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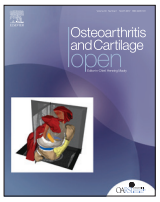

Mature and inactive microvessels are prominent in areolar synovium of femoroacetabular impingement and hip osteoarthritis patients

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ABSTRACT

Objective: To provide insight into the earliest changes in hip osteoarthritis (OA) pathogenesis. Histopathology of synovium was investigated in patients with femoroacetabular impingement (FAI), FAI with early hip osteoarthritis, and advanced hip osteoarthritis.

Methods: Synovium biopsies were collected from ten FAI, fourteen FAI with early osteoarthritis, and twelve advanced osteoarthritis patients. Histopathological grading allowed assessment of osteoarthritis-associated features. Microvessel density and maturity were determined through immunofluorescent labelling of CD31 and α-smooth muscle actin. Immunohistochemical staining was applied to calculate $CD105^{+}$ microvessel density, providing insight into microvessel activity.

Results: In all groups, vascularization was prominent, with a mean [95 % confidence interval] of 1.64 [1.40, 1.89]. In all three groups, mature microvessel density was greater than immature microvessel density (82.32 [62.92, 101.71] versus 14.84 [9.86, 19.83] microvessels/mm^2). Low $CD105^{+}$ microvessel density across all groups (3.14 [0.92, 5.37] microvessels/mm^2) suggests microvessel inactivity. Inflammatory composite scores were significantly greater in the advanced OA (1.08 [0.84, 1.32]) versus the FAI group (0.47 [0.26, 0.68]), and in the advanced OA versus the FAI with early OA group (0.69 [0.49, 0.89]) ($p < 0.017$).

Conclusion: Synovium from patients with FAI (with and without hip osteoarthritis) demonstrated synovitis and other OA-associated changes. Mature, inactive microvasculature was prominent in all three groups investigated. Histopathological similarities between FAI and hip OA synovium indicate that disordered synovium appears in FAI patients. These findings highlight a potential role of synovial changes in the progression from FAI to hip OA, underscoring the need for early intervention and further investigation into early disease mechanisms.

1. Introduction

Synovitis is an abnormal change seen in osteoarthritic joints and is associated with worse pain in early- and late-stage osteoarthritis (OA) patients [1–3]. Much of what we know regarding synovitis in OA comes from synovial tissue samples of knee rather than hip OA patients, which have shown increased immune cell infiltration [4], accumulation of apoptotic cells [5], along with microvascular dysfunction (MVD) marked by increased vascularization and perivascular edema [6]. Chronic synovitis leads to synovial fibrosis, which is observed in late-stage knee OA patients [7] and has been shown to negatively correlate with pre-operative range of motion in the knee [8]. Despite acknowledgement of abnormal changes in the synovium of early- and late-stage knee OA patients, there is limited investigation of these features in hip OA. Specific investigations of hip OA are necessary because despite also being a major cause of disability worldwide, much of what is assumed about hip OA is extracted from knee OA studies [9].

Although OA of the hip is underreported compared to that of the knee [9], the hip may offer a unique ability to observe the earliest manifestations of OA pathogenesis through patients with femoroacetabular impingement (FAI), a pre-arthritic condition. FAI is a hip disorder commonly observed in younger patients and is characterized by irregular morphology of the proximal femur and/or acetabulum [10], producing mechanical conflict between the two during physiologic range of motion.

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FAI commonly results in damage to the labrum and shear forces to the articular cartilage in the acetabulum. This chronic trauma to osteoarticular joint structures can lead to secondary hip OA [11]. Furthermore, FAI is often treated arthroscopically, making biopsy collection and therefore tissue analysis possible. Because of its pre-arthritic nature, the young population it affects, and its recommended surgical treatment, FAI may offer interesting insights into the earliest changes in synovium as they relate to hip OA pathogenesis.

Previous histopathology reports of synovium from FAI patients have not provided substantial insight into the earliest changes preceding hip OA development. One study demonstrated low synovitis scores for early FAI patients and increased scores for late FAI patients [12], while another reported inflammation in synovium from FAI patients without a description of tissue-specific changes [13]. Although these studies suggest synovial abnormalities are present in some FAI patients, the scoring system used in both reported an overall synovitis score derived from 3 features: thickened lining, stromal cell density, and leukocyte infiltration [14]. Though these are important features to be considered during the histopathological analysis of OA synovium, they do not include features of fibrosis or MVD — both of which are prominent in osteoarthritic synovium and are speculated to affect function [8,15].

The present study seeks to describe abnormalities and determine differences in synovial features across a spectrum of arthritic hip disorders, from FAI to early-stage hip OA (secondary to FAI), to advanced-stage hip OA. Its purpose is to determine the earliest synovial changes preceding the onset of hip OA, which may inform future diagnostic and therapeutic approaches. To accomplish this, three central aims will be addressed: (1) seven features known to manifest in OA synovium will be assessed — lining thickness, cellular infiltrate, fibrin deposition, vascularization, fibrosis, perivascular edema, and vasculopathy; (2) the status of microvascular maturity will be measured, providing insight into the nature of microvasculature across this spectrum of hip disorders; and (3) microvascular activity will also be investigated to determine whether active neovascularization contributes to the earliest changes observable in OA. We hypothesize the synovium of symptomatic FAI patients will demonstrate pathological tissue-level changes associated with changes in hip OA synovium.

2. Methods

2.1. Ethics approval

The study received approval from Western University's Research Ethics Board (122040). Permission was granted to include biobanked samples from an ongoing study (114679) in the present analysis.

2.1. Participants

Patients were considered for recruitment if they experienced symptomatic FAI and/or symptomatic hip OA diagnosed by an orthopaedic surgeon (RD or BL), based on a combination of clinical findings and standard views of plain radiographs [16]. In the case of FAI patients, radiographs were assessed by an orthopaedic surgeon for cam and pincer morphology. Patients that were scheduled to undergo hip arthroscopy or total hip arthroplasty were invited to participate.

Patients were eligible if they satisfied the following inclusion criteria: (1) radiographic evidence of unilateral or bilateral symptomatic FAI and/or OA; (2) undergoing arthroplasty or arthroscopy for their affected hip. Patients were considered ineligible if their upcoming surgery was a revision hip arthroplasty. Participants were placed into one of the following three groups based on clinical and radiographic findings: FAI, FAI with early secondary hip OA, and advanced hip OA. Patients with FAI and a Tönnis grade of 0 were placed in the FAI group. None of these patients had undergone prior arthroscopy. FAI patients with early secondary hip OA (Tönnis grade of 1 or 2) were placed in the second group. Advanced hip OA participants were those with a Tönnis grade of 3. All participants provided informed consent for collection of areolar synovium at the time of surgery, and for completion of patient-reported outcome measures (PROMs). Demographic data, including PROMs, were collected as descriptive data to provide contextual information regarding the study's population. The following PROMs were collected: modified Harris Hip Score, Hip Outcome Score, and the International Hip Outcome Tool-33 (iHOT-33). These three PROMs were selected due to validity and reliability in a variety of hip pathologies (mHHS) [17], and validity in assessing younger, active patients with hip pathologies (HOS, iHOT-33) [18,19]. Because advanced OA PROM data was collected in-person under time constraints rather than via web-based questionnaires, there is unfortunately some discrepancy in availability of PROM data from this group of participants (Table 1).

