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DEVELOPMENT AND EVALUATION OF NEW BIOMARKERS FOR PROGNOSIS, EARLY DIAGNOSIS AND MONITORING OF TREATMENT EFFICACY IN PERIPROSTHETIC INFECTIONS

SUMMARY OF THE DOCTORAL THESIS

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CONTENT

LIST OF PUBLISHED SCIENTIFIC WORKS

- in correlation with the topic of the doctoral thesis -

1. **Sandu EC**, Cursaru A, Iordache S, Serban B, Costache MA, Cîrstoiu C. *Utility of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Periprosthetic Joint Infection Diagnosis*. Cureus. 2024 Oct 1;16(10):e70650. FI-1, capitolul 3, pag. 39-40

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https://www.cureus.com/articles/290879-utility-of-matrix-assisted-laser-desorption-ionization-time-of-flight-mass-spectrometry-in-periprosthetic-joint-infection-diagnosis#!/

2. **Sandu EC**, Serban B, Iordache S, Cursaru A, Costache MA, Dumitru A, Cîrstoiu C. *Immunohistochemistry Study of Antimicrobial Peptides as a Future Diagnostic and Prognostic Tool for Periprosthetic Joint Infections*. Cureus. 2024 Sep 18;16(9):e69629. FI-1, capitolul 7, pag. 126-129

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3. **Sandu EC**, Cursaru A, Serban B, Iordache S, Costache MA, Cîrstoiu C. *Is the Presence of Sinus Tract in Periprosthetic Joint Infection Still One of the Main Deciding Factors for Septic Revision?* Cureus. 2024 Dec 9;16(12):e75379. FI-1, capitolul 6, pag. 109-112

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INTRODUCTION

RESEARCH FIELD

Periprosthetic joint infection (PJI) is considered one of the most serious causes of implant degradation, on the one hand due to the difficulty of establishing a correct and timely diagnosis, on the other hand due to the laborious treatment and reserved prognosis. For many years, there were no universally accepted defining diagnostic criteria, each author or scientific society using its own "gold standards", which may include clinical, paraclinical, analytical, radiological, microbiological or histopathological aspects. Despite these different research directions, the histological and genomic study of periprosthetic tissue and synovial fluid have always been considered key components in the attempt to confirm or deny a possible periprosthetic infection, and their importance is demonstrated by the inclusion of PJI as diagnostic criteria in the protocol proposed by the Musculoskeletal Infection Society (MSIS) in 2011 and revised in 2018, by the Infectious Diseases Society of America (IDSA) and the European Bone and Joint Infection Society (EBJIS).

Despite the establishment of a rigorous diagnostic protocol, differential diagnosis remains an important challenge, as underdiagnosed periprosthetic joint infections can have an impressive impact on the health and mobility of patients, but also on the healthcare system considering the high financial costs involved in case management.

Early diagnosis of periprosthetic infection is absolutely critical; delaying its establishment can negatively affect the final outcome and the ability to eradicate the infection.

Currently, multiple diagnostic tests are available to determine the cause of joint implant degradation. Although the clinical diagnosis of PJI is not always easy, the lack of a "gold standard" test makes diagnosis truly difficult. History, clinical examination, and laboratory data cannot always differentiate between septic and aseptic degradation of the prosthesis. For this reason, it is common to encounter cases presumed to be aseptic degradation but in fact these are periprosthetic infections that either were not investigated rigorously prior to revision or were simply not detected using the usual means currently available in the diagnosis of PJI.

I. GENERAL PART

1. Definition and characteristics of periprosthetic joint infection

1.1. General aspects of PJI

Periprosthetic infection is one of the most serious complications of arthroplasty, on the one hand due to the difficulty of correct and timely diagnosis, on the other hand due to the laborious treatment [1]. It is a major cause of arthroplasty failure, being the main reason for revision in the first five years after the initial operation, but also a negative prognostic criterion in the evolution of revision [2]. As a percentage, periprosthetic infections have been reported as a complication in 0.5-2.2% of cases benefiting from primary arthroplasty, the incidence being much higher for patients who have already undergone a revision arthroplasty operation [3]. Due to the increasing number of annual arthroplasty operations worldwide, the total number of periprosthetic infections is expected to increase [4]. The true incidence of periprosthetic infections reported in arthroplasty registries is likely to be much higher, as a proportion of patients initially diagnosed with aseptic component detachment who undergo revision arthroplasty are subsequently diagnosed with periprosthetic infection on intraoperative tissue analysis [5].

Periprosthetic infections are associated with poorer functional outcomes, prolonged hospital stays, and multiple complex revision surgeries, which in turn may increase the risk of developing severe postoperative complications; revision arthroplasty for septic detachments has a fivefold higher mortality rate compared with revision for aseptic detachments, and the reinfection rate may be as high as 20% [6].

