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Histological Evaluation of Periprosthetic Infection Using HOES and CD15 Expression Analysis in Hip Revision Arthroplasty

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Abstract

Background. The effectiveness improvement and standardization of the methods of histological diagnosing periprosthetic infection (PPI) is an urgent task in the treatment of complications after large joint arthroplasty. ***Purpose of the study*** — Histopathological evaluation of the infection involvement of periprosthetic tissues at the stage of revision arthroplasty for deep infection of the hip using HOES scale and immunohistochemical analysis of CD15 expression. ***Materials and Methods.*** A single-center prospective study was performed on the clinical intraoperative material obtained at the stage of revision arthroplasty of the hip in 27 patients at the age of 65 (55÷69) years. The group of examination included patients with acute and chronic forms of deep periprosthetic infection. Light-optical microscopic investigation of the samples of periprosthetic connective-tissue membrane and bone tissue from the foci of infectious involvement was made on paraffin sections stained with hematoxylin and eosin; with the immunohistochemical reaction to determine the expression of CD15 neutrophil granulocyte markers. HOES Scale for pathohistological assessment was used in order to objectify osteomyelitis signs in periprosthetic bone tissue. ***Results.*** The signs of acute and chronic stages of periprosthetic osteomyelitis were observed in 9/16 patients with PPI chronic course within 1–30 months of postoperative period, from one to 18 months after manifestation of the symptoms. The signs of subsided osteomyelitis were determined in 12/27 patients with PPI of acute and chronic forms. Infected periprosthetic membranes were found in 19/27 clinical cases in the early and long-term time periods after arthroplasty surgery. A direct significant correlation was revealed between histopathological signs of infecting the periprosthetic bone and the connective-tissue periprosthetic membrane, especially strong one in patients with acute and chronic PPI osteomyelitis. ***Conclusion.*** The use of HOES Scale and the analysis of CD15 expression ensure the objectivity of PPI histological diagnosing. The results obtained indicate an increased risk of osteomyelitis development in patients with chronic periprosthetic infection after the hip arthroplasty.

Keywords: hip revision arthroplasty, periprosthetic infection, histology, osteomyelitis, periprosthetic membrane.

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Background

Periprosthetic joint infection (PJI) is a catastrophic complication of large joints arthroplasty, occupying one of the leading positions in the structure of revision arthroplasty causes[1, 2, 3]. Due to the annual increase in the number of such patients, the number of concomitant purulent complications naturally increases [4, 5]. The frequency of PJI after primary arthroplasty is quite low and is about 1%, however, with repeated infection after revision arthroplasty varies from 14 to 33% [3, 6, 7]. According to various data, after the diagnosis of PJI, the overall survival of the implant with total hip arthroplasty is from 67 to 97% for five years [5, 8, 9, 10].

Deep PJI is characterized by the most severe course, since it is common not only in the skin and subcutaneous tissue, but also in the peri-implantation zone with the involvement of muscle and bone tissues [11]. An infectious lesion of the periprosthetic bone during arthroplasty of large joints is a relatively new manifestation of osteomyelitis, requiring an individual approach to the choice of treatment methods, taking into account the severity and duration of symptoms. The frequency of osteomyelitis detection after revision surgery can reach 20% [12, 13]. Based on this, clarifying the localization of the infectious inflammatory process at the stage of revision arthroplasty is a key point in choosing the tactics of surgical treatment of this disease [12, 14].

Pathohistological confirmation of infectious inflammation is included in the list of internationally recognized criteria and is an integral part of the diagnosis of PJI [13, 15, 16]. The pathomorphological conclusion about the presence of PJI is formed mainly on the basis of histological examination of periprosthetic/neosynovial biofilms [7, 13, 15, 17]. At the same time, osteomyelitic lesion of the paraprosthetic bone is implied [3, 8, 18], but special publications on this topic are extremely few [19]. Meanwhile, according to a retrospective population cohort study, the risk of developing chronic osteomyelitis after total hip arthroplasty is quite high [19].

The gold standard for the diagnosis of osteomyelitis is pathomorphological examination, which is still difficult due to the lack of accurate histological criteria [12, 20]. The use of the HOES score scale (Histopathological osteomyelitis evaluation score) objectifies and increases the accuracy of differential pathohistological diagnosis of acute and especially chronic osteomyelitis [20, 21]. Immunohistochemical determination of the surface marker of neutrophil granulocytes CD15 is recommended to improve the quality of diagnosis of infectious inflammation in PJI [15, 22, 23]. However, the shared use of these approaches for the diagnosis of PJI has not yet been practiced.

The aim of the study was a histopathological assessment of the periprosthetic tissues infectious lesion at the stage of revision arthroplasty for a deep infection of the hip joint using the HOES scale and immunohistochemical determination of CD15.

Materials and Methods

Study design

The nature of the study is a single-center prospective study conducted from 2016 to 2019.

