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Research Article

MALDI TOF IMS - A Tool for Diagnosis of Low-Grade Infection?

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Abstract

Introduction: With the rising success of alloplastic material in implantation surgery, implant-associated complications have increased. One of the most severe complications is infection of the implant. Especially diagnosis of low-grade infections is challenging due to absence of clear clinical signs of inflammation. Often, explantation of the implant and histopathological review of the tissue are required. However, many implants are not sectionable or severely hinder the cutting process of the tissue, resulting in poor sample quality with subsequent difficulties to detect polymorphonuclear granulocytes (PMNs). MALDI TOF IMS may provide additional information to diagnose infection of implants combining histopathological review with mass spectrometry.

Material and Methods: In this retrospective study, we analyzed tissue from 15 formalin-fixed and paraffin-embedded (FFPE) surgical hernia explants. The tissues were conventionally stained with hematoxylin and eosin (H&E) and by immunohistochemistry using CD15 to detect PMNs as indicator cells for infections. Areas with high number of PMNs were annotated. Subsequently, MALDI TOF IMS was performed, and specific (m/z) ion peptides were searched as biomarkers for infection.

Results and Conclusion: We were able to detect PMN peptides in mesh embedded tissue. Histologically annotated areas with high numbers of neutrophils showed high intensity for the neutrophil-associated peptides. As a proof of principle MALDI TOF IMS can detect PMN peptides across different tissue types and provide additional information for diagnosing low-grade infection in implants. Due to the small sample size further studies are necessary to substantiate the potential of MALDI TOF IMS in diagnosis of low-grade infection.

Keywords: Alloplastic Material; Biomarkers; Low-Grade Infection; Mesh; MALDI TOF IMS

Introduction

The use of alloplastic material in implantation surgery has become a standard procedure. Whether it involves mesh implantation in hernia surgery, heart valve replacement in cardiology or total joint implantation in orthopedic surgery, these procedures belong to the most frequently performed operations. Through the increasing use of alloplastic materials implant-associated complications have naturally risen. One of the most severe complications is infection of the implant. Diagnosing implant infection includes clinical examination, laboratory parameters, microbiological samples, radiological imaging and is followed by antibiotic and surgical treatment often leading to explantation of the implant [1]. While acute infections can be diagnosed relatively easy, diagnosing chronic low-grade infection is challenging [2]. In periprosthetic joint implantation surgery low-grade infections are known to cause severe complications such as loosening of the prosthesis, pain, pseudoarthrosis and immobilization leading to secondary complications [3]. This extends the hospital stay and increases financial costs for the health system and the patient's mortality [4,5]. In unclear cases and to secure diagnosis a biopsy of periprosthetic tissue is necessary, following histopathological

examination[1]. Histopathological examination thereby relies on the number of neutrophils per high-power-field [6]. Discriminating low-grade from high-grade infection is possible using the CD-15-Focus-Score [7]. However, applying this method to other implants and tissue presents notable challenges. While periprosthetic tissue is cuttable, alloplastic material embedded in the tissue impedes the sample preparation resulting in poor sample quality and suboptimal examination conditions. Especially in unclear cases poor sample quality is devastating since treatment of septic and aseptic implant failure differs.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) is a technique originally used in forensic science for identification of unknown substances and drugs [8,9]. In the following years MALDI TOF was used in microbiology to identify subspecies of bacteria as well as their antibiotic resistances [10,11]. In the progress MALDI TOF imaging mass spectrometry (IMS) emerged as a tool to unite histopathological examination with mass spectrometry, combining the molecular assessment with morphology of the tissue and showing their spatial distribution within the tissue. MALDI TOF IMS has shown promising results in tumor typing and classification, offering the possibility to use specific m/z values as biomarkers to discriminate tissue types [12]. Gravius et al successfully detected specific neutrophil peptides using MALDI TOF IMS in periprosthetic tissue [13].In the present study we used infected explanted meshes as an example for implants to investigate the potential of MALDI TOF IMS in diagnosis of low-grade implant infection.

