Synovial membrane immunohistology in early untreated juvenile idiopathic arthritis: differences between clinical subgroups

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ABSTRACT

Objective Juvenile idiopathic arthritis (JIA) consists of a heterogeneous group of inflammatory disorders, within which there are a number of clinical subgroups. Diagnosis and assignment to a particular subgroup can be problematical and more concise methods of subgroup classification are required. This study of the synovial membrane characterises the immunohistochemical features in early untreated, newly diagnosed JIA and compares findings with disease subgroup at 2 years.

Methods 42 patients with newly diagnosed untreated JIA underwent synovial biopsy before the administration of steroids or disease-modifying antirheumatic drugs. Patients were classified as either polyarticular, persistent oligoarticular or extended-to-be oligoarticular. The location and semiquantitative analysis of T-cell subsets, B cells, macrophages and blood vessels were determined using immunohistochemistry.

Results Synovial hyperplasia varied significantly between the three groups (p<0.0001). There was a significant difference in the CD3 T-cell population between the three groups (p=0.004) and between the extended-to-be and persistent group (p=0.032). CD4 expression was significantly higher in the poly and extended-to-be oligo groups (p=0.002), again the extended-to-be group had more CD4 T cells than the persistent group (p=0.008). B-cell infiltrates were more marked in the polyarticular group and were significantly higher in the extended-to-be group compared with the persistent group (p=0.005). Vascularisation was more pronounced in the polyarticular and extended-to-be oligoarticular groups, the extended-to-be group had significantly more vascularisation than the persistent group (p=0.0002).

Conclusions There are significant differences in the histomorphometric features of synovial tissue between patient subgroups. Immunohistological examination of synovial membrane biopsies may provide further insight into early disease processes in JIA.

Juvenile idiopathic arthritis (JIA) is the one of the most common rheumatological childhood diseases, with a prevalence of approximately one in 1000.1 JIA consists of a heterogeneous group of diseases defined as persisting for more than 6 weeks and commencing before the age of 16 years. The cause of JIA is still unknown, environmental and genetic factors do, however, seem to play a role in its pathogenesis; moreover, the heterogeneity of the disease suggests that multiple factors have a role to play in the pathogenesis of disease.2 Over time persistent joint inflammation can result in chronic pain and stiffness, joint damage and disability. Generally, the prognosis for children with JIA is relatively good; however, approximately half of the patients continue to have active joint disease into adulthood.3

The International League of Associations for Rheumatology (ILAR) criteria are the most commonly used classification system in which JIA is segregated into seven clinical subgroups.4 5 The largest subgroup, oligoarticular JIA, accounts for approximately 65% of all cases and refers to children with four or fewer joints involved within the first 6 months of disease. Oligoarticular disease is defined as persistent when it remains confined to four or fewer joints throughout the course of the disease, it is more prevalent in girls, with a peak age of onset of 1–3 years.6 In approximately 25% of patients with oligoarticular JIA the disease may spread to four or more joints after the first 6 months;7 this is defined as extended oligoarticular. It is not fully understood why the disease extends and it is not possible to predict in whom this will occur. Polyarticular JIA is defined as the involvement of four or more joints in the first 6 months of disease;8 it can be subdivided into rheumatoid factor (RF)-positive and RF-negative polyarticular JIA. This subgroup accounts for approximately 10–15% of all JIA cases.8

Across the spectrum of chronic arthritis cellular infiltrates within the synovium promote cytokine release and enhance their production during inflammation.10 Chronic synovial inflammation followed by invasion of synovial tissue into adjacent cartilage and bone results in joint deformity and ultimate disability;11 this has been well documented in adult disease;12 however, little is known about synovial pathology in early stage untreated JIA.

Joint disease in children with JIA is characterised by a persistent inflammatory cellular infiltrate into the synovium.13 Consequently, the accumulation of T cells14 15 encourages the production of pro-inflammatory cytokines.16–18 This results in subsequent over-production and secretion of synovial fluid followed by pannus formation. If persistent, synovial inflammation may ultimately lead to articular cartilage and bone damage.