Inclusion criteria: deep hip PJI that meets large and small diagnostic signs according to the decisions of the international consensus on PJI (2018) [16].

Exclusion criteria: bilateral total hip arthroplasty, periprosthetic fracture or recurrent dislocation, bone cancer, histiocytic proliferative diseases, sarcoidosis, osteomyelitis, cases of uninformative tissue samples.

Patients

In 2016-2019, 390 patients who were referred from other institutions for hip PJI were treated on at the clinic of the NMIC TO named after G.A. Ilizarov. In 304 (78%) patients, the

infection developed after primary arthroplasty, in 86 (22%) – after revision arthroplasty. Taking into account the above criteria, 27 out of 97 patients underwent surgery by one orthopedic surgeon were included in the study. The group included patients aged 33 to 79 years.

For the correct presentation of patient information and research results, the data were grouped in accordance with the summary classification of PJI proposed by us (Table 1). Subgroup 1 included patients with acute PJI; patients of subgroup 2 were diagnosed with chronic, and in subgroup 3 – chronic persistent/recurrent forms of PJI.

Table 2 presents demographic and some clinical data on admitted patients, as well as the outcomes of their treatment.

Pathogens of periprosthetic infection

Gram–positive microflora in monoculture was identified in 13 patients in the material obtained during surgery, gram-negative microflora in monoculture in 5 patients, microbial associations in 4 patients.

Associations of microorganisms were represented: in subgroup 1 - K. pneumoniae and S. haemolyticus; in subgroup 2 - S. epidermidis, P. aeruginosa and E. faecalis; V. species, E. coli, extended-spectrum β-lactamase (ESBL), P. aeruginosa and K. pneumoniae; in subgroup 3 – S. saprophyticus and S. capitis. In 5 patients in subgroups 2 and 3, the results of microbiological examination were negative. Generalized data on the species belonging of microorganisms in the group of patients with PJI are presented in Table 3.

Table 1

Pilot classification of periprosthetic infection [7, 23, 24, 25, 26, 27]

		Type of periprosthetic infection		
		Acute	Chronic	Chronic persistent/recurrent
Pathogenesis	Postoperative (<90 days after surgery)	< 21 days after the initial manifestation of symptoms in the period up to 3 months after surgery	> 21 days after the initial manifestation of symptoms in the period up to 3 months after surgery	Persistence or recurrence of symptoms for more than 24 months after manifestation
	Hematogenic (>90 days after surgery)	< 21 days. from the moment of the initial manifestation of symptoms after 3 months or more after surgery	> 21 days. from the moment of the initial manifestation of symptoms 3 months or more after the operation	Persistence or recurrence of symptoms for more than 24 months after manifestation
Surgical treatment		Sanation of an infectious focus with replacement of all mobile and preservation of fixed components of the endoprosthesis	Complete removal of the endoprosthesis (one-, two- or multi-stage replacement)	Complete removal of the endoprosthesis (one-, two- or multi-stage replacement, resection arthroplasty or arthrodesis)

Table 2

Demographic and clinical characteristics of patients

Parameter	Subgroup			
	1 (n = 5)	2 (n = 16)	3 (n = 6)	Bce (n = 27)
Age, years, Me(Q1–Q3)	55 (39–51)	67 (64–73)	52 (39–59)	65 (55–68)
Gender, Male / Female	3/2	3/13	5/1	11/16
The period from endoprosthetics to the onset of infection, Me (Q1-Q3)	24 (22–24) days.	12 (1–18) months.	42 (15–60)* months.	–
The period from the onset of infection to revision surgery, Me(Q1-Q3)	17 (15–17) days.	4 (1–11) months.	60 (60–60)* months.	–
Concomitant diseases				
Diabetes	1	2	2	5
Chronic kidney disease	1	1	1	3
Immunodeficiency conditions (AIDS, viral hepatitis)	2	–	2	4
Oncological diseases		1	–	1
Obesity	1	3	1	5
Anemia	4	12	1	16
Types of surgery and cases of re-infection				
Debridement	5	–	–	5
One-step revision	–	3	2	5
Two-stage revision	–	13	3	16
Resection arthroplasty	–	–	1	1
Recurrence of periprosthetic infection	–	5	1	6

* In three patients of subgroup 3, the onset of the inflammatory process was considered to be the manifestation of purulent arthritis preceding primary revision arthroplasty; clinical and laboratory signs of intra-articular infection were absent at the time of primary arthroplasty surgery.

