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Comparative study of cultures and next-generation sequencing in the diagnosis of shoulder prosthetic joint infections

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Background: Serum and synovial markers used to diagnose lower extremity prosthetic joint infection (PJI) have performed poorly for shoulder PJI. As a result, diagnosis is commonly reliant on the accuracy of positive or negative cultures. Next-generation sequencing (NGS) can sequence an entire bacterial genome. This study was conducted to determine the correlation between NGS and routine cultures in revision shoulder arthroplasty.

Methods: All patients undergoing revision shoulder arthroplasty were prospectively enrolled. In a standardized manner, tissue samples were transferred immediately into sterile specimen containers and transported for culture and NGS (MicroGen Dx, Lubbock, TX, USA). Infection definitions using culture and NGS were analyzed for concordance.

Results: There were 44 total revision arthroplasty cases included. There were no cases of polymicrobial culture results. *Cutibacterium* (formerly *Propionibacterium*) *acnes* was the most common bacterial species cultured (8 of 13 [61.5%]) and identified by NGS (12 of 17 [70.1%]) in cases of definite and probable infection. The concordance (κ) between the 2 diagnostic criteria for defining infection that included culture or NGS was 0.333 (fair). There were significantly more cases of probable contaminants when cultures (10 of 44 [22.7%]) were used in the definition of infection compared with NGS (0 of 44 [0%]; $P = .001$).

Discussion: Culture data from revision shoulder arthroplasty cases commonly yields monomicrobial results; whereas, NGS data suggests that bacterial loads in revision arthroplasty are most commonly polymicrobial. In addition, a definition of infection that uses cultures is more prone to "probable contaminants" than NGS. Significant uncertainty remains about our current methods of diagnosing shoulder PJI.

Level of evidence: Level II; Diagnostic Study

Keywords: Prosthetic joint infection; next-generation sequencing; cultures; shoulder surgery; *Cutibacterium acnes*; revision arthroplasty

Periprosthetic joint infection (PJI) of the shoulder remains a difficult diagnostic challenge. In a consensus statement from the Musculoskeletal Infection Society, Parvizi et al[25] developed criteria for clinically significant PJI among patients undergoing revision hip or knee arthroplasty. These criteria include:

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- a sinus tract communicating with the prosthesis;
- a pathogen isolated by culture from at least 2 separate tissue or fluid samples obtained from the affected prosthetic joint; or
- 4 of the following criteria: elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration, elevated synovial leukocyte count, elevated synovial neutrophil percentage, presence of purulence in the affected joint, isolation of a microorganism in one culture of periprosthetic tissue or fluid, or greater than 5 neutrophils per high-power field in 5 high-power fields observed from histologic analysis of periprosthetic tissue.

Given that *Cutibacterium* (formerly *Propionibacterium) acnes* (*C. acnes*), a low-virulence and slow-growing organism, colonizes the shoulder at increased rates compared with the knee and hip and is the most frequently cultured organism in patients with infections after open and arthroscopic shoulder surgery,[5,15,21,26] criteria that involve clinical or intraoperative signs of infection or serum markers are less commonly positive in revision shoulder arthroplasty.

Although alternative strategies for diagnosis, such as serum interleukin-6 and synovial leukocyte esterase that have shown promise in hip and knee arthroplasty have been studied in the shoulder, they have not demonstrated the sensitivity and specificity to substantially aid in the diagnosis of shoulder PJI.[23,32] As a result, diagnosis commonly relies on the accuracy or inaccuracy of positive or negative culture results that can be influenced by the number of specimens sent, the anatomic locations from which specimens are acquired, intraoperative specimen handling and transfer, the growth medium used, the duration that cultures are held, laboratory contamination, and interpretation.

Next-generation sequencing (NGS) has emerged as a technological innovation with the ability to sequence an entire bacterial genome from samples of tissue or fluid. The role of NGS in the diagnosis of PJI in the shoulder is unknown. This study was conducted to determine the correlation between NGS and routine cultures in revision shoulder arthroplasty.

