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BRIEF REPORT

Real-Life Experience and Diagnostic Utility of the BioFire Joint Infection PCR Panel in Bone and Joint Infections: Analysis of a Prospective Validation Study

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ABSTRACT

Introduction: Microbiological diagnosis is central for adequate treatment of bone and joint infections. Culture-based methods have a limited diagnostic sensitivity and a long turnaround time (TAT). The objective of this study was to compare the diagnostic performance of BioFire Joint Infection Panel Investigational Use Only version (hereafter BioFire)—a sample-to-result multiplex PCR panel—with culture-based methods and 16S ribosomal RNA (rRNA) PCR and sequencing, when available.

Methods: This study presents a retrospective analysis of a prospective validation study of the BioFire panel. Specimens were obtained from consecutive patients evaluated for suspected bone and joint infections and processed using culture, BioFire, and 16S rRNA PCR and sequencing. Final clinical diagnosis was used as the reference for definition of infection.

Results: Samples, including synovial fluid, bone and periarticular tissue, were obtained from 57 patients, 39 of whom were finally diagnosed with a bone or joint infection. Cultures were positive in 27/39 infected patients and in 3/18 uninfected patients (sensitivity 69%, specificity 83%). BioFire was positive in 22/39 infected patients and in none of the uninfected patients (sensitivity 56%, specificity 100%). Sensitivity for PCR panel organisms was 92% (22/24) and sensitivity for organisms identified by any microbiological modality was 69% (22/32). Gram stain results were positive in 13/39 infected patients and in none of the uninfected patients (sensitivity 33%, specificity 100%). 16S rRNA was positive in 20/28 infected patients and in 0/12 uninfected patients (sensitivity 71%, specificity 100%). Net machine time for BioFire—1 h—was shorter than the mean TAT for Gram stain results, which was 4 h.

Conclusion: BioFire offered equivalent diagnostic performance with superior TAT for bone and joint infections, compared with conventional methods.

Keywords: Bone and joint infections; Molecular diagnosis; Multiplex PCR; BioFire; 16S rRNA

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40121-023-00809-x.

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Key Summary Points

Microbiological diagnosis is central for adequate treatment of bone and joint infections.

Culture-based methods have a limited diagnostic sensitivity and a long turnaround time.

The objective of this study was to compare the diagnostic performance of the BioFire Joint Infection Panel Investigational Use Only version with culture-based methods and 16S ribosomal RNA PCR and sequencing.

The BioFire joint infection panel offered equivalent diagnostic performance with superior turnaround time for clinically defined bone and joint infections, compared with conventional methods.

INTRODUCTION

Bone and joint infections (BJIs) are associated with considerable disability and substantial mortality [1]. Despite the need for prompt decision-making regarding antimicrobials and surgical intervention, BJIs often pose a clinical dilemma due to their non-specific manifestations and diagnostic challenges. Traditional cultures have limited sensitivity due to obstacles in sample processing, fastidious organisms, and previous antimicrobial exposure, and have a relatively long turnaround time (TAT) [2]. Molecular microbiological methods yielded conflicting results due to variable investigational methodologies, and carry the risk of identifying nucleic acids of non-viable organisms or skin contaminants [3, 4]. We aimed to evaluate the clinical and microbiological yield of BioFire Joint Infection Panel Investigational Use Only version (BioFire Diagnostics, Utah, USA; hereafter BioFire) in real-life use. Although the intended use of the panel is qualitative identification of pathogens and resistance mechanisms in synovial fluid samples, we also tested its performance in off-label use, in bone and periarticular samples, as is often required in common clinical scenarios.

