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EXTENDED REPORT

An immunological biomarker to predict MTX response in early RA

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ABSTRACT

Objectives The therapeutic goal for patients with rheumatoid arthritis (RA) is clinical remission. This is best achieved by early diagnosis and appropriate therapeutic intervention. RA is associated with dysregulation of T-cell subsets (naïve, regulatory (Treg) and inflammation-related cells (IRC)) early in the disease. Our aim was to test the hypothesis that T-cell subset quantification can predict the achievement of clinical remission with early treatment in RA.

Methods T-cell subsets were quantified in 108 drug-naïve, early RA patients commencing methotrexate (MTX) or MTX+antitumor necrosis factor (anti-TNF) and in 105 healthy controls (HC). The primary outcome assessed was remission (DAS28<2.6). A pilot study used frozen cells (38 patients and 35 HCs, see online supplementary material) and was validated with fresh blood (70 patients and 70 HCs).

Results Immune dysregulation in early RA was confirmed with an association between age and reduced naïve cells compared with HCs (p=0.006), a lower age-adjusted Treg and higher IRC frequency (p=0.001). Anticitrullinated peptide antibody (ACPA) positivity was associated with lower naïve (p=0.031) and Treg frequencies (p=0.039). In 50 patients treated with MTX, ACPA/age-adjusted analysis demonstrated that higher naïve cell frequency (relative to HC) was associated with remission (OR 5.90 (1.66 to 20.98), p=0.006, sensitivity/specificity 62%/79%, Positive Predictive Value (PPV)/Negative Predictive Value (NPV) 66%/76%). Remission with MTX+anti-TNF (n=20) was not found to be associated with naïve cell frequency, and for patients with reduced naïve cells the remission rate increased from 24% (MTX) to 42% (MTX+anti-TNF).

Conclusions Baseline T-cell subset analysis has a value in predicting early RA remission with first therapy with MTX. Immunological analysis could be used in conjunction with clinical/serological features to predict response to MTX and help select the most appropriate therapy at disease presentation.

Rheumatoid arthritis (RA), one of the most prevalent autoimmune diseases affecting approximately 1% of the population, is a major cause of potentially treatable disability and produces a significant health burden and economic cost.[1] Early diagnosis and appropriate therapeutic intervention produces rapid disease control and improves the functional and structural outcomes.[2–4]

The modern therapeutic approach is to treat early and to aggressively target inflammation with the aim of inducing remission. In a recent study, it was shown that remission at 12 months in early RA can be achieved in 67% of patients (COMET trial) with the combination therapy of a conventional disease-modifying anti-rheumatic drug (DMARD) with an antitumour necrosis factor (anti-TNF) agent.[5] Furthermore, several systematic reviews and current clinical guidelines endorse optimal inflammation suppression in early disease.[6–8]

We have performed a number of studies highlighting the importance of T-cell parameters in early and established RA, recently reviewed.[9] We demonstrated reduced naïve CD4+ T-cell frequency in early RA, due to a combined effect of inflammation on the thymus and the concomitant differentiation of naïve cells into another T-cell subset related to their exposure to systemic inflammation (hence their name: inflammation-related cells (IRC)).[10 11] In early RA, successful withdrawal of TNF-blockade without flare after achieving remission was associated with significantly higher circulating naïve T-cell frequency.[12] Additionally, the frequency of naturally occurring regulatory T-cells (Treg, CD4+ $CD25^{high}$) is reduced in early RA.[13]

The aim of the current study was to examine the relationship between such immunological dysregulation, and the ability of patients to achieve remission in DMARD-naïve, early RA treated with methotrexate (MTX) alone or in combination with anti-TNF. Using frozen peripheral blood mononuclear cells (PBMC), we first tested the hypothesis that disturbance of T-cell subsets could predict remission and determined that the naïve T-cell subset had the best predictive value (the pilot data using frozen samples are presented in online supplementary material). In a second validation study, which also tested the feasibility of using fresh blood samples, we confirmed the pilot data and established the sensitivity and specificity of naïve T-cells as biomarker of response to MTX.