Materials and methods

All revision arthroplasties performed at a single institution from July 2016 until April 2017 were prospectively analyzed. All patients aged >18 years who were undergoing revision shoulder arthroplasty, regardless of clinical suspicion of infection, were approached for enrollment in the study, and consent was obtained. Five fellowship-trained shoulder surgeons performed all procedures.

Data variables

Preoperative clinical signs of infection, including swelling, sinus tract, redness, or drainage, were documented. Preoperative laboratory analysis, including white blood cell count, ESR, and CRP, were obtained in all patients. Intraoperative gross findings of infection, including purulent drainage or necrosis, were documented. Frozen sections for acute inflammation were not routinely obtained at our institution during revision surgery and were not obtained as part of this study.

Given that there is currently no gold standard or consensus definition for establishing the diagnosis of infection after shoulder arthroplasty, Frangiamore et al used a series of preoperative and intraoperative criteria to categorize patients into 4 groups: definite infection, probable infection, probably contaminant, and no evidence of infection.[5,11-14,18,31] For the purposes of this study, we used the criteria described by Frangiamore et al[11] (Table I, *A*) and also used a modified version of this criteria that included NGS results instead of culture results (Table I, *B*). Patients were monitored for 1 year after surgery for clinical signs of recurrent infection.

Sample acquisition

After a standardized skin preparation and surgical exposure, needle aspiration of the glenohumeral joint was attempted before the joint capsule was opened. When available, fluid was sent for culture and NGS. Once the joint was opened, tissue from the anterior capsule, inferior capsule, glenoid, humeral canal, and underneath the prosthetic humeral head was obtained using "fresh" instruments. Tissue samples were transferred immediately into sterile specimen containers and transported for culture and NGS (MicroGen Dx, Lubbock, TX, USA).

Culture protocol

Immediately after collection in the operating room, orthopedic fluids and tissues were placed into anaerobic fluid vials (20-mL serum stopper vials with 1.3 mL of prereduced peptone yeast extract broth, 0.5 g/L cysteine hydrochloride, and 1 mg/L resazurin indicator) or tissue vials (sterile 30-mL screw-top vials filled with CO_2), respectively. Tissues were homogenized using a Seward Stomacher 80 Biomaster (Seward Inc., Port St. Lucie, FL, USA) in 3 mL of brain heart infusion broth for 1 min. A 0.1-mL sample of tissue homogenate or fluid in anaerobic transport medium were inoculated onto Centers for Disease Control and Prevention anaerobic sheep blood agar and placed in a CO_2-flushed holding jar which, within 2 hours, was set up with an AnaeroPack (Thermo Fisher Scientific, Lenexa, KS, USA).

After the jar was opened for plate examination, subsequent incubation was in a glove box at 37°C for 14 days. A 1-mL sample of fluid or homogenate was also inoculated into an anaerobically prereduced hemin-thioglycolate broth, which was closed and incubated at 37°C for 14 days. The broth was examined daily or until positive, and cloudy broth was subcultured. The plate was examined every other day for the first week and then on days 7 and 13 or until positive.

NGS protocol

Total genomic DNA was isolated from tissue samples using TissueLyser (Qiagen, Valencia, CA, USA) and High Pure PCR Template Preparation Kits (Roche, Pleasanton, CA, USA). Samples were amplified for pyrosequencing using the 28F 16S rDNA forward primer constructed with a 5′-3′ Roche A linker and an 8- to 10-base pair (bp) barcode,[34] and the 519R 16S rDNA reverse fusion primer was

Table I (**A**) Prosthetic joint infection diagnostic criteria as described by Frangiamore et al[11] and (**B**) modified prosthetic joint infection diagnostic criteria for next-generation sequencing