METHODS

Presented is a retrospective analysis of a prospective evaluation of the BioFire panel, as part of the premarketing evaluation program supported by bioMérieux, performed at Sheba Medical Center, Israel, on consecutive patients evaluated for BJIs. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Sheba Medical Center Institutional Review Board (study protocol 8996-21-SMC). During the study period, specimens were obtained from patients suspected of having BJIs, and routinely processed in the microbiological laboratory using Gram stain, cultures, and 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR). Only samples remaining after routine laboratory processing were processed in the BioFire JI system as part of a laboratory validation study. No additional samples were obtained from patients for research purposes and BioFire results were not reported in patients' electronic medical records or delivered to the treating physicians in real time, and therefore had no effect on the management of the patients. This study presents retrospective analyses of the data obtained from the laboratory validation study. For the above-mentioned reasons, this study was approved by the Institutional Review Board with waiver of the requirement for patients' informed consent.

Synovial fluid, synovial tissue, bone, and soft-tissue samples were processed simultaneously using: Gram stain and cultures (aerobic, anaerobic, and fungal, mycobacterial per request, both solid media and enrichment broth were used per protocol), 16S rRNA PCR and sequencing, and BioFire, which detects 31 microorganisms and 8 antimicrobial resistance genes, with a net machine time of 1 h (https://www.BioFiredx.com/products/the-filmarray-panels/ji/). Specimens were processed for

cultures as follows: depending on the biological material, samples were either ground and streaked (tissue biopsies) or directly streaked (fluid specimens) onto solid media (blood and chocolate agar plates were incubated at 35 °C and 5% CO_2, and anaerobic plates were incubated in an anaerobic jar), and into liquid media (thioglycolate broth, and if volume and consistency allowed, also injected into pediatric and anaerobic blood culture bottles); aerobic plates were examined on days 1,2, and 5, anaerobic plates were examined on days 2 and 14, thioglycolate broth was examined daily up to day 7, organism identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, USA), and antimicrobial susceptibility testing was performed according to CLSI guidelines. The 16S rRNA laboratory procedure is detailed in Supplementary S6.

Patients were defined as having a BJI on the basis of a final diagnosis of the treating team and only if a course of definitive antibiotic therapy was administered with a subsequent response. Microbiological modalities were defined as concordant if they were either positive or negative and organism identification matched. BioFire was defined as affecting management if its result would have led to a decision to commence or halt antimicrobials, modify the antibiotic regimen, or perform a surgical intervention. Sensitivity and specificity of the tests were evaluated in relation to the final diagnosis (infected or uninfected).

For statistical analysis, descriptive data of continuous and ordinal variables are presented as means, and categorical variables are presented as numbers and percentages; chi-squared test, independent t test, and Wilcoxon rank-sum test were used to test for differences in characteristics between infected and uninfected patient groups. McNemar test was used to test for difference between test sensitivity and specificity rates; statistical significance was defined as $P < 0.05$. All data were analyzed using IBM SPSS Statistics for Mac, Version 28.0 (IBM Corp. Released 2020. Armonk, NY), and diagnostic test sensitivity and specificity were calculated using Microsoft Excel (Microsoft Corporation, 2019).

RESULTS

Fifty-seven patients suspected of having a BJI or a periarticular soft-tissue infection between March and August 2021 were included, 39 of whom (68%) were ultimately defined as infected (Tables S1 and S2). Cultures were positive in 27/39 infected patients and in 3/18 uninfected patients (sensitivity 69%, specificity 83%) (Table 1), with a median TAT to final antimicrobial susceptibilities of 47 h. BioFire was positive in 22/39 infected patients and in none of the uninfected patients (sensitivity 56%, specificity 100%) (Table 1), with a median TAT of 1 h. Of the 17 infected patients with negative BioFire results, 7 had negative results in all diagnostic modalities, 2 had BioFire panel organisms identified in cultures, and 8 had pathogens that were not included in the BioFire repertoire identified either by culture or 16S rRNA PCR (Table S3). Two patients, recently exposed to antimicrobials, were diagnosed with *Streptococcus dysgalactiae* using BioFire and 16S rRNA, undetected by culture (Table S4). Thus, sensitivities for infected patients were 92% (22/24) for panel organisms and 69% (22/32) for organisms identified by any modality. BioFire results were judged to have had an impact on clinical management of 22/39 (51%) of infected patients, and on 2/18 (11%) of the uninfected patients (Table 1).

