Evaluation of synovial fluid culture in patients with high suspicion for septic arthritis

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Abstract: Septic arthritis is the infection of joints and delay in treatment may lead to irrecoverable injuries such as joint destruction and dissemination of infection to other organs. The aims of this study were to evaluate synovial fluid cultures in patients with high suspicion for septic arthritis, their clinical and laboratory findings and to determine probable causes of true/false negative cultures. In this cross-sectional study, 25 patients with painful and swollen joint and high clinical suspicion for septic arthritis enrolled the study. Sterile synovial fluid aspiration was performed and specimens were evaluated direct smear for gram staining and 3 different cultures using chocolate agar, Mac-Conkey and blood agar for 24 hours. Blood samples were also obtained for culture. Data were analyzed using SPSS software (version 15). Fever, joint pain, swelling, warmth and tenderness were reported by all patients. Ten patients (40%) suffered from chilling and 24 patients (96%) from restricted movement. All synovial fluid gram staining and blood cultures were negative. But synovial blood cultures were positive in 3 patients (12%) showing Klebsiellapneumoniae, Candida albicans and Brucella infections. The results of our study were different from other studies. False negative cultures may be due to fastidious organisms, inadequate laboratory techniques, or prior antibiotic therapy.

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Introduction:

Septic arthritis refers to the infection of articular space and is an urgent medical problem, since any delay in diagnosis and cure can lead to irreversible damage to the joint, such as articular destruction and the spread of infection to other parts of the body (1). The prevalence of septic arthritis is about 2-5 per 100,000/year in normal population and 28-38 per 100,000/year in high risk population, like those having rheumatoid arthritis(RA)and also, 40-68 per 100,000/year in those having prosthetic joints (2). The mortality rate of septic arthritis is about 12-18%. Also, about 30-52% of the patients lose their joint activity (3).

Most causes of septic arthritis occur through hematogenous spread. Staphylococcus aureus is the most common cause of non-gonococcal septic arthritis in adults, accounting for about 2/3 of the cases (4). Also, about 80% of cases with septic arthritis in RA patients are due to Staphylococcus aureus. The prognosis of septic arthritis is poor in case of RA, age above 80 years, presence of prosthetic joints and use of immuno-suppressive drugs (5). Even a mortality rate reaching 50% has been reported in those having RA and concomitant poly-articular septic arthritis (5).

The most common form of septic arthritis is the mono-articular involvement and knee is the most common involved joint. The definite diagnosis is made by directly directly the organism in gram-stained smears of the synovial fluid, positive synovial fluid culture and/or showing the DNA of the micro-organism using polymerase chain reaction (PCR) (6, 7). Currently, diagnosis is often made according to synovial fluid cell count, smear and culture. If the patient has not recently used antibiotics, cultures would be positive in 70-90% of non-gonococcal bacterial arthritis (8). Cultures may show negative results in case of technical problems, fastidious organisms or recent antibiotic use by the patient (9).

PCR has some advantages including high sensitivity and rapidity, especially for fastidious organisms or in case

of antibiotic use. But the main problems are contamination with other organisms which can lead to false positive results and the high price of the technique (10). Also, it has not completely become standardized and is not commonly available.

The aims of this study were to evaluate synovial fluid cultures in patients with high suspicion for septic arthritis, their clinical and laboratory findings and to determine probable causes of true/false negative cultures.

Methods:

Twenty five patients with articular pain and swelling and high suspicion for septic arthritis, referring to Imam-Ali hospital of Zahedan, entered the study. The decision for classifying the patient as highly susceptible for septic arthritis was made according to the presence of some clinical symptoms including fever, articular pain, swelling, erythema, tenderness and/or stiffness and some laboratory findings including leukocytosis, elevated erythrocyte sedimentation rate (ESR) and/or elevated C-reactive protein (CRP) and high leukocyte count in synovial fluid with polymorphonuclear (PMN) predominance. In case of hip involvement, orthopedic consultation for articular drainage was also performed.

If the patients were using non-steroidal anti-inflammatory drugs (NSAIDs), methotrexate(MTX) or other cytotoxic medications, the drugs were stopped until the results of smear and culture got ready, but other drugs were continued. After synovial fluid aspiration, empirical antibiotic therapy was started according to common organisms (ceftriaxone 1gr q12 hours and cloxacillin 2gr q6 hours). The decision for continuation or discontinuation of antibiotics and using further treatments were made according to the results of smears and cultures.