Prahalad and Glass19 reported that the subtypes of JIA are distinct and have the potential to be etiologically and genetically distinctive. A number of studies has been undertaken to investigate synovial histopathology in JIA. These studies included material obtained at synovectomy, and some included children with a disease duration of up to 22.2 years.
Using ultrasound guidance as described previously. Biopsies were snap frozen in liquid nitrogen and stored at −80°C. Seven µm thick frozen sections were cut and allowed to air-dry at room temperature before histological and immunohistochemical staining.

### Initial microscopic analysis of biopsies

Synovial membrane sections were stained with haematoxylin and eosin using standard protocols. Three biopsies were analysed per patient, with 10 high power fields (HPF) studied per biopsy (×200 magnification) and were scored for the following parameters:

1. Evidence of general synovial hyperplasia and fibrosis were each scored on a scale of 0–10.
2. Proliferating capillaries were assessed such that one vessel per HPF was viewed as normal with a score of 0. Additional vessels were then counted and an average taken over 10 HPF.
3. Focal cell aggregates (FA) (aggregates greater than 10 cells in diameter, or those that did not involve a vessel) were scored as absent (A) or present (P).
4. Inflammatory infiltrates of lymphocytes (IL) were scored as a percentage of the total cell population per HPF, in which 1=10% and 10=100% cells.

#### Immunohistochemistry

To address the issue of the number of biopsies required to obtain representative data of the cellular infiltration into the synovial tissue, the maximal number of biopsies available for each child was utilised. An average of three biopsies per patient were analysed. Per biopsy, 10 HPF were studied (×200 magnification) for the presence and magnitude of positively stained cells, ie, the percentage of cells positively stained for each marker was recorded for 10 different HPF and averages were calculated. Antibodies were optimised in lymphoid tissue and isotype matched negative controls used for each immunohistochemical stain. Evaluation of stained sections was performed by two independent, blinded authors (SF and SC). Four patient samples could not be included in the study as the tissue was

### Methods

Children with newly diagnosed JIA of less than 2 years duration were recruited following informed consent. Medical ethics committee approval was obtained and patient assent and parent informed consent given (10/NIR01/11). For the purpose of this study children had to have at least one knee involved that required intra-articular steroid injection. They had to be steroid naive and never treated with DMARD. Children were classified according to ILAR criteria. Detailed clinical examination, biochemical and immunological data and imaging assessments were obtained. Clinical data included: joint count, degree of clinical inflammation in the knee (scored on a scale of 0–4; 0, no inflammation; 1, minimal; 2, mild; 3, moderate and 4, marked), degree of any flexion deformity of the knee, patients/parents pain scores on a visual analogue scale (VAS) and physician’s global disease activity score was recorded on a VAS. Children were followed for 2 years after biopsies were taken. During this period children whose disease went on to involve more than four joints (after they had the disease for a total of six or more months) were re-classified as extended oligoarticular, ie, they were not extended at the time the biopsy was taken, this subgroup is referred to as ‘extended-to-be’ from here on in.

### Tissue collection, preparation and storage

Under general anaesthetic, synovial fluid was aspirated and synovial membrane biopsies were obtained by blind needle biopsy using ultrasound guidance as described previously. Biopsies were snap frozen in liquid nitrogen and stored at −80°C. Seven µm thick frozen sections were cut and allowed to air-dry at room temperature before histological and immunohistochemical staining.

