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ORIGINAL ARTICLE

Evaluation of cytokine profiles in rheumatoid arthritis patients with clinically active disease and normal inflammatory indices

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Abstract

Objective To assess the potential utility of a cytokine measurement in rheumatoid arthritis (RA) patients with active joint disease but normal acute phase reactants (APR).

Methods RA patients in a longitudinal observational registry with available cytokine array data were included. Patients were categorized based on agreement/disagreement of physical examination and APR measurements: concordant high (CH) [high tender and/or swollen joint counts (TJC + SJC > 3) and APR (ESR ≥ 28 mm/h + CRP ≥ 1.5 mg/L)]; concordant low (CL) [TJC + SJC ≤ 3 and normal APR]. Discordant (D) [TJC + SJC > 3 and normal APR] patients were stratified into low, medium, and high-disease activity (DL, DM, DH). Weighted-average and log-transformed cytokine scores were calculated based on results of a cytokine array. Chi-square tests compared categorical variables by concordance status; *t* tests, Wilcoxon rank-sum tests, ANOVA models, and ordinary least squares (OLS) regressions were used to compare continuous measures.

Results RA patients ($n = 1467$) were predominantly male (91%). Compared to CH patients ($n = 174$), D ($n = 434$) were younger, less frequently seropositive, with lower TJC, SJC, and DAS28-3v scores ($p < 0.001$). Cytokine scores for DL, DM, and DH groups were lower than CH patients ($p < 0.001$) and did not differ between DL, DM, and DH subgroups and were similar to CL ($n = 356$) patients. In multivariable analyses including CH and D patients, log-cytokine score was associated with higher DAS28-3v scores ($p = 0.029$). In multivariable analyses including CL patients, concordance status ($p = 0.011$) and ACPA ($p = 0.013$) were predictors of higher log cytokine score.

Conclusion In this study, cytokine scores did not identify active joint disease in RA patients with normal APR.

Keywords Biomarkers · Cytokine profiles · Disease activity · Rheumatoid arthritis

Key message

- RA patients with clinical evidence of inflammatory arthritis but normal APR are more often younger, seronegative, and had lower TJC, SJC counts, and DAS28-3v scores.
- A multiplex cytokine array, measured at a single time point, did not accurately identify active inflammation in patients with discordant RA disease activity measures

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Introduction

Despite the plethora of therapeutic options and treatment algorithms currently available for rheumatoid arthritis (RA), the accurate prediction of optimum treatment selection, course of disease, and mortality risk for individual patients remains elusive. Both RA clinical parameters and acute phase reactants (APR) provide the core dataset of composite disease activity measures that often guide therapeutic decisions [1, 2]. The expected pairing of adverse disease outcomes and radiographic progression with high inflammatory status at disease presentation mandates vigilance and adherence to the treat-to-target paradigm. Yet, there are RA disease subsets that do not conform to this paradigm, such as radiographic progression in the presence of disease remission [3]. Various cytokine and chemokines that are more sensitive and specific to immune-mediated and RA disease processes have been identified and a commercial multi-biomarker disease activity

(MBDA) test such as VECTRA DA® are available for use in clinical practice. These panels further define RA patients by either demonstrating concordance with composite disease activity measures, or identify a subset with continuing inflammation despite fulfilling parameters of low disease activity or remission [4]. The utility of these MBDA assessment tools in predicting radiographic progression or the need for continuation of disease-modifying anti-rheumatic drug (DMARD) versus the addition of biologic agents at various stages of RA disease (early vs. late) remains to be defined [5].

The population of patients with active RA, however, is heterogeneous, with a significant subset (as many at 58% in one cohort) demonstrating active synovitis despite having normal APR [6]. Further, this subset is often excluded from clinical trials despite clinical findings of synovial proliferation, limiting guidance in treatment options and responses. To date, there are limited reports of biomarker assays in this discordant RA subset. Therefore, defining this subset by a specific cytokine profile compared to patients with concordant clinical and APR parameters may be useful in assessing RA treatment responses and outcomes. Herein, we compared clinical characteristics and cytokine assays in RA concordant and discordant clinical disease/APR subsets.

Patients and methods

Study participants

Participants were enrollees in the Veterans Affairs Rheumatoid Arthritis (VARA) registry, which has been well described previously [7]. In summary, VARA is a longitudinal, observational study of US veterans with RA, started in 2003 and with participation from rheumatology clinics at VA medical centers within the USA. All patients fulfilled the 1987 American College of Rheumatology RA classification criteria, had disease onset after 18 years of age, with no exclusion criteria, and all provided informed consent. All VARA sites have approval by their respective Institutional Review Boards (DC VAMC IRB #00842), as well as by the VARA Scientific Ethics Advisory Committee.