Synovial fluid aspiration was performed using sterile material and methods. Using a 10-cc syringe, 10cc of synovial fluid was sent to laboratory for cell count, gram smear, culture, antibiogram and also assessment for crystals. Cultures were performed in 3 different media (chocolate-agar, Mc-Conkey and blood agar; all from Merck Company, Germany) and were kept in an environment with 5-10% carbon dioxide at of 37°C for 48 hours. Also, some amounts of synovial fluids were injected in the blood culture media (Padtan-teb, Iran) and were kept in 37°C. If any turbidity was observed during the coming 3 days, cultures were performed in the chocolate-agar, Mc-Conkey and blood agar again. If no turbidity was observed during the first 3 days, cultures from blood culture media were performed every week for 4 weeks. If a colony was found, smears and other processes were performed as described above.

During the protocol, if any colony was found in a culture-plate, smears of the mentioned colonies were assessed using gram stain. In case of gram positive cocci, catalase test were performed to differentiate between staphylococcus and streptococcus (One drop of oxygenated water was put on a lam and 2-3 colonies of Mc-conkey or chocolate agar media were added and if bubbles were formed, it was assumed as positive catalase result, referring to the presence of staphylococcus). Then coagulase test and cultures in mannitol salt-agar were performed (5-6 colonies were added to 4cc of plasma and were kept in 37°C for 4 hours. This complex was evaluated every 0.5-1 hour and in case of clot formation, the presence of staphylococcus aureus was confirmed.). Also, cultures in media including 1/25000, 1/50,000 and 1/100,000 concentrations of thionin and fuchsin were performed for Brucella and germ-tube test was performed for Candida. In case of Brucella species, biochemical tests including agglutination test was also performed.

Results:

Among 25 participants, 6 (24%) were male and 19 (76%) were female. The mean age was 48.8 ± 10.8 years (ranging from 21 to 61 years). Three patients (12%) had diabetes mellitus, 12 (48%) had RA, 4 (16%) had history of renal transplantation, one (4%) had systemic lupus erythematosus (SLE), one (4%) had juvenile rheumatoid arthritis and one patient had both diabetes and RA, simultaneously. Three of the patients (12%) had no underlying disease.

Among those 12 patients with RA, 9 patients used MTX and all were taking prednisolone (7 patients used 7.5mg/day, 3 patients used 10mg/day and 2 patients used 15mg/day).

None of the patients had prosthetic joints or history of intra-articular injection. Three patients (12%) had previous history of joint trauma and 7 patients (28%) had previous history of septic arthritis.

All patients had fever, articular pain, warmth, swelling and tenderness. 40% of patients complained of chills and 96% had joint stiffness. Knee was the most common (92%) site of involvement (60% left knee and 32% right knee) and the remaining 8% had hip involvement. None of the patients had adjacent osteomyelitis in their X-ray evaluations.

The mean white blood cell (WBC) count was 12,172 ± 1,851 /ml. Fifteen patients (60%) had WBC > 11,000/ml. The mean percent of PMNs was 76.4% ± 13.8% and 10 patients (40%) had PMN > 80%.

The mean synovial fluid white cell count was 49,880 ± 17,609 /ml. Ten patients (40%) had synovial fluid cell count > 50,000.

The mean synovial fluid cell count had significant correlation with WBC ($p < 0.05$). Also, the mean synovial fluid cell count showed significant difference among different joints (52,666 ± 15,559 /ml for the left

knee, 39,125 ± 17,041 /ml for the right knee and 72,000 ± 21,174 /ml for the hip join) ($p< 0.05$) (figure 1).

The mean ESR level was 74.8 ±31.4 mm/first hour (ranging from 35 to 125 mm/first hour). The mean ESR levels according to the involved joints are shown in figure 2.

The mean percent of PMN in synovial fluid was 76.5 ± 15.8%. Fourteen patients had PMN > 80%.

Synovial fluid appeared turbid in 17 patients (72%). The mean white cell counts in turbid and clear fluids were 53,666 ± 15,807 /ml and 40,142 ± 19,463, respectively ($p< 0.05$) (figure 3).