Table 3

Species spectrum of microorganisms in the group of patients with deep periprosthetic infection

Microorganism	Number of patients (total)			
	Subgroup 1	Subgroup 2	Subgroup 3	All subgroupds
Gram - positive				
Staphylococcus aureus	1	3	–	4
Staphylococcus aureus MRSA	–	2	–	2
Staphylococcus epidermidis	–	1	–	1
Staphylococcus epidermidis MRSE	1	–	1	2
Staphylococcus saprophyticus	–	1	1	2
Staphylococcus haemolyticus	1	–	–	1

Table 3

Species spectrum of microorganisms in the group of patients with deep periprosthetic infection

Staphylococcus capitis	–	–	1	1
Enterococcus faecalis	–	2	1	3
Peptostreptococcus magnus	1	–	–	1
Actinomyces spp	–	1	–	1
Gram - negative				
Pseudomonas aeruginosa	1	4	1	6
Klebsiella pneumoniae	1	1	–	2
Klebsiella pneumonia ESBL	–	1	–	1
Escherichia coli ESBL	–	1	–	1
Vibrio species	–	1	–	1
Monocultures	4	11	3	18
Mixed cultures	1	2	1	4
There is no growth	–	3	2	5

MRSA — methicillin-resistant Staphylococcus aureus;
MRSE — methicillin-resistant epidermal staphylococcus;
ESBL — extended-spectrum β-lactamase.

Histological and immunohistochemical studies

Histological examination of each clinical case was performed on 3-5 samples of intraoperatively excised periprosthetic tissues of the inflammation focus. Bone fragments and connective tissue periprosthetic membrane (PPM) were fixed in 10% neutral formalin. The samples were decalcified in a SakuraTM TDE 30 tissue histological processing machine with TDETM 30 solution (Sakura Finetek Europe, the Netherlands). The processing of soft tissues with bone inclusions was performed for one day, bone fragments - up to 5 days. Next, the tissue samples were dehydrated in ethanol, compacted in paraffin and microtomized. Histological sections 5-7 microns thick were stained with hematoxylin and eosin.

Immunohistochemical staining of paraffin sections was performed manually using primary rabbit antibodies against CD15 antigen (Anti-CD15 antibody [SP159] ab135377) diluted with buffer (ab64211) in a ratio of 1:50 in accordance with the manufacturer's recommendation (Abcam, UK). To identify sites of specific binding of primary antibodies, a peroxidase detection system with diaminobenzidine with a micropolymer (ab236469 — Rabbit specific HRP/DAB Detection IHC Detection Kit-Micropolymer) was used. The main stages of the analysis were carried out in accordance with the protocols posted on the manufacturer's website (Abcam, UK). The changes concerned the incubation conditions of dewaxed histological sections with primary antibodies: the procedure was performed for 1 hour at a temperature of 4° C. Primary antibodies were not used in the formulation of a negative control reaction.

Automated digitization of histological preparations was performed in a scanning microscope for laboratory studies PANNORAMIC Midi II BF (3DHISTECH Ltd.,

Hungary) using Whole-slide imaging technology. When obtaining digital images of the fields of view with a lens x40, an option was used to improve the image quality by scanning several focal planes with their subsequent digital alignment. A descriptive study of digital histological preparations, an estimate of the number of cells in the field of view with an area of 0.1 mm^2 was performed using the software product PANNORAMIC Viewer, version 2.4 (3DHISTECH Ltd., Hungary). When visually assessing the number of CD15-positive neutrophil granulocytes in the field of vision, we were guided by the recommendations of F. Krenn et al. [15]. At the same time, CD15-positive cells were not counted in the lumen of blood vessels and red bone marrow [28].

To objectify the histopathological signs of osteomyelitis, the HOES score scale developed by A. Tiemann et al. was used [20]. On a point scale (from 0 to 3 points), the severity of the osteomyelitic process was assessed on digital preparations according to the criteria:

- acute condition - osteonecrosis (A1), soft tissue necrosis (A2), granulocyte infiltration (A3);

- chronic condition - neoplasm/bone fibrosis (C1), lymphocytic-macrophage infiltration (C2).

According to the proposed formulas, the correspondence of the total score to the stages of osteomyelitis was determined:

$A1 + A2 + A3 \geq 4$ – acute;

$A1 + A2 + A3 + C1 + C2 \geq 6$ – active chronic ("blooming");

$C1 + C2 \geq 4$ – chronic;

$1 < C1 + C2 < 4$ – in remission (subsided);

$C1 + C2 \leq 1$ – there are no signs of osteomyelitis.

Histological typing of PPM was performed in accordance with the classification of V. Krenn et al (2014). Abrasive (I), infectious (II), combined (III), and indifferent or fibrous (IV) types were distinguished [29].

The infectious process was verified using the Feldman criterion – more than 5 neutrophils in at least five fields of view at a microscopic magnification of 400 [22]. At least 10 visual fields were examined for each tissue sample. The identification of wear particles in periprosthetic tissues was performed in accordance with the diagnostic algorithm developed by G. Perino et al [30].

