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MAJOR ARTICLE

Plasma Microbial Cell-free DNA Next-generation Sequencing Can Be a Useful Diagnostic Tool in Patients With Osteoarticular Infections

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Background. Recent advances in shotgun metagenomic sequencing (sMGS) for detecting microbial cell-free DNA (mcfDNA) in peripheral blood have shown promise across various patient populations. This study evaluates the application of sMGS for diagnosing osteoarticular infections (OAIs), a condition with significant diagnostic challenges.

Methods. We conducted a retrospective analysis on 73 patients suspected of OAIs at the Mayo Clinic from 2019 to 2023, incorporating mcfDNA sMGS (Karius test [KT]) into their diagnostic evaluation. We categorized the clinical impact of KT on OAI diagnoses and management into 4 distinct outcomes. (1) KT was able to confirm an established diagnosis, (2) KT supported noninfectious diseases diagnosis, (3) KT established an unsuspected diagnosis, (4) KT did not add relevant information.

Results. In our cohort, KT was performed in 73 patients. Among the infected individuals, KT yielded positive results in 22 of 43 (51.2%) cases. Of these 22 cases, 11 (50%) showed agreement with conventional diagnostic workup, whereas in 5 (22.7%) cases, the KT established an unsuspected diagnosis. Native vertebral osteomyelitis diagnosis ($P < .001$) or OAIs with concomitant presence of endocarditis or endovascular infection ($P = .005$) were statistically associated with a definite, probable, or possible diagnostic certainty of KT result.

Conclusions. In complex OAIs, KT enhanced diagnostic accuracy by 11.6%, proving especially beneficial in diagnosing native vertebral osteomyelitis and infections with concurrent endocarditis or endovascular complications. Our findings underscore the utility of KT in the diagnostic workflow for challenging OAI cases, potentially altering clinical management for a significant subset of patients.

Keywords. cell-free DNA; osteoarticular infection; shotgun metagenomic sequencing; vertebral osteomyelitis.

Osteoarticular infections (OAIs) are a heterogeneous group of infections [1, 2] and present a substantial healthcare burden. Their incidence is increasing, particularly among the elderly, patients with diabetes mellitus or immunosuppression, and those with implanted orthopedic devices [3, 4].

Effective management of OAIs relies on the accurate identification of the culprit pathogens. Given the limited yield of blood cultures for these infections [5–8], the diagnosis often relies on invasive sampling for microbiological analysis. Nonetheless, an etiologic agent is not identified in approximately 35%–50% of cases [9, 10]. In recent years, advancements in pathogen identification techniques, such as polymerase chain reaction (PCR) and metagenomic sequencing, have significantly improved diagnostic accuracy [11]; however, the existing framework continues to depend extensively on invasive sampling and the quality of the collected sample. In light of these considerations, there is a pressing need for new culture-independent and noninvasive tests that provide rapid microbiological results in OAIs with high diagnostic performance.

In 2019, Blauwkamp et al [12]. reported on the validation and deployment of a shotgun metagenomic sequencing (sMGS) method that identifies the presence of microbial cell-free DNA (mcfDNA) in blood plasma, covering a range of more than 1250 bacteria, DNA viruses, fungi, and eukaryotic parasites (Karius, Karius Inc., Redwood City, CA). This is the first test of its kind implemented in the United States of America [12]. Since its introduction, this test has been studied in various conditions, such as bloodstream infections, sepsis, febrile neutropenia, pneumonia, and infective endocarditis in

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both immunocompetent and immunocompromised patients [13–19]. However, the clinical usefulness of Karius test (KT) in OAIs remains uncertain. Consequently, the purpose of our study was to assess the real-world usefulness of the KT for identifying organisms in blood or plasma and its impact on diagnosis and clinical management in a retrospective cohort of patients with OAIs at a quaternary care center.

METHODS

Study Population

We conducted a retrospective cohort study of adult patients who presented to the Mayo Clinic with suspected OAIs between 1 November 2019 and 31 November 2023, and for whom mcfDNA sMGS testing was ordered as part of their diagnostic workup. All patients were identified using the electronic health records at Mayo Clinic. The study data were collected and managed using the REDCap electronic data capture tool [20]. Data collected included demographics (age, sex), comorbidities (Charlson comorbidity score), immunosuppression (malignancy, hematopoietic or solid organ transplantation), and laboratory testing (blood and invasively collected culture results, biopsy results, C-reactive protein and erythrocyte sedimentation rate at the time of the KT). We also extracted data regarding KT results and diagnostic certainty and how it affected diagnosis and management of patients. A priori definitions of the syndromes involved can be found in Supplementary material.