The management of periprosthetic infections requires a complex therapeutic strategy that includes a multidisciplinary approach, laborious surgical procedures, and long-term antibiotic therapy. An accurate diagnosis with identification of the pathogenic microorganism and appropriate antibiotic therapy is an extremely important step in terms of prognosis and evolution of the infection. An undiagnosed or underdiagnosed periprosthetic infection can lead to persistence of the infection and multiple revision surgeries that considerably decrease the quality of life and increase the disability of the patients.

The treatment of periprosthetic infections requires the existence of a multidisciplinary team composed of an orthopedic surgeon, an anesthesiologist, an infectious disease physician, an anatomopathologist and a biologist. An interdisciplinary approach is crucial in obtaining optimal results [7]. In this chapter we try to provide the

reader with an overview of current concepts in the management of periprosthetic infections and the specific pathophysiology.

1.2.Infections classification:

The appropriate initial treatment of periprosthetic infections depends on the extent of the infection, its chronicity, the stability of the implant, and the patient's biological status. Although the treatment of deep infections in total hip or knee arthroplasty is most often surgical, the decision to retain or remove the prosthetic components may be influenced in part by the chronic status of the infection. Tsukayama classified periprosthetic infections into four broad categories [8]:

a) Acute postoperative infection – defined as an infection occurring within the first month postoperatively; the diagnosis in this case is established by identifying the pathogen and quantifying the cellularity in the intra-articular aspirate. The peculiarity of this stage of infection is represented by the conservative therapeutic conduct, thus reintervention will be attempted with aggressive debridement of non-viable tissues, abundant lavage, evacuation of the postoperative hematoma if present, with keeping the orthopedic implant in place, possibly changing removable components such as the femoral head, polyethylene insert, bacterial biofilm may be present on their surface, and parenteral antibiotic therapy for 2-6 weeks, a procedure known in the literature under the acronym DAIR (Debridement, Antibiotics, Implant Retention).

b) Late chronic infection – with insidious onset of symptoms more than one month postoperatively; revision of all prosthesis components is almost always necessary, this procedure can be performed in a single or two-stage operation.

c) Acute hematogenous infection – occurring more than one month postoperatively, but with acute onset of symptoms in a patient with a previously perfectly functional prosthesis and a distant location of another infection; the cause of this type of infection is most often bacteremia from another infectious source with remote localization. From the point of view of prophylaxis, it is necessary to screen for infections in the urinary tract, airways, gallbladder, teeth or skin lesions. The therapeutic consensus proposes to approach this type of infection as in the case of an acute postoperative infection if symptoms manifest for a period shorter than 3 weeks, otherwise according to the protocol for chronic infections.

d) Positive intraoperative culture – a positive culture obtained during the revision procedure in a patient without signs and symptoms of a preoperative septic process; the diagnosis is

established postoperatively by at least two positive cultures from tissues harvested intraoperatively. A single positive culture does not establish the absolute indication for specific treatment of a PJI [9]. To exclude contamination of the samples, it is recommended that at least five tissue samples be harvested and sent for microbiological and histopathological examination. Regarding treatment, targeted antibiotic therapy (intravenous or combined intravenous/oral) according to the antibiogram can extend from 3 days to 6 weeks.

1.3.Management of periprosthetic joint infections

1.3.1. Surgical treatment:

Debridement, antibiotics, implant retention (DAIR)

Although early studies investigating the application of this type of procedure in cases of septic revision have shown high chances of failure [10], the chances of success may be improved when the local and general condition of the patient meets certain conditions such as: stable implant, a pathogenic bacteria with a good response to antibiotics, lack of an active fistula or compromised soft tissues, and duration of symptoms of less than 3 weeks. This type of surgery is suitable for the treatment of acute, uncomplicated periprosthetic infections.

One step revision

As its name suggests, this type of revision involves the explantation of the old prosthesis and the implantation of a new prosthesis at the same time surgically. This type of intervention is suitable for patients who have good bone stock and uncompromised soft tissues, without active joint fistula and do not have infections with multidrug-resistant bacteria [11]. Although this type of procedure can be used successfully in the treatment of both septic decimations (when certain conditions are met) and aseptic decimations, in our clinic, the protocol unanimously accepted by the team reserves this surgical treatment for patients with aseptic degradation of the prosthesis, since most patients present to the hospital late, when the evolution of the infection exceeds the limitations of this type of procedure.

