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ORIGINAL RESEARCH

Diagnostic and prognostic role of synovial tissue analysis in juvenile idiopathic arthritis: a monocentric study

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ABSTRACT

Objectives This study investigates the diagnostic role of synovial tissue analysis in children presenting with arthritis and assesses its prognostic significance to predict clinical outcome in juvenile idiopathic arthritis (JIA).

Methods Synovial samples of paediatric patients undergoing synovial biopsy between 1995 and 2020 were analysed histologically and immunohistochemically. Relationships between histological/immunohistochemical parameters and clinical variables were assessed.

Results Synovial biopsy was performed for diagnosis in 65 cases allowing to correctly classify 79% of patients. At histological analysis on 42 JIA samples, any difference in the number of synovial lining layers, subsynovial elementary lesions, fibrin deposit, Krenn Synovitis Score, inflammatory infiltrate score and pattern emerged between JIA subsets or on treatment exposure. Synovial tissue analysis predicted outcome: higher number of synovial layers predicted worse disease course (>4 flares during follow-up; 4.5 vs 3.0, p=0.035), even after adjusting for age at diagnosis and observation time (OR 2.2, p=0.007); subjects who had switched>2 biological disease-modifying antirheumatic drugs had higher prevalence of subsynovial elementary lesions (55.6% vs 10.3%, p=0.005) and fibrin deposits in synovial lining (60.0% vs 22.6%, p=0.049), even after adjustment for observation time and age at diagnosis (OR 8.1, p=0.047). At immunohistochemistry on 31 JIA samples, higher CD3 expression was described in polyarticular compared with oligoarticular subset (p=0.040). Patients with severe disease course had higher CD20+ rate (OR 7, p=0.023), regardless of JIA subset and treatment exposure.

Conclusions Synovial tissue analysis might support the clinicians in the diagnostic approach of paediatric patients presenting with arthritis and guide the clinical management in JIA.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ To date, no study has addressed the role of synovial pathobiology to support the diagnostic approach and guide the clinical management in children suffering from juvenile idiopathic arthritis (JIA).

WHAT THIS STUDY ADDS

⇒ Synovial biopsy may support clinicians in the diagnostic approach of paediatric patients presenting with arthritis, especially in doubtful cases.

⇒ Synovial tissue analysis opens new frontiers about synovial tissue pathophysiology in JIA and highlights different synovial microenvironments.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Differences in histopathology may predict different clinical outcomes and may guide the therapeutic approach for patients with JIA.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) refers to any arthritis persisting for more than 6 weeks and presenting before 16 years, with incidence peaking among infants aged from 1 to 3 years.[1] A prevalence of 16–150 cases per 100 000 in developed countries renders JIA the most common childhood rheumatic disease.[1] Due to the wide clinical heterogeneity of such condition and the differential response to treatment, the International League of Associations for Rheumatology (ILAR) identifies seven different JIA subsets.[2] Unfortunately, the pathogenesis of JIA still remains to be elucidated, even though the interaction between environmental exposure and genetic factors, mainly located in both class I (HLA A-2 and HLA B27) and class II (HLA-DRB1 and HLA-DP) major histocompatibility complex (MHC) loci, is currently regarded as the main aetiological contributor.[3–6] In JIA, inflammation originates from the synovium, resulting in alterations of synovial architecture such as hyperplasia of the synovial lining membrane, hypervascularity and infiltration of the sublining by mononuclear cells. The inflammatory infiltrate consists mainly in T-lymphocytes, plasma cells and macrophages but also proliferating fibroblasts and macrophage-like

synoviocytes, with altered activation patterns of both resident and infiltrating cells.[7 8] In children, the synovial membrane may be the target of a plethora of additional disorders, such as storage, infectious, inflammatory and neoplastic diseases. All these conditions can lead to an arthritic phenotype, representing an important challenge for clinicians: the diagnostic algorithm takes into account the age at disease onset, the number and the type of involved joints and the associated symptoms or signs. In this context, synovial biopsy may be useful to correctly diagnose cases of uncertain aetiology, in particular when few joints are affected. Once the diagnosis of JIA has been formulated, the clinicians face challenges in tailoring the therapeutic approach on each patient's clinical features. Indeed, several different conventional and biological disease modifying antirheumatic drugs (cDMARDs and bDMARDs) are available in the paediatric rheumatological pharmacological armamentarium, but the rate of response depends on several clinical and biological variables while a not negligible rate of patients never obtains clinical remission.[9] In a similarly challenging scenario, seminal works on synovial tissue analysis have demonstrated its clinical utility when integrated in the decision process for routine management of adult patients with rheumatoid arthritis (RA). Indeed, in this setting, synovial tissue analysis allows identifying different pathotypes based on the relative enrichment of cell populations and gene expression signatures (lympho-myeloid, myeloid or pauci-immune): these pathotypes have been consistently demonstrated to contribute to the diverse clinical phenotypes, outcomes and response to treatment.[10 11]

To date, no study has addressed the role of synovial pathobiology to support the diagnostic approach and guide the clinical management in children presenting with arthritis. Therefore, the aims of the present study were (i) to investigate the role of synovial biopsy in the diagnostic approach to children presenting with arthritis and (ii) to assess the prognostic significance of synovial histology and immunohistochemistry pathological features in predicting clinical outcome and treatment response among patients with JIA.

