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ORIGINAL STUDY

Alpha-defensin for the intra-operative diagnosis of prosthetic joint infections

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A single-center prospective study was conducted over one-year period to determine the performance (sensitivity, specificity, positive and negative predictive values) of the synovasure test for the diagnosis of prosthetic joint infection using the MSIS consensus criteria as the reference.

The study included all patients admitted for resumption of hip or knee prosthesis whatever the reason, all couples of friction, patients under antibiotic treatment, immuno-compromised or with systemic inflammatory diseases. 62 consecutive patients were preoperatively distributed into three groups (infected, uninfected and questionable). In order to determine MSIS criteria, pre-operative blood tests, as well as bacteriological, cytological and histological analyses of intraoperative tissues were performed. The synovasure test was performed following the protocol on articular fluid intraoperatively and showed a sensitivity of 83.3%, a specificity of 95.7%, a positive predictive value of 83.3% and a negative predictive value of 95.7%.

Keywords : alpha-defensin ; synovasure ; prosthetic joint infection.

INTRODUCTION

Joint replacement significantly improves the quality of life of patients with degenerative joint disease and represents a major breakthrough in the surgical field *(1)*.Prosthetic joint infections are among the dreaded complications of this type of orthopedic procedure because of their heavy consequences in terms of morbidity and mortality *(2)*. Infections are estimated to account for 15 to 25% of prosthesis revision cases *(3,4)*.The diagnosis of a prosthetic joint infection is based on a set of arguments integrating clinical findings, blood biology and cytology, microbiology and histology of synovial fluid and intra-operative tissues *(5,6)*. All of these tests lack sensitivity and specificity, justifying the use of a combination of criteria for diagnosis of prosthetic joint infections in most studies *(7)*. The International Society of Musculoskeletal Infections (MSIS) has established a score based on a set of major and minor criteria *(8,9)* in order to facilitate diagnosis. However, the definition of a prosthetic infection is still controversial and there is currently no real gold standard *(10)*. In order to improve diagnostic tools, synovial markers have been developed in recent years ; their results seem to be promising for the diagnosis of prosthetic joint infections *(11,12)*. Alpha-defensin is an antimicrobial peptide secreted by neutrophils cells in the event of infection ; It responds to a wide spectrum of

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organisms *(13)* and is not affected by prior antibiotic treatment *(12)*. However, the production of alpha-defensin is controlled by numerous cytokines (IL-1β, IL-6, TNF-α), which are able to positively regulate its expression *(14)*. Alpha-defensin is measured in the synovial fluid by the mean of an enzyme-linked immunosorbent assay *(15,16)* ; the lateral flow alpha-defensin immuno-assay "synovasure" is a rapid test that allows an immediate diagnosis (waiting time of 10 minutes) of prosthetic joint infection. It has shown excellent results for the diagnosis of prosthetic joint infections *(17,18)*. To date, the use of this test is not yet widespread or proposed in the international recommendations. The aim of the study is first to determine the performance of the synovasure test (sensitivity, specificity, positive and negative predictive values) for the diagnosis of prosthetic infections and secondary to determine his place in the event of doubtful case as well as the economic impact of this test.

MATERIAL AND METHOD

A single-center prospective study was validated by the local ethics committee and conducted in the orthopedic department of Ambroise-Paré Hospital in Mons Belgium, over one-year period from 01/01/2016 to 31/12/2016. This study covered all patients over 18 years admitted for revision of hip or knee prosthesis for any reason. All couples of friction, patients under antibiotic treatment, immuno-compromised patients or with systemic inflammatory diseases were included. An informed consent was obtained for all participants. The exclusion criterias were the hemorrhagic aspect or the lack of joint fluid and a life expectancy less than 72 hours. Data about history of prosthesis, comorbidities, antibiotic treatment and civility were collected by one of the investigator physicians and reported on a Case Report Form (CRF) numbered from 1 to 62. Preoperatively, some laboratory analysis including white blood cells count, CRP, ESR, ionogram, liver tests, urea, creatinine, serology of hepatitis B, C and human immunodeficiency virus (HIV) were documented ; Two vials of haemocultures were collected in case of fever (axillary temperature> 38°C). Imaging was done on a case-by-case basis by the surgeon : radiography, ultrasound, CT scan, magnetic resonance (MRI), bone scan, labeled leukocytes or gallium bone scan.