### Table 1

Demographic and clinical characteristics at time of biopsy, mean value displayed (range)

<table>
<thead>
<tr>
<th></th>
<th>Poly RF*</th>
<th>Poly RF−</th>
<th>Persistent oligo</th>
<th>Extended-to-be* oligo</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients recruited</td>
<td>2</td>
<td>8</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Disease duration at time of biopsy (months)</td>
<td>4.5 (4–5)</td>
<td>3.25 (1–10)</td>
<td>5.6 (2–20)</td>
<td>6 (2–15)</td>
</tr>
<tr>
<td>Disease duration at time of extension (months)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>18 (8–39)¹</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16 (16–16)</td>
<td>4.75 (1–11)</td>
<td>5.5 (1.5–15.5)</td>
<td>6.98 (2–16)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>0/2</td>
<td>2/8</td>
<td>4/18</td>
<td>5/5</td>
</tr>
<tr>
<td>No of swollen joints³</td>
<td>20 (15–25)</td>
<td>9.5 (5–24)</td>
<td>1.82 (1–4)</td>
<td>2.8 (1–4)</td>
</tr>
<tr>
<td>Knee swelling/inflammation score (0–4)</td>
<td>3.5 (2–4)</td>
<td>3.25 (2–4)</td>
<td>3 (0–4)</td>
<td>3.3 (0–4)</td>
</tr>
<tr>
<td>VAS pain (parent)</td>
<td>8.15 (6.5–9.8)</td>
<td>6.94 (3.3–9)</td>
<td>4.34 (0–7.5)</td>
<td>5.74 (0.8–1.5)</td>
</tr>
<tr>
<td>VAS global physician</td>
<td>5.5 (3.8–7.4)</td>
<td>4.2 (1.5–7.5)</td>
<td>1.84 (0.8–3.3)</td>
<td>2.16 (0.4–3.2)</td>
</tr>
<tr>
<td>WCC</td>
<td>10.55 (9.3–11.8)</td>
<td>10.66 (7.7–12.9)</td>
<td>9.54 (5.8–15.7)</td>
<td>12.68 (6.6–20.5)</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>7.8 (7.2–8.4)</td>
<td>10.34 (8.3–14.1)</td>
<td>10.96 (8.9–14.1)</td>
<td>11.12 (9–12.8)</td>
</tr>
<tr>
<td>ESR</td>
<td>124 (110–138)</td>
<td>40.86 (20–96)</td>
<td>20.75 (2–63)</td>
<td>34.1 (7–111)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>126.5 (72–181)</td>
<td>30.64 (5.6–67.3)</td>
<td>9.23 (0–66.5)</td>
<td>8.34 (0–23.3)</td>
</tr>
<tr>
<td>PC</td>
<td>489.5 (401–578)</td>
<td>461.25 (314–601)</td>
<td>393.15 (222–600)</td>
<td>397.2 (243–646)</td>
</tr>
<tr>
<td>ANA (±)</td>
<td>1/2</td>
<td>5/44</td>
<td>9/11²</td>
<td>5/5</td>
</tr>
<tr>
<td>ANA titre</td>
<td>40 (0–80)</td>
<td>85.7 (0–320)</td>
<td>52.38 (0–640)</td>
<td>26 (0–80)</td>
</tr>
</tbody>
</table>

¹Patients were classified as oligoarticular at time of biopsy but were subsequently reclassified as extended oligoarticular if more than four joints became involved after the first 6 months of disease, ie, at biopsy they were oligoarticular, only later did they extend.

²Given are the number of swollen joints in extended-to-be patients at the time of biopsy (before they extended). When all 10 patients in this group were reclassified as extended (after an average of 16 months disease duration) the average number of swollen joints was 6.2 (range 5–10) (see supplementary figure 4, available online only).

³No data for one patient.

ANA, antinuclear antibodies; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PC, platelet count; RF, rheumatoid factor; VAS, visual analogue scale; WCC, white cell count.
either damaged during processing (by freeze fracture) or there was not enough synovial membrane present in the sample.

