Serum IL-7 as diagnostic biomarker for rheumatoid arthritis, validation with the EULAR 2010 classification criteria

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Abstract

Objective

Despite the well-established value of currently used classification criteria for the early diagnosis of rheumatoid arthritis (RA) there is a constant demand for novel biomarkers notably in autoantibody-negative patients. Interleukin 7 (IL-7) has been reported as a candidate diagnostic biomarker based on ACR-1987 criteria. However, clinical practice has moved to using the EULAR 2010 classification criteria. Therefore, to advance the use of IL-7 alongside the RA biomarker pipeline, we repeated the original study in a new cohort.

Methods

255 patients were recruited. IL-7 was quantified by ELISA. Univariate and regression analyses were used to model RA diagnosis.

Results

123 patients were diagnosed with RA (EULAR 2010) while 132 were classified as non-RA. In univariate analysis, RA was associated with autoantibodies and SE-positivity, higher joint counts, DAS28 (all $p<0.001$) and CRP ($p=0.024$). IL-7 was lower in RA ($p=0.05$). Logistic regression analysis in 227 patients with complete data set confirmed IL-7 was the second best predictive marker ($p=0.035$) following SJC ($p=0.007$) with good model fit (AUROC=0.889). A second model investigated 147 ACPA-negative patients: lower IL7 was the second best predictive marker ($p=0.075$) behind SJC ($p=0.013$).

Conclusion

This study validates our previous results from a UK cohort using EULAR 2010 criteria although the predictive power associated with IL-7 is lower than in the study using ACR 1987 criteria (both French/UK cohorts). IL-7 remains a potential biomarker for ACPA-negative RA although further validation with larger numbers of ACPA-negative patients is still needed notably to translate these results into clinical applicability.

Key words

interleukin-7, diagnostic biomarker, rheumatoid arthritis

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Introduction

RA is a common autoimmune disease that is associated with progressive disability, systemic complications, high premature mortality and a high cost burden for national health systems. Currently, initiation of aggressive therapy is recommended at an early phase as soon as diagnosis is established, followed by dose escalation and guided by assessments of disease activity. Nowadays this "treat-to-target" approach is a realistic goal for the achievement of remission in early RA (1) although the crucial step towards it, remains early diagnosis.

The well-established value of the presence of anti-citrullinated protein antibodies (ACPA) as a diagnostic criteria for RA, has brought about a change in practice as this test is now included in the EULAR 2010 classification criteria. Despite this progress, there is still a demand for novel biomarkers to further improve early diagnosis, notably as the presence of ACPA is only reported in 40–50% of patients attending an early arthritis clinic (EAC) and awaiting diagnosis (2, 3).

Cytokine production is central to the pathogenesis of RA (4). Interleukin-7 (IL-7) has consistently been detected in the synovial fluid and tissue of RA patients in high amounts (5-7). In contrast in the circulation, levels are reduced compared to health and to patients with other forms of inflammatory arthritis (3, 8). Animal models and human studies have provided further evidence that IL-7 is involved in perpetuating inflammation (6, 7, 9) and it is now a well-recognised therapeutic target for several autoimmune diseases (7, 10-12).

IL-7 has been reported as a candidate biomarker for the diagnosis of RA (using the ACR 1987 criteria), differentiating it from other forms of arthritis in patients with <6 months of symptoms duration in a French cohort (3). However, to demonstrate the value of any test and advance the RA biomarker pipeline, multiple studies need to be reported. Here, the issue is complicated due to the change in clinical practice towards using the EULAR 2010 criteria over past 5 years.

The objective of the present study was first, to validate the original findings in a UK population of patients attending the Leeds EAC using ACR 1987 criteria and second, to assess the value of IL-7 in the biomarker pipeline accounting for the change in clinical practice towards the use of EULAR 2010 criteria. Third, we needed to address the potential of IL-7 as a diagnostic biomarker for autoantibody-negative RA.