Two step revision

This type of procedure involves explantation of the old prosthesis and implantation of a waiting joint spacer in a first surgical step, then implantation of the final prosthesis in a second surgical step at a certain time interval. The period between the two operations can vary from 4 to 8 weeks depending on the virulence of the incriminated bacteria, resistant

strains requiring prolonged antibiotic therapy, and depending on the condition of the soft tissues [12]. While waiting for the infection to resolve, an antibiotic-impregnated polymethylmethacrylate joint spacer is implanted with the role of increasing the local concentration of antibiotic and preserving the length and mobility of the affected limb as can be seen in figure 1.1. This type of procedure is considered the gold standard of PJI treatment, especially in patients with extensive infections [13], the reinfection rate after this type of treatment being slightly lower than in the case of revision in a single surgical step [14].

2. Biofilm – central element of infectious pathology

2.1.Role and formation of the bacterial biofilm

Once the bacteria have been inoculated at the surgical site, they can exist in suspension, biofilm or invasive (intracellular) form [15]. The suspension form is represented by a solitary bacterium and is the form that is most easily identified and eradicated by the immune system and host antibodies. Biofilm is a three-dimensional colony of bacteria that is often associated with the materials that make up joint prostheses because their abiotic surface provides a perfect interface for biofilm attachment and maturation, thus playing a crucial role in periprosthetic infections [16]. Biofilm does not only form at the prosthesis level, in the context of an already established infection, it can be present on the acrylic cement, the bone itself or on the fibrous tissue. Intracellular bacteria, recently studied in the context of periprosthetic infections, are able to enter, survive and proliferate in host cells, especially in non-professional phagocytes such as endothelial cells and osteoblasts, thus avoiding the triggering of an immune response [17]. The similarity between the phenotypes of biofilm and intracellular bacteria such as small colony variants of S. aureus would indicate that intracellular pathogens develop from biofilm [15-17]. Thus, in order to eradicate a periprosthetic infection, all bacteria in the synovial fluid, on the surface of the implants or periprosthetic tissues must be removed; bacterial repopulation is likely to occur if lavage, debridement or explantation are insufficient [18]. Once established, biofilm is extremely difficult to eradicate, or even more problematically, it prevents the establishment of a correct diagnosis of periprosthetic infections by identifying pathogens. The minimum antibiotic concentration for biofilm eradication is generally 100–1000 times higher than the minimum for inhibition of planktonic bacteria [19]. Antibiotic resistance is a consequence of the limited penetration of antibiotics into the biofilm, the degradation of antibiotics in the “rough” areas at the periphery of the biofilm, the function of the polymeric extracellular matrix to act as a buffer by binding and degrading antibiotics, the innate resistance of

dormant cells, and the limited diffusion that creates a concentration gradient of antibiotics, thus exposing different bacterial subpopulations of the biofilm to non-lethal concentrations and increasing their tolerance to antibiotics, which is known from previous studies [20]. Biofilms also use conventional mechanisms of antibiotic resistance such as beta-lactamases, upregulation of efflux pumps to eliminate intracellular antibiotics, and the ability to transfer genes horizontally.

3. Current and emerging diagnostic methods

3.1. Special features and current diagnostic protocols of PJI

Early diagnosis of periprosthetic infection is absolutely critical. Delay in its establishment can negatively affect the final outcome and the ability to eradicate the infection. A detailed history of symptoms can provide information about the possibility of a septic process. In the case of acute postoperative onset, the attending physician should raise the suspicion of a periprosthetic infection if the patient presents with delayed wound healing, pain, limping, and the patient's inability to progress in regaining functionality [21]. On the other hand, hematogenous infections are most often characterized by a fulminant evolution, with the sudden onset of pain, swelling, and possibly cellulitis [22]. The presence of risk factors such as dental, urological, or other invasive surgical procedures should raise the suspicion of a periprosthetic infection, as well as the presence of less common signs in this pathology such as fever, chills, or altered general condition [23].

Clinically, the most prominent symptoms of an infectious pathology, fever, chills, active fistula with purulent drainage, are inconsistently encountered in periprosthetic infections [24]. More common symptoms at presentation are pain, swelling, local hyperthermia or synovitis, symptoms that are also present in aseptic degradation of implants, making the establishment of a definite diagnosis difficult [25]. One thing is certain, patients who present with evolving pain symptoms without clear reasons in the immediate postoperative period or with sudden onset of pain should be evaluated from an infectious point of view. As a general consensus, these patients should be considered infected until proven otherwise. Most patients with acute or chronic periprosthetic infections will develop pain at some point; cases in which they present without pain, only with fatigue or altered general condition are rare. Cellulitis is a variable clinical sign, but it may raise suspicion of a more serious diagnosis, especially in the case of hematogenous infections. It is considered that pain-free knee mobilization in such situations could signal the superficial nature of the

infection, but aspiration of the joint through an area without skin involvement is recommended [26].