A semiquantitative five-point scale was developed to represent the percentage of cells that were positively stained: 0 represented no cells; 1, 1–24%; 2, 25–49%; 3, 50–74%; and 4, 75–100% of cells expressing the markers. This scale was used for all cell markers, unless otherwise stated. As some histological markers are more abundant than others in synovial tissue, we adjusted the scoring system appropriately for each individual marker based on examining a representative number of biopsies for each marker, thus the sensitivity of the scoring system was different for some markers as described for CD20 expression (below).

CD20 expression appeared in a more focal arrangement than CD3, CD4, CD8 and CD68, and so scoring per HPF would not have given a true representation of its expression. Instead, CD20 expression was given an overall score based on its expression in an entire piece of synovial tissue, all tissues analysed were approximately the same size. It was scored as follows: score 0, no CD20 expression; score 1, 1 FA of CD20 cells; score 2, 2 FA of CD20 cells; score 3, 3 FA of CD20 cells; score 4, 4 or more FA of CD20 cells.

The thickness of the lining layer (LL) was assessed on CD68 stained sections based on the number of thick cells that were positively stained, the mean was calculated for 10 HPF. Vessel markers were counted starting at the top left hand corner of the specimen, and 10 HPF were selected as previously described.

### Statistical analysis

All statistical analysis was performed with the Analyse-it add-in statistics software package in Excel. Clinical data were not normally distributed therefore differences between the three subgroups were analysed using the Kruskal–Wallis one-way analysis of variance. Pearson correlation tests were used to evaluate tissue scores between the two blinded observers and correlations between particular histological features. The two observers were blinded to the clinical and diagnosis data. Scores awarded by the two observers SF and SC were highly correlated ($r=0.93$, $p<0.0001$).

### RESULTS

#### Patient demographics and clinical features

There were 33 females and nine males included in the study. The clinical and laboratory findings are shown in table 1.

#### General laboratory characteristics of JIA patients

By definition of the study inclusion criteria, all had at least one knee involved in disease. There was no significant difference in the mean inflammation/knee swelling score of the biopsied knees between subgroups: polyarticular (mean 3.3), persistent (mean 3), extended-to-be (mean 3.3) (see supplementary figure S1, available online only). There was no significant correlation between inflammation/knee swelling score and any of the immunohistological features. Within the polyarticular subgroup there was a tendency towards a correlation between CDS/CD4 expression ($r=0.45$ and 0.69, respectively) and inflammation/knee swelling score; however, the correlation did not reach statistical significance. There were significant differences in C-reactive protein levels between the polyarticular (mean 49.81) and extended-to-be oligoarticular subgroups (mean 8.34; $p=0.0017$) and between the polyarticular and persistent oligoarticular JIA subgroups (mean 9.23; $p=0.0036$). The erythrocyte sedimentation rate was significantly higher in the polyarticular (mean 59.33) compared with the persistent oligoarticular subgroup (mean 20.75; $p=0.006$). VAS pain scores (parent) were significantly higher in the polyarticular (mean 6.94) compared with the persistent oligoarticular subgroup (mean 4.34; $p=0.031$), and when assessed by a physician the VAS pain score was also significantly higher in the polyarticular (mean 4.46) compared with the persistent oligoarticular subgroup (mean 1.84; $p=0.0005$). There was a significant difference in the white cell count between the persistent (mean 9.54) and extended-to-be oligoarticular groups (mean 12.68; $p=0.055$) (table 2).

### Synovial hyperplasia and inflammatory infiltrates

The degree of synovial hyperplasia was assessed using standard methods on haematoxylin and eosin stained sections and was scored on a scale of 0–10. The degree of synovial hyperplasia and inflammation varied according to the disease subgroup: polyarticular subgroup (mean 9.45; range 8.5–10), extended-to-be oligoarticular subgroup (mean 8.35; range 5–10), and persistent oligoarticular subgroup (mean 3; range 0–10). There was a significant difference in the white cell count between the three subgroups ($p<0.0001$) (figure 1 and table 2). Fibrosis was scored on a scale of 0–10; it did not vary significantly between the three subgroups (table 2).