The study was approved by the institutional review board at Mayo Clinic under a waiver of informed consent (IRB 23-002189).

mcfDNA-Next-generation Sequencing Test

Peripheral blood samples were collected in a BD or a K2-ethylenediaminetetraacetic acid Vacutainer. The KT was created and validated within the Karius Clinical Laboratory Improvements Amendments-certified/College of American Pathologists-accredited/New York State Department of Health-approved laboratory in Redwood City, California. This test is designed to identify and measure mcfDNA in plasma. After blood collection, samples underwent processing, plasma was isolated, and sent to the Karius laboratory for next-generation sequencing, in adherence to the Karius Specimen Collection & Preparation Instructions protocol, as previously described (https://kariusdx.com/karius-test/karius-test-process#specimen-collection) [12].

Clinical Application of KT Results

As described previously [13], diagnostic certainty of KT result was defined, by adjudication, as definite, probable, possible, or unlikely. These categories were applied to KT results to assess their concordance with the conventional diagnostic workup. Definitions can be found in Supplementary material. An organism identified through either conventional diagnostic testing or KT was considered clinically significant if the clinical team found the result plausible, or if the positive test information was used to enact a clinical change in patient management.

Clinical changes to patient management made in response to KT results were assessed by manual chart review. The clinical impact categories of KT were divided into 4 categories: (1) KT was able to confirm an established diagnosis, (2) KT supported noninfectious diseases diagnosis, (3) KT established an unsuspected diagnosis, or (4) KT did not add relevant information. These categories were based on the treating team's interpretation of KT results and their subsequent management decisions. Definitions or the categories can be found in Supplementary material. On the other hand, the impact on antibiotic therapy was divided into 5 categories: KT results led to (1) starting antibiotics, (2) escalation of antibiotics, (3) no change, (4) deescalation, and (5) discontinuation of antibiotics.

Statistical Analysis

Data management and analysis were conducted using IBM SPSS version 28 (IBM, New York, NY, USA). Descriptive statistics were presented as count and percentage for categorical variables and as mean and standard deviation (SD) for continuous variables, or as median and interquartile range when the variables were not normally distributed. Additionally, independent-samples t-test (or Wilcoxon rank-sum test) and Pearson χ^2 (or Fisher exact) tests were used for continuous and categorical variables, respectively, to compare baseline characteristics for specific subgroups including KT outcome (KT definite/probable/possible vs KT unlikely/negative) and KT results (positive vs negative). For the analysis of infectious versus noninfectious versus unknown groups One-way analysis of variance or the Kruskal-Wallis test were used. Significance was set at $P < .05$.

RESULTS

Baseline Characteristics of the Study Population

We identified 73 patients with suspected OAIs who were tested with KT as part of their diagnostic workup. The mean age was 58.9 years (SD 15.7), with a mean Charlson comorbidity score of 4.3 (SD 3.2) (Table 1).

Overall, 43 (58.9%) of the patients tested with KT had an infective final diagnosis, compared to 26 (35.6%) noninfective and 4 cases (5.5%) with unknown final diagnosis. Clinical characteristics of patients in the infected cohort were similar to those in uninfected cohort for the factors analyzed (Supplementary Table 1). Death occurred in 7 (9.6%) patients, of whom 2 were attributed to the infection detected by KT.

Karius Impact on Diagnosis and Diagnostic Certainty

KT result was positive in 31/73 (42.5%) patients, including 22/43 (51.2%) with infection as a final diagnosis. Comparing characteristics of patients with positive and negative KT results,

Table 1. Baseline Characteristics of the Patients With Suspected Osteo-articular Infections, Including Demographics, Clinical Characteristics, Diagnosis, and KT Results

