RESEARCH ARTICLE

A More Positive Culture by Resin-containing Media Usage after Suspicious Arthroscopic Infections in Patients Receiving Antimicrobial Therapy

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Abstract

Background: Although infections following arthroscopic surgery of cruciate ligament and meniscus are uncommon, they have potentially serious consequences for the graft and articular cartilage. This study aimed to investigate the efficacy of correct sampling and appropriate media, especially resin-containing media, for the detection of infections in patients receiving antibiotics under suspicion of joint infection after arthroscopic anterior cruciate ligament (ACL) and meniscal surgery. In such cases, proper sampling and the use of suitable culture media that cause the neutralization of antibiotics are very effective in isolating microorganisms from the patient samples and positive cultures.

Methods: In total, 10 patients who had received antibiotics with suspected knee infection after arthroscopic ACL and meniscal surgery were identified after referral to surgeons during a period of 10 months and investigated in this study. The sample collection, culture on various media (i.e., resin-containing culture media), microbiological tests, and antibiotic susceptibility tests were performed in this study. The amplification of the mecA gene using PCR assay was accomplished for methicillin-resistant staphylococcus strains.

Results: This study was conducted on 10 patients who underwent arthroscopic procedures and had received antibiotics. Overall, joint fluid and tissue culture were positive in 60% of the patients. The resin-containing media revealed a trend toward increased detection of bacteria. Coagulase-negative staphylococcus strains were the most frequently isolated bacteria in arthroscopic ACL surgery infections. Out of five methicillin-resistant staphylococcus strains, four strains were found that were resistant to cefoxitin and positive-mecA designated as methicillin-resistant strains. Except for one case, the rest of the staphylococcal strains were resistant to methicillin but susceptible to vancomycin.

Conclusion: Despite uncommon and low percentage of infections after arthroscopic ACL and meniscal surgery, the results of our study showed that correct sampling, appropriate cultures, especially aerobic and anaerobic resin-containing media, and microbiological testing remained useful and valuable for diagnosing bacterial infections.

Level of evidence: II

Keywords: Anterior cruciate ligament and meniscal surgery, Arthroscopy, Infection, Resin-containing culture media

Introduction

An anterior cruciate ligament (ACL), meniscus lesions, and combined injuries are the most common knee injuries in sport and traumatic insult (1). These injuries predispose the knee to subsequent injuries and are regarded as the potential for the earlier onset of osteoarthritis (2, 3).

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Arthroscopic knee surgery is one of the most frequent orthopedic procedures conducted worldwide, especially ACL and meniscal surgery (4-7). Infection is one of the complications after arthroscopic surgery within the range from 0.1% to 3.4% (8, 9). The infection presentation after arthroscopic ACL surgery involves a painful knee joint with a limited range of motion, persistent effusion, local erythema, and unusually a fever of 38°C or greater. In this study, prompt detection and therapy of the infection after surgery is significant to avoid complications, such as injury to the cartilage and arthrofibrosis; moreover, a delay in therapy can lead to multiple additional operations, graft rejection, cartilage destruction, and joint dysfunction (10-12). After detection, it is of critical importance to decrease the risk of false-negative cultures, perform correct sampling and appropriate culture (resin-containing culture media), take multiple samples from different sites, and stop antibiotic administration at least two weeks prior to sampling (13, 14). The most frequently encountered organisms include coagulase-negative staphylococci (CoNS), Staphylococcus aureus, and methicillin-resistant staphylococcus (MRS) strains; however, Gram-negative bacteria are infrequently observed (4, 5). The MRS strains contain mecA, a gene that encodes the penicillin-binding protein 2a, which induces resistance to methicillin and other β-lactam antibiotics. It is worth mentioning that these strains are a global public health threat (15, 16). This study aimed to investigate the efficacy of correct sampling and appropriate media, especially resin-containing media, for the detection of infections in patients receiving antibiotics under suspicion of joint infection after arthroscopic ACL and meniscal surgery. This situation is considered complicated and chronic. The appropriate sampling and use of the culture media (aerobic and anaerobic resin-containing media) contributed to neutralizing antibiotic activities and isolation of bacteria.