PATIENTS, MATERIAL AND METHODS

Patients and healthy controls

One hundred and eight steroid and DMARD-naïve, early RA patients fulfilling 1987 American College of Rheumatology (ACR) diagnostic criteria were recruited from our Early Arthritis register (demographic and baseline data in table 1). They may,

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Table 1 Early RA cohort description at baseline

Pilot cohort (frozen PBMC)	*Early RA (n=38)*	*HC (n=35)*
Age (years)*	58 (45, 66)	48 (32, 60)
Gender (M/F)	14/24	15/20
Symptom duration (months)*	5 (3, 10)	
RF (pos/neg)	26/12	
ACPA (pos/neg)†	18/16	
CRP (mg/L)*	18 (9, 58)	
TJC*‡	12 (6, 23)	
SJC*‡	10 (5, 15)	
HAQ*‡	11 (6, 13)	
RAQoL*‡	17 (9, 22)	
DAS28*	6.21 (4.81, 6.69)	
Naïve (% CD4T-cells)	4.1 (2.7, 10.0)	11.2 (5.9, 13.9)
IRC (% CD4T-cells)*	29.0 (21.1, 35.5)	2 (0.9, 7.4)
Treg (% CD4T-cells)*	2.6 (1.7, 3.6)	5.4 (4.1, 8.0)
Validation cohort (fresh blood)	*Early RA (n=70)*	*HC (n=70)*
Age (years)	53 (44, 64)	46 (33, 52)
Gender (M/F)	20/50	29/41
Symptom duration (months)§	6 (4, 8)	
RF (pos/neg)	35/35	
ACPA (pos/neg)	53/17	
CRP (mg/L)	8 (0, 18)	
TJC	8 (3, 13)	
SJC	5 (2, 9)	
DAS28	4.51 (3.21, 5.61)	
Naïve (% CD4T-cells)	32.1 (18.9, 45.3)	36.1 (31.1, 46.2)
IRC(% CD4T-cells)	2.5 (1.2, 4.3)	1.3 (0.6, 2.3)
Treg (% CD4T-cells)	2.9 (1.7, 4.1)	4.8 (3.8, 8.2)

*Median (lower, upper quartile).
†Missing data in two patients.
‡Data available only in 28 patients.
§Only 2 of these 70 patients had longer than 12 months symptom duration.
ACPA, anticitrullinated protein antibody; CRP, C-reactive protein; DAS28, disease activity score; HAQ, health assessment questionnaire; HC, healthy controls; IRC, inflammation-related cells; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; RAQoL, RA quality of life questionnaire score; RF, rheumatoid factor; SJC, swollen joint count out of a possible 28; TJC, tender joint count out of a possible 28.

however, have used analgesia and nonsteroidal anti-inflammatory drugs before attending a rheumatology clinic for the first time. Healthy controls (HC) blood samples (n=105) were also obtained from the Yorkshire Blood Transfusion Service and from laboratory volunteers. Ethical approval was obtained from the Leeds Teaching Hospitals NHS Trust Local Research Ethics Committee. Informed consent was obtained from all individuals prior to drawing blood.

In a pilot study using frozen PBMC samples, 38 RA patients and 35 HCs were studied with outcome at 12 months. All patients had <24 months symptom duration. Nine of these RA patients received MTX+anti-TNF[4 14] and were matched with 10 patients receiving MTX for the longitudinal analysis of T-cell subsets over 38 weeks. All data related to this pilot cohort are displayed in online supplementary material.

In a validation study using fresh blood (also testing the feasibility of using flow cytometry for immediate reporting), 70 early RA patient (satisfying both ACR-1987 and EULAR-2010 criteria) and 70 HCs were studied with outcome at 6 months. All patients had <12 months symptom duration with the exception of 2 (14 months and 19 months). All treatments were initiated <1 month of diagnosis. Fifty patients received standard MTX therapy and 20 received MTX+anti-TNF.

DAS28 C-reactive protein (CRP) was used as outcome to classify response, using DAS28<2.6 to define remission. Anticitrullinated peptide antibody (ACPA) status was defined using anti-CCP2 tests.

Cell staining and flow cytometry strategies

In the pilot cohort, peripheral blood (6 mL) was collected into lithium-heparin tubes. PBMC were separated using a LymphoPrep (Nycomed Axis-Shield Diagnostic, Huntington, UK) step gradient and stored in liquid nitrogen for later analysis by flow cytometry (pilot cohort). In the validation cohort, flow cytometry was performed on 6 mL of fresh blood (lithium-heparin) within 2 h of collection, following red cell lysis.