II. SPECIAL PART

4. Research hypothesis and general objectives

Some of the most common reasons for joint prosthesis degradation are represented by aseptic loosening, instability (luxations), periprosthetic fractures or septic decimation (periprosthetic infection). Although the incidence of periprosthetic infections is extremely low, accounting for only 1% of postoperative complications, it is a much more serious and complex complication. Periprosthetic infections are associated with multiple surgical interventions for sanitation, a complex diagnostic algorithm, a long-term antibiotic treatment, prolonged hospitalization time and the need for a multidisciplinary therapeutic team, all of which translate into an extraordinarily high consumption of financial resources by the hospital, as well as increased morbidity and decreased quality of life for the patient.

Starting from the premise that the success of the treatment of such a pathology is strictly conditioned by the early establishment of a correct and rapid diagnosis, the current work aims to bring innovations in the diagnostic process of periprosthetic infections. The main objectives of this research direction have developed from the need to obtain new, more precise, faster, cheaper diagnostic criteria in order to simplify and improve the current diagnostic algorithm.

By monitoring demographic data, individual characteristics and paraclinical data, we wanted to establish the risk factors that lead to an increased chance of developing an infectious complication, the concurrent existence of several risk factors leading to the staging of a high-risk group.

5. General methodology of the research

In the prospective clinical study conducted over four years between 2020 and 2024 in the Orthopedics and Traumatology clinical department of the Bucharest University Emergency Hospital, 52 cases of revision hip or knee arthroplasty were included from 165 recruited patients, with the aim of bringing new information regarding the diagnosis and prognosis of periprosthetic infections. Consecutive patients who met our inclusion and exclusion criteria were selected.

All included patients agreed to participate in this study and signed the informed consent. The study was approved by the hospital ethics board.

Using the diagnostic algorithm proposed by MSIS in 2018, the included patients were divided into two groups: the periprosthetic infections group (n=23) and the aseptic dehiscence group (n=29). For early diagnosis, clinical data such as joint fistula, periarticular purulence, radiographs, as well as paraclinical data represented by serum C-reactive protein (CRP), serum leukocyte count, erythrocyte sedimentation rate (ESR) and synovial fluid cellularity analysis were used. Identification of pathogenic bacteria was obtained by performing bacteriological cultures from synovial fluid, periprosthetic tissues harvested during revision or from the prosthesis sonication fluid. Furthermore, the harvested periprosthetic tissues were also subjected to histopathological analysis in order to diagnose infectious pathology. Demographic data of the patients such as age, sex, environment of origin, presence of risk factors (smoking, diabetes mellitus, obesity, etc.) were also analyzed.

6. Analytical study of diagnostic methods, prognosis and factors that determined the evolution of patients with prosthetic revisions

The main objective of this first study was achieved by describing the main characteristics of the two subgroups of patients who underwent revision total hip or knee arthroplasty during the given period, the major differences between them being quantified following statistical analysis. Considering the immense impact that periprosthetic infections have on the patient's quality of life as well as on the medical staff and the hospital, Eka A. et al. identified the main risk factors for this pathology in a study published in 2015. Among these factors were listed such as obesity, malnutrition, hyperglycemia (diabetes mellitus), rheumatoid arthritis, preoperative anemia, associated cardiac pathology and smoking [27]. The results obtained from our study identified similar risk factors that were more frequently present in septic patients. It was observed that the concomitant association of diabetes mellitus, obesity and smoking was present only in patients with periprosthetic infections, moreover, this association had a very high weight in the subgroup, involving 41.67% of patients. Statistical analysis demonstrated a high intensity correlation (p=0.002) between the concomitant existence of these three risk factors and periprosthetic infections, indicating a class of patients with a high risk of developing such a complication.

The analysis of the most important paraclinical biomarkers revealed that C-reactive protein together with the erythrocyte sedimentation rate remain the most used markers in the

screening of paraclinical infections. This aspect is due on the one hand to their extremely low cost, accessibility and ease of use, on the other hand to the relatively high accuracy in identifying the pathology taking into account the other advantages mentioned above. Values above 10 mg/L for CRP and above 30mm/H for ESR were considered as the ideal cut-off for diagnosis.

Identification of pathogens was obtained after inoculation of different types of samples on various culture media. The highest accuracy was achieved by cultures from the sonication fluid and the lowest in the case of intraoperative cultures. These results can be explained by several factors. It can be observed from the statistical analysis that a large part of the patients with sterile intraoperative cultures consumed antibiotics before collection, significantly decreasing the sensitivity of this type of testing from 73.33% in the case of patients who were not administered pre-collection antibiotics to 52.17% (average sensitivity within the septic subgroup).