2.2. Biospecimen collection, preparation, and grading

Participants undergoing both surgery types agreed to donate biopsy samples of synovium. Orthopaedic surgeons were responsible for collecting biopsies from participants during arthroscopy and arthroplasty (RD or BL). Areolar synovium biopsies were collected from the intracapsular recess of the hip. Tissues were fixed in 4 % paraformaldehyde (PFA) overnight (16–24 h) and transferred to 70 % ethanol to be stored prior to tissue processing. Processed tissues were embedded in paraffin wax, sectioned in 5 μm increments, and collected on microscope slides.

Five slides per sample (each 100 μm apart) were selected, baked at 60 °C for 30 min, and deparaffinized and stained with hematoxylin and eosin (H&E). Histopathological scoring of synovium was conducted following the Minten grading system [6]. Six features, as described by Minten et al. (2019), were assessed: (1) synovial lining thickness, (2) sub-synovial infiltration, (3) fibrin deposition, (4) vascularization, (5) fibrosis, (6) perivascular edema [6]. A seventh parameter (vasculopathy) was also applied, as described in a publication by Sodhi et al. (2022) [7]. Five slides per sample were visualized under a Leica DM1000 LED microscope at 200 × magnification. Each slide was reviewed by an independent grader (RA). The 7 histopathologic parameters were graded for each of 5 regions of interest (ROI), and the score for each parameter in a given sample was determined as the mean average of each parameter across 5 slides. An inflammatory composite score was also determined for each ROI [6]. Slides were then reviewed by a second grader (DK) to ensure interrater reliability. The interrater reliability for each histopathological parameter was calculated using Cohen's Kappa [20], achieving the following: *synovial lining thickness* ($\kappa = 0.63$), *sub-synovial infiltration* ($\kappa = 0.75$), *surface fibrin deposition* ($\kappa = 0.55$), *vascularization* ($\kappa = 0.89$), *fibrosis* ($\kappa = 0.59$), and *perivascular edema* ($\kappa = 0.69$) [20]. These scores fall within the almost perfect (0.81–1.00), substantial (0.61–0.80), and moderate (0.41–0.60) agreement ranges as defined by Cohen [20]. Moderate agreement of fibrin and fibrosis were also achieved in the publication describing this system [6].

2.3. Assessment of microvessel maturity with immunofluorescence

Selected slides were deparaffinized and rehydrated, followed by antigen retrieval and permeabilization. Dual immunofluorescent labelling of endothelial cells and smooth muscle cells was achieved using the following primary antibodies, respectively: Rabbit Monoclonal Anti-CD31 (0.519 mg/mL, ab182981, Abcam) and Mouse Monoclonal Anti-α-Smooth Muscle Actin (α-SMA) (1.0 mg/mL, M0851, Agilent, USA). A master mix was created using a 1:500 dilution factor for anti-CD31 and 1:250 dilution factor for anti-α-SMA in blocking solution. Sections were incubated for 1 h with the following fluorescently labelled secondary antibodies (both diluted to 1:500 in 1 × PBS): Goat Anti-Rabbit Alexa Fluor 488 (C840N74, Jackson Immunoresearch, USA) and Goat Anti-Mouse Alexa Fluor 647 (C840X19, Jackson Immunoresearch, USA). Coverslips were mounted using Invitrogen ProLong Gold Antifade Mountant with DAPI (4′,6-diamidino-2-phenylindole) (P36941, Fischer Scientific, Canada) to label cell nuclei.

Table 1
Participant baseline demographics, clinical characteristics, and clinical outcome scores ($n = 37$).

Parameter	FAI ($n = 10$)	FAI w/Early OA ($n = 15$)	Advanced OA ($n = 12$)	Total ($n = 37$)
Sex, *n* (%)				
Male	5 (50)	10 (67)	8 (67)	23 (62)
Female	5 (50)	5 (33)	4 (33)	14 (38)
Age, years	28.4 ± 8.5 (17, 41)[d]	48.8 ± 8.0 (36, 61)[d]	67.2 ± 7.7 (56, 82)[d]	49.2 ± 17.0 (17, 82)
BMI, kg/m^2	28.1 ± 3.8 (23.2, 33.0)	28.8 ± 4.3 (21.4, 36.2)	29.0 ± 1.2 (26.4, 30.6)	28.7 ± 3.4 (21.4, 36.2)
Tönnis grade, *n* (%)				
0	10 (100)	–	–	10 (27)
1	–	13 (87)	–	13 (35)
2	–	2 (13)	–	2 (6)
3	–	–	12 (100)	12 (32)
Impingement type[a], *n* (%)				
Cam	2 (20)	5 (33)	–	7 (19)
Pincer	1 (10)	–	–	1 (3)
Mixed	7 (70)	10 (67)	–	17 (46)
Labral tear[a], *n* (%)				
Yes	10 (100)	14 (93)	–	24 (64)
No	–	1 (7)	–	1 (3)
VAS pain score	38.8 ± 22.1 (10.0, 80.0)	28.9 ± 18.7 (0, 78.0)	27.8 ± 24.2 (0, 79.0)	31.2 ± 21.4 (0, 80.0)
iHOT-33				
Total[c]	36.4 ± 16.9 (18.3, 58.5)	31.9 ± 17.0 (7.1, 61.6)	–	33.7 ± 16.7 (7.1, 61.6)
Symptoms & functional Limitations	52.1 ± 18.8 (30.0, 91.9)	38.0 ± 20.8 (5.9, 83.5)	40.7 ± 22.7 (14.25, 89.13)	42.7 ± 21.2 (5.9, 91.9)
Sports & recreation[c]	22.2 ± 14.2 (8.0, 53.6)	24.6 ± 19.3 (0.4, 58.5)	–	23.7 ± 17.2 (0.4, 58.5)
Job-related concerns[c]	43.4 ± 29.3 (10.0, 95.0)	39.1 ± 22.8 (0, 82.0)	–	35.3 ± 26.2 (0, 95.0)
Social, emotional, and lifestyle concerns[c]	31.6 ± 14.9 (8.6, 60.3)	25.9 ± 19.0 (0, 61.7)	–	28.2 ± 17.4 (0, 61.7)
HOS (Total)[b]	57.5 ± 19.3 (17.1, 81.7)	48.4 ± 19.3 (25.0, 68.3)	–	52.0 ± 19.4 (17.1, 81.7)
ADL[c]	64.9 ± 16.1 (32.5, 89.7)	55.9 ± 20.0 (25.0, 94.1)	–	59.5 ± 18.8 (25.0, 94.1)
SS[c]	40.5 ± 23.2 (0, 66.7)	33.4 ± 25.9 (0, 72.2)	–	36.2 ± 24.6 (0, 72.2)
mHHS[c]	61.2 ± 9.8 (46.2, 73.6)	53.9 ± 14.7 (26.4, 73.6)	–	56.8 ± 13.2 (26.4, 73.6)

