cells were isolated and the frequency and phenotype of B, Tfh and Tfr cells were evaluated by flow cytometry. Serum levels of APRIL, BAFF, IL-1α, IL-2, IL-4, IL-6, IL-10, IL-17A, IL-21, IL-22, IFN-γ, PD-1, PD-L1, sCD40L, CXCL13 and TNF were measured by multiplex bead-based immunoassay and/or ELISA in all groups included.

**Results:** The frequency of B, Tfh and Tfr cells was similar between JIA patients and controls. Children with eoJIA and pJIA, but not poJIA, had significantly lower frequencies of plasmablasts, regulatory T cells and higher levels of Th17-like Tfh cells in circulation when compared to controls. Furthermore, APRIL, BAFF, IL-6 and IL-17A serum levels were significantly higher in pediatric eoJIA and pJIA patients when compared to controls. These immunological alterations were not found in adult JIA patients in comparison to controls. Our results suggest a potential role and/or activation profile of B and Tfh cells in circulation when compared to controls. Furthermore, APRIL, BAFF, IL-1α, IL-2, IL-4, IL-6, IL-10, IL-17A, IL-21, IL-22, IFN-γ, PD-1, PD-L1, sCD40L, CXCL13 and TNF were measured by multiplex bead-based immunoassay and/or ELISA in all groups included.

**Conclusion:** Changes in B and Tfh cell subpopulations, but not in Tfr cells, were found in peripheral blood of children with eoJIA and pJIA when compared to controls. Our results suggest a potential role and/or activation profile of B and Tfh cells in the pathogenesis of eoJIA and pJIA, but not poJIA.

**REFERENCES:** NIL.

**Disclosures of Interests:** None Declared.

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**OP0032**

**MULTI-PARAMETRIC ANALYSIS EVIDENCES TRAFFICKING OF CIRCULATORY CXCR3+CCR6+ CD4+ T CELLS AS A SOURCE OF TH1- AND TH17-MEDIATED INFLAMMATION IN THE ENTHESITIS-RELATED ARTHRITIS (ERA) SYNOVIA

**Keywords:** Synovium, Adaptive immunity, Cytokines and chemokines

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**Background:** Enthesitis-related arthritis (ERA), a common subtype of juvenile idiopathic arthritis (JIA), carries a poor prognosis. Current therapies, including anti-TNF agents, are limited in controlling disease. There is a poor understanding of ERA immunopathogenesis, especially the ontogeny of synovial pro-inflammatory T cells, which limits prognostic and therapeutic targets.

**Objectives:** We aim to compare, using a high-dimensional approach, the synovial and circulatory immune architectures of active ERA patients. Through this, we aim to identify CD4+ T cell subsets strongly associated with synovial inflammation in active ERA patients. We also aim to assess differentiation, cytokine production and regulation of candidate CD4+ T cell subsets.

**Methods:** We interrogated, using mass cytometry, CD4+ immune cells from the synovium (n=10) and circulation (n=30) of ERA patients with active joint inflammation, as well from blood of healthy paediatric controls (n=30). FlowSOM clustering were performed with functionally and phenotypically important immune markers.

**Results:** Alteration in B cell subsets are present in patients with pSS compared to controls, with an expansion of atypical memory B cells and Tfh. The B cell abnormalities are not affected by treatment. Our data confirm a hyperactivation of the humoral immune system in patients with pSS and provide evidence for their development as biomarkers and to develop new therapeutic strategies aimed at controlling B cell hyperactivation in pediatric patients with SS.

**Acknowledgements:** NIL.

**Disclosure of Interests:** None Declared.

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**OP0033**

**ANALYSIS OF B CELL SUBSETS AND B CELL CYTOKINES IN PEDIATRIC SJÖGREN’S SYNDROME

**Keywords:** Sjögren syndrome, Cell biology, Adaptive immunity

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**Background:** Pediatric Sjögren’s syndrome (pSS) is a rare disorder that is often diagnosed late due to the lack of validated diagnostic criteria and validated biomarkers. The pathogenesis is largely unknown, but there is evidence of involvement of both the innate and adaptive branch of the immune system. Immunological overactivity is central in the pathogenesis of pSS. Several studies showed the presence of B cells abnormalities in patients with SS with an expansion of naïve B cells and a decrease in the frequency of memory B cells.

**Objectives:** We set out to investigate the distribution of B cell subsets and B cell cytokines in patients with pSS at disease onset and at follow up visits.

**Methods:** A monocentric retrospective cohort study was conducted on 23 patients with pSS enrolled at the department of Rheumatology of Bambino Gesù Children’s Hospital. Serum levels of CXCL13 and BAFF were analyzed by ELISA. B cell phenotype was assessed on peripheral blood mononuclear cells (PBMCs) by flow cytometry. Systemic disease activity was evaluated by ESSDAI (EULAR Sjögren’s syndrome disease activity index) and Clinical-ESSDAI score (Clin-ESSDAI), according to 2020 EULAR recommendations; active disease was defined by Clin-ESSDAI ≥1 and remission by Clin-ESSDAI<0. Analysis we selected age-matched people with no diagnosis of pSS or any other systemic autoimmune disease.

**Results:** Serum levels of CXCL13 and BAFF were significantly higher in patients with pSS than the control group (p<0.05) (Figure 1A). We correlated levels of biomarkers with clinical and laboratory parameters: we observed a positive correlation between hypergammaglobulinemia and BAFF (r=0.80). Analysis of B-cell subsets at disease onset revealed the expansion of a population of atypical memory B cells (p<0.00049) and a reduction in IgM memory B cells (p=0.0034) compared to the control group (Figure 1B). We then compared the distribution of B cell subpopulations at disease onset (pre) with samples obtained at follow-up (post) for each patient no significant differences were observed (Figure 1B). We also investigated the distribution of Th17 cells in patients with pSS and we observed a significant expansion of CXCR3+PD1+ T cells (Figure 1B).

**Conclusion:** Patients with pSS showed high levels of CXCL13 and BAFF at disease onset. Alteration in B cell subsets are present in patients with pSS compared to controls, with an expansion of atypical memory B cells and Tfh. The B cell abnormalities are not affected by treatment. Our data confirm a hyperactivation of the humoral immune system in patients with pSS and provide evidence for their development as biomarkers and to develop new therapeutic strategies aimed at controlling B cell hyperactivation in pediatric patients with SS.

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**Figure 1.**