Materials and Methods

Sample Preparation

15 formalin-fixed paraffin-embedded (FFPE) specimens with explanted meshes were cut as serial sections. For each case serial sections were cut for MALDI and mounted onto an ITO-slide, following two sections for CD-15 immunohistochemistry and histological examination, which were stained with hematoxylin and eosin. All 15 cases were histopathologically diagnosed as infected tissues and neutrophil-rich areas were annotated by a pathologist on the H&E slide (B.K.). Diagnosis of infection was performed by counting the number of neutrophils per high power field using the synovitis score established by Krenn et al. [14] Infected tissues were histologically distinguished in low-grade and high-grade infected tissues. For sample preparation for MALDI IMS we performed deparaffinization, antigen retrieval and in situ digestion with trypsin as shown in Figure 1.

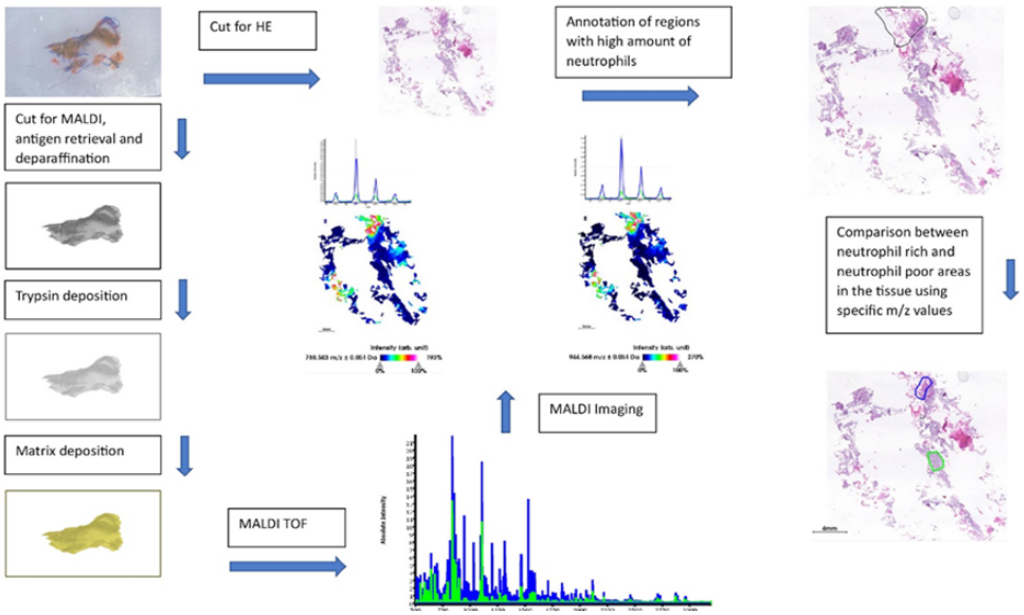

Figure 1: Workflow of MALDI TOF IMS: the specimen is cut as serial sections. The hematoxylin and eosin (H&E) samples and CD-15-immunohistochemistry samples are used for histopathological examination (HE) and annotation of areas with a high number of neutrophils. Parallel the serial section is used for MALDI TOF IMS. After sample preparation including deparaffinization, antigen retrieval, trypsin and Matrix deposition MALDI TOF IMS can be performed. Predictive m/z values (here shown for 788.405 and 944.533) working as biomarkers were applied to detect neutrophil activity in tissue. Afterwards we compared areas with high intensity for applied biomarkers to histologically annotated areas

MALDI Analysis

Following the instructor manual, CHCA matrix solution (7mg/ml in 50/50 acetonitrile/0,5% TFA) was applied onto the digested sections by the same ImagePrep devise. Afterwards MALDI imaging analysis was performed using rapifleX® (Bruker Daltonik, Bremen, Germany). Mass spectra within a range of mass to charge ratio (m/z values) from 500-4000 were acquired. After data processing, according to the manual including baseline subtraction, realignment, and normalization, we searched for specific m/z values (Table 1) as biomarkers to detect neutrophils with MALDI TOF and compared regions with high intensity for the applied m/z values to the histologically annotated areas.