Patient characteristics

Age, self-reported race/ethnicity, education level, date of RA diagnosis, smoking status (current, former, never), any disease-modifying anti-rheumatic drug (DMARD), prednisone and biologic use, and body mass index (BMI, kg/m^2) were collected at enrollment. Frequency of follow-up visits were at the discretion of the practicing rheumatologist, but for this study, only the enrollment visit was analyzed. At each visit, medications, three-variable Disease Activity Score (DAS28-3v) [8] and the multidimensional health assessment questionnaire [9] (MDHAQ) for function were collected. Anti-cyclic citrullinated peptide antibody (ACPA), rheumatoid factor (RF), and high-sensitivity C-reactive protein (hsCRP) were measured from banked enrollment serum.

In instances where values of erythrocyte sedimentation rate (ESR), hsCRP, tender joint count (TJC), swollen joint count (SJC), DAS28-3v, and/or prednisone use were missing at the date nearest enrollment, imputation of the most proximate non-missing value was performed ($n = 293$ had at least one value imputed). There was no difference in tender or swollen joint counts between subjects who had disease activity markers (ESR/CRP) imputed versus those that were not.

Boolean ACR/EULAR remission criteria of RA disease activity require ≤ 1 TJC and ≤ 1 SJC to meet definitions of remission [10]. Alternatively, a SDAI (simple disease activity index) score of < 3.3 also satisfies criteria for disease remission. Thus, a TJC/SJC of > 3, even with normal APR, results in evidence of disease activity based on either of these criteria. Hence, patients with high joint counts (TJC + SJC > 3) and APR (ESR ≥ 28 mm/h + CRP ≥ 1.5 mg/L) were categorized as concordant high (CH) and those with TJC + SJC ≤ 3 and normal APR (ESR < 28 mm/h + CRP < 1.5 mg/L) were concordant low (CL). Patients who had TJC + SJC > 3, but with ESR < 28 mm/h and CRP < 1.5 mg/L were categorized as discordant (D). Discordant patients were further stratified into low, medium, and high disease activity (DL, DM, DH), based on TJC + SJC counts of 4–5, 6–8, and 9 or higher, respectively. Patients with TJC + SJC < 3 with high APR were excluded due to the inability to adequately control for confounders, such as infection or other inflammatory disease, and in clinical practice, will unlikely lead to change in DMARD therapies.

Cytokine and chemokine assays

Cytokines and chemokines were measured from banked enrollment serum using a Bio-Plex Pro™ Human Cytokine 17-plex Assay (Bio-Rad) run on a Luminex 200 system. Seventeen analytes were examined: Interleukin (IL)-1β, IL-2, IL-4,IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon gamma (IFN-γ), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1β, and tumor necrosis factor (TNF)-α. Cytokines and chemokines were analyzed as a composite measure using a previously derived cytokine score [7] .The cytokine score was developed as a measure of overall inflammation and is calculated from the log-transformed, normalized, and weighted summation of individual cytokines with the following formula:

$$\sum_{i=1}^{17} B_i \frac{\log(C_i)}{\mathrm{SD}(C_i)}$$

(where B_i are regression coefficients and the C values represent the individual cytokines). Data processing was performed with Bio-Plex Manager software and analyte concentrations were interpolated from standard curves. To create an outcome variable that approximated a normal distribution, the natural log of cytokine scores rather than the original values were used for all analyses. The cytokine assays were optimized for RF status.

Statistical analyses

Baseline characteristics, clinical measures, and baseline drug use were compared between CH and D patients using chi-square tests for categorical measures and t tests and Wilcoxon rank sum tests, as appropriate, for continuous measures. Log cytokine scores were compared between CH, CL, and D groups and also between CH, CL, and the DL, DM, and DH groups using ANOVA models with post-hoc pairwise comparisons and Scheffe's method to adjust for multiple comparisons. An ordinary least squares (OLS) regression model was used to compare log cytokine scores between CH and D patients while controlling for age, body mass index, RF and ACPA positivity, and DAS28-3v score. A second OLS regression model was used to compare concordance status with the same control variables as the first, but which included CL patients in addition to CH and D patients.