Continuous data are reported as mean average ± standard deviation (minimum value, maximum value). Categorical data are reported as *n* (%).
BMI = body mass index (kg/m^2); VAS = visual analog scale; iHOT-33 = International Hip Outcome Tool (33-item) HOS = Hip Outcome Score; ADL = activities of daily living; SS = sport subscale; mHHS = Modified Harris Hip Score; w/= with Tönnis Grade is a score assigned to pelvic radiographs to classifying hip osteoarthritis on a scale of 0–3 based on the following qualitative criteria: (0) no osteoarthritis; (1) mild joint cavity narrowing, mild sclerosis of acetabulum and/or femoral head; (2) moderate joint cavity narrowing, small acetabular/femoral head cysts, moderate decrease in femoral head sphericity; (3) severe or complete joint cavity narrowing; prominent acetabular/femoral head cysts, severe femoral head deformation, avascular necrosis.

[a] Advanced hip OA patients were not assessed for evidence of femoroacetabular impingement or labral tears, as FAI morphology is difficult to determine in severely degenerated hips and inconsequential for diagnosis and treatment.

[b] Visual analog scale of pain was scored from 0 (extreme pain) to 100 (no pain).

[c] Measures in this category were not collected for the advanced hip OA cohort. Therefore, total cohort averages for these denoted categories are based on averages between the FAI and early OA with FAI cohorts ($n = 25$).

[d] Statistically significant differences were found between each of the three groups for mean age.

A total of 5 ROIs per positive section (and 1 ROI per negative control section) were imaged using a Leica TCS SP8 MP Multiphoton Microscope at 400 × magnification. The proportions of all synovial microvessels, mature synovial microvessels, and immature synovial microvessels to synovial area were calculated by a single observer (RA) and reported as microvessels/mm^2 of synovial tissue area.

2.4. Assessment of microvessel activity with immunohistochemistry

From each of the three groups, areolar synovium samples on slides were prepared for immunohistochemistry (IHC). Slides were deparaffinized and rehydrated, followed by antigen retrieval, permeabilization, and exogenous peroxidase blocking. Primary antibody incubation was performed to investigate endothelial cell activity, with the following primary antibody diluted with blocking solution: Rabbit Polyclonal anti-Endoglin (1:100, 0.20 mg/mL, HPA067440, Atlas Antibodies, Sweden). Slides were incubated with the secondary antibody, Goat Anti-Rabbit IgG H&L HRP (1:2000, ab205718, Abcam). A DAB (3,3′-Diaminobenzidine) solution was added to each section on the slides for an optimized time of 3.5 min, followed by quenching in distilled water. Cell nuclei were then stained with Harris hematoxylin and rinsed. A total of 5 ROIs per positive section (and 5 ROIs per negative control section) were visualized using a Leica DM1000 LED microscope at 200 × magnification. Endoglin-positive microvessels were considered to be any microvessels with brown staining of the endothelial cell layer.

2.5. Statistical analysis

For all demographic data and participant clinical characteristics, results were reported as mean ± standard deviation for continuous variables, and frequencies with percentages for categorical variables. Maximum and minimum values were also reported for all continuous variables of demographic and clinical data. All histopathological results (including semi-quantitative scoring parameters and microvessel densities) were reported as means with 95 % confidence intervals for each group, as follows: mean [lower CI, upper CI]. The means for all continuous data of the three groups were compared using a one-way ANOVA ($\alpha = 0.05$). Where significance ($p < 0.05$) was established between the three groups for any given variable, post-hoc two-tailed Student's t-tests followed by Bonferroni corrections were applied to identify significant differences ($p < 0.017$) between any two specific groups.

2.6. Microvessel density calculations

For microvessel calculations, a microvessel was considered to have a diameter no greater than 30 μm [21]. For all calculations, the 5 ROIs with the most microvessels were assessed. Mature microvessel density was calculated as the total number of CD31-positive vessels in the synovial intima and subintima that had >75 % coverage by α-SMA in all 5 ROIs, per total synovial tissue area of all 5 ROIs. Immature microvessel density was calculated as the total number of CD31-positive vessels in the

synovial intima and subintima that had ≤75 % coverage by α-SMA in all 5 ROIs. Endoglin-positive microvessel density was calculated as the total number of stained microvessels in all 5 ROIs, per total synovial tissue area of all 5 ROIs. Only intimal and subintimal areas of each ROI were considered in total area calculations. ImageJ was used to quantify synovial area and measure vessel diameters.

3. Results

3.1. Patient demographics

Thirty-nine patients were screened for eligibility, with one being excluded following surgical rescheduling (2.6 %) and one excluded due to a revision hip arthroplasty making quality sample collection infeasible (2.6 %). All patients approached agreed to participate. A total of 37 participants were included in the analysis of this study. Eight participants were enrolled in the separate, ongoing study (HSREB no. 114679). In total, 10 patients (27 %) were placed in the FAI group, 15 (41 %) in the FAI with early hip OA group, and 12 (32 %) in the advanced hip OA group. Age was determined to be significantly different ($p < 0.017$) between all three groups. All patients in the advanced hip OA group completed baseline visual analog scales (VAS) of pain, and the iHOT-33 *Pain and Functional Limitations* subscale. No significance was found between VAS measures of pain nor the *Pain and Functional Limitations* subscale between any group ($p > 0.017$). Pre-operatively, all patients in both the FAI and FAI with early OA groups completed the iHOT-33, HOS, mHHS, and VAS of pain. Between these two groups, no significant differences were discovered in these measures ($p > 0.017$). All baseline demographics, clinical characteristics, and clinical outcome scores are summarized in Table 1.