CD4+ T-cell subsets (gated on CD3+ CD4+ expression) were identified based on their expression of CD45RB-FITC (clone MEM-55, Serotec, Oxford, UK), CD45RA-PE (clone F8-11-13, Serotec) and CD62L-APC (clone 145/15 Coulter, High Wycombe, UK) as described previously.[10] Naïve and IRC CD4+ T-cells were identified and quantified as previously described[10–12 15] and as shown in figure 1A (top panels). Treg were quantified by cell surface staining for CD4–Pacific blue (clone RPA-T4, BD, Oxford, UK), CD25-APC (clone 2A3, BD), and CD127-PE (R34.34, Beckman coulter) performed as described above, followed by intracellular staining for FOXP3-FITC (clone PCH101 eBioscience, San Diego, California, USA) according to the manufacturer's instruction using the antihuman Foxp3 staining kit (Insight Biotechnology, Wembley, UK). Quantification of Treg was based on high expression of CD25 ($CD25^{high}$)[16] with the addition of Foxp3+ and $CD127^{low}$[17 18] (figure 1A top panels). Flow cytometry analysis was performed on a LSRII cytometer (BD), using BD Biosciences FACSDIVA software. Subset frequencies were reported as % of gated CD4+ T-cells. Additional details on antibody validation and optimisation of flow cytometry strategies are available in online supplementary material.

Statistics

Continuous measures were compared between groups using Mann–Whitney U tests and nominal measures with χ^2 tests. Linear regression with robust SEs was used to compare naïve cell frequency between RA patients and controls, adjusting for age. To identify potential confounders, associations between variables were assessed using Spearman's correlation; these tests were not corrected for multiplicity. Binary logistic regression models were constructed to verify whether T-cell subsets were associated with remission independently of potential confounders that were shown to have substantive association with either T-cell frequency or remission; where sample size was low (≤20), exact logistic regression was used. Full multivariable analysis was not attempted due to the small sample size. In the validation cohort, the difference between each patient's observed naïve cell level and the level that was predicted by the regression equation for HCs of the same age was calculated; non-parametric receiver operating characteristic (ROC) analysis was then used to investigate different cut-offs when predicting remission at 6 months. Analyses were conducted using Stata V.12.1.

RESULTS

T-cell analysis in early RA: remission and disturbance from health in pilot cohort

T-cell subsets analysis was performed as previously described[10 13] using frozen PBMCs in 38 early, DMARDs naïve RA patients and compared with 35 HCs (figure 1 shows our flow cytometry gating strategies). Overall, results confirmed previously reported data[10 11 13] with significantly lower frequencies of naïve CD4+ T-cells ($p<0.001$) and Treg cells ($p<0.001$), and higher frequencies of IRC ($p<0.001$) in RA compared with HC (data presented

