assessed the age of onset in relation to the presence of autoantibodies. The association with characteristics of the anti-citrullinated protein antibodies (ACPA)-response was also explored.

Objectives: 1) determine the association between age of RA-onset and the presence of ACPA, rheumatoid factor (RF) and anti-carbamylated protein (anti-CarP) antibodies, 2) study if age of onset was associated with characteristics of the ACPA-response, 3) substantiate previously reported associations between age of onset and clinical characteristics.

Methods: 3,321 1987-positive RA-patients included in the Leiden-EAC, BARFOT, ESPOIR, Umeå and Lund cohorts were studied at presentation on age of onset and the presence of ACPA, RF and anti-CarP antibodies. Logistic regression analyses were performed; effect sizes were summarized in inverse-weighted meta-analyses. Within ACPA-positive RA, ACPA-level was studied in all cohorts; ACPA-isotypes, ACPA-fine-specificity and ACPA-avidity index and clinical characteristics were studied in the Leiden-EAC.

Results: From the age of fifty onwards, the proportion of ACPA-negative RA-patients increased in Dutch, Swedish and French cohorts. Similar observations were done for RF and anti-CarP. The composition of the ACPA-response did not change with increasing age of onset with respect to titer, isotype distribution, fine specificity and avidity index. With increasing age of onset RA-patients smoked less often, had higher acute phase reactants and more often a sub(acute) symptom onset.

Conclusions: Data of five cohorts revealed that with higher age of onset ACPA-negative RA is more frequent than ACPA-positive RA, while characteristics of ACPA-positive RA as judged by the composition of the ACPA-response appeared not age-dependent. Although more biologic studies are needed to characterize the pathogenesis of ACPA-negative polyarthritis at older age, the present data can promote personalized treatment decisions in ACPA-negative patients in daily practice.

Disclosure of Interest: None declared

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AB0013 IS THERE IMMUNE DISREGULATION IN NON-SJÖGREN SICCA SYNDROME? A STUDY OF BLOOD LYMPHOCYTE SUBPOPULATIONS

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Background: A large number of patients with sicca syndrome not fulfilling Sjögren's syndrome (SS) classification criteria, present manifestations of autoimmunity, like arthritis, Raynaud's, rash or hematologic disturbances, and have anti-nuclear antibodies, lacking however more specific antibodies. The designation Undifferentiated Connective Tissue Disease was coined to refer to those patients, and some will eventually progress to a definite disease, of which SS would be a likely candidate. Immune cell disturbances could be progression marker, since diseases like pSS have distinct lymphocyte profiles.

Objectives: We aim to study the circulating lymphocyte subsets in non-Sjögren sicca patients (n-SS), and compare them with pSS and healthy controls.

Methods: We included 65 n-SS patients, 53 pSS patients (2002 AECG criteria) and 22 healthy controls. Lymphocyte subsets were characterized by flow cytometry, including follicular and regulatory T cells and naïve, mature, memory, plasmablasts and regulatory B cells. Statistical analysis was performed with GraphPad, and significance was considered for $p < 0.05$.

Results: Comparing to controls, n-SS patients had lower counts of T cells ($p=0.016$), with lower CD4 ($p=0.0028$), however that difference was not as pronounced as between SS and controls. n-SS patients had higher percentages of CD4 ($p=0.0005$) and lower CD8 percentages ($p=0.0009$) than pSS. Additionally, there was a decrease in absolute counts of Tregs ($p=0.0028$) in n-SS patients compared to controls, which was less pronounced than the comparison between SS and controls ($p=0.0008$). Th17 cells were decreased in SS compared to controls ($p=0.0005$), but not in SS patients. Compared with controls, both n-SS and SS patients presented decreased absolute count ($p=0.0001$ and $p<0.0001$, respectively) of CXCR5⁺ Tfh cells, with no differences between n-SS and SS patients. However, higher levels of IL21⁺CD4 T cells and Tfh1 cells were found comparing SS patients with both controls ($p=0.0209$ and $p=0.0092$ respectively) and n-SS patients ($p=0.0051$ and 0.0028 respectively).

Absolute counts of memory, unswitched and switched memory cells in n-SS patients present intermediate levels between controls with significantly higher levels, and SS patients with significantly lower levels. Accordingly, using the Bm1-5 classification, we have found decreased Bm1 ($p=0.004$), eBm5 (Abs, $p=0.0273$) and Bm5 cells (Abs, $p=0.0444$) in n-SS patients compared to controls. Though not significant, there was an increase in eBm5 (Abs, $p=0.063$) and Bm5 cells (Abs, $p=0.05$) in n-SS compared to SS patients. Again, CD24⁺CD27⁺ Bregs were also decreased in n-SS patients compared to controls ($p=0.036$), but increased in n-SS compared to SS patients ($p=0.0007$).