3.2. Areolar synovium histopathological grading

Biopsies of areolar synovium were successfully collected from 37 patients. For areolar synovium, a total of 7 samples were graded using 2–4 slides, rather than 5 slides. Representative images of H&E-stained areolar synovium samples for each group are shown in Fig. 1. Following one-way ANOVA, no significance was detected between any of the three groups for synovial lining thickness, cellular infiltrate, vascularization, and vasculopathy scores ($p > 0.05$). Fibrin deposition scores demonstrated a statistically significant increase between the FAI group and the advanced OA group ($p < 0.017$). Fibrosis scores were significantly higher in the advanced OA group compared to both the FAI group and the FAI with early OA group ($p < 0.017$). Perivascular edema scores were only significantly increased in the advanced OA group compared to the FAI group ($p < 0.017$). Finally, inflammatory composite scores were significantly greater in the advanced OA group versus the FAI group, and significantly higher in the advanced OA group versus the FAI with early OA group ($p < 0.017$). A summary of histopathological grading of areolar synovium is found in Table 2. Fig. 2 displays a visual representation of the data outlined in Table 2.

3.3. Total, mature, and immature microvessel densities in areolar synovium

Microvessel densities are reported as vessels per mm^2 of synovial tissue area. For the FAI, FAI with OA, and advanced OA groups, respective average total microvessel densities were 96.00 [46.60, 145.31], 95.13 [56.91, 133.34], and 104.10 [71.05, 137.09]. Likewise, mature microvessel densities were 81.20 [40.00, 122.31], 73.78 [35.72, 111.83], and 93.96 [64.79, 123.13]. Finally, immature microvessel densities were 14.79 [5.48, 24.11], 20.72 [10.50, 30.95], and 7.54 [2.92, 12.16]. Following one-way ANOVA, no statistically significant differences were detected between the individual averages of the three groups for any of total microvessel, mature microvessel, or immature microvessel densities. These individual group values for microvessel densities are summarized in Table 3. Representative immunofluorescent images used to calculate microvessel densities are provided in Fig. 3.

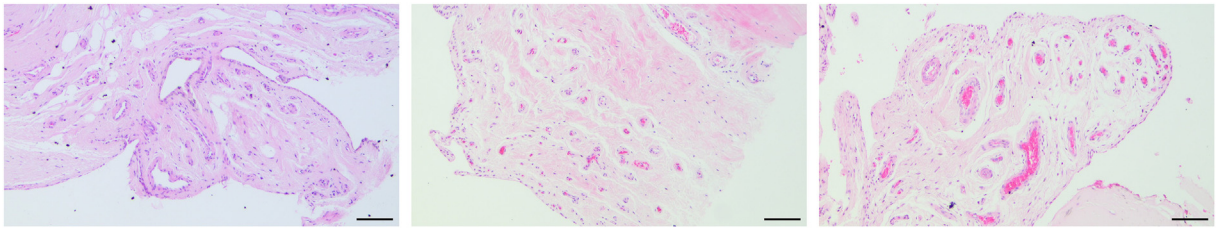

Fig. 1. Areolar synovium across a spectrum of hip disorders. Representative images of hematoxylin and eosin (H&E)-stained areolar synovium from FAI (*left*), FAI with early hip OA (*middle*), and advanced hip OA (*right*) patients which are close representatives for all 7 histopathological grading parameters and inflammatory composite scores for their respective cohort averages. Images were captured at 200 × magnification. Scalebars = 100 μm.

Table 2
Histopathological scoring of areolar synovium biopsies for features of inflammation, fibrosis, and microvascular dysfunction.

Parameter	FAI ($n = 10$)	FAI w/Early OA ($n = 15$)	Advanced OA ($n = 12$)	Total ($n = 37$)
Lining	0.06 [−0.01, 0.13]	0.37 [0.08, 0.66]	0.40 [0.19, 0.61]	0.30 [0.16, 0.43]
Infiltration	0.20 [−0.02, 0.42]	0.43 [0.21, 0.66]	0.77 [0.25, 1.29]	0.48 [0.28, 0.67]
Fibrin	0.43 [0.21, 0.65][a]	0.70 [0.43, 0.97]	0.88 [0.66, 1.09][a]	0.68 [0.54, 0.83]
Vascularization	1.35 [0.88, 1.81]	1.54 [1.17, 1.91]	2.02 [1.53, 2.51]	1.64 [1.40, 1.89]
Fibrosis	0.12 [−0.11, 0.35][a]	0.54 [0.28, 0.79][b]	1.68 [1.35, 2.00][a,b]	0.79 [0.54, 1.05]
Edema	0.14 [−0.07, 0.35][a]	0.42 [0.10, 0.74]	1.11 [0.59, 1.62][a]	0.57 [0.33, 0.81]
Vasculopathy	0.19 [−0.05, 0.43]	0.13 [−0.05, 0.31]	0.27 [0.02, 0.52]	0.19 [0.07, 0.31]
Inflammatory composite	0.47 [0.26, 0.68][a]	0.69 [0.49, 0.89][b]	1.08 [0.84, 1.32][a,b]	0.76 [0.62, 0.90]

Continuous data are reported as mean average [95 % CI].
FAI = femoroacetabular impingement; OA = osteoarthritis; CI = confidence interval; w/ = with.
Histopathological scoring for the following parameters were graded according to the criteria described by Minten et al. (2019): lining, infiltration, fibrin, vascularization, fibrosis, perivascular edema, inflammatory composite. Vasculopathy was graded following the criteria described by Sodhi et al. (2022). For all histopathological parameters, the following grades represent a range of severity: 0 = none, 1 = mild, 2 = moderate, 3 = severe.
[a] Denotes statistical significance between two values in the same row.
[b] Denotes statistical significance between two values in the same row.