METHODS

Medical records of paediatric patients who underwent a synovial biopsy in Pediatric Rheumatology/Orthopedic Department (G. Pini Hospital) between 1995 and 2020 were retrospectively reviewed. The following clinical details were collected in a dedicated database: observation time (defined as the time between diagnosis and last follow-up visit), 2011 Wallace remission criteria[12] and juvenile arthritis disease activity score[13 14] at the latest examination, the number of disease flares since the beginning of the disease to last visit, the number of previous cDMARDS and bDMARDs and histological/immunohistochemistry features. Response to therapy was defined as the achievement of clinical remission according to Wallace's criteria within 6 months from the beginning of treatment.

Patients with JIA were subclassified into two groups based on postbiopsy disease course: when more than 4 flares occurred during follow-up and patients had switched more than two bDMARDs, disease was defined as moderate–severe disease. In the remaining cases, disease was defined as mild.

Synovial biopsy

Arthroscopic biopsies were performed in surgical theatre under local anaesthesia and sterile conditions through a small-bore arthroscope that allows inspection and biopsy of synovial tissue under direct vision from different sites. Disinfection of the skin was performed with iso-betadine. Local anaesthetics included xylocaine 1% for the skin and joint capsule, and xylocaine 0.5% for the intra-articular joint space. Sodium chloride 0.9% was used for joint lavage. In patients candidate to elective orthopaedic surgery, synovial samples were retrieved during open sky procedures under spinal or general anaesthesia in the operating room, following different approaches and procedures based on the main surgical indication.

At least 6–8 synovial samples of macroscopically inflamed synovial membrane were collected for each patient.

All patients and/or both their parents provided written informed consent to the procedure.

Histological and immunohistochemical analysis

All synovial specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 3 µm. A minimum of 10 sequentially cut sections was obtained from each biopsy. The first section was stained for H&E.

The following parameters were evaluated in each biopsy: number of layers of synovial lining, inflammatory cell infiltrate and stromal cell density using a semiquantitative score from 0 (absent) to 3 (strong). The infiltrate was evaluated by the pattern of distribution (diffuse, cluster, perivascular, pseudofollicolar), predominant inflammatory infiltrates (lymphocytic, plasmacellular, neutrophilic, eosinophilic). The three items were then combined to calculate Krenn Synovitis Score (KSS) in order to discriminate between high grade versus low-grade synovitis. The maximum score is 9.[15]

Immunohistochemistry was performed by automated staining system (Dako Omnis, Agilent). The following lineage markers were used: CD3 (Agilent Technologies, Santa Clara, California, USA: polyclonal prediluted), CD20 (Agilent: clone L26, prediluted), CD103 (Gennova Biopharmaceuticals, Pune, India: EPR4166,[2] 1:50 dilution), CD69 (Gennova Biopharmaceuticals: EPR2184, 1:50), and Ki67-MIB1 (Agilent Technologies: MIB-1, prediluited).

Slides were examined using a light microscope by two independent pathologist (EA and AP), both expert in synovial tissue histology, blinded to clinical data of patients.

Statistical analysis

Descriptive statistics was used to summarise data. Categorical variables were expressed as percentages. The normality of variable distribution was tested by applying Shapiro-Wilk test. Continuous variables with non-parametric distribution were expressed as median values (IQR) while continuous variables with parametric distribution were expressed as mean (SD). Differences in non-parametric continuous variables between groups were assessed by Mann-Whitney test while associations between categorical predictors and outcomes were evaluated by χ^2 or Fisher's exact test, as appropriate. Logistic multivariable models with moderate–severe disease course as dependent variable was applied in order to measure the strength of association (OR) and related 95% CI, adjusted for potential confounders. P values ≤0.05 were considered as statistically significant. Analyses were performed with R commander and GraphPad Prism V.9.4.1.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research, also due to the retrospective design of the present study.

RESULTS

During the study period, 706 synovial biopsies were performed in paediatric patients. Patients with pure orthopaedic conditions, malignancies or missing clinical details were excluded leading to the inclusion in this study of 99 patients (65% female;online supplemental figure 1). Among these patients, the median age at onset of joint swelling was 8.5 years (IQR 8, range 1–17) while median age at synovial biopsy was 14 years (IQR 7, range 3–21), with a median follow-up time of 161 months (IQR 927, range 8–1160).

Synovial samples were obtained from the following joints: coxo-femoral (2), shoulders (2), wrists (6) and knees (89).

The diagnostic role of synovial tissue analysis in children presenting with joint swelling of unknown aetiology

Of the 99 included subjects, 34 individuals with an established JIA diagnosis underwent synovial biopsy in the course of an orthopaedic procedure (synovectomy and joint replacement). In all these patients, the synovial tissue analysis was consistent with JIA.

The remaining 65 patients were candidate to synovial biopsy for diagnostic purposes. In 40 cases, histological features were suggestive of a chronic synovitis and a final JIA diagnosis was formulated, in one case with a concomitant foreign body. In the other cases, the histological findings allowed a final diagnosis of other conditions. In particular, the synovial biopsy proved to be instrumental for the diagnosis in 11 patients (sarcoidosis n=1, osteomyelitis n=1, tenosynovial giant cell tumour n=6, osteochondromatosis n=1, foreign body n=2). In the remaining 14 patients, synovial biopsy allowed excluding inflammatory diseases, but the final diagnosis was reached thanks to other investigations (genetic and imaging tests): camptodactyly arthropathy, coxa vara, pericarditis (CACP) in two patients, Thiemann disease, skeletal dysplasia, osteochondrosis in one case each. In nine cases, diagnosis remained undefined (online supplemental figure 2).

