RHEUMATOLOGY

Original article

Elevated free secretory component in early rheumatoid arthritis and prior to arthritis development in patients at increased risk

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Abstract

Objectives. Considering growing evidence of mucosal involvement in RA induction, this study investigated circulating free secretory component (SC) in patients with either recent-onset RA or with ACPA and musculoskeletal pain.

Methods. Two prospective cohorts were studied: TIRA-2 comprising 452 recent-onset RA patients with 3 years of clinical and radiological follow-up, and TIRx patients ($n = 104$) with ACPA IgG and musculoskeletal pain followed for 290 weeks (median). Blood donors and three different chronic inflammatory diseases served as controls. Free SC was analysed by sandwich ELISA.

Results. Serum levels of free SC were significantly higher in TIRA-2 patients compared with TIRx and all control groups ($P < 0.01$). Among TIRx patients who subsequently developed arthritis, free SC levels were higher compared with all control groups ($P < 0.05$) except ankylosing spondylitis ($P = 0.74$). In TIRA-2, patients with ACPA had higher baseline levels of free SC compared with ACPA negative patients ($P < 0.001$). Free SC status at baseline did not predict radiographic joint damage or disease activity over time. In TIRx, elevated free SC at baseline trendwise associated with arthritis development during follow-up ($P = 0.066$) but this disappeared when adjusting for confounders ($P = 0.72$). Cigarette smoking was associated with higher levels of free SC in both cohorts.

Conclusion. Serum free SC levels are increased in recent-onset RA compared with other inflammatory diseases, and associate with ACPA and smoking. Free SC is elevated before arthritis development among ACPA positive patients with musculoskeletal pain, but does not predict arthritis development. These findings support mucosal engagement in RA development.

Key words: rheumatoid arthritis (RA), free secretory component, cyclic citrullinated peptide antibodies (ACPA), musculoskeletal pain, clinical progress

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Rheumatology key messages

- Free secretory component in serum is elevated in early RA and correlates with ACPA levels.
- Free secretory component is elevated before arthritis development in ACPA positive patients with musculoskeletal pain.
- The findings support a link between circulating ACPA and mucosal involvement in RA development.

Introduction

The triggers of RA development remain unknown, but both genetic and environmental factors are involved in a complex interplay [1]. ACPA, which are highly specific for RA, are often elevated in the circulation before patients show clinical symptoms such as arthralgia or swollen joints [2]. Further, even after the onset of arthralgia, there are no significant alterations in synovial histology [3]. Together, this has brought forward the hypothesis that early stages of RA development occur outside the

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joints and that mucosal immunity might contribute to RA induction [4]. ACPA IgG is analysed in routine clinical practice, but ACPA IgA also occur in a subpopulation of ACPA IgG positive patients [5]. We have shown that recent-onset RA patients positive for ACPA IgA and ACPA IgG at baseline suffer from a more severe disease course compared with patients testing positive only for ACPA IgG [5, 6]. In first degree relatives it has been demonstrated that circulating ACPA IgA is more frequently present compared with ACPA IgG, which instead are more frequent in established RA [7]. In addition, IgA plasmablast levels are elevated among subjects at risk for RA development [8]. These findings support that early RA might be associated with mucosal immunity.

Secretory IgA (SIgA) is formed at mucosal sites where dimeric or polymeric IgA, linked by a joining chain, are complexed with secretory component (SC) [9, 10]. Recently, we presented evidence that SC-containing ACPA (SC-ACPA) can be detected not only in mucosal fluids, but also in serum samples from recent-onset RA patients [11, 12]. SC is synthesized by epithelial cells [13] and transported to the cell membrane where it becomes available for binding to IgA or shedding in free form [14]. SC is considered to support stability and to reduce proteolytic cleavage of the SIgA molecule [9]. *In vitro*, free SC can act both anti-inflammatorily by inhibiting neutrophil migration [13, 15], and pro-inflammatorily as free SC can fix the complement fragment C3b and enhance local immune responses [16].

The present study was performed to investigate circulating free SC in relation to autoantibodies, smoking and disease progression in recent-onset RA and ACPA positive patients with musculoskeletal pain.

Methods

Subjects

Two observational cohorts formed the basis of this study. The TIRA-2 cohort (Swedish acronym for 'Timely Intervention in RA') comprises 452 recent-onset RA patients (<12 months since first observed joint-swelling according to the patient's judgement) recruited 2006–2009 in southeast and mid-Sweden, fulfilling either $\geqslant$4/7 ACR 1987 revised RA-classification criteria (ACR-87) [17] ($n = 429$), or morning stiffness $\geqslant$60 min, symmetrical arthritis, and arthritis of hands (wrists, metacarpophalangeal or proximal interphalangeal joints) or feet (metatarsophalangeal joints) ($n = 23$). Follow-up procedures in TIRA-2 have been detailed previously [12, 18].