Table 1

Applied Masses (m/z)	Predictive protein
660.273	Annexin-1
788.405	Calgranulin C (S100A12)
944.533	Histone H2A
1349.621	Calgizzarin (S100A11)

Results

Using meshes as an example for explanted alloplastic material we were able to demonstrate factors that are impeding the diagnosis of infection by standard histological and immunohistological procedures. Even though tissues with the embedded meshes are still cuttable, the sample preparation is challenging, sometimes resulting in poor sample qualtiy (Figures 2(a,b) and 3(a-c)). Due to squeezing artifacts and poor cutting quality, PMNs cannot be adequately identified using light microscopy. The visualization of PMNs is also difficult using CD15 immunohistochemical staining due to the strong background staining. A reliable determination of the density of PMNs for the diagnosis of a low-grade infection is therefore not possible. As a proof of principle, we demonstrate in the present study that detection of neutrophil peptides in tissue with explanted meshes is possible with MALDI TOF IMS independent from the section or tissue quality. Specific m/z values as biomarkers to detect neutrophil activity indicated high intensity in infected meshes. The histologically annotated regions matched with areas with high intensity for predictive proteins listed in Table 1, as well as with the spatial distribution of the neutrophils in the CD15 immunohistochemistry (Figure 4(a-c)). Comparing the intensity of applied m/z values from neutrophil-rich to neutrophil-poor areas significant differences were observed. Using SCiLS Lab software, we were able to automatically find a co-localized m/z value as presented in Figure 4b within the tissue. The co-localized m/z value has been identified as the Calgranulin B (S100A9). In addition to the already annotated regions we detected further regions with high numbers of neutrophils using MALDI TOF IMS.

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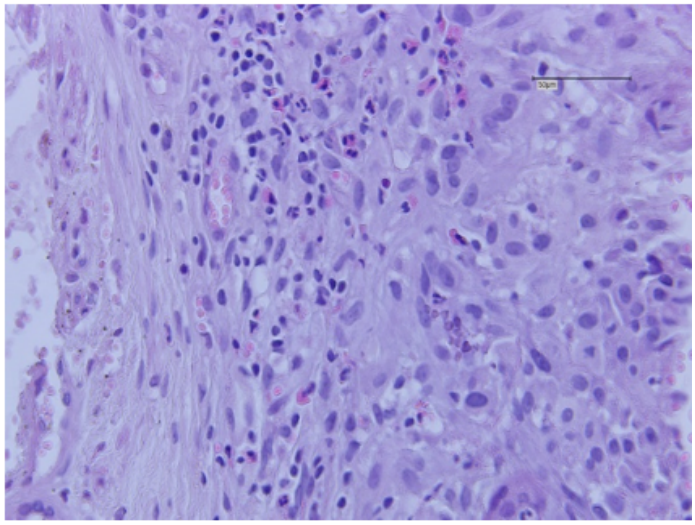

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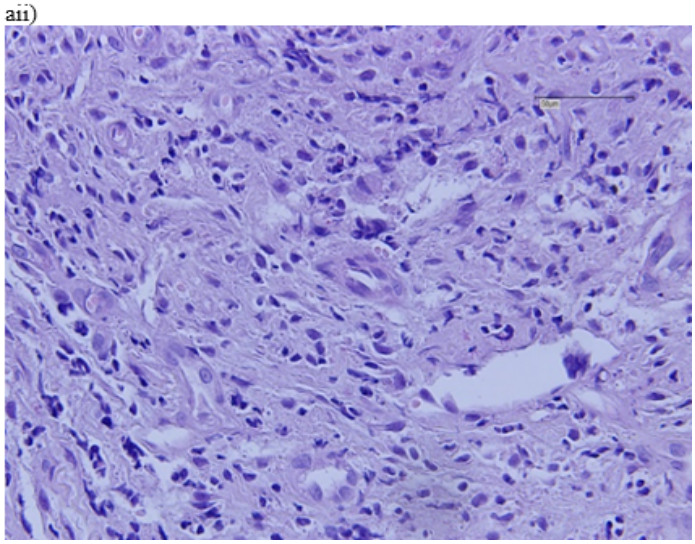

Figure 2a: hematoxylin-eosin section presenting the spatial distribution of neutrophil granulocytes in the investigated area of interest.

