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Contributions of human tissue analysis to understanding the mechanisms of loosening and osteolysis in total hip replacement

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Abstract

Aseptic loosening and osteolysis are the most frequent late complications of total hip arthroplasty (THA) leading to revision of the prosthesis. This review aims to demonstrate how histopathological studies contribute to our understanding of the mechanisms of aseptic loosening/osteolysis development. Only studies analysing periprosthetic tissues retrieved from failed implants in humans were included. Data from 101 studies (5532 patients with failure of THA implants) published in English or German between 1974 and 2013 were included. “Control” samples were reported in 45 of the 101 studies. The most frequently examined tissues were the bone-implant interface membrane and pseudosynovial tissues. Histopathological studies contribute importantly to determination of key cell populations underlying the biological mechanisms of aseptic loosening and osteolysis. The studies demonstrated the key molecules of the host response at the protein level (chemokines, cytokines, nitric oxide metabolites, metalloproteinases). However, these studies also have important limitations. Tissues harvested at revision surgery reflect specifically end-stage failure and may not adequately reveal the evolution of pathophysiological events that lead to prosthetic loosening and osteolysis. One possible solution is to examine tissues harvested from stable total hip arthroplasties that have been revised at various time periods due to dislocation or periprosthetic fracture in multicenter studies.

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Conflict of interest statement

The work has not been supported by commercial sources and we are not aware of any potential conflict of interests.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 2–4, are difficult to interpret in black and white. The full color images can be found in the on-line version, at http://10.1016/j.actbio.2014.02.003

Keywords

Aseptic loosening; Osteolysis; Total hip; Tissue analysis; Immunostaining

1. Introduction

It is estimated that about two million total hip arthroplasties (THAs) are performed worldwide each year and projections of rising demand are reported at least for the USA [1]. However, some THAs fail during the period of service and require revision surgery, which is more expensive than the primary operation and brings less satisfactory outcomes and increased risk for complications [2,3]. This causes a significant economic impact on the health care system. Therefore, understanding current failure mechanisms of primary THAs and especially the potential for prevention are crucial.

Although instability, infection, pain and periprosthetic bone fractures prevail as reasons for reoperation in the first five years after an index surgery, the most frequent cause of late failure is aseptic loosening accompanied by osteolysis [4]. Since the pioneering work of Willert et al. [5,6], there has been a tendency to associate these late complications with a local tissue response to large numbers of tiny particles generated from bone cement and articulating/non-articulating surfaces of THA. Small particles are phagocytosed by macrophages or stimulate cells in a non-phagocytic manner. These cells then release pro-inflammatory molecules that trigger pathways influencing the osteoclast–osteoblast coupling in bone multicellular units [7,8]. Particle-associated dysregulation of osteoclast–osteoblast coupling in favor of osteoclasts over-weight leads eventually to net bone resorption at the bone–implant interface. In support of this concept, studies have demonstrated inflammatory and osteolytic responses after cell/organ culture stimulation by polymethylmethacrylate, polyethylene and titanium particles [9–15].

Immediately after the surgery, mechanical factors influence the development of the bone–implant interface. These are associated with intermittent loading of the artificial hip during daily living activities, and later with hydrodynamics of the artificial joint fluid creating significant pressures in the adjacent tissues. From some time postoperatively, biological and mechanical pathways interact together, creating conditions appropriate to periprosthetic osteolysis and aseptic loosening. We have previously described these processes in detail [16,17]. Here, we summarize current evidence derived from analyses of tissues retrieved during the reoperation of THA performed due to aseptic loosening and periprosthetic osteolysis.

2. Search strategy and rules for evaluation

We included all research studies that examined human periprosthetic tissues retrieved either during the THA surgery or post-mortem using histopathological examination and immunostaining. Further, we used articles and resources focusing on this issue. One of the authors (J.V.) searched for potentially relevant studies in the PubMed database. Articles published between January 1974 and June 2013 were identified with the keywords and medical subject heading terms “aseptic loosening” and “periprosthetic osteolysis” and “total

hip arthroplasty" and "bone loss" or "immunohistochemistry" or "cytokines" or "RANKL" or "hypersensitivity" or "apoptosis" or "interleukin" or "infection". We reviewed all of the retrieved articles and extracted relevant data, which we incorporated in table in the Excel 2010 software package (Microsoft). Although 223 articles were identified, 82 were excluded (Table 1, Fig. 1) because they lacked data about histopathological examination of retrieved tissues. In agreement with recent requirements for research in biomedicine, data should be considered preliminary until replicated by a different center. Therefore, all molecules, pathways and cell groups reported only in an initial histopathological study were stated as being preliminary, while those also featuring in a replication study were considered as proven.

3. Results

Data from 101 studies (5532 patients with failure of THA implants) published between 1974 and 2013 were included. "Control" samples were reported in 45 of the 101 studies.

3.1. Methods used for investigation of periprosthetic tissues

The retrieved tissues (or derived cell/organ cultures) were studied histologically and histochemically, especially using immunostaining or molecular biology methods. Another set of methods aimed to detect the prosthetic particles. The aims of all of these investigations were: (i) to distinguish between septic and aseptic THA failures; (ii) to detect prosthetic by-products in periprosthetic tissues; (iii) to analyze cell/tissue structure; and (iv) to detect the signaling proteins and proteolytic enzymes in the periprosthetic tissues.

3.2. Protocols for tissue processing

Some authors examined results from cell/organ cultures derived from periprosthetic tissues [18–22], but their results were excluded because we focused on direct histopathologic examinations of the periprosthetic tissues. These were processed post-operatively using approximately 5–6 μm thick frozen tissue sections (41 of 101 studies) or 3–10 μm thick paraffin-embedded tissue sections (45/101), or both methods (10/101). Methylmethacrylate-embedded sections were used in seven studies [5,23–28], and a further two studies did not report the methodology [29,30]. Mostly, the sections were cut from periprosthetic tissues fixed immediately after harvesting in 10% buffered formalin and then embedded in paraffin (formalin fixed, paraffin embedded; FFPE), or were prepared as freshly frozen sections in the cryostat. The sections were stained for comprehensive microscopic evaluation after hematoxylin-eosin staining. For precise demonstration of the specific molecular components within the cells and in the proper tissue context, special histological stains (Table 2) and immunostains were used (Figs. 2 and 3).

Different staining methods and molecular and cellular biology techniques were used. The most frequently used methods capable of identifying specific RNA or DNA molecules are polymerase chain reaction (PCR) and in situ hybridization (ISH). Some authors also used PCR to detect bacterial DNA, or inflammatory cytokines and other mediators. In most cases, the results from the molecular biology methods were compared to immunostaining of the same tissue samples [31,32].

Immunostaining can specifically identify categories of cell lineage and their regulatory molecules (proteins), and detect the presence of specific antigens in cells with high sensitivity. The immunostains used were based on the reaction between antigen and primary and secondary antibodies, with one of them being labeled with an enzyme (horseradish peroxidase, alkaline phosphatase, biotin), the fluorophore fluorescein isothiocyanate [33,34] or tetramethylrhodamine isothiocyanate [34].