Conclusions: Our data showed that n-SS patients present immune dysregulation, represented by alterations in the B cell compartment but also in Tfh subset, known to modulate the humoral immune response. Although less pronounced, these modifications resemble the ones found in SS patients. Whether n-SS is a stage in the evolution to SS remains to be clarified. The identification of a characteristic

dysregulation of the immune system in n-SS could be useful for diagnostic and prognostic purposes.

Disclosure of Interest: None declared

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AB0014 DIFFERENTIAL EFFECTS OF A SINGLE DOSE OF CYCLOPHOSPHAMIDE ON CIRCULATING CELL SUBPOPULATIONS IN PATIENTS WITH VASCULITIDES AND AUTOIMMUNE SYSTEMIC DISEASES

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Background: High-dose cyclophosphamide (CFA) is used for treatment of patients with severe manifestations of systemic autoimmune diseases. The knowledge about the effect of CFA on circulating cell populations in these diseases is still limited.

Objectives: To identify the effect of a single dose of CFA on circulating cell populations in patients with vasculitides and autoimmune systemic diseases.

Methods: We immunophenotyped T lymphocytes (CD3, CD4, CD8, CD25, CD127, HLA-DR), B lymphocytes (CD19, CD20, CD27), NK cells (CD3, CD16, CD56, CD69), neutrophils (CD15, CD11b, CD16, CD54, CD62L, CD64), and monocytes (CD11b, CD14, CD16, CD64, HLA-DR) using 6-color cytometer BD FACSCanto II (Becton Dickinson) in peripheral blood of patients with vasculitides ($n=6$) and systemic disorders of connective tissue ($n=6$). From each patient, we obtained paired samples before and one month after a single CFA dose. Statistical tests were performed using GraphPad Prism (GraphPad Software, Inc). P -value < 0.05 was considered as significant.

Results: Single dose of CFA resulted in increase of percentage of CD8+ T lymphocytes ($P=0.002$), leading to marked decrease of CD4+/CD8+ ratio ($P=0.009$). Except three patients, overall percentage of neutrophils decreased after the treatment ($P=0.01$). Although CFA pulse did not influence the percentage of NK cells, the percentage of NK cells carrying stimulatory receptor CD69 increased after CFA ($P=0.037$). Similarly, CFA enhanced the percentage of co-stimulatory molecule CD27 on B lymphocytes ($P=0.048$). Among subsets of monocytes, CFA treatment increased percentage of CD14-CD16+ monocytes ($P=0.006$) and increased expression (MFI) of Fc fragment CD64 ($P=0.02$). Moreover, increase of HLA-DR was observed on CD14-CD16+ monocytes ($P=0.037$). The investigations whether the changes in immune cell subpopulations in treated patients have prognostic potential are ongoing.

Conclusions: Single dose of CFA resulted in increase of CD8+ T lymphocytes, activation of NK, B lymphocytes and monocytes as well as in some patients decrease in neutrophil counts. Further investigation of selected markers may lead to identification of new prognostic markers and predict the effectiveness of the treatment.

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AB0015 CAPTURE OF IGA IMMUNE COMPLEXES AND ENRICHMENT IN IGA IG GENE EXPRESSION BOTH SUGGEST A ROLE FOR FCRL4+ B CELLS IN THE LINK BETWEEN MUCOSAL AND JOINT INFLAMMATION

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Background: Increasing evidence points to the autoimmune process of rheumatoid arthritis (RA) originating at mucosal surfaces. Previous work from our group described a subset of B cells in the synovium and synovial fluid (SF) of RA patients which can be distinguished by their expression of the Fc-like receptor 4 (FcRL4) and elevated expression of RANKL, indicating a unique pathogenic function^{1,2}. B cells expressing FcRL4 were originally described as a distinct memory B cell subset in human tonsils where they accumulate in the epithelium³. We have recently shown that they are enriched in cells recognizing citrullinated autoantigens (Amara, K. et al. under revision). Recent *in vitro* work suggested that FcRL4 is a low affinity receptor for aggregated IgA⁴.

Objectives: 1) To investigate the interaction of RA synovial fluid FCRL4+ B cells with IgA

2) To examine the distribution of Ig classes by flow cytometry and PCR

Methods: SF mononuclear cells were isolated, labelled for FCRL4, IgA and CD19 and analysed by flow cytometry. In experiments identifying IgA B cell receptors, SF mononuclear cells were briefly incubated in an acidic buffer to remove surface receptor bound antibodies before staining. Heat-aggregated purified human IgA

was added to assess IgA binding to FcRL4+ B cells. Single B cells were sorted from SF of RA patients and their constant region genes probed for identification of their Ig subclass by PCR.