Results

Participants and characteristics

There were 1467 patients in VARA with cytokine and chemokine data and an outpatient visit between 60 days prior to enrollment date and the end of data collection (January 1, 2015). The cohort was primarily male, with mean age and disease duration of 64 and 12 years, respectively (Table 1). Eighty percent reported either former or current tobacco exposure. A majority were seropositive for either RF or ACPA (80% and 77% respectively) and the cohort, on average, had moderate disease activity [mean (SD) DAS28-3v = 3.7 (1.4)]. Sixty percent of patients recorded TJC + SJC of greater than 3 despite 83% receiving DMARD therapy and 28% receiving a biologic agent. Normal APR values were recorded in 54% of patients.

There were 174 CH patients, 356 CL patients, and 434 D patients. Compared to CH patients, D patients were significantly younger, less frequently seropositive, had lower TJC, SJC, and DAS28-3v scores and were more likely to be on DMARD therapy. However, there were no differences in gender, tobacco use, disease duration, or biologic use between these two subsets (Table 1).

Comparison of cytokine score between concordant high (CH), concordant low (CL), and discordant (D) RA patients

Log cytokine scores were significantly higher in CH patients (CH [mean (SD)] = 2.55 (1.0) vs. CL 1.99 (0.95) and D 2.13 (0.98), $p < 0.001$ for both comparisons). There was no significant difference in log cytokine scores between the CL and D groups ($p = 0.081$), and this suggests that the cytokine scores might parallel APR rather than TJC + SJC count. However, Spearman correlations for log cytokines with ESR (0.121), CRP (0.188), and total swollen and tender joint count (0.168), showed no significant associations. When the CH and CL disease subsets were compared with discordant subsets based on disease activity (SJC + TJC), log cytokine scores were significantly greater in the CH group compared to all levels of discordant disease (DL, DM, DH, $p \leq 0.001$), but were not significantly different in the CL group compared to the DL, DM, and DH groups (Table 2). Furthermore, there were no significant differences in log cytokine scores between the discordant disease subsets.

In a multivariable model comparing CH and D status, age, BMI, RF and ACPA status, and DAS28-3v, the only independent predictor of log cytokine score was the DAS28-3v ($p = 0.029$, Adj. $R^2 = 0.066$). A second multivariable model was also run for comparison which included CL patients (CH, CL, and D). In this second model (Adj. $R^2 = 0.059$) concordance status ($p = 0.011$), and ACPA ($p = 0.013$) were the only significant independent predictor of higher log cytokine scores (Table 3).

When comparing levels of individual cytokines in CH vs. D patients, we found that nearly all were significant (data not shown), with values being notably higher for all cytokines in the CH group, supporting our log cytokine score results.

Discussion

The expanding armamentarium of therapeutic options and the treat-to-target strategy for RA require frequent assessment of disease activity, utilizing clinical, laboratory, and radiographic parameters. Biomarkers that can provide reliable, objective information beyond that provided by traditional APR measures may assist treatment decisions. To this end, we evaluated the utility of a panel of cytokines and chemokines similar to that available commercially, in a subset of RA patients with clinically active joint disease despite normal APR levels. We did not find additional benefit of a multi-biomarker cytokine assay score in delineating various levels of active disease in the presence of normal APR values.

The frequent assessment of disease activity via core and composite measures is the cornerstone of the treat-to-target strategy in RA. Yet, in the absence of objective laboratory