The TIRx cohort (Swedish acronym for 'xtra early rheumatology follow-up') comprises 104 patients enrolled 2010–2013 at the Rheumatology unit of Linköping University Hospital, Linköping, Sweden. Inclusion criteria were seropositive ACPA IgG test in clinical routine; and musculoskeletal pain of any sort and duration, but maximally one palpable synovitis upon clinical examination. Exclusion criteria were previous rheumatic disease, treatment with oral corticosteroids within 6 weeks, or >1 swollen joint. Follow-up visits to a rheumatologist were scheduled at 3, 12, 24 and 36 months, followed by scheduled visits every second year, but patients were instructed to contact the Rheumatology unit without delay in case of increased joint symptoms between visits. In this study, we analysed follow-up data until 1 September 2017, resulting in a median follow-up time of 290 (range 200–368) weeks.

Arthritis was defined upon clinical examination by an experienced rheumatologist (J.C., Å.R.O., T.S., A.K.). At inclusion, 22 (21%) of the TIRx patients had one palpable synovitis, while 82 (79%) had none. Among the patients without arthritis at baseline, 39/82 (48%) developed at least one during follow-up [after median 25 (range 5–307) weeks].

The study protocol was approved by the review board in Linköping, Sweden (decision numbers M168-05, M220-09, 2015/236–32, M75-08/2008, 2010/205–31, M177-07 and 2010/182–32). All participating subjects gave written informed consent.

In both TIRA-2 and TIRx, cigarette smoking habits were assessed either by questionnaire provided by the Epidemiological Investigations of RA Study [19] or by chart review. Patients were classified as ever smokers if they stated at baseline that they were, or had been, smoking cigarettes. There was no minimum duration or quantity in this definition. Those who had been smoking cigarettes within 1 year of inclusion were classified as current smokers, while those who stated no current or previous cigarette smoking were classified as never smokers [19].

Pharmacotherapy was initiated as found appropriate by the treating physician. Neither cohort had received DMARDs prior to baseline serum sampling. Serum samples from inclusion (0 months), 3 months (TIRA-2) and 12 months (TIRx) were analysed after storage at −70°C. Baseline patient characteristics are summarized in Table 1.

One hundred healthy blood donors (mean age = 46 years, 50% women) and three groups of patients with chronic inflammatory diseases [AS, $n = 50$, mean age = 49 years, 32% women; ANCA-associated vasculitis (AAV), $n = 49$, mean age = 67 years, 27% women; and SLE, $n = 70$, mean age = 52 years, 88% women] served as controls. The AS patients fulfilled the modified New York criteria [20] with a disease duration of 15.1 (10.3) years. AAV patients met the 2012 revised international Chapel Hill consensus nomenclature of vasculitis criteria [21] [disease duration of 7.7 (5.6) years]. All SLE patients fulfilled the 2012 SLICC criteria [22] [disease duration of 18.8 (7.5) years].

Free SC analyses

Free SC was analysed using an in-house sandwich ELISA [23]. Serum samples were diluted 1: 25, added in duplicate to microtitre plates pre-coated with 10 µg/ml mouse mAb anti-human free SC (clone 6B3) and incubated overnight at 4°C. Following washing and 90 min at 37°C min incubation with diluted serum samples and subsequent washing, horseradish peroxidase (HRP)-conjugated mouse mAb anti-human SC (clone 5D8), diluted 1: 100, was added to the wells for 60 min at 37°C for detection of

TABLE 1 Baseline characteristics in the cohorts TIRA-2 ($n = 452$) and TIRx ($n = 104$)