Immunoenzyme protocols with many different principles were applied for antibody-aided detection, including: (i) the avidin-biotin complex method [19,25,35–51]; (ii) the labeled streptavidin-biotin method [32,33,52–59]; and (iii) the polymer-based detection method [60–67]. The presence of antigen was most often visualized by chromogen 3,3′-diaminobenzidine tetrachloride, which produces a brown reaction that can be seen with a light microscope. Occasionally, a fast red TR salt [35], aminoethylcarbazole [38] or fuchsine [39,52], giving a red stain, or chromogen, with the blue-colored precipitate nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate [41], was used. Finally, sections were counterstained with hematoxylin prior to mounting.

In some cases, double immunostains were used, which can identify two sets of antigens in the same section when the antibodies are applied in sequence or at the same time. Some authors followed an immunofluorescence staining protocol [38,40,50,61,68], while others preferred antigens labeled by enzymes [38,48,50,61,68,69].

Histological analysis was mostly carried out at 2–500 × magnification, using a light or fluorescence microscope. Polarized light, electron or transmission electron microscopy was used to identify various sizes of wear particles and the intracellular pathology induced by prosthetic by-products [70]. Polyethylene wear debris is strongly birefringent in polarized light, unlike ceramic or metal particles.

3.3. Distinction between aseptic and septic failure

This analysis is based on the detection of the number of polymorphonuclear neutrophil leukocytes (PMNs) in the examined tissues. The higher the number of PMNs observed, the higher the probability that the examined tissue is affected by infection. Generally, the minimum criterion for infection is more than five PMNs in five separate high-power fields, using 400 or 500 × magnification [71]. Others have proposed a criterion of 23 PMNs in 10 high-power fields [72]. In fact, the key prerequisite for accurate interpretation is a very experienced pathologist, ideally blinded.

To identify PMNs, routine hematoxylin-eosin staining (of polymorphic nuclei) and immunostains using CD15 are performed on FFPE or frozen sections. Using the classification system of Morawietz et al. (Table 3) [56], analysis of frozen sections has a high correlation to results from FFPE specimens [73]. Hence, the intraoperative histological analysis of frozen sections is recommended as a valid diagnostic tool for the prompt confirmation of infection [74–76]. Although sensitivity of frozen sections can vary, the specificity of intraoperative examination is excellent [73,75,76]. In addition, a number of studies examined the contribution of Gram staining to the diagnosis of prosthetic joint

infection [77,78]. However, the evidence for the routine use of this method is limited [79,80].

Some investigators have shown that “particle disease” is more likely when bacterial molecules are absorbed on the prosthetic particles [81]. Participation of bacterial substances in the processes of aseptic loosening can be demonstrated either by finding these products (e.g. teichoic acids, endotoxins, protein A) or signs of a bacteria-specific host response in periprosthetic tissues. In favor of this hypothesis, several studies have shown bacterial by-products, including bacterial DNA, in periprosthetic tissue homogenates [82]. Additionally, septic membranes have a significantly higher number of CD45+ leukocytes, while aseptic membranes show a preponderance of CD68+ macrophages and histiocytes [57,69]. However, tissues with low-grade infection can appear like tissues retrieved from patients with aseptic loosening.

Bacteria and bacterial products have been found to cause up-regulation of interleukin-1 receptor-associated kinase (IRAK-M), which regulates toll-like receptor (TLR) signaling [51]. Several studies show that TLRs, sensors for detection of bacteria, are activated in periprosthetic tissues [57,83]. However, microbe-oriented sensors like TLRs, nucleotide-binding oligomerization-domain protein-like receptors, RIG-like receptors and AIM-2-like receptors are stimulated not only by exogenous pathogens, but also by intrinsic signals of tissue/cell damage serving generally as inducers of inflammation [84]. The detection of specific antibacterial proteins secreted by innate immunity cells after contact with bacteria seems to be a promising approach [85,86].

However, to date, no one study has analyzed the microbe-specific responses in aseptic periprosthetic tissues in detail. As a result, the histological evidence of the participation of bacteria in aseptic loosening and periprosthetic osteolysis is still considered insufficient.

3.4. Evidence for aseptic inflammatory response

Local tissue resident cells orchestrate the tissue response to prosthetic by-products via regulation of macrophage polarization into an M1 or M2 phenotype, and balance between fibrocytes and fibroblasts [16,87]. The foreign body response to wear debris and the abundance of macrophages and giant cells show a direct relationship to the degree of bone resorption [37,42,88]. Polyethylene particles smaller than 5–7 μm can be observed within macrophages [42,47], while larger particles induce a foreign body giant cell reaction – with the amount of particles correlating with the number of foreign body giant cells [19,32]. Generally, tissues with increased numbers of wear particles have the appearance of foreign body granuloma [19,66,69,88,89].

Samples from osteolytic tissue show higher proliferative cellular responses, including predominantly macrophages, fibroblasts, highly vascularized fibrous tissue with rare occurrence of PMNs (Fig. 4) and high expression of inflammatory cytokines [37,43,88,90,91]. Histological findings show various differences in the profiles of cell populations and biological mediators dependent on the type of fixation and articulation bearings (Table 4), which may reflect different mechanisms of aseptic loosening and osteolysis [92–95]. The amount and distribution of T cells, and their specific role in aseptic

loosening, were explained by the cytokines which they produce, the most important ones being interferon (IFN)-γ and interleukin (IL)-17. However, the former can also be produced by other cells, such as natural killer T cells [39], and IL-17A and IL-17F are also produced, for instance, by mast cells [96]. Much less is known about the other important cell types, such as fibroblasts [97], mast cells [44,90], eosinophils, platelets, adipocytes, vascular endothelial cells [98], lymphatics [60] and neurons [99]. Several histological studies show that fibroblasts release similar factors as macrophages [32,41,42,48,50,63,100,101].

Periprosthetic tissue was found to express increased levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1α, IL-1β, IL-6, IL-8 and macrophage colony-stimulating factor (M-CSF) [18,42,88,102]. The most commonly observed cytokines expressed in periprosthetic tissue are IL-1β, IL-6 and TNF-α. Some studies showed high levels of prostaglandin E2 (PGE_2), inducible nitric oxide synthase (iNOS) and other mediators of inflammation in periprosthetic tissues [40,66]. Also investigated were regulators of tissue homeostasis participating potentially in development of osteolysis and aseptic loosening, such as vascular endothelial factors, IL-10 or transforming growth factor (TGF)-β [95,103–106]. Importantly, it was shown how wear debris up-regulates IRAK-M, which plays a critical role in innate immunity and the down-regulation of foreign body reactions [51].

In aseptic loosening, a periprosthetic membrane develops at the interface between the implant and the bone bed. A wide variation was observed in the macroscopic appearance of this membrane. Many authors use the term “synovial-like interface membrane” (SLIM), while others prefer the term “granulomatous tissue” [18,107,108]. The term “synovial-like” simply refers to the presence of synovial lining on the interface membrane as a result of the expansion of the “effective joint space” [26]. Hyaluronan-containing synovial fluid induces a connective tissue synovial lining composed of macrophage-like type A cells and fibroblast-like type B cells [25]. The synovial-like membrane has a villous architecture and contains three distinct zones: first, a layer adjacent to the implant consisting of synovial-like cells supported by fibrovascular tissue; second, a vascular layer composed of dense fibrous tissue with sheets of activated macrophages, foreign body giant cells, polymorphonuclear leukocytes and a large accumulation of wear debris; and third, the poorly vascularized fibrous layer adjacent to the bone, in part covered by osteoclasts and osteoblasts, and showing signs of accelerated bone remodeling [42,90,109,110].