### Inflammatory infiltrates

Inflammatory infiltrates of lymphocytes were scored on a scale of 0–10. There was a significant variable degree of inflammation between the three groups ($p=0.0054$). When the polyarticular (mean 8.8; range 8.5–10) was compared with the persistent oligoarticular group (mean 6.4; range 1.7–9) there was a significant difference between the two ($p=0.0025$). B-cell and T-cell focal aggregates were observed in 60% of polyarticular patients, 73% of extended-to-be patients and 48% of persistent patients (table 2).
There was a significant difference in CD3 T-cell expression (p=0.004) between the three subgroups, with polyarticular (mean 3.1; range 1.71–4) and extended-to-be oligoarticular (mean 2.88; range 2–3.75) groups having higher CD3 scores. When the extended-to-be oligoarticular group was compared with the persistent oligoarticular group (mean 2.04; range 0.5–3.75) there was a significant difference in CD3 T-cell expression (p=0.032) (figure 2A and table 3). Similarly for CD4 there was also a significant difference in expression between the three subgroups (p=0.002), and again there was a significant difference in CD4 expression between the persistent (mean 1.35; range 0.3–2.3) and extended-to-be oligoarticular group (mean 2.24; range 1.2–3.68) (p=0.008) (figure 2 and table 3).

CD8 expression was not significantly different between the subgroups.

**B-cell expression**
Distinct clusters of CD20 B cells were observed in all three subgroups. In general, B cells demonstrated a focal appearance rather than a diffuse expression pattern, with distinct perivascular groups of CD20 cells found surrounding vessels in some biopsies (figure 3). There was a significant difference in CD20 expression between the three groups (p=0.004), and when the polyarticular group (mean 2.55; range 1–4) was compared with the persistent oligoarticular (mean 0.98; range 0–5) there was a significant difference in CD20 expression (p=0.0014) and there was also a significant difference in expression between the persistent and extended-to-be oligoarticular (mean 1.95; range 0.5–4) groups (p=0.005) (table 3).

**Macrophage expression**
There were marked macrophage populations in the lining layer and the sublining layer in all three subgroups, with all three displaying moderate/strong CD68 expression in the lining layer, with less pronounced but still marked expression in the sublining layer (table 3).

As the number of cells in the synovial lining layer are known to increase with synovitis we measured the average cell depth of this layer; no significant difference was found between subgroups (table 3).

**Angiogenesis**
Inflamed synovial tissue for all patients in the study displayed an increased number of blood vessels beyond the normal threshold.
Figure 2  Distribution of CD3, CD4 and CD8 regulatory T cells in synovial tissue. Representative sections of biopsied hyperplastic synovial tissue from children with juvenile idiopathic arthritis stained with antibodies to CD3 in (A) polyarticular synovial tissue, (B) persistent oligoarticular synovial tissue, (C) extended-to-be oligoarticular tissue and (D) tonsil-positive control. CD4 expression in (E) polyarticular synovial tissue, (F) persistent oligoarticular synovial tissue, (G) extended-to-be oligoarticular tissue and (H) tonsil-positive control. CD8 expression in (I) polyarticular synovial tissue, (J) persistent oligoarticular synovial tissue, (K) extended-to-be oligoarticular tissue and (L) tonsil-positive control. Sections were counterstained with haematoxylin.
value of one vessel/HPF. Factor VIII⁺ was used as a vessel marker, the degree of vascularisation varied significantly with disease subgroup (p<0.01), with higher numbers of factor VIII⁺ vessels in extended-to-be (mean 25.2 vessels/HPF; range 18–32 vessels/HPF) and polyarticular (mean 20.5 vessels/HPF; range 11.1–31.8 vessels/HPF) groups. There was also a significant difference in factor VIII⁺ expression between the persistent (mean 12.2 vessels/HPF; range 1–25.2 vessels/HPF) and extended-to-be subgroups (p=0.0002) (table 3 and figure 4). We also examined the expression of αβ3, which is expressed on endothelial cells during the early stages of angiogenesis; its expression in both the persistent and extended oligoarticular groups was quite low (mean 3.7 vessels/HPF) but we found a significant difference in expression between the three subgroups, with the polyarticular group showing the highest expression (mean 10.5 vessels/HPF; p=0.006) (figure 4).