Results: *Ex vivo*, FcRL4+ B cells in SF of RA patients have a higher load of IgA bound to their surface compared to their FcRL4- counterparts. After in vitro removal of surface bound IgA, they can bind heat-aggregated IgA ($p=0.0313$). We also demonstrate that a significantly higher proportion of FcRL4+ B cells use IgA BCRs ($p=0.0061$) by flow cytometry and further more by probing constant region genes by PCR an enrichment for Ig genes for coding the IgA1 isotype ($p=0.009$) was found.

Conclusions: Both their ability to capture IgA immune complexes through binding to FcRL4 and their enrichment in IgA Ig gene expression suggest a potential role for synovial fluid FcRL4+ B cells in the mucosal origin of joint inflammation.

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AB0016 B-CELL SUBSETS DIFFERENCES IN INFLAMMATORY RHEUMATIC DISEASES

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Background: Targeting humoral immunity has been proved effective in several

Table 1. Patient and healthy controls demographics, clinical variables and absolute cell counts.

	AS (n=22)	RA (n=20)	SLE (n=18)	HC (n=12)
Patients				
Male, Female; n (%)	11 (50); 11 (50)	9 (45); 11 (55)	5 (27.8); 13 (72.2)	3 (30); 7 (70)
Age; median (IQR)	56 (45.8-65.5)	55 (51-65.5)	44 (37.5-52.5)*	60.5 (32-64.5)
ESR mm/hour	14 (10-29.3)	21 (10.3-37.8)	23 (6-34.5)	11 (8-22.5)
CRP mg/dL	1.1 (0.9-1.7)	0.98 (0.7-3.2)	0.6 (0.5-1.6)	-
HLA B27+: n (%)	11 (68.8)	-	-	-
With csDMARDs, n (%)	6 (30)	18 (90)	15 (83.3)	0
Ig seric levels, mg/dl; median (IQR)				
IgG	1165 (881.3-1247.5)	1021 (830.3-1265)	1140 (1003-1325)	1033.5 (823.3-1231)
IgA	230 (158.8-340)	250.5 (164.3-315.3)	261 (205-323)	236.5 (150.3-339.5)
IgM	95.3 (65.6-119.5)	112 (67.7-164.8)	103 (60.6-131.5)	122 (71.5-158.3)
Absolute cell counts/μl blood; median (IQR)				
Lymphocytes	1685 (1217-2007.5)	1555 (1097.5-2085)	1250 (625-1892.5)	2170 (1830-2377)*
Total B-cells (CD20 ⁺)	186.3 (110.9-238.6)	96.2 (50.3-180.6)***	65.8 (20.9-116.1)***	182.1 (100.9-269.1)
Immature Transitional B-cells (CD5⁺CD27⁺IgD⁺), median (IQR)				
CD24 ⁺⁺⁺ CD38 ⁺⁺⁺ (T1)	2.8 (1.8-3.9)	0.6 (0.1-2.7)**	1.5 (0.2-2.8)**	4.3 (2.3-6.5)
CD24 ⁺⁺ CD38 ⁺⁺ (T2)	8.3 (5.1-14.9)	2.6 (0.2-8.0)**	3.0 (1.0-8.0)**	13.7 (5.7-18.8)
CD24 ⁺ CD38 ⁺ (T3)	9.0 (5.5-17.7)	2.2 (0.3-4.6)**	1.9 (0.2-3.7)**	7.7 (4.1-12.3)
Mature B-Cells, median (IQR)				
CD27 ⁺ IgD ⁺ (naïve)	73.2 (49.7-121.7)	40.1 (19.2-76.9)**	27.2 (11.9-57.1) †	78.6 (48.4-163.4)
CD27 ⁺ IgD ⁺ (mem. MZ-like)	25.9 (13.6-39.9)	13.0 (3.5-27.2)**	2.6 (1.8-9.6) †	18.9 (11.2-27.1)
CD27 ⁺ IgD ⁺ (switch mem.)	18.8 (12.2-37.9)	13.5 (3.9-37.6)	4.9 (2.2-17.2)***	29.3 (14.51-37.9)
CD27 ⁺ IgD ⁺ (double neg.)	2.4 (1.8-5.2)	3.1 (2.0-6.4)	2.9 (1.0-5.0)	5.2 (2.6-8.1)

Mann-Whitney and the Fisher's exact test were used for comparison between AS and other groups

* $p<0.05$; ** $p<0.02$; *** $p<0.01$; † $p<0.0001$

inflammatory rheumatic diseases (IRD). Though clinical trials have shown some efficacy of B-cell depletion in ankylosing spondylitis (AS), results are less convincing. Other studies have revealed an association between mutations and expression of immune regulatory genes suggesting B-cell dysfunction in the development and progression of AS. Yet, there is still lack of data describing B-cell subsets in AS, how these compare to other IRD and an evaluation of B cell compartment homeostasis in the pathophysiology of this disease.