Blood cultures were negative in all patients, but synovial fluid cultures were positive in 3 patients, including:

- Klebsiellapneumoniae in 45 year old woman with SLE. The patient had no history of cytotoxic or steroid use. She had referred with fever, chills and articular pain and stiffness. WBC count of the patient was 12,000/ml and white cell count in her synovial fluid was 42,000/ml.
- Brucella in a 48 year old woman with RA. She was taking prednisolone (15mg/day) and had positive history for using non-pasteurized milk. She had also previous history of septic arthritis. Her WBC count was 12,300/ml and the synovial fluid white cell count was 48,000/ml. The synovial culture showed the organism in the chocolate-agar environment.
- Candida albicans in a 59 year old woman with diabetes mellitus. She did not have previous history of septic arthritis. Her WBC was 8,000/ml and her synovial fluid cell count was 22,000/ml.

All the 3 mentioned patients had left knee arthritis.

Discussion:

Although most cases of septic arthritis are due to hematogenous spread of the infection to the articular space, in some cases, bacterial arthritis can be due to bites, trauma, direct inoculation of bacteria during articular surgeries and rarely, due to spreading from adjacent osteomyelitis (11).

Patients with RA are at high risk for bacterial arthritis (12). Intra-articular injection and immuno-suppressive drugs play role in increasing the susceptibility for septic arthritis (13). Therefore, in our study, about 90% of the enrolled patients had risk factors for septic arthritis.

Patients with septic arthritis usually refer with acute pain and swelling of one or more joints (14). Fever is another important finding in patients with septic arthritis (15). According to a meta-analysis performed by Margaretten et al, articular pain (85% sensitivity for predicting septic arthritis), swelling (78% sensitivity) and fever (57% sensitivity) were the only findings in more than 50% of the patients. The less common findings were sweating (27% sensitivity) and stiffness (19% sensitivity) (16). In our study, all the enrolled participants had fever and articular pain, swelling, warmth and tenderness.

All the enrolled patients had mono-articular involvement and left knee was the most common involved joint. According to other studies, knee involvement has been reported in more than 50% of patients with septic arthritis (11). Deesomchok reported knee involvement as the most common site of involvement (52.5%) during studying 101 patients with non-gonococcal septic arthritis (17).

During our study, the synovial fluid white cell counts of 10 patients were more than 50,000, but none of them had positive blood culture results. The normal synovial fluid is almost without cells. Inflammatory or infected fluids have increased white cell counts, reaching to even 50,000-150,000/ml in bacterial arthritis, mostly consisted of PMNs (18). According to a study performed by McGillicuddy on 49 septic arthritis patients who had positive synovial culture results, 39% of patients had synovial fluid white cell counts less than 50,000/ml (19).

According to the meta-analysis performed by Margaretten, the most important laboratory findings in septic arthritis are synovial fluid cell count and the percentage of PMNs, so that PMN>90% was significantly correlated with the presence of septic arthritis (16). But according to our study, all the 3 patients with positive synovial culture results had white cell counts less than 50,000. Therefore, lower synovial fluid cell counts cannot rule out the diagnosis of septic arthritis.

During our study, synovial fluids appeared to be turbid in 72% of the cases. Although turbidity can be due to non-cellular materials, it is mostly due to increases in cell counts of synovial fluid (20).

The viscosities of synovial fluids in our study were all decreased. Although purulent synovial fluids can rarely be viscous, the presence of proteolytic enzymes in the synovial fluid usually decreases the viscosity (15).

According to our results, synovial fluid smears could not show any organism in any patient. Previous studies have shown that smears can show the organism in most of the cases with positive culture results. The sensitivity of smears in showing the organism is accounted to be about 29-50% (15). However, the presence of violet crystals and mucin can lead to false positive results due to mimicking cocci organisms in gram staining (20).

According to our results, 12% of patients had positive synovial fluid culture results, including Klebsiellapneumoniae, Brucellaand Candida. Although our results are much different from other studies, the important point was the presence of underlying disease in all of them.

According to the study performed by Deesomchok on 101 patients with non-gonococcal septic arthritis, synovial fluid cultures were positive in 72.3% of the

patients. The most common organism were Staphylococcus aureus (47.5%), beta-hemolytic Streptococcus (28.7%) and gram-negative bacilli (13.9%) (17).

According to a study performed by Von Essen on 47 patients with bacterial arthritis, using blood culture environments for cultivating synovial fluid led to positive results in 21% of patients in whom traditional culture environments could not previously show the organism (21).

Staphylococcus aureus and Streptococcus have higher tendency than gram-negative bacillus to involve joints. About 80% of cases with septic arthritis in those who have RA are due to Staphylococcus aureus (2). But no one of our patients showed Staphylococcus infection. This may be due to technical problems or may be due to previous antibiotic consumption by the patients who did not exactly know the drugs theyhad used, although we asked the patients to bring all the drugs that they had used during the previous 2 weeks.