Table 1 Patient characteristics defined by joint count and acute phase reactant

	Mean (SD) or % $N = 1467$	Concordant high TJC + SJC > 3 and ESR > = 28 mm/h, CRP > =1.5 mg/L $N = 174$, mean (SD) or %	Concordant low TJC + SJC < = 3 and ESR < 28 mm/h, CRP < 1.5 mg/L, $N = 356$, mean (SD) or %	Discordant TJC + SJC > 3 and ESR < 28 mm/h, CRP < 1.5 mg/L, $N = 434$, mean (SD) or %	CH v D *p* value	CL v D *p* value
Male	91	93	91	91	0.397	0.999
Age (years)	64 (11)	66 (11)	65 (12)	62 (11)	< 0.001[a]	< 0.001[a]
Disease duration (years)	12 (12)	12 (12)	12 (11)	12 (11)	0.340[b]	0.780[b]
Body mass index, kg/m^2	28 (6)	28 (6)	29 (5)	29 (5)	0.066[a]	0.876[a]
Smoking status (ever)	79	79	76	80	0.768	0.121
RF +	80	91	75	75	< 0.001	0.913
ACPA +	77	86	73	72	< 0.001	0.663
DAS28-3v	3.7 (1.4)	5.5 (1.1)	2.2 (0.7)	3.8 (1.1)	< 0.001[a]	< 0.001[a]
TJC (0–28)	5.3 (6.9)	10.0 (8.0)	0.5 (0.8)	8.1 (7.1)	0.009[b]	N/A[c]
SJC (0–28)	4.3 (5.6)	9.5 (6.9)	0.4 (0.8)	6.1 (5.4)	<0.001[b]	N/A[c]
ESR mm/h[c]	27 (23)	58 (25)	13 (8)	12 (7)	N/A[c]	0.018[b]
CRP mg/dl[c]	1.3 (2.0)	4.3 (3.1)	0.4 (0.3)	0.4 (0.3)	N/A[c]	0.001[b,d]
TJC > 3	42	73	0	71	0.568	N/A[c]
SJC > 3	41	80	0	63	< 0.001	N/A[c]
TJC + SJC > 3	60	100	0	100	N/A[c]	N/A[c]
ESR < 28 mm/h	63	0	100	100	N/A[c]	N/A[c]
CRP < 1.5 mg/dl	75	0	100	100	N/A[c]	N/A[c]
ESR + CRP (< 28 mm/h,< 1.5 mg/dl)	54	0	100	100	N/A[c]	N/A[c]
Prednisone use	43	50	36	44	0.216	0.019
DMARD use	83	78	86	85	0.035	0.868
Biologic use	28	20	31	27	0.059	0.278

TJC tender joint count, *SJC* swollen joint count, *ESR* erythrocyte sedimentation rate, *CRP*, C-reactive protein, *RF* rheumatoid factor, *ACPA* anti-citrullinated protein antibody, *DAS* disease activity score, *DMARD* disease modifying anti-rheumatic drug

[a] t test, [b] rank sum test, [c] these values are same/different between groups by definition, [d] values are higher for D group

Table 2 Associations of concordance status with log cytokine scores in rheumatoid arthritis patients

Group	Group abbreviation	*N*	Cytokine score median (IQR)	Log cytokine score mean (SD)
TJC + SJC > 3, ESR > 28, CRP > 1.5 (concordant high)	CH	174	11 (6–27)	2.5 (1.0)
TJC + SJC ≤ 3, ESR < 28, CRP < 1.5 (concordant low)	CL	356	6 (4–14)	2.0 (1.0)
TJC + SJC ≥ 3, ESR < 28, CRP < 1.5 (discordant)	D	433	7 (4–17)	2.1 (1.0)
JC + SJC > 3–< 6, ESR < 28, CRP < 1.5 (discordant, low activity)	DL	77	6 (4–11)	1.9 (0.8)
TJC + SJC ≥ 6–< 9, ESR < 28, CRP < 1.5 (discordant, moderate activity)	DM	91	6 (4–15)	2.1 (0.9)
TJC + SJC ≥ 9, ESR < 28, CRP < 1.5 (discordant, high activity)	DH	267	7 (4–20)	2.2 (1.0)

Overall ANOVA p values were < 0.001 for two models: the first (M1) compared CH, CL, and D, the second (M2) compared CH, CL, DL, DM, and DH. For M1, significant pairwise associations were CHvD ($p < 0.001$) and CHvCL ($p < 0.001$). For M2, significant pairwise associations were CHvCL ($p < 0.001$), CHvDL ($p < 0.001$), CHvDM ($p = 0.017$), and CHvDH ($p = 0.012$). All other pairwise associations were not statistically significant ($p > 0.050$)

