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Clinical trial

## Comparative analysis of late-stage rheumatoid arthritis and osteoarthritis reveals shared histopathological features



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### SUMMARY

**Objectives:** Osteoarthritis (OA) is a debilitating and heterogeneous condition, characterized by various levels of articular cartilage degradation, osteophytes formation, and synovial inflammation. Multiple evidences suggest that synovitis may appear early in the disease development and correlates with disease severity and pain, therefore representing a relevant therapeutic target. In a typical synovitis-driven joint disease, namely rheumatoid arthritis (RA), several pathotypes have been described by our group and associated with clinical phenotypes, disease progression, and response to therapy. However, whether these pathotypes can be also observed in the OA synovium is currently unknown.

**Methods:** Here, using histological approaches combined with semi-quantitative scoring and quantitative digital image analyses, we comparatively characterize the immune cell infiltration in a large cohort of OA and RA synovial tissue samples collected at the time of total joint replacement.

**Results:** We demonstrate that OA synovium can be categorized also into three pathotypes and characterized by disease- and stage-specific features. Moreover, we revealed that pathotypes specifically reflect distinct levels of peripheral inflammation.

**Conclusions:** In this study, we provide a novel and relevant pathological classification of OA synovial inflammation. Further studies investigating synovial molecular pathology in OA may contribute to the development of disease-modifying therapies.

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### Introduction

Osteoarthritis (OA) affects over 500 million people worldwide with a huge socio-economic burden<sup>1,2</sup> and is mainly characterized by the degradation of articular cartilage, the formation of osteophytes, and various degrees of synovial inflammation collectively leading to loss of joint function. Although OA has long been considered a degenerative “wear and tear” disease predominantly

related to the individual aging process, increasing evidence demonstrates that complex and heterogeneous pathophysiological mechanisms underline the development of this disease. Indeed, various OA phenotypes, based on clinical, laboratory, imaging, or etiologic features, with distinct possible underlying aetiologies and pathogenic pathways have been defined over the years.<sup>3–5</sup> Recognized clinical phenotypes of knee OA include, for instance, patients with chronic pain, metabolic syndrome, or inflammatory features. Overall, the implementation of standardized definitions, statements, and reporting recommendations provides a useful framework for the design of phenotype-specific interventions.<sup>6</sup>

Among the various tissues affected by OA, clinical, imaging, histological, and molecular analyses have now identified the synovial tissue and its inflammation as major players in both pain and structural progression of the disease.<sup>7</sup> Importantly, strong evidence suggests that synovitis is observed prior to the development of

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radiographic OA<sup>8</sup> and correlates with radiographic severity, cartilage damage, and pain throughout disease progression.<sup>9,10</sup> A recent study from the Osteoarthritis Initiative analyzing 4-year magnetic resonance imaging (MRI) data from 249 patients further confirmed that sustained synovitis is associated with progressive OA.<sup>11</sup>

The OA synovium is characterized by a variable infiltration of innate and adaptive immune cell types. Particularly macrophages and CD4<sup>+</sup> T-cells have been shown to be predominant in this tissue and to correlate with either disease activity and progression or pain.<sup>12,13</sup> In addition to producing a variety of pro-inflammatory and pro-catabolic mediators that sustain local cell activation and drive cartilage degradation, OA synovial immune cells establish a complex crosstalk with stromal cells, such as chondrocytes or fibroblasts like-synoviocytes.<sup>14</sup> The Krenn's synovitis score is the best-known histological assessment of synovial inflammation in arthropathies, including OA and rheumatoid arthritis (RA).<sup>15</sup> However, although it is based on three reproducible histopathological criteria, including the thickness of the lining layer, the cellular density of the synovial stroma, and the presence of a leukocytic infiltrate, it does not take into consideration the individual features and distributions of the synovial immune cells. In RA, a prototypical synovitis-driven disease, our group previously described three synovial histological pathotypes: (1) lympho-myeloid, characterized by the presence of B/T cells forming highly organized ectopic lymphoid structures (ELS), plasma cells, and macrophages in the sublining; (2) diffuse-myeloid, marked by a predominant infiltration of CD68<sup>+</sup> synovial cells (monocytes, macrophages) without distinctly organized follicular structures; and (3) pauci-immune, defined by a scant immune cell infiltrate and a fibroblast-rich stroma. Importantly, these pathotypes are associated with clinical phenotypes and can stratify clinical response to therapy and predict the radiographic progression in RA.<sup>16–18</sup> Whether these pathotypes can be also observed in the OA synovium is currently unknown.

Here, for the first time, we aimed to characterize the immune cell infiltration in end-stage OA synovium in comparison with RA and its relationship with clinical features. We suggest a new classification of OA synovial inflammation based on histological pathotypes and provide hypotheses for future target identification and novel therapeutic development.

## Method

### *Patients' recruitment and tissue processing*

Patients with a diagnosis of OA (n = 185) or RA (n = 29),<sup>19</sup> undergoing knee, hip (OA and RA), or wrist (RA only) arthroplasty at Newham University Hospital (Barts Health NHS Trust) were enrolled in the EMR Biobank study (referred as “end-stage” OA and RA) [REC Ref. No. 07/Q0605/29]. Synovial tissue was detached from the whole joint and removed during arthroplasty. Clinical data, including age, sex, site of the procedure, and complete blood counts were collected for each patient. Ultrasound-guided synovial biopsies collected from large joints (knees) from RA patients at different disease stages were also analyzed: i) Early (< 12 months) Disease Modifying Anti-Rheumatic Drugs (DMARDs)-naïve patients (referred as “treatment-naïve RA”) were enrolled as part of the “Pathobiology of Early Arthritis Cohort” (PEAC, <http://www.peac-mrc.mds.qmul.ac.uk>, REC 05/Q0703/198)<sup>16</sup>; ii) Conventional synthetic (cs) DMARDs-inadequate responder (IR) RA patients (referred as “csDMARDs IR RA”) were enrolled as part of the “Stratification of Biologic Therapies for RA by Pathobiology” (STRAP, <https://matura-mrc.whri.qmul.ac.uk/>) [REC 14/WA/1209]; iii) patients who received csDMARDs and at least one anti-tumour necrosis factor (TNF) (referred as “anti-TNF IR RA”) were enrolled as part of the “rituximab vs tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis” R4RA trial (<https://r4ra-nih.whri.qmul.ac.uk/>) [REC 12/WA/0307].<sup>20</sup> To avoid the potential bias related to different joint sizes, the analysis

involving disease stage comparison in OA and RA was performed on a subselection of OA (n = 117) and RA (n = 19) end-stage patients whose synovial tissue was retrieved from the knee joint only. All patients gave written informed consent. Following fixation, all synovial samples spanning different locations within the joint were embedded in paraffin for histological characterization. Two to three blocks per patient were prepared.

### *Histology and immunohistochemistry*

Sequential 3- $\mu$ m-thick sections of synovial tissue underwent hematoxylin and eosin (H&E) and immunohistochemical staining to check the quality of the tissue, determine the level of inflammation, and the degree of cellular infiltration by B cells (CD20<sup>+</sup>, Agilent Technologies, Santa Clara, CA, USA), T cells (CD3<sup>+</sup>, Agilent Technologies), plasma cells (CD138<sup>+</sup>, Agilent Technologies), and lining (L) or sublining (SL) monocytes/macrophages (CD68<sup>+</sup>, Agilent Technologies). Sections were digitally scanned using Nanozoomer S210 (Hamamatsu Photonics, Hamamatsu City, Japan). Synovitis scores were attributed according to the Krenn's classification as follows: no synovitis (scores 0 or 1), low-grade synovitis (scores 2–4), or high-grade synovitis (scores 5–9).<sup>15</sup> Synovial samples were also categorized into three pathotypes (pauci-immune, diffuse-myeloid, and lympho-myeloid) following semi-quantitative scoring of CD20, CD138, CD3, CD68-L, and CD68-SL staining according to the previously published reports<sup>16,21</sup> and to the following criteria: i) lympho-myeloid: CD20  $\geq$  2) and/or CD138  $\geq$  2; ii) diffuse-myeloid: CD68-SL  $\geq$  2, CD20  $\leq$  1 and/or CD3  $\geq$  1, CD138  $\leq$  2, and iii) pauci-immune: CD68-SL < 2 and CD3, CD20, CD138 < 1. Krenn's scoring and CD markers semi-quantitative scoring were performed by two independent observers. The percentage of concordance between the observers was very high and if the pathotype attributed by each observer was different, a consensus was reached. In addition, but if two distinct histopathological categorizations were attributed for two distinct blocks of the same patient, we always followed the same rule and the worst score/pathotype was considered for further analysis.