Synovial fluid cultures are mostly positive in patients with non-gonococcal bacterial arthritis. Negative results can be due to recent antibiotic use or contamination with Streptococcus or Mycoplasma (20).

During our study, a 59 years old patient with diabetes was found with Candida arthritis. Fugal arthritis should be kept in mind when encountering a patient with mono-arthritis in whom immune system is suppressed. In these patients, special culture environments should be used.

Blood culture environments have also been offered for cultivating synovial fluid to help identifying the organismin case of negative results by traditional synovial fluid culture environments (21). False negative results are encountered while cultivating fastidious organisms, laboratory technical problems and prior antibiotic use. During our study, those who had history of recent antibiotic use were excluded from the study. Therefore, the two remaining reasons might have been the main causes of high negative results during our study.

Recently, PCR has been offered as a method for definite diagnosis of septic arthritis. According a study performed on 121 patients with septic arthritis, the sensitivity and specificity of PCR in identifying the organism in synovial fluid were 95% and 97%, respectively. However, this technique is not available world-wide and is very expensive.

Conclusion:

According to our results, diagnosing septic arthritis according to clinical symptoms and signs of the patients are probably more reliable than smear and culture results in hospitalized patients. Also, it seems better to start therapy according to the patient's clinical status.

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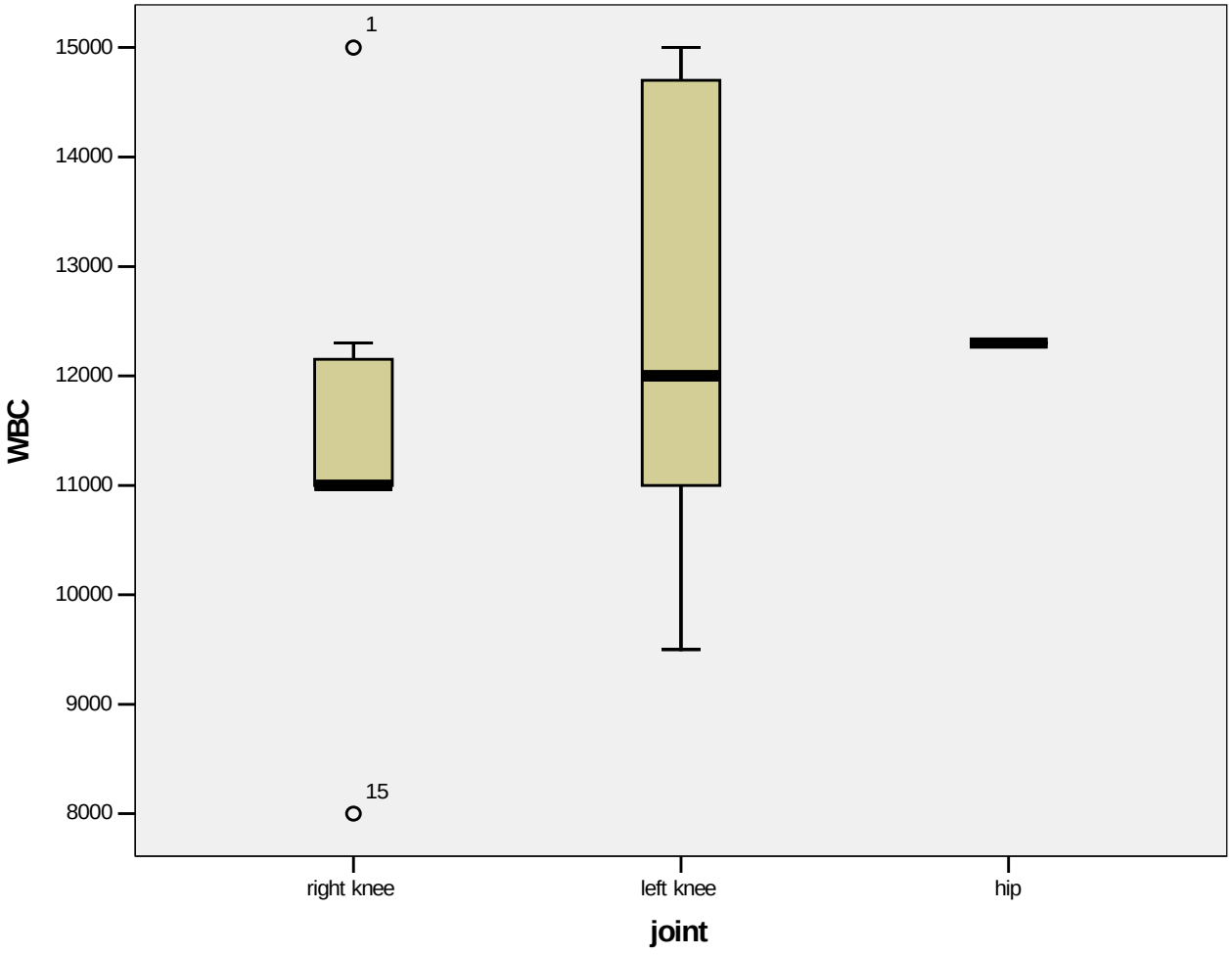