A pre-test probability of prosthetic joint infection based on the clinical, biological analysis (ESR, CRP

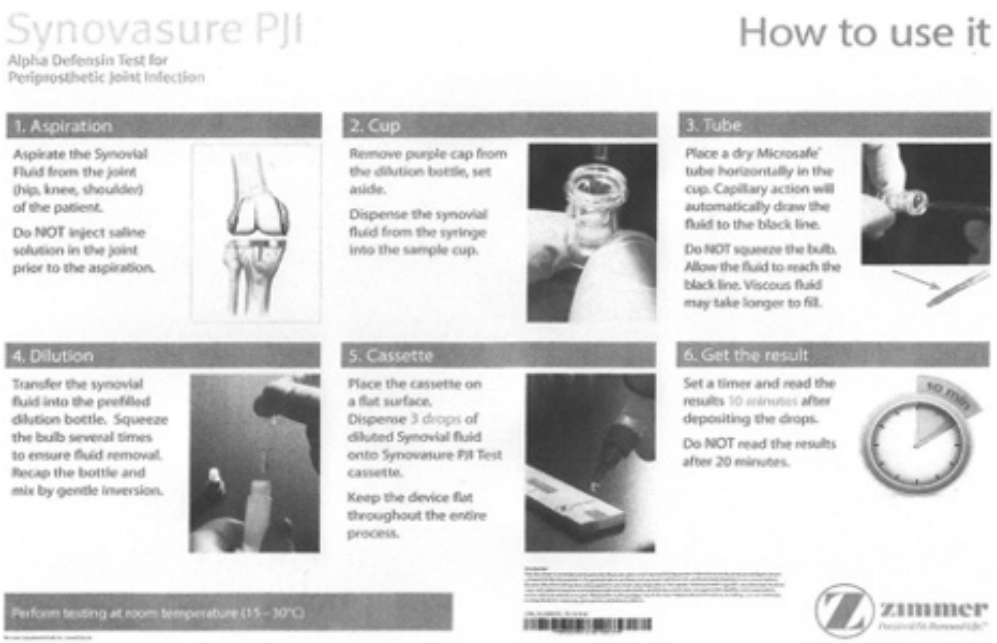

Figure 1

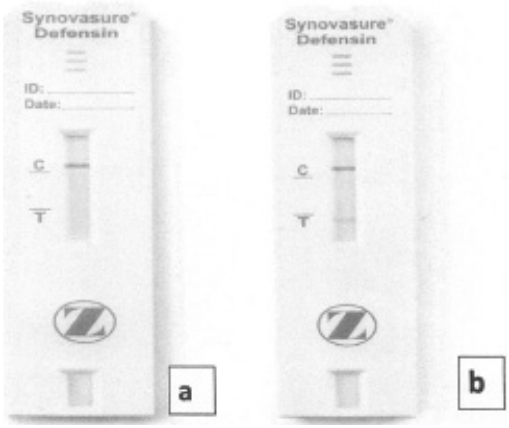

Figure 2

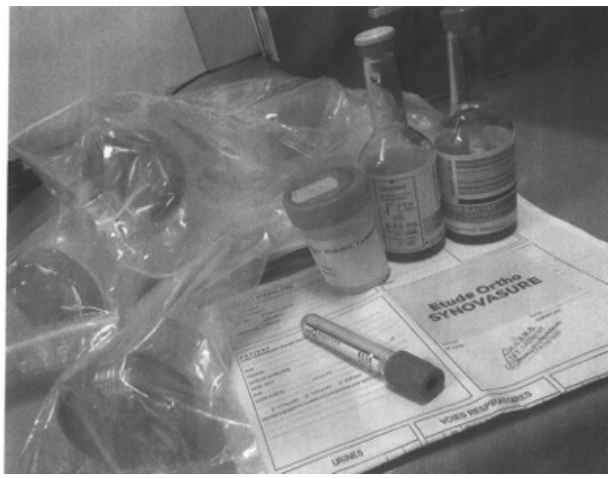

Figure 3

and white blood cells count) and imaging findings (collection, gallium or labeled leukocyte bone scan) was established for each patient ; then patients were classified into three preoperative categories : infected (in the event of fistula or sepsis associated with imaging of collection or a positive gallium or leucocyte-labeled bone scan), uninfected (in the absence of fistula, sepsis and collection, or other reasons for prosthetic resumption), questionable (when the clinical, biological and radiological signs did not allow distinguishing between infected and uninfected).