**DISCUSSION**

The results presented in this study demonstrate differences in the immunohistochemical characteristics of polyarticular, persistent oligoarticular and extended-to-be oligoarticular synovial biopsy specimens taken at an early disease stage (mean disease duration 5.33 months—see supplementary figure 2, available online only) and before the initiation of any steroid or DMARD. Within the polyarticular group only two patients were RF positive. We have presented results in the tables for both the RF-positive and negative groups separately; however, due to these small sample numbers we analysed the polyarticular group overall as a whole. Statistically significant higher scores for infiltration by CD3, CD4, CD20 cells were observed between the three groups. There was also significantly more angiogenesis in the extended-to-be oligoarticular and polyarticular groups. Synovial tissue from the polyarticular and extended-to-be oligoarticular groups also had higher scores for synovial hyperplasia; there was also a tendency for more focal aggregates of lymphocytes to be present in the polyarticular and extended-to-be oligoarticular tissue. We found no significant correlation between disease duration and any of the immunohistochemical parameters. We postulate that highly inflamed synovial tissue with marked hyperplasia scores, high CD3/CD4 expression and a high angiogenesis score in children who initially present with oligoarticular JIA could be an early indication that disease may spread to other joints. The formation of new blood vessels from an early stage in disease is a critical process in the perpetuation of the inflammatory response in the synovium of patients with rheumatoid arthritis (RA) and JIA. This then facilitates the active infiltration of the synovial membrane into cartilage, ultimately destroying the cartilage and leading to bone deformity. We found significantly more angiogenesis in the extended-to-be and polyarticular subgroups; these initial results would suggest that higher angiogenesis scores may imply a more severe disease phenotype (polyarticular) and/or the possibility of disease extension. To our knowledge this is the first study to demonstrate this relationship; it would be interesting in future studies to examine further the relationship between angiogenesis and disease extension. Ultimately, more patients would be required to validate these initial results.

We also investigated the expression of αβ3, which is upregulated on endothelial cells during angiogenesis. We found αβ3 localised around small vessels with much less intensity than factor VIII⁺. There was significantly more αβ3 expression in the polyarticular subgroup than the other two subgroups. αβ3 is only found on early undifferentiated vessels and is lost as vessels

**Table 3** Immunohistochemical analysis of the synovial membrane in JIA

<table>
<thead>
<tr>
<th></th>
<th>Poly (RF⁺) n=2</th>
<th>Poly (RF⁻) n=8</th>
<th>Persistent oligo n=22</th>
<th>Extended-to-be oligo n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>3.49 (0.035)</td>
<td>3.1 (0.34)</td>
<td>2.04 (0.21)</td>
<td>2.88 (0.26)</td>
</tr>
<tr>
<td>CD4</td>
<td>2.98 (0.254)</td>
<td>2.99 (0.35)</td>
<td>1.35 (0.15)</td>
<td>2.24 (0.28)</td>
</tr>
<tr>
<td>CD8</td>
<td>3 (0.25)</td>
<td>1.98 (0.46)</td>
<td>1.22 (0.19)</td>
<td>1.37 (0.42)</td>
</tr>
<tr>
<td>CD20</td>
<td>3.5 (0)</td>
<td>2.31 (0.33)</td>
<td>0.93 (0.18)</td>
<td>1.95 (0.33)</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>18.36 (2.58)</td>
<td>21.91 (2.52)</td>
<td>12.22 (1.75)</td>
<td>25.2 (1.57)</td>
</tr>
<tr>
<td>αβ3</td>
<td>18.2 (5.8)</td>
<td>7.4 (2.1)</td>
<td>3.7 (0.7)</td>
<td>3.8 (1.5)</td>
</tr>
<tr>
<td>CD68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sublining layer score</td>
<td>3.7 (0.1)</td>
<td>3.3 (0.4)</td>
<td>2.04 (0.22)</td>
<td>2.57 (0.29)</td>
</tr>
<tr>
<td>Lining layer thickness</td>
<td>3.7 (2.47)</td>
<td>2.8 (0.24)</td>
<td>2.5 (0.16)</td>
<td>2.7 (0.22)</td>
</tr>
</tbody>
</table>