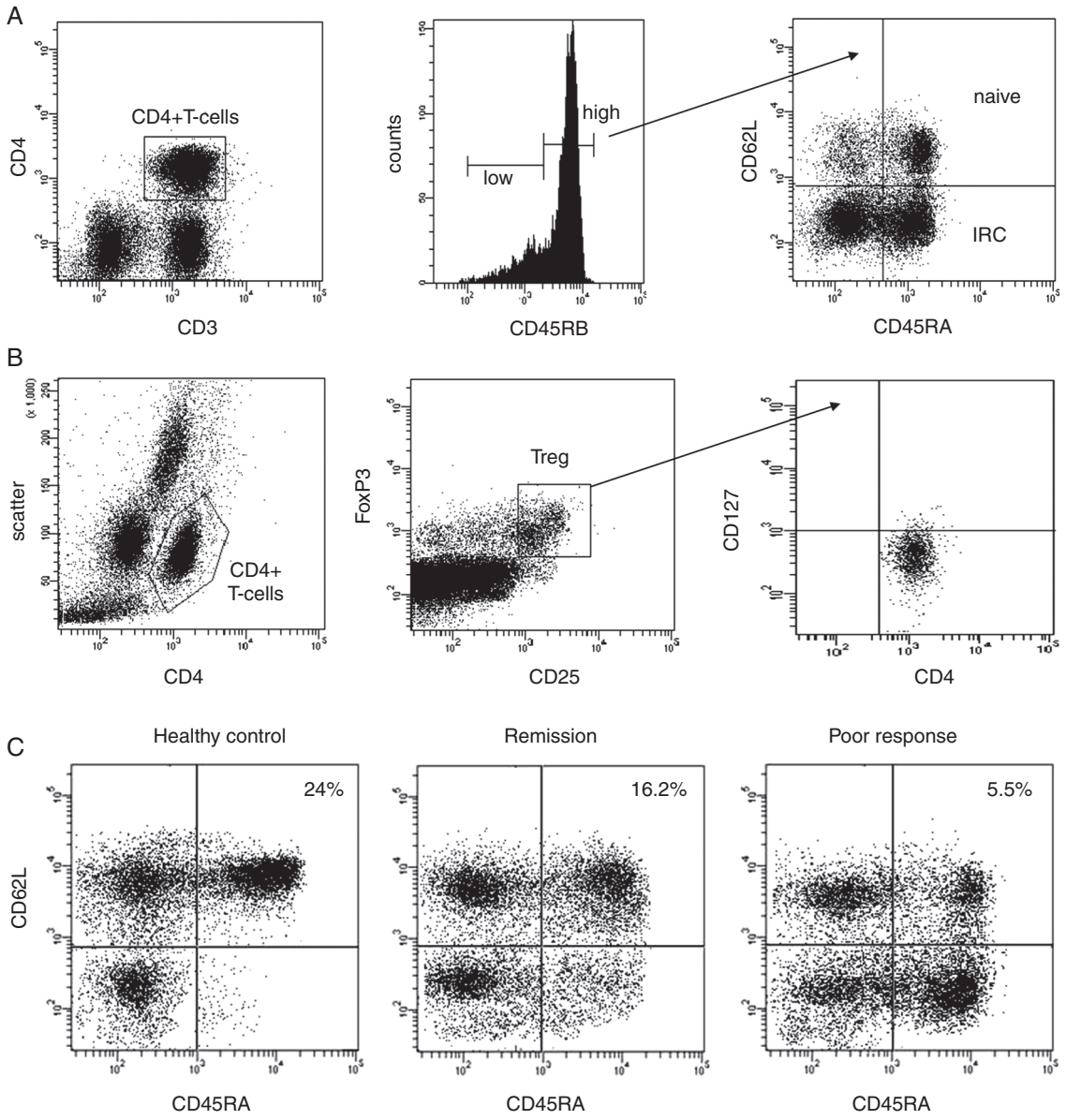

Figure 1 Flow cytometry gating strategies.(A) Representative flow cytometry analysis of naïve / inflammation related cells (IRC) cell frequency and regulatory T-cells. Peripheral blood mononuclear cells were stained for cell surface markers followed by intracellular marker detection. For naïve and IRC cell subsets, CD4+ T-cell were gated using double positivity for CD3/CD4. High expression of CD45RB was used to gate CD4+/CD45RB^{high} cells and the gate applied to dual plot of CD45RA and CD62L. Naïve cell were quantified as CD4+/CD45RB^{high}/ CD45RA+/CD62L+ and IRC as CD4+/CD45RB^{high}/ CD45RA+/CD62L−. (B) For Treg, CD4+ T-cell were gated using scatter and CD4. Treg were quantified using high expression of CD25 and Foxp3. The T-reg phenotype was confirmed using low levels of CD127 expression. Expression of CD62L was further analysed as a proportion of Treg. (C) Representative flow cytometry plot for naïve cell evaluation in a healthy control (left), an early rheumatoid arthritis patient achieving remission (middle) and one with only poor response (right).

in see online supplementary figures and in table S1). Distribution of data in early RA patients for each T-cell subset (see online supplementary figure 1S) suggests patients have a variable disturbance compared with health, except for the regulatory subset where abnormalities appear consistently in all patients.

Achieving remission at 12 months was associated with higher naïve cell frequency (OR 2.23 (1.38 to 4.84), p<0.001) and lower IRC (OR 0.91 (0.83 to 0.97), p=0.005) but not with any clinical parameters.

Validation of our novel flow cytometry strategy using fresh blood samples

Naïve and IRC cells appearing the most interesting subsets, we developed a flowcytometry protocol to analyse subsets within 2 h of blood collection, the lowest naïve cell frequencies approaching the limit of detection due to low cells viability in frozen samples (see full validation of the strategy in online supplementary material).

We analysed 70 HC and 70 early RA patients (table 1). Viability was considerably increased (over 95%), and the numerical ranges of frequencies for naïve were higher. Treg frequency was less affected. The direct relationship between naïve cell frequency and age in HC was maintained (n=70, r=−0.697, p<0.001). The correlation between IRC and CRP previously reported[10] was verified using 13 patients with established RA and higher CRP (data not shown).