Materials and Methods

During 10 months, 10 patients receiving antibiotics with suspected knee infection following arthroscopic ACL and meniscal surgery were referred to surgeons in tertiary care hospitals and enrolled in this study. This cross-sectional study included samples from patients with suspected knee infection following arthroscopic ACL and meniscal surgery for 10 months. Comparative statistical analyses to the assay of parameters were conducted using the Chi-square and Fisher's exact test analyses. All patients (six athletes and four non-athletes) had symptoms of swelling, pain, warmth, erythema, and effusion in the knee at the time of referral. None of the patients was diabetic and immunosuppressed. Hamstring tendon autografts were used for all patients with arthroscopic ACL surgery (n=6), arthroscopic ACL and meniscal surgery using hamstring tendon autografts (n=3), and arthroscopic meniscal surgery (n=1).

Out of 10 patients who were injured in sport or by traumatic insult, nine male and one female patients (age range: 29-37 years) were qualified to be included in the study. The patients' characteristics covered such information as age, gender, blood inflammatory markers (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]), white blood cell (WBC), neutrophil percent (Neut %) or polymorphonuclear cell percentage (PMN%), fever, antibiotic consumption (in ≤ 2 to 4 weeks preceding surgery), and time to the presentation (days after the operation) of patients [Table 1]. Arthroscopic knee surgery and sampling were performed on patients who had clinical signs and received antibiotics. All 10 patients of arthroscopic ACL and meniscal surgery with high CRP and ESR had taken multiple antibiotics before sampling. According to the days after arthroscopic surgery until the onset of the signs of arthritis, the infections were considered acute (≤14 days), subacute (>14 days and ≤30 days), or delayed (>30 days).

In sample processing, synovial fluid (at least one sample) and biopsy (at least two samples) specimens were taken from the synovial membrane of patients with suspected knee infections who had received antibiotics and underwent arthroscopic knee surgeries.

The synovial fluids were inoculated into aerobic and anaerobic resin-containing culture bottles, and biopsies were inoculated into thioglycollate broth. The culture processes were performed in the operating room, and the diagnostic tests were accomplished in the microbiology laboratory. The aerobic and anaerobic resin-containing culture bottles containing synovial fluids were incubated at 37°C for 48 h in different conditions (aerobic, microaerophilic atmosphere composed of 5%-7% CO2, and anaerobic conditions). The obtained synovial tissues were homogenized using a sterile Ten Broeck tissue grinder, and its suspensions were inoculated into aerobic and anaerobic resin-containing media. Subsequently, they were incubated at 37°C after exposure times of 1-7 days in different conditions.

If there is no turbidity and growth, they will keep for 14 and 21 days under aerobic and anaerobic conditions, respectively. After growth and turbidity, part of the liquid medium was cultured on different media of agar, such as Blood, Chocolate, MacConkey, and Schaedler agar supplemented with vitamin k1 at 37°C; moreover, the incubation time was prolonged to 14-21 days for slow-growing and fastidious microorganisms. The identification of bacterial isolates was performed using biochemical assays and VITEK 2 Compact system (BioMerieux, USA). The susceptibility of bacterial isolates to various classes of antibiotics was determined by the disk diffusion method (Kirby Bauer) with antibiotic discs (MAST Company, United Kingdom) according to the Clinical and Laboratory Standards Institute guideline (17).