	TIRA-2					TIRx		
	Total	ACPA IgG		Smoker		Total	Smoker	
		Positive	Negative	Ever	Never		Ever	Never
Women, n (%)	303 (67)	199 (69)[a]	91 (31)[a]	105 (71)[b]	65 (77)[b]	83 (79)	38 (81)	45 (79)
Age, mean (S.D.), years	59 (14)	58 (14)[c]	61 (15)[c]	58 (11)[b]	51 (15)[b]	52 (15)	55 (13)	50 (16)
Rheumatoid factor positive, n (%)	269 (60)	240 (82)[c]	24 (17)[c]	98 (66)[b]	54 (64)[b]	35 (34)	21 (45)	14 (25)
Swollen joint count, mean (S.D.)	8.1 (5.4)[d]	7.1 (4.6)[e]	10.3 (6.2)[e]	8.2 (5.6)[b]	7.7 (5.0)[b]	0.2 (0.4)	0.2 (0.4)[f]	0.3 (0.4)[f]
Anti-CCP IgG positive, n (%)	303 (68)[g]	—	—	103 (69)[b]	52 (61)[b]	104 (100)	47 (45)	57 (55)
Anti-CCP SC positive, n (%)	81 (19)[h]	81 (28)[c]	0[c]	38 (26)[b]	12 (14)[b]			
DAS28, mean (S.D.)	5.1 (1.3)[l]	5.0 (1.2)[j]	5.3 (1.3)[j]	5.1 (1.1)[k]	5.0 (1.2)[k]			
HAQ, mean (S.D.)	1.0 (0.6)[l]	1.0 (0.6)[m]	1.0 (0.6)[m]	1.0 (0.6)[n]	1.0 (0.6)[n]			
Larsen score, 36 months progression, mean (S.D.)	2.0 (3.7)[o]	2.4 (4.1)[p]	1.3 (2.8)[p]	1.7 (3.2)[q]	2.7 (4.9)[q]			
Larsen score at month 36, mean (S.D.)	4.9 (5.9)[r]	5.3 (5.6)[s]	4.0 (6.5)[s]	4.5 (5.4)[t]	5.2 (7.6)[t]			
Oral corticosteroids, n (%)	282 (63)[u]	182 (62)[c]	93 (64)[c]	93 (62)[b]	48 (57)[b]			
csDMARD monotherapy, n (%)	384 (85)[u]	252 (86)[c]	122 (84)[c]	128 (86)[b]	70 (82)[b]			
csDMARD combination therapy, n (%)	31 (7)[u]	19 (6.5)[c]	11 (7.6)[c]	11 (7.4)[b]	8 (9.4)[b]			
bDMARD therapy, n (%)	1 (0.2)[u]	1 (0.3)[c]	—	—	—			

[a]Data available from 290 patients. [b]Data available from 234 patients. [c]Data available from 438 patients. [d]Data available from 442 patients. [e]Data available from 429 patients. [f]Data available from 16 patients. [g]Data available from 444 patients. [h]Data available from 439 patients. [i]Data available from 419 patients. [j]Data available from 407 patients. [k]Data available from 216 patients. [l]Data available from 398 patients. [m]Data available from 386 patients. [n]Data available from 215 patients. [o]Data available from 250 patients. [p]Data available from 248 patients. [q]Data available from 145 patients. [r]Data available from 261 patients. [s]Data available from 258 patients. [t]Data available from 149 patients. [u]Data available from 446 patients. Due to missing serum samples, radiographs, or clinical information, some variables have incomplete data. bDMARD: biological DMARD; csDMARD: conventional synthetic DMARD; SC: secretory component.

free SC. Tetramethylbenzidine (TMB; Merck, Darmstadt, Germany) was added as the substrate for an incubation of 10 min, and the reaction was stopped with 1 N H_2SO_4 and read at 450 nm (TECAN Sunrise, Tecan Nordic AB, Mölndal, Sweden). A seven-step serial diluted standard curve was used to calculate the concentrations. Cut-off for positivity was set at the 99th percentile of the 100 healthy controls (32 ng/ml). Analyses were conducted to verify the specific detection of free SC and not Ig-bound SC. Purified SIgA (Bio-Rad Antibodies, Kidlington, UK) added to pre-coated wells gave rise to an average OD_{450} of 0.298, which was similar to blank controls (OD_{450} of 0.238). Recombinant human free SC (kind gift from Blaise Corthésy, Lausanne, Switzerland [24]) was added to RA samples, positive or negative for free SC, to verify the accuracy of the binding of free SC. The detected concentration of recombinant free SC in spiked samples differed only by 7% from the calculated sum of the serum and recombinant free SC analysed separately. This strongly supports that the assay does not detect secretory immunoglobulins. The intra- and interassay variation was 2% and 9%, respectively.

Autoantibody analyses

All ACPA isotypes were detected by immunoassays using the second generation CCP as antigen. SC-ACPA and ACPA IgM were measured by modifying commercially available anti-CCP ELISA kits (Euro-Diagnostica, Malmö, Sweden) [25]. Serum samples were diluted 1: 25, added to pre-coated CCP microtitre plates and incubated for 60 min at room temperature. Following washing, HRP-conjugated polyclonal goat anti-human SC and IgM antibodies, respectively (Nordic Biosite, Täby, Sweden), were used to detect SC anti-CCP (dilution 1: 2000) and IgM anti-CCP (1: 10 000). Incubation with the secondary Ab for 30 min was followed by addition of TMB and incubation for another 30 min. The reaction was arrested and the plate read at 450 nm (TECAN Sunrise). A seven-step serial dilution was used for standard curve calculations using patient sera with high levels of SC-ACPA and ACPA IgM, respectively. The intra- and interassay variation in the SC-ACPA ELISA was 5% and 10%, respectively. For the ACPA IgM ELISA the intra- and interassay variations were 2% and 17%. Cut-off values were set at the 99th percentile among the healthy controls (SIgA 3089 AU/ml and IgM 6032 AU/ml).