and radiographic parameters, the clinical assessment can be confounded by non-inflammatory conditions such as fibromyalgia and osteoarthritis [11]. In a VARA cohort, a vast majority (90%) failed to meet ACR/EULAR disease remission criteria, and in our study cohort, ~ 30% had discordant clinical and APR measures [12]. Confirming active disease is challenging when synovitis accompanies normal APR and other clinical or laboratory parameters of inflammation are absent. To provide an alternate objective disease measure in clinical care, the commercially available VECTRA DA® assay was formulated. VECTRA DA® is a multi-biomarker disease activity (MBDA) test that utilizes 12 serum protein biomarkers including YKL-40 (human cartilage glycoprotein 39), interleukin-6 (IL-6), leptin, tumor necrosis factor receptor I (TNFRI), vascular endothelial growth factor A, epidermal growth factor, vascular cell adhesion molecule 1, serum amyloid A (SAA), matrix metalloproteinase 1 (MMP-1), MMP-3, resistin, and CRP [13]. Various studies and trials indicate VECTRA DA® to predict radiographic and clinical progression of the disease and thereby guide treatment decisions [14]. While the VECTRA assay was clinically validated in the CAMERA (Computer-Assisted Management in Early Rheumatoid Arthritis) [15] and BeST (Behandel Strategieen) [4] studies and shown to correlate with multiple clinical parameters (DAS28-CRP, DAS28-ESR, SDAI, CDAI, and HAQ), the AMPLE (Abatacept versus Adalimumab Comparison in Biologic-Naive RA Subjects with Background Methotrexate) study did not reveal a similar association with DAS28, CDAI, or RAPID3 parameters, neither was it predictive of radiographic outcomes [16]. Hence, there appears to be a need for additional biomarker studies to provide consistency and guidance in evaluation of inflammatory RA.

Table 3 Multivariable model of Ordinary Least Squares Regression of Log Cytokine Score

Model term	Beta coefficient (SE)	*p* value
Model excluding CL group (Adj. $R^2 = 0.066$)		
Concordant high vs. discordant	0.204 (0.115)	0.077
Age (years)	0.005 (0.004)	0.171
Body mass index	0.009 (0.008)	0.258
RF positivity	0.247 (0.128)	0.053
ACPA positivity	0.203 (0.120)	0.091
DAS28-3v	0.083 (0.038)	0.029
Model including CL group (Adj. $R^2 = 0.059$)		
Concordant high vs. discordant	0.274 (0.108)	0.011
Concordant low vs. discordant	− 0.068 (0.088)	0.440
Age (years)	0.004 (0.003)	0.230
Body mass index	0.006 (0.006)	0.315
RF positivity	0.176 (0.093)	0.058
ACPA positivity	0.222 (0.089)	0.013
DAS28-3v	0.050 (0.033)	0.132

In our predominantly male, seropositive cohort with moderate disease activity, we found that discordant patients had lower cytokine scores than concordant high patients but there was no significant difference in the cytokine score between different levels of clinical disease activity within the discordant subset, implying our cytokine panel had no advantage over APR in delineating discordant patients with active disease. The discrepancy in cytokine assays among these cohorts is unclear, but differences in study populations and degree of seropositivity may be contributing factors. Moreover, a distinct cytokine panel may not capture inflammatory pathology in all clinical disease subsets, differentiate local joint versus systemic processes, or encompass a specific cytokine/chemokine that optimally detects inflammation in discordant cohorts. Our cytokine panel did however parallel active disease in patients that had concordant high joint counts, elevated APR levels, and DAS28-3v scores.

The identification of the discordant RA subset of patients has implications for clinical care. Longitudinal data indicate that discordant RA patients have slower disease progression. In a large US study cohort, RA patients with CDAI > 2.8 but low APR at baseline, had lower MDHAQ and CDAI 1 year

later compared to those with increasing APR values [6], suggesting less aggressive RA therapies may be needed. The utility of imaging modalities with biomarker assays in RA disease subsets remains unclear. In a small study of 52 established RA patients, the correlation of imaging modalities (ultrasound power Doppler, MRI bone edema, and synovitis) with serial VECTRA measurements was not linear, but did, however, verify radiographic progression in those with higher scores [17]. Both long-term follow-up and the utility of imaging, to include discordant RA subsets, are needed to further stratify outcomes.

We recognize that our study had limitations. Despite the large RA cohort, it was comprised of predominantly male smokers, which could limit the general applicability of our results. Additionally, our study was cross-sectional and lacked prospective, serial measurements and comparisons of the clinical, laboratory, radiographic, and cytokine scores that may have yielded greater insight. Ideally, cytokine assays are most accurate if measured at consistent time points and with multiple sampling, and the specific action of each best studied in tissue culture stimulation and deposition with mRNA [18], but are all impractical in the clinic setting. We also did not control for cancer as a potential confounder. Although the log cytokine score we utilized has not yet fully been validated in RA patients, it has been utilized in two previously published studies [19, 20]. However, our study included core data components (TJC, SJC, and APR) that provide useful clinical scenarios in daily practice where uncertainty arises regarding RA treatment adjustments. Our findings suggest the need for more reliable and valid cytokine assays to help identify all subsets of RA patients that need treatment adjustments, whether escalation or de-escalation of therapies with regards to clinical remission.

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Compliance with ethical standards

Disclosures None.

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