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Acute Musculoskeletal Infection: Comparison of Different Methods for Intraoperative Bacterial Identification

Akutní infekce pohybového ústrojí: srovnání peroperačních metod pro detekci bakteriální infekce

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ABSTRACT

PURPOSE OF THE STUDY

Various techniques are used for detection of pathogens in musculoskeletal infection. These methods differ with respect to reliability and ease of handling. A prospective study was performed to evaluate the efficacy of three intraoperative techniques.

MATERIAL AND METHODS

In 20 cases (18 patients) with clinically confirmed acute musculoskeletal infections, intraoperative collected swab samples, tissue samples and fluid samples injected into standard blood culture vials were used for microbiological diagnosis. Identification of bacteria, time necessary for detection and ease of handling during surgery was evaluated.

RESULTS

In 19 cases bacterial growth was demonstrated using either intraoperative swabs or blood culture technique (95% sensitivity),whereas 18 tissue biopsies were positive (90% sensitivity. 27 bacterial species were isolated. In 18 instances for the swab technique, 14 instances for the tissue biopsy and 4 operations for the blood culture vials, ease of handling was rated as excellent.

DISCUSSION

The study demonstrated differences between the three tested methods with respect to ease of handling. With respect to the number of detected organisms and time for their detection there are no significant differences. These last findings are in contrast to of the results of other authors. The reason for this could be that during operative dissection an accurate and specific collection of specimens from the acute deep infected soft tissues and bones independent from the type of surgical procedure is possible. Therefore, even with the swab method a high amount of microorganisms can be recovered. Especially for intraarticular infections, fluid samples injected into standard blood vials is a practical method for the surgeon. In acute musculoskeletal infections other than joint infections, there is less benefit for the blood culture vials.

CONCLUSION

Intraoperative swab technique yields valid results comparable to other techniques and is an accurate technique for detection of pathogens from acute musculoskeletal infections.

Key words: implant, infections, bacteriological techniques, comparative study.

INTRODUCTION

Acute postoperative surgical site infections remains a major source of morbidity in the surgical patient leading to longer hospitalisation and higher costs. Therefore, immediate and meticulous debridement is the most important step in the surgical management of traumatic and infected wounds ranging from open fractures to joint infections and revisions after infected joint arthroplasties.

Accurate identification of pathogenic organisms is essential for the treatment of infection of the musculoskeletal system. Intraoperative detection of microorganisms is important for diagnosis of infection and for the initiation of precise antimicrobial therapy immediately after surgery. The use of an intraoperative culture technique with a high sensitivity and specificity is essential. Protection of anaerobic bacteria from exposure to oxygen during the transport of surgical specimens to the microbiological laboratory is crucial for the survival of

these organisms. Wound culture methods are the subject of considerable discussion (1, 4, 8, 11, 20, 25, 29, 30). Routinely during treatment of acute infection of the musculoskeletal system wounds are swabbed with sterile culturette swabs. This technique is plagued with inherent limitations (specimen transportation times, specimen storage media and plating problems, high rate of false negative results) that can lead to confusing microbiology results (5). Aspirates of fluids and tissue biopsies specimen are superior to samples collected on swabs. They are recommended for intraoperative diagnostics especially in prosthetic joint infections (3, 17). Recent studies have demonstrated the utility of blood culture methods over standard plate and broth methods for the isolation of microorganisms from synovial fluid or decubitus ulcers (3, 12, 32, 34, 35).

The objectives of this study were to evaluate potential differences between three intraoperative methods (swab culture, tissue culture and fluid samples injected into standard blood culture vials) for detection of microorganisms in acute deep musculoskeletal infections. Therefore identification of bacteria, time necessary for unspecific/specific detection, sensitivity and ease of handling during surgery of methods were evaluated.