Quantitative digital image analyses were also performed on a subset of OA patients spanning various synovial histological features based on prior semi-quantitative scoring (n = 37, including 7 pauci-immune, 15 diffuse-myeloid, and 15 lympho-myeloid) to determine the percentage of CD20, CD3, CD138, or CD68-positive cells within the tissue using QuPath software v0.3.0.<sup>22</sup>

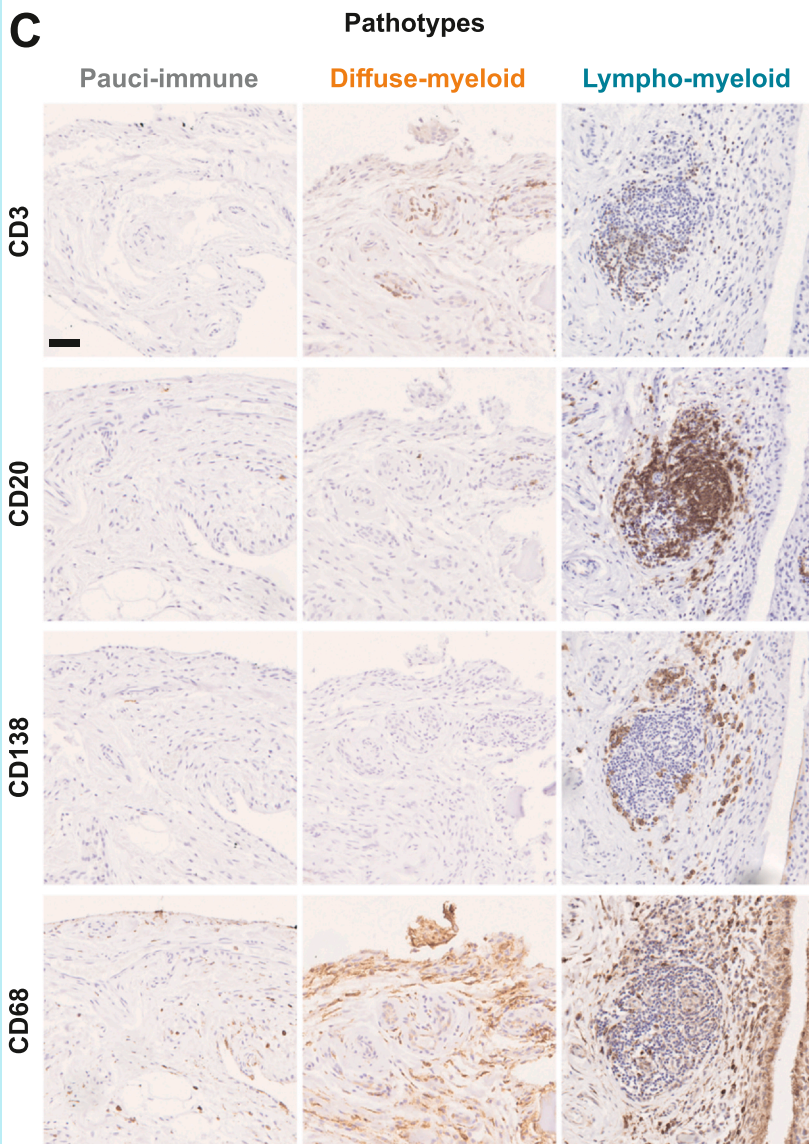
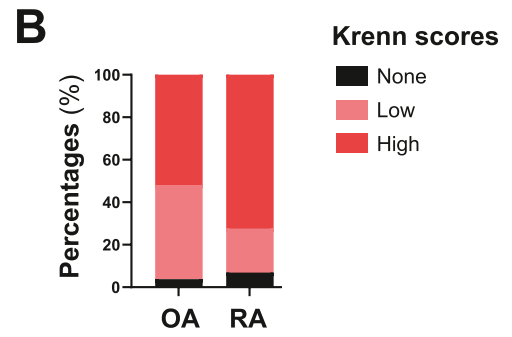
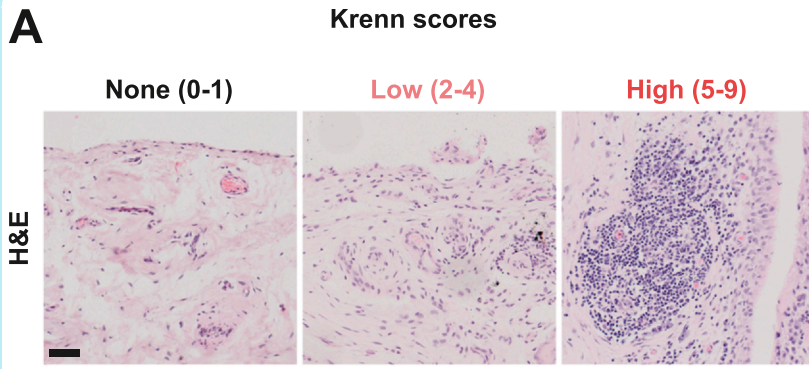
### *Statistical analyses*

Differences in continuous variables were evaluated by the non-parametric Mann Whitney U test (two groups) or the parametric Student's t test (two groups) or Kruskal Wallis with Dunn's post-test (multiple groups). Chi-square/Fisher's exact test was used to evaluate associations of categorical variables. Correlations were evaluated by Spearman's bivariate analysis. Statistical analyses were performed using GraphPad Prism-v9 software (Graphpad, San Diego, CA, USA). Data are shown as median and interquartile range and p-values < 0.05 were considered significant.

## Results

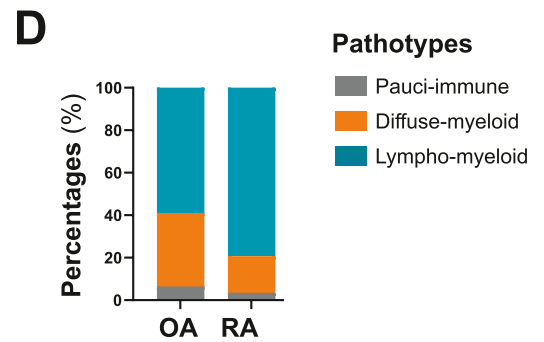
### *Synovial tissue from end-stage OA can be categorized into three pathotypes*

First, we compared end-stage OA and RA synovial tissues collected during joint replacement. Clinical and demographic features of the 185 OA and 29 RA patients included in this study are summarized in [Supplementary Table 1](#). Following H&E staining of OA and RA synovium