Methods

Patients

The Inflammatory Arthritis Disease Continuum (IACON) register, in-house longitudinal study run between 2010 and 2016 comprises over 2000 patients of whom 225 were selected for this analysis, on the basis of having early arthritis with <6 months symptoms duration and being naïve for disease-modifying anti-rheumatic drug (DMARD) at inclusion. Demographic and clinical data included: swollen and tender joint counts (SJC, TJC), C-reactive protein (CRP), disease activity score (DAS-28), autoantibody status (ACPA and RF) (Table I). HLA Shared Epitope (HLA-SE) status was collected although with missing data. Patients were followed for 2 years until fulfilling the EULAR 2010 classification criteria for RA or exiting the study when diagnosed with a non-RA type of arthritis (*i.e.* connective tissue disease, fibromyalgia, gout, osteoarthritis (OA), non-inflammatory arthritis, reactive arthritis, psoriatic arthritis, undifferentiated arthritis (UA)) or with self-resolving diseases. Fulfilling the ACR 1987 criteria was recorded over the first 3 years of recruitment (n=197).

To increase the numbers recruited into our study, we included 30 patients enrolled in similar registers (SpARRO and MIROR), of early psoriatic arthritis (PsA) ankylosing spondylitis (AS) or osteoarthritis (OA) patients. Patients with less than 12 months symptoms duration were selected. The clinical data recorded were similar although not identical with JC/CRP/SE and HAQ missing in several cases.

Protocols were approved by the local ethics committee: IACON REC: 09/H1307/98; SpARRO REC 04/Q1205/65 and 03/028; MIROR REC 02/120. All patients gave written consent.

Table I. Characteristics of patients based on EULAR 2010 criteria (n=255).

Variable	non-RA n=132	RA n=123	*p*-value
Age (years)	50 (41-63.3)	60 (47-70.5)	0.0003
ACPA (pos/neg)	6/123	76/47	<0.0001
RF (pos/neg)	9/112	68/55	<0.0001
SE (pos/neg)	41/82	74/45	<0.0001
SJC	1.00 (0.00-3.00)	3.00 (1.00-9.00)	<0.0001
TJC	3.00 (1.00-7.00)	6.00 (2.00-12.00)	0.0012
CRP	5.00 (<5.00-17.55)	11.15 (<5-26.75)	0.0023
DAS28 CRP	3.15 (2.28-3.93)	4.20 (3.13-5.10)	<0.0001
BMI	27.53 (24.30-32.72)	27.64 (23.98-31.31)	0.530
HAQ*	6.00 (2.00-10.00)	9.00(3.50-12.50)	0.044
Smoking (current/never/previous)	22/42/38	28/46/49	0.845
Gender (F/M)	89/43	84/39	0.988
IL-7	18.60 (13.82-26.57)	16.90 (13.95-22.60)	0.108
(Removing 3 outliers)	*18.60 (13.82-26.57)*	*16.80 (13.95-22.60)*	*(0.05)*

Data are median and interquartiler range (IQR). *Data missing in 53 cases.