7. Immunohistochemical study of antimicrobial peptides as a future method for the diagnosis and prognosis of periprosthetic infections

7.1. Introduction

Understanding the mechanism and importance of antimicrobial peptides in bacterial infections, more and more studies have been published on their role in periprosthetic infections. The most relevant AMPs discovered were α-defensins 1-3, human β defensin 2 and 3 (HBD-2, HBD-3), and cathelicidin LL-37. Some of the results will be discussed in more detail in the discussion section of this study. Detection of α-defensins in synovial fluid has been successfully implemented and approved as a diagnostic criterion for periprosthetic infections in the form of a rapid test kit (Synovasure) or by ELISA technique [28].

The histopathological staging of the periprosthetic membrane proposed by Morawietz et al. is still considered one of the most important criteria in the diagnosis of PJI [29]. Although the identification of inflammatory cells in the examined tissue shows good results in diagnosing pathology, there is room for improving the accuracy of this type of testing. In the present study, we attempted to optimize and facilitate the histopathological diagnosis of periprosthetic infections using immunohistochemical analysis of periprosthetic tissues for the detection of antimicrobial peptides HBD3 and LL37, hoping to improve the outcome and prognosis of patients undergoing revision arthroplasty.

7.2.Methods and materials

7.2.1. General data of the batch

This study used the same inclusion and exclusion criteria as the previously presented study, using the same population. Patients who underwent revision joint replacement surgery were again divided into two subgroups, patients with aseptic dehiscence (n=29) and those with periprosthetic infections (n=23) using the diagnostic criteria proposed by MSIS and revised in 2018.

7.2.2. Semi-quantitative analysis of the immunohistochemical reaction

The slides obtained were analyzed by optical microscopy using 10x, 20x and 40x objectives. The most representative areas from 3-5 slides per patient were selected and evaluated using the immunoreactivity score (IRS) proposed by Remmele [30]. This score is the result of multiplying the score of positive cells by the intensity of staining.

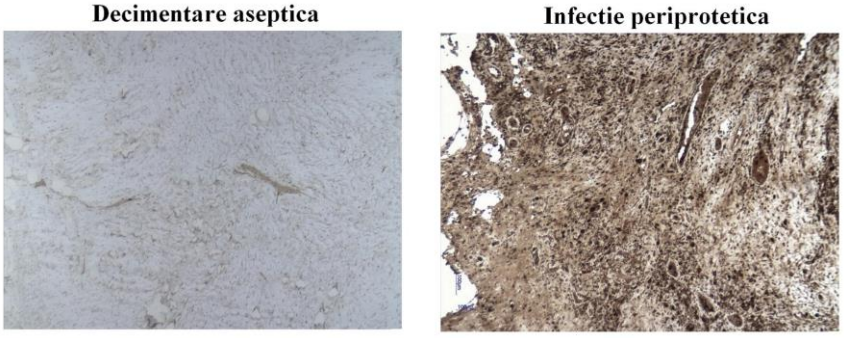
With a score that can take values between 0 and 12, we considered values 0 and 1 as negative results and values between 2 and 12 as positive to interpret the immunohistochemical reaction in both the subgroup of patients with aseptic decimations and the subgroup of periprosthetic infections.

7.3.Results

7.3.1. Immunohistochemical testing results

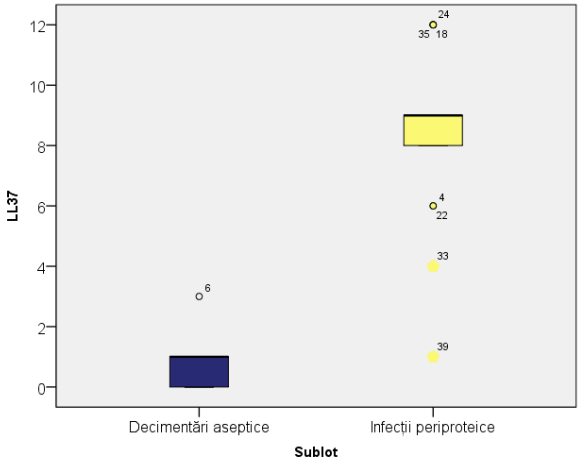
The slides resulting from the automated immunohistochemical process described above were analyzed and described. Several representative images were selected (Fig. 7.2 and 7.3) where the obvious visual difference in the DAB chromogen staining (brown staining) between the two subgroups was observed, the subgroup of periprosthetic infections presenting intensely positive results of the immunohistochemical reaction with both the HBD3 antibody and the LL37 antibody, as well as the absence of reaction in the subgroup of aseptic decimations.