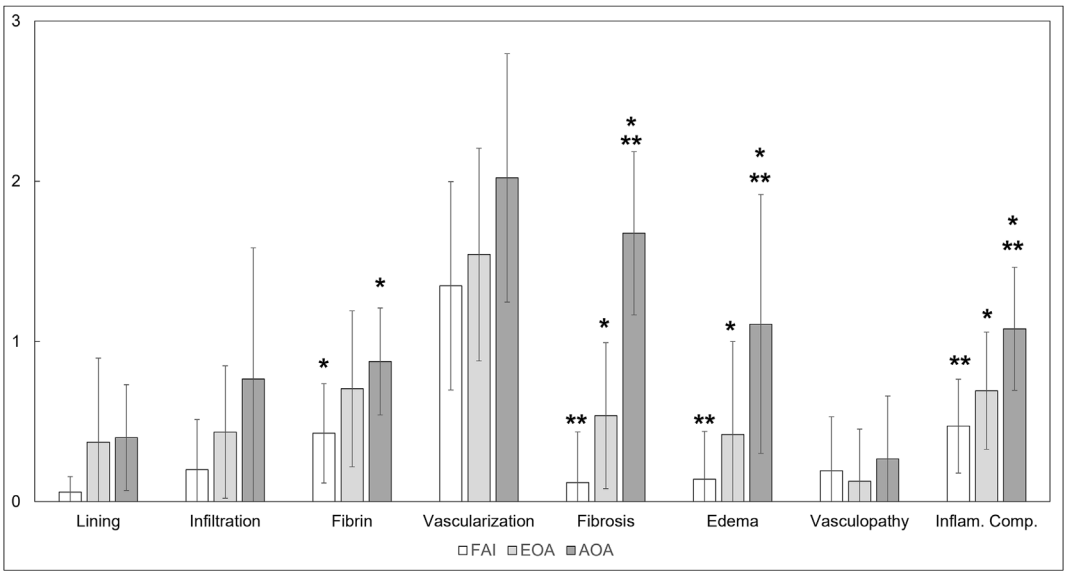

Fig. 2. A visual summary of histopathological scores for areolar synovium of hip pathology patients. This figure is a visual representation of the data presented in Table 2. All scores are reported as the cohort mean ± standard deviation. Parameters were graded from 0 to 3 according to the criteria defined by Minten et al. (2019) and Sodhi et al. (2022). FAI = femoroacetabular impingement, EOA = FAI with early osteoarthritis, AOA = advanced osteoarthritis. Asterisks (*, **, ***) represent significant differences determined by Bonferroni-corrected post-hoc Student t-tests ($p < 0.017$) following one-way ANOVA ($p < 0.05$).

Table 3
A summary of microvasculature in areolar synovium samples of hip pathology patients.

Parameter	FAI ($n = 10$)	FAI w/Early OA ($n = 15$)	Advanced OA ($n = 12$)	Total ($n = 37$)
Microvessel density	96.00 [46.60, 145.31]	95.13 [56.91, 133.34]	104.10 [71.05, 137.09]	98.25 [77.47, 119.03]
Mature microvessel density	81.20 [40.00, 122.31]	73.78 [35.72, 111.83]	93.96 [64.79, 123.13]	82.32 [62.92, 101.71]
Immature microvessel density	14.79 [5.48, 24.11]	20.72 [10.50, 30.95]	7.54 [2.92, 12.16]	14.84 [9.86, 19.83]
ENG^+ density	0.84 [−0.15, 1.83]	1.54 [−0.26, 3.34]	7.06 [0.53, 13.59]	3.14 [0.92, 5.37]
ENG^+ Samples[a]	5 (50)	4 (27)	6 (50)	15 (41)

Continuous data are reported as mean average [95 % CI]. Categorical data are reported as *n* (%).
FAI = femoroacetabular impingement; OA = osteoarthritis; CI = confidence interval.
All microvessel densities are reported as vessels per mm^2 of synovial tissue area.
Mature microvessels were counted as those CD31-positive microvessels with >75 % coverage by α-SMA. Immature microvessels were counted as those CD31-positive microvessels with ≤75 % coverage by α-SMA. There is no statistical significance between microvessel densities.
[a] Endoglin (ENG)-positive synovium samples are those with ≥1 microvascular endothelial lining demonstrating endoglin positivity through immunohistochemistry.

3.4. Endoglin-positive areolar synovium microvessel density

The endoglin-positive microvessel densities for the FAI, FAI with early OA, and advanced OA groups were 0.84 [−0.15, 1.83], 1.54 [−0.26, 3.34], and 7.06 [0.53, 13.59], respectively. One-way ANOVA followed by post-hoc two-tailed Student's t-tests revealed no significant differences between any two groups for mean ENG-positive microvessel density. Endoglin positivity is summarized in Table 3. Representative images of areolar synovium samples stained immunohistochemically for endoglin are provided in Fig. 4.

4. Discussion

We compared the histopathology of synovium between FAI patients, FAI patients with early hip OA, and advanced hip OA patients. Our findings suggest that OA-associated features of synovitis are observed in non-arthritic and arthritic FAI. To our knowledge, just two studies have reported histopathology from the synovia of FAI patients [12,13]. In the present study, average synovial lining thickness and inflammatory infiltrate were scored as less than 1 in each group, with no significant differences between the mean scores of the three groups. The limited evidence of increased lining thickness in our FAI samples suggests this feature may not be notable in FAI patients, with or without OA. Similarly, the FAI samples investigated (with and without early OA), had limited evidence of inflammatory infiltrate overall, suggesting this may not be a prominent feature in FAI synovium (Table 2). Although "enlargement of the synovial lining cell layer" and "inflammatory infiltrate" are two of the three equally weighted parameters of the Krenn synovial scoring system, neither of the previous reports on FAI synovium histopathology explicitly reported the individual scores for these parameters [12,13]. However, both studies reported synovitis in their FAI patients based on their overall synovitis scores [14]. This is compatible with our report of significant increases in synovial inflammatory composite scores between our FAI (both with and without early OA) and our advanced hip OA groups (Fig. 2), which suggests synovitis worsens with radiographic severity of hip OA.

Unique to our study is the assessment of other OA-related synovial features in FAI patients, namely fibrin deposition, vascularization, fibrosis, perivascular edema, and vasculopathy. Fibrin deposition scores increased significantly between the FAI only group (i.e., no evidence of OA) and the advanced hip OA group, suggesting this feature may increase in prominence with the severity of OA and synovitis. Fibrosis scores were not prominent in either FAI group, although they were mild-to-moderate in severity in our advanced OA group (Fig. 2). This is in keeping with what is hypothesized regarding synovial fibrosis in OA, namely that it tends to manifest following prolonged synovitis toward the end stages of the disease [8,22,23]. Although statistical significance could not be established between the means for all parameters, pathological scores

