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Revision Total Knee Arthroplasty: Infection should be Ruled Out in All Cases

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Abstract:

We hypothesized that some aseptic revision knee arthroplasty (TKA) failures are indeed due to occult infection. This prospective study recruited 65 patients undergoing revision TKA. Mean follow-up period was 19 months. Collected synovial fluid was analyzed by Ibis T5000 biosensor (a multiplex PCR technology). Cases were considered as infected or aseptic based on the surgeon's judgment and Ibis findings. Based on Ibis biosensor, 17 aseptic cases were indeed infected that had been missed. Of these 17 cases, 2 developed infection following the index revision. A considerable number of so called aseptic failures seem to be occult infections who were not adequately investigated and/or miss-categorized as aseptic failure. We recommend that all patients undergoing revision arthroplasty need to be investigated for PJI.

Key words: Revision knee arthroplasty, Aseptic failure, Periprosthetic joint infection, Ibis T5000 biosensor, Diagnosis

Introduction

Periprosthetic joint infection (PJI), that occurs following 1 to 3% of TKAs [1, 2] is the most common cause of failure after total knee arthroplasty (TKA) [3-5]. Diagnosis of PJI continues to pose a challenge to the medical community because of lack of a "gold standard" [6]. It is, however, critical that aseptic cases be distinguished from PJI, as treatment for these conditions is vastly different [7, 8].

History taking, physical examination, and radiographic findings can be similar in PJI and aseptic loosening and may not allow distinction in most cases [7]. Joint aspiration and serologic tests such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are usually performed during the work-up of a patient with suspected PJI. Although a very high sensitivity has been reported for ESR and CRP, these laboratory tests have their own limitations in the diagnosis of PJI [9]. Even intraoperative culture and pathology have limited sensitivity in diagnosis of PJI particularly in those receiving antibiotics [10, 11]. It has been demonstrated that intraoperative culture yields false negative results in 6.6% [12] to 17.7% [13] and false positive in 13% of cases [14]. Imaging modalities such as nuclear scans (tc-99, indium 111, gallium) have also been employed in diagnosis of PJI but these tests are also unable to accurately differentiate between aseptic loosening and septic failure after joint arthroplasty [15-17].

One of the main reasons for inability to isolate the pathogen relates to the presence of a biofilm [8, 18] and/or internalization of organism by osteoblasts [19]. Biofilm is a complex structure comprising microorganisms enveloped in macromolecules of glycocalyx and other protective films [18, 20]. As a result, it is probable that some of PJIs to be missed and treated as aseptic failure which subsequently cause recurrent failure [21].

Using molecular techniques may improve diagnosis of PJI as these methods have high sensitivity and are culture independent [11]. Polymerase chain reaction (PCR) has been used in several studies to diagnose PJI [22-25]. Using a specific PCR or a broad-range (16S ribosomal DNA) PCR which are respectively able to detect only a single microorganism or previously unknown organisms were limitations of these studies. Compared to the specific PCR, the sensitivity and specificity of the broad-range PCR is lower, needs subsequent sequencing for bacterial identification, and fails to detect mixed infections [11]. Recently, the Ibis T5000 universal biosensor has been introduced as a sensitive and specific method for identification of bacteria, viruses, fungi, and protozoa. The system operates based on broad-range PCR and high-performance mass spectrometry and seems to be more accurate than conventional PCR. [26] However, it has not yet been approved by the Food and Drug Administration (FDA) for routine use in clinical practice.

The hypothesis of this study was that some cases of aseptic failure were indeed due to infection that had either escaped diagnosis using conventional modalities or had not been adequately investigated. This prospective study was set up to examine the postoperative course of a consecutive cohort of patients undergoing revision TKA in whom an intra-articular tissue and/or fluid sample was also sent for analysis by Ibis T5000 biosensor.