Serum ACPA IgA and IgG were analysed at the accredited Clinical Immunology Laboratory at Linköping University Hospital using an automated fluoroenzyme immunoassay (EliA™Thermo Fisher Scientific, PhaDia AB, Uppsala, Sweden), with identical antigen as with the manually performed SC-ACPA and ACPA IgM (as

described previously [26]). The cut-off level for ACPA IgA test was set at the 99th percentile of healthy blood donors (2 µg/l). For ACPA IgG analyses the manufacturer's cut-off point was used (7 U/ml).

Agglutinating RF tests were performed in a clinical routine setting at each local laboratory associated with the participating rheumatology unit. An interassay variability of ⩽10% was accepted. RF results in the TIRA-2 cohort were reported as positive or negative. In the TIRx cohort, RF was analysed at the Clinical Immunology Laboratory at Linköping University Hospital and the numerical values were reported.

Radiographic analyses

Baseline and 3-year follow-up radiographs of hands and feet were available from 250 TIRA-2 patients. Joint damage was evaluated in chronological order by one experienced reader (M.Z.) according to the Larsen–Dahle method [27]. Grading of joint damage was performed without knowledge of disease characteristics or free SC results.

Statistical analyses

The Mann–Whitney test was used to analyse differences in free SC levels between patients and different groups, and to test differences according to anti-CCP status and smoking. The Wilcoxon signed-rank test was used to analyse free SC and ACPA isotype changes between baseline and follow-up at 3 months. Correlation between free SC levels and/or relative free SC level changes and ACPA isotype, RF, disease activity variables and radiographic damage was analysed using the Spearman test. The relative change in free SC levels in relation to treatment regime was analysed using the Mann–Whitney test. Disease activity measures *vs* free SC status were analysed using a general linear model with repeated measures and radiographic damage *vs* free SC status using the Mann–Whitney test. Cox regression was used to analyse free SC and potential confounders (smoking, ACPA IgG, gender, age and RF) in relation to arthritis development in TIRx. Linear regression was used to analyse free SC levels and confounder (ACPA IgG) in relation to radiographic damage. $P < 0.05$ was considered statistically significant. To reduce the risk of false-positive results, corrections for multiple comparisons were performed according to the method of Bonferroni following two or more comparisons. The intra-assay variation was analysed by dividing the s.d. of three duplicate samples with the mean of the duplicate sample where the average of the three samples gives the variation. The same method was used for interassay variation with the difference that it was performed using three samples repeated at three different occasions. The statistical analyses were performed using SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA).

Results

Free SC in patients and controls

Mean levels of circulating free SC were significantly higher among TIRA-2 patients [34.3 (93.4) ng/ml, mean (s.d.)]

compared with TIRx [16.1 (83.4) ng/ml] as well as with each control group, including healthy blood donors ($P < 0.01$, Fig. 1 with *P*-values in inset table). A trend towards higher levels of free SC was seen in the total TIRx cohort [16.1 (83.4) ng/ml, mean (s.d.)] compared with blood donors [3.5 (8.1) ng/ml, $P = 0.069$], AAV [7.7 (24.5) ng/ml, $P = 0.054$] and SLE patients [5.3 (13.1) ng/ml, $P = 0.068$], but not compared with AS [7.4 (9.1) ng/ml, $P = 0.81$] (Fig. 1).

In TIRA-2, baseline ACPA positive patients displayed higher mean baseline levels of free SC compared with ACPA negative patients ($P < 0.001$, Fig. 2A). Also, a larger proportion of ACPA positive patients tested positive regarding free SC (107 out of 303, 35%) compared with ACPA negative patients (7 out of 134, 5%, $P < 0.001$). Patients classified as ever smokers had higher levels of free SC compared with never smokers, reaching statistical significance in TIRx [29.4 (123) *vs* 5.1 (11.4) ng/ml, mean (s.d.), $P = 0.036$] but did not remain significant following correction for multiple comparisons ($P_{corrected}$ (P_c) = 0.07) and borderline in TIRA-2 [39.2 (87.4) *vs* 18.8 (34.7) ng/ml, mean (s.d.), $P = 0.06$; Fig. 2B]. Furthermore, levels of free SC were significantly higher among current smokers compared with never smokers in both TIRA-2 [64.8 (133) *vs* 18.8 (34.7) ng/ml, mean (s.d.), $P = 0.003$] and TIRx [60.3 (197) *vs* 5.1 (11.4) ng/ml, $P = 0.008$] (Fig. 2B). The outliers observed in the ever smoking subset, with a free SC concentration above 750 ng/ml, were within the average age span and they did not show increased disease activity at baseline compared with the average levels displayed in Table 1.

