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Acute peri-prosthetic joint infection: improving diagnosis through the novel alpha-defensins test

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Summary

Background. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been proposed as novel alpha-defensins test for diagnosis of peri-prosthetic joint infections (PJIs). The aim of the current study is to assess the diagnostic accuracy of alpha-defensins MALDI-TOF MS in case of acute PJIs.

Methods. This prospective study included a series of 10 consecutive patients affected by PJIs according to the 2018 MSIS criteria. Synovial fluids were assessed for routine synovial fluid tests and alpha-defensins measurement with MALDI-TOF MS. Sensitivity, specificity, positive, and negative predictive values (PPV and NPV) were assessed.

Results. Six females and 4 males with acute PJIs were included. The mean age was 72.5 (range 67-79), and the mean time elapsed from primary arthroplasty to PJIs was 52 days (range 13-90). Eight TKA and 2 THA were evaluated. The involved bacteria were *Staphylococcus aureus* in 4 patients, coagulase-negative Staphylococcus species in 3 patients, Enterococcus species in 3 patients, and *Pseudomonas aeruginosa* in 1 patient. Three cases were characterized by polymicrobial infection. The MALDI-TOF alpha-defensin test correctly identified all 10 patients with acute PJIs. According to the current preliminary results the MALDI-TOF assay showed a 100% sensitivity, specificity, PPV, and NPV.

Conclusions. The novel alpha-defensins MALDI-TOF MS test showed promising results with high sensitivity and specificity in the diagnosis of acute PJIs. The reliability of the test in case of acute PJI could allow surgeons to manage the infection with a less invasive procedure with several advantages for patients. The findings of the present study seemed to confirm that the novel assay, which needs only few milliliters of sample, provides rapid results, and has substantial cost-effectiveness, and thus may be a useful diagnostic tool in clinical practice, even in case of acute PJIs. Further studies are needed to confirm these results on a larger series of patients.

Key words: peri-prosthetic joint infections, PJIs, alpha-defensins, biomarker, TKA, THA

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Introduction

Nowadays, periprosthetic joint infections (PJIs) represent the most frequent cause of revision in total knee arthroplasty (TKA) accounting for 16.8% of all the cases, and the third cause in total hip arthroplasty (THA) accounting for 14.8% of all hip revisions [1-3]. PJI is considered the most fearsome complication after total joint

arthroplasty (TJA). In fact, management of PJIs leads to higher financial and social costs compared with primary arthroplasties or aseptic revisions [4] with a 5-fold higher mortality rate [5,6].
In this regard, reliable and timely PJIs diagnosis is essential for successful treatment. However, diagnosis of PJI still represents a serious challenge for orthopedic surgeons because it relies on a battery of clinical findings and laboratory exams, and there is no single test with absolute accuracy [7,8]. This problem is even more relevant in acute onset infection in which some clinical findings may be confounding, and many laboratory tests may be altered. In fact, in case of suspected early post-operative infections, different cut-off values have been proposed for serum C-reactive protein (CRP), D-dimer and erythrocyte sedimentation rate (ESR), while synovial white blood cell count (WBC) and leukocyte esterase strip test are not recommended during the first six post-operative weeks [9,10].
A novel and promising strategy to treat acute cases of PJIs was recently proposed, which consists in the combination of debridement, antibiotic pearls, and retention of the implant (DAPRI) [11,12]. DAPRI allows to remove only the polyethylene or modular component of the prosthesis, reducing the time of the revision surgery and the impact on patient recovery. In this regard, it can be crucial to timely diagnose PJIs in the acute phase.
In the last few years, the efforts of the orthopedic community sought to improve diagnosis of PJIs by introducing a diagnostic algorithm and proposing innovative tests and biomarkers [13-15]. Among these, synovial fluid alpha-defensins have shown promising results [16-28], and their detection has been introduced in the most recent definition of PJIs developed by the Musculoskeletal Infection Society (MSIS) [29].
Measurement of alpha-defensins in synovial fluid may be challenging if performed with the two commercially available methods (i.e. the enzyme-linked immunosorbent assay [ELISA] laboratory-based test [® α-defensin immunoassay – Citrano Medical Laboratories, CD Diagnostics] and the lateral flow test [Synovasure-Zimmer Biomet, Warsaw, Indiana]. In fact, the ELISA is expensive, and time-consuming, while the lateral flow test has less accuracy and is also costly [16-27]. Novel diagnostic tests for detection of alpha-defensins in synovial fluids have been recently proposed with interesting results [28,30,31]. Among these, the method based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is expected to be readily introduced in the clinical practice. In fact, MALDI-TOF MS is nowadays widely diffuse in clinical laboratories for the diagnosis of several microbiological and metabolic conditions. Thanks to its accuracy in detecting small molecules in biological fluids with high specificity, the method has been proposed as a novel and reliable aid for diagnosis of PJIss, but has never been used in the management of acute PJIs.
This study aimed to assess the diagnostic accuracy of alpha-defensins measurement using MALDI-TOF MS in synovial fluids of patients with acute TKA or THA septic failure. The hypothesis was that alpha-defensins measurement with MALDI-TOF MS assay may be a highly sensitive and specific test for PJI diagnosis compared with the 2018 MSIS criteria. Its utility in clinical practice in comparison with the other available alpha-defensins tests is also discussed.

