isolation. Most of the patients were diagnosed based on the leukocyte count in the synovial fluid. Of 56.5% patients diagnosed as septic arthritis, leukocyte count was above 50,000/mm^3 in the synovial fluid.

Symptoms of septic arthritis include increased temperature, redness, tenderness, and limitation of movement. Systemic fever may also be present. In most cases, symptoms develop rapidly within two weeks. Symptoms occur more slowly in those infected with microorganisms and mycobacteria with low pathogenicity [2].

In two prospective studies performed by Shemerling and Jeng, the rate of detection of septic arthritis in patients who applied for monoarthritis was between 8-27% [6,7]. It was 39% in the study of Morgenstern et al. [5]. In our study, this rate was 43.3% (23/53). In our study, the detection rate of septic arthritis seems to be higher than in the literature. However, in other studies, not only cases with a prediagnosis of septic arthritis but also all cases with monoarthritis were included in the study. In our study, those with a clinical diagnosis of septic arthritis were included. Therefore, we believe that our rate is high.

Septic arthritis is more common under 15 years of age and above 55 years of age [3]. Patients over the age of 18 were included in our study. The average age of our patients was 61.7±18.96; 65.2% of them are over the age of 55. In the study of Clerc et al., the mean age was 57.6 in cases over 16 years old [9]. In our study, the mean age of adults diagnosed with septic arthritis was found to be over 55, similar to the literature.

Septic arthritis is more common in men. The effect of gender on the clinical course of natural septic arthritis is unknown [10]. However, although arthroplasty is a more common operation in women, postoperative septic arthritis is more common in men [8]. In the study of Berthoud et al. 80% of the cases and 61% in the study of Baran et al. were men [11,12]. In our study, 52.2% (12/23) of the cases were male, in compliance with the literature.

Sixty % of septic arthritis cases are observed in large joints such as hip and knee. It is frequently observed in a single joint, but in 22% of cases, more than one joint involvement occurs [2]. Polyarticular involvement is often observed in patients with underlying rheumatoid arthritis, collagen tissue disease, and in patients with sepsis [3]. The most commonly involved joints in the study of Morgenstern et al. and in the study of Yıldırım et al. were knee and hip joints [4,8]. In our study, the most commonly involved joints were knee and hip joints, respectively. While only three of our patients with a preliminary diagnosis of septic arthritis had more than one joint involvement, all patients with septic arthritis had a single joint involvement. Our results are similar with the literature.

Risk factors identified in the literature for septic arthritis were

degenerative osteoarthritis, alcoholism, intravenous drug (IV) addiction, DM, Human Immunodeficiency Virus (HIV) infection, previous intraarticular injection or joint surgery (such as arthroscopy, prosthesis) and advanced age [2]. In the study of Nissim et al., previous joint surgery, DM, immunosuppression and osteoarthritis were stated as the most common risk factors [10]. Similarly, joint operation, DM and degenerative joint disease were the most commonly detected risk factors in our study. In addition, one patient had arthroscopy and one patient had a history of trauma. A patient was receiving immunosuppressive therapy for rheumatological disease.

The most common route of entrance of bacteria is the hematogenous route. Infection can also develop with direct inoculation after an insect bite, trauma, surgical intervention or intraarticular injection. Rarely, there may be spread from the adjacent infected focus such as skin-soft tissue infection or osteomyelitis [2,3]. Seven of our patients had joint prosthesis, one had a history of arthroscopic intervention, and one had a history of trauma. We think that these patients developed infections as a result of direct inoculation. However, it is difficult to comment on this subject as the study is retrospective.

S. aureus is the most common cause of septic arthritis in adults. *Streptococci* are other im-portant gram positive bacteria. Gram-negative bacilli are more common in the elderly. Immunosuppression, trauma, and intravenous drug usage are risk factors. *Pseudomonas spp.* may also be a factor in immunosuppressive people and IV drug users. Small joint involvement is often polymicrobial and can be caused by *streptococci*, anaerobes and Eikenella [3]. In our patients, *S. aureus* was determined as the only agent. In a patient with CNS growth, the patient was not evaluated as septic arthritis, since no cells were observed in the leukocyte count and other diseases were also present in the preliminary diagnosis. Growth was interpreted as contamination. In a study conducted by Fowler et al. to investigate the presence of contamination in patients who underwent synovial fluid culture with a pre-diagnosis of septic arthritis, the most common contamination factor was determined as CNS [14].