Association between the number of synovial layers and severe clinical course in patients with JIA

Overall, 74 patients in our study cohort received a JIA diagnosis in agreement with current classification criteria for JIA.[1] Twenty-three histological samples were not available and 9 were deteriorated; thus, the relationship of histological parameters and clinical outcome was assessed in a sample of 42 cases, all obtained from knee joints but 4 (2 from the shoulders and 2 wrists). Demographics and clinical features of these 42 patients are summarised in table 1.

In this JIA cohort, the median disease duration at the time of synovial biopsy was 43 months (IQR 14.7–147.2) while median time from synovial biopsy to the latest visit was 102 months (IQR 41.5–151.3). The number of layers of synovial lining, subsynovial elementary lesions, fibrin deposit, KSS, inflammatory infiltrate score and pattern were recorded for each sample (figure 1). Any differences in these histological parameters could be described across different JIA subgroups (table 2) and on age at diagnosis, with a threshold set at 7 years of age.

Only 9 patients (21%) were naïve to steroids or cDMARDs and bDMARD therapy at the time of biopsy, while 11 patients received only intra-articular steroid injection (26%) 6–12 months before biopsy. Treatment-experienced subjects had been followed up for a significantly longer period than the 9 treatment-naïve children (mean 17.1 (SD 9.0) vs 6.0 years (SD 4.6), p=0.001). All treatment-naïve patients reached clinical remission and had a good final prognosis (100% in treatment-naïve subject vs 56% in those treatment experienced; p=0.035). However, no differences in histological features between treatment-naïve and treatment-experienced patients were found (table 3).

At the last follow-up visit, all patients were on medication: 27 subjects (64%) were in clinical remission or displayed low disease activity, while in 15 patients (36%) disease was active. Synovial tissue analysis allowed identifying patients with subsequent moderate–severe disease course (table 4).

Disease course was defined as moderate–severe in case of requirement of more than 2 biologics and more than 4 flares in the course of follow-up, from disease onset to the latest visit. All remaining patients were classified as mild disease. Subjects who experienced more than 4 flares during postbiopsy follow-up showed a significantly higher number of layers of synovial lining (median 4.5 (IQR 3.0) vs 3.0 (IQR 2.5), p=0.035, figure 2). The number of layers remained significantly predictive of follow-up flares even after adjusting for age at diagnosis and observation time (OR 2.2, 95% CI 1.3 to 3.9, p=0.007). Subjects who had

Table 1 Demographic and clinical features of patients with a definite diagnosis of juvenile idiopathic arthritis (JIA) subgrouped on disease subset at the time of synovial biopsy

	JIA subsets					
	Oligo n=27	RF- Poly n=6	ERA n=5	PsA n=1	Systemic n=3	Overall population n=42
Age at the time of biopsy (years), mean (SD)	13.4 (4.7)	12.8 (5.5)	15 (3.2)	15	16.7 (2.5)	13.8 (4.5)
Disease duration at time of biopsy (months), median (IQR)	42 (155.5)	22.5 (53.3)	27 (34)	1	157 (70)	43 (132.5)
Gender, %F (n)	74 (20)	67 (4)	40 (2)	100 (1)	67 (2)	69 (29)
Age at the onset of symptoms, mean (SD)	7.1 (4.1)	8.4 (4.7)	12.6 (2.4)	4	4.3 (3.5)	7.6 (4.4)
Age at confirmed diagnosis, mean (SD)	7.5 (4.3)	9.3 (4.9)	12.8 (2.7)	4	4.7 (4.0)	8.1 (4.6)
Diagnostic delay (months), median (IQR)	6 (4.5)	10.5 (15.3)	9 (6)	1	4 (2)	5.5 (8.8)
ANA positivity, % (n)	52 (14)	17 (1)	40 (1)	100 (1)	0 (0)	43 (16)
Cumulative treatment at the time of biopsy						
Treatment naïve % (n)	22 (6)	33 (2)	20 (1)	0 (0%)	0 (0%)	21 (9)
Steroidal injection % (n)						
Alone	30 (8)	17 (1)	20 (1)	1 (100%)	0 (0%)	26 (11)
In association with other treatment	74 (20)	33 (2)	20 (1)	0 (0%)	0 (0%)	62 (26)
csDMARDs % (n)	44 (12)	33 (2)	60 (3)	0 (0%)	100 (3)	48 (19)
bDMARDs % (n)	22 (6)	33 (2)	20 (1)	0 (0%)	67 (2)	26 (11)

ANA, antinuclear antibodies; bDMARDs, biological disease-modifying antirheumatic drugs.; csDMARDs, conventional disease-modifying antirheumatic drugs; ERA, enthesitis-related arthritis; JIA, juvenile idiopathic arthritis; Oligo, oligoarticular; PsA, psoriatic arthritis; RF- Poly, rheumatoid factor negative polyarticular.

switched more than 2 bDMARDs had a higher prevalence of elementary lesions of the subsynovia, both of fibrotic and edematous nature (55.6% vs 10.3%, p=0.005; figure 3A) and a higher prevalence of fibrin deposits at the level of the synovial lining (60.0% vs 22.6%, p=0.049; figure 3B). Fibrin deposits remained significant