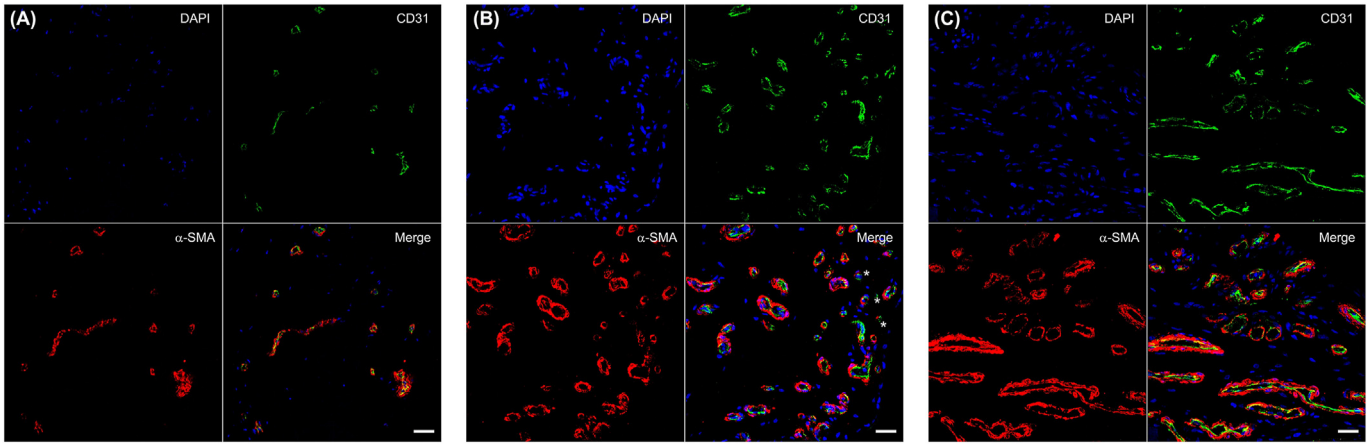

Fig. 3. Areolar synovium of patients with FAI and/or hip OA is marked by a high density of mature microvessels in the intimal and subintimal regions. Patients with FAI (left), FAI with early hip OA (middle), and advanced hip OA (right) showed similarly high densities for microvasculature in areolar synovium samples. In all three cohorts, immature microvessel density was relatively low compared to mature microvessel density. The top right channel in each image (CD31, green) was used to count the total number of microvessels. The top right and both bottom channels were used in conjunction to determine microvessel maturity status, with mature vessels being at least 75 % wrapped by α-SMA (red), and immature vessels ≤75 % wrapped by α -SMA. Nuclei are represented by blue. *Denotes immature microvessels (seen in the middle, bottom-right panel). Scalebars = 50 μm.

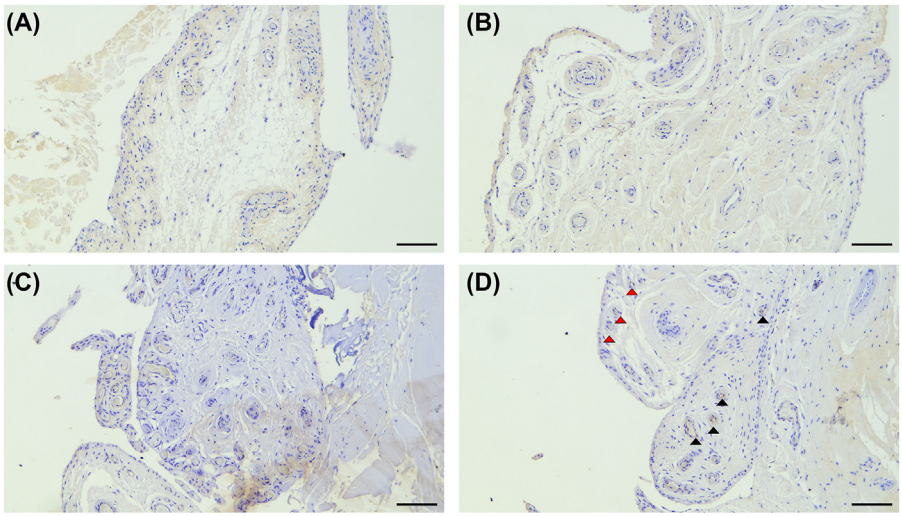

Fig. 4. Immunohistochemical staining for endoglin (ENG) in areolar synovium samples of hip pathology patients. In areolar synovium samples from FAI (A), FAI with early hip OA (B), and advanced hip OA patients (C), the density of microvessels positive for ENG was relatively low compared to total microvessel densities. (D) Shows a field of view from the same sample as (C), but with ENG^{+} vessels. Synovial ENG positivity is marked by brown staining of the endothelial lining of vasculature (denoted by black arrows). Red arrows denote ENG^{-} vessels within proximity to ENG^{+} vessels. Scalebar = 100 μm.

tended to increase from FAI to FAI with early OA, to advanced OA, regardless of significance (Fig. 2).

To the best of our knowledge, this is the first study to report on synovial microvasculature in FAI patients. We assessed MVD in FAI and in hip OA, investigating vasculopathy, vascularization, and perivascular edema. Vasculopathy (i.e., where the vascular wall is thicker than the lumen it surrounds) was unremarkable in all three groups. Perivascular edema scores increased significantly between the FAI and advanced OA groups, and FAI with early OA and advanced OA, and insignificantly between FAI only and FAI with early OA (Fig. 2). This suggests that perivascular edema presents within the hip synovium in a more severely dysfunctional intraarticular environment. The most prominent histopathological feature we discovered was increased vascularization (based on Minten's criteria) in all three hip disorder groups (Fig. 2). Mild-to-moderate vascularization was notable in FAI only, FAI with early hip OA, and advanced hip OA patients, with no significant differences between the three groups (Fig. 2). If the vascularization observed in the synovia of FAI patients is the product of increased angiogenesis, this would suggest MVD is one of the earliest detectable changes in synovium preceding the development of radiographic hip OA (especially secondary to FAI). Given the important role of angiogenesis in wound healing, it is possible that the prominent vascularization observed in these FAI patients is in response to the chronic, injurious, shearing forces experienced by the intraarticular soft tissues of the hip.

We report similar total microvessel densities, with predominantly mature and minimally immature microvessel densities in all three groups (Table 3). This is the first report on synovial microvessel density in FAI, and comparisons to other findings in the hip are limited. However, our results differ from those of Kennedy et al. (2010) who reported no evidence of microvessel immaturity in the synovium of knee OA patients [24]. In another knee study, immature microvessels were detected in low quantities in OA synovium, even lower than our own [25]. These differences in total microvessel densities and immature microvessel densities may indicate differences in synovial microvasculature distribution between synovium of the hip and knee. As with the previously mentioned differences, there may be further joint-specific differences in synovial microvasculature of the hip versus the knee. Grieshaber-Bouyer et al. (2019) discovered that the inflammatory profiles of knee versus hip OA synovium differ greatly, with knee synovium showing nearly four times higher synovial mononuclear cell levels than the hip [26]. Mast cell

counts have also proven to be higher in knee synovium versus hip synovium, along with synovitis scores [27]. Furthermore, $CD14^+$ macrophages and $CD8^+$ cytotoxic T cell counts were also elevated in the knee [26]. Grieshaber-Bouyer et al. (2019) suggest that these differences may explain the tendency for hip OA progression to accelerate at a higher rate than knee OA, with early increased mononuclear cell levels in OA causing inflammatory cytokine elevation, followed by decreased levels of mononuclear cells as hip OA progresses. Given the negligible inflammatory infiltrate in both FAI groups, we propose that this increase in mononuclear cell activity may occur later in the pathophysiological trajectory of hip OA as a result of a decompensated joint microenvironment. Given that patients can experience FAI symptoms for many years before end-stage hip OA manifests, a younger and healthier hip microenvironment may be compensating against the onset of these rapidly destructive mechanisms.