MATERIALS AND METHODS

After approval of the study protocol by the university ethics committee, all patients signed an informed consent for study participation. The prospective study was performed at the BG Trauma Center, University of Tuebingen, Germany, which is a Level I trauma center in the southwest of Germany. History, physical examination, routine radiography and laboratory (CRP, leukocyte count) were done in all patients. For each case a clinical diagnosis of the patient's infection status was made on the basis of data and in accordance with Centers for Disease Control and Prevention recommendations (22). Leukocyte counts greater than 10^3/ml and CRP reactive values greater than 5 mg/l were considered suspicious of infection. Based on review of the patient's history, physical examination and analysis of the laboratory data, a clinical diagnosis for acute infection was made and the patient was included into the study. Therefore the patients had surgery after diagnosis of an acute deep infection of the musculoskeletal system. Antibiotics were discontinued at least two days before the operation. All procedures were performed within a 6 months period from January 2004 to June 2004. Two patients required more than one operation due to recurrent infection. The same aseptic technique was used in all surgical cases. This included preparation of the operative field using an alcohol-based antiseptic (Softasept^R N, B. Braun Melsungen AG, D-34209 Melsungen, Germany) and sterile non-woven drapes. All surgeries were performed in standard-ventilated, non-laminar airflow operating rooms with similar personnel present for each case. Altogether 7 surgeons were involved in the study, three of which were attending and 4 who were senior resident physicians. All had ample experience with the used methods for intraoperative microbiological diagnostics. In all cases, three types of intraoperative cultures (swabs (Copan Italy/Hain Lifescience, Art. Nr. 108), tissue biopsies (BD Port-A-Cul^{TM} Transport Jar Sterile Pack), fluid from the operative side injected into blood culture vials (BD Bactec^{TM} Lytic/10 Anaerobic/F Aerobic/F) were obtained by the senior surgeon during the operation. The process of sample collection and the details of each culture techniques are as follows: Tissue biopsies of the involved areas were performed using a sterile rongeur, scalpel or forceps. These instruments were only used for tissue collection during the procedure, to avoid the risk of potential sample contamination. Swab cultures were obtained by passing a sterile swab over the deepest area of infected tissue, bone and fluid suspected of infection. Collection of vial culture specimens involved aspiration of 5 to 10 mL of purulent material and fluid suspected for infection from the surgical site using a sterile syringe with a needle. All intraoperative samples were obtained, placed into their respective transport media and processed by the microbiology department within 6–8 hours. After samples were taken, therapy with a third-generation cephalosporin was started during surgery. The intraoperative application of the sampling techniques was recorded by the surgeon after the procedure using a scale from "1" to "3", with "1" being excellent, "2" as fair and "3" as poor. If the method is burdened with extensive packaging, complicated intraoperative presentation of the transport media to the surgeon and difficult collection of a high volume for diagnostics, "poor" application was recorded. An "excellent" application is achieved if the method is offered to the surgeon with minimal fear of contamination during presentation and if a large amount of material is collected and packaging of the transport-media in the operation theatre is simple.

Each of the surgeons who were involved in the study had to classify the intraoperative application of the sampling technique in every single case according to the data mentioned above.

Tissue samples and swab samples were plated in the microbiology laboratory (accredited acc. to ISO 15189) on various media and cultured in fastidious broth for 12 days. We used sheep blood agar, Endo agar and Tarozzi-broth as backup medium for culturing aerobic and Chocolate-agar (brain heart infusion with 5 % sheepblood) and kanamycin-vancomycin agar for culturing anaerobic bacteria. Enrichment cultures were also kept for 12 days. Positive enrichment cultures were plated on Chocolate-agar (brain heart infusion with 5 % sheep-blood) and incubated under aerobic and anaerobic conditions. Positive cultures were sent for organism identification and sensitivity testing in a similar manner, irrespective of the initial culture technique.

Statistical methods

Statistical analysis was performed with JMP 5.1 (www.JMP.com). We used the sign test to compare the

scores for the evaluation of intraoperative handling after combining score "2" and "3" and paired t-tests (for evaluation of bacterial numbers and time for unspecific and specific bacterial identification). We calculated sensitivity (the proportion with positive test results of those infected). For all statistical analyses, the level of significance was set at $p < 0.05$.