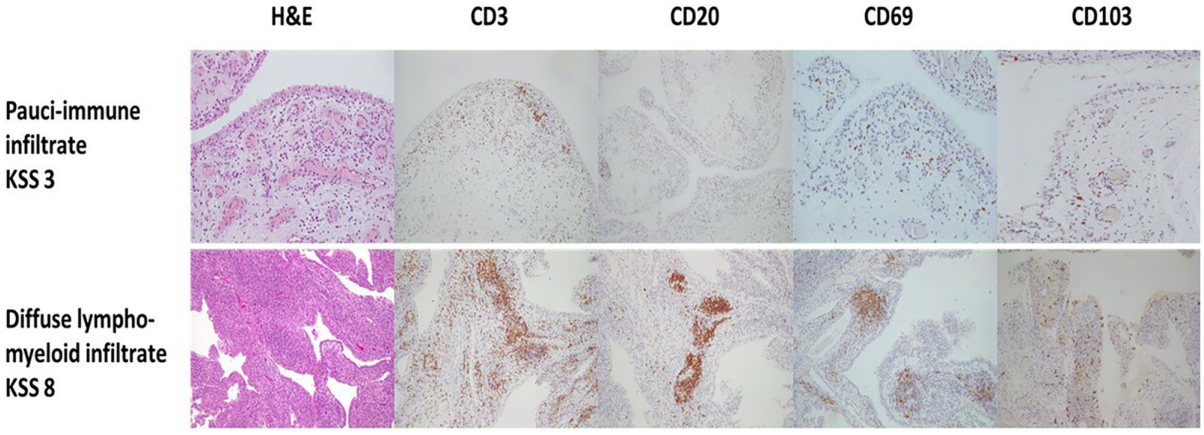

Figure 1 H&E staining and immunohistochemistry of synovial tissues. H&E staining and immunohistochemistry of synovial tissues for CD20+ B cells, CD3+ T cells, CD69+ activated lymphocytes and CD103+ intraepithelial lymphocytes in synovial lining/sublining layers in patients with low-grade inflammatory infiltrate (KSS 3) versus high-grade inflammatory infiltrate (KSS 8). KSS, Krenn Synovitis Score.

Table 2 Synovial morphological and immunohistochemical features in the overall population and within juvenile idiopathic arthritis (JIA) subsets

	JIA subsets					
	Oligo JIA n=27	**RF- Poly JIA n=6**	**ERA n=5**	**PsA n=1**	**Systemic JIA n=3**	**Overall population n=42**
Morphologic						
Layers of synovial lining (score), median (IQR)	3.0 (2.0) (n=26)	2.8 (2.4) (n=6)	6.0 (3.0) (n=4)	5.5 (0.0) (n=1)	4.0 (1.0) (n=3)	3.5 (2.6) (n=40)
Subsynovial elementary lesions, % (n)	n=24	n=6	n=4	n=1	n=3	n=38
Oedema	33 (8)	50 (3)	25 (1)	0 (0)	0 (0)	32 (12)
Fibrosis	50 (12)	50 (3)	50 (2)	0 (0)	33 (1)	47 (18)
Oedema+fibrosis	17 (4)	0 (0)	25 (1)	100 (1)	67 (2)	21 (8)
Fibrin deposit, % (n/N)	33 (9/27)	17 (1/6)	25 (1/4)	0 (0/1)	67 (2/3)	32 (13/41)
Krenn Synovitis Score, median (IQR)	6.0 (1.5) (n=24)	5.0 (3.5) (n=6)	6.0 (2.3) (n=4)	7.0 (0.0) (n=1)	7.0 (2.5) (n=3)	6.0 (3.0) (n=38)
Inflammatory infiltrate (score), median (IQR)	2.0 (1.0) (n=27)	1.5 (1.0) (n=6)	2.0 (1.0) (n=5)	2.0 (0.0) (n=1)	2.0 (1.0) (n=3)	2.0 (1.0) (n=42)
Immune infiltrate pattern% (n)	n=27	n=6	n=5	n=3	n=3	n=42
Scattered	96 (26)	100 (6)	100 (5)	100 (1)	100 (3)	98 (41)
Perivascular	48 (13)	33 (2)	40 (2)	0 (0)	0 (0)	40 (17)
Pseudofollicular	11 (3)	0 (0)	0 (0)	0 (0)	0 (0)	
Cell infiltrate	n=27	n=6	n=5	n=1	n=3	n=42
Lymphomonocytic	100 (27)	100 (6)	40 (2)	100 (1)	67 (2)	90 (38)
Plasma cells	52 (14)	0 (0)	40 (2)	100 (1)	33 (1)	43 (18)
Granulocytes	41 (11)	50 (3)	20 (1)	0 (0)	67 (2)	40 (17)
Eosinophilic	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed	59 (16)	50 (3)	60 (3)	100 (1)	100 (3)	62 (26)
Immunohistochemistry						
Number of samples	**n=22**	**n=5**	**n=3**	**n=1**	**n=0**	**n=31**
CD3≥40% % (n)	41 (9)	100 (5)	33 (1)	0 (0)	–	48 (15)
CD20≥40% % (n)	32 (7)	20 (1)	67 (2)	0 (0)	–	32 (10)
CD69≥40% % (n)	23 (5)	40 (2)	0 (0)	0 (0)	–	23 (7)
CD103≥40% % (n)	5 (1)	0 (0)	0 (0)	0 (0)	–	3 (1)
Ki67≥15% % (n)	27 (6)	20 (1)	33 (1)	0 (0)	–	26 (8)

ERA, enthesitis-related arthritis; Oligo, oligoarticular; PsA, psoriatic arthritis; RF- Poly, rheumatoid factor negative polyarticular.

predictors of requirement of more than 2 bDMARDs even after adjustment for observation time and age at diagnosis (OR 8.1, 95% CI 1.03 to 64.2, p=0.047).