To further investigate the nature of the microvasculature, endothelial cells were stained immunohistochemically with endoglin (CD105), a marker of active angiogenesis [28], with evidence suggesting its expression is necessary for regular VEGF-induced angiogenic activity [29]. Following IHC, endoglin staining was notably absent in 59 % of all our areolar synovium samples (Table 3). The samples that did demonstrate some level of endoglin expression had very low endoglin-positive microvessel densities (Table 3). The low detection of endoglin, or its absence entirely, in FAI, FAI with early OA, and advanced hip OA synovia could have several possible explanations. Endoglin (ENG) expression has previously been investigated immunohistochemically in synovia of hip OA patients [30,31]. Gurzu et al. (2017) reported ENG expression by endothelial cells of all small vessels observed in primary hip OA, and by the larger vessels observed in hip OA secondary to avascular necrosis [30]. They reported the presence of synovial hyperplasia with inflammatory infiltrate in primary hip OA, suggesting that as a result of these features, ENG-positive synovium-derived mesenchymal stem (or *stromal*) cell-like cells (SD-MSCs) are partially responsible for differentiation to ENG-positive endothelial cells comprising small vessels [30], as MSCs are capable of differentiating into vascular endothelial cells [32]. The absence of, or early nature of OA in our FAI synovial samples, along with the scarcity of cellular infiltrate and lining hyperplasia could indicate SD-MSC levels are low in these FAI synovia, accounting for the limited expression of endoglin in the endothelial cells we observed.

This study is subject to limitations, including the use of small group sizes, absence of healthy controls, and failure to include patients with advanced hip OA secondary to FAI. The descriptions of clinical data do not include a measurement of symptom duration and FAI deformity size or location. Finally, a single biopsy per tissue type was collected from each patient. To account for the heterogeneity of synovium, multiple biopsies would prevent the underreporting of OA-associated histopathological features. However, obtaining samples from healthy controls and patients with advanced hip OA secondary to FAI would be challenging given the highly invasive nature of collecting samples from the intracapsular environment of the hip and the relatively recent awareness of FAI diagnosis, respectively.

In summary, our findings indicate that OA-associated changes may already be present in the synovia of symptomatic FAI patients, including those without clinically diagnosed OA. Given the variability of results within each group, these changes appear to be heterogeneous, which is consistent with prior observations in OA synovium [33]. We observed prominent vascularization in the synovia of patients across different stages of hip pathology, suggesting that early vascular changes could be a feature of FAI synovium. Perivascular edema was also notable in synovial samples from advanced hip OA patients. Our microvascular analysis suggests that the synovium of FAI patients is predominantly composed of mature and inactive vessels. We reiterate the need for hip-specific OA studies rather than assuming direct parallels with knee OA. Future research should focus on histopathological changes in end-stage hip OA patients with a documented history of FAI to better understand disease progression and decompensation in hip OA secondary to FAI.

Contributions

RA contributed to study design, data collection, data and statistical analysis, interpretation of results, and writing of the manuscript. BL contributed to study design, data collection, and revision of the manuscript. TA contributed to conceptualization and design of the study, supported the study, and contributed to analysis and writing of the manuscript. RD contributed to conceptualization and design of the study, supported the study, and contributed to analysis and writing of the manuscript.

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Declaration of competing interest

No competing interests.

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References

[1] B.J.E. de Lange-Brokaar, A. Ioan-Facsinay, E. Yusuf, A.W. Visser, H.M. Kroon, G.J.V.M. van Osch, et al., Association of pain in knee osteoarthritis with distinct patterns of synovitis, Arthritis Rheumatol. 67 (2015) 733–740, https://doi.org/10.1002/art.38965.

[2] T. Neogi, A. Guermazi, F. Roemer, M.C. Nevitt, J. Scholz, L. Ardent-Nielsen, et al., Association of joint inflammation with pain sensitization in knee osteoarthritis: the multicenter osteoarthritis study, Arthritis Rheumatol. 68 (2016) 654–661, https://doi.org/10.1002/art.39488.

[3] H.T. Philpott, T.B. Birmingham, R. Pinto, C.A. Primeau, D. Arsenault, B.A. Lanting, et al., Synovitis is associated with constant pain in knee osteoarthritis: a cross-sectional study of OMERACT knee ultrasound scores, J. Rheumatol. 49 (2022) 89–97, https://doi.org/10.3899/jrheum.210285.

[4] M.J. Benito, D.J. Veale, O. FitzGerald, W.B. van den Berg, B. Bresnihan, Synovial tissue inflammation in early and late osteoarthritis, Ann. Rheum. Dis. 64 (2005) 1263–1267, https://doi.org/10.1136/ard.2004.025270.

[5] L. Del Sordo, G.B. Blackler, H.T. Philpott, J. Riviere, L. Gunaratnam, B. Heit, et al., Impaired efferocytosis by synovial macrophages in patients with knee osteoarthritis, Arthritis Rheumatol. 75 (2023) 685–696, https://doi.org/10.1002/art.42412.

[6] M.J.M. Minten, A. Blom, G.F. Snijders, M. Kloppenburg, F.H.J. van den Hoogen, A.A. den Broeder, et al., Exploring longitudinal associations of histologically assessed inflammation with symptoms and radiographic damage in knee osteoarthritis: combined results of three prospective cohort studies, Osteoarthr. Cartil. 27 (2019) 71–79, https://doi.org/10.1016/j.joca.2018.10.014.

[7] E.U. Sodhi, H.T. Philpott, M.M. Carter, T.B. Birmingham, C.T. Appleton, Sex-differences and associations between complement activation and synovial vascularization in patients with late-stage knee osteoarthritis, Front. Immunol. 13 (2022) 890094, https://doi.org/10.3389/fimmu.2022.890094.

[8] S. Hodgeson, S. O'Brien, J. Simkin, E. Plakotaris, C. McCarthy, V. Dasa, et al., Differences in synovial fibrosis relative to range of motion in knee osteoarthritis patients, J. Orthop. Res. 40 (2022) 584–594, https://doi.org/10.1002/jor.25061.

[9] M. Hall, M. van der Esch, R.S. Hinman, G. Peat, A. de Zwart, J.G. Quicke, et al., How does hip osteoarthritis differ from knee osteoarthritis? Osteoarthr. Cartil. 30 (2022) 32–41, https://doi.org/10.1016/j.joca.2021.09.010.

[10] R. Ganz, J. Parvizi, M. Beck, M. Leunig, H. Nötzli, K.A. Siebenrock, Femoroacetabular impingement: a cause for osteoarthritis of the hip, Clin. Orthop. Relat. Res. 417 (2003) 112–120, https://doi.org/10.1097/01.blo.0000096804.78689.c2.

[11] T.J. Menge, N.W. Truex, Femoroacetabular impingement: a common cause of hip pain, Physician Sportsmed. 46 (2018) 139–144, https://doi.org/10.1080/00913847.2018.1436844.