Statistical analysis

Digital data was statistically processed using Microsoft Excel spreadsheets. Information about the age of patients, the duration of periods before and after the detection of PJI relative to the stages of primary and revision arthroplasty are presented in the form of medians (Me) and their lower and upper quartiles (Q1-Q3). The correlation between the detection of osteomyelitis signs and infection with PPM was evaluated using the nonparametric Spearman coefficient r with a statistical power of the criterion of 0.8-0.9 at a confidence level of 95% [31, 32].

Results

According to the HOES histological score scale, signs of acute osteomyelitis were noted only in one patient of subgroup 2 28 days after the manifestation of the infectious process (Table 4).

Morphological signs of acute osteomyelitis included a complex of necrotic changes in the bone and soft tissues of the lesion. The bone trabeculae were deformed and sequestered, had a rugged contour, and visually empty bone lacunae. Fibrin, tissue and cellular detritus, and reactively altered granulation tissue were detected in the intertrabecular spaces. Pronounced peritrabecular edema, hyperemia and thrombosis of the microcirculatory bed vessels were noted. Immunohistochemically determined the abundant infiltration of CD15-positive granulocytes (Fig. 1 a, b).

Chronic active ("blooming") osteomyelitis was diagnosed in 4 patients of subgroup 2 in the period from 4 to 10 months after the manifestation of infectious inflammation

Table 4

Evaluation of osteomyelitis stages and histological types of periprosthetic membranes in patients with deep hip periprosthetic infection

Subgroup	Number of patients	Osteomyelitis stage*					PPM type			
		нет	AO	CAO	CO	SO	I	II	III	IV
1	5	1	0	0	0	4	0	2	2	1
2	16	2	1	4	4	5	3	5	7	1
3	6	3	0	0	0	3	3	2	1	0
Total	27	6	1	4	4	12	6	9	10	2

* Osteomyelitis stages: AO — acute; CAO — chronic active; CO — chronic; SO — subsided.

signs (see Table 4). Bone microsequesters surrounded by reactively altered loose connective tissue infiltrated by polymorphonuclear leukocytes, lymphocytes, plasma cells, and monocytes were determined in the lesion. In the matrix of bone trabeculae there were numerous gluing lines, neglected lacunae and necrobiotically altered osteocytes. Reparative processes were weakly expressed. Multifocal microcirculation disorders were observed — vascular stasis, microthrombosis and peritrabecular edema (Fig. 1 c). CD15-positive neutrophil granulocytes were diffusely distributed and accumulated in microabsesses (Fig. 1 d).

Signs of the chronic stage of osteomyelitis were determined in four clinical cases of subgroup 2, signs of PJI persisted from 4 to 18 months (see Table 4). Histologically, a sign of productive inflammation was observed - bone marrow fibrosis with infiltration of reactively altered connective tissue mainly by lymphocytes, plasma cells, eosinophils, monocytes and macrophages. Bone tissue remodeling was noted: endosteal and periosteal osteogenesis, osteoclastic resorption. In the loose connective tissue of the intertrabecular spaces, phenomena of peritrabecular edema, uneven blood filling and microthrombs in the vessels of the intraosseous microcirculatory bed were observed. No more than 5 CD15-positive neutrophils were detected in most fields of view (Fig. 1, e, f).

In 12 treated patients in three subgroups, the histological assessment corresponded to subsided osteomyelitis (see Table 4). The trabecular network of the spongy bone substance was hypoplastic, the bone marrow was subjected to focal fibrosis and fatty degeneration. In its composition, cellular-inflammatory elements were determined: lymphocytes and mononuclear phagocytes, plasma cells. CD15-positive neutrophil granulocytes were rare, no more than one in the field of vision. Uneven blood filling of intraosseous blood vessels, edema of peritrabecular spaces were noted (Fig. 1, g, h).

Histological signs of osteomyelitis were absent in 6 patients underwent surgery on at various times after the manifestation of periprosthetic inflammation. Bone trabeculae formed a developed large-cell network with a typical red bone marrow of intertrabecular spaces. Nucleated osteocytes were detected in the bone lacunae, the surface of the trabeculae was lined mainly with resting cells, active osteoblasts were found (Fig. 1, i). A large number of CD15-positive cell elements were immunohistochemically stained, but they were not counted due to the impossibility of differential detection of granulocytes at the early and terminal stages of myelopoiesis (Fig. 1 j).