The association between CD20+ infiltrate and severe disease course in patients with JIA

Immunohistochemistry analysis was performed in 31 samples from 22 patients with oligoarticular JIA, 5 with polyarticular disease and 4 with enthesitis-related arthritis (ERA) or psoriatic arthritis (PsA). Samples of patients with ERA and PsA were of limited size thus were grouped together. The 31 samples were obtained from knee joints, except 4 (2 from the shoulders and 2 wrists). Any difference in immunohistochemistry features emerged across different JIA subgroups (table 2), except for a higher prevalence of CD3 positive lymphocyte infiltrate in polyarticular JIA compared with oligoarticular JIA

Table 3 Synovial morphological and immunohistochemical features in treatment-naïve versus treatment-experienced patients with juvenile idiopathic arthritis (JIA)

	Treatment experienced n=33	Treatment naïve n=9	Overall population n=42	P value
Layers of synovial lining (score), median (IQR)	4.0 (2.6) (n=33)	3.0 (2.0) (n=7)	3.5 (2.6) (n=40)	0.885
Subsynovial elementary lesions, % (n)	n=31	n=7	n=38	
Oedema	29 (9)	43 (3)	32 (12)	0.682
Fibrosis	48 (15)	43 (3)	58 (18)	0.702
Oedema+fibrosis	23 (7)	14 (1)	21 (8)	1
Fibrin deposit, % (n/N)	24 (8/33)	63 (5/8)	32 (13/41)	0.083
Fibrinoid necrosis, % (n/N)	39 (13/33)	63 (5/8)	44 (18/41)	0.267
Krenn Synovitis Score, median (IQR)	6.0 (3.0) (n=31)	6.0 (3.0) (n=7)	6.0 (3.0) (n=38)	0.938
Inflammatory infiltrate (score), median (IQR)	2.0 (1) (n=33)	2.0 (1.0) (n=9)	2.0 (1.0) (n=42)	0.908
Immune-infiltrate pattern % (n)	(n=33)	(n=9)	(n=42)	
Scattered	97 (32)	100 (9)	98 (41)	1
Perivascular	42 (14)	44 (4)	43 (18)	1
Pseudofollicular	6(2)	22 (2)	10 (4)	0.195
Cell infiltrate	(n=33)	(n=9)	(n=42)	
Lymphomonocytic	100 (33)	100 (9)	00 (42)	1
Plasma cells	45(15)	44 (4)	45 (19)	1
Granulocytes	42 (14)	22 (2)	38 (16)	0.442
Eosinophilic	0 (0)	0 (0)	0 (0)	–
Mixed	63 (21)	56 (5)	62 (26)	0.710
Immunohistochemistry	**n=23**	**n=8**	**n=31**	
CD3≥40%% (n)	46 (11)	57 (4)	48 (15)	0.685
CD20≥40%% (n)	43 (10)	25 (2)	39 (12)	0.432
CD69≥40%% (n)	17 (4)	43 (3)	23 (7)	0.302
CD103≥40%% (n)	4% (1)	0% (0)	3 (1)	1
Ki67≥15%% (n)	29 (7)	14 (1)	26 (8)	0.641

(p=0.040; figure 4A). No differences were found between persistent and extended oligoarticular subsets (p=0.670; figure 4B).

Any statistically significant difference in molecules expression could be observed in patients subgrouped on age at diagnosis (greater or lower than 7 years). Treatment-naïve patients and those treatment-experienced were similar in terms of immunohistochemistry findings on synovial tissues (table 3).

Subjects with a moderate–severe disease course presented a higher percentage of CD20+ B cells (OR 7, 95% CI 1.4 to 35.5, p=0.023) compared with patients with a milder course of disease, regardless of the subtype of JIA (figure 4C and 4D). CD20+ infiltrate remained significant in predicting disease course severity even after adjusting for observation time and age at diagnosis and when considering exclusively the oligoarticular group (OR 10, 95% CI 1.5 to 61.3, p=0.052).

DISCUSSION

The present study offers several novel insights into the potential role of synovial pathobiology in JIA, significantly advancing current knowledge.

First of all, the potential contribution of synovial tissue analysis in the diagnostic approach to children presenting with swollen joints of unknown aetiology was assessed: in our cohort, synovial biopsy allowed to correctly classify 79% of patients undergoing biopsies for diagnostic purpose, being in the other subjects instrumental for formulating the correct diagnosis. In particular, synovial biopsy is mandatory in case of clinically suspected sarcoidosis, malignant tumour or tenosynovial giant cell tumour.