Category	Criteria*
(**A**)	
Definite infection	≥1 positive preoperative or intraoperative finding of infection and >1 positive culture with the same organism identified
Probable infection	≥1 positive preoperative or intraoperative finding of infection and 1 positive culture, or 0 preoperative or intraoperative findings of infection and >1 positive culture with the same organism identified
Probable contaminant	0 preoperative or intraoperative findings of infection and 1 positive culture
No evidence of infection	0 preoperative or intraoperative findings of infection and 0 positive cultures
(**B**)	
Definite infection	≥1 positive preoperative or intraoperative finding of infection and >1 positive NGS result with the same organism identified
Probable infection	≥1 positive preoperative or intraoperative finding of infection and 1 positive NGS result, or 0 preoperative or intraoperative findings of infection and >1 positive NGS result with the same organism identified
Probable contaminant	0 preoperative or intraoperative findings of infection and 1 positive NGS result
No evidence of infection	0 preoperative or intraoperative findings of infection and 0 positive NGS results

NGS, next-generation sequencing.
* Preoperative finding of infection = clinical signs (swelling, sinus tract, redness, or drainage), positive erythrocyte sedimentation rate, or positive C-reactive protein. Intraoperative finding of infection = gross findings (purulent drainage or necrosis).

constructed with (5′-3′) a biotin molecule and the Roche B linker.[34] Reactions were performed in 25 µL volumes containing 12.5 µL of HotStarTaq master mix (Qiagen), 9.5 µL of water, 1 µL of each primer (diluted to 5µM), and 1 µL of template DNA and were amplified using an ABI Veriti thermocycler (Applied Biosystems, Carlsbad, CA, USA) under the following conditions: 95°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 54°C for 40 seconds, and 72°C for 1 minute; and a final extension at 72°C for 10 minutes.

Amplification products were visualized using eGels (Life Technologies, Grand Island, NY, USA), pooled in equimolar amounts, and subjected to size selection using an Agencourt AMPure XP system (Beckman Coulter, Inc., Indianapolis, IN, USA) according to Roche 454 protocols. Size-selected pools were then quantified with NanoDrop 1000 spectrophotometer (Thermo Fisher), and 150 ng of each DNA sample was hybridized to Dynabeads M-270 (Life Technologies, Carlsbad, CA, USA) to generate single-stranded DNA. Single-stranded DNA was diluted and analyzed by emulsion-based polymerase chain reaction (PCR), and the resulting amplification products were subsequently enriched and sequenced. All methods were performed according to the manufacturer's protocols (454 Life Sciences; Roche, Branford, CT, USA).

Sequences generated during 454 pyrosequencing have a per base accuracy rate of 99.5%.[16,29] Correction of these errors and removing chimeras from the sequencing was done by first trimming sequences back using a running average of Q25. Trimmed sequences were then run through USEARCH[7] to cluster the sequences at 4% divergence. Cluster selection, chimera depletion, and sequence mapping were completed using the USEARCH UPARSE operational taxonomic unit (OTU) selection algorithm.[6] Mapped sequences were then grouped by OTU and quality scoring-based sequence correction was performed.

Corrected sequences were then run through the Research and Testing Laboratory Genomics taxonomic analysis pipeline to determine the taxonomic classifications and abundance for each sample. The first step of this pipeline was to perform quality analysis on each corrected sequence to check for and remove primers and ensure that each sequence was a minimum of 300-bp in length. OTU selection was then performed using the UPARSE OTU selection pipeline.[6,7] Selected OTUs were then aligned using MUSCLE[8,9] and a phylogenetic tree generated using FastTree.[27,28] The selected OTU sequences were then globally aligned using USEARCH[7] against a database of classified 16S sequences. Confidence values were assigned to each OTU classification, and the lowest common ancestor was determined based on these confidence values. The top hit and lowest common ancestor was then reported for each OTU.