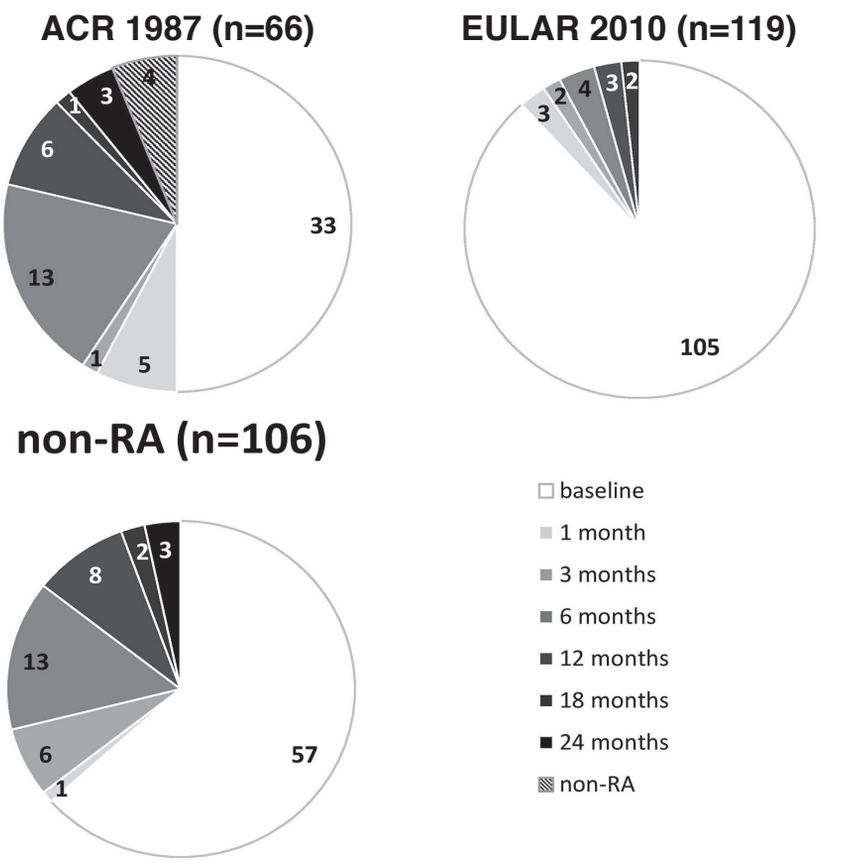

Fig. 1. Time delays to the classification of patients from the early arthritis clinic (EAC). Overall 225 patients were recruited from the EAC (between 2010–2016) and followed up for 24 months. Diagnosis was established at district visits (0, 1, 3, 6, 12 and 24 months).
a) 119/123 patients fulfilled the EULAR 2010 classification criteria for RA (4 missing data for a recorded time of classification);
b) 66 patients (recruited between 2010–2013) were classified as RA using ACR 1987 criteria;
c) 106/132 patients were classified as non-RA (missing data in SpARRO and MIRROR).

IL-7 ELISA

255 serum samples were tested with a high sensitivity commercial ELISA (R&D Systems, Abingdon, UK) according to manufacturer's instructions. The sensitivity of the assay was <0.1 pg/ml.

Statistical analysis

Variables were not normally distributed and therefore non-parametric tests were used throughout. To explore the data, univariate MWU and Chi square tests were used to compare 2 independent diagnostic groups. Covariance was explored using Pearson correlation and Chi square tests. The level of significance for *p*-values was set at 0.05. No adjustment was made for multiple testing in this exploratory phase.

Unadjusted odds ratios (OR) were calculated from cross tabulations for categorical risk factors and from simple logistic regressions for continuous risk factors. Multivariable logistic regression models were built to provide adjusted OR using only variables with an exploratory $p<0.150$ value.

The statistical package R v. 3.2.0 (R core team 2015) was used throughout the analysis.

Results

Over the 6 years of recruitment, practice changed with respect to switching the use of ACR 1987 towards and only recording EULAR 2010 classification criteria (from 2013). When using the new criteria, diagnosis was achieved much more rapidly at inclusion for 88% of RA and 63% of non-RA patients (Fig. 1), while it took over 6 months to achieve similar results using ACR 1987. Four patients classified as RA by EULAR 2010 remained undifferentiated arthritis by ACR 1987. Furthermore, of the ACPA-positive RA patients (n=76, 62% of the RA group), 94% were classified at baseline while for ACPA-negative (n=47), 22% of patients needed more than an additional 3 months to fulfil the EULAR 2010 criteria. These data suggest a need for additional markers for seronegative RA, particularly as this could help differentiate them from other types of seronegative arthritis.