Second, an intriguing experimental hypothesis was addressed: the heterogeneity in clinical presentation that exists across different JIA subsets might be mirrored in synovial pathobiology. We evinced that patients with polyarticular JIA display a significantly higher CD3 T-cell

Table 4 Demographic and clinical features of 31 patients with juvenile idiopathic arthritis (JIA) available immunohistochemical samples subgrouped on disease course

	Mild disease n=20	Moderate–severe disease n=11	Overall population n=31	P value
Follow-up time after biopsy, (months) median (IQR)	56.5 (102.3)	129 (121)	97 (106.5)	0.075
Gender % (n)	70 (14)	82 (9)	74 (23)	0.675
Age at onset of symptoms, mean (SD)	8.6 (4.2)	5.0 (4.0)	7.3 (4.4)	0.020
Number of csDMARDs used, median (IQR)	1 (0.3)	2.0 (2)	1.0 (1)	0.005
Number of bDMARDs used, median (IQR)	0.0 (1)	3.0 (1.0)	1.0(3)	0.000
Type of bDMARDs, median (IQR)				
Anti-tumour necrosis factor α (adalimumab, etanercept, infliximab, golimumab, certolizumab)	0.0 (1)	3.0 (1.0)	1.0 (2.0)	0.000
Others (tocilizumab, abatacept, anakinra)	0.0 (0)	1.0 (1.5)	0.0 (1.0)	0.002
Number of flares % (n)				
<4	65 (13	0 (0)	42 (13)	0.000
4–8	35 (7)	0 (0)	23 (7)	0.033
>8	0 (0)	100 (11)	35 (11)	0.000
Number of articular prothesis % (n)	5 (1)	27 (3)	13 (4)	0.115

bDMARDs, biological disease-modifying antirheumatic drugs; cDMARD, conventional disease-modifying antirheumatic drugs.

expression, whereas no differences were found between persistent and extended oligoarticular groups. These findings are in partial agreement with the seminal work by Finnegan, who observed higher rate of CD3+ cells in the polyarticular and extendend-to-be oligoarticular groups in 42 treatment-naïve patients with JIA.[16] In addition, in our study, synovial hyperplasia and infiltrate subsets and distribution were not significantly dissimilar across JIA subgroups. Even though this finding might be possibly due to the under-representation of some JIA subtypes in our cohort, our results are consistent with the work by Kruithof *et al*, where synovial histology did not differ between JIA subgroups in treatment-experienced patients.[17] Additional data can be retrieved from the study by Finnegan. By assessing immunohistochemical synovial features, these authors observed a greater synovial hyperplasia in the polyarticular group, while vascularisation and B-cell and T-cell infiltrates were more pronounced in the polyarticular and extended-to-be oligoarticular groups.[16] Conversely, a UK group analysed

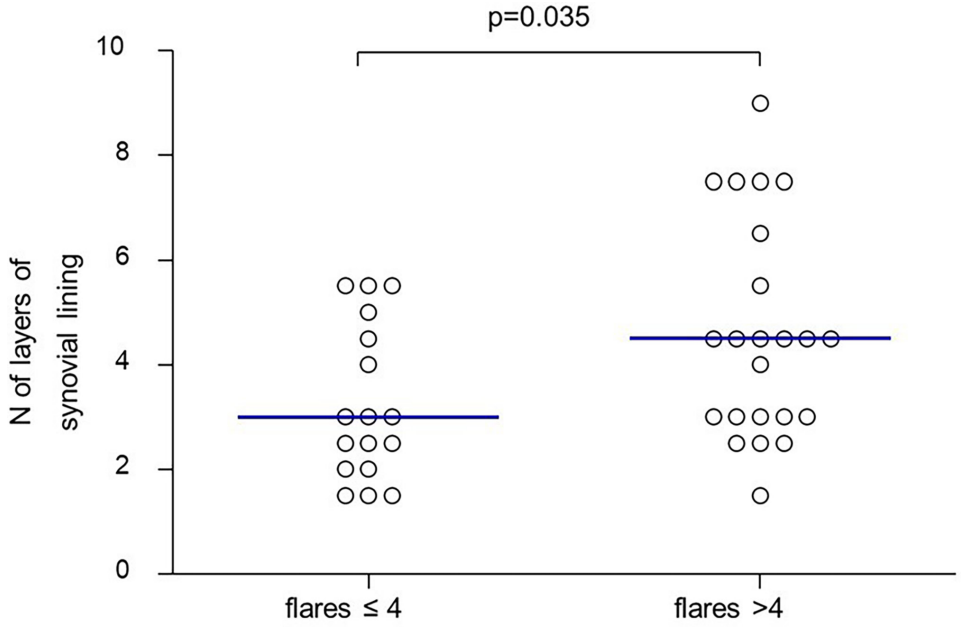

Figure 2 Number of layers of synovial lining in relation with the numbers of clinical flares.