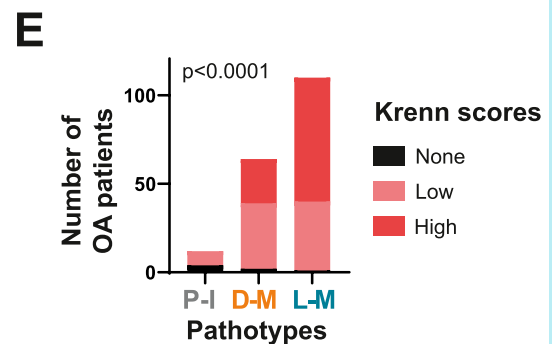
	OA n=185	RA n=29
None (0-1)	3.78% (7)	6.90% (2)
Low (2-4)	44.32% (82)	20.69% (6)
High (5-9)	51.90% (96)	72.41% (21)

p-value      0.05; ns



	OA n=185	RA n=29
Pauci-immune	6.49% (12)	3.45% (1)
Diffuse-myeloid	34.59% (64)	17.24% (5)
Lympho-myeloid	58.92% (109)	79.31% (23)

p-value      0.11; ns



(caption on next page)

## Fig. 1

## Osteoarthritis and Cartilage

A novel histopathological pathotypes classification of synovial tissues from osteoarthritis (OA) knees and hips. **(A)** Sections of OA synovium were stained with hematoxylin and eosin and classified as per Krenn's score as "none" (0–1), "low" (5–4), and "high" (5–9) inflammatory tissues. Representative images are shown for each category. Scale bar = 50  $\mu$ m. **(B)** Bar chart (upper panel) and table (lower panel) representing the distribution of each Krenn's score category ("None" in black, "Low" in pink, and "High" in red) in end-stage OA (n = 185) in comparison with rheumatoid arthritis (RA, n = 29). ns, non-significant, as assessed by the Fisher's exact test. **(C)** Sections of OA synovium were stained with CD3 (T cells), CD20 (B cells), CD138 (plasma cells), and CD68 (monocytes/macrophages) antibodies. Representative images are shown for each pathotype (pauci-immune, diffuse-myeloid, and lympho-myeloid). Scale bar = 50  $\mu$ m. **(D)** Bar chart (upper panel) and table (lower panel) representing the distribution of each pathotype ("pauci-immune" in gray, "diffuse-myeloid" in orange, and "lympho-myeloid" in blue) in end-stage OA (n = 185) and RA (n = 29). ns, non-significant, as assessed by the Fisher's exact test. **(E)** Bar chart representing the distribution of Krenn's categories ("none" 0–1, "low" 5–4, and "high" 5–9) in each pathotype group ("P-I" pauci-immune, "D-M" diffuse-myeloid, "L-M", lympho-myeloid) in synovium from patients with end-stage OA (n = 185). p-value was calculated using the Fisher's exact test.

represented in Fig. 1A, in keeping with previously published data,<sup>15</sup> we observed that the proportion of RA synovial tissue exhibiting a high Krenn's score was increased, although not significantly ( $p = 0.05$ , in end-stage RA compared to OA tissues), with a higher proportion of tissues presenting no synovitis or a low-grade synovitis in the OA group (respectively 48.10% and 27.59% of synovium belonging to the "None" or "Low" group in OA and RA) (Fig. 1B).

Next, we investigated the presence of synovial pathotypes in OA, namely pauci-immune, diffuse-myeloid, and lympho-myeloid, defined based on CD3<sup>+</sup> T cells, CD20<sup>+</sup> B cells, CD138<sup>+</sup> plasma cells, and CD68<sup>+</sup> monocytes/macrophages synovial infiltration (Fig. 1C), as described in Methods section. The pathotype distribution showed no statistically significant difference between end-stage OA and RA synovium ( $p = 0.11$ ) (Fig. 1D), with a significant enrichment of the lympho-myeloid pathotype, characterised by infiltration of B and T cells, in RA synovia (79.31%) compared to OA (58.92%) ( $p$ -value L-M compared to non-LM 0.04). Importantly, we confirmed the non-redundancy between both classification systems, as each pathotype group was composed of patients from different Krenn's categories (Fig. 1E).

Notably, when evaluating the prevalence of each pathotype in both OA and RA according to the site of procedure (knee, hip, wrist), there was no difference in the location-specific distribution of the pathotypes at this end-stage disease (Supplementary Table 2).

#### Comparison of Krenn's and pathotypes synovial classifications in knees of OA and RA patients reveals disease and stage-specific features

Following the definition of a new application of synovial pathotype classification to OA synovium, we next compared both Krenn's score and pathotypes distribution in OA with various stages of RA. The histological features of RA tissues harvested from the knees of early treatment-naïve patients (PEACohort), csDMARDs IR (STRAP trial), and anti-TNF IR (R4RA trial) patients were compared with end-stage OA and RA. To avoid the potential bias related to different joint sizes, we only selected biopsies from large joints (knees) for this comparison. Although the groups were homogeneous according to sex distribution, the age was significantly different across disease stages, as one would expect (Supplementary Table 3).

When looking at the degrees of synovial inflammation in OA vs RA, Krenn's scores were variable in the distinct RA-stages groups and relatively different compared to the distribution observed in OA, especially for csDMARDs IR ( $p = 0.05$ ), anti-TNF IR ( $p = 0.02$ ) and end-stage RA ( $p = 0.06$ ) (Fig. 2A). As for the pathotype distribution, we observed a relative increase of the lympho-myeloid pathotype prevalence in RA synovial tissue from early to late-stage disease in the knee joints (Fig. 2B, not significant between naïve and end-stage RA). Except for the csDMARDs IR group ( $p = 0.01$ ), the pathotype distribution did not show significant difference between OA and RA

(Fig. 2B), but our analysis may be underpowered due to the design of the study and the differences in group size.

Interestingly, the histopathological categorization of end-stage OA synovial tissues revealed that the frequency of each synovitis score group and pathotype category was close to the one observed in early treatment-naïve RA, although our analysis may be underpowered to draw a definitive conclusion (Fig. 2A, B).

#### Semi-quantitative scoring of OA synovial immune cell infiltration is confirmed by digital image analysis and distinguishes synovium from different diseases

To approve the semi-quantitative scoring system of immune cells infiltrate established by our group on RA synovial sections,<sup>16</sup> and also applied to other inflammatory diseases such as psoriatic arthritis,<sup>23</sup> in the context of OA, we next selected OA synovial tissues spanning various histological features (including 7 pauci-immune, 15 diffuse-myeloid, and 15 lympho-myeloid) to perform digital image analysis. Following cell segmentation, we defined the percentage of positive and negative cells for each selected marker of immune cells, namely CD3 for T cells, CD20 for B cells, CD138 for plasma cells, and CD68 for monocytes/macrophages (Fig. 3A). Importantly, we showed a significant positive correlation between each CD semi-quantitative score and the respective percentage of positive cells assessed by digital image analysis (Fig. 3B), therefore approving our semi-quantitative scoring methodology also for OA tissues. Both the percentages of CD3<sup>+</sup>, CD20<sup>+</sup>, CD138<sup>+</sup>, and CD68<sup>+</sup> cells, as well as semi-quantitative scores were compared between the three pathotypes (Fig. 3C). Both methods allowed an accurate distinction of the immune cell infiltrate between either pauci-immune and lympho-myeloid, or diffuse-myeloid and lympho-myeloid pathotypes. However, on this selection of patients, the semi-quantitative scoring system was more powerful than the digital image analysis to distinguish the pauci-immune and diffuse-myeloid infiltrate.

Finally, we compared the semi-quantitative scores for each CD marker in the full cohort (n = 185 OA and 29 RA) and noted that the level of synovial immune cell infiltration in pauci-immune and diffuse-myeloid appeared similar in OA and RA (Fig. 3D). Moreover, not only the lympho-myeloid pathotype was relatively enriched in RA synovia compared to OA, although not significantly, (Fig. 1D), but we also revealed that within this pathotype, among the different immune cells analyzed, the T cells and the sublining macrophages were significantly more represented in the RA tissues compared to OA (Fig. 3D).