Antimicrobials used against the Gram-positive bacteria in this study were cefoxitin (30μg), penicillin (10IU), ampicillin (10μg), vancomycin (30μg), clindamycin (2μg), erythromycin (15μg), tetracycline (30μg), ciprofloxacin (5μg), trimethoprim-sulfamethoxazole (1.25-23.75 μg), linezolid (30μg), rifampin (5μg), cefazolin (30μg), and

Table 1. Characteristics of patients suspected knee infection after arthroscopic ACL and meniscal surgery

Patients	Age (yr.)	Sex	Concomitant procedures	Previous knee surgery	Graft	Empirical antibiotics (in ≤ 2 to 4 weeks preceding surgery	Time to presentation (days after operation) with clinical signs	Fever	Synovial fluid WBC/ mm^3	Synovial fluid %Neut	ESR (mm/h)	CRP (mg/L)	Identified bacteria
1	29	M	ACLR	Yes	Autograft- HT	CFX	30	+	544000	98	18	100	MRSA+ P.M
2	29	M	ACLR	Yes	Autograft- HT	RF,VA	14	+	640000	80	34	68	MRCNS
3	32	M	ACLR	Yes	Autograft- HT	RF,TS	60	-	39400	91	44	32	E.f
4	27	M	ACLR , MeniscusR	Yes	Autograft- HT	Lvx ,CZ(IV)	90	-	450000	94	84	81	MRSA
5	35	M	ACLR	Yes	Autograft- HT	Lvx ,CZ(IV)	40	+	24000	98	40	96	MSCNS
6	31	M	ACLR	Yes	Autograft- HT	CFX	25	+	2430	90	74	110	MRCNS
7	35	M	ACLR	Yes	Autograft- HT	VAN,KAZ,GM,MEM	58	+	5300	51	100	60	No growth
8	35	M	ACLR , MeniscusR	Yes	Autograft- HT	CFX, CFM	180	-	49000	90	49	40	No growth
9	37	M	ACLR , MeniscusR	Yes	Autograft- HT	CIP,CFX	365	-	2170	65	70	280	No growth
10	24	F	MeniscusR	No	-	CFX,CD AMP	120	-	640	51	80	31	No growth

RF: Rifampin, VA: Vancomycin, TS: Trimethoprim-sulfamethoxazole, Lvx: levofloxacin, CZ: Cefazolin, CFM: Cefixime, CFX: Cephalexin, MEM: Meropenem, KAZ: Ceftazidime, CIP: Ciprofloxacin, GM: Gentamicin, CD: Clindamycin, AMP: Ampicillin. IV: Intravenous, WBC: peripheral white blood cell count, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein. MRSA: methicillin-resistant Staphylococcus aureus, MRCNS: methicillin-resistant coagulase-negative Staphylococci (Methicillin resistant Staphylococcus epidermidis), MSCNS: methicillin-susceptible coagulase-negative staphylococci (methicillin-susceptible Staphylococcus epidermidis), E.F: enterococcus faecalis, P.M: Proteus mirabilis, ACLR: Anterior cruciate ligament reconstruction, MeniscusR: Meniscus reconstruction, HT: hamstring tendon

cephalexin (30µg).

On the other hand, ciprofloxacin (5µg), gentamicin (10µg), piperacillin-tazobactam (100-10µg), cefepime (30µg), ceftazidime (30µg), imipenem (10µg), meropenem (10µg), trimethoprim-sulfamethoxazole (1.25-23.75 µg), tetracycline (30µg), and ampicillin (10 µg) were used against the Gram-negative bacteria. The vancomycin minimum inhibitory concentrations (MIC) were determined using the E-test (MIC test strip, liofilchem, Italy) for MRS spp. The MRS-Screen test was performed using cefoxitin resistance and was confirmed using the amplification of the mecA gene by PCR assay. Molecular assay of mecA gene was performed, and after the extraction of bacterial DNA, PCR assays for mecA gene were performed in a reaction volume of 25 µl. Primers were mecA-F: 3'- ACTGCTATCCACCCTCAAAC-5' as forward and mecA-R: 3' CTGGTGAAGTTGTAATCTG-5' as reverse with annealing at 58°C for 45 sec, and the expected amplicon was 163 bp (18, 19).