Free SC vs RA-related autoantibodies

Baseline levels of free SC in TIRA- 2 correlated moderately with baseline levels of SC-ACPA ($\rho = 0.555$,
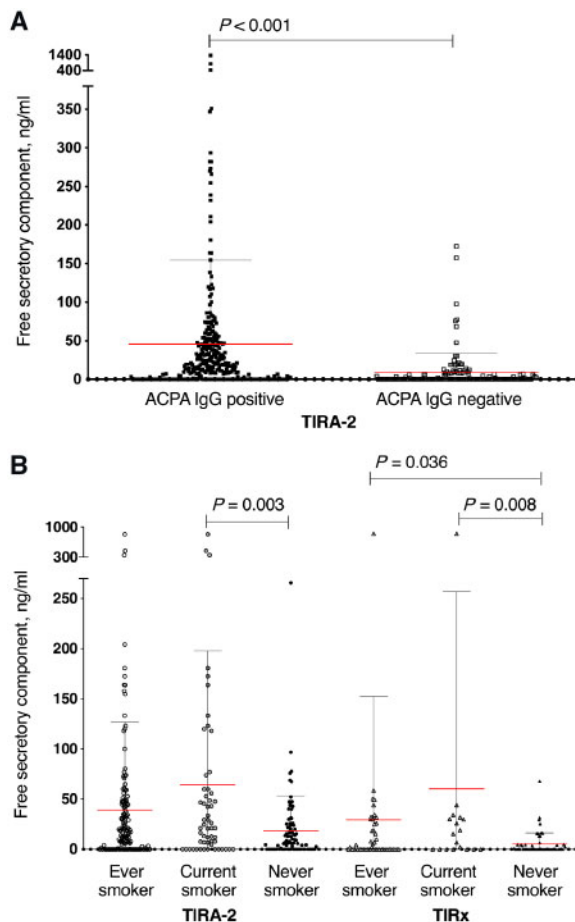
Fig. 1 Serum free SC in recent-onset RA (TIRA-2), ACPA-positive patients with musculoskeletal pain (TIRx) and controls
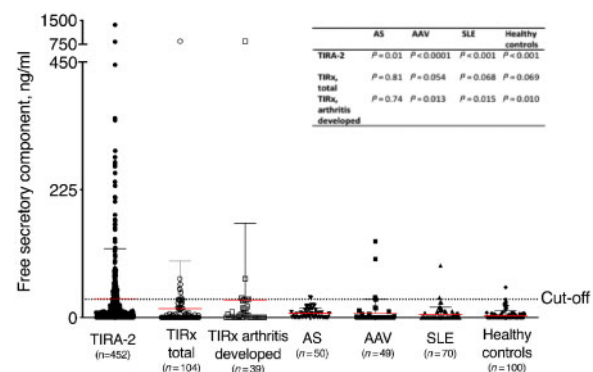
Control groups comprise SLE, AAV, AS and healthy blood donors. Bars denote mean (red) and s.d. (black). Comparisons between the different groups (analysed using Mann–Whitney test) with corresponding *P*-values are displayed in the inset table. AAV: ANCA-associated vasculitis; SC: secretory component.

Fig. 2 Serum free SC in relation to ACPA IgG and smoking

(**A**) Serum levels of free SC according to ACPA IgG status among patients with recent-onset RA (TIRA-2). (**B**) Free SC levels according to smoking status in TIRA-2 and ACPA-positive patients with musculoskeletal pain (TIRx). Differences were tested by Mann–Whitney test. Bars denote mean (red) and s.d. (black). SC: secretory component.