#### OA synovial pathotypes, but not Krenn's scores, reflect different degrees of peripheral inflammation

To assess the clinical relevance of this pathotype classification in OA, we next investigated the relationship between pathotypes and

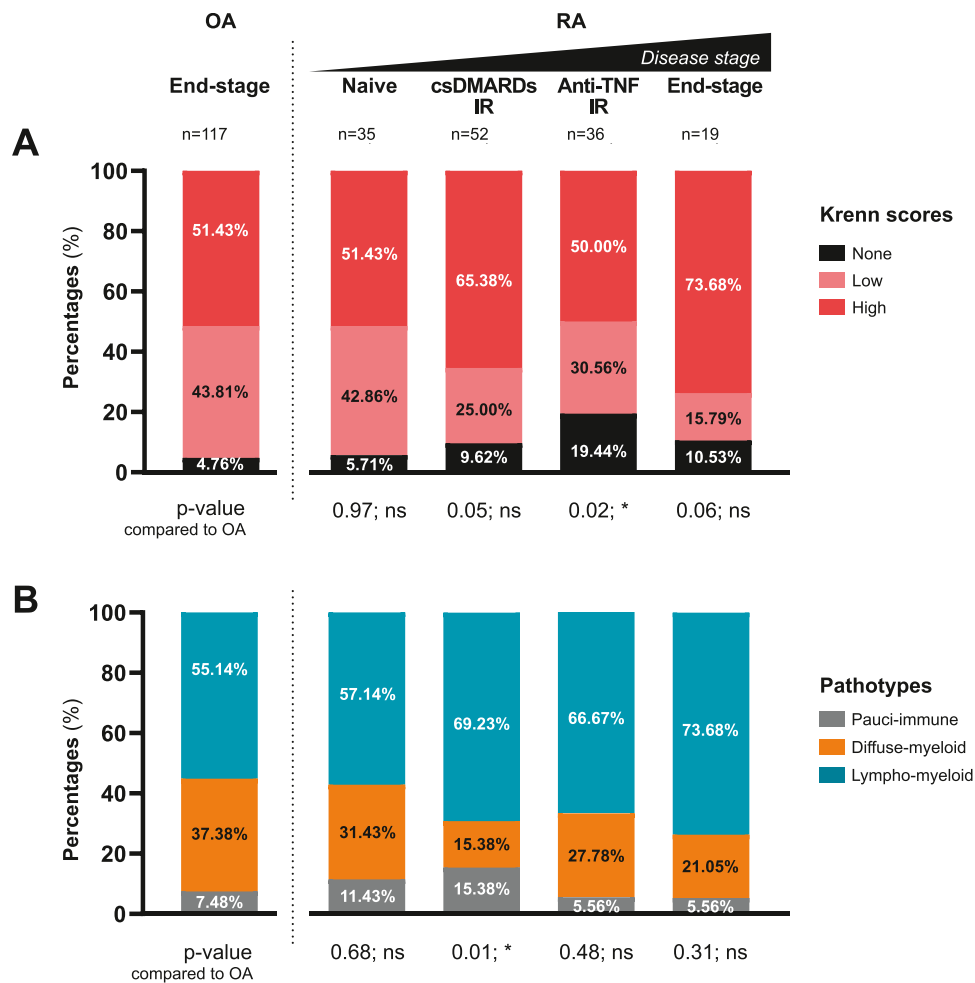


Fig. 2

Comparison of Krenn's and pathotypes synovial classification in knees of osteoarthritis (OA) and rheumatoid arthritis (RA) patients across disease stages. (A–B) Bar charts representing the distribution of each Krenn's score category (panel A, “None” in black, “Low” in pink, and “High” in red) and pathotype (panel B, “pauci-immune” in gray, “diffuse-myeloid” in orange, and “lympho-myeloid” in blue) in synovium from patients with end-stage OA knees (n = 117) and RA patients who were, at the time of the tissue collection, either treatment-naïve (n = 35), conventional synthetic (cs) Disease-Modifying Anti-Rheumatic Drugs (DMARDs, n = 52) inadequate responders (IR), anti-Tumor Necrosis Factor (TNF) IR (n = 36), or at the end-stage of their disease (n = 19). \*p < 0.05, ns non-significant, p-values were calculated using Fisher's exact test by comparing the end-stage OA group and each RA stage.

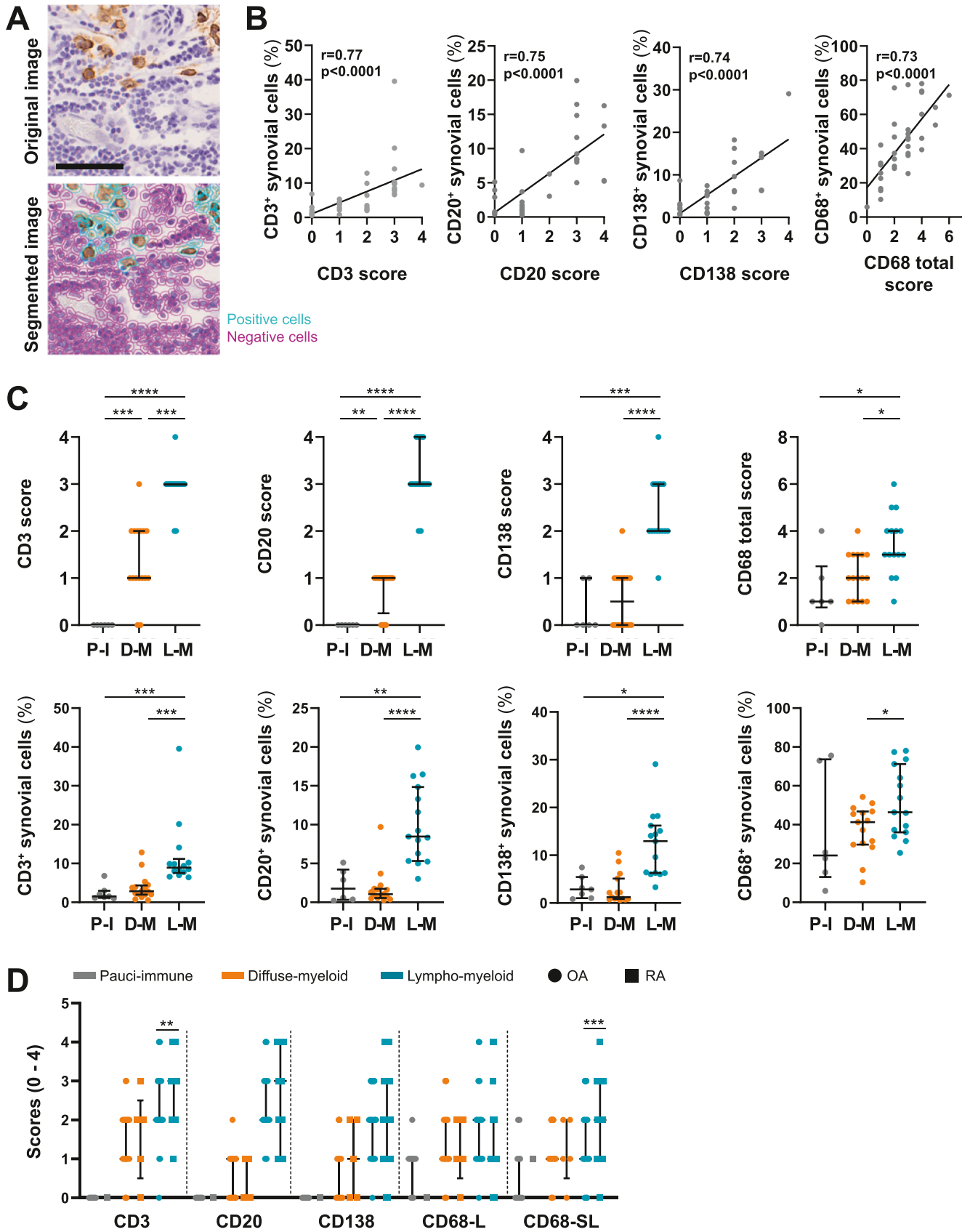
clinical variables, such as age, sex, and complete blood count. Neither the Krenn's nor the pathotype classification associated with the age (Fig. 4A, B) or the sex (Fig. 4C) of the patients. While the Krenn's synovitis classification did not associate with blood cell counts (Fig. 4D, upper panels), the patients with distinct synovial pathotypes exhibited significant changes in their blood cell composition (Fig. 4D, lower panels). The presence of a lympho-myeloid synovial pathotype was indeed associated with a lower hemoglobin values and lymphocytes count compared to the pauci-immune pathotype. Accordingly, both the circulating monocytes-to-lymphocytes ratio (MLR) and the neutrophils-to lymphocytes ratio (NLR) were increased for these patients. Of note, patients with a diffuse-myeloid pathotype presented an intermediate profile with a reduced count of lymphocytes and reduced MLR compared to pauci-immune patients (Fig. 4D, lower panels). Moreover, OA patients with a lympho-myeloid pathotype had similar or higher hemoglobin levels,