Characteristic	N	Overall (N = 73)
Demographics	…	…
Sex at birth: female (%)	73	29 (39.7%)
Age (years; mean, SD)	73	58.9 (±15.6)
Patient characteristics	…	…
BMI (kg/m^2; median, IQR)	73	29 (25.4–34.2)
Immunosuppression	73	27 (37%)
Dialysis	73	2 (2.4%)
Previous surgery on the site of infection	73	16 (21.9%)
Previous injections or percutaneous procedures at the site of infection	73	11 (15.1%)
Previous radiotherapy at the site of infection	73	2 (2.7%)
Charlson comorbidity score (mean, SD)	73	4.29 (±3.17)
Presence of endocarditis or endovascular/device infection	73	11 (15.1%)
Bloodwork	…	…
C-reactive protein at the time of diagnosis (±72h, mg/L; median, IQR)	45	41.8 (7.6–125)
C-reactive protein at time of KT (±72h, mg/L; median, IQR)	51	51.9 (12.5–142.9)
Total leukocyte count, at time of KT (±72h, 10^9/L; mean, SD)	64	8.26 (±3.92)
Erythrocyte sedimentation rate at time of KT (±72h, mg/L; median, IQR)	43	41.0 (10.0–66.0)
Clinical characteristics	…	…
Suspected diagnosis	73	…
Native vertebral osteomyelitis	…	32 (43.8%)
Hardware-associated vertebral osteomyelitis	…	2 (2.7%)
Hardware-associated nonvertebral osteomyelitis	…	1 (1.4%)
Native nonvertebral osteomyelitis	…	9 (12.3%)
Prosthetic joint infection	…	1 (1.4%)
Native septic arthritis	…	18 (24.7%)
Septic bursitis	…	1 (1.4%)
Other[a]	…	10 (13.7%)
Final diagnosis	73	…
Infective	…	43 (58.9%)
Noninfective	…	26 (35.6%)
Unknown	…	4 (5.5%)
Final infective diagnosis	43	…
Native vertebral osteomyelitis	…	23 (53.5%)
Hardware-associated vertebral osteomyelitis	…	2 (4.7%)
Native nonvertebral osteomyelitis	…	5 (11.6%)
Hardware-associated nonvertebral osteomyelitis	…	0 (0%)
Prosthetic joint infection	…	0 (0%)
Native septic arthritis	…	7 (16.31%)
Septic bursitis	…	1 (2.3%)
Other[b]	…	5 (11.6%)
Microbiological workup	…	…
Positive blood culture	51	3 (5.9%)
Positive culture from first biopsy	42	7 (16.7%)
Positive culture from second biopsy	22	5 (22.7%)
Positive culture from arthrocentesis	22	3 (13.6%)
Positive culture from surgery	39	8 (20.5%)
Patients with positive PCR result	57	5 (8.7%)
Duration of antibiotics before KT (days; median; IQR)	43	6 (3–13)
Antimicrobial agents administered ≥ 7 d before KT	73	17 (23.2%)
Antimicrobial agents administered < 7 d before KT	…	26 (35.6%)
No antimicrobial agents beforeKT	…	31 (42.5%)
KT	…	…
ID consultation at Mayo Clinic	73	68 (93.2%)
Result	73	…
Positive	…	31 (42.5%)
Negative	…	42 (57.5%)
Number of pathogens identified (mean, SD)	31	1.71 (±1.57)
Positive KT considered clinically significant	31	16 (51.6%)
Diagnostic certainty	…	…
Definite	31	11 (35.5%)
Probable	…	4 (12.9%)
Possible	…	4 (12.9%)
Unlikely	…	12 (38.7%)
Clinical management	…	…
Number of patients for whom T changed management	73	6 (8.2%)
Impact on diagnosis	73	…
Confirmed an established diagnosis	…	11 (15.1%)
Supported non-ID diagnosis	…	14 (19.2%)
Established an unsuspected diagnosis	…	5 (6.8%)
Did not add relevant information	…	43 (58.9%)
Impact on antibiotic therapy	73	…
Started	…	2 (2.7%)
Escalation/broadened	…	2 (2.7%)
No change	…	67 (91.8%)
Deescalation	…	1 (1.4%)
Discontinuation	…	1 (1.4%)
Patient outcome	…	…
Death	73	7 (9.6%)
Death attributed to present infection	7	3 (42.9%)
Death attributed to infection detected by KT	…	2 (28.5%)
Patients treated with antibiotics	73	55 (75.3%)
Patients treated with surgery	73	35 (47.9%)

Abbreviations: BMI, body mass index; ID, infectious diseases; IQR, interquartile range; KT, Karius test; PCR, polymerase chain reaction; SD, standard deviation.

[a]Other suspected diagnosis: inflammatory arthropathies (n = 2), chronic recurrent multifocal osteomyelitis (n = 1), tenosynovitis (n = 5), wet gangrene (n = 1), synovitis (n = 1).