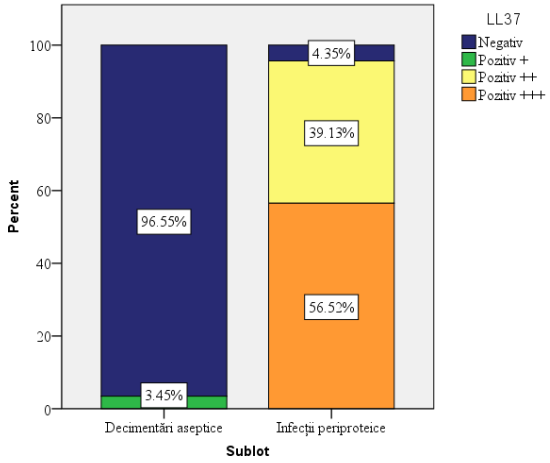
Coloratie imunohistochimica folosind anticorpul LL37



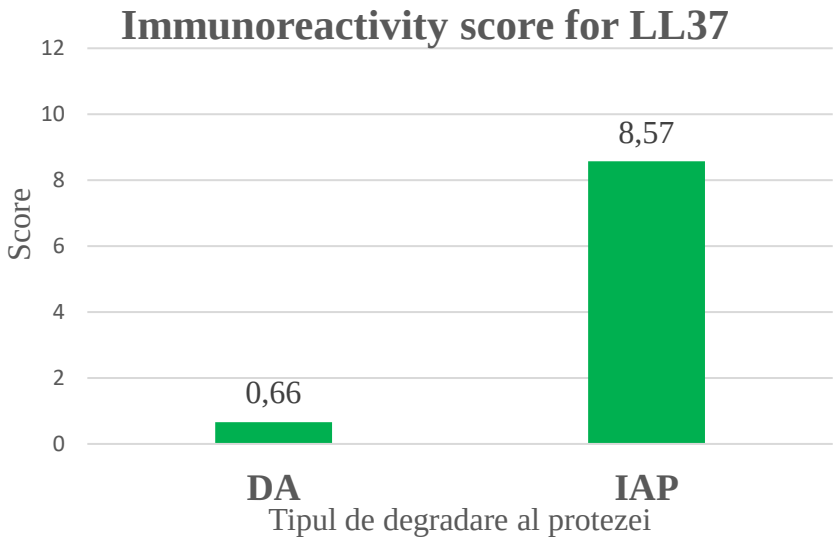
7.3.2. The results of the semiquantitative analysis of the immunohistochemical examination (immunoreactivity score – IRS)

Fig 7.4. si 7.5.: *Cases ditribution depending of LL37 results (0-1 negative, 2-3 positive + (weak), 4-8 positive ++ (moderate), 9-12 positive +++ (strong))*





Following the semiquantitative analysis of the immunohistochemical examination, significant differences were noted between the IRS scores (staining with LL37 and HBD3 antibodies) of the two subgroups.



Thus, the mean IRS score was approximately 13 times higher in periprosthetic infections compared to aseptic patients when the antibody for LL37 was used and almost 6 times higher when the antibody for HBD3 was used ($p<0.001$).

The accuracy of diagnosing periprosthetic infections using immunohistochemical testing with LL37 and HBD3 antibodies of periprosthetic tissues was evaluated using the AUC value obtained from ROC curve analysis. This type of testing shows excellent diagnostic potential of PJI ($p<0.001$), with an AUC value of 0.987 for LL37 detection (fig 7.10) and 0.925 for HBD3 detection (fig 7.11).