In accordance with the instructions, the synovasure test was achieved intra-operatively by the chief investigator on joint fluid after opening the capsula (Fig. 1).The test was negative in presence

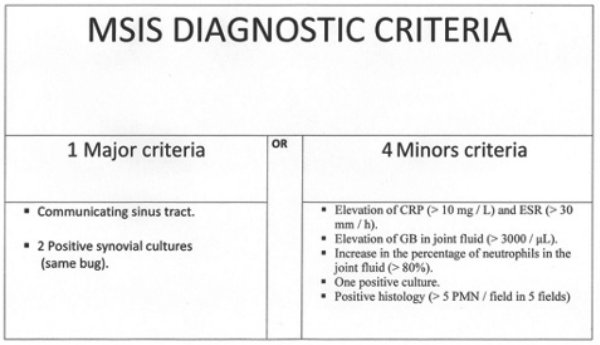

Figure 4

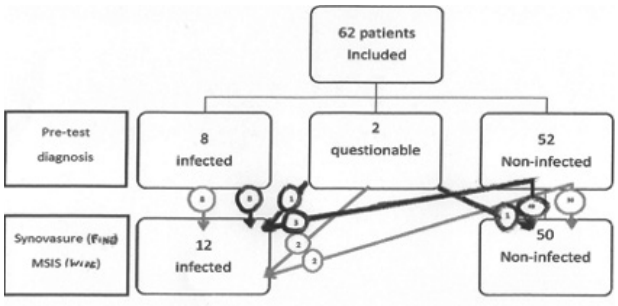

Figure 5

of single control line (Fig 2-a) and positive after appearance of a second line (Fig 2-b).Once this test has been conducted, the MSIS criteria were completed with the harvesting of one biopsy of the synovium (set in formalin for histology), five bacteriological specimens including joint fluid, synovium and bone (three placed in sterile containers and two in vials of blood culture) and at last one sample of an heparinized tube for cytology (Fig.3). These samples were quickly sent to the laboratory and immediately sown. The cultures of the surgical intraoperative biopsies were kept for 14 days.

The evaluation of the results was carried out by a college of investigators associating two orthopedists, two infectious diseases specialists and a statistician from the same hospital in a meeting two weeks after surgery. The MSIS consensus criteria (Fig. 4) were used to classify prostheses into septic and non-septic categories. The performance of the synovasure lateral flow test (sensitivity, specificity, positive predictive values and negative) was calculated matching the synovasure test results with the MSIS criteria (Fig.5). The study was conducted

Table 1. — General caracteristics of patients

Caracteristics	All patients (N=62)
Age. Mean-number	69 [43-90]
Gender male- number (%)	21 (33,9)
Smoker -number (%)	10 (16,1)
Alcoholism -number (%)	7 (11,3)
Body Mass Index	
<18.5	2 (3,2)
18.5-24.9	15 (24,2)
25-29.9	22 (35,5)
>30	19 (30,6)
Missing values	4 (6,5)
Malnutrition- number (%)	4 (6,5)
Diabetes - number (%)	15 (24,2)
Peripheral vasculopathy - number (%)	2 (3,2)
Heart Failure - number (%)	5 (8,1)
Hepatic Insufficiency - number (%)	0 (0)
Pulmonary insufficiency - number (%)	13 (21)
Chronic renal insufficiency - number (%)	0 (0)
Dementia - number (%)	3 (4,8)
Systemic Diseases - number (%)	8 (12,9)
Solid tumors - number (%)	7 (11,3)
Hematologic Diseases - number (%)	3 (4,8)
Immunodepression - number (%)	5 (8,1)
Dermatological diseases – number (%)	3 (4,8)

> : superior ; < : lower ; %: percentage.

