Patient burden and joint inflammation during development of RA from arthralgia: is it similar in ACPA-positive and ACPA-negative disease?

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Clinical science

Patient burden and joint inflammation during development of RA from arthralgia: is it similar in ACPA-positive and ACPA-negative disease?

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Abstract

Objectives: ACPA-positive and ACPA-negative RA differ in underlying risk factors but have a similar clinical presentation at RA diagnosis. It is unknown what the ACPA-associated differences or similarities are during the symptomatic at-risk stage of RA, i.e. clinically suspect arthralgia (CSA). To deepen insights into these differences/similarities, we compared the course of symptoms/impairments and subclinical joint inflammation in the CSA phase during progression to inflammatory arthritis (IA) or to CSA resolution.

Methods: A total of 845 CSA patients were followed for a median of 24 months; 136 patients developed IA and an additional 355/505 patients had resolution of CSA according to rheumatologists. Patient burden (pain, morning stiffness, fatigue, functional disabilities, presenteeism) was assessed at baseline and 4, 12 and 24 months and at IA development. Subclinical joint inflammation in the hands and feet was assessed over time with 1.5T MRI. Linear and Poisson mixed models were used.

Results: In both ACPA-positive and ACPA-negative patients, patient burden increased towards IA development and decreased towards CSA resolution. However, patient burden was lower in ACPA-positive *vs* ACPA-negative disease at all timepoints. Conversely, subclinical joint inflammation tended to increase more rapidly during development of ACPA-positive IA [incidence rate ratio (IRR) 1.52 (95% CI 0.94, 2.47), $P = 0.089$] and remained higher over time in ACPA-positive CSA patients achieving resolution compared with ACPA-negative patients [IRR 1.52 (95% CI 1.07, 2.15), $P = 0.018$]. Although correlation coefficients between changes in patient burden and subclinical joint inflammation during progression to IA were weak, they were consistently higher in ACPA-positive than ACPA-negative disease, e.g. $\rho = 0.29$ *vs* 0.12 for functional disabilities.

Conclusion: During RA development and CSA resolution, ACPA-positive CSA patients have lower patient burden but more subclinical joint inflammation than ACPA-negative CSA patients. These data strengthen the notion that the development of ACPA-positive and ACPA-negative RA is pathophysiologically different and encourage further research on these differences.

Keywords: patient burden, joint inflammation, anti-citrullinated protein antibody, clinically suspect arthralgia, rheumatoid arthritis.

Rheumatology key messages

- During RA development and CSA resolution, ACPA-positive CSA patients have a lower disease burden but more subclinical joint inflammation than ACPA-negative CSA patients.
- The correlation between symptoms and subclinical joint inflammation was consistently stronger in ACPA-positive than in ACPA-negative disease.
- These data strengthen the notion that the development of ACPA-positive and ACPA-negative RA is pathophysiologically different.

Introduction

RA is a systemic autoimmune disease characterized by polyarthritis of the small joints. Autoantibodies including ACPA are present in a substantial percentage of RA patients. Interestingly, clues increasingly emerge about ACPA-positive and ACPA-negative disease being distinct entities of RA [1, 2]. As such, differences exist in genetic and environmental risk factors between ACPA-positive and -negative RA [3, 4]. Similarly, multiple extensive studies have observed differences in long-term outcomes such as drug-free remission, joint damage and mortality [2, 5–9]. Despite all these differences in initial risk factors and long-term outcomes, intriguingly the clinical picture at RA diagnosis is similar for ACPA-positive and ACPA-negative patients [10, 11].

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The development of RA can be divided into an asymptomatic phase (in which autoimmune responses arise and mature) and a subsequent symptomatic phase that can be recognized as clinically suspect arthralgia (CSA). CSA is a complex of clinical symptoms and signs that is identified by rheumatologists using pattern recognition for imminent RA. It is unknown whether the course of patient burden and joint inflammation in the latter symptomatic phase differs between ACPA-positive and ACPA-negative CSA (i.e. in line with identified differences in risk factors) or is the same (i.e. in line with the clinical similarities seen at diagnosis). Although it has previously been observed that the symptomatic at-risk stage is somewhat shorter during development of ACPA-positive *vs* -negative RA [12], ACPA-associated differences in disease burden and the relation with local joint inflammation in CSA patients during progression to clinically apparent inflammatory arthritis (IA) are unexplored. In addition, only part of CSA patients develops IA or RA; another long-term outcome at the other end of the spectrum is the spontaneous disappearance ('resolution') of the CSA phenotype. How symptoms/impairments and joint inflammation progress over time in this situation is also completely unknown.