lymphocytes count, and MLR/NLR compared to RA patients exhibiting the same synovial pathotype (Supplementary Figure 1).

Considering the known link between sex and OA development,<sup>24</sup> we performed the same analysis on females and males separately. As expected, females had lower hemoglobin level, red blood cells, and monocytes counts compared to men (*data not shown*). The percentage of females in each pathotype group was similar (Supplementary Table 4), and interestingly, the significantly decreased hemoglobin level previously highlighted in OA patients with a lympho-myeloid pathotype was only observed in females (Figure 4E). However, lymphocytes count, MLR, and NLR results were not affected by the sex (Fig. 4E).

## Discussion

Synovial tissue presents significant changes over the course of OA and synovitis correlates with OA severity and pain,<sup>7</sup> therefore



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Fig. 3

Quantitative and semi-quantitative scoring of immune cells infiltration distinguish osteoarthritis (OA) synovial pathotypes. **(A)** Digital image analysis was performed on OA synovial tissue sections using QuPath software.<sup>22</sup> A representative picture of both the immunohistochemistry original (upper panel; 3,3'-Diaminobenzidine (DAB)-positive cells in brown, nuclei in blue) and segmented (lower panel; DAB-positive cells are circled in light blue, DAB-negative cells in pink) images is shown. Scale bar = 50  $\mu$ m. **(B)** Correlation between the percentage of CD3<sup>+</sup>, CD20<sup>+</sup>, CD138<sup>+</sup>, and CD68<sup>+</sup> positive cells and the semi-quantitative scores for inflammatory cells markers CD3, CD20, CD138, and CD68 (total score of both lining and sublining monocytes/macrophages), respectively. p-values were calculated using Spearman correlation test. The analyses were performed on a selection of 37 OA synovial sections, including n = 7 pauci-immune, n = 15 diffuse-myeloid, and n = 15 lympho-myeloid. **(C)** Percentage of CD3<sup>+</sup>, CD20<sup>+</sup>, CD138<sup>+</sup>, and CD68<sup>+</sup> positive cells (upper panels) and CD3, CD20, CD138, and CD68 (total score of both lining and sublining monocytes/macrophages) semi-quantitative scores (lower panels) in pauci-immune (P-I, n = 7, gray dots), diffuse-myeloid (D-M, n = 15, orange dots), and lympho-myeloid (L-M, n = 15, blue dots) OA synovial sections. \*\*\*p < 0.001, \*\*\*\*p < 0.0001, as assessed by the Kruskal–Wallis test with Dunn's post-test. Individual dots represent each patient, and median and interquartile range are represented for each condition. **(D)** CD3, CD20, CD138, lining CD68 (CD68-L), and sublining CD68 (CD68-SL) semi-quantitative scores in synovial tissues from OA (circle dots) and rheumatoid arthritis (RA, square dots) patients presenting a pauci-immune (P-I, n = 12 OA, n = 1 RA, gray dots), diffuse-myeloid (D-M, n = 64 OA, n = 5 RA, orange dots), or lympho-myeloid (L-M, n = 109 OA, n = 23 RA, blue dots). \*\*p < 0.01, \*\*\*p < 0.001, as assessed by the Mann-Whitney test comparing OA and RA conditions exclusively in the D-M and L-M groups for each marker. Individual values, median and interquartile range are shown.

representing a relevant target for therapeutic intervention. OA is a phenotypically heterogeneous disease driven by a variety of etiological and pathological factors<sup>3</sup> and little is known about the relationship between synovial histopathological features and clinical features. Moreover, the best-known histological assessment of synovial inflammation, represented by the Krenn's score categorization, cannot distinguish specific patterns associated with clinical presentation. Here, we proposed a categorization of OA synovial inflammation based on histological pathotypes described and validated in RA, and provided novel insights for future consideration of the relationship between synovitis and OA clinical features.

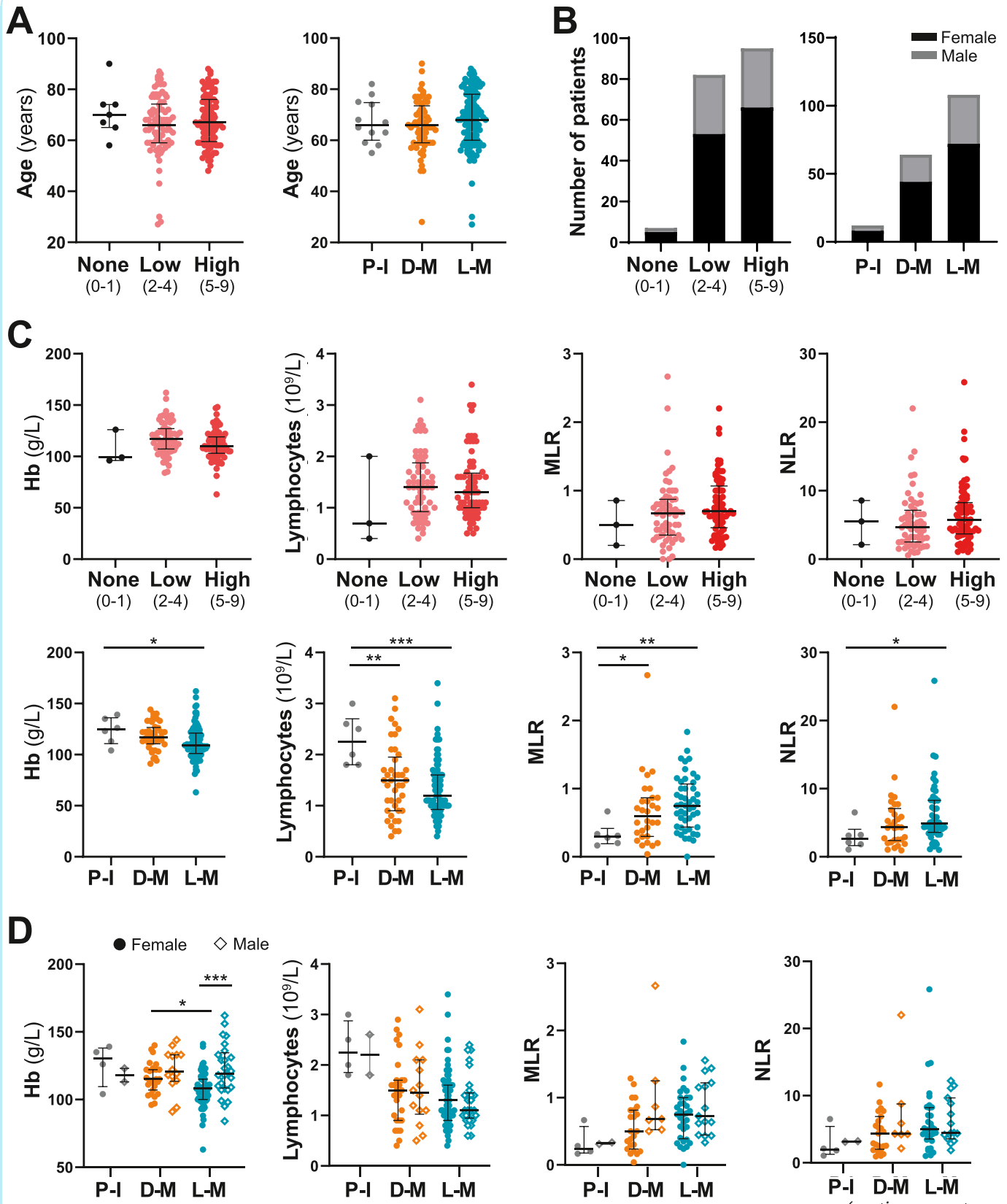
First, we observed recurrent patterns of synovial inflammation, or pathotypes (pauci-immune, diffuse-myeloid and lympho-myeloid) in a large cohort of OA patients, as previously highlighted by our group in the context of RA, a chronic synovitis-driven joint disease<sup>16,18</sup>. We described a robust categorization of OA synovial tissues, based on semi-quantitative scores of immune cells infiltration corroborated by digital image analysis. In line with previous publications, we found that a high Krenn's score was more common in RA compared to OA,<sup>15</sup> with, respectively, 72.41% of RA and 51.89% of OA synovium belonging to this category. Although the lympho-myeloid pathotype was significantly more represented in the RA synovia, over half of the OA patients presented this pathotype characterized by the presence of ELS. ELS are highly organized structures able to sustain autoimmunity in RA and are associated with a more aggressive and erosive disease.<sup>25–27</sup> However, the role of ELS in OA has not been investigated and several hypotheses, such as the presence of debris resulting from joint failure and cartilage degradation in the joint environment, or the chronic joint inflammation may explain the development of the lymphocytic aggregates. Whether ELS could participate in the formation of recently described autoantibodies found in OA<sup>28,29</sup> also deserves further investigation. In studies focusing on various chronic inflammatory joint diseases including RA, OA is often considered as a negative control (seronegative and non/low inflamed synovium), however, the full spectrum of OA synovial inflammatory features must now be considered.