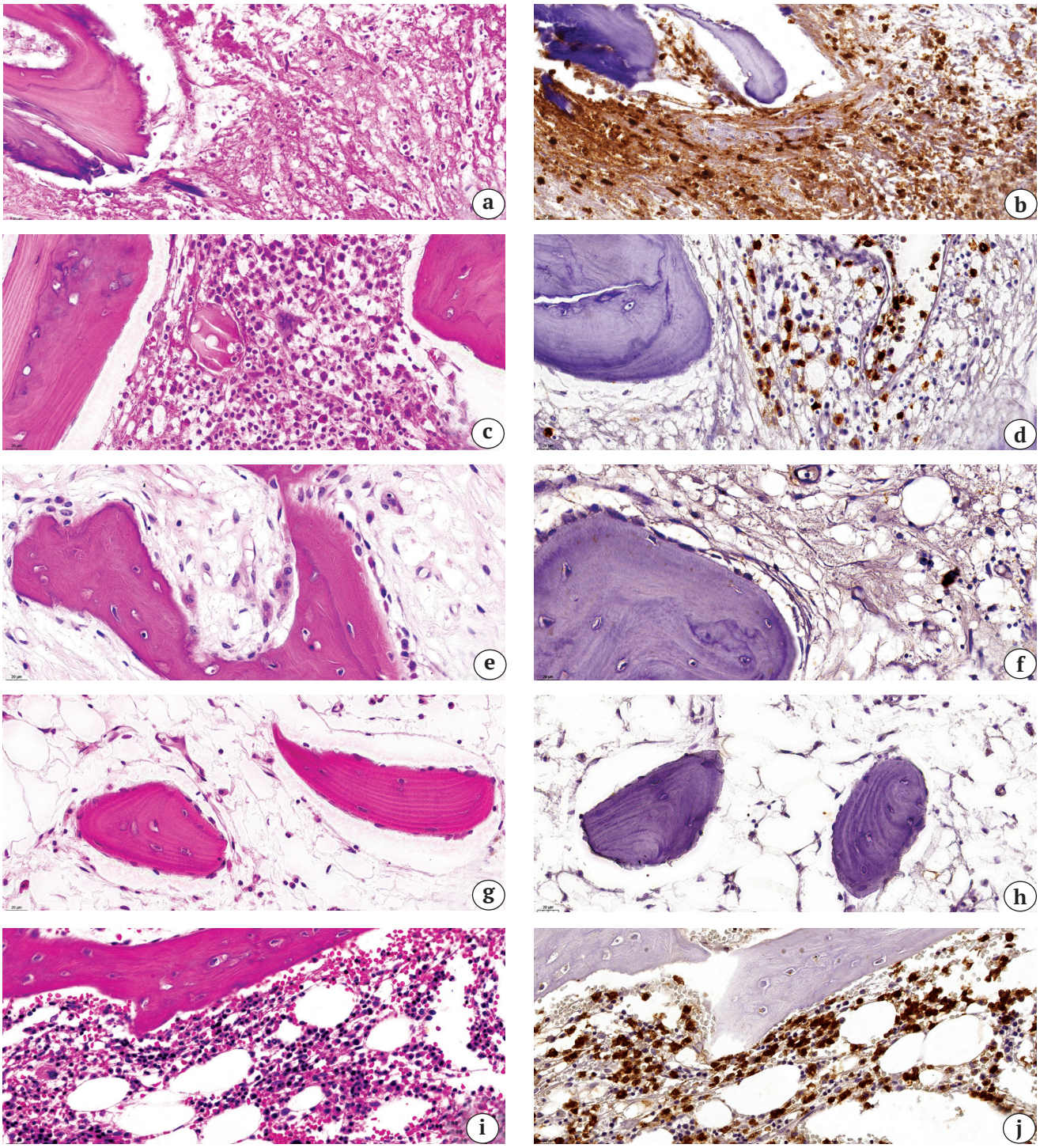

Fig. 1. Histology of periprosthetic cancellous bone in patients with deep periprosthetic infection of the hip at the stage of revision arthroplasty. Histological and immunohistochemical signs of acute (a, b), chronic active (c, d), chronic (e, f), subsided (g, h) osteomyelitis; spongy bone without signs of osteomyelitic involvement (i, j).
Paraffin sections. Staining with hematoxylin and eosin (a, c, e, g, i).
Immunohistochemical detection of CD15 antigen (b, d, f, h, j). Original mag. ×400; scale bar — 50 μm

Abrasive type I PPM was determined in six clinical cases in subgroups 2 and 3 more than 1 month after arthroplasty surgery. In the early postoperative period, the reactively altered fibrous connective tissue of the PPM was rich in fibroblast-like cells and elements of the monocyte-macrophage series, circulatory lymphocytic infiltration was noted in some fields of view. The vessels of the microcirculatory bed were wide, full-blooded, there were foci of hemorrhages. At a later date, fibrous components of the tissue matrix prevailed, cell density was reduced. Wear particles differing in morphological features were detected in many fields of view. Among them there were transparent polygonal polyethylene particles with a diameter of about 10 microns; conglomerates of black metal particles with a diameter of about 1 microns or less, as well as loose clusters of small grayish-brown ceramic particles (Fig. 2, a, b). Giant multinucleated cells were visualized near large foreign particles. Small particles, hemosiderin were phagocytized by macrophages with weakly CD15-positive cytoplasm (Fig. 2, b). Single CD15-positive polymorphonuclear granulocytes were observed in separate fields of vision.