RESULTS

18 patients (20 cases) with an acute deep infection of the musculoskeletal system were available for analysis. The patient's ages ranged from 42 to 76 years (median age 64 years). There were 9 females and 9 males. All patients had an acute deep bone or joint infection on the basis of clinical, radiographic and laboratory findings. One patient with a hematogenous bone infection had no history of surgery. 17 patients were suffering from a postoperative or posttraumatic infection of the musculoskeletal system. In 6 cases surgery was performed with a prosthesis or osteosynthesis material still in situ. 12 patients had a removal of osteosynthesis material or prosthesis in previous surgeries. In five cases a procedure had been performed within the last 6 weeks. 14 patients had prior surgeries longer than 6 weeks before surgery.

All cases showed abnormal values for C reactive protein. In 5 out of 20 cases leukocyte levels were elevated.

A total of 20 aerobic and 7 anaerobic bacterial species were isolated from the 20 procedures. From 20 cases (18 patients) intraoperative swabs and blood culture vials were positive in 19 cases (17 patients). Intraoperative tissue biopsies were positive in 16 patients (18 cases). The most common bacteria found were *Staphylococcus aureus*, followed by *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Table 1).

Only one case out of 20 had negative intraoperative cultures with all three techniques.

There was no difference between swab and vial cultures according to sensitivity (Table 2).

With intraoperative swabs a single species was isolated in 15 cases (n=75%). Two bacterial species were detected in 5 cases. 15 single isolates were diagnosed with the blood culture vials. Two species were found in 4 cases. 14 single bacterial species were diagnosed with the tissue biopsies, followed by 3 cases with two and one case with three bacterial species detected intraoperatively. Within medians of 1 day for all three methods and ranges of 1 to 8 days for the blood culture, 1 to 4 days for the tissue biopsies and 1 to 7 days for the swab culture, unspecific confirmation of bacterial growth was possible. Specific identification of bacteria was possible after a median of 4 days (range 1-11 days) for the swab, 2 days (range 2–8 days) for the blood culture and 4 days (range 1–7 days) for the tissue biopsy technique.

According to the paired t-test there were no significant differences among the three methods with respect to the time for unspecific and specific identification and number of species detected per case.

In 8 cases the intraoperative antibiotic therapy that was initiated was changed upon confirmation of the definitive organism.

Table 1. Cultured organism and the number of times grown with each method.

Organism name	Swab	Vials	Tissue
Methicillin-sensitive Staphylococcus aureus	10	10	10
Staphylococcus epidermidis	4	5	4
Pseudomonas aeruginosa	3	3	2
Peptostreptococcus spp.	2	1	1
Enterococcus faecalis	1	1	1
Methicillin-resistant Staphylococcus aureus	1	1	1
Clostridium difficile	1	2	1
Clostridium butylicum	1	1	1
Enterobacter cloacae			1
ropionibacterium acnes	1		1
Positive cultures overall	24/20	24/20	23/20
No growth	1	1	2

Table 2. Comparison of the three different methods for intraoperative bacterial identification according to sensitivity.

Culture method	Sensitivity (%)
Swab cultures	95
Tissue biopsies	90
Vial cultures	95

Table 3. Intraoperative application: Ease of handling of the techniques. Joined distribution of scores for ease of intraoperative handling (1=Excellent, 2=Fair or Poor) by method.

Score: Blood culture samples	Score: Tissue samples	Score: Swab samples	Cases (n=20)
1	1	1	4
2	1	1	10
2	2	1	4
2	2	2	2

In 18 of 20 instances for the swab ease of handling of this technique was rated as excellent. In 14 instances for the tissue biopsy and in 4 instances for the blood culture the intraoperative method of collection was rated as excellent. For the blood culture technique intraoperative rating for ease of application was fair in 10 cases and poor in 6 cases.

The blood culture received a worse score than the swab in 16 cases and in zero cases a better score. According to the sign test this result is highly significant ($p < 0.0001$). There was no significant difference between swab and tissue. The score for tissue was 4 times worse and never better than for swab ($p = 0.125$). The blood culture received a worse score in 14 cases and never a better score than the tissue ($p = 0.00012$) (Table 3).

DISCUSSION

We prospectively evaluated three different methods for intraoperative detection of bacteria in acute deep musculoskeletal infections. With the exception of intraoperative handling we found no significant differences

between the tested methods (swabs, tissue samples and fluid samples injected into standard blood vials).