Statistical analysis

Patients were categorized as infected (definitely infected or probably infected) or aseptic (probable contaminant or no evidence of infection) using the previously described criteria. Groups were further subdivided by whether culture results were positive. The Student *t* test was used to calculate difference in continuous variables between groups, and χ^2 analysis was used to measure differences in categorical variables. Concordance between culture and NGS was also examined. Concordance was defined as NGS and culture diagnosing a case as definitely infected/probable infection or probable contaminant/definitely not infected.

Results

Overall cohort

The cohort included 44 patients who underwent revision arthroplasty consisting of 32 single-stage revisions and 12 antibiotic spacer placements. Mean age was 68.7 ± 9.7 years (range, 47.8-89.3 years), 26 patients (59.1%) were men, and mean body mass index was 29.6 ± 5.7 kg/m^2 (range,

19.0-46.9 kg/m^2). The mean ESR and CRP for the entire cohort were 20.9 ± 18.4 mm/h and 2.8 ± 6.7 mg/L, respectively. There was no difference in ESR (17.5 ± 10.5 vs. 22.0 ± 20.4 mm/h; $P = .49$) or CRP (1.9 ± 2.1 vs. 3.2 ± 7.8 mg/L, $P = .53$) values for single-stage revision vs. antibiotic spacer placement. No patients had preoperative clinical signs of infection (swelling, sinus tract, redness, or drainage), 7 patients (15.9%) had elevated laboratory markers (ESR or CRP), and 5 patients (11.4%) had positive intraoperative findings of infection (purulent drainage or necrosis).

At least 1 positive culture specimen was present in 23 patients (52.3%), and 12 (29.5%) of these patients had ≥2 positive culture specimens with the same organism. There were no polymicrobial culture results. *C. acnes* (13 of 23 [56.5%]) and coagulase-negative *Staphylococcus* (CNS; 9 of 23 [39.1%]) were the most commonly cultured bacteria (Table II). NGS identified at least 1 positive specimen in 17 patients (38.6%), and in 16 (36.4%) of these, ≥2 specimens identified the same organism. A mean of 6 organisms was identified in the cultures that were NGS-positive. *C. acnes* (12 of 17 [70.6%]), *Acinetobacter radioresistens* (6 of 17 [35.3%]), and CNS, specifically *S epidermidis* (5 of 17 [29.4%]), were the most commonly identified bacteria by NGS. *C. acnes* was identified by both culture and NGS in only 4 cases (9.1%).

Infected cases

When cultures were considered in the definition of infection, 7 cases were definitely infected and 6 cases were probably infected. *C. acnes* was the most common bacterial species cultured in cases of definite and probable infection (8 of 13 [61.5%]). When the modified criteria with NGS were used, 8 cases were definitely infected, and 9 cases were probably infected. *C. acnes* was the most commonly identified bacterial species in cases of definite and probable infection (12 of 17 [70.1%]). An antibiotic spacer was placed in 7 of the 17 patients (41.2%) with positive NGS results and in 6 of the 23 patients (26%) with positive culture results ($P = .49$).

Eight patients were diagnosed as definitely or probably infected by both culture and NGS. The mean ESR and CRP for patients who were considered to be infected by both culture and NGS were 20.8 ± 15.5 mm/h and 2.6 ± 1.9 mg/L, respectively. The concordance (κ) between the 2 diagnostic criteria for defining infection that included culture or NGS was 0.333 (fair).

Synovial fluid was obtained in 31 patients (70.6%). Organisms in fluid specimens were identified by culture in 3 patients and by NGS in 6 patients. The 3 culture cases identified the following organisms, *Bacteroides fragilis*, methicillin-resistant *S aureus*, and *S epidermidis*. All 3 positive culture cases also identified the same organisms by NGS and were considered definitely infected by both culture and NGS definitions ($\kappa = 0.617$). An additional 3 cases had positive synovial fluid samples by NGS only and all were identified as infected by NGS criteria.