As observed in the pilot cohort, in fresh blood from early RA patients, naïve cells (p=0.005) and Treg (p<0.001) frequencies

were lower compared with HC. IRC were significantly increased (p=0.001) however, gender and age ranges were not fully matched between RA and HCs in this group (table 1). Previous studies having showed that naïve cell frequency decreases with age[10 11] we performed an age-adjusted comparison between the two groups (see full details in online supplementary material). We found that while in HCs, naïve cell frequency directly decreased in relation with age (figure 2A), such direct association was lost in RA (figure 2B). Similar data were obtained in the pilot cohort (see online supplementary material). IRC and Treg frequency were not previously related to demographic parameters.

T-cell subsets analysis in early RA

We previously demonstrated an increased IRC frequency in RA as a result of aberrant differentiation of T-cells under the influence of inflammation[10]; however due to low levels of CRP (table 1, median 8 g/mL, range <5–228) the association between IRC frequency and CRP was not verified in the validation cohort (r=0.162, P=0.347), but was observed in the pilot cohort (r=0.362, P=0.007, higher median levels of CRP: 18 mg/L). There was no other parameter associated with IRC (table 2). Treg frequency was not related to age, naïve T-cells or IRC (table 2).

Of the other demographic or clinical parameters, only ACPA positivity was found to be associated with lower naïve cell frequency (table 2, p=0.031) and with lower Treg frequency (p=0.039). Lower Treg frequency was also associated with female gender (p=0.041). We therefore performed an adjusted analysis by log-transforming Treg and IRC, then using linear regression to assess the association between Treg and IRC frequency, and between Treg and naïve cell frequency, while adjusting for gender and ACPA, which did not alter the conclusion that Treg was not associated with either of the other subsets (data not shown).

Altogether in early RA patients, the distribution of data for each T-cell subset in the validation cohort confirmed that some patients, particularly those aged <50, had a more profound disturbance from health with fewer naïve cells and presence of IRC, whereas Treg were consistently lower in all patients. This suggests that disturbances of the naïve and IRC subsets can occur independently of that of the Treg subset.

Table 2 Associations between demographic and clinical variables with T-cell frequencies (validation cohort n=70)

Variables	Naïve T-cell	IRC T-cell	Treg
Gender			
Male	25.0 (18.0, 43.0)	3.0 (1.1, 4.8)	3.7 (3.0, 4.6)
Female	32.1 (22.6, 46.5)	1.9 (0.9, 4.1)	2.4 (1.5, 3.5)
	z=0.98 (p=0.328)	z=−1.30 (p=0.194)	z=−2.04 (p=0.041)
RF			
Positive	27.1 (17.5, 42.3)	2.5 (1.1, 4.3)	2.5 (1.6, 4.2)
Negative	36.0 (23.0, 47.7)	2.2 (0.9, 6.8)	2.8 (1.5, 3.4)
	z=−1.34 (p=0.181)	z=−0.07 (p=0.948)	z=0.16 (p=0.872)
ACPA			
Positive	29.0 (18.6, 40.2)	2.5 (1.1, 4.3)	2.4 (1.6, 3.5)
Negative	42.5 (24.9, 50.5)	1.8 (0.9, 4.9)	3.4 (2.6, 5.2)
	z=−2.15 (p=0.031)	z=0.42 (p=0.678)	z=−2.07 (p=0.039)
Age (years)	−0.09 (p=0.450)	0.22 (p=0.073)	0.19 (p=0.172)
Symptom duration	0.02 (p=0.845)	−0.11 (p=0.378)	−0.07 (p=0.627)
CRP (mg/L)	−0.12 (p=0.314)	0.09 (p=0.474)	−0.07 (p=0.635)
TJC	−0.04 (p=0.761)	0.05 (p=0.675)	−0.17 (p=0.224)
SJC	−0.16 (p=0.192)	0.10 (p=0.425)	0.09 (p=0.520)
DAS28	0.00 (p=0.996)	0.10 (p=0.405)	−0.15 (p=0.290)
Naïve T-cell	NA	0.05 (p=0.657)	−0.05 (p=0.752)
IRC T-cell	0.05 (p=0.657)	NA	0.03 (p=0.828)
Treg	−0.05 (p=0.752)	0.03 (p=0.828)	NA

Between-group comparisons were performed; gender and autoantibody status: median T-cell frequency (lower, upper quartile) is provided for each group followed by the Mann–Whitney U test z-statistic and significance. For all other variables, correlations were performed using Spearman's r and significance is provided.
ACPA, anticitrullinated protein antibody; CRP, C-reactive protein ; IRC, inflammation related cells; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

Remission is only associated with naïve T-cell frequencies but not with demographic or clinical data (validation cohort n=70)