We performed this study with the ultimate aim of deepening our knowledge of differences and similarities during disease development in ACPA-positive and ACPA-negative RA. We specifically studied differences between ACPA-positive and ACPA-negative CSA in the course of symptoms/impairments and local joint inflammation (and their correlation) during progression to RA or to resolution of CSA.

Patients and methods

Patients

We longitudinally studied CSA patients who were consecutively included between April 2012 and June 2022 in the Leiden CSA cohort. The Leiden CSA cohort is an inception cohort including patients with arthralgia of the small joints that is considered clinically suspicious for progression to RA according to the expertise of the rheumatologist. Patients were not included if clinically apparent arthritis was already present or if the rheumatologist considered another explanation for the arthralgia (e.g. osteoarthritis or fibromyalgia) was more likely than imminent RA. In addition, we also studied patients in the placebo group of the TREAT EARLIER trial (NL4599). This trial included CSA patients with presence of subclinical joint inflammation on MRI [13]. The study was conducted in compliance with the Helsinki Declaration. Research protocols for the Leiden CSA cohort (P11.210) and the TREAT EARLIER trial were approved by the local Medical Ethical Committee of the Leiden University Medical Center. Written informed consent was obtained from all patients before taking part.

Importantly, the Leiden CSA cohort includes patients based on symptom pattern and without knowledge of ACPA status and therefore offers an opportunity to study differences in ACPA-positive and ACPA-negative at-risk patients. Similarly, the Dutch TREAT EARLIER trial did not consider ACPA status for inclusion. Since general practitioners (GPs) in the Netherlands are discouraged to perform any serological tests, and inclusion in the CSA cohort occurs at the first visit to the outpatient clinic, identification and inclusion of CSA patients are based on the clinical presentation (Supplementary Table S1, available at *Rheumatology* online) [14]. The availability of both ACPA-positive and ACPA-negative CSA patients is unique, as many other cohorts with persons at risk of RA only include autoantibody-positive individuals.

Follow-up visits in the CSA cohort were performed at 4, 12 and 24 months after baseline. During follow-up, CSA patients were not treated with DMARDs (including corticosteroids). Follow-up visits in the TREAT EARLIER trial occurred every 4 months for 2 years. In both the CSA cohort and TREAT EARLIER trial, patients were seen between visits if patients perceived more symptoms, to verify whether they had developed IA.

IA development outcome

In the CSA cohort, IA was defined as joint swelling at physical examination by the rheumatologist. In the TREAT EARLIER trial, the primary outcome was also IA at physical examination that persisted for at least 2 weeks and fulfilled the 2010 RA classification criteria or involved two or more joints [13]. For the current study, one patient of the TREAT EARLIER trial who had persistent arthritis of only one joint and who did not fulfil the 2010 criteria was also considered as IA. In this way, IA in the current study was defined similarly for all patients.

CSA resolution outcome

Patients who did not develop IA were studied regarding whether they had achieved resolution of CSA. Resolution was defined according to the rheumatologists' expertise. These data were collected with rheumatologists answering the following question during follow-up visits: 'Is there still CSA?' The answer options were: 1. No, because pain complaints have disappeared; 2. No, because the pain has changed in nature and is no longer clinically suspicious; 3. Yes, because there is inflammatory arthralgia; 4. Yes, because morning stiffness is >60 min and 5. Yes, because other. Both 'no' answers (answer options 1 and 2) were considered as CSA resolution. If rheumatologists had not completed this question, medical files were studied to assess CSA resolution by both S.J.H.K. and A.v.d.H.-v.M. This was assessed according to the conclusions of the rheumatologists about the absence of CSA or the absence of arthralgia and morning stiffness. CSA resolution was not evaluated in patients with <1 year of follow-up, because the CSA cohort is an ongoing study. This means that not everyone has yet had an equal follow-up duration. To prevent defining CSA resolution too soon after CSA inclusion, we assessed CSA resolution consistently after 1 year of follow-up for all patients. By doing so, we aimed to prevent potential false-positive or false-negative evaluation of CSA resolution.

