



Alpha defensin accuracy and pitfalls in diagnosing septic arthritis in native joints

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Purpose: This study aimed to test the accuracy of the synovial fluid biomarker, α -defensin, in diagnosing septic arthritis in native joints and highlight the pitfalls of its utilization for primary joints. We also discuss the cases where false results were demonstrated.

Methods: A retrospective review of 10 patient's records, who underwent α -defensin testing for suspected primary joint infections prior to performing arthroplasty surgery, was performed.

Results: 6 primary knee and 4 primary hip joints were evaluated for septic arthritis utilizing the α -defensin test after arthrotomy was performed, as the patient had turbid synovial fluid or atypical bony features suggestive of septic arthritis. 2 of the primary hip joints had previous cephalomedullary nail insertions that cut-out and 1 knee joint had previous distal femur fixation for peri-articular fracture. There were 2 false positive α -defensin results in native knee joints that were eventually attributed to inflammatory arthritis after extensive cultures and microbial sequencing results returned negative. All other patients had negative α -defensin results that correlated with antimicrobial testing.

Conclusion: The utilisation of the α -defensin test in primary joints should be interpreted with caution. The elevated levels of α -defensin may be attributable to inflammatory arthritis and further studies of its levels in native joints are required.

Keywords : Alfa defensin ; Joint infection ; Septic Arthritis ; Periprosthetic Joint Infection ; Arthroplasty.

INTRODUCTION

Interest in utilizing intra-synovial fluid biomarkers to diagnose Prosthetic Joint Infections (PJI) has increased after synovial white blood cells (WBCs) were found to have unique gene expression profiles in response to bacterial infection (1). Deirmengian (3) screened 43 biomarkers based on previous studies and found 5 biomarkers; to be 100% sensitive and specific for diagnosis of PJI utilizing MSIS criteria. Defensins are antimicrobial peptides that are released by polymorphonuclear cells (PMNs), monocytes and lymphocytes as part of the innate immune system to neutralize invading pathogens without prior sensitisation.

The α -defensin test has, in numerous subsequent studies, been found to have consistent sensitivity and specificity, ranging from 95% to 100% (5), not

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affected by antibiotics or systemic inflammatory joint diseases and to respond to a wide variety of organisms including fungi (3,4). The test is commonly utilized as a cassette embedded, visual immunochromatographic assay (Synovasure) that can provide a diagnosis of PJI in 10 minutes. It is increasingly being added to the armamentarium of the arthroplasty surgeon who may be interested to stretch the indications for its utilisation. The purpose of this study is to highlight the pitfalls of its utilisation in native joints and discuss the factors that may lead to a false result.

METHODS

This study was approved by the Institutional Review Board. A retrospective review of 10 cases of suspected joint infection that utilized the Synovasure test (CD Diagnostics) between Nov 2015 and Nov 2017 was performed. Institutional review board approval was granted for this study and patient's clinical notes, operative records and perioperative radiographs were analysed.

All synovial fluid was collected preoperatively or intraoperatively via needle aspiration or after arthrotomy under aseptic conditions in the operating theatre. The synovial fluid was tested using the Synovasure (CD Diagnostic) test which utilizes an enzyme-linked immunosorbent assay which defined 5.2mg/L as a positive result. Multiple fluid and tissue specimens were obtained and processed for full examination of microscopic elements and bacterial cultures, 72 hours for aerobic cultures and 120 hours for anaerobic cultures. Patients with high suspicion of septic arthritis were further tested with bacteria genome polymerase chain reaction (PCR) if initial results returned negative and culture specimens were kept for an extended period of a week before disposal. Patients were deemed to have septic arthritis if the cultures or polymerase chain reaction returned positive.

RESULTS

Six primary knee and four primary hip joints were evaluated for septic arthritis with Synovasure,

as the patient had turbid synovial fluid or atypical bony features suggestive of septic arthritis. These included two primary hip joints which had had previous cephalomedullary nail insertions that had cut-out and 1 patient who had had previously distal femur fixation complicated by subsequent aseptic loosening. All three cases had true negative results.