Fifty of these 70 patients were treated with MTX and were evaluated at 6 months for remission status. Twenty-four patients (48%) achieved remission. As seen in the pilot cohort, remission

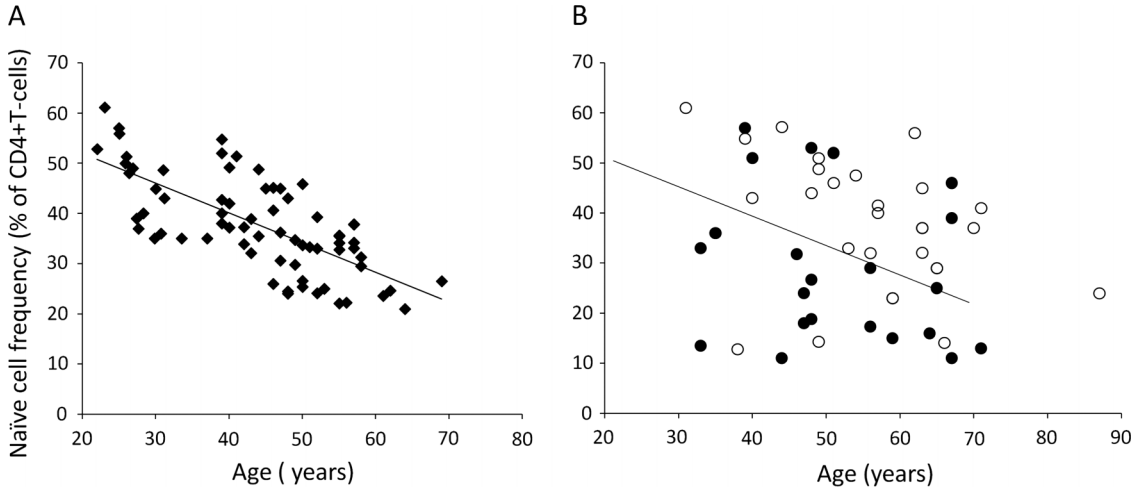

Figure 2 T-cell subset frequency in early rheumatoid arthritis (RA) (validation cohort on methotrexate (MTX), n=50). (A) Association between age and naïve cell frequency measured in fresh blood from 70 healthy controls (naïve cell frequency=63.71−0.59 (age); R^2=0.48, $F_{(1,68)}$=82.19, p<0.001). (B) Naïve cell frequency in relation with age is displayed with respect to outcome at 6 months in 50 early RA patients treated with MTX (open symbols represent patients achieving remission and closed symbols those who did not). The association between age and naïve cell frequency in healthy controls is represented by the line.

Table 3 Association between demographic, clinical and immunological variables and remission status

	MTX (n=50)		MTX+anti-TNF (n=20)	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Age per year	1.01 (0.97 to 1.06)	0.583	1.04 (0.97 to 1.13)	0.309
Female	0.73 (0.22 to 2.40)	0.606	0.31 (0.00 to 4.23)	0.379
Symptom duration/month	1.03 (0.89 to 1.20)	0.681	1.05 (0.80 to 1.38)	0.749
RF positive	1.50 (0.42 to 5.32)	0.530	1.47 (0.19 to 12.40)	1.000
ACPA positive	0.62 (0.19 to 1.99)	0.424	6.07 (0.49 to 353.84)	0.238
CRP per mg/L	0.99 (0.97 to 1.01)	0.205	1.09 (0.97 to 1.25)	0.160
TJC per joint	0.97 (0.88 to 1.06)	0.518	0.94 (0.83 to 1.05)	0.303
SJC per joint	0.94 (0.85 to 1.05)	0.295	0.99 (0.81 to 1.21)	0.987
DAS28 per unit	0.85 (0.57 to 1.27)	0.418	0.83 (0.44 to 1.53)	0.557
Naïve				
Unadjusted	1.04 (1.00 to 1.09)	0.045	1.00 (0.94 to 1.07)	0.983
Adjusted for age/ACPA	1.05 (1.00 to 1.09)	0.042	1.02 (0.94 to 1.11)	0.660
IRC	0.99 (0.90 to 1.09)	0.812	0.58 (0.27 to 0.98)	0.038
Treg				
Unadjusted	1.05 (0.85 to 1.31)	0.635	1.66 (0.53 to 6.18)	0.418
Adjusted for gender/ACPA	1.02 (0.81 to 1.28)	0.883	1.00 (0.00 to 58.59)	1.000

Results of binary maximum likelihood (MTX) or exact (MTX+anti-TNF) binary logistic regression models. All results are unadjusted unless otherwise stated.
ACPA, anticitrullinated protein antibody; anti-TNF, antitumour necrosis factor; CRP, C-reactive protein ; IRC, inflammation related cells; MTX, methotrexate; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

itself was not associated with any demographic, clinical or immunological parameters, except from higher naïve T-cell frequency (table 3, p=0.045) which, furthermore, remained associated with remission independently of age and ACPA by logistic regression (p=0.042).