Mean histological score (+SEM).

T cells and B-cell positive cells were scored on a scale of 1 (1–24%), 2 (25–49%), 3 (50–74%) and 4 (75–100%) of the expressed marker. The numbers of cells thick within the lining layer, labelled with CD68 were counted, and the mean of 10 HPF calculated. Macrophages (within the sublining layer) were scored on a scale of 1 (1–24%), 2 (25–49%), 3 (50–74%) and 4 (75–100%) of the expressed marker. The numbers of cells thick within the lining layer, labelled with CD68 were counted, and the mean of 10 HPF calculated. HPF, high power field; JIA, juvenile idiopathic arthritis; RF, rheumatoid factor.
Our results imply that not only are there increased numbers of mature vessels in polyarticular tissue, but the process of angiogenesis is ongoing within this subgroup, as demonstrated by the presence of an early vessel marker. Of interest is the fact that even though the extended-to-be oligoarticular group had a higher mean score for factor VIII expression, its score for \( \alpha V \beta 3 \) was minimal as in the persistent subgroup. Interestingly, Barnes et al.\(^{32} \) found specific genes related to angiogenesis were upregulated in polyarticular synovial fluid and antiostatic factors were increased in oligoarticular samples. They suggest this may tip the balance towards greater angiogenic activity in polyarticular disease when compared with oligoarticular disease, and this may have a role to play in the more severe phenotype of polyarticular JIA.

Figure 4  The highly specific endothelial cell marker von Willebrand factor (factor VIII) was used to demonstrate that inflamed juvenile idiopathic arthritis (JIA) synovial tissue contained an increased number of blood vessels. There was significantly higher factor VIII expression in the polyarticular (A) and extended-to-be oligoarticular (C) subgroups when compared with the persistent oligoarticular (B) subgroup. Representative sections of synovial membrane are shown for each subgroup. Sections were counterstained with haematoxylin. The bar chart illustrates the mean number of factor VIII + vessels per high power field (HPF) for the three JIA subgroups (+SEM). \( \alpha V \beta 3 \) expression on serial sections of the same biopsy from the same patient. This tissue is from a patient with polyarticular JIA, there are many mature vessels present as shown by the presence of factor VIII (D), it is evident that angiogenesis is ongoing as there are also many early vessels as shown by the expression of \( \alpha V \beta 3 \) (E). Arrows indicate vessels that have stained positive for \( \alpha V \beta 3 \) and not for factor VIII. Both sections have been counterstained with haematoxylin. The bar chart illustrates the mean number of \( \alpha V \beta 3 ^+ \) vessels per HPF for the three JIA subgroups: polyarticular (P), persistent oligoarticular (O) and extended-to-be oligoarticular (EO) (+SEM).
A number of studies has previously been undertaken to characterise synovial T-cell infiltrates in patients with RA and JIA; however, these studies were all undertaken in children with established treated disease, when medications known to alter the synovial microenvironment had been used. Murray et al.\(^{23}\) found higher levels of CD4 and CD8 T cells in oligoarticular childhood-onset chronic arthritis when compared with polyarticular disease; however, the study had preferentially selected samples that displayed significant inflammation and high CD3 and CD68 expression, thus introducing a selection bias of the patient group for disease activity.Gattorno et al.\(^{35}\) analysed synovial tissue from patients with an average disease duration of over 3 years who had all been treated with methotrexate. In a later study by Kruithof et al.\(^{34}\) in which the authors concluded that synovial histology did not correspond with any particular JIA subgroup, these patients too had been receiving treatment in the form of DMARD and/or corticosteroids before biopsy; these treatments are known to alter the synovial microenvironment significantly.\(^{21}\) In another study by Gattorno et al.\(^{36}\) the synovial tissue from six patients with a mean age at the time of biopsy of 15.8 years was used. There was no mention of disease duration in these patients, and it is also unclear if the patients had been receiving any DMARD before biopsy.