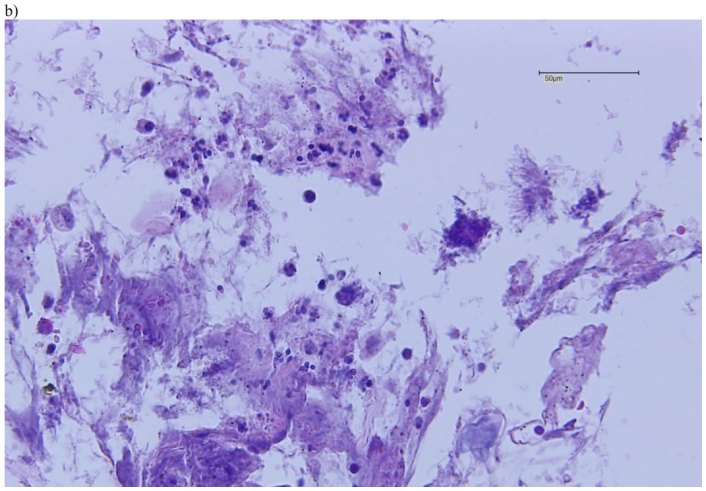

Figure 2b: hematoxylin-eosin section of a different sample, showing the varying quality of the sample.

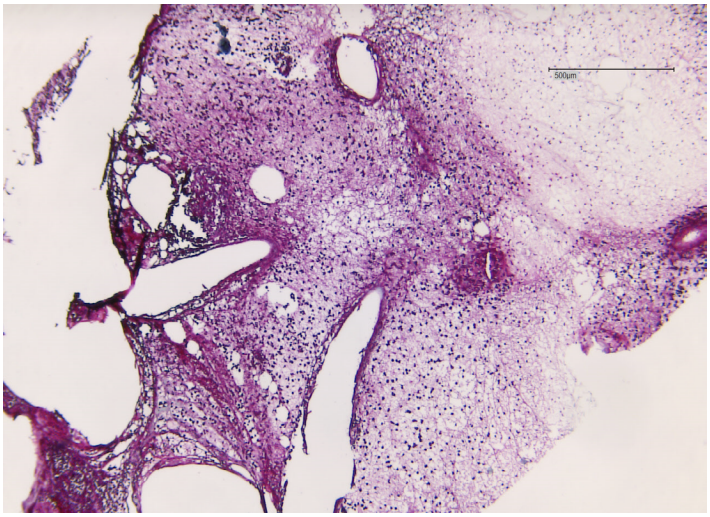

Figure 3a: CD-15-Immunohistochemistry section with tissue folding.