[b]Other final infective diagnosis: tenosynovitis (n = 4); wet gangrene (n = 1).

* We considered as index procedure the nearest invasive diagnostic procedure (eg, open or percutaneous biopsy, arthrocentesis) at the site of infection to the date when KT sample was drawn, that provided any result from the microbiology laboratory. If an invasive procedure was not performed for any reason, we considered the nearest blood cultures to the KT sample (7/73 patients).

variables significantly associated with KT positivity were native vertebral osteomyelitis (NVO) as a final diagnosis ($P = .004$) and presence of infective endocarditis or endovascular infection ($P = .045$). Other variables analyzed were not significantly associated with KT positivity (Supplementary Table 2).

Out of the 43 infectious cases, the KT proved beneficial in identifying a new infective diagnosis in 5 infective cases (Table 2) and validating a preexisting infective diagnosis in 11 cases (Supplementary Table 3), improving the diagnostic power from 25.6% (11/43) to 37.2% (16/43). All 5 cases were ultimately identified as infectious, and their treatment was guided by KT results. Subsequent follow-up assessments by treating physicians revealed clinical improvement in all cases after

Table 2. Relevant Microbiologic Studies of Patients With Unsuspected Diagnoses Established by the KT (N = 5)

Patient	KT Result	Blood Culture Results	PCR Results	Microbiology From Biopsy/fluid Results	Source Where Definitive Pathogen Identified	Suspected Diagnosis	Impact On Antibiotic Therapy	Final Diagnosis
9	*Streptococcus agalactiae* (MPM = 5991)	Negative	Not done	Not done	Culture negative	Native vertebral osteomyelitis	Deescalation of antibiotics	Infectious
10	*Escherichia coli* (MPM = 367)	Not done	Negative	Negative (micro negative, histo negative)	Culture negative + same pathogen on urine culture	Knee native septic arthritis	No change	Infectious
25	*Gardnerella vaginalis* (MPM = 738)	Not done	Negative	Negative (micro negative, histo negative)	Culture negative	Native vertebral osteomyelitis	Started antibiotics	Infectious
44	*Acinetobacter haemolyticus* (MPM = 156)	Negative	Negative	Negative (micro negative, histo negative)	Culture negative	Native vertebral osteomyelitis	Escalation of antibiotics	Infectious
53	*Lawsonella clevelandensis* (MPM = 545)	Negative	*Lawsonella clevelandensis*	Not done	Broad range PCR in biopsy tissue	Hardware associated nonvertebral osteomyelitis	No change	Infectious

Abbreviations: KT, Karius test; MPM, DNA molecules per microliter; PCR, polymerase chain reaction.

KT-guided antibiotic therapy. Nonetheless, it did not add relevant information in 43/73 (58.9%) cases because 28/43 (65.1%) of them had negative results and 15/43 (34.9%) of them had positive KT results that were considered not clinically significant and yielded a negative result in 19/26 (73.1%) patients with noninfectious diagnosis.

Distribution of the KT results and their respective outcomes can be found in Figure 1.

Factors Associated With Diagnostic Certainty of KT

Based on the diagnostic certainty categories mentioned in the methods section, 19/73 (26.0%) patients had either definite, probable, or possible KT results, whereas 54/73 (73.9%) had either unlikely or negative KT results. In a univariate analysis comparing definite, probable, and possible KT results group to negative and unlikely KT results group, variables significantly associated with KT diagnostic certainty were NVO as final diagnosis (68.4% in the definite/probable/possible group vs 16.7% in the unlikely/negative group; $P < .001$), and presence of infective endocarditis or endovascular infection (36.8% vs 7.4%; $P = .005$). However, there was no significant difference in sex ($P = .76$), age ($P = .68$), immune status ($P = .57$), and biologic markers, when comparing KT diagnostic certainty between the 2 groups (Table 3).

KT Impact on Management and Antibiotic Therapy

Overall, KT influenced antibiotic therapy in 6 cases (8.2%). Within this subset, antimicrobial therapy was initiated in 2 patients (2.7%), broadened in 2 (2.7%), deescalated in 1 (1.4) and discontinued in 1 (1.4%) of the cases (Table 4). Notably, all patients, except for patient 54, exhibited a favorable response to changes in antibiotic therapy. Therefore, 5/73 (6.8%) of the patients had a positive change in management of antibiotic therapy.