Results

During the study period, 10 patients with suspected knee infections who had received antibiotics were identified in tertiary care hospitals. These 10 autograft recipient patients were enrolled after arthroscopic ACL and meniscal surgery with clinical signs and suspected infection. The use of multiple patient samples for culture instead of only one sample contributed to culture-positive results. The findings of culture on broth media (aerobic and anaerobic resin-containing media) and thioglycollate broth, were valuable for synovial fluid and tissue suspensions. There were four and six culture-negative and positive patients, respectively. Acute (n=1), subacute (n=2), and delayed (n=3) infections were observed in six culture-positive patients. In this study, the time of referral of patients with clinical signs in the knee after arthroscopic knee surgery was 14 to 365 days. Furthermore, six patients with positive cultures encountered the clinical signs on days 14-90 after surgery, and four patients with negative cultures noticed these signs on days 58-120, except for one case that observed the signs 365 days after surgery.

Table 1 tabulates the characteristics of the patients with suspected infection after arthroscopic ACL and meniscal surgery. This cross-sectional study included a small number of samples; therefore, there were no statistically significant differences among them regarding the parameters. Out of 10 patients with arthroscopic ACL and meniscal surgery, the culture-positive patients had arthroscopic ACL surgery (n=5), as well as arthroscopic ACL and meniscal surgery (n=1). Consequently, joint fluid and tissue culture were positive in 60% of the patients.

Among positive-culture patients, methicillin-resistant coagulase-negative staphylococci (MRCNS) was isolated in two arthroscopic ACL surgery cases, and methicillin-resistant Staphylococcus aureus (MRSA) was isolated in one arthroscopic ACL and meniscal surgery case; furthermore, methicillin-susceptible coagulase-negative staphylococcus (MSCNS) was isolated in one arthroscopic ACL surgery case, and one Enterococcus faecalis (E.f) was isolated in one arthroscopic ACL surgery case. In addition, both MRSA and Proteus mirabilis (P.m) were isolated in one arthroscopic ACL surgery case.

The most common isolated bacteria were the MRCNS and MRSA which were outlined in Table 1. Antibiotic susceptibility testing of seven bacterial isolates was also determined in this study. Table 2 summarizes the antibiotic susceptibility obtained from the testing data. All five staphylococcus isolates were susceptible to linezolid and rifampin; however, they were resistant

Table 2. Arthroscopic ACL and meniscal surgery-related infections, Gram positive and Gram negative pathogens antimicrobial susceptibility testing results; *Staphylococcus sp, Enterococcus faecalis, Proteus mirabilis*

	Susceptibility and resistance to antibiotics						
Antibiotics / Bacteria	***S.aureus 1***	***S.aureus 2***	***CoNS 1***	***CoNS 2***	***CoNS 3***	***E. faecalis***	***P.mirabilis***
Gram-positive							
Ampicillin	NA	NA	NA	NA	NA	S	NA
Penicillin G	R	R	R	R	R	S	NA
Cefoxitin	R	R	R	S	0	NA	NA
Cefazolin	R	S	S	S	S	NA	NA
Cephalexin	R	R	S	S	S	NA	NA
Clindamycin	R	R	S	S	S	NA	NA
Ciprofloxacin	S	R	S	S	R	NA	NA
Erythromycin	S	R	S	S	R	NA	NA
linezolid	S	S	S	S	S	S	NA
Rifampin	S	S	S	S	S	R	NA
Tetracycline	S	S	R	S	S	NA	NA
Trimethoprim-sulfamethoxazole	S	R	R	S	S	NA	NA
Vancomycin	S	S	S	S	S	S	NA
Gram-negative							
Ampicillin	NA	NA	NA	NA	NA	NA	R
Ceftazidime	NA	NA	NA	NA	NA	NA	S
Ciprofloxacin	NA	NA	NA	NA	NA	NA	R
Ceftriaxone	NA	NA	NA	NA	NA	NA	S
Cefepime	NA	NA	NA	NA	NA	NA	S
Gentamicin	NA	NA	NA	NA	NA	NA	R
Imipenem	NA	NA	NA	NA	NA	NA	S
Meropenem	NA	NA	NA	NA	NA	NA	S
Piperacillin-tazobactam	NA	NA	NA	NA	NA	NA	S
Tetracycline	NA	NA	NA	NA	NA	NA	R
Trimethoprim-sulfamethoxazole	NA	NA	NA	NA	NA	NA	R