Table 2. — Orthopedic caracteristics of patients

Characteristics	All Patients
Knee prosthesis -number (%)	13 (21)
Hip prosthesis - number (%)	49 (79)
Reason for placing a prosthesis	
Degenerative-number (%)	59 (95,2)
Inflammatory rheumatism-number (%)	2 (3,2)
Traumatic-number (%)	1 (1,6)
Reason for revision	
Mechanical - number (%)	54 (87)
Infectious - number (%)	8 (13)
Equipment	
Polyethylene-metal - number (%)	42 (67,7)
Titanium - number (%)	16 (25,8)
Metal-metal - number (%)	2 (3,2)
Ceramic-polyethylene (%)	2 (3,2)
Clinical Symptoms	
Pain - number (%)	60(96)
Fistula – number (%)	6(9,5)
Fever - number (%)	5(8)
Flow - number (%)	6 (9,5)
Wound dehiscence - number	1 (0,01)
Inflammation – number (%)	6 (9,5)
Hematoma – number (%)	5 (8)
Sepsis – number (%)	3(5)

independently, without additional cost or procedure for patients except for performing the synovasure test. A statistical analysis was carried out with the SPSS Statistics 22 (IBM) program descriptive statistics and frequency. The chi-square test was used for the categorical variables, with a p-value considered significant at <0.05.

RESULTS

Of a cohort of 65 consecutive patients enrolled, three were excluded (one for lack and two for hemorrhagic aspect of joint fluid) remaining 62 for the study. Tables 1 and 2 illustrate the general and orthopedic characteristics of patients. The majority of the resumption cases (49 patients) concerned hip prostheses and there was a majority of women (41 patients).

The synovasure test was performed only by the chief investigator. The test was easy to perform and the reading was clear for all patients. There were no doubtful cases. Neither ESR in18 cases (29%), nor CRP in 10 cases (16%) was available and the cytology of joint fluid was missing in 17 patients (27%). We obtained histology for all but one patient.

Preoperatively, eight infected, two questionable and fifty-two uninfected patients were identified (Fig.1). Of the eight assumed infected patients, six had fistula, three had sepsis with an imaging of collection in two cases. All but one patient had positive bacterial cultures. Of the two questionable patients, the first had hip pain and a history of the same hip prosthesis resumption for infection three years earlier, with a negative gallium bone scan. The second was a building worker who used machines that produced vibrations. There were soft tissue collections on its imaging but a negative gallium bone scan. Eight patients had a general inflammatory disease, one in the infected group and seven in the uninfected group. Four patients of the infected

group were immune-suppressed. Two patients had prostheses with a metal-metal friction couple ; one of them belonged to the infected category and the second to the uninfected category. Seven patients were or had been on antibiotic treatment less than two weeks prior to surgical revision ; all but one belonged to the infected group.

Postoperatively, infection was confirmed by synovasure test and the MSIS criteria in the entire infected group. There were a predominance of Staphylococcus aureus (62.5%), Enterobacteriaceae (25%) and Streptococcus dysgalactiae (12.5%) .

Regarding the questionable group, the first patient was confirmed infected by the synovasure test and the MSIS criteria ; the found micro-organism was Staphylococcus cohnii. The second patient presented positive synovasure test with negative MSIS criteria despite a mixed flora of Staphylococci (Capitis + Saccharolyticus) in two different intra-operative samples. He did not receive any antibiotic treatment.

Among the uninfected group, one patient was confirmed to be infected by the synovasure test and the MSIS criteria, constituting a fortuity discovery. This patient had a loosening of the acetabulum following a fall. All intra-operative cultures were positive for pseudomonas aeruginosa. Another patient who was admitted for resumption of uni-compartimentale knee prosthesis had a positive synovasure test, while the infection was not confirmed by the MSIS criteria. Two other patients from the same group had negative synovasure tests with two late-positive bacterial cultures (day 10) of Staphylococcus epidermidis after enrichment. One had been admitted for recurrent dislocations of a hip prosthesis, the other for acetabulum loosening with history of hip prosthetic resumption for dislocations. All other patients who were assumed to be uninfected were confirmed negative by synovasure and MSIS criteria postoperatively.