DISCUSSION

To our knowledge, this is the first retrospective study aiming to assess the real-world usefulness of the KT on the diagnosis and clinical management of patients with OAIs. In our cohort, among the infected individuals, the KT yielded positive results in 22 of 43 cases. Of these 22 cases, 11 (50%) showed agreement with conventional diagnostic workup, whereas in 5 (22.7%) cases, the KT established an unsuspected diagnosis, improving the diagnostic power of 11.6%. In the remaining 6 (27.3%) cases, KT did not add relevant information. Moreover, we found that NVO diagnosis and concomitant presence of endocarditis or endovascular infection were statistically significantly associated with a definite, probable, or possible certainty of KT result. Although KT results did not influence antibiotic therapy in most cases, it led to the initiation, escalation, or deescalation of antibiotic treatment in 6 patients (8.2%).

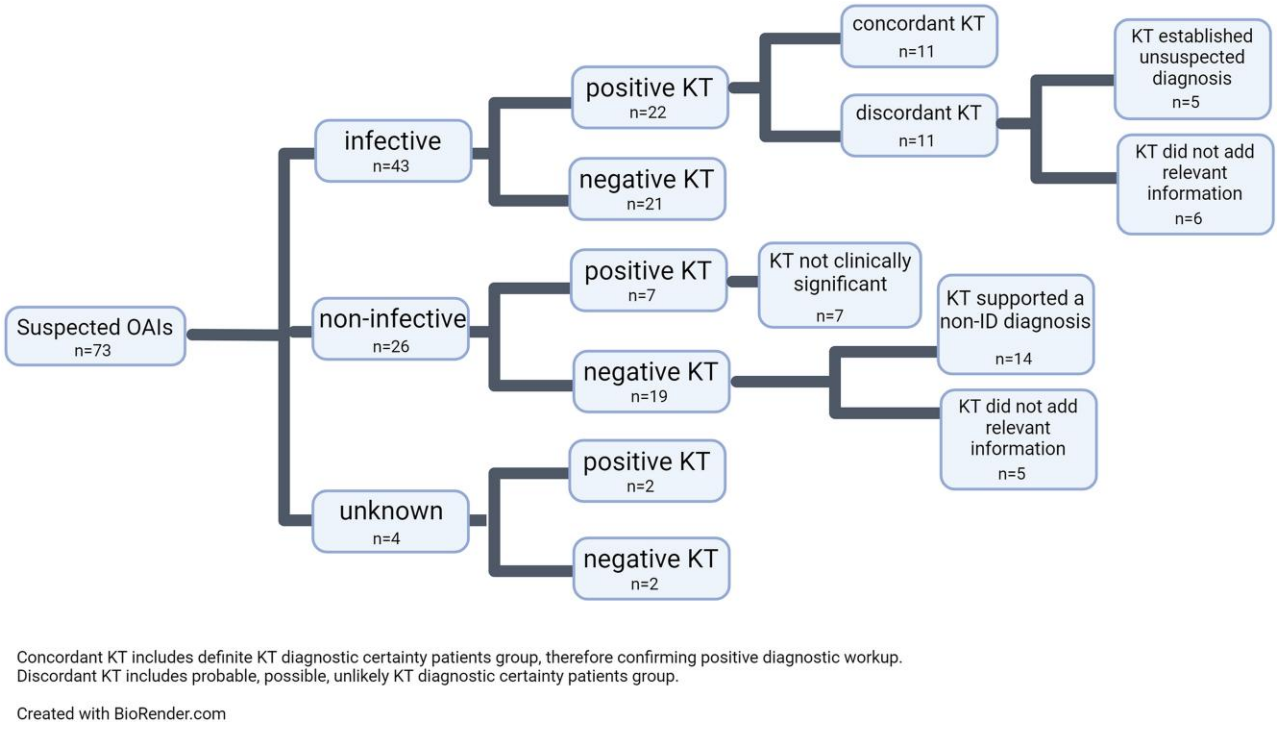

Figure 1. Distribution of Karius test (KT) results and their respective outcome according to the final diagnosis of suspected osteoarticular infection (OAI) cases.

To our knowledge, only 2 prospective studies to date employed KT in the context of prosthetic joint infection (PJI) [21] or musculoskeletal infections [22]. In the former, KT from plasma successfully identified the pathogen in 66% of PJI patients, enhancing perioperative pathogen detection by 7%. Although these findings closely align with our own, the interpretation of their significance requires caution because of differences in case ascertainment and study design. However, our series is distinct, marked by a diverse range of OAIs, particularly NVO, without any case of PJI. Similarly, in the latter, where children were the target population, only 1 case involved vertebral infection.