Infectious type II PPM were registered in 9 clinical cases, in each of the observation subgroups (see Table 4). Reactively altered fibrous connective tissue was determined in the intraoperative samples. Along with the loosening of the collagen bundles, a high density of fibroblasts and the circulatory localization of lymphohistiocytic infiltrates were observed (Fig. 2, c). Uneven blood filling, stasis, and neoangiogenesis were noted in the microcirculatory vascular network. Numerous foci of tissue and cellular destruction were detected in combination with inflammatory edema and abundant infiltration by CD15-positive polymorphonuclear leukocytes (Fig. 2, d).

Combined type III PPM was identified in 10 patients, mainly in subgroup 2 (see Table 4). Its histological characteristics combined signs of type I and II PPM. In the reactively altered connective tissue, loosening and destruction of collagen fiber bundles, high numerical density of fibroblasts, foci of neoangiogenesis, inflammatory edema, infiltration by lymphocytes, monocytes, CD15-positive polymorphonuclear leukocytes were noted. Clusters of wear microparticles were detected in many fields of view (Fig. 2, i, f).

PPM of indifferent type IV was observed in two clinical cases in the period up to 2 months after arthroplasty surgery (see Table 4). In the studied samples, fibrous tissue with a low content of cells and vessels of the microcirculatory bed, rich in collagen fibers, was determined. Loose connective tissue layers between fibrous strands were vascularized by full-blooded capillaries, infiltration by inflammatory cell elements was not observed (Fig. 2, g, h).

The analysis of the data obtained allowed us to establish a link between the detection of histological signs of periprosthetic osteomyelitis and infection with PPM in patients with PJI. Figure 3 shows that infectious and combined types of PPM were determined mainly in combination with signs of acute, chronic active, chronic and subsided osteomyelitis. At the same time, abrasive type I PPM was found both in patients with signs of subsided osteomyelitis and without signs of intraosseous infection. PPM of indifferent type IV was observed only in the absence of an inflammatory process in the bone tissue.

Statistical analysis of the correlation between histological assessments of osteomyelitic lesions of the periprosthetic bone and infection of connective tissue PPM revealed a direct statistically significant relationship, especially pronounced in cases of acute, chronic active and chronic stages of osteomyelitis (Table 5). The correlation between histologically confirmed osteomyelitis and positive results of microbiological testing of intraoperative samples was also direct and significant, but generally less close.