The definitive diagnosis of septic arthritis is synovial fluid analysis and culture. The diagnosis is performed by observation of bacteria in gram staining and isolation of bacteria in culture. It is stated in the literature that isolation of bacteria from synovial fluid can be performed in more than 60% of cases in bacterial arthritis [3]. However, in some studies, it is reported that the agent could not be isolated in 64% of the cases [2]. The reasons for not being able to isolate the bacteria may be due to the patient's use of antibiotics, insufficient synovial fluid taken for culture, and keeping the incubation time short after inoculation in the culture [15]. In this case, it becomes difficult to confirm the diagnosis and to choose the appropriate antibiotic [2]. When bacteria cannot be demonstrated by gram staining and culture in synovial fluid, the diagnosis can also be performed by the presence of a purulent appearance on direct macroscopic examination (the equivalent of this appearance in microscopic examination is the leukocyte count of 50,000-150,000/mm^3) [3].

In our study, the leukocyte count was over 100,000/mm^3 in all patients whose pathogen microorganism was isolated and accepted as septic arthritis. In septic arthritis, the agent isolation rate was 12% when the cut-off value for the leukocyte count is taken as >20,000/mm^3, 18,7% when the cut-off value is taken as >50,000/mm^3, and 33,3% when the cut-off value is taken as >100,000/mm^3. The difference between high leukocyte count and bacterial isolation was statistically significant (p=0.031).

Before antibiotherapy is initiated in patients with a diagnosis of septic arthritis, a rapid synovial fluid analysis should be performed. Bacteriological examination and leukocyte count should be performed on the taken fluid. On microscopic examination, the sensitivity was 77%, 62%, 29%, respectively and the specificity is 73%, 92%, 99%, respectively for the leukocyte count >25,000/mm^3, >50,000/mm^3 and >100,000/mm^3 [2]. In gonococcal arthritis, in the early period of infection, in the presence of an additional disease causing leukopenia, and in the presence of prosthetic joints, the leukocyte count may be below 20,000/mm^3 [1]. The fact that the leukocyte count is below 20,000/mm^3 considerably reduces the possibility of infection [2].

Septic arthritis is one of the orthopedic emergencies. Antibiotherapy should start in the early period [4]. In our study, all patients (n=16) with a leukocyte count above 50,000/mm^3, 55% (5/9) of patients with a leukocyte count between 20,000-50,000/mm^3, and 8.3% (2/24) of patients with a leukocyte count below 20,000/mm^3 were diagnosed with septic arthritis and empirical antibiotherapy was initiated. All patients whose leukocyte count was less than 50,000/mm^3 and who were accepted as septic arthritis had joint prosthesis. Since the leukocyte count may be less in patients with joint prostheses, these patients were accepted as septic arthritis and treatment was initiated.

The total duration of antimicrobial treatment is 4-6 weeks. At least the first two weeks of this treatment should be administered parenterally. Later, transition to oral treatment may be performed [2]. Empirical treatment should start according to gram staining results. In cases where bacteria are not observed in Gram staining, vancomycin is recommended in natural septic arthritis if there is no complicating factor such as trauma. Those with a history of trauma should be administered vancomycin in combination with a third generation cephalosporin. In immunosuppressive people, vancomycin and a cephalosporin combination with antipseudomonal efficacy should be started [3]. Ceftriaxone treatment is recommended for those who are young and have a history of recurrent sexually transmitted diseases [2].

Successful treatment of septic arthritis is possible with the removal of purulent material from the joint. For this purpose, serial needle aspirations, open joint debridement, arthroscopic joint debridement and continuous joint irrigation after arthroscopy can be used in addition to systemic antibiotherapy [4]. In a study comparing serial needle aspiration and open arthrotomy, which are methods used in the surgical treatment of septic arthritis, no statistically significant difference was found between these two methods in the treatment of uncomplicated septic knee arthritis in terms of length of hospital stay, mortality rates, and treatment success [16].

Since our study was designed retrospectively, it has some limitations. Not all of the antibiotic choices used in the treatment could be accessed through the electronic file records. Only information regarding initiation of antibiotics could be accessed for some patients. Therefore, antibiotic choices were not included

in the study. Not all surgical records were also available. Therefore, the results of surgical treatment also could not be included in the study. The lack of biochemical analysis results of acute phase reactants and synovial fluid is another limitation of our study. Biochemical analysis of synovial fluid was not studied in any of the patients. Since acute phase reactants were not studied in more than one third of the patients, these data were not included.

Conclusion

In conclusion, septic arthritis is a disease that requires a multidisciplinary approach. Synovial fluid analysis should be performed rapidly in patients with suspected septic arthritis. Direct microscopic examination of the liquid, gram staining and culture should be performed. Incubation time should be extended after inoculation for culture to increase the chance of bacterial isolation. Empirical antibiotherapy should not be initiated without performing synovial fluid analysis because it will cause false negativity in the future synovial fluid examination. Microscopic examination of the leukocyte count is a valuable diagnostic parameter for the diagnosis of septic arthritis in secondary healthcare institutions where diagnostic possibilities are limited and where automated culture systems are not available.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was approved by Tokat Gaziosmanpaşa University, Non-Invasive Clinical Re-search Ethical Committee (date: April 26, 2021, No: 2020/04 and Project No: 20-KAEK-062).

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