Replication of the ACR 1987 criteria analysis

We first explored the data reproducing our initial analysis using the ACR 1987 criteria (3). Due to the change in clinical practice during the course of this study, diagnosis based on the ACR 1987 criteria was only available in 197 cases (Supplementary material, Table S1). We confirmed the value of IL-7 as a diagnostic biomarker (Table S2, univariate $p=0.056$) in this UK-based population; however with slightly less statistical significance in the regres-

Table II. Regression models for RA patients diagnosed according to EULAR 2010 RA.

	Unadjusted	Adjusted		Adjusted (no autoantibody)	
Covariate	OR (95% CI)	OR (95% CI)	Adjusted *p*-value	OR (95% CI)	Adjusted *p*-value
Age	1.03 (1.01, 1.05)	1.04 (1.01, 1.06)	0.0033	1.03 (1.01, 1.05)	0.0071
ACPA	$8.59x10^8$ ($4.38 x10^7$, $1.51 x10^{10}$)	$7.97x10^8$ ($4.75 x10^7$, $3.02 x10^{11}$)	<0.0001	NA	NA
SJC	1.16 (1.09, 1.25)	1.10 (1.00, 1.23)	0.1044	1.10 (1.01, 1.20)	0.0372
TJC	1.06 (1.02, 1.10)	1.06 (1.00, 1.14)	0.1656	1.02 (0.97, 1.08)	0.6451
CRP	1.02 (1.01, 1.03)	1.01 (1.00, 1.03)	0.2135	1.02 (1.00, 1.03)	0.0171
IL-7	0.97 (0.94, 1.01)	0.95 (0.90, 1.00)	0.0376	0.92 (0.88, 0.97)	0.0006
AUROC		0.9210		0.7520	
Seronegative patients					
Age	1.04 (1.02, 1.07)	1.04 (1.01, 1.07)	0.0050		
SJC	1.19 (1.10, 1.30)	1.09 (0.99, 1.22)	0.0903		
TJC	1.09 (1.04, 1.14)	1.06 (0.99, 1.14)	0.0890		
CRP	1.01 (1.00, 1.03)	1.01 (0.99, 1.03)	0.3128		
IL-7	0.99 (0.95, 1.04)	0.95 (0.90, 1.01)	0.1012		
AUROC		0.7995			

AUROC: area under the receiver operating curve.

sion model (Table S2, p=0.042, AUROC=0.889) compared to the French cohort (3).

Validation with EULAR 2010 criteria

123/255 patients had RA diagnosed using EULAR 2010 classification criteria at follow-up (Table I), while 132 were classified as non-RA (62 persistent UA, 55 other diagnosis and 15 non-persistent inflammation). This cohort was similar to many other such early inflammatory arthritis (IA) cohorts and representative of the patient population attending EACs. Exploring the diagnosis outcome in univariate analysis, RA was highly associated with ACPA, RF and SE positivity (p<0.0001). RA patients also had significantly higher SJC, TJC, DAS28 (all p<0.0001) and CRP (p=0.0023), all variables being well-known for their association with RA and likely biased towards it as contributing to the EULAR 2010 score. Correlations were explored and observed between SE/ACPA/RF (p<0.0001) and not surprisingly between the DAS and its components (p<0.001). IL-7 tended to be lower in RA compared to non-RA (p=0.108) due to 3 outliers. After removal of these data without altering the median/IQR, IL-7 was significantly lower (Table I, p<0.05).

We then used a logistic regression analysis (Table II) including age, IL-7, ACPA (accounting for covariance with RF and SE), and SJC, TJC, CRP (as component of disease activity) and including 227 patients with complete data set (due to lack of JC/CRP in OA patients). IL-7 contributed to the prediction (p=0.036) more than SJC (p=0.104) but less than age (p<0.003), and ACPA (p<0.0001) while CRP and TJC were no longer contributing. The AUROC was 0.9210 suggesting a good model fit.

However, ACPA is overwhelming in this model with an OR of $7.97x10^8$ due to the rare occurrence of ACPA-positivity in the non-RA category. Furthermore, ACPA is also part of the EULAR 2010 classification criteria. We therefore re-analysed the data without the autoantibodies status (ACPA/RF). In this second model, IL-7 was the largest contributor to prediction (p=0.0006), followed by age, SJC and CRP while TJC was non-significant (Table II). AUROC was 0.7520 still representing a good fit.