In the 5 patients for whom KT established an unsuspected diagnosis, atypical pathogens were identified. Specifically, *Escherichia coli* was identified in a patient with native knee septic arthritis, *Gardnerella vaginalis, Acinetobacter haemolyticus*, and *Streptococcus agalactiae* in patients with NVO, and *Lawsonella clevelandensis* in a patient with sternal osteomyelitis with extension to aortic root and concomitant prosthetic aortic valve endocarditis. The diagnosis of the first and fourth patients was aided by their history of previous infections; specifically, the first patient had a prior isolation of *E. coli* in urine, and the fourth patient had a recent episode of *S. agalactiae* bloodstream infection. 16S rDNA PCR and sequencing on a tissue biopsy corroborated the KT findings for the final patient. In 3 cases, antibiotic therapy was adjusted based on the test results. Meanwhile, for 2 patients, the existing antibiotic regimen already provided adequate coverage for the identified pathogen. The value of KT in detecting unusual or unexpected pathogens when conventional microbiological techniques have failed was similarly underlined in a previous study on febrile neutropenia [13].

Additionally, KT confirmed an established diagnosis in 12 patients, thereby reinforcing the microbiological diagnosis and affirming the need for directed antibiotic therapy. This was particularly pertinent for cases involving atypical pathogens, such as *Aerococcus urinae, Candida albicans, S. agalactiae* and *dysgalactiae, Pseudomonas aeruginosa*, and *Dolosigranulum pigrum* NVO cases, and *Coxiella burnetii* tenosynovitis. In 2 cases, KT confirmed the diagnosis of *Corynebacterium striatum* native knee septic arthritis and *Staphylococcus epidermidis* NVO. These pathogens, known for their difficulty to be classified as either pathogens or contaminants in OAIs, were identified through concordant results from cultures and the KT.

Native vertebral osteomyelitis, representing 53.5% of the infected cases in this cohort, was the primary infectious condition. A breakdown of all cases diagnosed with NVO can be found in Supplementary Table 4. This trend may be attributed to clinicians' preference for using the KT, given that spine biopsies identify pathogens involved in NVO in approximately only 50% of cases, and a second biopsy only marginally increases sensitivity [23–25]. This traditional diagnostic approach poses its challenges because of the invasiveness and variable

Table 3. Factors Associated With Diagnostic Certainty categories

…	Total Number of Patients	KT Definitive\Possible \Probable	KT Negative \unlikely	*P* Value
Sex (female) (n, %)	29/73 (39.7%)	7/19 (36.8%)	22/54 (40.7%)	.76
Age (mean ± SD)	58.9 (±15.6)	60.16 ± 13.53	58.41 ± 16.35	.68
CCI	4.29 (±3.17)	4.47 ± 3.06	4.22 ± 3.24	.77
Syndrome (NVO)	22/73 (30.1%)	13/19 (68.4%)	9/54 (16.7%)	<.001
Erythrocyte sedimentation rate (± 72 h from KT, mm/h, Mean rank, $Z = -1.32$)	41.0 (10.0–66.0)	26.89	20.71	.19
C-reactive protein (± 72 h from KT, mg/L, mean rank, $Z = -0.71$)	51.9 (12.5–142.9)	26.31	23.12	.47
WBC	8.26 (±3.92)	7.14 ± 2.62	8.71 ± 4.27	.15
Immune status (immunosuppression)	27/73 (36.9%)	6/19 (31.6%)	21/54 (38.9%)	.57
Antimicrobial agents administered for	…	…	…	
≥ 7 d before KT	17/73 (23.2%)	5/17 (29.4%)	12/17 (70.6%)	.836
< 7 d before KT	25/73 (34.2%)	7/25 (28.0%)	18/25 (72.0%)	
None	31/73 (42.5%)	7/31 (22.6%)	24/31 (77.4%)	
Endovascular infection/presence of endocarditis	11/73 (15.1%)	7/19 (36.8%)	4/54 (7.4%)	.005

Abbreviations: CCI, Charlson comorbidity index; KT, Karius test; NVO, native vertebral osteomyelitis; SD, standard deviation; WBC, white blood cell.

diagnostic accuracy, particularly with prior or ongoing antimicrobial therapy. In this context, the KT offers significant potential as a noninvasive diagnostic alternative, minimizing the risks associated with conventional biopsy procedures and providing a crucial diagnostic aid in cases where ongoing antibiotic therapy might interfere with the yield of traditional testing methods. However, larger studies must be conducted to assess this specific use before recommending any practice change.