Synovial immune cells infiltrating a relatively high proportion of OA synovium, through their secretion of a plethora of inflammatory and catabolic factors, can undeniably participate in local and low-grade peripheral inflammation characterizing OA patients<sup>30</sup> and may lead to the later development of other chronic conditions, such as metabolic syndrome.<sup>31</sup> Here, for the first time, we highlighted a potential link between synovial pathotypes in OA, in particular the presence of a lympho-myeloid pathotype, with markers of low-grade inflammation, such as relative anemia and lymphopenia. These results emphasize that OA may be considered as a whole-body disease

instead of joint- or even cartilage-restricted condition.<sup>32</sup> Other studies indeed highlighted a compromised immune cell composition in the blood of OA patients, which may also be accentuated with aging.<sup>33</sup> Additional peripheral parameters linked to synovial histopathology in RA,<sup>16</sup> such as the erythrocyte sedimentation rate or C-reactive protein levels, may be included in future studies to better assess the relationship between OA pathotypes and peripheral changes. Our results also highlighted that both MLR and NLR, two parameters associated with OA and with the Kellgren-Lawrence score of x-ray OA intensity,<sup>34–36</sup> were significantly different in distinct synovial pathotypes, calling for further investigations of the relationship between histopathology and both OA joint inflammation and degradation. As such, the relationship between synovial pathotype and the level of joint cartilage damage, assessed by the radiographic Kellgren-Lawrence score, or the Osteoarthritis Research Society International (OARSI) scoring of Safranin-O Fast green cartilage staining should be considered in future studies. In addition, metabolic changes and obesity have been shown to influence synovitis features in RA,<sup>37</sup> and, considering the importance of metabolic changes in OA and the impact of obesity on the transcriptomic profile and distribution of synovial cells, it appears important to further dissect the relationship between synovial histopathology and the body mass index. Finally, as pain is a major driver of OA and specific features of synovitis have been associated with distinct levels of pain for the patients,<sup>38,39</sup> collecting indications on pain, stiffness, and physical function (e.g., Western Ontario and McMaster Universities Arthritis Index) will be of particular importance in the future. Follow-up studies should therefore be carefully designed to validate our findings and to further investigate the above-mentioned aspects. Importantly, in line with the established link between sex and OA development,<sup>24</sup> we highlighted that OA female patients were more prone to exhibiting a low hemoglobin level associated with the presence of the lympho-myeloid pathotype, emphasizing that sex-specific parameters should be monitored in OA studies.

We further analyzed the distribution of Krenn's and pathotype categories in synovial tissues harvested from the knees of patients with OA and various stages of RA. Importantly, as our work was based on sample availability and not on power calculations a priori, the number of patients included in each group (185 OA, 29 RA) was very different, and our analysis may be underpowered to draw definitive conclusions about disease comparison. In line with what we previously published,<sup>17</sup> we confirmed that the lympho-myeloid pathotype is increasingly represented as the RA stage progresses toward long-standing disease. Here, we showed similar synovial pathotypes distribution between OA and early treatment-naïve RA





(caption on next page)

**Fig. 4**

Osteoarthritis (OA) synovial pathotypes, but not Krenn's scores, are associated with distinct blood cell counts and reflect different grades of peripheral inflammation. **(A)** Age (in years) of the OA patients presenting a synovial tissue classified as “none” (score 0–1,  $n = 7$ ), “low” (score 2–4,  $n = 82$ ), or “high” (score 5–9,  $n = 96$ ) according to the Krenn scoring system (left panel) or as pauci-immune (P-I,  $n = 12$ ), diffuse-myeloid (D-M,  $n = 64$ ), or a lympho-myeloid (L-M,  $n = 109$ ) synovial pathotype (right panel). p-value was calculated using the Kruskal-Wallis test with Dunn's post-test and was not significant. **(B)** Bar chart representing the distribution of female and male patients in each Krenn's score category (left panel, “none” 0–1, “low” 5–4, and “high” 5–9) and pathotype group (right panel, “P-I” pauci-immune, “D-M” diffuse-myeloid, and “L-M” lympho-myeloid) in synovium from patients with end-stage OA ( $n = 184$ ). p-values were calculated using the Fisher's exact test and were not significant. **(C)** Peripheral hemoglobin level (g/L), lymphocyte count ( $10^9/L$ ), monocyte-to-lymphocyte ratio (MLR), and neutrophil-to-lymphocyte ratio (NLR) in patients with end-stage OA ( $n = 185$ ) presenting each Krenn's score category (upper panels; “None” in black,  $n = 7$ ; “Low” in pink,  $n = 82$ ; “High” in red,  $n = 96$ ) or pathotype group (lower panels; pauci-immune “P-I” in gray,  $n = 12$ ; diffuse-myeloid “D-M” in orange,  $n = 64$ ; lympho-myeloid “L-M” in blue,  $n = 109$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , as assessed by the Kruskal-Wallis test with Dunn's post-test. **(D)** Peripheral hemoglobin level (g/L), lymphocyte count ( $10^9/L$ ), MLR, and NLR in female ( $n = 124$ , black circle dots) and male ( $n = 60$ , white diamond-shaped dots) patients with end-stage OA presenting a pauci-immune (“P-I” in gray,  $n = 12$ ), a diffuse-myeloid (“D-M” in orange,  $n = 64$ ), or a lympho-myeloid (“L-M” in blue,  $n = 108$ ) pathotype. \* $p < 0.05$ , \*\*\* $p < 0.001$ , as assessed by the Kruskal-Wallis test with Dunn's post-test. **(A, C, D)** Individual dots represent each patient, median, and interquartile range are represented for each condition.

synovial tissue, calling for further analysis to confirm this result with two groups of balanced size. Of note, we demonstrated here that knee and hip joints exhibited similar pathotypes repartition at late stage of OA. Unfortunately, our retrospective study only included OA synovial samples harvested at the time of total joint replacement, when pain and mechanical restrictions caused by OA are established and therefore considered as late/end-stage. Various synovial cell composition comparatively in early and late OA have been recently uncovered<sup>38</sup> and the encouraging results presented herein should now prompt further clinical studies to investigate the influence of disease stage on synovial histopathology and establish a potential relationship with early clinical manifestations. In addition, considering the possible histopathological variation overtime, influenced for example by treatment exposure, as previously described by our group in the context of RA,<sup>17</sup> should also prompt the development of longitudinal histopathological analysis in the context of OA.