Gregorio et al.\(^{20}\) investigated the pattern of lymphoid organisation in JIA; however, the mean disease duration was 6.7 years and the majority of patients had all been treated with DMARD, some with anti-tumour necrosis factor, some with methotrexate and some with steroids. In another study by Nistala et al.\(^{36}\) the children were all receiving either methotrexate or corticosteroids before sample collection. Those studies are distinct from the current study, which focused specifically on early, untreated disease (mean disease duration at the time of biopsy was 5.33 months) as none of the patients in our study had received any DMARD before biopsy.

In a more recent study by Hunter et al.\(^{34}\) synovial fluid samples from treatment-naive JIA patients were studied. The authors found the CD4:CD8 ratio in synovial fluid of children with extended JIA was significantly lower than those with persistent disease. In our study we found an increased CD4:CD8 T-cell ratio in the synovial membrane of children with extended-to-be disease. The study by Hunter et al.\(^{34}\) was carried out using cells within synovial fluid, which could account for the discrepancy between the findings of our study. Furthermore, a previous study by Ravlic-Gulan et al.\(^{37}\) found that RA patients had a decreased CD4:CD8 ratio in synovial fluid but an increased ratio of CD4:CD8 in the synovial membrane when compared with the ratio in peripheral blood, possibly implying that there are differences in the local immunological events occurring in synovial membrane and synovial fluid. It would be interesting to investigate further this reciprocal relationship. Could it be possible that the higher CD4:CD8 ratio in the synovial fluid is as a result of trafficking out of the inflamed tissue?

Of interest within the polyarticular JIA subgroup is one patient who had below average scores for CD3/CD4/CD8. They initially presented with only 1 month of symptoms and were classified as oligoarticular JIA. After 3 months of the initial diagnosis, however, this patient was re-classified as polyarticular. It would be interesting to follow up this patient and investigate the synovial histopathology at the end of the 2-year study to determine if pathology had become more pronounced.

As the diagnosis of JIA is essentially down to clinical assessment and no laboratory test can be conclusive for JIA diagnosis, there are undoubtedly some flaws within the classification system. Most often, joint count is used as an initial diagnostic criterion to determine subgroup classification. Inherently there will be some error as joint counts may differ between observers and it also depends on parental and child recall; however, ILAR is currently the accepted international classification and thus the one we have to work with. There is without doubt a lack of correlation between immunochemistry, genetics and histopathology and specific JIA subgroups. The current ILAR classification system is often regarded as an ongoing work in progress, which will be amended as future studies provide more insight into JIA classification.\(^{38}\)

At present, there is no definitive way to predict whether disease will spread to more than four joints in a child who initially presents with oligoarticular JIA. It is therefore imperative to try and devise new methods that could help predict disease extension, so that more effective treatment could be implemented at an earlier stage in the disease process. Indeed, what we have shown is that there are already differences at the level of synovial histology before extension occurs; these results could be the basis for further research to help determine specific criteria in order to stratify JIA patients better. We postulate that the timely prediction of clinical outcome could be facilitated by the characterisation of cellular infiltrates present in early untreated inflammatory JIA.

References


Synovial membrane immunohistology in early untreated juvenile idiopathic arthritis: differences between clinical subgroups

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