Gram stain was positive in 13/39 infected patients and in none of the uninfected patients (sensitivity 33%, specificity 100%; Table 1), with a median TAT of 4 h. Among 26 infected patients with negative Gram stain, BioFire was positive in 13, providing the only indication for infection prior to culture results in these patients.

16S rRNA PCR results were available for 40 patients in total—28 infected patients and 12 uninfected patients. Results of 16S rRNA are missing in 17 cases, due to insufficient specimen quantity or a technical failure. Of these 40 patients, results were positive in 20 infected patients and in none of the uninfected patients (sensitivity 71%, specificity 100%) (Table 1).

Diagnostic modalities performance rates according to specimen type, sampling method, infection site, and recent antibiotic exposure are detailed in Table S5.

Table 1 Diagnostic modalities performance rates according to infection status

	Total (*n* = 57)	**Infected (*n* = 39)**	**Uninfected (*n* = 18)**
Positive BioFire (%)	22 (39)	22 (56)	0
BioFire sensitivity	56%		
BioFire sensitivity for panel organisms in culture-positive cases	92%		
BioFire sensitivity for organisms identified in any modality	69%		
BioFire specificity	100%		
BioFire result effect on management (%)	22 (39)	20 (51)	2 (11)
Positive Gram stain (%)	13 (23)	13 (33)	0
Gram stain sensitivity	33%		
Gram stain specificity	100%		
BioFire–Gram stain concordance rate (%)	44 (77)	26 (67)	18 (100)
Positive culture (%)	30 (53)	27 (69)	3 (17)
Culture sensitivity	69%		
Culture specificity	83%		
BioFire–culture concordance rate (%)	45 (79)	30 (77)	15 (83)
	Total (*n* = 40)	**Infected (*n* = 28)**	**Uninfected (*n* = 12)**
Positive 16S rRNA[1] PCR (%)	20 (50)	20 (71)	0
16S rRNA PCR sensitivity	71%		
16S rRNA PCR specificity	100%		
BioFire–16S rRNA PCR concordance rate (%)	34 (85)	22 (79)	12 (100)

[1]*rRNA* ribosomal ribonucleic acid

DISCUSSION

Rapid and accurate diagnosis of BJIs and soft tissue infections is critical for timely medical and surgical intervention, to reduce the risk of disability and systemic spread of virulent pathogens [5]. Nevertheless, these infections can be diagnostically challenging, as systemic manifestation may be subtle or non-specific and other bone and joint disease can mimic infectious conditions. Definite and prompt microbiological diagnosis has a pivotal role in decision-making regarding patient hospitalization, antimicrobial therapy and urgency, and extent of surgical interventions. Gram stain, still the most common rapid assay for diagnosing BJIs, has limited sensitivity and requires skilled laboratory personnel. Cultures, the gold-standard of microbiological diagnosis, are slow, and although more sensitive than Gram stain, may be hampered by previous antimicrobial therapy and technical challenges [2]. The role of molecular methods for hastening results and improving the yield of BJI diagnosis was evaluated in several studies. Several studies have reported superior sensitivity of 16S rRNA PCR and next-generation sequencing compared with

culture [6, 7]. However, both are labor-intensive, have a long TAT, and require highly skilled personnel and expensive infrastructure. Other commercial platforms were reported to yield a wide range of performance rates, probably due to variable patient populations, clinical specimens, comparator microbiological assays, and panel pathogen repertoires [8–10]. The performance of BioFire was previously reported on synovial fluid samples only, reporting sensitivity and specificity of 90% and 100%, respectively, compared with cultures [1]. In this study, BioFire had an overall sensitivity and specificity of 56% and 100%, respectively, for infection rates similar to those of conventional cultures. The majority of infected cases with negative BioFire results were either negative in all diagnostic modalities or caused by pathogens not included in BioFire repertoire, mainly *Cutibacterium acnes* or coagulase-negative staphylococci. Whether these low-virulence organisms are true pathogens in all of these cases is debatable, as they are also the most common skin contaminants. Nevertheless, their absence in BioFire panel, presumably aimed to minimize false-positive results, mandates the use of additional diagnostic modalities when these pathogens are suspected, e.g., in cases involving infection of shoulder joints, in infections involving foreign material, and in cases of chronic and indolent infections. Despite the absence of these pathogens in BioFire panel, the specificity rates reported in this study were similar to those reported in studies that examined the Unyvero PCR kit, which includes these two organisms [9, 10]. As for laboratory flow, the 1-h TAT of BioFire was significantly shorter than the 4-h TAT for Gram stain. Gram stain requires more skilled personnel, and thus is not available at all times.