[12] M. Haneda, M.F. Rai, L. Cai, R.H. Brophy, R.J. O'Keefe, J.C. Clohisy, et al., Distinct pattern of inflammation of articular cartilage and the synovium in early and later hip femoroacetabular impingement, Am. J. Sports Med. 48 (2020) 2481–2488, https://doi.org/10.1177/0363546520935440.

[13] G.D. Abrams, A. Luria, J. Sampson, R.A. Madding, W.H. Robinson, M.R. Safran, et al., Decreased synovial inflammation in atraumatic hip microinstability compared with femoroacetabular impingement, Arthroscopy 33 (2017) 553–558, https://doi.org/10.1016/j.arthro.2016.09.007.

[14] V. Krenn, L. Morawietz, G.R. Burmester, R.W. Kinne, U. Mueller-Ladner, B. Muller, et al., Synovitis score: discrimination between chronic low-grade and high-grade

synovitis, Histopathology. 49 (2006) 358–364, https://doi.org/10.1111/j.1365-2559.2006.02508.x.
[15] Y. Henrotin, L. Pesesse, C. Lambert, Targeting the synovial angiogenesis as a novel treatment approach to osteoarthritis, Ther Adv Musculoskelet Dis 6 (2014) 20–34, https://doi.org/10.1177/1759720x13514669.
[16] D.R. Griffin, E.J. Dickenson, J. O'Donnell, R. Agricola, T. Awan, M. Beck, et al., The Warwick Agreement on femoroacetabular impingement syndrome (FAI syndrome): an international consensus statement, Br. J. Sports Med. 50 (2016) 1169–1176, https://doi.org/10.1136/bjsports-2016-096743.
[17] P. Kumar, S. Ramesh, S. Aggarwal, S. Agarwal, R.K. Rajnish, Reliability of modified Harris Hip Score as a tool for outcome evaluation of total hip replacements in Indian population, J Clin Orthop Trauma 10 (2019) 128–130, https://doi.org/10.1016/j.jcot.2017.11.019.
[18] R.L. Martin, B.T. Kelly, M.J. Philippon, Evidence of validity for the hip outcome score, Arthroscopy 22 (2006) 1304–1311, https://doi.org/10.1016/j.arthro.2006.07.027.
[19] N.G.H. Mohtadi, D.R. Griffin, M.E. Pedersen, D. Chan, M.R. Safran, N. Parsons, et al., The development and validation of a self-administered quality-of-life outcome measure for young, active patients with symptomatic hip disease: the International Hip Outcome Tool (iHOT-33), Arthroscopy 28 (2012) 595–605, https://doi.org/10.1016/j.arthro.2012.03.013.
[20] M.L. McHugh, Interrater reliability: the kappa statistic, Biochem. Med. 22 (2012) 276–282, https://doi.org/10.11613/BM.2012.031.
[21] L. Haywood, D.A. Walsh, Vasculature of the normal and arthritic synovial joint, Histol. Histopathol. 16 (2001) 277–284, https://doi.org/10.14670/hh-16.277.
[22] S.A. Ali, P. Datta, M. Kapoor, R. Gandhi, Adiponectin is a potential mediator of synovial fibrosis from early to late knee osteoarthritis, Osteoarthr. Cartil. 27 (2019) S479–S480, https://doi.org/10.1016/j.joca.2019.02.527.
[23] V. Venerito, G. Cazzato, G. Lopalco, M. Fornaro, G. Righetti, L. Urso, et al., Histopathologic features of fibrotic knee synovitis in a young adult with seronegative rheumatoid arthritis, J. Clin. Rheumatol. 27 (2021) e73–e74, https://doi.org/10.1097/rhu.0000000000001247.
[24] A. Kennedy, C.T. Ng, M. Biniecka, T. Saber, C. Taylor, J. O'Sullivan, et al., Angiogenesis and blood vessel stability in inflammatory arthritis, Arthritis Rheum. 62 (2010) 711–721, https://doi.org/10.1002/art.27287.
[25] E. Izquierdo, J.D. Cañete, R. Celis, B. Santiago, A. Usategui, R. Sanmartí, et al., Immature blood vessels in rheumatoid synovium are selectively depleted in response to anti-TNF therapy, PLoS One 4 (2009) e8131, https://doi.org/10.1371/journal.pone.0008131.
[26] R. Grieshaber-Bouyer, T. Kämmerer, N. Rosshirt, T.A. Nees, P. Koniezke, E. Tripel, et al., Divergent mononuclear cell participation and cytokine release profiles define hip and knee osteoarthritis, J. Clin. Med. 8 (2019) 1631, https://doi.org/10.3390/jcm8101631.
[27] L. Farinelli, A. Aquili, M. Mattioli-Belmonte, S. Manzotti, F. D'Angelo, C. Ciccullo, et al., Synovial mast cells from knee and hip osteoarthritis: histological study and clinical correlations, J Exp Orthop 9 (2022) 13, https://doi.org/10.1186/s40634-022-00446-2.
[28] P. Wikström, I.F. Lissbrant, P. Stattin, L. Egevad, A. Bergh, Endoglin (CD105) is expressed on immature blood vessels and is a marker for survival in prostate cancer, Prostate 51 (2002) 268–275, https://doi.org/10.1002/pros.10083.
[29] Z. Liu, F. Lebrin, J.A. Maring, S. van den Driesche, S. van der Brink, M. van Dinther, et al., ENDOGLIN is dispensable for vasculogenesis, but required for vascular endothelial growth factor-induced angiogenesis, PLoS One 9 (2014) 86273, https://doi.org/10.1371/journal.pone.0086273.
[30] S. Gurzu, S.G. Turdean, S.T. Pop, A. Zazgyva, C.O. Roman, M. Opris, et al., Different synovial vasculogenic profiles of primary, rapidly destructive and osteonecrosis-induced hip osteoarthritis. An immunohistochemistry study, Int. Orthop. 41 (2017) 1107–1112, https://doi.org/10.1007/s00264-016-3302-4.
[31] S.G. Turdean, I. Jung, S. Gurzu, A. Zazgyva, A. Fetyko, C.O. Roman, et al., Histopathological evaluation and expression of the pluripotent mesenchymal stem cell-like markers CD105 and CD44 in the synovial membrane of patients with primary versus secondary hip osteoarthritis, J. Invest. Med. 65 (2017) 363–369, https://doi.org/10.1136/jim-2016-000244.
[32] G.V. Silva, S. Litovsky, J.A.R. Assad, A.L.S. Sousa, B.J. Martin, D. Vela, et al., Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model, Circulation 111 (2005) 150–156, https://doi.org/10.1161/01.cir.0000151812.86142.45.
[33] A. Thomson, C.M.U. Hilkens, Synovial macrophages in osteoarthritis: the key to understanding pathogenesis? Front. Immunol. 12 (2021) 678757 https://doi.org/10.3389/fimmu.2021.678757.