Objectives: To assess and compare the immature, naïve and antigen differentiated subsets of peripheral B-cell compartment in AS with those in healthy controls (HC) and other IRD

Methods: Patients (pts) with AS, RA and SLE according to respective classification criteria were included in this study. Pts under biologic DMARDs were not included. Sociodemographic and clinical variables were recorded. Blood samples were collected for quantification of inflammatory markers (ESR and CRP), immunoglobulin serum levels and assessment of B-cell immature transitional stages and mature subsets by flow cytometry (figure). Mann-Whitney and Fisher's exact test were used for comparison of AS with other groups

Results: Overall, 60 pts and 12 HC were included (Table). All patient groups presented similar and rather low levels of inflammation, as measured by CRP, ESR and immunoglobulins, in addition to a decreased lymphocyte count by comparison with HC. There were no differences in the B-cell counts between AS pts and HC, with both groups having higher B-cell counts than RA and SLE pts. Regarding B-cell subsets, the immature transitional compartment of AS pts was found in normal range, but not in the RA and SLE groups. In fact, the latter presented a significant decrease in all transitional cell maturity stages (T1-T3). The next step in B-cell differentiation is mature naïve cells, also found in normal levels in AS and decreased in RA and in particular in SLE. AS pts presented slightly higher counts of CD27+IgD+ MZ-like and class able to switch memory cells with reference to HC and these cell numbers were found to be low in RA and even lower in SLE pts. Switched memory CD27+IgD- B-cells were reduced in all patient groups, however, only SLE pts presented highly decreased cell levels.

Conclusions: We found that while a severe dysfunction is present in the homeostasis of the B-cell compartment in RA and in particular SLE pts, which are lymphopenic in both immature and mature B-cell compartments, it appears that AS pts are not affected in the same way. At this stage, functional studies appear to be necessary in order to identify differences in key mechanisms of B cell development and differentiation that may play a role in the aetiology and progression of these inflammatory rheumatic diseases. Our first results, however, establish that pathophysiological mechanisms involving B-cells clearly differentiate AS from RA and SLE

Disclosure of Interest: None declared

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AB0017 THERAPEUTIC EFFECT OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS TRANSPLANTATION IN A MOUSE MODEL OF PRIMARY BILIARY CIRRHOSIS

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Background: Primary biliary cirrhosis (PBC) is a cholestatic liver disease characterized by slowly progressive non-suppurative cholangitis with immune mediated destruction of intrahepatic bile ducts. Previous studies have shown beneficial effects of mesenchymal stem cells (MSCs) transplantation in many autoimmune diseases. However, few studies have focused on the effects of MSCs on PBC.

Objectives: In our study, we investigated the therapeutic effect of umbilical cord-derived mesenchymal stem cells (UC-MSCs) transplantation in a well-defined mouse model of PBC and explored the potential mechanism.

Methods: After 2OA-BSA-induced autoimmune cholangitis was created in C57BL/6 mice, cultured human UC-MSCs or a vehicle control was administered. Liver injury severity was assessed by clinical and histologic analysis. The immunity suppression effects and mechanism of UC-MSCs were tested.

Results: UC-MSCs administration alleviated bile duct damage and intrahepatic inflammatory cell infiltration in C57BL/6 mice that had undergone 2OA-BSA immunization. Serum levels of ALT and ALP were significantly decreased. Also, UC-MSCs reduced the production of anti-mitochondrial autoantibodies (AMA) in PBC mice. UC-MSCs downregulated inflammatory cytokine, such as IFN- γ , TNF- α , IL-12 and IL-17A production both in peripheral blood and local liver. Notably, Infusion of UC-MSCs resulted in an increase in regulatory T cells (Tregs) in peripheral blood but a decrease of this kind of cells in liver tissues. Furthermore, UC-MSCs significantly suppressed Th1- and Th17-cell responses and these alternations could be detected in spleen, peripheral blood and liver in PBC mice.

Conclusions: In summary, the current study shows that UC-MSCs based therapy has profound inhibitory effects on inflammatory responses and immunoregulatory effects both in local and systemic abnormalities in PBC. These findings also provide further evidence regarding the role of MSCs in the clinical trials of PBC.

Disclosure of Interest: None declared

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