$P < 0.001$), ACPA IgM ($\rho = 0.448$, $P < 0.001$), ACPA IgA ($\rho = 0.412$, $P < 0.001$) and ACPA IgG ($\rho = 0.355$, $P < 0.001$) (Fig. 3). This correlation was sustained when only analysing RF negative TIRA-2 patients for SC-ACPA ($\rho = 0.335$, $P = 0.001$), ACPA IgA ($\rho = 0.205$, $P = 0.039$) and ACPA IgG ($\rho = 0.209$, $P = 0.035$, not shown in figure). In TIRx, baseline levels of free SC did not correlate with ACPA IgG ($\rho = 0.169$, $P = 0.087$), but weakly with levels of RF ($\rho = 0.314$; $P = 0.001$). When stratifying TIRx patients according to presence of arthritis at baseline, we found a significant correlation between ACPA IgG and free SC among patients with one baseline arthritis ($n = 22$; $\rho = 0.431$, $P = 0.045$), but not among patients without ($n = 82$; $\rho = 0.086$, $P = 0.440$). Regarding RF, we found a correlation with free SC among those without baseline

arthritis ($\rho = 0.255$, $P = 0.021$), as well among those with one arthritis at baseline ($\rho = 0.467$, $P = 0.028$) (Supplementary Table S1, available at Rheumatology online). In TIRA-2 patients, levels of free SC and all ACPA isotypes declined significantly between inclusion and month 3 ($P < 0.0001$, Supplementary Fig. S1, available at Rheumatology online). Also, the relative change in free SC levels correlated weakly, but significantly, with the relative change in all ACPA isotypes analysed [SC-ACPA ($\rho = 0.195$, $P = 0.005$), ACPA IgG ($\rho = 0.251$, $P < 0.001$), ACPA IgA ($\rho = 0.166$, $P = 0.017$) and ACPA IgM ($\rho = 0.282$, $P < 0.001$)].

Levels of free SC in relation to treatment

To investigate the influence of anti-rheumatic therapy on free SC levels, we chose to relate changes in free SC levels between baseline and 3 months' follow-up to prescribed treatment in TIRA-2. Regardless of treatment regime, mean levels of circulating SC declined from 34.3 (93.4) to 27.3 (80.1) ng/ml mean (s.d.) ($P < 0.001$) during the initial 3 months in all TIRA-2 subjects. Patients treated with a combination of conventional synthetic DMARDs (csDMARDs, $n = 17$) had a more pronounced relative decline in free SC levels during the initial 3 months compared with those treated with csDMARD monotherapy ($n = 199$; -61% vs -38%; $P = 0.038$; actual levels are illustrated in Supplementary Fig. S2, available at Rheumatology online). There was no significant difference in free SC level change between patients who were treated with oral corticosteroids (-32%, $n = 137$) and those who were not (-16%, $n = 93$; $P = 0.10$, not shown in figure). The relative change in free SC levels was more pronounced among patients treated with SSZ ($n = 22$), given as monotherapy or in combination with other csDMARDs, compared with those who were not ($n = 194$; -66% vs -39%; $P = 0.027$; Supplementary Fig. S2, available at Rheumatology online). However, the statistical significance did not remain following correction for multiple comparisons (combo vs mono: $P_c = 0.15$; and SSZ vs other: $P_c = 0.11$).

In the TIRx cohort, where only 34 (33%) of the patients were prescribed csDMARDs during the first year, free SC levels decreased by 29% if csDMARDs had been prescribed, compared with 11% among those without, but the difference was not statistically significant ($P = 0.97$; Supplementary Fig. S2, available at Rheumatology online). Statistical power was insufficient to investigate specific treatment modalities in TIRx.

Free SC in relation to disease course

In the TIRx cohort, there was no difference in baseline free SC levels between patients with arthritis at baseline and those without [8.4 (15.2) vs 18.1 (93.6) ng/ml, mean (s.d.), $P = 0.91$]. We then compared free SC levels in TIRx patients without signs of arthritis at baseline, but who developed arthritis during follow-up, with all control groups. In this subgroup of TIRx patients ($n = 39$), baseline free SC levels were significantly increased compared with healthy blood donors ($P = 0.010$), AAV ($P = 0.013$) and SLE ($P = 0.015$), but not compared with AS ($P = 0.74$) (Fig. 1).

