OP0011

INTEGRATION OF FLOW AND DIGITAL CYTOMETRY IN EARLY TREATMENT-NAÏVE RHEUMATOID ARTHRITIS IDENTIFIES DISTINCT IMMUNOPHENOTYPES IN PERIPHERAL BLOOD AND DISEASE TISSUE

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Background: The study of synovial tissue in patients with Rheumatoid Arthritis (RA) has led to the identification of synovial patterns of immune cell infiltration and specific cellular subsets associated that have been disease activity and clinical outcomes (1–3). However, the relationship of circulating and synovial immune cell sub-sets with histopathological features and clinical outcomes remains to be defined.

Objectives: To assess the relationship of peripheral blood and synovial immune cells with RA histopathology and clinical outcomes, by performing flow and digital cytometry in matched peripheral blood and synovial samples from patients with early RA.

Methods: 70 patients with early (<12 months) untreated RA (2010 criteria) recruited in the pathobiology of early Arthritis Cohort (PEAC) at the Barts Health NHS Trust were included (1). Peripheral blood mononuclear cells (n=70) were analysed by flow cytometry. Matched synovial tissues (n=70) obtained by minimally invasive ultrasound-guided synovial biopsy underwent semi-quantitative scoring (0–4) of immune cell infiltration and classification into lympho-myeloid (LM), diffuse-myeloid (DM) and pauci-immune (PI) pathotypes, as previously described (7). 49 synovial and 36 matched peripheral blood samples underwent RNA-sequencing and were analysed by digital cytometry (Xcell) (4) and Singular Value Decomposition (SVD).

Results: Circulating B cells and their subsets showed significant inverse correlations with inflammatory markers (ESR, CRP), disease activity (swollen joints, four components and two components (5) DAS28) and ultrasound scores (Fig 1A). Among T cell subsets, CXCR5-CD11b^{hi}CD4⁺ T cells (T peripheral helper cells, Tph) had strong positive correlations with inflammatory markers (ESR and CRP), disease activity (DAS28) and ultrasound scores (Fig 1B). Tph in the peripheral blood also correlated with immune cell infiltration in synovia (Fig 1C) and were significantly higher in patients with a LM pathotype (Fig 1D). Accordingly, circulating Tph were associated with synovial LM pathotype independently of clinical features such as DAS28, ACPA positivity, Body Mass Index (BMI) and age (AUC 0.821). By applying digital cytometry in matched synovial and peripheral blood samples, synovial B and T cells were significantly higher in patients with a LM pathotype, in line with the histological definition of the LM pathotype

Figure 1

Figure 1.

– rich in B and T cells. On the contrary, circulating B cells and total CD4 and CD8 T cells were significantly lower in patients with a synovial LM pathotype (Fig 1E). The Tph signature in synovia derived by Singular Value Decomposition (SVD) correlated with baseline ESR (R 0.38, p<0.0001) and DAS28 (R 0.35, p<0.0001) and with delta-DAS28 after 6 months of treatment with conventional synthetic DMARDs (R 0.27, p 0.026). Finally, the baseline synovial Tph signature was significantly higher in patients who progressed to the use of biologics and was predictive of future biologic DMARDs use, independently of baseline DAS28, ACPA positivity, BMI and age (AUC 0.703).

Conclusion: By combining conventional flow cytometry in the peripheral blood and digital cytometry on matched synovial and peripheral blood samples, we highlight diverging associations of circulating immune cell subsets with synovial inflammation and pathotypes. Tph cells, in particular, emerge as predictors of lympho-myeloid synovial inflammation and disease progression.

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OP0012

ASSOCIATION BETWEEN PASSIVE SMOKING IN CHILDHOOD AND ADULTHOOD, AND RHEUMATOID ARTHRITIS: RESULTS FROM THE FRENCH E3N-EPIC COHORT STUDY

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Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease of multifactorial aetiology, which preferentially affects women. To date, active smoking has been the most reproducibly reported risk factor for anti-citrullinated protein antibodies (ACPA) positive RA, particularly persons who carry the HLA-DRB1-shared epitope (SE) alleles.

Objectives: We aimed to investigate the relationships between passive smoking in childhood (PSc) or in adulthood (PSa), and the risk of incident RA in a large prospective cohort of healthy French women.