Five other patients had negative α -defensin results that correlated with anti-microbial testing. Two patients had severe avascular necrosis of the femoral head with severe collapse and end stage arthritis. Three primary knees had atypical features of end stage arthritis. One patient had a valgus knee with severe arthritis, 1 patient had synovial overgrowth and 1 patient had turbid synovial suggestive of infection. The patient profiles, comorbidities, surgical indications, intraoperative findings and Synovasure results are shown in Table I.

There were two false positive α -defensin results in native knee joints that were eventually attributed to inflammatory arthritis after extensive cultures and microbial sequencing results returned negative.

The 1st patient was a 54 year old Chinese gentleman with a past history of refractory rheumatoid arthritis, on Prednisolone and Cyclosporine. Previous therapies with sulphasalazine, hydroxychloroquine, GOLD, Infliximab and adalimumab had failed. He was a Hepatitis B carrier on Lamivudine and Adefovir for 18 years and suffered from ischaemic heart disease post percutaneous intervention, hyperlipidemia, gastric polyps, colonic tubular adenomas and latent tuberculosis (TB), for which he had completed 4 months of Rifampicin treatment 8 years previously. He complained of worsening right knee pain over the past 6 months and was only able to walk less than 1 bus stop on a level surface. He had had no recent fever or exacerbation of his pain. He had had intra-articular injections of hydrocortisone and lignocaine for pain relief, at 5 months and 15 months prior to surgery. Physical examination of his knee revealed minimal effusion with mild warmth with range of motion from 10 to 100 degrees. Pre-operative total white cell count was 14.9×10^9 per liter and radiographs of his right knee showed tricompartmental osteoarthritis with reduction in joint space, marginal osteophytes, subchondral sclerosis and subchondral cysts. A

Table I. — Summary of patient demographics, white cell count, intraoperative findings and synovasure results when used in native joints

Patient	Comorbidities	Surgical Indication	Pre-op White Cell Count (10 ⁹ /L)	Intraoperative Findings	Synovasure Results
83/F	DM, HTN, HLD, Lt Breast Cancer	Left Hip Cephalomedullary Nail Cut-out	8.2	Cephalomedullary nail cut-out	Negative
63/M	DM, HTN, HLD, Anaemia	Right Distal Femur Fixation Failure	10.5	Distal femur plate fixation loose.	Negative
47/M	HTN, HLD, Gastric Ulcer	Right Knee OA	7.4	Valgus knee with severe arthritis	Negative
74/M	HTN, Lung Asbestosis	Left hip AVN	9.4	Femoral head collapse with end stage arthritis	Negative
68/M	HTN, HLD, Gout, Chronic Kidney Disease	Right knee OA	8.9	Medial and lateral tibia condyle wear and patella synovium overgrowth	Negative
55/M	Previous Left Hip Osteomyelitis, Psoas Abscess	Left hip AVN	10.4	Femoral head collapse with end stage arthritis	Negative
60/M	DM, HTN, HLD, Anaemia, Schizophrenia	Left knee OA	5.3	Turbid synovial fluid	Negative
66/F	DM, HTN, HLD, Chronic Kidney Disease, Asthma, Multiple Myeloma	Left Hip Cephalomedullary Nail Cut-out	7.2	Cephalomedullary nail cut-out	Negative
54/M	HLD, Rheumatoid Arthritis, Bronchiectasis, Hep B Carrier, Latent TB	Right knee Rheumatoid Arthritis	11.6	Turbid synovial fluid	Positive
67/M	HTN, HLD, Inflammatory Arthritis, Aortic Valve Regurgitation post metallic valve replacement	Left knee Inflammatory Arthritis	9.8	Synovium pale with turbid synovial fluid	Positive

DM : Diabetes Mellitus, HTN : Hypertension, HLD : Hyperlipidemia, TB : Tuberculosis, OA : Osteoarthritis, AVN : Avascular Necrosis.

suprapatellar effusion and a lateral patellar tilt was also noted on the radiograph.