Fɪɢ. **3** Correlations between baseline levels of free SC and different ACPAs
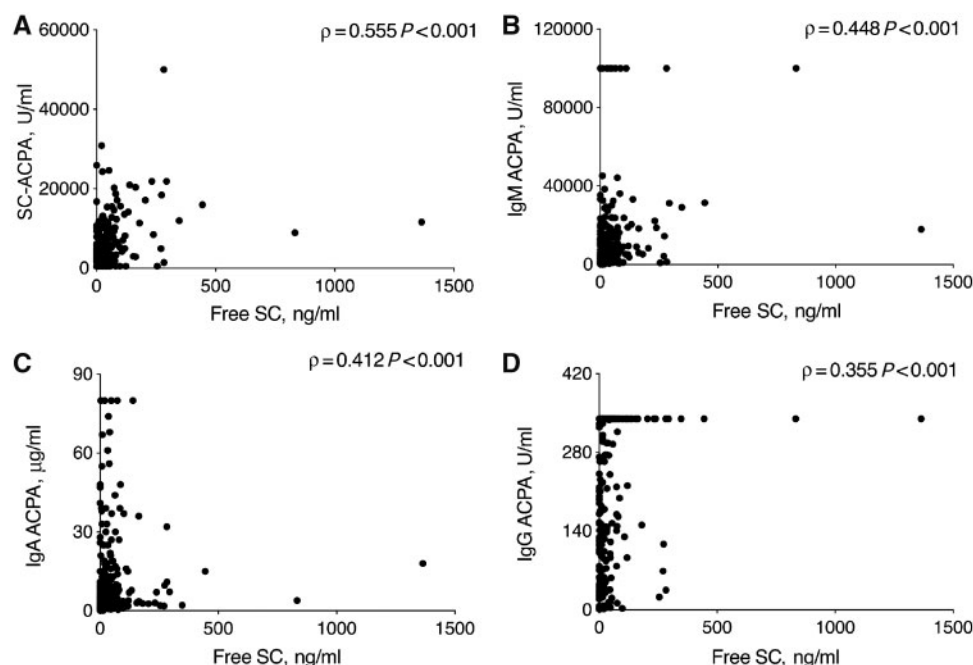
Correlation test between free SC and SC-ACPA (**A**), ACPA IgM (**B**), ACPA IgA (**C**) and ACPA IgG (**D**) in recent-onset RA (TIRA-2). Correlation statistics were obtained from Spearman tests. SC: secretory component; SC-ACPA: SC-containing ACPA.

Following corrections for multiple comparisons, all results remained significant except for the comparison between TIRx patients who developed arthritis *vs* the SLE control group ($P_c = 0.06$). There were no significant differences between TIRx patients without signs of arthritis at baseline who did not develop arthritis during follow-up [$n = 43$, 6.3 (13.4) ng/ml, mean (s.ᴅ.)] and any of the control groups ($P > 0.53$), except for AS [7.4 (9.1) ng/ml, $P = 0.013$]. Mean baseline levels of free SC were numerically higher among patients developing arthritis [31.1 (134.7) ng/ml, mean (s.ᴅ.)] compared with those who did not [6.3 (13.4) ng/ml]; however, the groups were not statistically significantly different ($P = 0.066$) and Cox-regression analysis with relevant adjustments (age, sex, RF, ACPA IgG levels and smoking) revealed no association between free SC and progression to arthritis ($P = 0.72$).

In the TIRA-2 cohort, neither disease activity variables (ESR, CRP, DAS28, HAQ) nor radiographic damage (evaluated by Larsen score [27]), differed significantly over the 36-month period, when compared with baseline free SC status (i.e. positive *vs* negative; Supplementary Fig. S3, available at *Rheumatology* online). No statistically significant correlation was seen between baseline levels of free SC and baseline data from the disease activity variables ESR, CRP and DAS28 (data not shown). In contrast, a weak but statistically significant correlation was seen when comparing the levels of free SC at inclusion with radiographic joint damage at month 36 (Larsen score, $\rho = 0.142$, $P = 0.022$), and almost with 36-month

progression of joint damage (Larsen progress, $\rho = 0.124$, $P = 0.051$), but as expected due to the weak correlation, this association disappeared when adjusting for ACPA IgG in a linear regression ($P = 0.95$). The change in free SC levels during the initial 3 months correlated weakly with the change in DAS28 score during the same period ($\rho = 0.309$, $P < 0.001$).

Discussion

This study presents the novel finding of increased serum levels of the mucosa-related protein free SC in patients with recent-onset RA and in ACPA positive patients subsequently developing arthritis. These results provide indications that mucosal immunity may be involved in RA development. The increased risk of ACPA positive RA associated with smoking is one of the suggested roles for mucosal involvement in ACPA development. We have previously shown that circulating SC-ACPA, containing IgA, is linked to smoking [12], and now extend this finding by the association between smoking and higher levels of circulating free SC in at-risk individuals. In recent-onset RA, we found that serum levels of free SC were elevated predominately among ACPA positive patients, and the levels correlated with ACPA of several isotypes and with SC-ACPA. Thus, ACPA formation could be part of the link between mucosae and RA. Recent findings by van Delft *et al.* [28] provided evidence that IgM, and not IgA as might have been expected, is the prominent

isotype of SC-ACPA. Thus, the previously described findings of circulating secretory ACPA [12] likely reflected SIgM ACPA rather than SIgA ACPA. A limitation in the present study is that the isotypes of SC-ACPA were not specifically addressed, but such results would not alter the main finding of elevated free SC levels among ACPA positive early RA patients and at-risk individuals.