Together, these observations provide strong evidence that macrophages and fibroblasts orchestrate inflammatory, immunomodulatory and osteolytic pathways, leading to the formation of chronic fibrosis and periprosthetic osteolysis in aseptically loosened THA. Histopathological studies have demonstrated a number of factors typical of chronic inflammation and fibrosis, including chemokines, cytokines, nitric oxide metabolites and metalloproteinases.

3.5. Influence of biomaterial combinations on histology of periprosthetic tissues

A number of studies investigated the specific biomaterial-dependent variability in the periprosthetic tissues trying to clarify how the type of prosthetic particles or ions (in the case of metal components) influences the structure of periprosthetic membranes

[18,24,93,94,109,111–114]. However, the answer to this question is not straightforward because the status of periprosthetic tissues at the time of revision is not simply a function of the prosthetic particles [16]. There is some evidence for the difference between polyethylene-on-zirconia-, CoCr-on-CoCr- and ceramic-on-ceramic-bearing materials not being substantial 1 year after surgery [67]. However, with the amount of particles and tissue damage growing with time after surgery, a slightly wider spectrum of histological findings has been observed even with identical implants and in different areas of individual patients [100,115]. Undoubtedly, the “non-material” characteristics of the particles (their size, number, shape, charge, etc.) play a key role in the development of histological findings, together with other factors not related to biomaterial particles, like the stability of an implant, parameters of the effective joint space, the volume of joint fluid and its pressure patterns [116,117]. In relation to the latter, the synovial lining layer develops at the sites in contact with fluid and/or relative motion. Taken together, tissues retrieved during revision arthroplasty exhibit a relatively limited macroscopic variability, at least in terms of tissue color (basically metallic vs. non-metallic), tissue consistency (ranging from rigid firm fibrotic to soft fragile tissue) and volume (atrophy vs. abundant hypertrophic periprosthetic tissues). In this line, histopathological studies have revealed a trend suggesting that there could be differences associated with particular biomaterial combinations in terms of inflammatory response (in relation to the presence of specific cell populations, mediators, etc.), type of fibrosis, extent of tissue necrosis or presence of apoptotic events (Table 4). For example peri-implant tissues from cemented THA (a polyethylene cup and a stainless steel or CoCr alloy stem) often consist of a dense fibrotic tissue with variable areas of necrosis. With regard to the cellular profile, macrophages predominate, together with fibroblasts and myofibroblasts, followed by the scattered to frequent presence of foreign body multinuclear cells and perivascular infiltrates of lymphocytes [5,110]. Membranes retrieved from uncemented CoCr or titanium alloy femoral components are very similar. Both types of membrane were composed of a dense fibrous tissue stroma with a moderate to large number of macrophages and fibroblasts [90,109]. This is due at least in part to very similar bearing materials (here metal on polyethylene). Ceramic-on-ceramic THAs exhibit very low wear rates, and ceramic wear particles also have much lower specific and functional biological activity than polyethylene particles [118]. Therefore, under normal conditions (i.e. without gross surface damage or fracture of ceramic components associated with metallosis), membranes taken from loosened ceramic-on-ceramic THA are generally hypotrophic in comparison to the former ones and contain decreased numbers of macrophages and lymphoplasmacytic cells, with the scattered presence of foreign body multinuclear cells [94,112]. Adverse reaction to metal particles/ions is described elsewhere in this review.

3.6. Significance of necrosis/apoptosis ratio around aseptically failed THA

Apoptosis can contribute to the resolution of inflammation, the normalization of tissue turnover and the processes of cell renewal accompanying tissue proliferation [69,84]. In contrast, insufficient apoptosis could contribute to the growth of granulomatous tissue and prolonged production of proteolytic enzymes. In fact, a number of studies have shown apoptotic activity of various cells in peri-implant tissues around loosened THAs [59,62,63,66,69]. The low immunopositivity of the apoptotic markers in fact correlated with the degree of osteolysis, and with the proliferation of macro-phages and fibroblasts [63].

Importantly, the type of implant, and the gender and age of patients did not influence the level of apoptosis or the number of T lymphocytes [61].

Apoptosis is characterized in hematoxylin–eosin staining by dark eosinophilic and dense cytoplasm, dense purple condensation of nuclear chromatin, DNA fragmentation and the formation of apoptotic bodies, which are quickly phagocytosed by macrophages or adjacent cells. Current studies were designed to identify the intrinsic and extrinsic pathways of apoptosis by immunostaining (Table 5). The strong expression of iNOS, cyclooxygenase-2, nitrotyrosine, caspase-4, GRP78 and GADD153 was demonstrated in macrophages [66]. Macrophages, giant cells and fibroblasts also expressed important apoptotic regulators, including p53 and BAK [62].

Immunostaining of tissues from aseptic loosened THAs showed DNA-cleaving active caspase-3 and pro-apoptotic BAK positive macrophages, fibroblasts, giant cells and T lymphocytes [62,63]. The immunopositivity of Fas ligand (FasL), which is able to bind to its death receptor FasR, was found in macrophages, fibroblasts and giant cells [62]. The precursor and active forms of caspase-1 (an interleukin-converting enzyme) were found in the macrophage-like, fibroblast-like cells and some vascular endothelial cells in the synovial-like membrane [101]. Fibroblasts showed caspase-8 positivity located downstream from FasL and FasR, and no reaction with caspase-6 [69,119].

TUNEL assays (TdT-mediated dUTP–biotin nick-end labeling) can be used to detect cells undergoing apoptosis. This method in fact also detects necrotic cells in some cases because it extends and labels the free terminal ends of the cleaved deoxyribonucleic acid. The results of TUNEL analysis suggest high numbers of apoptotic macrophages in the interface membrane with metal and polyethylene debris [66], but only a few TUNEL-positive fibroblasts in aseptic loosening of cementless implants [69]. On the other hand, the anti-apoptotic marker Bcl-2 was not found in macrophages, giant cells or fibroblasts [62,69].

Taken the above findings together, it appears that apoptosis may be a key element in understanding the mechanism of aseptic loosening and periprosthetic osteolysis.

3.7. Evidence for osteoclast-driven periprosthetic bone resorption

To determine whether osteoclasts play a key role in periprosthetic osteolysis requires their identification in areas of bone resorption, together with a demonstration of increased concentrations of the chemokines that can attract osteoclast precursors and promote their differentiation in the periprosthetic tissues. M-CSF has been detected in macrophages, fibroblasts and vascular endothelial cells in periprosthetic tissues [50]. High levels of receptor activator of nuclear factor kappa-B ligand (RANKL) were detected in macrophages, multinucleated giant cells adjacent to wear particles and endothelial cells [38,39]. RANKL was also found in fibroblast-like stromal cells in periprosthetic tissues and soluble RANKL was found to bind $RANK^+$ cells [34]. In another study, histiocytes, multinucleated giant cells and fibroblast-like cells in the membranous tissue expressed RANKL and TNF-α converting enzyme [32]. Fibroblasts and macrophages also expressed IL-11, which participated in osteoclast formation [48].

An opposite role is played by osteoprotegerin (OPG), which can down-regulate osteoclastogenesis by binding to and neutralizing RANKL. OPG was reduced in the tissues located near failed implants [38]. As a result, the RANKL/OPG ratio was increased in periprosthetic tissues from sites with osteolysis and with a high number of wear particles [34,53,65].