Measurements of patient burden

The following symptoms and impairments were studied over time: pain, morning stiffness, fatigue, functional disabilities and presenteeism. These were completed at every study visit (i.e. baseline, follow-up visits and upon IA development).

Pain, morning stiffness and fatigue were assessed uniformly by a trained research nurse and were expressed on a scale from 0 (no symptoms) to 100 (extreme symptoms); phrasing of these questions is provided in Supplementary Table S2, available at *Rheumatology* online.

Functional disabilities were assessed using the HAQ Disability Index. The HAQ is the average of 20 questions,

with 0 indicating no disabilities and 3 indicating inability to perform a task [15].

Presenteeism is the percentage of impaired productivity at work due to joint symptoms and was assessed using the Work Productivity and Activity Impairment Questionnaire [16]. Previous research in CSA has shown that presenteeism is more impaired than absenteeism [17]. Therefore, this study focused on presenteeism.

Measurement of subclinical joint inflammation

To assess subclinical joint inflammation, patients underwent a unilateral contrast-enhanced 1.5T MRI of the hand and foot. MRI was performed at study entry and at the end of the study (24 months or moment of IA development). In the TREAT EARLIER trial, MRI was additionally performed at 4 and 12 months. Wrist, MCP(2–5) and MTP(1–5) joints were evaluated for subclinical joint inflammation (sum of synovitis, tenosynovitis and osteitis) and were scored according to the RA MRI scoring system and the Haavardsholm method (Supplementary Tables S3 and S4, available at *Rheumatology* online).

Sensitivity analyses

Two sensitivty analyses were performed to evaluate the robustness of the results. Firstly, analyses on symptom severity and subclinical joint inflammation were repeated in patients who developed RA. RA was defined as IA plus a clinical diagnosis of RA with fulfilment of the 1987 and/or 2010 RA classification criteria and/or the start of DMARD treatment at the moment of IA development. Secondly, the analyses were performed in autoantibody-positive (ACPA and/or RF) *vs* autoantibody-negative CSA patients towards IA development.

Statistical analyses

All analyses were performed separately in patients who developed IA or CSA resolution. Analyses were additionally stratified for the presence or absence of ACPA.

The course of symptoms/impairments towards IA development was analysed using separate linear mixed models (LMMs). In these models, all measurements of a symptom/impairment were added as a dependent variable. The date of IA was considered as $t = 0$, and the time towards IA development was calculated per measurement and used as a covariate in the model. The courses of symptoms/impairments in CSA resolution were similarly analysed using LMMs, but now using 'time after study entry' as a covariate. These timelines are graphically depicted in Supplementary Table S5, available at *Rheumatology* online.

The courses of subclinical joint inflammation towards IA development or CSA resolution were analysed using a Poisson mixed model, because MRI scores are regarded as count data. The output of a Poisson mixed model is represented on a multiplicative scale as an incidence rate ratio (IRR). An IRR <1 indicates relatively less MRI inflammation, whereas an IRR >1 indicates more MRI inflammation. Because MRI-detected inflammation is age dependent, we corrected the analyses on subclinical joint inflammation for age [18]. The best model fit was assessed by comparing different models (e.g. evaluating interactions and knots). Eventually, for the linear and Poisson mixed models, a random intercept model was used with independent and unstructured covariance matrices, respectively. Restricted maximum likelihood was used to fit the models and model assumptions were checked graphically by inspection of residuals.

To statistically evaluate whether the courses of patient burden and subclinical joint inflammation differed over time between ACPA-positive and ACPA-negative patients, the analyses were repeated per symptom/impairment and subclinical joint inflammation in one analysis including ACPA-positive and ACPA-negative patients and by evaluation of an interaction between time and ACPA status. If this interaction was not statistically significant, indicating that the course was comparable for both ACPA groups, the mean difference in symptom/impairment or subclinical joint inflammation over time between ACPA groups was calculated.

Finally, the correlation between the change in patient burden and concomitant change in subclinical joint inflammation was analysed using Spearman's correlation coefficient. Considering the non-linear course of subclinical joint inflammation during 2 years of follow-up in patients with IA development, but a linear increase in the last 12 months, this analysis was performed in patients with IA development in the period of 12 months prior to IA development (Supplementary Table S6, available at *Rheumatology* online). For patients who achieved CSA resolution, analyses were performed during total follow-up and only in ACPA-negative patients, because of low numbers of ACPA-positive CSA patients with resolution ($n = 20$).