In accordance with the use of clinical diagnosis as gold standard for infection, this study also reported the potential impact of BioFire on clinical decision-making. In 51% of infected patients, antimicrobial use and surgical interventions would have been altered following the BioFire results. Of the ultimately uninfected patients, 11% would have been affected by the BioFire results, as its result would have supported a decision to withhold empirical antibiotic therapy. These results support the use of BioFire for the initiation and selection of appropriate antimicrobials. Since empirical antimicrobial regimens for bone and joint infections commonly include combinations of broad-spectrum agents, BioFire may also serve as an antimicrobial stewardship aid, despite the lack of complete antimicrobial susceptibility data, as its results may lead to discarding coverage for Gram-positive or Gram-negative bacteria when only one is detected, or to de-escalation of antimicrobial agents with narrower spectrum when common antimicrobial resistance genes are not detected.

There are several limitations to this study. This is a single-center study with an off-label use of the kit, as it is intended for synovial fluid only. Moreover, only one sample per patient was used for molecular diagnosis, which may impact the sensitivity and specificity. In addition, the patient population, infectious diagnoses, and sample types were heterogeneous.

CONCLUSION

BioFire panel offers a rapid modality for the detection of common pathogens in BJIs, with a short TAT and minimal skill required, and similar sensitivity and specificity as conventional cultures. In our study, a substantial clinical impact was noted in 51% of infected cases, but the clinical setting and pathogens selection mandate a tailor-made diagnostic approach. Further studies should address the yield and clinical utility of this kit in different and specific patient groups, infection types, specimen types, and anatomical sites.

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Author Contributions. Tomer Hoffman: conceptualization of the study, methodology design, data analysis, writing of the original

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Or Kriger: performance and validation of laboratory procedures, data analysis, review and editing of the final manuscript, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Shoshana Cohen: performance and validation of laboratory procedures, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Shiraz Gefen Halevi: performance and validation of laboratory procedures, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Dafna Yahav: methodology design, writing of the original draft, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Sharon Amit: conceptualization of the study, methodology design, performance and validation of laboratory procedures, writing of the original draft, review and editing of the final manuscript, final approval of the version to be published, project supervision, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosures. Tomer Hoffman, Or Kriger, Shoshana Cohen, Shiraz Gefen Halevi, Dafna Yahav, and Sharon Amit have nothing to disclose.

Prior Presentation. This study has been presented as an abstract and a poster at the ECCMID conference 2022, Lisbon, Portugal, 23–26 April 2022.

Compliance with Ethics Guidelines. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Sheba Medical Center Institutional Review Board (study protocol 8996–21-SMC).

During the study period, specimens were obtained from patients suspected of having bone and joint infections, and routinely processed in the microbiological laboratory using Gram stain, cultures, and 16S rRNA PCR. Only samples remaining after routine laboratory processing were processed in the BioFire JI system as part of a laboratory validation study. No additional samples were obtained from patients for research purposes and BioFire results were not reported in patients' electronic medical records or delivered to the treating physicians in real time, and therefore had no effect on the management of the patients. This study presents retrospective analyses of the data obtained from the laboratory validation study. For the above-mentioned reasons, this study was approved by the Institutional Review Board with waiver of the requirement for patients' informed consent.

Data Availability. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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