Aseptic cases

When cultures were considered in the definition of infection, 10 patients (22.7%) were probable contaminants, and 21 (47.7%) had no evidence of infection (Table III). All cases of probable contamination were culture positive for *C. acnes* (5 of 10 [50%]) or CNS (5 of 10 [50%]). When the modified criteria with NGS were used, 0 cases were probable contaminants, and 27 (61.4%) had no evidence of infection. There were significantly more cases of probable contaminants when cultures were used in the definition of infection compared with NGS ($P = .001$).

Reoperation

There was 1 reoperation (2.3%) for infection during the 1-year follow-up period. This patient was initially treated with revision of an anatomic total shoulder arthroplasty to a reverse arthroplasty and was not considered infected by culture or NGS definitions. There was 1 reoperation for hematoma (2.3%), 1 reoperation to perform a latissimus transfer (2.3%), and 1 revision of an anatomic total shoulder arthroplasty to a reverse arthroplasty for instability (2.3%). Of the 12 patients who underwent placement of an antibiotic spacer, 9 (75%) were revised to a reverse arthroplasty within the 1-year follow-up period.

Discussion

In this study, we included patients with and without preoperative clinical signs of infection. Positive cultures were present in more than 50%, and positive NGS results were present in almost 40% of revision arthroplasty cases. This is consistent with the literature. In a study of 221 patients without obvious infection who underwent revision total shoulder arthroplasty, Lucas et al[19] reported a 53% positive culture rate. It is clear that positive culture results are common and should not be considered "unexpected" in cases of revision shoulder arthroplasty. In fact, positive superficial and deep cultures are also commonly observed in cases of primary shoulder arthroplasty and arthroscopy, indicating that bacteria are omnipresent during surgical intervention.[20,22] The challenges faced by shoulder surgeons rest in the interpretation and treatment of positive culture results.

In this study, *C. acnes* and CNS (*S epidermidis*) were the 2 most commonly identified organisms by culture. This is consistent with culture data from revision shoulder arthroplasties performed at a number of institutions.[10,19,24] Similarly, our reported results indicate that shoulder arthroplasty cases with positive culture results commonly involve a single bacterial species. This is consistent with the literature that reports uncommon identification of polymicrobial infection by culture analysis.[10,24] Foruria et al[10] indicated that multiple microorganisms were identified in only 9% of revision cases that had positive cultures. In contrast, NGS testing indicated that more