The new application of synovial pathotype classification to OA synovium described herein allows to take into consideration the major synovial immune cells proportion and to compare disease-specific features. The main disease-specific changes regarding immune cell infiltrate were observed in the lympho-myeloid group. We found that  $CD3^+$  T cells and  $CD68^+$  monocytes/macrophages located in the sublining part of the synovium were present in higher proportions in RA compared to OA. According to previous studies reporting a key role for macrophages in OA pathophysiology and the benefit of targeting these cells in OA mouse models,<sup>40</sup> the large spectrum of macrophage population described in the context of chronic joint disease should be considered for future preclinical investigations.<sup>41</sup> Moreover, a distinct distribution of macrophages within the sublining layer of OA and RA synovium described by Mucke and colleagues<sup>42</sup> may also have pathophysiological implications. We did not show significant differences in the total  $CD20^+$  B cells or  $CD138^+$  plasma cells infiltrate between OA and RA. While these results confirmed recent findings on 5 OA and 5 RA synovium,<sup>43</sup> the authors also highlighted that specific immunoglobulin G4 (IgG4)-secreting  $CD138^+$  plasma cells were more represented in RA,<sup>43</sup> in line with the close link between IgG4 and autoimmunity.<sup>44</sup>

Although promising therapeutic strategies have been proposed over the years and encouraging results obtained in pre-clinical studies to avert or slow down the progression of OA, the inability to adequately treat OA with disease-modifying drugs and prevent the joint damage remains still a major unmet need.<sup>45</sup> The failure to identify subgroups of patients who may better respond to either chondroprotective, or anti-inflammatory drugs may be the reason

why trials have failed so far. Here, we proposed a novel and relevant classification of OA synovial inflammation based on histopathological pathotypes, which may facilitate OA patients' stratification and the development of more personalized therapeutic strategies in the future.

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#### CRediT authorship contribution statement

M.-A.B. analyzed the data and wrote the manuscript; A.N., L.F.-J., R.H., F.R. managed the clinical data collection and storage and participated in the data analysis; M.A. processed and stored the samples and performed histological and immunohistological staining; A.N., L.F.-J., F.R., and C.P. raised the funds, critically contributed to study conception and revised the manuscript.

#### Conflict of interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.joca.2023.10.009](https://doi.org/10.1016/j.joca.2023.10.009).

## References

- Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: a Lancet Commission. *Lancet* 2020;396(10264):1711–2. [https://doi.org/10.1016/S0140-6736\(20\)32230-3](https://doi.org/10.1016/S0140-6736(20)32230-3)
- Safiri S, Kolahi AA, Smith E, Hill C, Bettampadi D, Mansournia MA, et al. Global, regional and national burden of osteoarthritis 1990–2017: a systematic analysis of the Global Burden of Disease Study 2017. *Ann Rheum Dis* 2020;57(1):153–64. <https://doi.org/10.1136/annrheumdis-2019-216515>
- Mobasheri A, Saarakkala S, Finnilä M, Karsdal MA, Bay-Jensen AC, van Spil WE. Recent advances in understanding the phenotypes of osteoarthritis. *F1000Res* 2019;8:2091. <https://doi.org/10.12688/f1000research.20575.1>
- Deveza LA, Melo L, Yamato TP, Mills K, Ravi V, Hunter DJ. Knee osteoarthritis phenotypes and their relevance for outcomes: a systematic review. *Osteoarthritis Cartilage* 2017;25(12):1926–41. <https://doi.org/10.1016/j.joca.2017.08.009>
- Deveza LA, Nelson AE, Loeser RF. Phenotypes of osteoarthritis: current state and future implications. *Clin Exp Rheumatol* 2019;37(5):64–72.
- Van Spil WE, Bierma-Zeinstra SMA, Deveza LA, Arden NK, Bay-Jensen AC, Kraus VB, et al. A consensus-based framework for conducting and reporting osteoarthritis phenotype research. *Arthritis Res Ther* 2020;22(1):54. <https://doi.org/10.1186/s13075-020-2143-0>
- Mathiessen A, Conaghan PG. Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis Res Ther* 2017;19(1). <https://doi.org/10.1186/s13075-017-1229-9>
- Atukorala I, Kwok CK, Guermazi A, Roemer FW, Boudreau RM, Hannon MJ, et al. Synovitis in knee osteoarthritis: a precursor of disease. *Ann Rheum Dis* 2016;75(2):390–5. <https://doi.org/10.1136/annrheumdis-2014-205894>
- Guermazi A, Hayashi D, Roemer FW, Zhu Y, Niu J, Crema MD, et al. Synovitis in knee osteoarthritis assessed by contrast-enhanced magnetic resonance imaging (MRI) is associated with radiographic tibiofemoral osteoarthritis and MRI-detected widespread cartilage damage: the MOST study. *J Rheumatol* 2014;41(3):501–8. <https://doi.org/10.3899/jrheum.130541>
- Baker K, Grainger A, Niu J, Clancy M, Guermazi A, Crema M, et al. Relation of synovitis to knee pain using contrast-enhanced MRIs. *Ann Rheum Dis* 2010;69(10):1779–83. <https://doi.org/10.1136/ard.2009.121426>
- Ramezanpour S, Kanthawang T, Lynch J, McCulloch CE, Nevitt MC, Link TM, et al. Impact of sustained synovitis on knee joint structural degeneration: 4-year MRI data from the osteoarthritis initiative. *Magn Reson Imaging* 2022;57(1):153–64. <https://doi.org/10.1002/jmri.28223>
- Klein-Wieringa IR, de Lange-Brokaar BJE, Yusuf E, Andersen SN, Kwekkeboom JC, Kroon HM, et al. Inflammatory cells in patients with endstage knee osteoarthritis: a comparison between the synovium and the infrapatellar fat pad. *J Rheumatol* 2016;43(4):771–8. <https://doi.org/10.3899/jrheum.151068>
- Xie J, Huang Z, Yu X, Zhou L, Pei F. Clinical implications of macrophage dysfunction in the development of osteoarthritis of the knee. *Cytokine Growth Factor Rev* 2019;46:36–44. <https://doi.org/10.1016/j.cytogfr.2019.03.004>
- Chou CH, Jain V, Gibson J, Attarian DE, Haraden CA, Yohn CB, et al. Synovial cell cross-talk with cartilage plays a major role in the pathogenesis of osteoarthritis. *Sci Rep* 2020;10(1):10868. <https://doi.org/10.1038/s41598-020-67730-y>
- Krenn V, Morawietz L, Burmester GR, Kinne RW, Mueller-Ladner U, Muller B, et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* 2006;49(4):358–64. <https://doi.org/10.1111/j.1365-2559.2006.02508.x>
- Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Ann Rheum Dis* 2019;78(6):761–72. <https://doi.org/10.1136/annrheumdis-2018-214539>
- Nerviani A, Di Cicco M, Mahto A, Lliso-Ribera G, Rivellesse F, Thorborn G, et al. A pauci-immune synovial pathotype predicts inadequate response to tnfa-blockade in rheumatoid arthritis patients. *Front Immunol* 2020;11:845. <https://doi.org/10.3389/fimmu.2020.00845>
- Lewis MJ, Barnes MR, Blighe K, Goldmann K, Rana S, Hackney JA, et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Reports* 2019;28(9):2455–70. <https://doi.org/10.1016/j.celrep.2019.07.091>
- Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. *Rheumatology* 2012;51(suppl 6):vi5–9. <https://doi.org/10.1093/rheumatology/kes279>
- Humby F, Durez P, Buch MH, Lewis MJ, Rizvi H, Rivellesse F, et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. *Lancet* 2021;397(10271):305–17. [https://doi.org/10.1016/S0140-6736\(20\)32341-2](https://doi.org/10.1016/S0140-6736(20)32341-2)
- Pitzalis C, Kelly S, Humby F. New learnings on the pathophysiology of RA from synovial biopsies. *Curr Opin Rheumatol* 2013;25(3):334–44. <https://doi.org/10.1097/BOR.0b013e32835fd8eb>
- Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: open source software for digital pathology image analysis. *Sci Rep* 2017;7(1):16878. <https://doi.org/10.1038/s41598-017-17204-5>
- Boutet MA, Nerviani A, Lliso-Ribera G, Lucchesi D, Prediletto E, Ghirardi GM, et al. Interleukin-36 family dysregulation drives joint inflammation and therapy response in psoriatic arthritis. *Rheumatology* 2020;59(4):828–38. <https://doi.org/10.1093/rheumatology/kez358>
- Tschon M, Contartese D, Pagani S, Borsari V, Fini M. Gender and sex are key determinants in osteoarthritis not only confounding variables. A systematic review of clinical data. *JCM* 2021;10(14):3178. <https://doi.org/10.3390/jcm10143178>
- Nerviani A, Pitzalis C. Role of chemokines in ectopic lymphoid structures formation in autoimmunity and cancer. *J Leukoc Biol* 2018;104(2):333–41. <https://doi.org/10.1002/JLB.3MR0218-062R>
- Corsiero E, Nerviani A, Bombardieri M, Pitzalis C. Ectopic lymphoid structures: powerhouse of autoimmunity. *Front Immunol* 2016;7:430. <https://doi.org/10.3389/fimmu.2016.00430>