Methods

Study population

The consecutive series of the first 10 patients referring to our Institute for suspicion of acute PJIs since June 2020 were prospectively enrolled in the study. The acute onset of PJIs was defined if suspicion occurred during the first three post-operative months according to the 2014 MSIS definition [10]. Each patient was evaluated for the following: pre-operative serum CRP, D-dimer, ESR, synovial fluid WBC, leukocyte esterase test strip, percentage of synovial fluid polymorphonuclear neutrophils (PMN%), and alpha-defensins; microbiological analysis of synovial fluid sample; presence of a sinus tract, purulence, or clinical suspicion of joint infection. Sufficient synovial fluid was needed to perform both routine synovial fluid tests and alpha-defensins measurement through MALDI-TOF MS. To be included in the study, diagnosis of PJIs had to be satisfied according to the two major 2018 MSIS criteria (i.e. presence of sinus tract or two positive cultures isolating the same pathogen) [29]. Once a diagnosis of PJI was confirmed, patients underwent revision surgery with DAPRI if infection occurred in the first 3 post-operative weeks or two-stage revision if the infection occurred between the third week and the third post-operative month.
Exclusion criteria consisted of the following: suspected infection occurring after three post-operative months; insufficient amount of synovial fluid to perform all diagnostic exams; contamination of the synovial fluid sample during aspiration or transport to the laboratory; and insufficient data or an inconclusive diagnosis according to the 2018 MSIS score.
All participants gave informed consent to their inclusion in the study, which was approved by the University's Ethics Committee.

Synovial fluid analysis

Synovial fluid samples were obtained from the affected joint in either the operating room or as outpatients through needle arthrocentesis under aseptic conditions. Microbiological analysis on synovial fluid was performed by culturing at 37°C for up to 14 days by using BD Bactec Plus aerobic and anaerobic bottles (Becton Dickinson, Franklin Lakes, NJ, USA). A few milliliters of synovial fluid were reserved for the intra-operative alpha-defensins lateral flow test when it was needed. Next, the synovial fluid sample was immediately delivered to the Pathology and Clinical Microbiology Laboratory of our

Institute for further evaluation (i.e., synovial WBC, leukocyte esterase strip test, and PMN%). The remaining synovial fluid was assessed for alpha-defensins using MALDI-TOF MS as described by Petrucca et al. [30]. Centrifugation at 15,000 rpm for five minutes was performed to remove eventual contamination of tissue debris or blood cells. A volume of 10 µL of the supernatant obtained was diluted 1:100 in a standard MALDI-TOF solution composed of acetonitrile and 0.1% of trifluoracetic acid in ultrapure water (ratio 50:2.5:47.5). Then, 1 µL of the dilution was spotted onto the MALDI target plate and allowed to air dry at room temperature. Subsequently, the dried sample was layered with 1 µL of MALDI-TOF matrix solution composed of 10 mg/mL α-cyano-4-hydroxycinnamic acid (HCCA). The sample was transferred into the MALDI-TOF instrument (Bruker Daltonics) under the control of FlexControl 1.4 software (version 3.4; Bruker Daltonics) and irradiated with a pulsed nitrogen laser at 337 nm to obtain the representative mass spectra in the range of m/z 1000-5000. External calibration was performed using the Peptide Calibration Standard II (Bruker Daltonics; m/z mass range 757.4-3147.47) comprising angiotensin I and II, substance P, bombesin, ACTH (1-17 and 18-39), and somatostatin 28 for calibration in the mass range between ~ m/z 700 and 3500, which is expected to cover the range of molecular weights of alpha-defensins. A calibration curve was also obtained with purified alpha-defensins solutions (HNPs; Sigma-Aldrich, St Louis, MO, USA). Mass spectra analysis was carried out automatically using FlexAnalysis software (version 3.4; Bruker Daltonics) with the following parameter: signal-to-noise (S/N) ratio of 3, which is comparable to the cut-off level of 5.2 mg/L adopted for the other alpha-defensins tests.