Materials and Methods

After approval of the Institutional Review Board of the Thomas Jefferson University, all patients who underwent TKA revision from February 2009 to May 2010 were recruited for this study. The study consists of 65 patients of whom 33 were men. The mean age of the patients was

65 ± 11 years. All patients underwent appropriate preoperative work-up based on the recommendation of the treating surgeon and then categorized as infected or uninfected based on these investigations and surgeon's judgment. In our center, patients who are suspicious for PJI are evaluated by measurement of serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), intraopeartive culture and synovial fluid analysis. However, frozen section isn't use as a part of PJI work-up in our institute. Intraoperatively tissue and/or fluid samples were collected and analyzed using the Ibis T5000 biosensor. After discharging from the hospital, patients are followed-up based on the protocol which is used routinely in our institution at 6 weeks, 6 months and 2 years after the revision surgery. In this study, all patients were followed-up for a minimum of six months with a mean follow-up of 19 months (range; 12 to 26). In particular, "aseptic" patients in whom the Ibis T5000 biosensor had detected an infecting pathogen were followed-up closely for development of subsequent failure after the index revision.

Preoperative work-up

Detailed history taking, examination, and routine radiographs were performed in all patients in this cohort. It is institutional policy that all patients undergoing revision arthroplasty at our institution have preoperative Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) measured. In addition, and based on the findings of serology, majority of patients (n=57) underwent joint aspiration with the fluid sent for analysis for neutrophil count, neutrophil differential, and culture. As this was an observational and not interventional study, we did not make any changes to the preoperative work-up of any patients. Collection of synovial fluid and/or tissue sample for analysis by Ibis T5000 biosensor was a requirement of the study.

Sample collection and the Ibis T5000 method

Joint fluid and/or tissue were collected intraoperatively. Joint fluid was aspirated prior to the arthrotomy and was also sent for WBC count (if indicated) and culture. Tissue-sampling was performed from areas that were considered by the surgeon that to be most suggestive of infection. In addition to the Ibis analysis, tissues were sent for histopathologic assessment and culture. The samples were not sent for measurement of inflammatory markers for this study. Samples then processed appropriately for later analysis by Ibis T5000 in batches. Appropriate cyrogenic vials were used to store fluid samples in a Styrofoam container. The vials were transferred in ice bags from the operating room to liquid nitrogen. About 0.5 to 1 mL of the liquid was stored for further analysis in liquid nitrogen. The syringe was changed in order to minimize possibility of accidental microbial contaminations before synovial fluid was transferred to the labeled vials. These vials were transferred to -140o F freezer where they were stored until they were shipped in batch to the Center for Research and Genomic Studies in Allegheny, PA.

For DNA extraction, 1 ml of the aspirate was centrifuged at 10,000 rpms for 3 min and 900uL of supernatant was removed. Then, ATL lysis buffer and proteinase K were added and the samples were incubated at 56°C until lysis occurred. The Qiagen DNeasy Tissue kit (Qiagen. Inc. cat # 69506) was used to extract nucleic acid from the lysed sample. After DNA was extracted, 10 uL of sample was loaded per well into each of 16 wells on the BAC detection PCR plate that each contained a different primer pair (Abbott Molecular. cat # PN 05N13-01). The BAC detection plate is a 96 well, 6 sample plate which contains 16 primers that identify all bacterial organisms, Candida species, and determines the presence of several key antibiotic resistance markers such as van-A and van-B (vancomycin resistance) in Enterococcus species, KPC (carbapenem resistance) in Gram-negative bacteria, and mec-A (methicillin resistance) in

Staphylococcus species. Once PCR was completed, the plate was loaded onto the Ibis T5000 machine. The products from the PCRs were desalted in a 96-well plate format and sequentially electrosprayed into the time-of-flight mass spectrometer. The resultant spectral signals were then processed to determine the masses of each of the PCR products present with sufficient accuracy that the base composition of each amplicon could be unambiguously deduced. Using combined base compositions from multiple PCRs, the identities of the pathogens and their relative concentrations in the starting sample were determined.

The isolated microorganism from the Ibis biosensor was considered as an "orthopedic pathogen" based on extensive search of the available literature. In other words, if there was any evidence even a case report that shows the isolated microorganism is able to cause bone and/or joint infection, that microorganism was defined as an "orthopedic pathogen". However, Ibis biosensor results did not change the treatment strategy and all patients were treated based on results obtained from conventional diagnostic tests and surgeon's judgment.