At the end, the synovasure test and the MSIS criteria were discordant in four patients (fig. 1) ; then there were two false positive and two false negative cases. Regarding the performance of the synovasure test, the statistical analyzes revealed a sensitivity of 83.33% (95% CI : 51.59% -97.91%), a specificity of 95.74% (95% CI : 85.46%). % -99.48%), as well as positive predictive value of 83.33% (95% CI : 55.72% -95.21%) and 95.74% negative value of (95% CI : 86.38%) -98.76%).

DISCUSSION

The most important finding of our study is that alpha-defensin showed a good accuracy (sensibility and specificity) taking into account MSIS criteria for Prosthetic joint infection. The advent of synovial markers in the diagnosis of PJI seems to be a progress *(10,19)*. The synovasure test seems to be the ideal candidate as a diagnostic marker since it is simple to perform ; the results are available immediately and are not distorted by antibiotics treatment *(12)*. We were able to easily perform the synovasure test in all patients included. Reading the results was clear for all patients and we did not have any doubtful cases. As part of the study, we decided to perform the test intra-operatively in order to obtain uniformity in the results. However, it can be done pre-operatively on joint puncture *(16)*.The results of the synovasure test show excellent specificity (95.74%) and excellent negative predictive value (95.74%). The sensitivity and positive predictive values are also good (83.33%) in both cases. These performances are lower than those of the alpha-defensin Elisa test, whose sensitivity and specificity are 97% as reported by other authors *(15)*. Using the synovasure test, we were able to make a fortuitous discovery of prosthetic infection. We found two false negatives and two false positive cases.

False negative cases concerned patients with late-positive cultures (day10) of *Staphylococcus epidermidis* after seeding. Dermeingian et al reported that the synovasure test is sensitive to a wide range of microorganisms *(13)*. However recent numerous studies have reported false negatives cases in the presence of fistula or when infection is caused by low virulent microorganisms such as *coagulase-negative staphylococci* and *mmuneacterial*. Theses microorganisms may cause a limited inflammatory response with consequently low level of alpha defensin *(18,20)*. In our study, the synovasure test was not influenced either by metallosis, inflammatory diseases, or antibiotics. Spangehl et al *(12)* reported that antibiotics do not

influence synovasure test results and Scholten et al *(21)* published a cohort of 37 patients including one with a metal-to-metal prosthesis without impacting the synovasure test results.

The first false positive case concerned a building worker patient using machines that produce vibrations. The relationship between synovasure test and vibration has not yet been described in the literature. The second case concerned a patient admitted for revision of an uni-compartmental knee prosthesis. Explanation could derive either in the contamination of the joint fluid by tissue debris as Kaspareck et al reported *(22)* or in a possible reaction with polyethylene *(23)*.

It should be noted that the test is expensive (about 200 Euros per unit) ; we therefore think that it is probably not useful in all patients, especially those who have a clear diagnosis of infection. On the other hand, it would be indicated in case of doubt, when the bundles of preoperative arguments do not make it possible to decide. A puncture should then be performed preoperatively with the achievement of the synovasure test and bacterial cultures. The same attitude should also be applied concerning patients under antibiotics treatment for who doubt remains.

Our study is limited by the size of the sample (62 patients), the mono-centric aspect, the fact that lot of analysis (ESR, CRP and cytology) could not be performed in all patients as well as that the study concerned a majority of hip prostheses (79%). The study has the advantage to be prospective and include immuno-compromised patients, those with systemic inflammatory diseases, under antibiotic treatment as well as all prosthetic materials and friction couples.

We had to exclude 3 patients because of hemorrhagic aspect or the lack of joint fluid

CONCLUSION

Our study shows synovasure test results that are consistent with those obtained in the literature *(20)*. Synovasure is a reliable test and provided a good sensitivity and specificity. The synovasure test is easy to perform and the result is available quickly. We therefore have an additional tool available for the diagnosis of prosthetic joint infections. The place of the test in the algorithm diagnosis remains to define. The test is expensive and we think that it should be reserved for the particular cases with a doubt diagnosis.

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