The 20 remaining patients were treated with MTX+anti-TNF and 9 (45%) achieved remission. Power to detect associations was low in this small group and adjusted analyses could not be performed; the unadjusted odds were largely consistent with those calculated in the group treated with MTX (table 3), with the possible exception that patients with higher IRC levels were less likely to respond, although this finding would need to be verified in a larger cohort. The results suggest that ACPA-positive patients may have been more likely to respond to anti-TNF (OR 6.07), however, the CI was wide and the association was not statistically significant.

Using naïve T-cells frequency to predict response on an individual patient basis (validation cohort)

To develop a predictive biomarker based on naïve T-cell subset analysis on an individual basis, we used the association between naïve T-cell frequency and age in HCs (figure 2A, n=70, naïve=63.71−0.59 (age); R^2=0.48, $F_{(1,68)}$=82.19, p<0.001) to characterise each patient's naïve cell frequency relative to that expected for a HC of their age. The differences between the expected and observed values were calculated in the 70 patients with early RA from the validation cohort.

In MTX-treated patients (n=50), the area under the ROC curve when using deviation from expected naïve frequency to predict remission was 0.68 (95% CI 0.53 to 0.84). Using a convenient cut-off of zero, that is, the point at which naïve cell frequency matched that expected in HC, which was close to the cut-off which concurrently maximised sensitivity and specificity (see online supplementary material), 29 patients demonstrated higher naïve T-cell levels and 21 lower frequencies (figure 2B). Sensitivity was 79% (19/24; 57% to 92%) and specificity 62% (16/26; 41% to 79%). The PPV was 66% (19/29; 46% to 81%) and the NPV 76% (16/21; 52% to 91%). In all, 72% of the patients were correctly classified overall in terms of remission status at 6 months (figure 3). This association was observed consistently across different age groups (sensitivity and specificity ages 31–40 years: 75% and 60%|41–50: 80% and 86%|51–60: 83% and 67%) with the exception of patients aged 61 years and over, in whom sensitivity remained high at 79% but specificity was reduced to 38%.

There was clear association between having higher naïve T-cells on an individual age-corrected basis and ability to achieve remission (unadjusted OR 6.08 (1.72 to 21.50), p=0.005) which remained significant when adjusting also for ACPA status (ACPA adjusted OR 5.90 (1.66 to 20.98), p=0.006).

Short duration is generally an important determinant of the ability to achieve remission, [5] however, this was not replicated

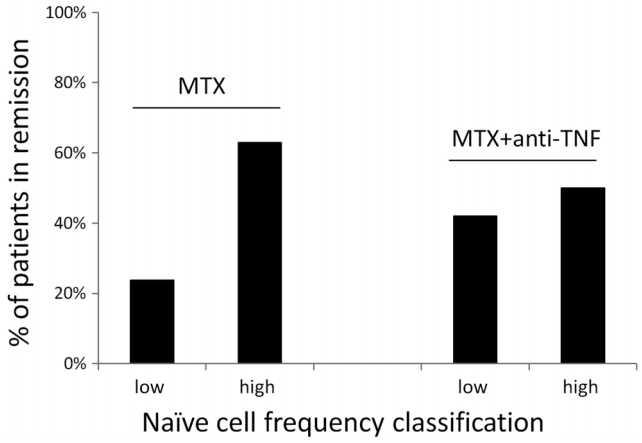

Figure 3 Proportion of patients achieving remission according to naïve cell status. Percentage of patients achieving remission (%) from the methotrexate (MTX) (n=50) or MTX+ anti-tumour necrosis factor (n=20) groups at 6 months in relation to each individual's frequency of naïve cells relative to that expected in healthy controls (high=higher frequency and low=lower frequency).

in this particular group (table 3). We investigated whether patients with <3 months symptom duration (n=10) were different from patients with longer symptoms (n=40), despite the fact that the reduction of naïve cell frequency was not associated with longer symptom (as reported in table 2, p=0.845). The two groups (<3 and >3 months) had the similar frequency of patients with age/corrected reduced naïve T cells (4/10 (40%) in <3 months and 17/40 (42.5%) in the >3 months). Patients achieving remission despite reduced naïve cell frequency was 2/4 (50%) in the <3 months but 4/17 (24%) in the >3 months. Patients achieving remission with normal naïve cell frequency was 5/6 (83%) in <3 months and 14/23 (61%) in the >3 month groups. Therefore, despite the low number of patients in the <3 months group, using naïve T-cell subset as biomarker appears to have value in both very early and early RA.