Results of the MALDI-TOF MS analysis were compared with the MSIS criteria used as the gold standard. The alpha-defensins MALDI-TOF MS assessment did not contribute to diagnosis of PJI and did not modify the clinical decision in any case.

Statistical analysis

The sensitivity, specificity, positive, and negative predictive values (PPV and NPV) were calculated for alpha-defensins analysis with MALDI-TOF MS using the 2018 major MSIS criteria as the reference standard. A $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS Statistics 24 software (IBM Corp., Armonk, New York).

Results

The study population included the first 10 patients referred to our Institute with a confirmed diagnosis of acute PJI who met the inclusion criteria. Six females and 4 males were included. The mean age was 72.5 (range 67-79), and the mean time elapsed from primary arthroplasty to PJI was 52 days (range 13-90). Eight TKA and 2 THA were evaluated. According to the 2018 major MSIS criteria, PJI diagnosis was defined by the presence of a sinus tract in 4 patients and by the presence of two positive cultures for the same pathogen in the remain 6 cases. The involved bacteria were *Staphylococcus aureus* in 4 patients, coagulase-negative Staphylococcus species in 3 patients, Enterococcus species in 3 patients, and *Pseudomonas aeruginosa* in 1 patient. Three cases were characterized by polymicrobial infection. Demographic data are reported in Table I. The MALDI-TOF alpha-defensin test correctly identified all 10 patients with acute PJI. The signal of the three alpha-defensin molecules was registered in all cases. Figure 1 shows the analysis of three samples which were positive. According to the current preliminary results, the MALDI-TOF assay showed a 100% sensitivity, specificity, PPV, and NPV.

Table I. Demographic Data

Age – years, mean (range)	72.5 (67-79)
Sex (females n/%)	6/60%
Implant (TKA n/%) Time to revision – days, mean (range)	8/80% 52 (3-90).

Discussion

The main finding of this prospective study is that the novel method to assess alpha-defensins with MALDI-TOF MS in synovial fluid of patients affected with acute PJI showed high diagnostic reliability and may be an effective tool in the management of acute infection after TKA and THA. Thus, the hypothesis of the study was confirmed.

The financial and clinical impact of PJIs is considerable. Management of PJIs requires 4-fold higher costs compared to primary arthroplasty [3], due to prolonged hospitalization, antibiotic therapy, and associated morbidity [4,5]. Furthermore, PJIs are associated with a 5-fold higher mortality rate than their aseptic revision counterparts [6]. Proper and timely diagnosis is crucial to achieve treatment success and subsequently reduce the aforementioned issues [32]. In the recent years, novel biomarkers have been employed to aid in diagnosis of PJI [33] (e.g. serum D-dimer [34], synovial LE[35], synovial alpha-defensins [23], synovial CRP [23]) as well as molecular techniques, such as next generation sequencing and mass spectrometry.

Although the 2018 MSIS criteria, which firstly included alpha-defensins assessment in the scoring system, showed a sensitivity of 97.7% and a specificity of 99.5%, up to 16% of patients were reported to present an inconclusive diagnosis (score of 4 or 5 points) [36]. As recommended by the 2018 ICM, in such cases, further tests can be useful to diagnose or rule out PJI. In this regard, Petrucca et al. [30] have recently published an innovative test to identify alpha-defensins using MALDI-TOF MS. In their preliminary study on 18 patients undergoing revision