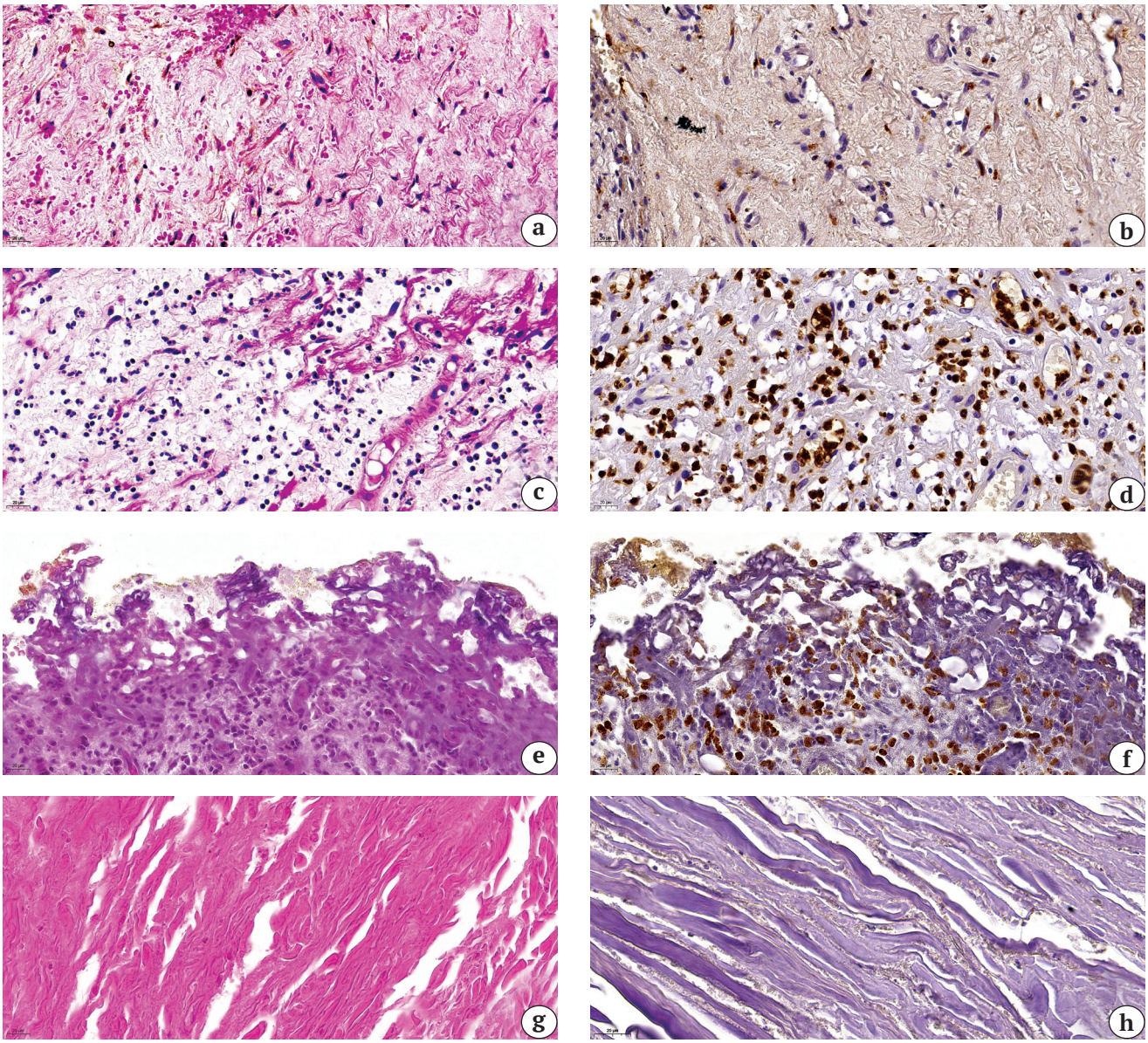

Fig. 2. Histological types of periprosthetic membranes in patients with deep periprosthetic infection of the hip at the stage of revision arthrroplasty: abrasion-induced type I (a, b), infectious type II (c, d), combined type III (e, f), indifferent (fibrous) type IV (g, h). Paraffin sections. Staining with hematoxylin and eosin (a, c, d, g). Immunohistochemical detection of CD15 antigen (b, d, f, h).
Original mag. ×400; scale bar — 50 μм

Table 5

Correlation of histological assessments of periprosthetic osteomyelitis with histological signs of infection of periprosthetic membranes and the results of microbiological testing

Stage of osteomyelitis	Spearman's coefficient r		Tightness of communication on the Cheddock scale		Criterion power*		The direction / significance of communication**
	PM	MT	PM	MT	PM	MT	
All results	0,85	0,60	High	Notable	0,9	<0,8	Direct / significant
Acute and chronic	0,99	0,83	Very high	High	0,9	<0,8	Direct / significant
Subsided	0,72	0,79	High	High	<0,8	0,8	Direct / significant
No signs	0,78	0,55	High	Notable	<0,8	<0,8	Direct / insignificant

* — the power value of the criterion at p<0.05; ** - the dependence of signs is statistically significant at p<0.05; PM - histological signs of infection of the periprosthetic membrane; MT - the results of microbiological tests.

Relapses of PJI after treatment were noted in 6 out of 22 patients in subgroups 2 and 3 (see Table 2). All 6 patients had signs of osteomyelitis of various stages (subsided, chronic, chronic active), infected with type II or III PPM. Aggressive gram—positive or gram-negative microflora (Staphylococcus aureus MRSA, Pseudomonas aeruginosa) was found in 5 patients with chronic PJI, and Enterococcus faecalis in monoculture was found in a patient with recurrent PJI.

Discussion

Histological examination of periprosthetic tissues is an important analytical tool for assessing the body's response to the implant and possible infectious complications. Its standardization is necessary to obtain accurate and reproducible data in complex clinical diagnostics, when conducting comparative scientific research and entering information into arthroplasty registers [33]. The authors of several clinical studies have found that the study of tissue samples obtained intraoperatively or as a result of an open biopsy is more informative in comparison with the analysis of an aspirate or needle biopsy [7, 34, 35, 36]. According to the current Russian and international clinical guidelines, histological examination of intraoperative samples of intraarticular and periarticular tissues is mandatory in case of suspected PJI [7, 11, 15, 16, 36, 37]. In accordance with these recommendations, we performed a single-center prospective histological examination of bone and connective tissue biopsies obtained during revision arthroplasty surgery in 27 patients with deep hip PJI.

Counting the number of polymorphonuclear neutrophil granulocytes in the tissues of the periprosthetic/neosynovial biofilms is one of the generally accepted diagnostic criteria for PJI according to the recommendations of the American Academy of Orthopaedic Surgeons (AAOS) and conciliatory conferences on musculoskeletal infection. It is recommended to analyze threshold values or the maximum concentration of neutrophils in high-power fields of view using different staining techniques [16, 28, 29, 37]. In our study, the use of this technique made it possible to identify infected PPM of types II and III in 19 out of 27 clinical cases in the early and long-term after arthroplasty surgery.