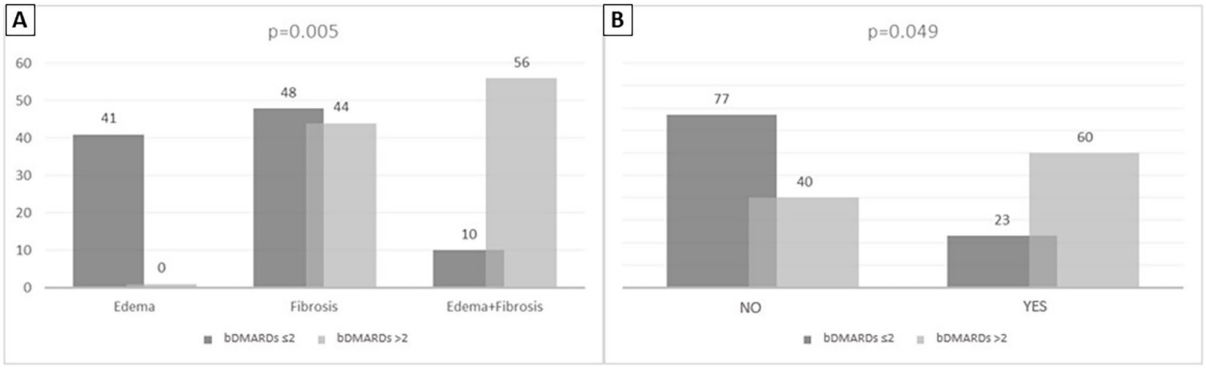

Figure 3 Prevalence of subsynovial elements (A) and fibrin deposits (B) in children who had switched more than two biological disease-modifying antirheumatic drugs (bDMARDs) and children who had switched at most two bDMARDs.

proteins extracted from 15 newly diagnosed, treatment-naïve JIA synovial membrane biopsies and reported distinct proteome profiles between JIA subgroups at an early stage in the disease process.[16]

In our cohort, histological and immunohistochemistry findings did not differ on the age at biopsy, thus clashing with the emerging concept of age at disease onset as a robust predictor of JIA clinical features and pathogenic mediators: patients with early-onset disease had been shown to display a peripheral blood mononuclear cell gene expression enriched in genes related to B cells but not cells of the myeloid lineage.[18] Furthermore, our pioneer data investigate whether in future paediatric rheumatology it will be feasible to stratify JIA severity and predict disease evolution on synovial pathobiology.

In our cohort, patients with JIA who had more than f4 disease flares during follow-up had a statistically significant higher number of layers of synovial lining. To note, an increase of one unit in the number of layers raised by twofold the risk of having a more severe course of disease. In addition, patients who had used more than two bDMARDs had a higher prevalence of elementary lesions of the subsynovia, both fibrotic and oedematous type, and a higher prevalence of fibrin deposits at the level of the synovial lining, even after adjusting for observation time and age at diagnosis. Conversely, in our cohort, the semiquantitative evaluation of synovitis by KSS did not predict clinical outcome. KSS was developed to harmonise the histopathological assessment of synovial membrane and to support the pathologist when

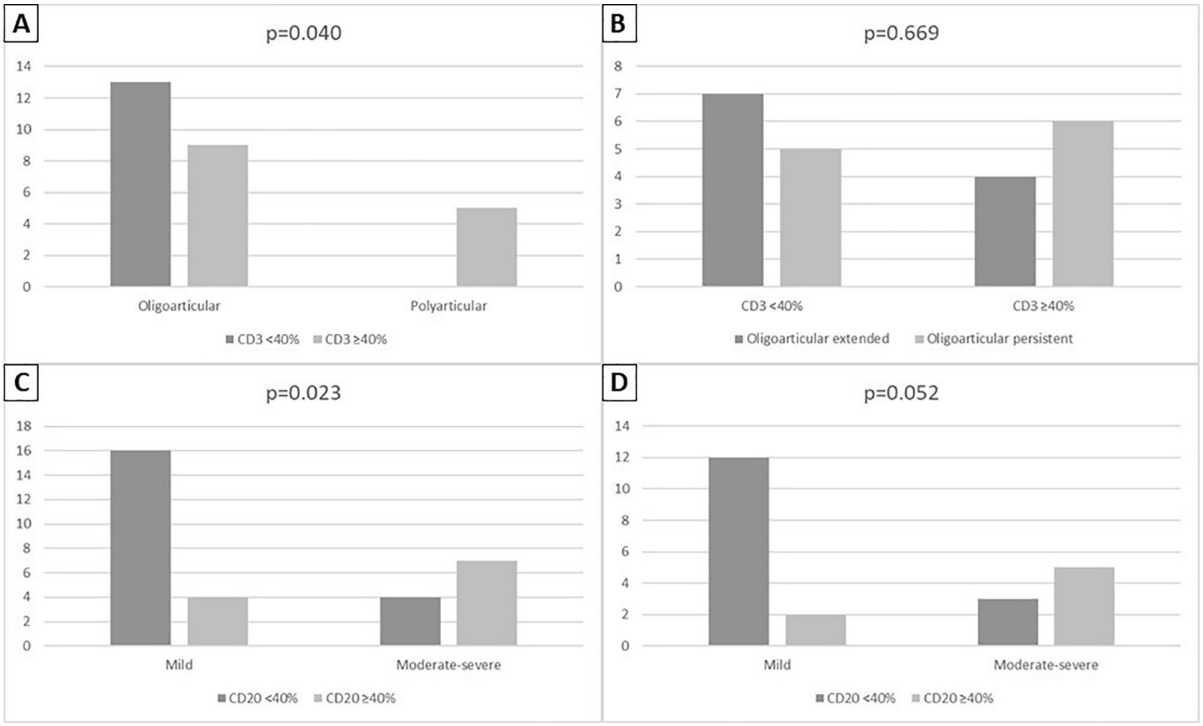

Figure 4 (A) CD3+ lymphocyte infiltrate in oligoarticular and polyarticular juvenile idiopathic arthritis (JIA). (B) CD3+ lymphocyte infiltrate in persistent and extended oligoarticular JIA. (C) Percentage of CD20+ B cells in patients subgrouped on the severity of disease in all JIA subtypes. (D) Percentage of CD20+ B cells in patients subgrouped on the severity of disease in oligoarticular group only.