In the 20 patients treated with MTX+anti-TNF, no association was detected between remission and higher levels of naïve T-cells (unadjusted OR=1.40 (0.23 to 8.46), p=0.714), however, 41.7% (5/12) of patients from the lower naïve cell frequency group achieved remission compared with 23.8% (5/21) from the same group treated with MTX, again suggesting that combination therapy can overcome greater immune dysregulation.

In a subgroup of 19 patients from the pilot cohort followed longitudinally,[4 14] patients who achieved remission when treated with MTX+anti-TNF (n=9) showed an increase in naïve T-cells over time (from mean 6.9±2.9% at baseline to 11±2% at week 38, see online supplementary figure S2), whereas patients on MTX only (n=10) had a minimal increase (from 5.1±2.9% to 5.5±2.9%, respectively).

DISCUSSION

In the modern era, the goal of therapy for patients with early RA is remission, with biologic therapies producing the highest rate.[4 5] Predicting the response to MTX is, however, an important clinical issue, as identifying which patients will do well on MTX would allow expensive therapy to be avoided, while the ability to predict non-response to MTX would avoid a period of harmful inflammation and enable early alternative treatment. A recent review[8] suggested multiple predictors of remission with DMARDs including: male sex,[19–21] shorter symptom duration[22 23] absence of autoantibodies (Rheumatoid Factor and ACPA[24–26]), clearly defining a critical window of opportunity for qualitatively and quantitatively superior response.[26–28]

The current study examined whether T-cell phenotyping could predict remission in DMARD-naïve early RA treated with MTX. We used a pilot cohort (frozen PB<C samples) to test the hypothesis that a disturbance of the T-cell subset could be used to predict remission at the population level, and found that reduced naïve T-cell numbers were most predictive. However, a limitation of frozen samples is the lower viability of PBMC. Therefore, a second validation study using fresh blood was undertaken. Remission with MTX therapy was achieved in 48% of patients with no clinical features predictive of the attainment of remission (table 3). This may be partly explained by small numbers (n=50). Notably, 66% of patients with a higher naïve T-cell frequency (measured on an individual basis) achieved remission compared with only 24% with a lower naïve T-cell level achieved this state (figure 3).

Immunological disturbances in RA patients have long been known and notably associated with abnormal T-cell function.[29–35] Our previous work over the past 10 years[10 12 13 15 36] has highlighted that such perturbations of T-cell differentiation (fewer naïve and regulatory T-cells, appearance of IRC) occur early during the disease progression.[10] However, contrasting with early RA, in established disease we have previously shown that the disturbance of naïve T-cell subsets was more consistent[10] although higher levels were seen in remission compared with active disease.[10 12 15] These data suggest that naïve T-cell frequency could be important in determining clinical responses in early disease.

In 50 MTX-treated patients, a reasonable PPV (66%) of individual naïve cell status for remission was observed; importantly, no other clinical parameter was able to predict remission in this group. The NPV was higher at 76% which, importantly, suggests that it is possible to predict at baseline which patients are unlikely to achieve the goal of remission with MTX monotherapy. This group of patients was not different from our general early RA register population. While this study will need replication, these biomarkers are now part of our routine research strategy of early disease patients, and more insight into their usefulness should be available in the future. While flow cytometry is used routinely for hospital tests, the development and validation of a naïve T-cell subset quantification test will require optimisation before it becomes routine.

The association between remission and higher naïve T-cells in patients treated with combination therapy (MTX+AntiTNF) was not observed and high naïve cells showed equal remission rate (4/8, 50%) compared with those who did not, however, the small sample size (n=20) must be taken into consideration, and this preliminary conclusion needs confirmation.

In conclusion, despite small patient numbers, the present data suggest for the first time that measurement of naïve T-cells at presentation in RA may help predict (lack of) response to MTX and potentially guide choice of therapy. The study of T-cell subsets could therefore be included in the assessment of patients presenting with new onset RA.

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An immunological biomarker to predict MTX response in early RA

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Notes