Intravenous Cefazolin was administered prior to induction but the patient did not receive any other antibiotics prior to surgery. Upon arthrotomy, turbid synovial fluid was noted, which was tested using the Synovasure test kit. The test returned positive and extensive cultures were taken before a synovectomy and washout was performed. The synovial fluid examination had 2778 nucleated cells, 89% neutrophils and no crystals. The synovium and fluid tested negative for aerobic, anaerobic, fungal and mycobacterial cultures. 6SS ribonucleic acid sequencing for bacteria, internal transcribed spacer (ITS) sequencing for fungal infection and heat-shock protein (HSP) 65 sequencing returned negative. Histology showed sections of synovium

with fibrinous exudate and moderate acute on chronic inflammation along with aggregates of plasma cells. Fibrosis, calcifications and osseous metaplasia were also noted. Stains for acid fast bacilli and fungi were negative. The patient was initially treated empirically for septic arthritis with intravenous cefazolin for 4 weeks but was later treated as per inflammatory arthritis and was planned for surgery at a later date.

The 2nd patient was a 67 year old Malay with a history of hypertension, hyperlipidemia and aortic valve regurgitation for which he had undergone a metallic valve replacement more than 10 years previously. He complained of left knee pain for the past 10 years with no recent exacerbations of pain, swelling or fever. Examination revealed medial joint line tenderness with good range of motion from 5 to

100 degrees without effusion. Pre-operative white cell count was 9.8×10^9 per litre and radiographs showed medial compartmental osteoarthritis with reduction in joint space, subchondral sclerosis and subchondral cysts. Intra-operatively, the synovium was pale and there was surrounding turbid synovial fluid. Decision was made to proceed with total knee replacement after extensive synovectomy and washout. Cultures and molecular testing for bacteria, fungi and mycobacterium returned negative. Additional serology for Brucella, Coxiella and Melioidosis was also negative. Histology showed chronic synovitis with focal granulomatous inflammation which favoured a diagnosis of autoimmune conditions rather than infective TB. Serology testing showed anti-cyclic citrullinated peptide (Anti-CCP) levels of 85u/ml and rheumatoid factor of 200 IU/ml. Radiographs of his hands showed reduction in joint space of bilateral carpal bones and joint space narrowing of the right lateral 3 carpometacarpal joints, of the proximal interphalangeal joints of both little fingers and all distal interphalangeal joints. He had a right 1st carpometacarpal joint subluxation and a left 1st metacarpal phalangeal joint medial subluxation. A diagnosis of inflammatory arthritis was made and he was referred to a rheumatologist for control of his condition.

DISCUSSION

The α -defensins are small cysteine rich peptides that are concentrated in the granules of neutrophils, constituting part of the humoral innate inflammatory response to foreign pathogens (5). The α -defensin test has been found in subsequent numerous studies to have consistent sensitivity and specificity ranges from 95% to 100% in PJI, is not affected by antibiotics and responds to a wide variety of organisms including fungi. The test has outperformed the Leukocyte Esterase (LE) colorimetric strip test in accuracy and reliability even in samples contaminated with blood.

While α -defensins are expressed in response to a diverse range of microorganisms, its levels

in native joints have previously been described to also be elevated in patients with autoimmune disease (2). Even with extensive history taking, physical examination and laboratory testing, some patients with inflammatory arthritis may still go undiagnosed prior to surgery.

As a point of care test, the Synovasure test may allow for expedient decision making if an arthroplasty surgeon suspects septic arthritis after arthrotomy has been performed. Additionally, this point of care test (POCT) may provide an expedient diagnostic test for the emergency department physician to rule out septic arthritis of the joint, and prevent unnecessary admissions. Our results show that the high false positive rates preclude its utilisation in such scenarios. Further studies however are required in evaluating its use in joints with peri-articular fixation with metallic implants.

CONCLUSION

While α -defensin testing has been shown to be highly predictive of PJI, the results of its utilisation in primary joints should be interpreted with caution.

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