Results

Based on preoperative investigations and surgeon's judgment, of the 65 patients recruited for this study, 21 patients were undergoing revision arthroplasty for PJI and the remaining 44 patients had aseptic failure. Among the 21 patients with PJI, synovial culture was negative in 11 cases. In the remaining 10 patients the isolated organisms were coagulase negative *Staphylococcus* (5 cases), *Staphylococcus aureus* (3 patients), *Streptococcus mitis* plus *Streptococcus sanguis* (1 case) and *Peptostreptococcus* species (1 case). Ibis identified a pathogen with confidence ≥ 0.7 in total of 36 cases. Ibis T5000 isolated an organism in 19 PJI cases and failed to isolate any organism in 2 cases that were categorized as infected. The isolated organism by Ibis was coagulase negative *Staphylococcus* in 10 patients and *Staphylococcus*

aureus in 4 patients. In the PJI group, comparison of the isolated organisms from the culture and the detected organism by the Ibis T5000 biosensor showed the same pathogens in 9 cases samples whereas in 11 cases, the Ibis biosensor found additional pathogen. Table 1 demonstrates comparison between isolated organism by culture and Ibis T5000 results in patients with PJI. On the other hand, the Ibis T5000 found additional non-pathogen organisms in 3 cases in which the Ibis T5000 had also detected an orthopedic pathogen.

All cultures in the aseptic group were negative whereas in 17 cases the Ibis T5000 found orthopedic pathogens. In 27 patients, the biosensor failed to find any orthopedic pathogens. Table 2 demonstrates more details on aseptic cases. During the follow-up period, 2 patients failed and needed re-revision who were both initially revised for aseptic failure. The cause of failure in these 2 patients was subsequent PJI with the same organism (Coagulase negative S*taphylococcus*) as one isolated by the Ibis T5000. At the latest follow-up which ranged from 12 to 26 months after the index revision, all the remaining patients appear to be doing well with no evidence of infection.

Discussion

Given the completely different management of aseptic loosening and PJI as well as the importance of early diagnosis of PJI for establishment of a more effective treatment, distinguishing between these two conditions needs special attention. Absence of a "gold standard" for diagnosis of PJI [27] in addition to various defensive mechanisms of pathogens such as biofilm production [8] make this differentiation more difficult. The infecting organism that segregate in biofilm evade detection by conventional culture as the latter relies on isolation of planktonic organisms. As a result it is suggested that some cases of PJI escape detection and

153 erroneously are categorized as aseptic failures [8]. Although many factors contribute to
154 development of PJI after revision surgery, the latter point may be considered as one of the
155 contributing factor for the much higher incidence of PJI after revision arthroplasty than that after
156 primary replacement.

157 The Ibis T5000 universal biosensor is a promising technology that has been used to
158 identify a wide spectrum of pathogens in sepsis [28] and it may cover limitations of PCR method
159 for diagnosis of PJI. Because of reliance on mass specterometry and further "purification" of
160 DNA it is assumed that Ibis does not suffer the same extreme sensitivity as conventional PCR.
161 Further, because of pan-genomic amplification, Ibis may be able to detect infecting organisms
162 that could be missed by conventional PCR.

163 This prospective study was designed to examine the possibility of escaping some cases of
164 TKA failures which are assumed to be "aseptic" from conventional diagnostic tests. These cases
165 may be indeed infections. that have escaped diagnosis and have been miss-categorized as
166 "aseptic" failures. The study relied on Ibis T5000 for isolation of organism. Although we did not
167 accept Ibis as the "gold standard" for diagnosis of PJI, we were interested to know in what
168 percentage of patients with aseptic failure Ibis T5000 biosensor was able to isolate a pathogen.
169 Further, we sought to examine the correlation between conventional culture and Ibis in terms of
170 their ability for isolation of a pathogen and its resistance profile.

171 At our institution since 2006, we have utilized an algorithmic approach for work-up of
172 patients with failed arthroplasty which includes determination of ESR and CRP prior to revision
173 arthroplasty and selective aspiration of the failed joint in those with abnormal serology or high
174 index of suspicion for PJI [7]. In addition, intraoperative culture is performed for all cases

undergoing revision arthroplasty. In spite of employing such a comprehensive and strict approach, the present study revealed that a few PJIs cannot be detectable by using routine diagnostic tools. It appears that reliance on conventional investigations is likely to miss occult PJI at least in 30% of patients (13 out of 44 if *Enterococcus faecalis* cases were considered as contamination). The 2 patients who were originally assumed to have aseptic failure, developed infection shortly after the index revision by organisms that had been isolated by the Ibis T5000 but failed to be detected by conventional culture. The infecting organism in one case was low-virulence, but a recognized pathogen [29, 30] namely *Staphylococcus Caprae.* Although these patients did not receive any treatment for isolated additional pathogens from the Ibis biosensor, our findings may indirectly indicate the clinical importance of isolated pathogens from the Ibis biosensor. However, we are not able to make a statement about effect of treatment on outcome of these patients with negative culture in whom the Ibis biosensor isolates additional pathogens. It is possible that the conventional culture may have identified these organisms if supplemented culture was utilized or the culture was kept for an extended period of time.