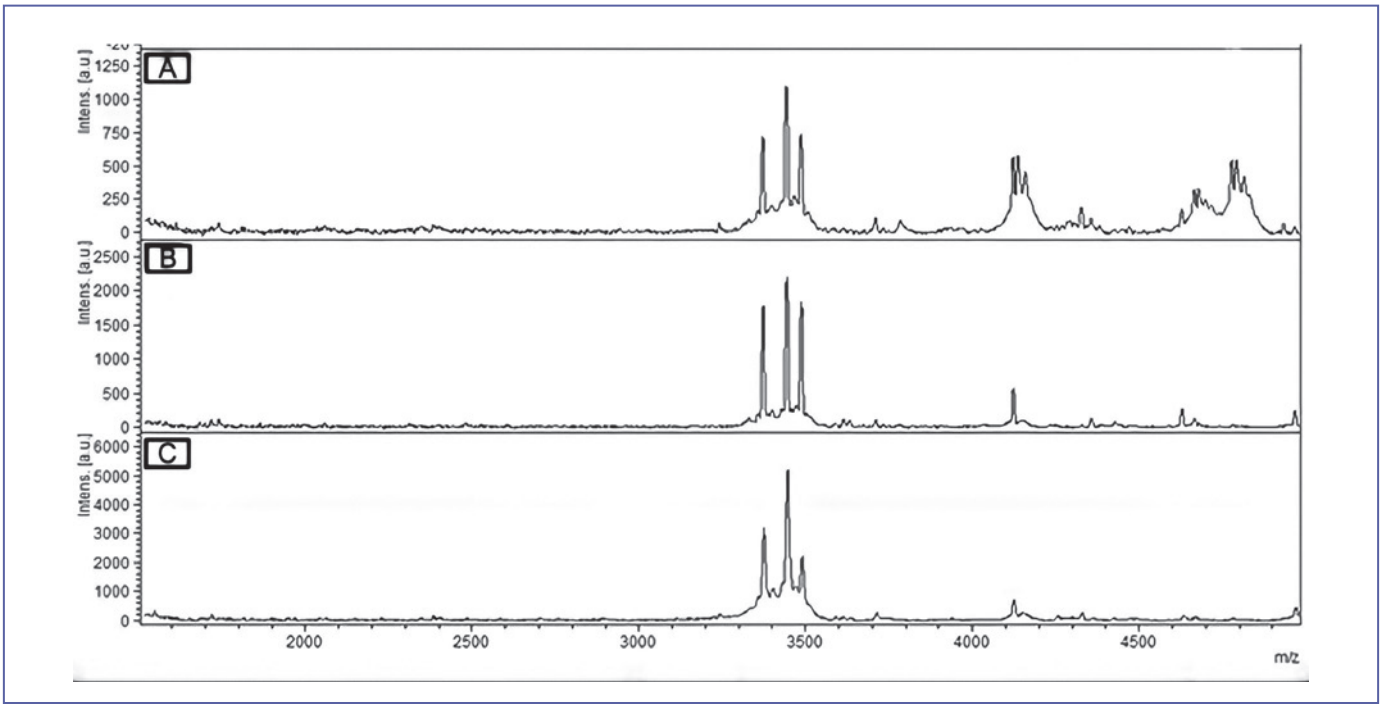

Figure 1. Synovial fluids MALDI-ToF analysis of three patients affected by acyte PJIs. Mass spectra analysis reveals three peaks around 3500 m/z corrisponding to the alpha-defensins molecular weight.

of TJA and classified as septic or aseptic according to the 2013 MSIS criteria, they found a higher overall diagnostic accuracy (in term of sensitivity and specificity) with MALDI-TOF MS than with the commercially available alpha-defensins lateral flow test. Their investigation also described the technical details of the procedure, and the pros and cons of the novel test.

We subsequently published a recent study to assess the clinical utility of MALDI-TOF MS as an innovative diagnostic test for PJI on a large series of patients undergoing THA or TKA revision [28]. An overall accuracy of 94.9, 93% sensitivity, and 96% specificity were found. The novel test did not seem to be influenced by concurrent antibiotic therapy, blood contamination, or low-virulence organisms, even though two false positive results were reported in case of rheumatoid arthritis (which may false the measurement similar to reports in other alpha-defensin available tests) [31,37].

