incompletely understood. Making use of an existing dataset, we investigated the CD4+ T-cell transcriptome in early RA patients, seeking biomarkers for drug survival on MTX monotherapy and associated pharmacological insights.

Methods In previous work [1] RNA was rapidly extracted from peripheral blood CD4+ T-cells of 173 early arthritis clinic attendees, at which time patients had been symptomatic for a median of 12 weeks, and were naïve to immunomodulatory treatments. Transcriptional profiling was undertaken using Illumina WG6v3 BeadChip oligonucleotide array technology. For this study a sub-cohort of RA patients was retrospectively identified, whose initial treatment with MTX monotherapy was continued for as long as deemed successful between patient and consulting rheumatologist. Intra-muscular steroid bolus administration (but not oral steroid therapy) was permitted during the study at the discretion of the consulting rheumatologist. MTX monotherapy survival after ≥1 year follow-up (median 28 months) was used as a surrogate outcome for efficacy, and bioinformatics analysis was performed using GeneSpring XI (Agilent).

Results Amongst 31 eligible patients, 19 (61%) remained on MTX monotherapy at the end of follow-up, but the treatment strategy was unsuccessful (and required modification) for the remaining 12 (39%). Baseline characteristics and final methotrexate doses were comparable between the two groups. 133 CD4+ T-cell transcripts were identified as being differentially expressed between comparator groups at baseline (>1.2 fold-change; $p < 0.05$) and a metric derived from their normalised expression values demonstrated a promising discriminatory utility with respect to MTX monotherapy survival (area under ROC curve 0.91). Functional analysis identified an over-representation of genes involved in apoptosis (11/133 genes; hypergeometric $p = 0.000045$).

Conclusions Although limited by its reliance on a surrogate efficacy outcome, our pilot study has identified potential transcriptional biomarkers for drug survival on MTX monotherapy amongst early RA patients. Alongside their potential clinical applicability, they suggest that this treatment's efficacy may depend on its ability to regulate CD4+ T-cell survival. Validation amongst a clinically well-characterised, independent early RA cohort is now on-going.

Reference

1. Pratt AG *et al*, *ARD* 2012.

A3.3 AN IMMUNOLOGICAL WINDOW OF OPPORTUNITY DEFINES THE ABILITY OF EARLY RA PATIENT TO ACHIEVE REMISSION WITH FIRST ANTI-RHEUMATIC TREATMENT

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Background and Objectives The therapeutic goal for patients with rheumatoid arthritis (RA) is clinical remission. This is best achieved by early diagnosis followed by appropriate therapeutic intervention. RA is associated with dys-regulation of T-cell subsets early in the disease with naïve cells and regulatory T-cell losses and acquisition of abnormal subset in realtion with inflammation (IRC). Our aim was to test the hypothesis that T-cell subset quantification can predict the achievement of clinical remission in patients with early RA.

Materials and Methods T-cell subsets (naive, Treg and IRC) were quantified using flow cytometry in 108 DMARDs-naïve, early RA patients (<24 months) symptom duration commencing methotrexate MTX or MTX + anti-tumour necrosis factor agents (anti-TNF) and in 105 healthy controls (HC). The primary outcome assessed was remission (DAS28 < 2.6). The pilot analysis was performed on frozen PBMC obtained from 38 RA patients and 35 HC. These results were validated using fresh blood samples on a cohort of 70 RA patients and 70 HC.

Results In the pilot study, T-cell subset analysis in early RA confirmed immune dysregulation compared to HC with reduced frequency of naïve CD4+ T-cells and Treg and increased IRC (all $p < 0.001$) compared to HC. Naive T-cell above median was associated with remission ($p = 0.001$). In the validation study, 50 patients were treated with MTX and showed the same relationship with naïve cell frequency above median being associated with remission ($p = 0.011$). Individual analysis on each patient's naïve cell frequency deviation from expected (using 70 HC) demonstrated that "normal" naïve cell frequency (observed in 30 patients) was associated with remission whereas reduced naïve cell frequency was more frequently observed in patients with poor response to MTX ($p = 0.03$). Patients with poor immunological status were not prevented to achieve remission when treated with MTX + anti-TNF (n = 20 including 10 patients with normal and 10 with reduced naïve cells) raising the rate of remission from 20% in the MTX group (n = 4 of the 20 patients with reduced naïve cells at baseline) to 60% in the MTX + anti-TNF group (n = 6 of the 10 patients).

Conclusions These data show that baseline naïve T-cell subset analysis has a value in predicting early RA MTX treatment outcome. Immunological analysis could be used in conjunction with clinical/serological features to predict response to MTX and select the most appropriate therapy at disease presentation.

A3.4 $CD3^+CD4^-CD8^-$ DOUBLE NEGATIVE TH17 CELLS: NEW INSIGHTS IN THE PATHOGENESIS OF PRIMARY SJÖGREN'S SYNDROME

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Background and Objectives IL-17 axis is widely recognised to be involved in the pathogenesis of autoimmune disorders. Besides conventional $CD4^+$ Th17 cells, a small IL-17 producing T-cell population, that lacks of both CD4 and CD8 molecules, defined as double negative (DN) was recently found to be expanded in the peripheral blood (PB) and to accumulate in the kidney in patients with lupus nephritis. Since IL-17 production is enhanced in minor salivary gland (MSG) infiltrates of patients with primary Sjögren's syndrome (pSS), we sought to investigate whether DN T cells may be involved in pSS pathogenesis.

Materials and Methods Thirty patients with pSS and 16 normal controls (NC) were studied. PBMCs were separated by density gradient and phenotypic characterisation was performed by flow cytometry on both freshly isolated cells and after culture (24, 48, 72 and 96 hours). Total PBMCs were cultured in anti-CD3 coated plates in presence or absence of dexamethasone (Dex) at different concentrations. In selected experiments, real time PCR at the same time-points was performed. The study of pSS-MSGs was performed by immunofluorescence.

Results Total circulating DN T cells were increased in pSS compared to NC. NC and pSS freshly isolated DN T cells expressed RORγt, activation markers (CD25, CD69, HLA-DR) and produced consistent amounts of IL-17. Despite IL-6/TGFβ ratio became abnormal in pSS patients after 72 hour-culture, Dex was able to down-regulate IL-17 in vitro production in NC and pSS $CD4^+$Th17 cells and in NC DN T cells from this time-point on. Surprisingly, IL-17 production by pSS-DN T cells was not affected at all by Dex at any time-point. Dex could also reduce the expression of activation markers on $CD4^+$ cells, but not in pSS and NC-DN T cells. Among DN T cells, those expressing αβTCR were expanded in patients with active pSS compared to those with inactive pSS. DN T cells were present in pSS-MSG infiltrate.

Conclusions To our knowledge, this is the first study identifying and characterising DN T cells in pSS. It shows that DN T cells are