Figure 1: comparing mean WBC counts according to the involved joints

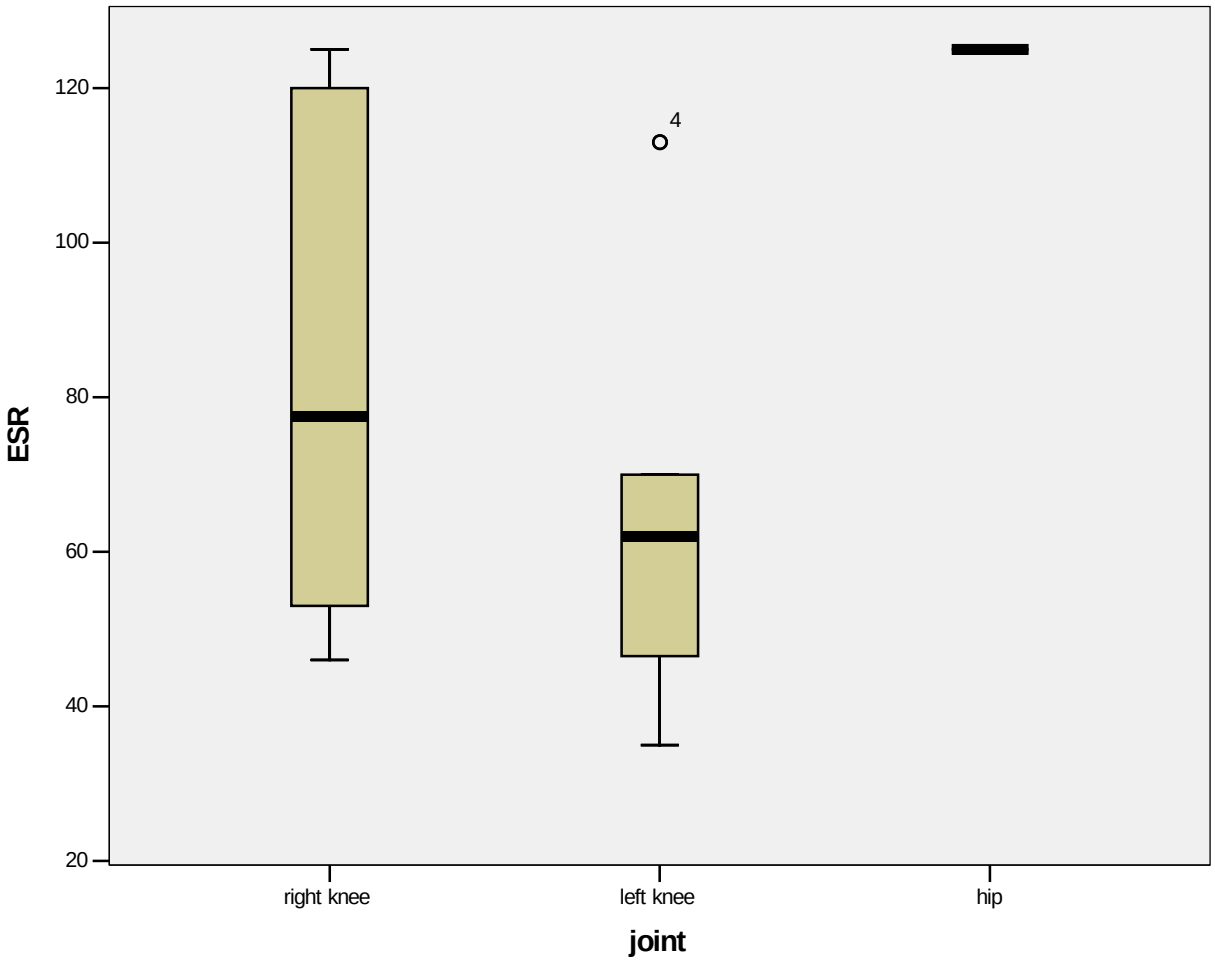

Figure 2: comparing mean ESR levels according