Table II Cases of positive cultures and next-generation sequencing

Case #	Surgery	Clinical signs of PJI	Elevated lab markers	Intra-op signs of PJI	Cultures		Next-generation sequencing	
		(yes/no)	(yes/no)	(yes/no)	Organism(s)	No. positive	Organism(s)*	No. positive
2	Single-stage	No	No	No	*C. acnes*	1		
6	Single-stage	No	No	No	*C. acnes*	4		
7	Single-stage	No	No	No	CNS (*S epidermidis*)	1		
9	Single-stage	No	No	No	CNS (*S epidermidis*)	1		
10	Dual-stage	No	No	Yes	CNS (*S epidermidis*)	1	***E coli, C. acnes,*** *R insidiosa, E hormaechei, C paradoxus, A junii, A tetradius, C tuberculostearicum, C hominis, K palustris, S hominis, L crispatus, S maltophilia*	3
11	Dual-stage	No	No	Yes			***E coli, C. acnes,*** *R insidiosa*	2
12	Single-stage	No	No	No	CNS (*S epidermidis*)	1		
13	Single-stage	No	No	No			***C. acnes, A radioresistens,*** *C chromoreductans, C quinii, C hveragerdense, C acidisoli, C circulans, S agalactiae, R picettii, P aeruginosa*	3
14	Single-stage	No	No	No			***A radioresistens, C. acnes,*** *G ruanii, C tuberculostearicum, S pettenkoferi, C vibrioides, B aggregatus, S cohnii*	2
15	Single-stage	No	No	No	*C. acnes*	1	***A radioresistens, C. acnes, S melonis, A calcoaceticus,*** *S condimenti, A rhizogenes, B cepacia, S aureus, S epidermidis, C tuberculostearicum, C striatum, S mitis, B fungorum*	3
16	Single-stage	No	No	No	*C. acnes*	1	***C. acnes, A calcoaceticus,*** *S hominis, B mycoides, S aureus, P aeruginosa, C tuberculostearicum, P saccharophilia, S melonis, S epidermidis*	3
17	Single-stage	No	No	No	*C. acnes*	1		
18	Dual-stage	No	No	No	CNS (*S epidermidis*)	1		
20	Single-stage	No	No	No			***S agalactiae, P aeruginosa,*** *A radioresistens, L albida*	2
21	Dual-stage	No	Yes	Yes	*B fragilis*	5	***B fragilis****, B nordii, B thetaiotaomicron, B virosa, A radioresistens*	5
22	Single-stage	No	No	No	*C. acnes*	5		
23	Dual-stage	No	No	No	*C. acnes*	5	***C. acnes, M luteus,*** *B dorei, B casei, C xerosis, A ferrireducens, R gnavus, S pettenkoferi, K rosea, B cepacia*	4
25	Dual-stage	No	Yes	Yes	*S aureus* (MRSA)	5	***S aureus* (MRSA)**	5
26	Dual-stage	No	No	No	CNS	1	***S epidermidis, K pneumoniae***	2
27	Single-stage	No	No	No	*C. acnes*	1		
28	Single-stage	No	Yes	No	*C. acnes*	2		
30	Single-stage	No	No	No	*C. acnes*	1	***C. acnes, A radioresistens,*** *B cepacia, S maltophilia, E coli, K oxytoca, M granosa*	2
31	Single-stage	No	Yes	No			***C. acnes, C diptheriae, L agilis,*** *B cepacia, K oxytoca, C testosteroni, A radioresistens*	2
34	Single-stage	No	No	No			***P agglomerans, P aeruginosa,*** *B thermosphacta, S parasanguinis, L manihotivorans, C. acnes, P aeruginosa, C aurimucosum*	2
36	Single-stage	No	No	No	*C. acnes*	3	***M catarrhalis, S mitis, C kroppenstedtii, C. acnes,*** *S piscifermentans, S lugdunensis, S pettenkoferi, S sanguinis*	3
37	Dual-stage	No	No	No	*C. acnes*	2		
40	Single-stage	No	No	No	*C. acnes*	4		
41	Dual-stage	No	Yes	Yes	CNS (*S epidermidis*)	2	***S epidermidis,*** *A excentricus*	4
44	Dual-stage	No	Yes	No	*S epidermidis*	2	*S epidermidis*	1

PJI, prosthetic joint infection; *CNS*, coagulase-negative *Staphylococcus; MRSA,* methicillin-resistant *Staphylococcus aureus.*

* Bold font indicates that the bacterial species was identified in at least 2 specimens.

Table III Infection classifications by culture and next-generation sequencing

Variable	Definitely infected	Probably infected	Probably contaminant	No evidence of infection
Culture, No.	7	6	10	21
NGS, No.	8	9	0	27
P value	1.0	0.57	.001	.28

NGS, next-generation sequencing.

than 90% of positive cases were polymicrobial. This finding was also reported in a recently published study from our institution by Tarabichi et al[30] in which NGS was used in the diagnosis of hip and knee PJI. Because treatment of polymicrobial infection in the lower extremity has been shown to have lower success rates compared with monomicrobial infection,[33] the importance of polymicrobial results in revision shoulder arthroplasty remains to be determined. Whether certain bacterial species predominate and other bacterial species are upregulated microbiota or whether all identified microorganisms should be specifically treated is unclear.