Shishido et al [26]. noted a beneficial effect of mcfDNA testing in patients who had received antimicrobial agents for less than 7 days before it. However, our study did not yield similar findings. This discrepancy can be interpreted positively because it suggests that in our patient group, antimicrobial treatment did not influence the impact of KT. Additionally, our cohort differed significantly from theirs: although only 4 of 80 patients in their study had OAIs, ours predominantly comprised patients with subacute or chronic conditions. These differing patient profiles likely entail different bacterial burdens that may be affected differently by antimicrobial administration, making direct comparisons challenging.

Similarly, our cohort has a high percentage of patients with concomitant infective endocarditis or endovascular infection. KT has already shown promising results in these settings [15, 16, 27, 28]. The strong association found between concordance of KT results with standard microbiological testing and NVO diagnosis or the presence of infective endocarditis (IE) and/or endovascular infection has plausible biological explanation (eg, higher bacterial burden in peripheral blood) and strengthen the hypothesis that KT might be especially useful in infectious syndromes where hematogenous seeding is the main pathogenic mechanism. This association was also corroborated in a secondary analysis including KT-positive or KT-negative results.

We notice the tendency from non-ID physicians to use KT as a tool to rule out infective causes of inflammatory arthritis of unknown origin without extensive conventional microbiologic workup. Using KT alone to expedite the process of ruling out infectious causes is not a recommended approach. However, further studies are needed to explore this setting because using negative KT as a tool to deescalate unnecessary antibiotics may support both antimicrobial stewardship and the maintenance of patient microbiome stability while reducing toxicity.

Although the KT has its benefits, the prevalence of false positives necessitates vigilant diagnostic stewardship. This underscores the critical role of ID physicians in distinguishing between actual pathogens and bacterial background noise resulting from contamination, commensalism, mucosal barrier injury, or occult bacteremia related to minor procedures. The potential of quantifying bacterial burden with DNA molecules per microliter (MPM) as a tool for discriminating between contamination and infection, such as cases involving *Cutibacterium acnes*, coagulase-negative staphylococci and corynebacteria, remains unexplored in this study and warrants further investigation in future research.

In one case, the KT inadvertently led to unnecessary broadening of antibiotic treatment. This occurred in a patient undergoing treatment for suspected NVO, where the test detected a variety of pathogens, including *Bacteroides* spp., *E. coli*, and *Clostridium innocuum*, prompting broadening of the antibiotic regimen. Final diagnosis was deemed noninfectious (degenerative spinal disease vs ankylosing spondylitis). This case underscores the need for careful interpretation of KT results to avoid overtreatment.

A positive impact of KT in establishing unexpected diagnosis in 5/43 (11.6%) patients, as we showed, should be seen as an improvement in an era where the microbiological diagnostic tools are vast and potentially allow for pathogen identification in nearly all infective cases now [28]. These results are in line with previous published research in other settings, such as pneumonia, where Bergin et al [14]. found an additive

Table 4. Relevant Microbiologic Studies of Patients in Whom KT Changed Antibiotic Therapy (N = 6)

Patient	KT Result	Blood Culture Results	PCR Results	Microbiology From Biopsy/fluid Results	Source Where Definitive Pathogen Identified	Suspected Diagnosis	Impact On Antibiotic Therapy	Final Diagnosis
9	*S. agalactiae* (MPM = 5991)	Negative	Not done	Not done	Culture negative	Native vertebral osteomyelitis	Deescalation/narrowing of antibiotics	Infective
18	*Aerococcus urinae* (MPM = 17 699)	*Aerococcus urinae*	*Aerococcus urinae*	Negative (micro negative, histo negative)	Broad range PCR on biopsy tissue and blood cultures	Native vertebral osteomyelitis	Started antibiotics	Infective
25	*Gardnerella vaginalis* (MPM = 738)	Not done	Negative	Negative (micro negative, histo negative)	Culture negative	Native vertebral osteomyelitis	Started antibiotics	Infective
28	*S. agalactiae* (MPM = 1112)	Negative	Not done	Not done	Culture negative	Native vertebral osteomyelitis	Discontinued	Infective
44	*Acinetobacter haemolyticus* (MPM = 156)	Negative	Negative	Negative (micro negative, histo negative)	Culture negative	Native vertebral osteomyelitis	Escalation/broadened	Infective
55	*Phocaeicola vulgatus* (*Bacteroides vulgatus*) (MPM = 96) *Bacteroides ovatus* (*fragilis* group) (MPM = 78) *E. coli* (MPM = 66) *Clostridium innocuum* (MPM = 43)	Negative	Negative	Negative (micro negative, histo negative)	Noninfectious	Native vertebral osteomyelitis	Escalation/broadened	Noninfectious (KT led to unnecessary treatment)