S: Susceptible, R: Resistant, S. aureus: Staphylococcus aureus, CoNS: Coagulase-negative Staphylococci, E. faecalis: Enterococcus faecalis, P. mirabilis: Proteus mirabilis

to penicillin G. Additionally, out of five staphylococcal strains, two isolates were resistant to erythromycin and tetracycline, and three isolates were resistant to trimethoprim-sulfamethoxazole, clindamycin, and ciprofloxacin. Except for one case, the rest of the staphylococcal strains were resistant to methicillin but susceptible to vancomycin, and the MIC for vancomycin among MRS strains was ≤ 1 µg/ml. The four MRS strains resistant to cefoxitin were mecA-positive staphylococci. It is noteworthy to mention that the antibiotic choice in two patients treated with empirical antibiotics (cefazolin and rifampin) was incorrect.

Discussion

The clinical signs following arthroscopic ACL and meniscal surgery included swelling, pain, warmth, knee effusion, and loss of knee flexion. In some patients, fever and erythema can indicate sepsis. Synovial fluid analysis including WBCs level, PMN %, as well as blood CRP and ESR levels can help diagnose sepsis (2, 3, 20, 21). On the other hand, positive-culture results definitely indicate infection (22). The use of antibiotics plays an important role in reducing the positive-culture results. In this study, all patients had consumed more than one type of antibiotic, except for two patients who used only one antibiotic. Despite

the fact that patients had received antibiotics and the expected lower percentage of positive cultures, 60% of synovial fluid and tissue cultures were positive. In a study conducted by Jabalameli et al., joint fluid culture in patients with knee septic arthritis after ACLR was positive in 40% of the patients (23). Similarly, Fowler et al. conducted a study on 115 patients with consecutive ACLRs using allografts.

According to the results, the clinical signs of infection were observed with no additional antibiotics; however, intravenous antibiotics were given within 1 h before skin incision, and positive allograft culture was 2.6% (17). Totally, two factors were effective in the high percentage of positive-culture results in this study, compared to other studies. Firstly, the use of various media, especially aerobic and anaerobic resin-containing culture, which can reduce the activities of several antibiotics present in the body fluids and increase the number of positive cultures. Secondly, the use of several patient samples for culture instead of only one sample (24, 25). Resin-containing culture media is commonly used for blood infection detection and neutralizing antimicrobial activities from the blood due to empirical initiation of antibiotic treatment before carrying out blood culture (24). The present study used this media for synovial fluid culture and tissue suspensions culture in patients consuming antibiotics, and the positive culture findings were 60%. The superiority of resin-containing culture bottles (BACTEC Peds Plus/F bottle) for detecting microorganisms in synovial fluid, compared to conventional agar plate and broth methods for patients suspected of having infection was reported by Hughes et al. They reported that the resin-containing culture detected significantly fewer contaminants (1 versus 11 contaminants) and more pathogens, compared to the conventional method (62 versus 51 pathogens).

The results of a study carried out by Hughes et al. revealed that from a total of 805 synovial fluids, microbial growth was produced by 74 cultures (9.2%) with 77 microorganisms from 60 patients (26). The use of multiple samples can be contributed to earning positive-culture results (13). In this study, the referral time after arthroscopic knee surgery of positive-culture patients with clinical signs was 14 to 90 days; however, it was 7 to 89 days in a study performed by Kim et al. (5). According to the days after arthroscopic surgery until the onset of the signs of arthritis, acute (n=1), subacute (n=2), and delayed or chronic (n=3) infections were observed in six culture-positive patients. On the other hand, Erice et al. demonstrated 30 patients with septic arthritis; moreover, 57% and 40% had acute and subacute infections, respectively. In addition, one patient had a delayed infection (27).