One of the criticisms of molecular techniques for the identification of infecting organisms has been the propensity of some techniques to identify multiple bacteria in cases of both septic and aseptic revision.[2,3,35] Prior study in hip and knee arthroplasty used PCR-based electron spray ionization time-of-flight mass spectrometry and reported a high organism detection rate in culture-negative cases that were presumed to be infected but also detected organisms in many revisions presumed to be aseptic.[17] In this study, there were no cases in which NGS identified an organism in only a single specimen. As a result, when NGS was used in the definition of infection, there were no cases of "probable contamination." This demonstrates a clear difference between NGS and traditional PCR-based molecular techniques previously reported in the literature. NGS generates thousands of individual sequences from a single broad-range PCR and, as a result, provides information on the organisms occupying the joint (microbiota).[30] Because of this, further data regarding the "normal" shoulder joint microbiota are necessary to determine which organisms are truly pathogenic and which organisms are commensal. Without this understanding, the value of NGS results in defining infection is unclear.

We report fair concordance between cultures and NGS. This indicates that most cases in which culture results indicated infection were not the same cases in which NGS indicated infection. In hip and knee arthroplasty, Tarabichi et al[30] reported substantially better concordance between NGS and cultures, potentially reflecting the challenges with identifying and defining infection with less virulent microorganisms such as *C. acnes* and CNS. Certainly, the limited concordance that we report adds substantial confusion to the definition of PJI in the shoulder and leads to 4 possibilities:

1. Cultures results are more accurate than NGS results and we should believe our cultures.
2. NGS results are more accurate that culture results and we should believe NGS.
3. Both are necessary for identifying different cases of PJI and should be combined in our definition of PJI.
4. Both are poor tools for identifying cases of PJI.

Investigators have demonstrated that culture results are substantially influenced by the number of samples obtained and the source of these specimens (explant, soft tissue, or fluid).[1] In addition, a semiquantitative value has been assigned to *Cutibacterium*-positive cultures (Specimen Propi Value).[1] NGS also provides the opportunity to define the relative abundance of organisms within a sample; however, threshold values for defining pathologic bacterial loads are unknown. We believe that quantifying bacterial loads and improving our understanding of the normal joint microbiota are the next steps toward defining PJI in the shoulder. In addition, distinguishing between surface (epidermal and dermal) *C. acnes* organisms that are present at the time of revision surgery and deep organisms that act in a pathogenic manner would be necessary to truly define infection.

Interestingly, cases in which synovial fluid identified organisms by culture or by NGS were all considered to be true infections and demonstrated much better concordance between tests. This likely indicates a high specificity for positive synovial fluid analysis but poor sensitivity and may indicate some value for routine preoperative aspiration of revision arthroplasty cases. This is similar to the data reported by Dilisio et al[4] in which they reported 16.7% sensitivity and 100% specificity for aspiration in the diagnosis of shoulder PJI.

This study has a number of limitations. Given the lack of a standardized definition of shoulder PJI and the uncommon presence of clinical signs of infection, it is not possible to report sensitivity, specificity, positive predictive value, or negative predictive values for the 2 definitions for infection. Our sample size was also limited and restricted by funding for NGS testing. Patients were treated with single-stage or dual-stage revision based on surgeon preference and not via a specific protocol. Patients were monitored for 1 year for infection and longer-term follow-up could provide a greater understanding of cases of true infection. Finally, and perhaps most importantly, given our limited understanding of the normal shoulder joint microbiota, it is difficult to interpret both the NGS and culture data and to define PJI.

Conclusions

Given the poor concordance between culture and NGS data, there remains significant uncertainty about our current methods of diagnosing shoulder PJI. Bacteria were commonly identified in cases of revision shoulder arthroplasty

by both culture and NGS. Although culture data suggest that bacterial loads in revision cases are most commonly monomicrobial, NGS data suggest that bacterial loads in revision arthroplasty are most commonly polymicrobial. Future study will be necessary to determine the role of NGS in the diagnostic algorithm.

Disclaimer

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