Because of the revision of criteria to include ACPA, seronegative diseases have become the main area of need for biomarker(s). A third model (Table II, bottom part) was therefore developed investigating only ACPA-negative patients (n=147, complete data set) including again age, IL-7, SJC, TJC and CRP. All variables (but CRP) appeared to contribute with similar small effects, although with a good model fit (AUROC=0.7950).

Discussion

The classification criteria for RA released by the EULAR 2010 task force are now frequently used as a diagnostic tool in patients with recent-onset arthritis (13). Their diagnostic ability in early arthritis has recently been reviewed and was suggested to be sub-optimal. A comparative overview of the criteria performance summarising sensitivities and specificities from different studies was performed (14). 58–91% of patients were identified early, similar to our data with 88% of patients identified at first visit. Importantly, due to the low specificities observed in these studies (47–60%), a large proportion of patients may be misclassified (14, 15), with a significant risk of over-diagnosis that may become an issue if the criteria are used to recommend disease-modifying anti-rheumatic drugs (14).

Our data suggests that IL-7 serum levels are lower in patients with early inflammatory arthritis symptoms pro-

gressing to RA. Our data confirmed the potential value of IL-7 as a diagnostic test, for EAC patients, particularly for those seronegative for ACPA. Using the EULAR 2010 classification criteria, however, the value of IL-7 is less impressive than when using the ACR 1987 criteria (3), but nonetheless reproducing the initial finding in a UK-based cohort. In ACPA-negative patients, IL-7 is still the best second predictive factor for RA using EULAR 2010 (or ACR 1987) similarly to our previous results (3).

Many candidate biomarkers have been proposed for RA over the recent years, including autoantibodies against carbamylated and oxidised peptides (16-18), cytokines (TNF-alpha, IL-6) (4, 19), cellular biomarkers (T-cells, Th17 cells) (20, 21), genetic susceptibility markers notably HLA-DR, PTPN22 and CTLA4 (22, 23), or gene expression signatures for type-I IFN related genes(24). Imaging in seronegative patients has also been proposed (25). None however have undergone the full replication and validation that are required to move candidates biomarkers forward in the biomarker pipeline towards clinical practice, provided they also demonstrate sufficient economic impact for the early diagnosis of RA.

Altogether, IL-7 appears a likely candidate for RA with this replication study in a different population. Studies combining candidates may prove necessary to achieve sufficient sensitivity and specificity to move the field towards a new set of criteria again. Furthermore, reduced levels of IL-7 in serum were observed in patients with other autoimmune diseases such as multiple sclerosis (26), scleroderma (27) or Graves' disease (28) as well as in active inflammatory bowel disease compared to patients who achieved remission (29). Reduced levels of circulating IL-7 (3, 8) in contrast to the joints where levels are high (6, 7) were recently associated with T-cell dysfunction contributing to a vicious cycle perpetuating inflammation(9, 30) and confirming the potential of IL-7 as a therapeutic target (10).

The limitation to our study includes the loss of patient between our initial cohort for the ACR1987 criteria replication study (2010–2013, Fig. 1) as clinical practice changed. Therefore we could not use the full recruitment from our EAC (2010–2016). Furthermore, we limited our group to <6 months symptom duration which may have biased our recruitment towards ACPA+ IA while seronegative milder disease patients may present with >6 months of duration to EAC, as suggested by the SpARRO and MIROR studies.

In conclusion, this study validates previous results using the ACR 1987 criteria as well as the currently used EULAR 2010 classification criteria for RA, however the predictive power associated with IL-7 is lower than in the initial study using ACR 1987 criteria (3). Importantly IL-7 remains an interesting candidate for ACPA-negative RA, a group of patients that still need biomarkers although further international validation with larger numbers may still be needed to further advance the biomarker pipeline for this particular group.

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