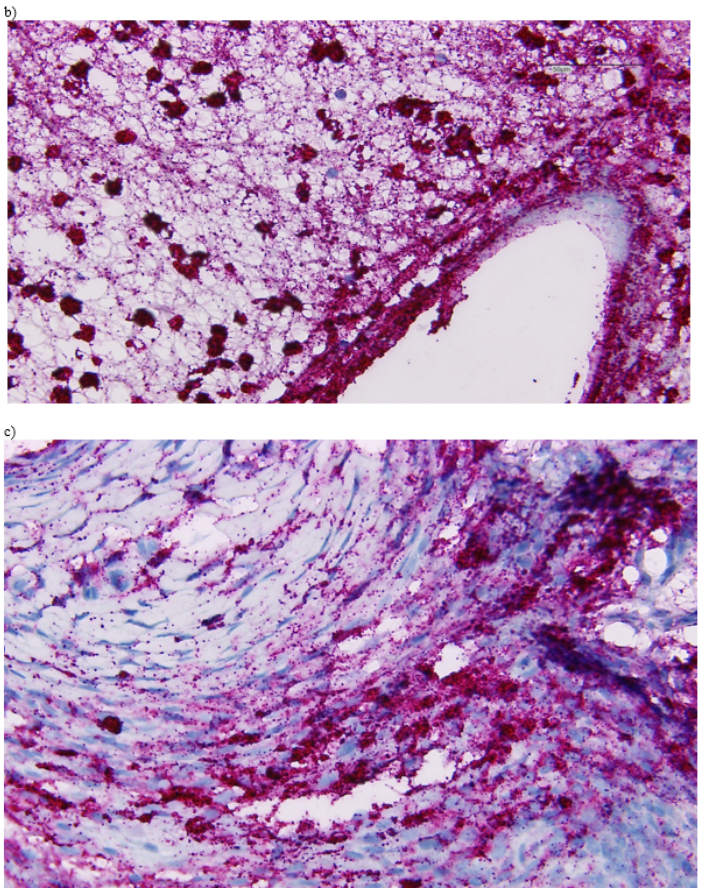

Figure 3b and Figure 3 c: CD-15 immunohistochemistry section in different samples, showcasing the varying quality of the staining.