to the involved joints

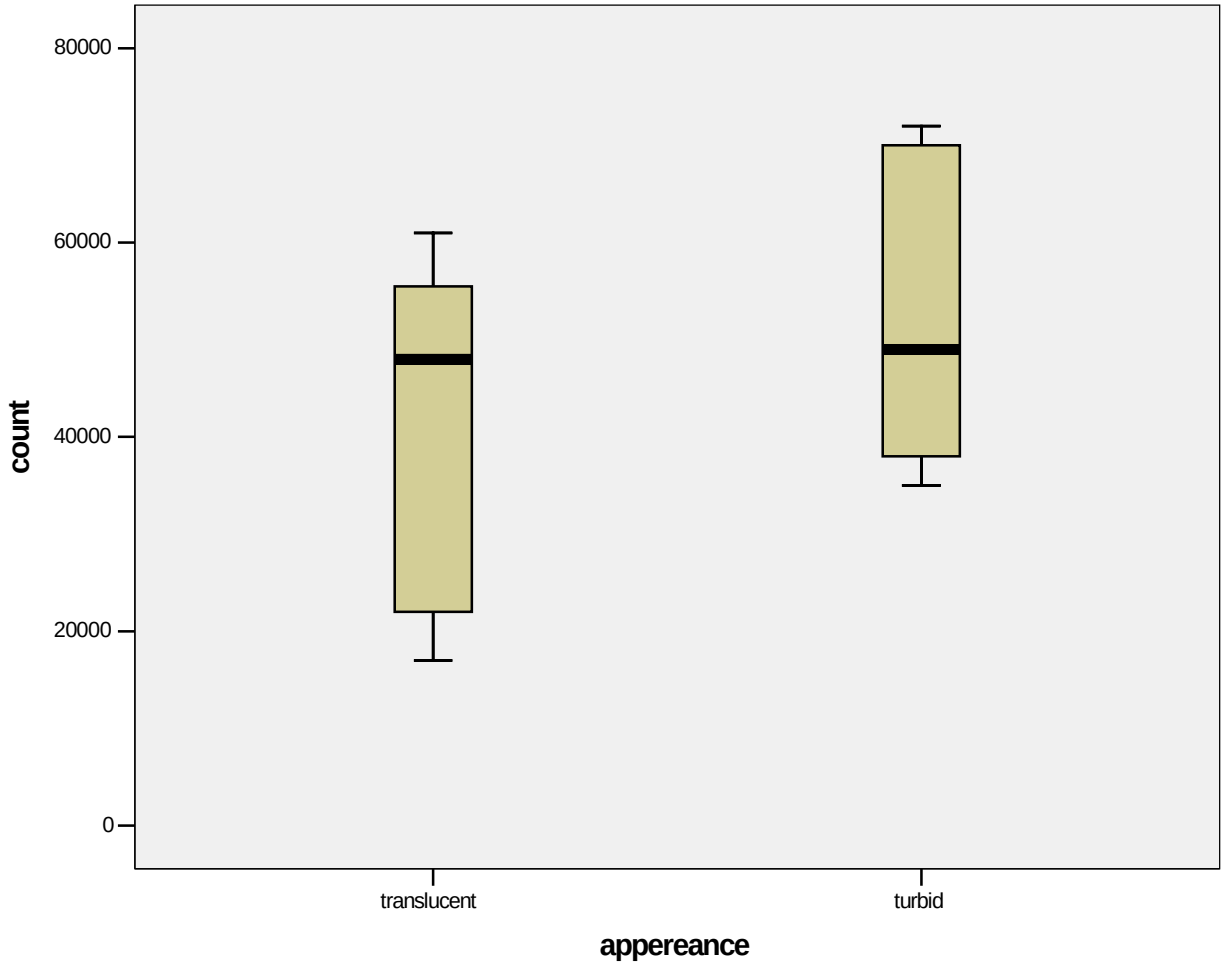

Figure 3: comparing mean synovial fluid cell counts according to the appearance of the fluid