In the present study, disease controls consisted of three different chronic inflammatory diseases. A potential limitation is that these were largely not recent-onset cases and, although not all in remission, they were likely to be less active than the RA patients. However, free SC does not appear to be a disease activity marker or acute phase reactant in RA, since no significant correlations with baseline ESR/CRP/DAS28 were found. Also, TIRx patients without arthritis at baseline, but who developed such during follow-up, still had raised levels of free SC at baseline. Taken together, we therefore assume that disease activity is of minor importance with regard to free SC levels also among controls. SLE patients can suffer from manifestations involving oral cavity [29], but mucosal immunity is not regarded as a mechanism for SLE induction [30], which is in line with the present findings of low levels of free SC in SLE patients. In AAV we previously showed that secretory PR3-ANCA could be detected in serum, but is unrelated to mucosal disease manifestations [31]. AS and intestinal inflammation show a strong genetic and clinical overlap [32], and both SIgA and free SC have been shown to be increased in AS [33]. A previous study showed equally increased levels of serum free SC in AS and established RA [33]. This contrasts to our findings, possibly reflecting different patient selection and treatment strategies.

The relative levels of free SC decreased during the 3 first months of early RA, and this correlated with improved disease activity. SC transcription can be enhanced by the glucocorticoid receptor [34], but in the present study there were no differences in mean free SC levels between patients receiving oral corticosteroids or not. However, treatment with a combination of csDMARDs and/or SSZ was associated with a greater reduction in free SC levels (although the difference did not remain after correction for multiple testing). This could be an interesting observation since SSZ is efficacious in inflammatory bowel disease, and may suggest that the intestinal mucosa is a main source of circulating free SC. SSZ can inhibit NF-κB [35], which is vital to SC transcription [36], and the marked reduction in free SC in early RA during the first 3 months could possibly be linked to SSZ treatment inhibiting NF-κB activation.

Among ACPA positive patients with musculoskeletal pain, we found free SC levels to be elevated prior to arthritis development. Although the mean baseline levels of free SC were numerically higher among patients subsequently developing arthritis compared with those who did not, we found no significant association between free SC and progression to arthritis in survival analysis.

It is known that RFs may interfere with immunoassays. In this study, we found the correlation between RF levels and free SC to be weak, and free SC levels remained significantly correlated with ACPA also in RF negative patients. Thus, we consider the free SC–RF correlation to rather represent an association with autoantibody positive disease phenotype.

In conclusion, free SC levels are higher among recent-onset RA patients compared with patients with other inflammatory diseases, and circulating free SC levels associate with ACPA and smoking. Compared with controls, circulating free SC levels were elevated among ACPA IgG positive patients later developing arthritis, but did not predict arthritis development. Taken together, we find limited diagnostic and prognostic value of serum free SC as a biomarker, but the results provide evidence for a link between presence of circulating ACPA and mucosal involvement in early RA development.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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Clinical Vignette

Digital ulcers secondary to radial artery thrombosis following arterial cannulation in systemic sclerosis

A 82-year-old lady with known lcSSc, pulmonary hypertension, primary biliary cirrhosis and paroxysmal atrial fibrillation was admitted for elective total hip arthroplasty. Her regular prescribed medications included apixaban, sildenafil and ambrisentan. She had a history of RP but with no previous digital ulceration. A left radial arterial line was placed (without immediate complications) postoperatively for invasive blood pressure monitoring and was *in situ* for <48 h. Three weeks postoperatively she developed new exquisitely painful unilateral ulceration on her left fingertips, which rapidly progressed to dry gangrene (Fig. 1). Arterial Doppler US revealed an occluded radial artery, which was not considered amenable to surgical intervention, and with good flow in the ulnar artery. Her medical management consisted of i.v. prostanoid (iloprost) infusions, optimal wound care and opioid-based analgesia.

Irreversible ischaemia following transradial access is rare, affecting 0.09% of individuals in critical care/anaesthetic settings [1]. In patients with SSc, disastrous thrombotic ischaemic complications—digital gangrene, hand autoamputation and below-elbow amputation— have been reported after radial arterial line placement [2]. Therefore, in patients with SSc the necessity and site of radial cannulation should be carefully weighed against the potential risk of developing severe ischaemic disease.

Disclosure Statement: The authors have declared no conflicts of interest.

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