In this study, despite the improvement of phenotypic methods and the use of resin-containing media, three of the patients who had taken multiple antibiotics before sampling had a negative culture that could be due to the low number of organisms in the infection or fastidious bacteria which had not grown. Accordingly, molecular methods were used in these cases. These three patients were without fever but with high levels of CRP and ESR. On the other hand, positive-culture response in six out of 10 patients who had taken multiple antibiotics before sampling could be due to the use of different culture media, such as resin-containing media, to absorb antibiotics and different conditions. In the present study, according to culture and antibiogram results, antibiotic choice in two patients treated with empirical antibiotics (e.g., cefazolin and rifampin) were incorrect. Since the isolated bacteria in these patients were resistant to selective antibiotics, antibiotics should be changed based on culture and antibiogram results. In the current study, only one Gram-negative bacterium (P.m) was isolated, and the CoNSs were the most frequently isolated bacteria in ACL reconstruction infections which were consistent with the results of a study carried out by Gobbi et al (28).

Infection after arthroscopic ACL and meniscal surgery is rare, and there are multiple risk factors that could cause infection (septic arthritis) after arthroscopic ACL and meniscal surgery including, previous knee operation, contamination during preparation of the graft or allograft usage, use of hamstring grafts versus bone-patellar tendon-bone grafts, peri-operative wound contamination, contamination of surgical instruments, an implant for graft fixation, environmental contamination of surgical equipment or hospital material, and presence of intra-articular foreign bodies (5, 29). There are important treatment options for infections after arthroscopic ACL and meniscal surgery to prolong intravenous antibiotics, such as arthroscopic or open washout and debridement, continuous joint irrigation, and graft retention or elimination with or without reimplantation. The most commonly used therapy is arthroscopic irrigation and debridement with graft retention. Fast removal of the infected ACL graft is recommended; however, some studies support graft removal only in cases of chronic infections (30).

Antibiotic susceptibility test is important for the treatment of infectious diseases. In this study, screening of MRS isolates was performed by cefoxitin disc diffusion, and PCR was conducted to confirm the presence of the mecA gene. One of the best choices of treatment for MRS isolates is vancomycin which is determined by resistance to cefoxitin (29). The correct antibiotic treatment is effective in faster recovery of patients, and antimicrobial resistance is one of the most pressing difficulties in modern medicine. Most of the time, empirical antibiotic therapy was continuing after debridement or synovial fluid aspirate until culture sensitivities are obtained (31). Furthermore, in some of the studies, it was revealed that bacteria were resistant to empirical antibiotic treatment (22).

Carney et al. and Wyatt et al. showed which prophylactic antibiotics were effective in preventing septic arthritis following knee arthroscopy (8, 32). In general, whether prophylactic antibiotics should be administered before knee arthroscopy is controversial (8). In the present study, the failure of antibiotic therapy in patients taking the correct antibiotics is likely due

to colonization by biofilm-forming bacteria in knee deeper tissues that the antibiotic failed to affect, and it can cause the failure of arthroscopic ACL and meniscal surgery (33). Further studies are recommended to use appropriate sampling and suitable culture media for the detection of the infection causative agents after knee arthroscopic surgeries in patients who received antibiotics following antibiotic correct treatment.

To the best of our knowledge, despite antibiotic prescribing in patients before arthroscopic knee surgery and sampling, the results of our study showed that correct sampling, appropriate cultures, especially aerobic and anaerobic resin-containing media, and microbiological testing remained useful and valuable for diagnosing bacterial infections.

Ethical considerations: Consent was obtained from all individual participants included in the study.

Conflict of interest: The authors declare that they have no conflict of interest.

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