An important role in the destructive bone process is attributed to collagenases and other members of the matrix metalloproteinase (MMP) family [46,120]. The expression of collagenase-3 (MMP-13) was found in endothelial cells, macrophages and fibroblasts of the synovial-like membrane [41]. Local mRNA expression profile showed that MMP-1, −9, −10, −12 and −13 were strongly elevated in aseptic loosening compared to controls; MMP-2, −7, −8, −11, −14, −15, −16, −17 and −19 were moderately expressed, whereas MMP-3 expression was lower and MMP-20 very low [121]. Furthermore, collagen degradation in periprosthetic tissue correlated significantly with the number of local MMP-1, MMP-13 and cathepsin K-positive cells [54].

Most studies report data on the cellular responses in soft periprosthetic tissue, rather than in bone samples. A key histomorphometric work in this field showed a high turnover in periprosthetic bone remodeling and immature bone formation around loosened cemented THA [27]. The total eroded surface was increased at the implant–bone interface in loose compared to well-fixed implants (14.7 ± 5.1 vs. 9.8 ± 2.4%, $p = 0.027$). However, the osteoid surface was increased in cases with loosening (25.8 ± 8.7 vs. 16.8 ± 3.4%, $p = 0.017$). In addition, in loosened implants, the volume value of the mature mineral was low (54.6 ± 12.9 vs. 80.6 ± 10.0%, $p < 0.001$), whereas the volume of low-mineralized bone was increased (39.3 ± 12.2 vs. 17.6 ± 9.1%, $p < 0.001$), which might be related to the low pH and high cathepsin K values in the interface membrane [33]. Shen et al. [45] examined specimens of soft tissue and demineralized bone to analyze the phenotype of cells associated with polyethylene particles or the bone surface. Expression of β3-integrin and calcitonin receptors was predominantly found in cells associated with the bone surface, and their activity rose after induction of cathepsin K and tartrate-resistant acid phosphatase (TRAP). Although TRAP and cathepsin K are used as markers of osteoclast lineage, several studies detected high activity of these enzymes in macrophages and giant cells located at the bone-oriented surface of the interface tissue [32,33,45,52].

Taken together, there is growing evidence for the role of osteoclasts in periprosthetic osteolysis and aseptic loosening in THA. On the other hand, the contribution of other cells of the periprosthetic bone multicellular unit to bone resorption still remains to be elucidated.

3.8. Evidence for localized delayed-type hypersensitivity reaction

Some authors believe that painful THA and aseptic loosening could be explained, at least partially, by hypersensitivity to chemicals or metals used in the implant [122–124]. Prosthetic particles and metallic ions can indeed induce type IV hypersensitivity reactions, as exemplified by allergic contact dermatitis overlying the implant. However, the putative participation in tissue damage and eventually in implant loosening is currently unclear. Metal ions, or haptens such as acrylates, primarily form covalent bonds between the electrophilic components of the hapten and the amino acid nucleophilic side chains of the

target proteins. This complex then binds to the surface of dendritic cells, which migrate to the draining local lymph nodes via the lymphatic vessels and present the antigen to T cells, leading to sensitization [125]. Memory T cells then migrate back to the site of the implant and cause an immune reaction, in particular with (antigen-responsive) lymphocyte infiltrates in the deep fibrous tissue and high endothelial venules for lymphocyte recruitment from the circulation [39,111,122,126– 128]. Pertinently, direct non-peptide-dependent activation of TLRs can also occur with some metals [129]. If this lymphocyte-mediated hypersensitivity response occurs in peri-implant tissues, which are already affected by a chronic foreign body reaction, multinuclear foreign body giant cells or granulomas can also be seen [123]. The typical histopathological picture in metal-on-metal arthroplasties demonstrates a layer of fibrin on the synovial lining, granulomatous inflammation and connective tissue necrosis [111,130,131]. The widespread infiltration in areas of tissue necrosis has been described as adverse reactions to metal debris – metallosis participating at least partially in the development of pseudotumors [30,132].

Key cell populations associated with hypersensitivity include lymphocytes and dendritic cells. The presence of large numbers of lymphocytes forming perivascular cuffs and synovial ulceration is called lymphocytic vasculitis-associated lesion (ALVAL), and demonstrates a lymphocyte-dominant delayed-hypersensitivity-type immunopathological reaction. The highest ALVAL score is found in association with suspected metal hypersensitivity [29,111,132]. The diffuse perivascular lymphocytic infiltration is associated with the presence of visible metal particles and aseptic loosening [89,133]. Importantly, cell-mediated immune responses have not been confirmed to be involved in aseptic loosening in metal-on-polyethylene THAs [59,91]. Other cell groups participating in tissue hypersensitivity are mast cells and eosinophils. The former were detected in periprosthetic tissues less frequently compared to in control knee synovium, despite their being more often degranulated in aseptic loosening [44]. However, mast cells play a role in immediate type I hypersensitivity reactions, which are characterized by Th2 skewing of the immune system, as seen in the atopic disorders.

In summary, the concept of local hypersensitivity remains to be proved in prospective studies with greater numbers of patients with aseptic loosening of different implants. In addition, rigorous standardized rules for tissue processing and interpretation of immunostainings have to be established [108]. Notably, metal hypersensitivity can be a secondary phenomenon due to inflammation-based corrosion of the metal alloys.

3.9. Limitations of histopathological studies of aseptic loosening and periprosthetic osteolysis

The obvious limitations include the origin and location of tissue samples, the size of the observed groups, their homogeneity, at least in terms of age, gender, primary diagnosis, type of implant and type of samples (pseudosynovial, interface membrane, osteolytic tissue, etc.), and the lack of appropriate control groups. The control samples involved in almost all studies are synovial tissues from primary osteoarthritis taken before THA/total knee arthroplasty surgery, rarely synovial tissue from around a well-fixed THA, tissues from mandibular or maxilla fractures or biological ingrowth membranes

[27,31,38,40,41,48,50,52,53,66,68,88,90– 92,101,106,134]. The periprosthetic tissue from deceased patients with THA was also used [5,23,24,61–63,130].

One important factor potentially influencing the results from tissue analysis is the location of the harvested specimen [115]. Some studies report an increased cellular response in synovial-like membrane of periprosthetic tissue and suggest that SLIM is the best material available for histopathological analysis [50,73]. However, some studies show that there is a large variability within the sampled tissues even when the same kind of retrieved tissue is sampled [42,115,135]. Discrepancies may be even in the single sample [42]. This points to the vast diversity in the process of aseptic loosening even in a single patient, at least in terms of the cell types and numbers present and the different levels of activation among cells, which may represent different stages of loosening in different topological areas.