Analyses were performed using SPSS version 25 (IBM, Armonk, NY, USA) and Stata version 16 (StataCorp, College Station, TX, USA). Two-sided P-values <0.05 were considered statistically significant. A Bonferroni correction was applied in the analyses on the course of the five symptoms/impairments to account for multiple testing. This implies that the cut-off for statistical significance was more stringent here and thus was $P < 0.01$ instead of $P < 0.05$.

Results

Patient characteristics

In total, 845 patients were studied (728 patients from the CSA cohort and 117 patients from the placebo arm of the TREAT EARLIER trial; baseline characteristics of both patient groups are presented in Supplementary Table S7, available at *Rheumatology* online). A total of 76% of patients were female, the mean age was 44.2 years (S.D. 12.6), 14% of patients were ACPA positive and 21% were RF positive.

From this total group of 845 patients and during a total median follow-up of 24 months [interquartile range (IQR) 14–26], 136 patients developed IA, of whom 66 (49%) were ACPA positive. An additional group of 355/505 patients developed CSA resolution, of whom 20 (6%) were ACPA positive (see Methods for a detailed description of IA development and CSA resolution). The remaining patients had no CSA resolution and thus persisting symptoms of CSA, ongoing CSA follow-up or were lost to follow-up (Supplementary Table S8, available at *Rheumatology* online). These patients were not studied further in the current study.

Baseline characteristics of patients with IA development ($n = 136$) or CSA resolution ($n = 335$; total $n = 491$) are shown in Table 1, stratified for ACPA. CSA patients who progressed to IA more often had increased acute phase reactants and had more MRI-detected subclinical joint inflammation at baseline compared with patients who achieved CSA

Table 1. Baseline characteristics of the study populations

Characteristics	ACPA positive with IA development ($n = 66$)	ACPA negative with IA development ($n = 70$)	ACPA positive with resolution of CSA ($n = 20$)	ACPA negative with resolution of CSA ($n = 335$)
Female, *n* (%)	46 (70)	49 (70)	17 (85)	256 (76)
Age, years, mean (S.D.)	48.6 (13.3)	46.6 (13.1)	52.2 (8.2)	44.3 (12.2)
TJC (68 joints), median (IQR)	3 (1-5)	5 (4-11)	3 (0-5)	5 (2-10)
Symptom duration, weeks, median (IQR)	25 (13-53)	16 (8-37)	19 (11-60)	22 (10-49)
RF positive, *n* (%)	61 (92)	14 (20)	12 (60)	39 (12)
CRP increased (≥5 mg/L), *n* (%)	24 (38)	24 (34)	5 (25)	578 (17)
ESR increased[a], *n* (%)	15 (23)	18 (26)	2 (10)	33 (10)
Inflammation on MRI[b], median (IQR)	5.5 (3.0-13.0)	5.5 (3.0-10.8)	4.0 (2.0-8.0)	2.0 (0.5-3.9)
NRS pain, mean (S.D.)	44 (25)	51 (25)	34 (25)	45 (23)
NRS morning stiffness, mean (S.D.)	47 (27)	58 (25)	49 (33)	55 (25)
NRS fatigue, mean (S.D.)	42 (30)	46 (30)	46 (32)	49 (29)
HAQ score, mean (S.D.)	0.6 (0.5)	0.9 (0.6)	0.3 (0.3)	0.6 (0.5)
Presenteeism, mean (S.D.)	32 (29)	39 (30)	25 (24)	33 (28)

NRS: numerical rating scale; TJC: tender joint count.
[a] ESR was considered elevated with a reference for age and sex (<50 years: male > 15 mm/h, female > 20mm/h; >50 years: male > 20mm/h, female > 30mm/h).
[b] Inflammation score on MRI is the sum of synovitis, tenosynovitis and osteitis (Supplementary Tables S2 and S3, available at *Rheumatology* online). Data were missing on MRI (18%), NRS pain (4%), NRS morning stiffness (12%), NRS fatigue (4%), HAQ (9%) and presenteeism (9%).

resolution. In both patients with IA development and CSA resolution, ACPA-positive patients presented with a lower mean tender joint count compared with ACPA-negative patients (both 3 *vs* 5).