Methods: The E3N-EPIC (*Etude Epidémiologique auprès des femmes de la Mutuelle générale de l'Education Nationale*) is a French prospective cohort study that investigates environmental factors associated with chronic diseases. It follows 98,995 healthy French women since 1990 covered by a national health insurance primarily involving teachers. RA cases have been previously identified with specific questionnaires and medication reimbursement database. Women were considered exposed to PSc if they self-declared staying in a smoky room several hours a day during childhood, and to PSa if they self-declared being exposed at least one hour a day to passive smoking while adults. We used Cox multivariable regression models with age as the timescale (model 1), adjusted on smoking status (never, current, or former smoker) and on the two types of passive smoking (model 2), and on educational level, and BMI (model 3). Stratified analyses were conducted depending on the active smoking status (never or ever-smoker).

Results: 79,806 women were included in the study. Mean ($\pm$ SD) age at cohort entry was 49.0 ($\pm$ 6.4) years. Among them, 698 incident RA cases were identified, diagnosed after a mean of 11.7 ($\pm$ 5.8) years after baseline. In the whole cohort, 10,810 (13.5%) women were exposed to PSc, 42,807 (53.6%) to PSa, 6,581 (8.25%) were exposed to both, and 47,036 (58.9%) were exposed to either. In the whole population, PSc was positively associated with the risk of RA in all three models (HR 1.24; 95% CI [1.01 to 1.51] in Model 3). In stratified analyses

on smoking status, PSc was associated with RA among never-smoking women (HR 1.42; 95% CI [1.07 to 1.88]), but not among ever-smoking women (HR 1.10; 95% CI [0.83;1.46]).

In the whole population, PSa was also positively associated with the risk of RA in all three models (HR 1.19; 95% CI [1.02 to 1.40] in Model 3). In stratified analyses on the smoking status, PSa was associated with an increased RA risk only among never-smoking women (HR 1.27; 95% CI [1.02 to 1.57]) and not among ever-smoking women (HR 1.16; 95% CI [0.93;1.44]).

Conclusion: In this large population-based prospective cohort study of French women, we reported that passive exposure to smoking during childhood or adulthood increased the risk of RA. The association was principally observed among never smoking women. These results suggest that smoking by-products, whether actively or passively inhaled absorbed, could generate autoimmunity, at least towards antigens involved in RA pathogenesis.

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OP0013

SEX DIFFERENCES IN AUTOIMMUNE DISEASE SUSCEPTIBILITY; A MULTI-OMIC APPROACH

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Background: Males and females have altered immune responses resulting in variation in autoimmune and cardiovascular disease risk (CVR). Recently, these differences have played a role in the inflammatory response to COVID-19. Sex differences exist in the frequency and activity of immune-cell subsets but mechanisms underlying sexual dimorphism remain unknown. Juvenile-onset systemic lupus erythematosus (JSLE) is an autoimmune disorder that commonly emerges during puberty, has a strong female prevalence (female:male ratio, 4.5:1) and results in an increased CVR. JSLE is characterised by chronic inflammation and dyslipidaemia, where cardiovascular disease is a leading cause of mortality for patients. Our previous work identified a link between immune cell function and lipid metabolism in adult-onset SLE. We hypothesised that sex hormones could influence both lipid metabolism and immune cell function and this could determine sex-specific susceptibility to JSLE and associated CVR.

Objectives: We investigated the role of sex hormones in modifying systemic lipid metabolism and inflammation.

Methods: Nuclear magnetic resonance spectroscopy based serum metabolomics measuring over 130 lipoproteins (14-subsets with lipid compositions), flow cytometry measuring immune-cells, and RNA-sequencing were used to assess the metabolic and immune profile in young, pre/post-pubertal males (n=10/17) and females (n=10/23) and in individuals with gender-dysphoria (GD) under cross-hormone treatment (trans-male/female, n=26/25). This analysis was also performed on a cohort of post-pubertal male (n=12) and female (n=23) JSLE patients. Data was analysed by logistic regression, balanced random forest machine learning (BRF-ML), differential gene expression (DEG) and pathway analysis.

Results: Post-pubertal males had significantly reduced cardio-protective high-density lipoprotein (HDL) subsets (p<0.0001) and increased cardio-pathogenic very-low-density lipoprotein subsets (p<0.0001) compared to females. These differences were not observed pre-puberty and were reversed significantly by cross-hormone treatment in GD individuals, suggesting that sex hormones regulate lipid metabolism *in-vivo*.