27. Humby F, Bombardieri M, Manzo A, Kelly S, Blades MC, Kirkham B, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS Med* 2009;6(1), e1. <https://doi.org/10.1371/journal.pmed.0060001>
28. Xie X, van Delft MAM, Shuweihdi F, Kingsbury SR, Trouw LA, Doody GM, et al. Auto-antibodies to post-translationally modified proteins in osteoarthritis. *Osteoarthritis Cartilage* 2021;29(6):924–33. <https://doi.org/10.1016/j.joca.2021.03.008>
29. Camacho-Encina M, Balboa-Barreiro V, Rego-Perez I, Picchi F, VanDuin J, Qiu J, et al. Discovery of an autoantibody signature for the early diagnosis of knee osteoarthritis: data from the Osteoarthritis Initiative. *Ann Rheum Dis* 2019;78(12):1699–705. <https://doi.org/10.1136/annrheumdis-2019-215325>
30. Chow YY, Chin KY. The role of inflammation in the pathogenesis of osteoarthritis. *Mediators Inflamm* 2020;2020:1–19. <https://doi.org/10.1155/2020/8293921>
31. Swain S, Sarmanova A, Coupland C, Doherty M, Zhang W. Comorbidities in osteoarthritis: a systematic review and meta-analysis of observational studies. *Arthritis Care Res* 2020;72(7):991–1000. <https://doi.org/10.1002/acr.24008>
32. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis Cartilage* 2013;21(1):16–21. <https://doi.org/10.1016/j.joca.2012.11.012>
33. Ponchel F, Burska AN, Hensor EMA, Raja R, Campbell M, Emery P, et al. Changes in peripheral blood immune cell composition in osteoarthritis. *Osteoarthritis Cartilage* 2015;23(11):1870–8. <https://doi.org/10.1016/j.joca.2015.06.018>
34. Gao K, Zhu W, Liu W, Ma D, Li H, Yu W, et al. Diagnostic value of the blood monocyte–lymphocyte ratio in knee osteoarthritis. *J Int Med Res* 2019;47(9):4413–21. <https://doi.org/10.1177/0300060519860686>
35. Taşoğlu Ö, Bölük H, Şahin Onat Ş, Taşoğlu İ, Özgirgin N. Is blood neutrophil-lymphocyte ratio an independent predictor of knee osteoarthritis severity. *Clin Rheumatol* 2016;35(6):1579–83. <https://doi.org/10.1007/s10067-016-3170-8>
36. Cai C, Zhang R, Xu X, Li G, Gou H. Diagnostic values of NLR and miR-141 in patients with osteoarthritis and their association with severity of knee osteoarthritis. *Exp Ther Med* 2020;21(1):74. <https://doi.org/10.3892/etm.2020.9506>
37. Alivernini S, Tolusso B, Gigante MR, Petricca L, Bui L, Fedele AL, et al. Overweight/obesity affects histological features and inflammatory gene signature of synovial membrane of Rheumatoid Arthritis. *Sci Rep* 2019;9(1):10420. <https://doi.org/10.1038/s41598-019-46927-w>
38. Nanus DE, Badoume A, Wijesinghe SN, Halsey AM, Hurley P, Ahmed Z, et al. Synovial tissue from sites of joint pain in knee osteoarthritis patients exhibits a differential phenotype with distinct fibroblast subsets. *eBioMedicine* 2021;72, 103618. <https://doi.org/10.1016/j.ebiom.2021.103618>
39. Vincent TL. Peripheral pain mechanisms in osteoarthritis. *Pain* 2020;161(Suppl 1):S138–46. <https://doi.org/10.1097/j.pain.0000000000001923>
40. Zhang H, Cai D, Bai X. Macrophages regulate the progression of osteoarthritis. *Osteoarthritis Cartilage* 2020;28(5):555–61. <https://doi.org/10.1016/j.joca.2020.01.007>
41. Boutet MA, Courties G, Nerviani A, Le Goff B, Apparailly F, Pitzalis C, et al. Novel insights into macrophage diversity in rheumatoid arthritis synovium. *Autoimmun Rev* 2021;20(3), 102758. <https://doi.org/10.1016/j.autrev.2021.102758>
42. Mucke J, Hoyer A, Brinks R, Bleck E, Pauly T, Schneider M, et al. Inhomogeneity of immune cell composition in the synovial sublining: linear mixed modelling indicates differences in distribution and spatial decline of CD68+ macrophages in osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther* 2016;18(1):170. <https://doi.org/10.1186/s13075-016-1057-3>
43. Dolzani P, Manferdini C, Meliconi R, Lisignoli G, Pulsatelli L. Preliminary study on immune cells in the synovium of end-stage osteoarthritis and rheumatoid arthritis patients: neutrophils and IgG4-secreting plasma cells as differential diagnosis candidates. *Acta Histochem* 2022;124(5), 151909. <https://doi.org/10.1016/j.acthis.2022.151909>
44. Maslinska M, Dmowska-Chalaba J, Jakubaszek M. The role of IgG4 in autoimmunity and rheumatic diseases. *Front Immunol* 2022;12, 787422. <https://doi.org/10.3389/fimmu.2021.787422>
45. Delplace V, Boutet MA, Visage CL, Maugars Y, Guicheux J, Vinatier C. Osteoarthritis: from upcoming treatments to treatments yet to come. *Joint Bone Spine* 2021;88(5), 105206. <https://doi.org/10.1016/j.jbspin.2021.105206>