The process of harvesting tissue and the type of fixation play crucial roles in the results of the diagnostic methods involved [136–138]. The samples used for immunostaining may be frozen and paraffin-embedded sections harvested right at the time of surgery. Although FFPE sections are suitable for immunohistochemical analysis of most antigens, many authors prefer snap-frozen cryostat sections [25,33,35,37–44,48,50,54,64,88,91,98,100,101,106,115, 126,139]. The preparation of fresh-frozen sections takes less time than FFPE sections; moreover, the technique of processing is simpler and the activity and epitope of antigens is better preserved. However, the use of frozen sections also has disadvantages: (i) the tissue morphology is not as clear as it is in the FFPE section; (ii) the fresh tissue for other diagnostic purposes must be stored, preferably at −70/80 °C; and (iii) freezing must be done very rapidly to minimize the formation of ice crystals. Immunostaining of FFPE samples after defrosting may show incorrect results and is of low histomorphological value. The tissue to be processed as an FFPE sample must be fixed in buffered formalin immediately after harvesting. The advantages of FFPE sections are: (i) applicability to routinely processed material, even if it is stored for long period; (ii) possibility of correlation with the morphologic parameters; and (iii) workability even in decalcified material (bone).

The critical factor for the interpretation of the results of immunostaining is their validity and verification of antibody specificity and sensitivity [136,137,140]. Despite the high popularity of immunostaining, there are also potential limitations with respect to limited availability of some sensitive or specific antibodies to the required antigens [136,137]. These may be demonstrable by PCR or ISH. Therefore, molecular techniques can provide superior diagnostic information and should be used together with immunostaining.

The results of immunohistochemical staining are mostly reported qualitatively, though some specific antibodies and numerous regulatory molecules require quantitative analysis [141,142]. Even though well-established criteria often exist for characterization of individual cells under light microscopy, inter-individual variation is also of concern. Computer-assisted analysis of digital images might prove to be more reliable [141–143].

3.10. Directions of future research

On the basis of the above limitations, one may speculate that further models of the bone-implant interface and periprosthetic tissues may be more descriptive of the mechanical and biological processes responsible for integration and loosening of THA in humans.

The clinical criteria for inclusion and exclusion of surgical cases and specimens need to be further defined. Other factors contributing to aseptic loosening, such as mechanical factors and/or implant deficiencies, must be elucidated. The key problem lies in the complexity of THA because a number of patient-, surgery- and implant-related variables could potentially influence the stability of the THA, making the interpretation of the cause of failure difficult in an individual case.

Further progress in developing valid control groups is needed. Samples from all patients with functional and stable THA could be collected and offered for research, for instance, via international collaboration (analogous to a tissue bank). If possible, samples should be collected from both capsular and interface membranes. Perhaps the optimal method for archiving of periprosthetic tissues is deep-freezing, for future distribution to specialized centers of excellence.

Research in fields related to material science, biochemistry, physiology, and cellular and molecular immunology could stimulate further investigation of periprosthetic tissues in terms of target cells, pathways and proteins. For instance, there is a lack of information on the role of fibroblasts, mast cells, dendritic cells and osteocytes, as well as lining cells of pseudosynovial membrane in periprosthetic tissue homeostasis and aseptic loosening.

The role of hypersensitivity and adverse tissue reactions to metal debris in aseptic loosening needs further investigation. First, it is necessary to identify the mechanisms of tolerance to metallic implants induced in most patients with THA. Secondly, the evaluation of hypersensitivity reactions should be based on more specific morphologic criteria, using immunostaining and the analysis of lymphocyte clonality and specificity and their cytokines. There is a need to clarify the role and presence of lymphocytes, their subsets and products. The relationship between delayed lymphocyte-mediated hypersensitivity reactions and in vivo and in vitro allergy tests, such as cutaneous skin patch tests (eliciting skin induration/lymphocyte infiltrates and erythema) or the ELISPOT test of peripheral blood lymphocytes (production of IFN-γ or other relevant cytokines), upon exposure to specific metals is extremely controversial. More definitive proof would require the demonstration of an oligoclonal (antigen- or allergen-driven) lymphocyte activation pattern locally in peri-implant tissues without any in vitro cellular expansion. This could be used to demonstrate discriminative antigen-driven local expansion of antigen-specific T cells in situ.

4. Conclusion

Histology and immunostaining of periprosthetic tissue have contributed significantly to the knowledge of the mechanisms of aseptic loosening and periprosthetic osteolysis. The key role of innate immunity sentinel cells and their receptors has emphasized the host response to prosthetic by-products and subsequent tissue damage. Histology and immunostaining also

contribute to the understanding of the mechanisms of prosthetic integration vs. the adverse outcomes associated with chronic inflammation, fibrosis and allergy in the processes of periprosthetic tissue maladaptation.

Limitations of histological analysis of retrieved periprosthetic tissues include: (i) the heterogeneity in patient, surgeon and implant characteristics; (ii) the inability to harvest tissues at different stages of tissue maladaptation before aseptic loosening; (iii) the lack of standardized sampling and processing methodologies; and (iv) the absence of true control tissues (tissues loaded by implant-related signals but without even early signs of tissue maladaptation). Further research in multiple specialized collaborative centers could address these issues more effectively.

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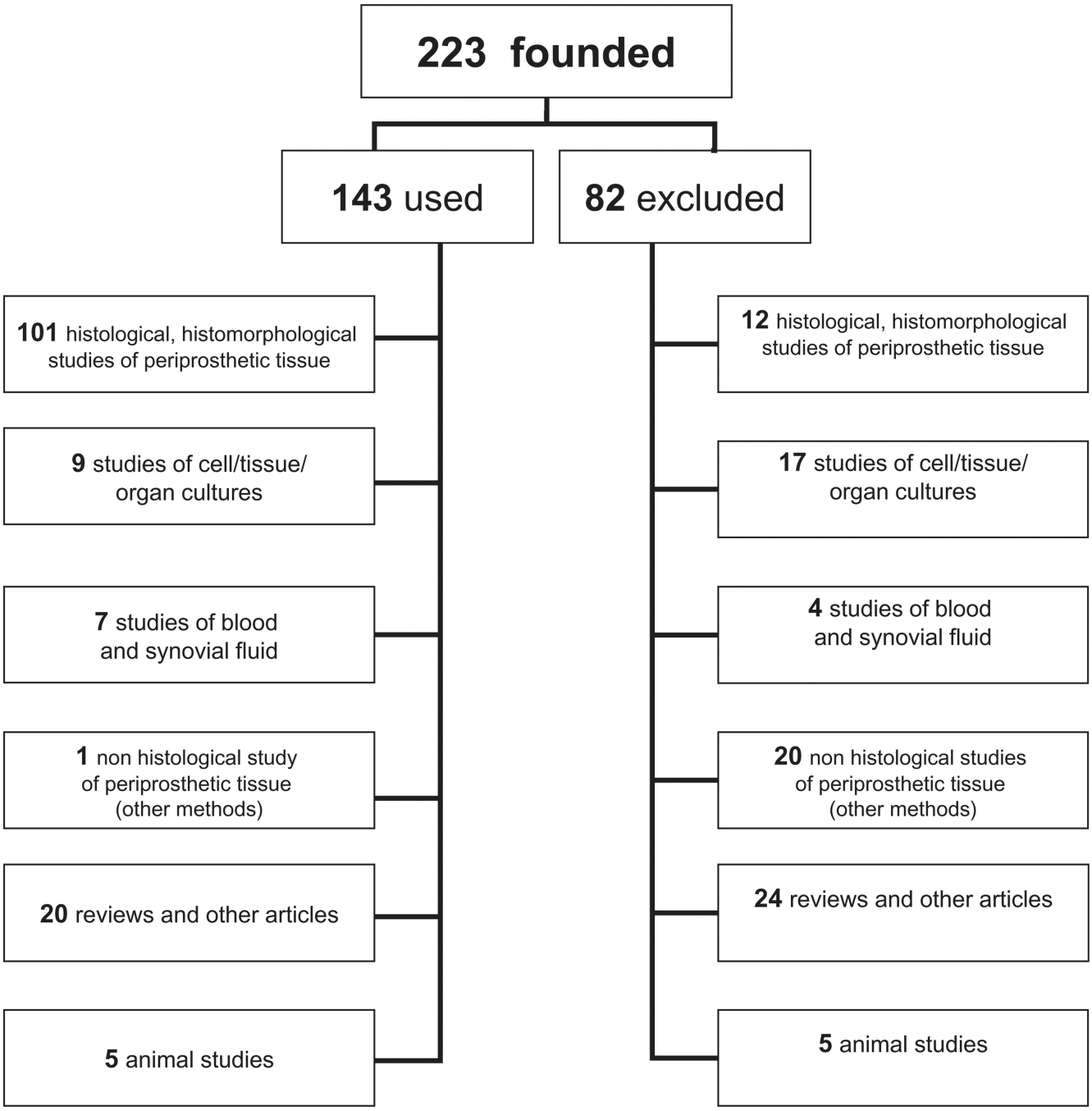