Course of patient burden

Pain, morning stiffness, fatigue, functional disabilities and presenteeism all increased in the 2 years prior to IA development for both ACPA-positive and ACPA-negative CSA patients (Fig. 1, upper part). These changes in patient burden towards IA development are described per year in Table 2A. All increases were statistically significant, except for pain and fatigue in ACPA-positive patients. For example, a β of 20 for ACPA-positive patients with IA development indicates that morning stiffness increased 20 points per year prior to IA development.

Patients who presented with CSA and achieved resolution had decreased symptoms and impairments over time (Fig. 1, lower part). In ACPA-positive patients, part of these decreases did not reach statistical significance. In contrast, within ACPA-negative CSA patients achieving resolution, all symptoms and impairments deceased significantly over time (Table 2B). For example, a β of −8 for morning stiffness in ACPA-positive patients with CSA resolution indicates that morning stiffness decreased 8 points (on a scale 0–100) per year after presentation with CSA.

Fig. 1 shows that symptoms and impairments were consistently higher in ACPA-negative patients than in ACPA-positive patients. This is also shown in the raw data (Supplementary Table S9, available at *Rheumatology* online) and in Table 3 by the mean differences between ACPA-negative and ACPA-positive CSA patients >0 over time. In CSA patients who developed IA, the ACPA difference in severity over time was statistically significant for morning stiffness [$\beta = 11$ (95% CI 3, 10), $P = 0.006$] and functional disabilities [$\beta = 0.2$ (95% CI 0.1, 0.4), $P = 0.005$; Table 3A]. In CSA patients who achieved resolution, the severity of symptoms and impairments was also higher in ACPA-negative patients compared with ACPA-positive patients. Although statistical significance was not achieved in the group with CSA resolution, the ACPA differences were roughly similar to those observed for pain, fatigue and functional disabilities in patients with IA development (Table 3B).

Course of subclinical joint inflammation

In patients with IA development, subclinical joint inflammation increased over time in both ACPA groups (Fig. 2, upper part). However, subclinical joint inflammation increased more rapidly towards IA development in ACPA-positive patients. Also, at the time of IA development, inflammation scores were higher in ACPA-positive patients. The interaction between time prior to IA development and ACPA status was positively associated with the course of subclinical joint inflammation [IRR 1.52 (95% CI 0.94, 2.47), $P = 0.089$]. This implies that inflammation scores increased 1.52 times faster per year for ACPA-positive *vs* ACPA-negative patients.

In patients with CSA resolution, subclinical joint inflammation decreased after presentation with arthralgia (Fig. 2, lower part). The IRR of 0.87 (95% CI 0.80, 0.95; $P = 0.001$) implies that the total inflammation score decreased 0.87 times per year of follow-up. However, subclinical joint inflammation remained higher in ACPA-positive CSA resolution (Fig. 2, lower part). Comparing the course of subclinical joint inflammation in ACPA-positive and ACPA-negative patients with resolution revealed that ACPA-positive patients had higher inflammation scores at all timepoints [IRR 1.52 (95% CI 1.07, 2.15), $P = 0.018$]. This implies that inflammation scores over time in ACPA-positive patients were 1.52 times higher than in ACPA-negative patients with CSA resolution.

Correlation between changes in patient burden and changes in subclinical joint inflammation

Correlation coefficients between changes in patient burden with simultaneous changes in subclinical joint inflammation in the 12 months prior to IA development were weak in both ACPA groups (Fig. 3). Nonetheless, the correlation coefficients were nominally and consistently higher in ACPA-positive disease than ACPA-negative disease: pain ($\rho = 0.15$ *vs* −0.18), morning stiffness ($\rho = 0.12$ *vs* −0.42), fatigue ($\rho = 0.30$ *vs* 0.07), functional disabilities ($\rho = 0.29$ *vs* 0.12) and presenteeism ($\rho = 0.33$ *vs* 0.11).