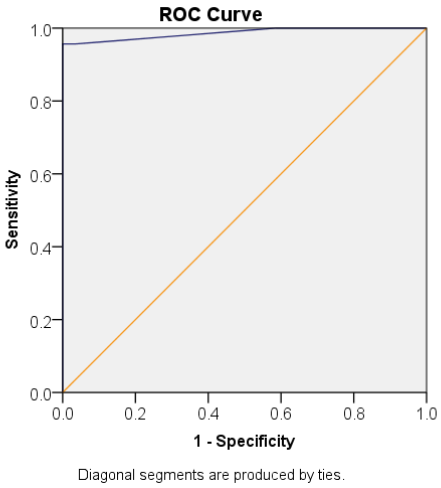


Fig 7.10. *ROC curve for LL37 and PJI*

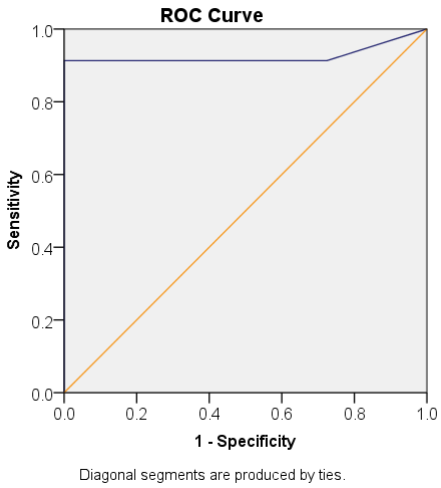
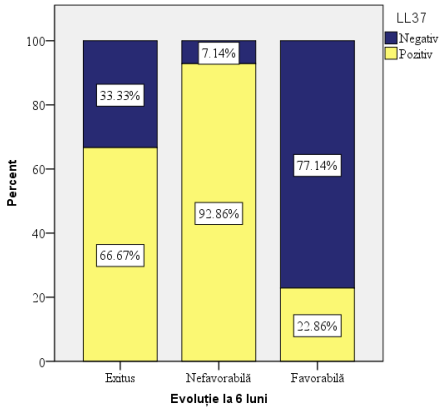


Fig 7.11. *ROC curve for HBD3 and PJI*

7.3.3. Patient evolution according to immunohistochemical testing



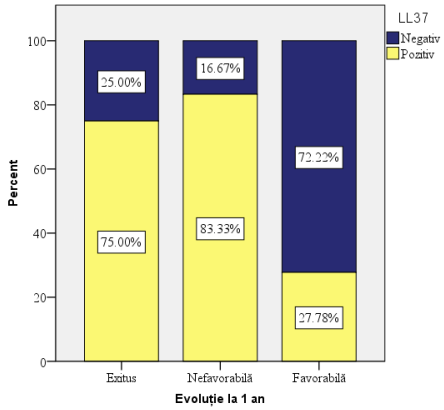
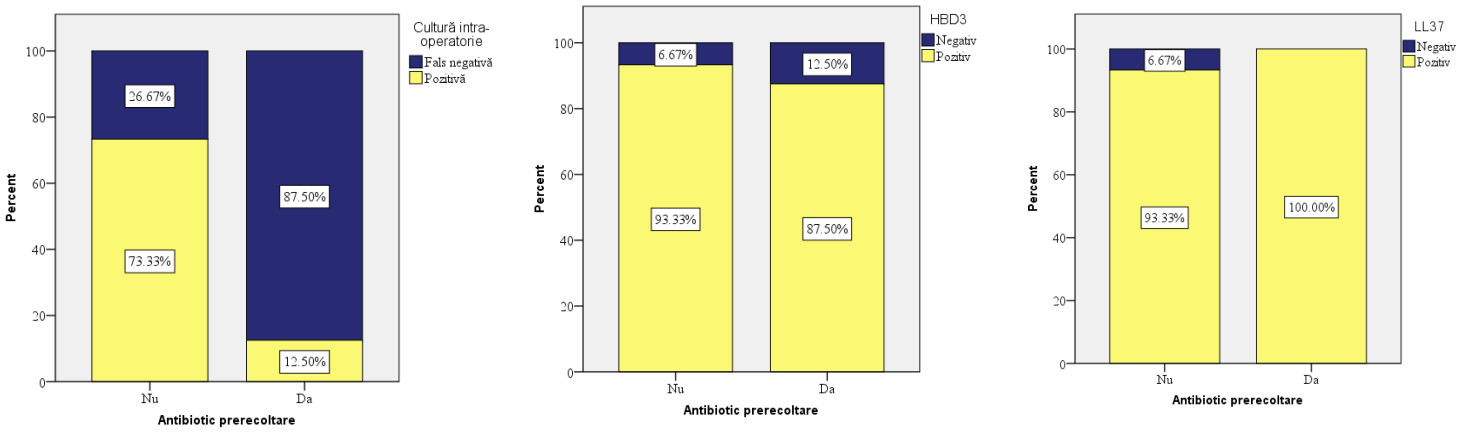


Fig 7.12 si 7.13: *distribution of cases according to the evolution at 6 months and 1 year related to the presence of the LL37 marker in the periprosthetic tissues*

7.3.4. Influence of pre-harvest antibiotic treatment on cultures and immunohistochemical testing

Fig 7.16.,7.17. si 7.18.: *Comparative graphs of intraoperative culture sensitivity with immunohistochemical biomarker sensitivity depending on preoperative antibiotic administration in the periprosthetic infection subset*



7.4.Discussions and partial conclusions

The main challenge in the management of periprosthetic infections remains the difficult diagnosis, especially in the case of chronic "low-grade" infections with weakly virulent bacteria [31]. Rapid and accurate identification of the pathogenic bacteria is the key to successful treatment. Standard bacteriological culture is the most widely used method for identifying the bacteria. Unfortunately, the sensitivity of this type of testing remains very low in the case of periprosthetic infections [32], as was also observed in our group. Preoperative cultures performed from synovial fluid or fistula secretions were able to identify only 15 of the 23 cases of periprosthetic infections. Moreover, cultures inoculated with periprosthetic tissues harvested intraoperatively identified only 12 positive cases. This decrease in sensitivity can be explained by the initiation of antibiotic treatment before sampling in some cases, which led to an increase in false-negative results [33]. Identification of pathogenic microorganisms was performed with much greater success when bacteriological cultures were inoculated with the fluid resulting from sonication of explanted prosthesis components.