Fig. 1.
Flowchart of the methodology of the study.

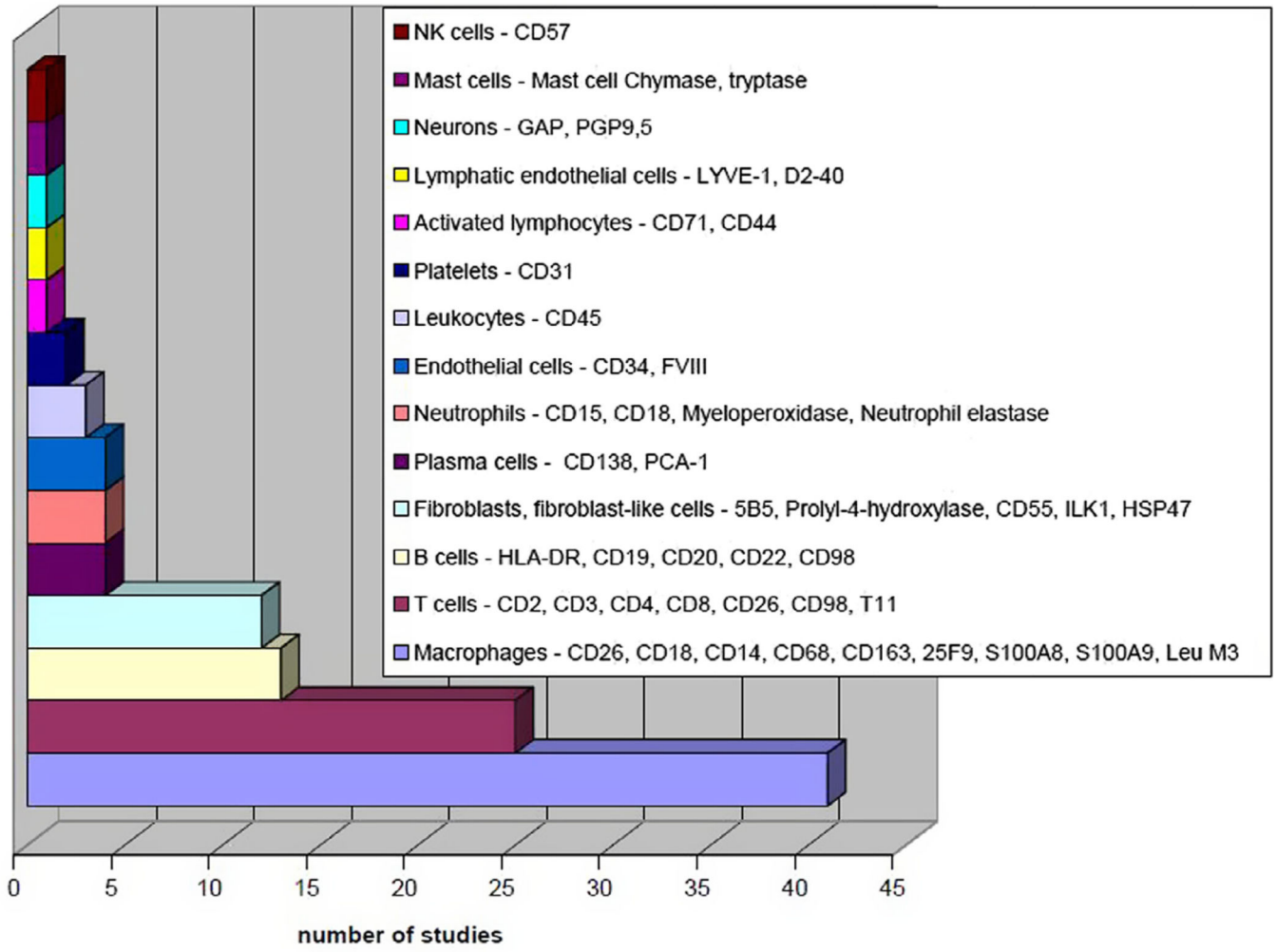


Fig. 2.
Cell types identified by immunostains.