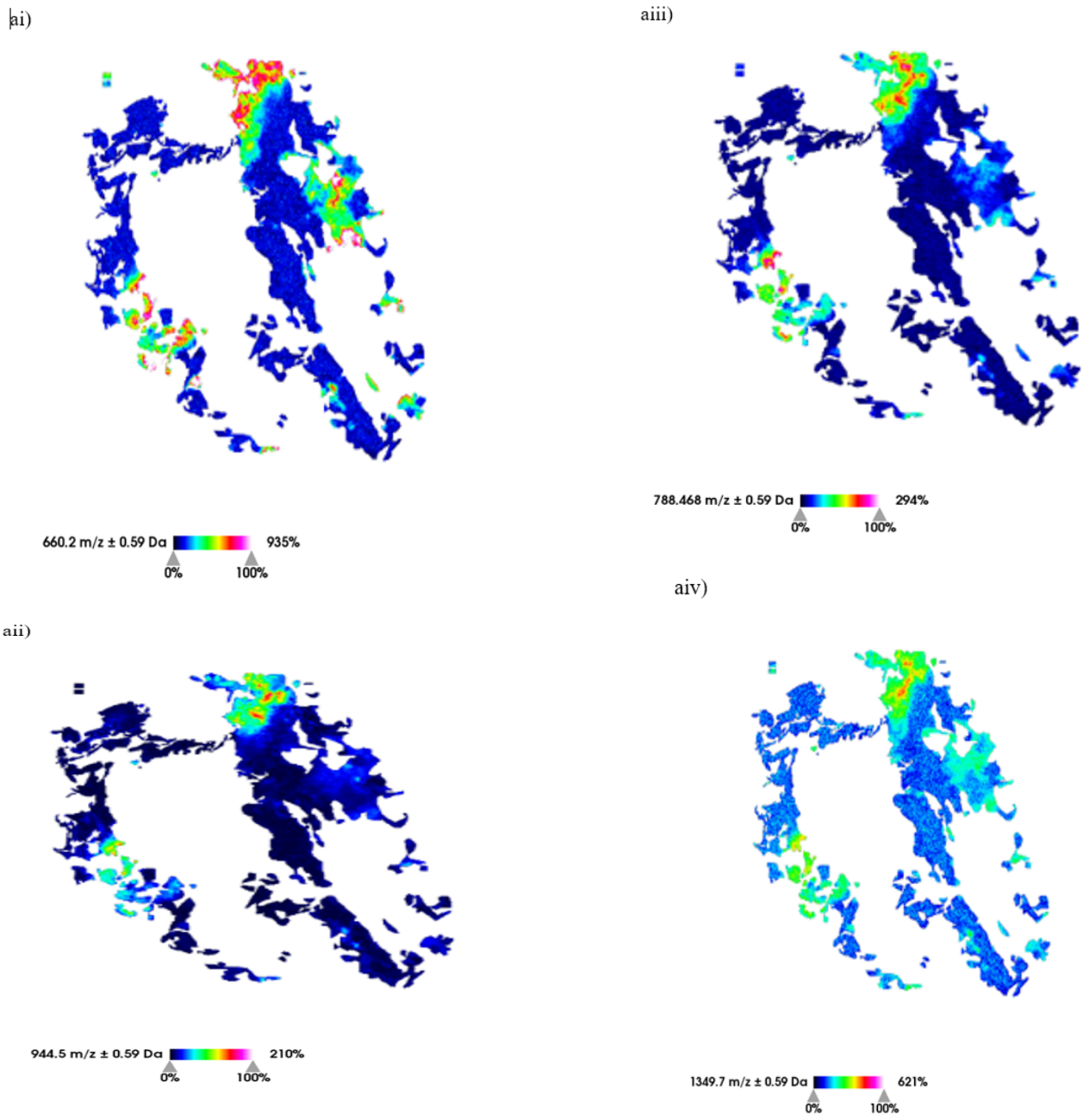

Figure 4a: Distribution of the applied m/z values, working as biomarkers for neutrophil activity and infection (m/z 660.2 ± 0.59, 788.468 ± 0.59, 944.5 ± 0.59 and 1349.7± 0.59).

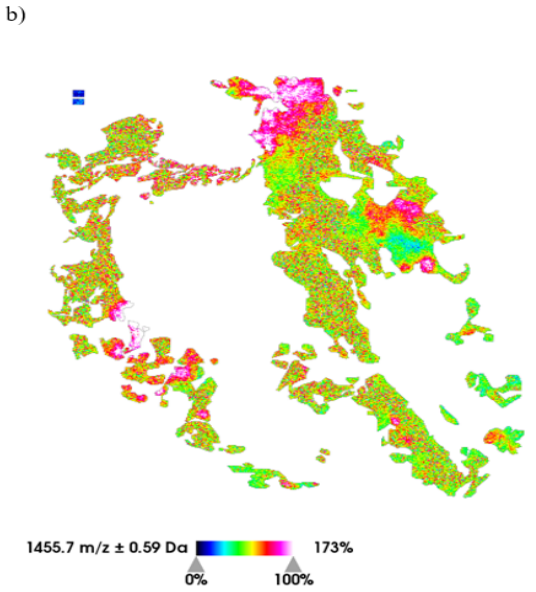

Figure 4b: Distribution of an additional m/z value 1455.7± 0.59, that showed co-localization to the applied biomarkers and the histologically annotated neutrophil-rich areas, which was identified as Calgranulin B.

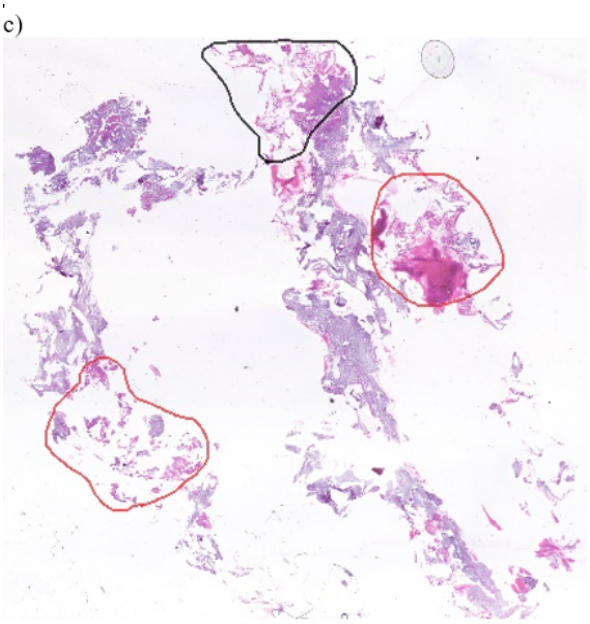

Figure 4c: tissue with low-grade infection in H&E staining with annotated areas with high number of neutrophils after initial (black) and second histological examination (red).