differentiating rheumatic versus non-rheumatic conditions as it allows a well-defined and reproducible evaluation of synovial tissues. In the literature, KSS has been rarely exploited in the histological analysis of samples from patients with JIA, although it is a feasible tool. Even though we could not evidence any association with disease course and severity, KSS could support the clinicians in the diagnostic approach to JIA. In the present study, the median KSS was above 5 across the different JIA categories, documenting high-grade synovitis.

Similarly, infiltrate subgroups based on cellular predominance (lympho-myeloid, B cells predominance, eosinophilic or neutrophilic) did not correlate with clinical outcome in our cohort. Such observation is in conflict with the current state of the art in RA, which envisages that the cellular synovial patterns can inform disease severity as well as progression.[19] Interestingly, in our cohort patients with a more severe disease course, regardless of the subtype of JIA and previous treatment exposure, presented a higher rate of CD20+ B cells. Data from JIA as well as adult patients with RA strongly suggest the relevance of in situ B cells, which contribute to synovial inflammation by producing autoantibodies, secreting proinflammatory and osteoclastogenic cytokines and acting as antigen-presenting cells.[16 20] Noteworthy, none of our patients had received anti-CD20 drugs, which might be potentially beneficial in such subset of patients refractory to common therapeutic lines. Indeed, synovial tissue analysis could even drive therapeutic intervention and treatment response, a mostly relevant issue that was for the first time explored in the setting of JIA in the present study. However, in our cohort, no significant differences in histological and immunohistochemical features were found when treatment-naïve and treatment-experienced patients were compared. At this regard, it should be highlighted that in our cohort few patients were treatment naïve, and this might have impacted the reliability of our findings. The option of synovial pathobiology as a driver in the therapeutic management might be regarded as futuristic in JIA, but is currently matter of research in adult patients.[19 21] The accumulating burden of evidence in RA is so convincing that a biopsy-driven randomised trial comparing the effect of tocilizumab or rituximab in patients with RA stratified for synovial B-cell status has been recently completed.[20]

Synovial tissue analysis could thus not only support the clinicians in the diagnostic approach to children presenting with arthritis, but also potentially inform about future disease course and treatment response in those diagnosed with JIA (online supplemental figure 3). Such scenario would require the future implementation of synovial biopsies in the routine care of patients with JIA. It should be acknowledged that currently obtaining synovial samples in paediatric patients can be rather challenging, especially in case of disease onset in the younger age. As general anaesthesia is warranted, synovial sampling could be performed in case of joint therapeutic injection, a procedure that is frequently pursued in younger patients with JIA.

Limitations of this study relate to the retrospective design and the lack of prospective longitudinal data. Some JIA categories were under-represented in our cohort preventing us to draw definitive conclusions about synovial pathobiology peculiarities across disease subsets. JIA is indeed a highly heterogeneous disease but our study, recruiting mostly patients classified as oligoarticular and polyarticular JIA, allowed us to investigate potential differences merely between these two subsets. On the other hand, this study envisaged the largest sample size of synovial tissue analysis ever published in JIA. Patients were not homogeneous in terms of disease duration and treatment exposure, with many subjects undergoing synovial sampling years after disease onset. Possibly, the timing of synovial biopsy in the course of disease evolution might affect the histology and immunochemistry characterisation of synovial tissues. Ideally, synovial samples should be obtained at disease onset in treatment-naïve patients. In our cohort, some synovial biopsies were performed during joint replacement surgery, thus not reflecting the initial phases of the disease; most of our patients had been exposed to treatment(s) before synovial sampling but treatment with DMARDs is known to influence the synovial membrane findings.[22–24] Patients with mild disease have been followed for a lower follow-up compared with subjects with moderate–severe disease; such limitation is due to the retrospective nature of our study: indeed, patients with mild disease course have a higher chance of achieving remission off treatment not requiring strict rheumatological follow-up.

Bioptic synovial samples were obtained from a single joint per each patient, questioning the inter-site comparability of findings. Even though there is no available study in children with JIA, data raised in adult population from multiple matched joints suggest that synovial specimens from a single joint are representative of the patient' overall synovial environment.[25] Unfortunately, we could not investigate the so-called pathotypes in our cohort. Synovial samples were collected during arthroscopic procedure, which allows to macroscopically evaluate synovial tissue and obtain adequate amount of tissue.[26] We are currently planning another study to prospectively evaluate disease course and response to treatment in relation to synovial pathobiology as well as relevant clinical and biological parameters, such as circulating CD20+ cells, in patients with JIA.

As a whole, this study opens new frontiers of research about synovial tissue pathobiology in JIA scenario, which might lead to further unravel the pathogenic mechanisms of this intriguing disease, to revolutionise the current taxonomy of the condition

by characterising the diverse clinical phenotypes in relation to synovial microenvironments and to optimise the therapeutic approach for our patients.

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