In that study, patients undergoing early revision in the first 8 post-operative weeks were excluded. In fact, diagnosis of acute PJI may have several pitfalls. Alijanpoour et al. [9] investigated the overall accuracy of serum CRP and ESR in acute and chronic infections and proposed different threshold levels in these two settings, because a higher level of both markers is "physiologically" present in the first post-operative phase. Similarly, synovial WBC count is considered unreliable in the first six post-operative weeks and according to the 2014 MSIS criteria [10] it should not be used for PJI diagnosis during that period. Furthermore, clinical findings of infections may be disguised in the first post-operative weeks and the clinician may also hesitate in aspirating the synovial fluid for microbiological cultures.

At the same time, a prompt diagnosis in the acute phase may pave the way for a less invasive surgical procedure and positively affect the success of treatment. Recently, DAPRI has been proposed as a promising procedure to treat acute post-operative and early hematogenous PJIs. This technique aims to remove the intra-articular biofilm by the combination of a tumor-like debridement of peri-prosthetic tissues, and argon beam application plus chlorhexidine gluconate brushing on the implant surfaces. This, combined with a higher and prolonged local antibiotic concentration by using calcium sulphate antibiotic-added beads, might enhance the disruption of the infection with an 80% rate of success in two recent consecutive preliminary studies [11,12], which is higher than that reported with DAIR [38]. At our Institute, patients were routinely treated with DAPRI if the infection occurred in the first 3 post-operative weeks or in the first seven days after late hematogenous PJI.

To the best of our knowledge, this is the first study which investigates the effectiveness of the alpha-defensin MALDI-TOF MS test in the diagnosis of acute PJI. This represents the main strength of the study. Thanks to its ability to detect a small number of molecules in biological fluids with high sensitivity and specificity, MALDI-TOF MS has been successfully for several years in pathogen identification, diagnosis of au-

toimmune diseases, metabolic screening, and other clinical uses [39-41]. Nowadays, MALDI-TOF MS is an increasingly available technology in clinical laboratories, and it has been recently validated as an innovative test for the diagnosis of PJI. Compared to the other two commercially available alpha-defensin tests, MALDI-TOF MS has shown not be affected by blood contamination or cellular debris, and is markedly less expensive than the others (0.73 euro is the estimated cost per sample) [28]. Cost-effectiveness represents a major advantage of the test, which may contribute to the widespread use of MALDI-TOF MS for diagnosis of PJI. Furthermore, similar to the lateral flow alpha-defensin test, MALDI-TOF MS allows to obtain results within 20 minutes after receipt in the laboratory, which is theoretically compatible with an intra-operative setting. The main disadvantage of the test which should be noted is its semi-quantitative nature. The importance of a quantitative assay for alpha-defensins measurement, as the laboratory-based immunoassay, can be deduced by the finding that also in non-infective conditions (e.g. inflammatory arthritis), low levels of alpha-defensins can be found [31].

This study also presents some limitations. Firstly, a direct comparison between MALDI-TOF MS and the other currently available tests (either the immunoassay or the lateral flow test) was not performed. However, the purpose of the current paper was to assess the utility and reliability of the method in diagnosis of acute PJI, which has never been investigated before. Thus, we adopted major MSIS criteria (representing the gold standard) to diagnose PJIs and to compare the accuracy of the test. Secondly, the investigation was limited to TKA and THA and did not consider other TJAs. However, this is a preliminary study which does not aim to assess the clinical impact of the test on the overall PJI diagnostic approach or algorithm. Furthermore, it is well known that TKA and THA represent most of the implants worldwide, and this likely mitigates the limitation.

Conclusions

The novel alpha-defensins MALDI-TOF MS test showed promising results with high sensitivity and specificity in the diagnosis of acute PJI. The reliability of the test in case of acute PJI could allow surgeons to manage the infection with less invasive procedure with several advantages for patients. The findings of the current study appear to confirm that the novel assay, which needs only few milliliters of sample, provides rapid results, and has substantial cost-effectiveness, and thus may be a useful diagnostic tool in clinical practice, also in case of acute PJIs. Further studies are needed to confirm our results on a larger series of patients.

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Conflict of interest statement
The Authors declare no conflict of interest.

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Authors' contributions
RI, EV: conceptualization; DM, AP, EV, IS, MB: data curation; RI, EV, AF: writing-original draft preparation; EV, AF: writing-review and editing; AF: supervision.
All Authors have read and agreed to the published version of the manuscript.

Ethical consideration
All patients were treated according to the ethical standards of the Helsinki Declaration, and were invited to read, understand, and sign the informed.

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