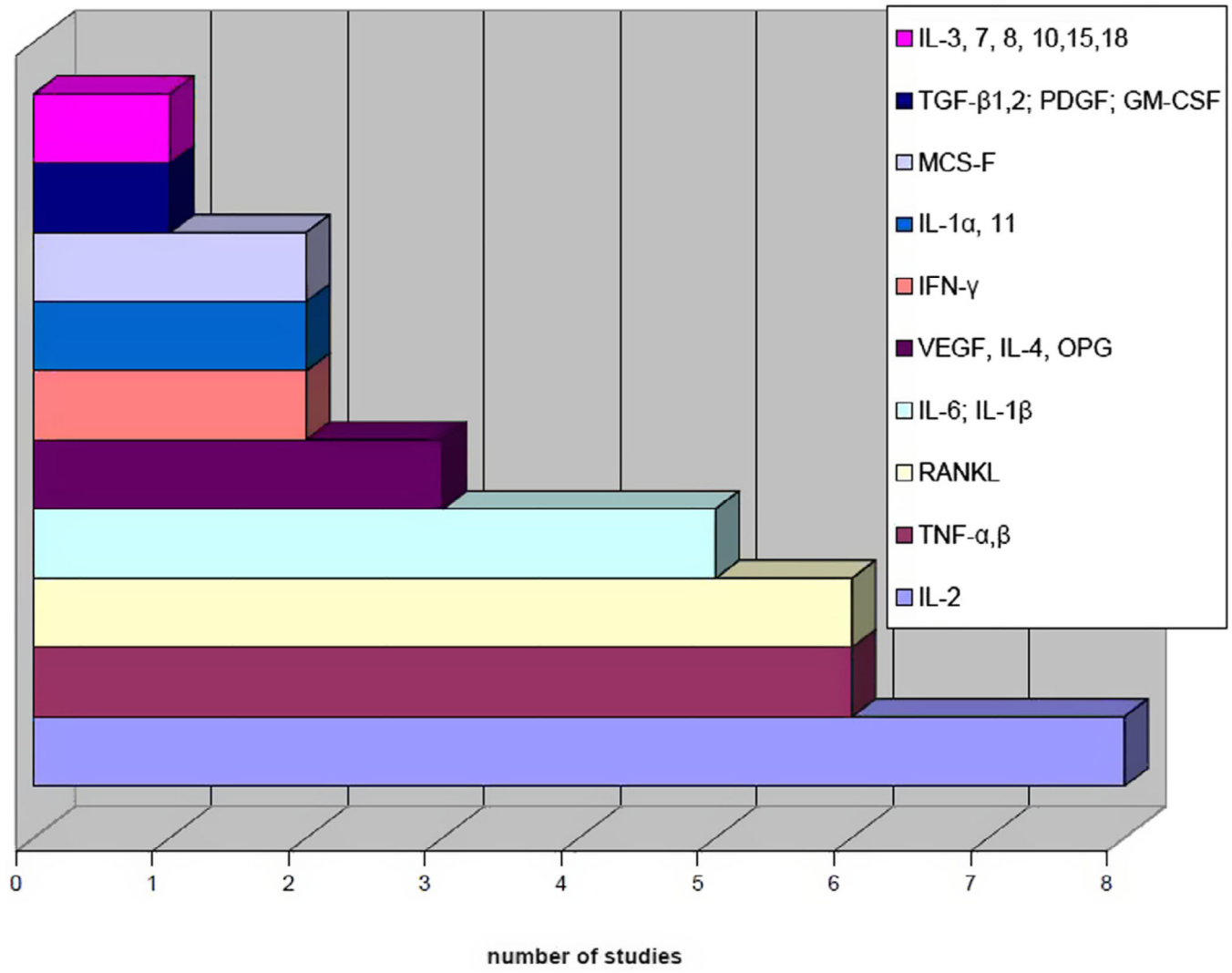
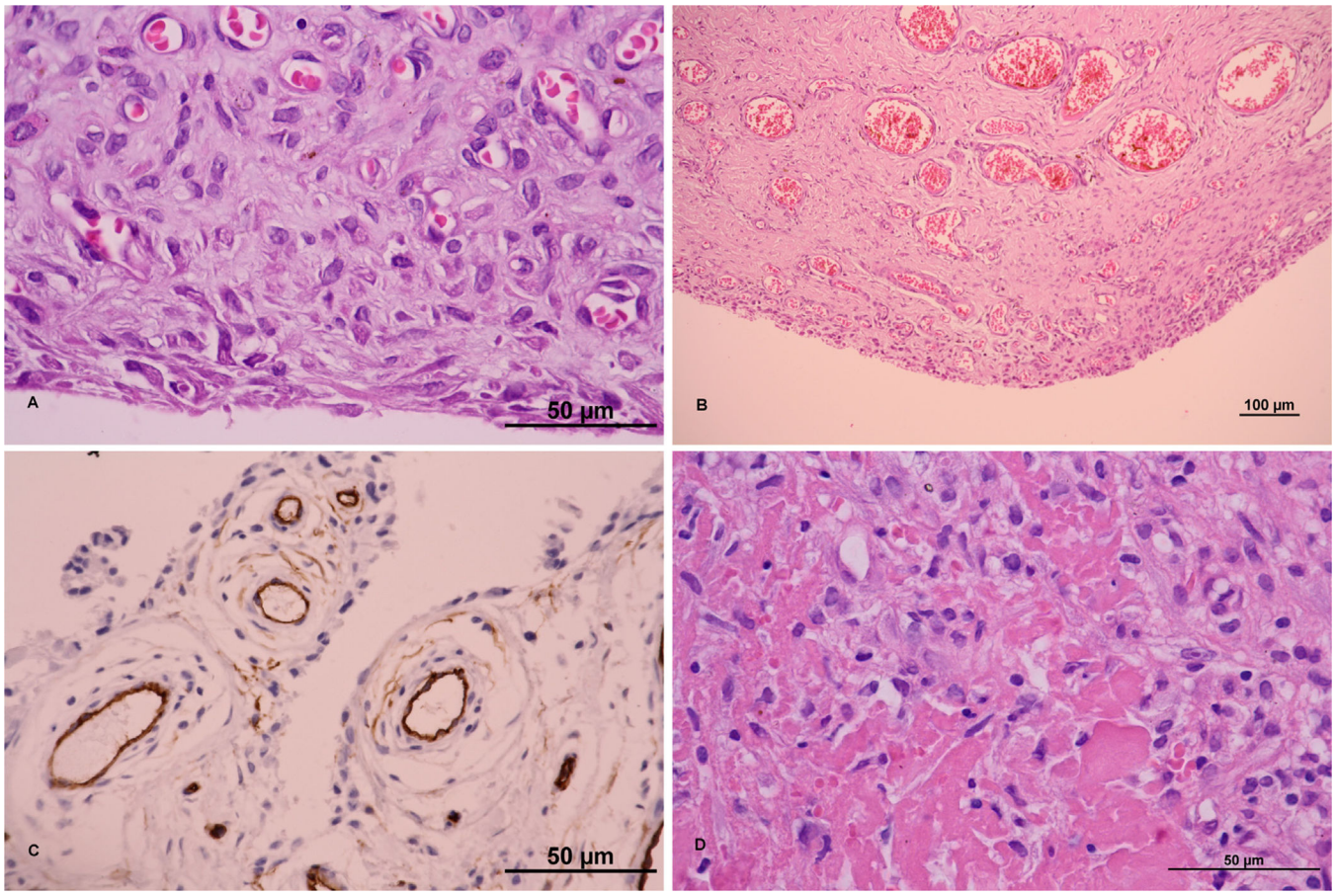


Fig. 3.
Studies focused on cytokines produced by harvested tissues.