According to A. Tiemann et al., histopathological assessment is an indispensable condition for the diagnosis of infectious bone lesion, since microbiological examination is associated with an unacceptably high fre-

quency of false negative results (up to 30%). In addition, histopathological examination of bone tissue provides differential diagnosis of neoplastic diseases [20]. V. Krenn and G. Perino consider pathohistological examination of periprosthetic bone lesion mandatory and define it as type VII implant-associated local pathology [33]. The HOES histological assessment scale, developed to provide a standardized and reproducible diagnosis of osteomyelitis, has confirmed its adequacy in clinical models of acute and chronic osteomyelitis of various localization [20, 21].

Using the HOES assessment scale for the analysis of surgical material, we found that signs of acute, active and inactive chronic periprosthetic osteomyelitis were observed in 9 out of 16 patients with chronic course of PJI in the period from 1 to 30 months postoperative period, from 1 to 18 months after the manifestation of symptoms. In 12 out of 27 patients with acute, chronic and persistent/recurrent forms of PJI, signs of subsided osteomyelitis were identified. A direct significant correlation was revealed between the histopathological signs of infection of the periprosthetic bone and connective tissue PPM. This relationship was extremely strong (functional) in the case of acute and chronic stages of osteomyelitis. At the same time, the correlation between the histological signs of osteomyelitis and the results of microbiological testing was less close, which corresponds to the published data on the high frequency of false negative results of the latter [20].

To date, it has been established that immunohistochemical examination of CD15 antigen on the surface of neutrophils significantly increases the diagnostic accuracy of pathohistological diagnosis of bacterial infection. Published papers report intensive CD15 labeling on the neutrophil surface in infected periprosthetic/neosynovial membranes [15, 17, 20]. The results of our studies also demonstrate the informative value of CD15 immunohistochemical analysis for the diagnosis of PJI on the material of PPM. However, we have not found any publications on histopathological studies of periprosthetic bone tissue using this technique.

According to the data obtained by us, the infectious inflammatory process in the intertrabecular spaces of the periprosthetic bone leads to the replacement of the red bone marrow with reactively altered connective tissue infiltrated by inflammatory cellular elements. Immunohistochemical analysis of CD15 makes it possible to accurately identify the localization of neutrophil granulocytes in histological sections of the affected bone, which contributed to the standardization of the osteomyelitis signs assessment on the HOES scale. PJI diagnostic algorithms are in the active stage of development [38], and our results can be useful for improving the methodological base.

The information we obtained about the presence of periprosthetic osteomyelitis histological signs at the stage of revision hip arthroplasty is consistent with the results of a retrospective cohort study by D.Z. Hung et al., which showed that total hip arthroplasty is associated with a significant risk of developing periprosthetic chronic osteomyelitis - 12.3% during the first year after surgery [19]. In our study, the acute stage and escalation of the osteomyelitis chronic stage were diagnosed in 5 out of 27 patients with PJI during the first year after primary arthroplasty. The chronic course of osteomyelitis was observed in 4 out of 27 patients in the period up to two years after the previous endoprosthesis implantation.

In the light of the data obtained, the recommendation of T. Winkler et al. to consider each case with pain syndrome in the area of hip arthroplasty as a potential infectious complication, especially during the first 2-3 years after surgery [7], acquires a deeper meaning. The correlation revealed by us between the infectious lesion of connective tissue PPM and paraprosthetic bone tissue confirms the rationality of choosing revision

surgery with the removal of all elements of the infected endoprosthesis or resection arthroplasty as the main methods of treatment [7, 8, 39].

Limitations of the study

The limitation of the study is the small sample size of patients with deep PJI at the stage of revision hip arthroplasty. For this reason, the analysis of the relationship of the identified pathohistological signs of PJI with the comorbid background, age and sex of patients was not performed. No intraoperative histological examination was performed on frozen sections of PPM. The immunohistochemical analysis procedure was not automated. It is possible that some of the results of histological analysis of PJI were false negative due to mistakes in intraoperative tissue sampling. Despite the limitations, the study has potentially clinical significance. The results obtained indicate a high risk of osteomyelitis in patients with chronic PJI after hip arthroplasty.

Conclusion

Currently, no PJI diagnostic method is absolutely reliable due to its inherent features that affect sensitivity and specificity. The proposed set of techniques provides an objective increase in the reliability of histological analysis as an integral part of measures for the diagnosis of PJI. It is advisable to study the manifestations of PJI in the tissues of the bone bed and connective tissue PPM using the standardized scale of pathohistological assessment of osteomyelitis HOES and immunohistochemical determination of the surface marker of neutrophil granulocytes CD15 as additional research methods.

Ethical expertise

The study was approved by the Ethical Council of the institution (Protocol No. 2 (57) of 17.05.2018).

Informed consent

All patients gave written informed consent to be included in the study.

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Conflict of interest:

The authors declare that there is no conflict of interest.