Abbreviations: KT, Karius test; MPM, DNA molecules per microliter.

diagnostic value of 12% [14]. The help of KT in this setting is also underscored by the fact that metagenomic techniques are theoretically independent from antibiotic administration [15]. Our study was not powered enough to study the impact of timing of KT relative to blood cultures on concordance and yield, as hypothesized by some authors [15, 22, 28]—specifically because the vast majority of our patient cohort was sampled only once because of logistic constraints and clinical decision, or the correlation between microbial burden and metastatic infections [27, 29, 30]. Moreover, how the discordance in positivity and lower diagnostic yield increases over time as time on antibiotics elapses or how KT can be used to track infection clearance over the course of treatment, still must be determined in this setting. Additionally, our study is subjected to selection bias because of its retrospective design, as previously mentioned, because of the strict selection of most complex cases in which KT was ordered. Finally, another limitation of this study is the absence of a formal analysis on KT's cost-effectiveness. This was not the main purpose of the study, but we believe that these data can suggest that in the setting of OAIs, strictly selecting cases with a high pretest probability of true positive result (eg, patients with NVO and/or OAIs with concomitant endovascular infections, early in the course of disease with inconclusive blood culture results and/or biopsy) would still be considered cost effective for complex cases with OAIs. This might not be the case considering the pretest probability of true negative results, but more data are needed. As already pointed out [31] the cost of metagenomic Next Generation Sequencing (mNGS) has decreased dramatically over time from approximately $14 million in 2006, to below $1000 a decade later [32] and KT costs approximately $2000 per test [33]. Analysis of cost-effectiveness of this technique in settings other than OAIs has already been performed, showing significant cost savings for KT even with a 0% reduction in hospitalization [33].

Our study has some strengths. First, case ascertainment bias was at least partially corrected by providing a priori definitions of the syndromes described, aiming at standardization and homogeneity of the results. Moreover, follow-up at our center was long, with a mean of nearly 7 months, allowing for a correct ascertainment of the outcome and of the distinction between infective and noninfective causes of the diseases. Second, the clinical-oriented design of this study allows us to see the real-world application of this test, a novel technique about which just a handful of papers have been published to date. Third, our cases originate from a quaternary center experience, in which patients frequently seek second or subsequent opinions, giving a wide variety of the epidemiological context. Finally, KT was performed with a median of 1 day after the index diagnostic procedure (interquartile range –2; 4). We believe that this nearly simultaneous sampling constitutes a strength of our study because it shows consistency and let us make stronger conclusions on the accuracy of the results

of both tools, KT and other conventional microbiological technique, applied concomitantly.

In conclusion, in challenging cases involving highly complex OAIs, where traditional microbiology methods failed to identify the responsible pathogen, KT contributed to a 11.6% increase in diagnostic power by revealing unexpected diagnoses. Patients with NVO or concomitant endocarditis or endovascular infections notably gained from KT testing as a crucial component of their diagnostic evaluation. Further research in larger studies is essential to fully understand the potential impact of KT role in clinical practice.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. The authors confirm contribution to the paper as follows. Conceptualization, methodology: F.P., O.K.M., N.R., S.E.Z., O.A.S., E.F.B., M.F.; Formal analysis: F.P., O.K.M.; Investigation: F.P., O.K.M., N.R., S.E.Z.; Writing—original draft: F.P., O.K.M.; Writing—review and editing: F.P., O.K.M., N.R., S.E.Z., O.A.S., E.F.B., M.F.; Supervision: N.R., S.E.Z., O.A.S., E.F.B., M.F.

Data availability statement. The data that support the findings of this study are available upon reasonable request.

Patient consent statement. The design of the work has been approved by local ethical committees and includes the name of the authorizing body.

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