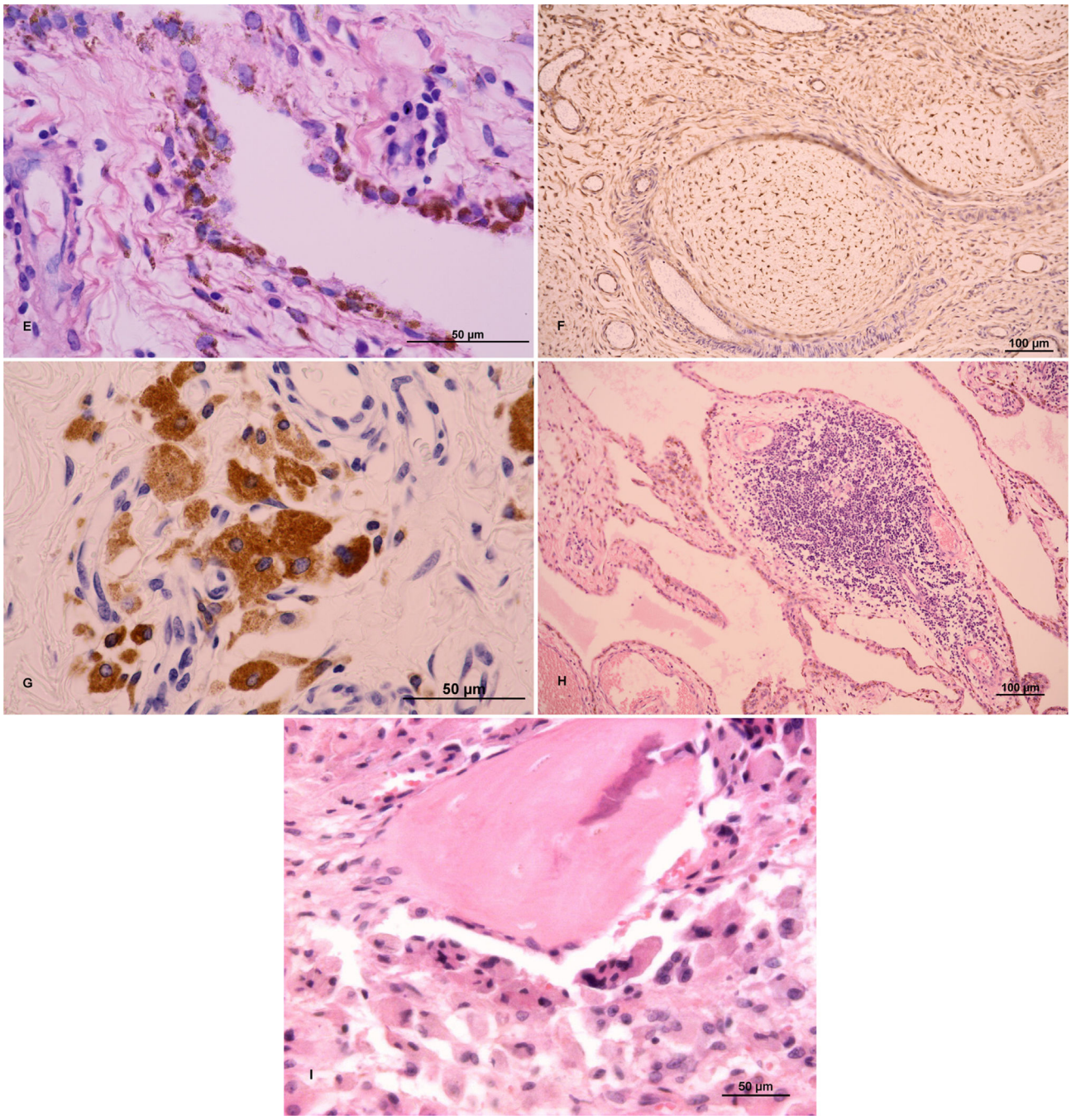


Fig. 4.

(A) Hyperplasia of synovial membrane, fibroproliferative changes in subsynovial stroma, reactive neovascularization; hematoxylin and eosin (H&E); ×1000. (B) "Neovascularization" in the synovial membrane; H&E; ×200. (C) Highly vascularized synovial membrane; CD34-positive vessels; ×1000. (D) Fibroproliferative changes with fibrinoid degeneration of connective tissue; H&E; ×1000. (E) Synovial-like cells containing metallic debris; H&E; ×1000. (F) Nodular hyperplasia with reactive changes; vimentin-positive mesenchymal cells; v200. (G) Reactive changes with macrophages containing

granules of exogenous material; CD68; ×600. (H) Lymphocytic infiltration typical for chronic inflammatory changes around an implant; H&E; ×1000. (I) Bone covered by osteoblasts and osteoclasts with multiple nuclei; HE; ×200. A scale bar of 50 or 100 μm is included in each particular figure. Sources of figures: (A–H) Department of Pathology, University Hospital Ostrava, Czech Republic; (I) Department of Pathology, Faculty of Medicine and Dentistry, University Hospital, Palacky University Olomouc, Czech Republic.

Table 1

The sample size of studies.

No. of patients	No. of studies
≤20	55
21–50	20
51–100	9
101–150	5
151–200	5
201–300	2
301–551	3

Table 2

Special histological stains.

Special stain	To demonstrate
Fite	microorganisms [30]
Gram	microorganisms [74,77–80]
Gomori	fungi [30]
Giemsa	mast cells, parasites, fungi, wear particles [122,127,128]
Grocott	microorganisms [70]
Masson's trichrome	collagen [24]
Oil red O	lipid and wear particles [90]
PAS reaction	mucin, basement membrane, fungi, inclusions [70,72,92,127,134]
Pearl's reaction	hemosiderin, wear particles [30,92,112,122,127,134]
Sudan III	lipid and wear particles [5,90]
Toluidin blue	mast cells [74,90]
Van Gieson	elastic fibers and collagen [5,92,113,122,127]
Ziehl–Neelsen	mycobacteria [70]

Table 3

Histopathological classification of synovial-like interface membrane.

Moravietz et al. [56]		**Krenn et al. [108]: the revised classification with the additional pathologies**	
Type I Wear particle induced	- abundance of macrophages and multinuclear giant cells	Allergic alteration	- lymphocytic infiltration
			- positive allergy tests
	- rarely lymphocytes	Type I Wear particle induced	- abundance of macrophages and multinuclear giant cells
			- rarely lymphocytes
		+Necrosis	- wear debris, metal on metal implant
Type II Infectious type	- activated fibroblasts, proliferation of small blood ves-sels, oedema	Type II Infectious type	- activated fibroblasts, proliferation of small blood ves-sels, oedema
	- abundance of neutrophilic granulocytes		- abundance of neutrophilic granulocytes
	- plasma cells		- plasma cells
Type III Combined type	- combination of the histomorphological changes in types I and II	Type III Combined type	- combination of the histomorphological changes in types I and II
Type IV	- connective tissue rich in collagen fibers	Type IV	- connective tissue rich in collagen fibers
Indetermine type	- fibrin or fibroblasts and macrophages sometimes on the membrane surface	Indetermine type	- fibrin or fibroblasts and macrophages sometimes on the membrane surface
		Implant-associated arthrofibrosis	- fibrosis and β-catenin positivity
		Osseous pathologies	- osteomyelitis, osteonecrosis, ossification, osteopenia

Table 4

Histopathological differences associated with type of prosthesis.

Type of fixation	cemented [18,24,35,41,42,48,50,52,62,74,77,82,90,91,100,106,120]	- abundance of macrophages, greater histiocytic reaction
		- well-developed synovial-like interface membrane
		- wear debris
	cementless [28,40–42,47,52,61,62,66,74,82,88]	- organized fibroblastic tissue
		- fibroblasts forming bundles or sheets associated with collagen fibrils
Articulation surface	metal-on-metal [29,30,47,70,92,93,89,104,111,113,122,127,128,130–132,134]	- more ulcerated, granulomatous pseudotumors and connective tissue necrosis
		- perivascular and diffuse lymphocytic infiltration
		- predominantly T lymphocytes
		- macrophages phagocytosing metallic wear-debris particles
	metal-on-polyethylene [38,63,93,94,133,135]	- predominantly histiocytic inflammation, loose connective tissue, fibrous and synovial cells
	ceramic-on-ceramic [58,94,112]	- necrosis, abundance of macrophages, neutrophils and lymphocytes
		- particles in agglomerates
	ceramic-on-polyethylene [92,132]	- lower degree of diffuse perivascular lymphocytic infiltration than in metal on metal bearing
		• extensive necrosis

Table 5

Immunohistochemical analysis of apoptosis.

Apoptotic markers	FAS [63]
	BAK [62,63]
	BAX [62,69]
	TUNEL [66,69,119]
	cytochrome c [66]
	iNOS [40,66]
	Nitrotyrosine [40,66]
	p53 [62]
Inhibitors of apoptosis	Survivin [69]
	B-cell CLL/lymphoma 2 [62,69]
	XIAP [69]