The most common species found were *Staphylococcus aureus*, followed by *Staphylococcus epidermidis* and *Pseudomonas aeruginosa.*

Infection after total joint arthroplasty and musculoskeletal infection, especially osteomyelitis are a major problem for the patient in addition to costly diagnosis, treatment and rehabilitation. To minimize the risk of recurrence, treatment must include thorough surgical debridement and precise antimicrobial therapy directed against the pathogen involved in the infection. Most experts agree that chronic osteomyelitis is actually a curable disease provided that thorough surgical debridement is done, osteosynthesis material is removed and appropriate antimicrobial therapy is instituted for at least 4–6 weeks (33).

The distribution of pathogens isolated from surgical site infections has not changed drastically during the last decades. The most frequently isolated pathogens are still *Staphylococcus aureus*, coagulase negative staphylococci, *Enterococcus* spp. and *Escherichia coli* especially in intestinal infections (7). However, an increasing proportion of infections are caused by methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa* (28).

Numerous preoperative and intraoperative tests are avaliable for diagnosis of musculoskeletal infection.

Preoperative investigations include aspiration especially in infected total joint arthroplasties, plain radiography and radionucleotide imaging studies. Furthermore hematological screening tests like white blood-cell-count, measurement of the erythrocyte sedimentation rate and level of C-reactive protein are recommended. There are no preoperative tests that are consistently 100% sensitive and specific (30).

Culture methods, defining characteristics of wound infection and classification of postoperative -prosthetic-infections are subject of considerable controversies (1, 4, 8, 11, 20, 25, 29, 30).

Microbiological diagnosis of infection generally depends upon the isolation of a pathogen from a clinical specimen. Therefore, appropriate intraoperative cultures considered and accurate identification of the pathogen are the gold standard for conclusive antimicrobial therapy (13, 18, 33).

Intraoperative investigations are analysis of synovial fluid, gram-staining and histological evaluation (of frozen sections) of inflamed tissue specimens and intraoperative cultures. These include quantitative and semi quantitative swab (21), curettage (31), histological punch biopsy (23), irrigation-aspiration and needle aspiration (9, 16). For septic arthritis, inoculation of synovial fluid into aerobic or anaerobic blood culture bottles is implemented. Several studies stated an improvement of this technique in comparison with conventional plating cultures (15, 35). The lack of sensitivity of the conventional plating may relate to the larger amounts of synovial fluids into blood culture media. The quantity of microorganisms in synovial fluids is often low. Culture of larger amounts of synovial fluid, which is possible by inoculation of specimen into blood culture vials as opposed to agar plates, results in theory in a higher level of recovery (15).

Irrigation-aspiration is an alternative, painless way to detect microorganisms in draining decubitus ulcers and other soft tissue infections in comparison to biopsy cultures (9).

In osteomyelitis and prosthetic joint infections bone cultures should be taken at time of debridement or deep bone biopsies. The isolation of a microorganism from three or more independent specimens, to be taken as part of a standard set of five or six specimens, is therefore an accurate and practical definition of infection especially in revision arthroplasty (2, 3).

The successful isolation of anaerobes largely depends on proper specimen collection and transport to the microbiology laboratory. False negative cultures of specimens from infected processes occur and may be due to small inoculums of infecting bacteria, concurrent antimicrobial therapy, technical problems in obtaining, transporting and plating of the material to be cultured. Especially, protection of anaerobic bacteria from desiccation and oxygen exposure is a critical step in the recovery of these organisms (14, 17, 26).