BRF-ML (28 immune-cell subsets) identified an increased frequency of anti-inflammatory regulatory T-cells (Tregs) in post-pubertal males compared to females (p=0.0097). These Tregs were also more suppressive in males compared to females. Differences in Treg frequency were seen pre-puberty and were not altered by sex hormone treatment in GD individuals. However, Treg DEGs and functional transcriptomic pathways altered between post-pubertal males and females, including those involved in inflammatory signalling, overlapped with those altered by hormones in GD, suggesting hormones may also drive Treg functional changes. In addition, HDL metabolites modified by hormones showed differential associations with Treg phenotypes between post-pubertal males and females.

Strikingly, sex differences in lipoproteins and Tregs were lost in JSLE, suggesting hormone signalling could be dysregulated in the pathogenesis of autoimmunity and could increase CVR for patients.

Conclusion: Sex hormones drive altered lipoprotein metabolism and functional transcriptomic pathways in Tregs. Males have a lipoprotein profile associated with increased CVR, but a more anti-inflammatory immune profile compared to females. Together, this could explain sex differences in inflammatory disease susceptibilities and inform future sex-specific therapeutic strategies for the management of both JSLE and CVR.

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Genomics, genetic basis of disease and functional genomics

OP0014

HLA ASSOCIATIONS IN PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS ASSOCIATED UVEITIS AND CLINICAL SUBTYPES

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Background: Juvenile idiopathic arthritis (JIA) is a childhood onset rheumatic disease which is classified into seven different clinical subtypes based upon the ILAR classification criteria. The most common extra articular manifestation of JIA is its associated uveitis (JIAU); particularly chronic anterior uveitis (CAU). Uveitis is a serious complication with the potential to lead to visual impairment and blindness. The rheumatoid factor negative polyarthritis and oligoarthritis ILAR subtypes, often referred to as the “polygo” subgroup, are at a higher risk for developing JIAU, with up to 30% of polygos afflicted by CAU. The HLA region has long been reported as a genetic risk factor for JIA susceptibility, with evidence suggesting that different amino acids of HLA genes infer risk to different JIA subtypes.

Objectives: Investigate the association of amino acids and genetic variants in the HLA region with susceptibility to JIAU and the ILAR clinical subtypes.

Methods: Samples were genotyped using the Illumina Infinium CoreExome and Infinium Omniexpress arrays. Samples were excluded based on <98% call rate, discrepancy between genetically inferred sex and database records, inferred relatedness (identify-by-descent) and ancestral outliers based on principal component analysis (PCA). SNPs were excluded based on <0.01 minor allele frequency (MAF), and call rate <98%. SNP2HLA was used to impute HLA amino acids, SNPs and alleles. Analysis was then executed on markers with an information score >0.9 and MAF >.01 using logistic regression or an omnibus test for multiallelic markers, including 3 PCs as covariates. Independent associations were identified using forward stepwise logistic regression including previously identified variants as covariates. Comparison of regression models was performed using a likelihood ratio test (LRT).

Results: We analysed 7425 markers within the HLA region in 450 JIAU and 2024 JIA cases without uveitis. The most significant association was to amino acid positions 13 of HLA-DRB1 (p=2.9×10⁻³⁰). Conditional analysis on DRB1 position 13 revealed an independent signal at DRB1 position 67 (p=2.4×10⁻⁶). Conditioning on all DRB1 alleles revealed an independent signal at HLA-DPB1 position 69 (p=5.3×10⁻⁷). As expected, ILAR subtype was found to be associated with JIAU (p=1.58×10⁻⁶). We used LRT to test if genetics provided further information above ILAR subtype alone and found that including residues at DRB1 position 13 significantly improved the fit of a model based on ILAR subtype alone (LRT p = 3.6×10⁻²⁷). The reciprocal analysis, adding ILAR subtype to a model based on DRB1 position 13 alone, did not significantly improve the fit of a model (LRT p=0.83). Exploring associations in the polygo subgroup (n=1646) we found significant associations to the three previously described amino acids and JIAU (DRB1 position 13 p=3.4×10⁻²⁰, DRB1 position 67 p=3.3×10⁻⁴, DPB1 position 69 p=2.2×10⁻⁶).

Conclusion: This is largest analysis of HLA markers in JIAU patients to date and we identify two independent associations to amino acids in HLA-DRB1 and a further independent association to HLA-DPB1. This analysis demonstrates that including data on genetic risk factors adds further information to that captured