PCR has been used previously for the purpose of isolating organism in cases of suspected PJI [22, 23, 31]. However, PCR methods suffer several limitations, most important of which is the high incidence of false positive results [11]. The technique is so sensitive that it may amplify contaminating and non-infecting organism such as those residing on the skin that may have been picked up by aspirating needle [25]. Ibis T5000 is a multiplex PCR technology that was designed to overcome some of the limitations of conventional PCR.

Compared to conventional PCR, the Ibis T5000 utilizes a pan-genomic amplifier that is capable of isolating atypical bacteria and even non-bacterial pathogens such as fungi. Unlike conventional PCR, Ibis does not rely on universal primers for amplification of DNA which may

198 detect contaminating organism. Instead Ibis T5000 biosensor uses multiple pairs of species-
199 specific primers to amplify regions of an organism's genome. This process is followed by the
200 identification of that region's base composition using mass spectrometry, the results of which are
201 compared to a database which matches it to the closest microorganism [26]. The Ibis T5000
202 universal biosensor technology combines nucleic acid amplification to high-performance
203 electrospray ionization mass spectrometry and base-composition analysis. The system can
204 identify and quantify all known bacteria, all major groups of pathogenic fungi, and the major
205 families of pathogen viruses in humans and animals. Moreover, the system is capable of
206 detecting virulence factors and antibiotic resistance markers [26].

207 Despite its appeal, Ibis T5000 may still be a victim of high sensitivity. Ibis isolated a
208 "pathogen" in 17 cases (38%) of "aseptic" cases. Of the latter 2 patients have failed so far due to
209 infection which we believe was missed during the index revision arthroplasty. It is possible that
210 occult PJI may have been present in a few more cases that were either eradicated during index
211 revision arthroplasty, effectively with the patient undergoing a one stage exchange arthroplasty,
212 or are likely to manifest a failure with further follow-up. It is unlikely that the isolated organism
213 by Ibis in all 17 aseptic cases represent a true pathogen. Thus, this technology, despite its appeal,
214 should be reserved for patients in whom high index of suspicion or PJI exists but no organism
215 can be isolated. In other words the indication for use of Ibis, in our opinion, is for cases of
216 culture negative PJI.

217 The study suffers a few limitations. Perhaps the most important limitation of this study is
218 the relatively short follow-up. As mentioned above, it is possible that with longer surveillance we
219 are likely to encounter more patients who may fail. Although plausible, the latter is unlikely to
220 alter the message of this study. The study highlights the importance of routine preoperative

work-up using conventional serology for all and selected aspiration for some, in line with the recent American Academy of Orthopedic Surgeons guidelines for diagnosis of PJI [6, 32]. It also highlights the fact that a sophisticated technology is available for use by orthopedic surgeons to isolate the infecting organism in cases of culture negative PJI. Another limitation of the study relates to lack of a "standard" definition for PJI. It is possible that using a different diagnostic criteria, some of the PJI cases in our cohort may have been considered as uninfected and vice versa. The latter is unfortunately a limitation inherent to any studies related to topic of PJI as various definitions for PJI exist and depending on the definition used the percent of infected versus uninfected cases in a given cohort may change. It is hoped that orthopedic societies in collaboration with other organizations may be able to address this shortfall in the future.

Despite the aforementioned limitations, the present study demonstrated that some aseptic loosenings are not "truly" aseptic and are low grade PJIs that remain undiagnosed using conventional modalities. Some of these cases may fail early for a subsequent infection. This may explain the relatively high incidence of infection following revision arthroplasty, compared to primary, and also the high rate of early failure of revision cases. It is thus recommended that all patients undergoing revision for failed arthroplasty should be subjected to routine conventional work-up which includes routine serology (ESR and CRP) and joint aspiration in patients with abnormal serology tests and high index of suspicion for PJI. With further refinements of molecular techniques such as multiplex PCR, the true nature of some of these so called "aseptic" failures is likely to be revealed.

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