Some authors compared different methods for intraoperative detection of infection. *Levine* and co-workers evaluated in a retrospective study three different intraoperative methods (swab cultures, tissue cultures and vial cultures) and their diagnostic value in determining clinical infection in patients after total joint replacements for intraoperative detection of infection. 25 (74%) of their 34 cases were diagnosed as clinically infected. Of these infected joints, 92% had positive vial cultures and 76% had positive swab cultures and 77% (n=13) had positive tissue biopsies. After discounting potential contaminants, they found a significant decrease in the number of correctly identified cases comparing the results of swab and tissue cultures with vial cultures. They found a significant increase of accuracy and sensitivity of vial cultures in comparison to swab and tissue cultures. The authors also emphasized two additional advantages. The vial cultures require minimal hand contact and with respect to the isolation of anaerobes. They also have minimal exposure time to the aerobic environment. The vial cultures provide anaerobic, growth promoting conditions at the time of inoculation until growth is detected spectophotometrically. Thus the risk of contamination through the plating process for swab or tissue biopsies cultures is considerably reduced. With reference to the arguments mentioned above, the authors pointed out that the blood culture vials are more cost-effective for determining septic versus mechanical joint failure (19). *Rudensky* and co-workers compared three different methods for bacterial identification from pressure sores. They evaluated aspiration biopsy versus swab and deep biopsy cultures. 43 pressure sores were simultaneously studied by the three different methods. Of these, 98% of the swab specimens were cultured positive, while only 53% and 63% of aspiration and biopsy

specimens, respectively, were positive. They conclude that the aspiration method tended to underestimate the number of pathogens really present in infected pressure sores. They also found swab specimens to be unreliable, representing mostly superficial colonization. They concluded that only deep biopsy specimens taken during surgical debridement accurately indicate infection (27).

CONCLUSIONS

We have demonstrated differences between the three tested methods with respect to ease of handling. With respect to the number of detected organisms and time for their detection there are no significant differences. These last findings are in contrast to of the results of other authors. The reason for this could be that during operative dissection an accurate and specific collection of specimens from the acute deep infected soft tissues and bones independent from the type of surgical procedure is possible. Therefore, even with the swab method a high amount of microorganisms can be recovered.

The data from the literature for fluid samples injected into standard blood vials is convincing. Especially for intraarticular infections, this is a practical method for the surgeon. In acute musculoskeletal infections other than joint infections, we see less benefit for the blood culture vials.

All methods achieve comparable results for diagnostics of acute deep musculoskeletal infections.

ZÁVĚR

Pro detekci patogenů vyvolávajících infekce pohybového aparátu se používá řada technik. Ty se liší ve své spolehlivosti a snadnosti provedení. V této prospektivní studii byla hodnocena účinnost tří peroperačních technik.

Ve dvaceti případech (18 pacientů) klinicky potvrzené akutní infekce pohybového ústrojí byly během operace pro mikrobiologickou diagnostiku použity vzorky tkání a vzorky tekutin odebrané do standardních nádobek pro hemokultivaci. Bylo hodnoceno určování bakterií, čas potřebný k detekci a snadnost provedení odběru během chirurgického zákroku.

V 19 případech byl bakteriální růst prokázán kultivací stěrů nebo technikou používanou u hemokultivace (citlivost 95 %), zatímco u biopsií bylo pozitivních 18 tkání (citlivost 90 %). Bylo izolováno 27 druhů mikroorganismů. Snadnost provedení odběru byla hodnocena jako výborná v 18 případech odběrů stěrů, ve 14 případech biopsie tkání a u čtyř operací při odběru pro techniku používanou u hemokultivace.

Studie prokázala rozdíly v hodnocení snadnosti provedení mezi třemi testovanými metodami. V počtu určených mikroorganismů a v čase nutném pro jejich určení nebyly signifikantní rozdíly. Tato dvě zjištění jsou v rozporu s výsledky jiných autorů. Důvodem může být to, že během chirurgické preparace je možný přesný a cílený odběr vzorků měkkých a kostních tkání s akutní hlubokou infekcí bez ohledu na to, o jaký typ chirurgického zákroku se jedná. Tudíž i metodou stěrů lze získat vysoké počty mikroorganismů. Zejména u nitrokloubních infekcí je pro chirurga odběr tekutých vzorků do standardních hemokultivačních nádobek praktickou metodou. Kromě kloubních infekcí je výhoda metody používané pro hemokultivaci u akutních infekcí pohybového systému menší.

Technika stěrů během operace dává spolehlivé výsledky srovnatelné s jinými technikami a poskytuje přesnou metodu pro určování patogenů vyvolávajících akutní infekce pohybového systému.

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