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Utility of 16S rRNA PCR in the Synovial Fluid for the Diagnosis of Prosthetic Joint Infection

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Dear Editor,

Conventional culture has been the mainstay for diagnosing prosthetic joint infections (PJIs), with synovial fluid and periprosthetic tissue samples being the preferred sample types [1]. However, culture-based methods often give false-negative results in patients with a high likelihood of PJI [2]. To overcome the limitations of culture methods, molecular techniques like universal 16S rRNA gene and pathogen-specific PCRs were developed for PJI diagnosis [2, 3]. Although PCR assays have shown satisfactory results in tests of periprosthetic tissue samples and sonication fluid, the diagnostic utility of PCR is less clear in tests of the synovial fluid [2-4]. Most studies that evaluated the utility of 16S rRNA PCR in the synovial fluid for diagnosing PJI have not used the Musculoskeletal Infection Society (MSIS) consensus criteria as the gold standard for PJI [2, 5]. Therefore, we evaluated the utility of 16S rRNA PCR in the synovial fluid for diagnosing PJI using MSIS criteria.

A total of 85 patients (40 females) who underwent revision arthroplasty (87 procedures: 55 hip and 32 knee revisions) at All India Institute of Medical Sciences (AIIMS), New Delhi, India, between June 2013 to June 2017 were prospectively enrolled. Synovial fluid (N=87) and three to five periprosthetic tissue samples were collected intraoperatively from each patient (N=296) and were processed in accordance with standard protocols [3]. Briefly, tissue samples were aseptically disrupted using a sterile mortar and pestle with saline solution for one minute. Aliquots of synovial fluid and tissue samples were inoculated onto sheep blood agar (SBA), MacConkey agar (MA), and brain heart infusion agar (BHIA) plates, and in Robertson's cooked meat broth (RCM). Both SBA and MA plates were incubated aerobically at 37°C for 2–4 days. BHIA plates were incubated anaerobically at 37°C for 7–14 days. RCM broth was subcultured if cloudy or systematically on the 7th and 14th day. Organisms were identified using the matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system (Vitek MS; BioMérieux, Marcy-L'Etoile, France). PJI was confirmed according to MSIS guidelines [1]. This study was approved by the AIIMS institutional review board (Ref. No. IESC/T-419/01.11.2013). Informed consent was obtained from all patients.

A PCR assay targeting a partial region of the16S rRNA gene [6] was carried out for both synovial fluid and periprosthetic tissue samples. To eliminate any exogenous bacterial contamination, prior to amplification, the master mix (without the dNTP mix and primers) was incubated for 15 minutes with 0.1 IU of DNaseI enzyme (DNase I RNase-free, Thermo Fisher Scientific, Waltham, MA, USA). Bacterial species were identified by sequencing the PCR amplicons. A positive result from synovial fluid culture (SFC) or PCR was defined as a true positive when

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Table 1. Comparison of conventional culture and 16S rRNA PCR for diagnosing PJI according to the MSIS guidelines

Sample	Positives from PJI group (N=47)	Positives in AF group (N=40)	Discrepant results* (N)	Sensitivity % (CI)	Specificity % (CI)	Positive predictive value % (CI)	Negative predictive value % (CI)
Synovial fluid culture (N=87)	33	0	− − + − −	70.2 (56–81.3)	100 (91.2–100)	100 (89.5–100)	74 (61–83.8)
Synovial fluid PCR (N=87)	32	0	+ + − − −	68.1 (53.8–79.6)	100 (91.2–100)	100 (89.2–100)	72.7 (59.7–82.7)
Periprosthetic tissue culture (N=87)	37	0	+ − + + −	78.7 (65.1–88)	100 (91.2–100)	100 (90.5–100)	80 (66.9–88.7)
Periprosthetic tissue PCR (N=87)	41	0	+ + + + + 2 3 6 2 4	87.2 (74.8–94.2)	100 (91.2–100)	100 (91.4–100)	86.9 (74.3–93.8)

*Discrepant results between culture and 16S rRNA sequencing of both synovial fluid and periprosthetic tissue; +, positive; −, negative.
Abbreviations: PJI, prosthetic joint infection; CI, confidence interval; MSIS, Musculoskeletal Infection Society.

there was concordance with the result from periprosthetic tissue culture (PTC) or PCR. Sensitivities of culture and PCR assays of both sample types were compared by McNemar's method. $P<0.05$ was considered statistically significant. Statistical analyses were performed with Stata version 14.2 (Stata Corp LLC, College Station, TX, USA).

A definitive diagnosis of PJI was confirmed in 46 (54%) patients, and the remaining 39 (45%) patients were classified as aseptic failure (AF). Of the 87 synovial fluid samples, 32 and 33 showed positive synovial fluid PCR results and positive SFC results, respectively, with similar sensitivities (68.1% and 70.2%, respectively; $P=0.79$; Table 1).

The culture and PCR results of the periprosthetic tissue were positive in 37 (78.7%) and 41 (87.2%) of the 47 confirmed PJI samples (sensitivities, 78.7% and 87.2%, respectively; $P=0.04$) (Table 1). Table 1 summarizes the discrepant results in both sample types.

Molecular techniques have proven to be sensitive and specific diagnostic tools for many diseases; however, they have shown varying sensitivities and specificities in the diagnosis of PJIs [7]. Indeed, the sensitivity of synovial fluid PCR was relatively low in our study; better sensitivities were reported by Gallo *et al* [2] and Panousis *et al* [5]. Lack of amplification due to a small amount of bacterial DNA, the presence of polymerase enzyme inhibitors, and heterogenous distribution of bacteria in the synovial fluid could be potential reasons for the low sensitivity of the assay [8].

A major drawback of 16S rRNA-based assays is the potential for false-positive results [8, 9]. The inclusion of DNase treatment in our PCR protocol for contaminant removal [10] resulted insignificantly better specificity than in previous studies [3, 5]. However, DNase treatment also reduces Taq DNA polymerase activity, which could be responsible for the reduced sensitivity of our PCR protocol. Despite the low sensitivity of synovial fluid PCR, it successfully detected five PJI cases that were deemed to be negative by SFC.

Overall, our results do not support the routine use of 16S rRNA PCR of the synovial fluid in the diagnosis of PJI. However, the excellent specificity of our PCR protocol suggests that this assay may be useful for confirming a diagnosis of PJI.

Authors' Disclosures of Potential Conflicts of Interest

The authors declare no conflicts of interest.

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