Discussion

The purpose of this study was to evaluate the possibility to use MALDI TOF IMS as a tool for diagnosing low-grade infection. As a proof of principle and an initial step to establish MALDI-TOF IMS in diagnostics of low-grade infection, we demonstrated its significant potential going forward. The annotated area of the low-grade infected tissue matched with the region with high intensity for applied m/z-values (Figure 4 (a&c)).

Comparing MALDI TOF IMS to CD-15 immunohistochemistry and histopathological examination it not only shows the spatial distribution of specific cells but also the presence of certain peptides, which are included in inflammatory response. Calgranulin C (S100A12) belongs to the group of calcium-binding proteins, which are mainly secreted by neutrophil granulocytes in inflammatory processes [15]. S100A12 has been used as a biomarker for pro-inflammatory diseases like inflammatory bowel disease and is discussed as a potential marker for local and systemic inflammatory processes [16]. Furthermore, S100A12 has also shown participation in directly combating bacterial infection [17]. In this study S100A12 has been highly presented in areas, which were annotated and showed high numbers of neutrophils compared to areas, that were not. Calgizzarin, also known as S100A11 is another member of the S100 protein family and plays an integral role in progression of osteoarthritis. Furthermore, increased levels of S100A11 have been found in the synovial fluid of rheumatoid arthritis patients. In a present study it is suggested, that S100A11 might be released by neutrophils in patients with rheumatoid arthritis, increasing the inflammatory reaction [18,19]. Other studies showed, that S100A11 can be used as a biomarker for myositis and infective endocarditis [20,21].

The release of S100A11 depends on NETosis [19]. Neutrophil Extracellular Traps (NETs) can be formed by neutrophils in a response to bacteria, parasites, fungi or virus and consist of neutrophil chromatin and proteins. Histones like H2A, H2B, H3 and H4 thereby contribute to approximately 70% of the protein mass [22]. As an immunomodulating protein, annexin A1 binds with proteins of the S100-family, underlining the co-localizing of both proteins in this present study [23,24]. Another peptide, that co-localized with described proteins was calgranulin B. Calgranulin B is also known as S100A9 usually binding with S100A8, forming a heterodimer calprotectin, which is contained in immunocytes and contributes to 45% of all cytoplasmic proteins in neutrophils [24-28]. S100A9 has been shown to modulate different inflammatory processes like in myocardial infarction and Alzheimer disease [29,30]. Moreover, elevated levels of S100A12 and S100A9 have been detected in patients with septic shock [31]. Wang et al. claim that neutrophils express and secrete the

heterodimer to modulate inflammatory processes, especially due to infection induced inflammation [28]. Furthermore, we were able to elaborate, that MALDI TOF IMS can detect additional areas with high number of neutrophils, that were not histologically annotated initially, demonstrating the holistic capture of neutrophil activity in the tissue using this new method. We propose that MALDI TOF IMS can provide an additional tool for diagnosis in unclear samples, offering further information to distinguish between low-grade infection and aseptic complications like degradation of the implant. The relative intensity of specific peptides could potentially serve as a discriminating factor between septic and aseptic complications. In this study design we analyzed meshes that were already diagnosed with mesh infection. However, using MALDI TOF IMS by applying biomarkers before histopathological review could be the next step establishing this procedure in diagnostics. Especially analysis of explants, that were histopathological examined and showed no clinical or histopathological signs of infection, could improve the process of establishing MALDI TOF IMS in diagnostics to differ septic from aseptic implant failure. Additionally, this could provide a better understanding of foreign body reaction and degradation of different alloplastic materials.

Conclusion

To our knowledge, this is the first study using MALDI TOF IMS on explanted mesh embedded tissue. Furthermore, this is the first try to apply biomarkers on the whole tissue to distinguish between infected and non-infected samples, as a trial to establish MALDI TOF IMS in diagnostics of implant infection. In addition, we were able to show, that there are other co-localizing peptides like Calgranulin B, that might expand the pool of possible biomarkers for infection. MALDI TOF IMS seems to provide additional information to the already existing diagnostic tools and might help to distinguish between aseptic and septic implant failure. Due to the small sample size further studies are needed to underline the potential of MALDI TOF IMS in diagnostics.

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