The use of antimicrobial peptide detection as a diagnostic test for periprosthetic infections was subsequently investigated more extensively. A study conducted by Banke et al. in 2020 demonstrated the presence of elevated levels of HBD2, HBD3, and LL37 in the synovial membrane of patients with septic degeneration [34]. The study population included

only patients previously diagnosed with coagulase-negative Staphylococcus infections, considering that this bacterium is most commonly associated with chronic low-virulent infections. Another study conducted by Paulsen et al. using PCR (polymerase chain reaction) testing demonstrated the presence of defensins and cathelicidins in pyogenic arthritis and their absence in healthy synovial membrane [35].

Using the IRS score for immunohistochemical stainings performed with HBD3 and LL37 antibodies, we were able to demonstrate a significant increase in these antimicrobial peptides in periprosthetic tissues in the periprosthetic infection subgroup. It was also observed that pre-reclosure antibiotic therapy did not influence the results obtained, the sensitivity of the test remaining almost constant in the entire group of patients. Results can be obtained on the first or second postoperative day, and a positive immunoreaction may lead to a more extensive investigation of the patient's septic status.

The results of the ROC curve analysis demonstrated excellent accuracy of immunohistochemical testing in identifying infectious pathology, with positive HBD3 and LL37 staining being almost exclusively associated with periprosthetic infections [36]. Taking this information into account together with the fact that PJIs are more frequently associated with increased morbidity and mortality, as well as a worse prognosis for the patient compared to aseptic degradations [37], we can understand why a large proportion of patients who presented positive results of the two immunohistochemical markers had an unfavorable evolution one year after the revision surgery procedure.

Conclusions and personal contributions

Doctoral thesis conclusions

One of the objectives achieved was to demonstrate the importance of a precise and rapid preoperative or intraoperative diagnosis for periprosthetic infections and its influence on the patient's evolution and the success of revision arthroplasty. This correlation is also supported by the fact that the treatment of aseptic degradations is completely different from the treatment of septic degradations, underdiagnosis of a periprosthetic infection being almost always associated with an unfavorable evolution of the patient.

The differential diagnosis can be facilitated by the presence or absence of risk factors for infections in patients with joint prostheses. Thus, a patient who presents risk factors such

as smoking, diabetes mellitus or obesity should be investigated more thoroughly to exclude a possible periprosthetic infection even if the symptomatology or other usual paraclinical data are not sufficient for such a diagnosis.

The statistical analysis demonstrated a strong association between the period of time elapsed from the implantation of the primary joint prosthesis to the time of revision and the type of degradation. Thus, patients with periprosthetic infections required surgical revision of the implant after a much shorter period than those with aseptic degradation.

Another factor that influenced the evolution of the patients was the duration of hospitalization. A shorter hospitalization was correlated with the tendency of both septic patients and patients with aseptic degradation to evolve favorably at 6 and 12 months postoperatively.

The main objective of the second study conducted was to develop and evaluate new biomarkers for the early diagnosis and prognosis of periprosthetic infections. It started from the success of histopathological examination, which is one of the gold standards in identifying infectious pathology, and wanted to improve it through an immunohistochemical analysis of periprosthetic tissues. The presence of antimicrobial peptides HBD3 and LL37 in periprosthetic tissues was closely correlated with periprosthetic infections. Semiquantitative immunohistochemical study of tissues, an easy-to-perform, semi-automated, accessible and cost-effective process, has proven to be the optimal method for identifying antimicrobial peptides and, implicitly, periprosthetic infections. Analysis of the evolution of patients in the group at 6 and 12 months postoperatively according to the results of these markers showed a tendency for patients to have an unfavorable evolution when the results of the immunohistochemical reaction are positive, emphasizing that these markers have not only diagnostic importance but also prognostic value.

With the advancement of molecular and proteomic technologies, we hope that the diagnosis of periprosthetic infections using antimicrobial peptides will become faster and more accurate. The ultimate goal of the research is the implementation of these biomarkers in the diagnostic algorithm of septic degradations, as obtaining a rapid diagnosis can influence the therapeutic decision in orthopedic implant pathology.

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