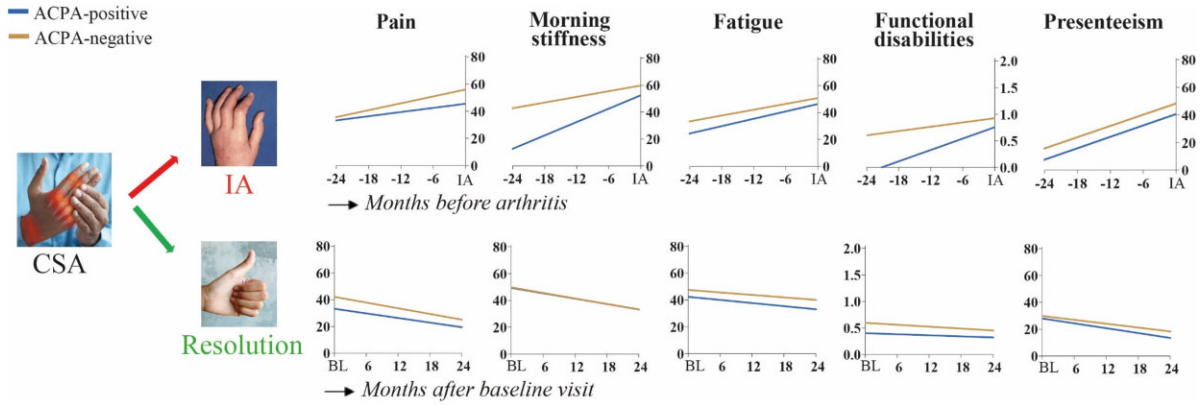

Figure 1. The course of patient burden in CSA over time during progression to IA and resolution of CSA, stratified for ACPA

Table 2. The change in disease burden in CSA patients over time during **(A)** IA development and **(B)** resolution of CSA, stratified for ACPA.

(A) Patients with IA development				
Burden	**ACPA positive**		**ACPA negative**	
	Increase/year, β (95% CI)	***P*-value[a]**	**Increase/year, β (95% CI)**	***P*-value[a]**
Pain (0–100)	+6 (−5 to 17)	0.26	+10 (4 to 17)	**0.002**
Morning stiffness (0–100)	+20 (9 to 31)	**<0.001**	+8 (1 to 16)	0.020
Fatigue (0–100)	+11 (0.04 to 22)	0.049	+9 (2 to 16)	**0.013**
Functional disabilities (0–3)	+0.4 (0.2 to 0.6)	**<0.001**	+0.2 (0.04 to 0.3)	**0.008**
Presenteeism (0–100)	+17 (5 to 28)	**0.006**	+16 (7 to 25)	**<0.001**
(B) Patients with CSA resolution				
Burden	**ACPA positive**		**ACPA negative**	
	Decrease/year, β (95% CI)	***P*-value[a]**	**Decrease/year, β (95% CI)**	***P*-value[a]**
Pain (0–100)	−7 (−13 to −1)	0.016	−9 (−10 to −7)	**<0.001**
Morning stiffness (0–100)	−8 (−14 to −2)	**0.006**	−8 (−10 to −7)	**<0.001**
Fatigue (0–100)	−5 (−10–1)	0.11	−4 (−5 to −2)	**<0.001**
Functional disabilities (0–3)	−0.04 (−0.1 to −0.036)	0.31	−0.07 (−0.09 to −0.05)	**<0.001**
Presenteeism (0–100)	−7 (−11 to −3.0)	**0.001**	−6 (−7 to −4)	**<0.001**

The (A) increase or (B) decrease in symptoms and impairments are shown per year. These are derived from separate and ACPA-stratified LMM analyses per symptom/impairment, including all measurements of a symptom/impairment as a dependent variable and the time of the measurement prior to (A) development of IA or (B) after study entry as covariates. For the analyses in patients with IA development, the moment of inflammatory arthritis was considered as $t = 0$. For example, a β of 20 for ACPA-positive patients with IA development indicates that morning stiffness increases by 20 points per year prior to IA development. For the analyses in patients with CSA resolution, the date of study entry was considered as $t = 0$.
Bold values are statistically significant.
[a] *P*-values were corrected for multiple testing by applying the Bonferroni correction, implying that the cut-off for statistical significance is $P < 0.01$ (corrected for five studied symptoms/impairments).

Table 3. Mean differences in patient burden over time between ACPA-negative and ACPA-positive CSA patients with **(A)** IA development and **(B)** resolution of CSA.

Burden	**(A) ACPA difference in patients with IA development (95% CI)**	***P*-value[a]**	**(B) ACPA difference in patients with resolution of CSA (95% CI)**	***P*-value[a]**
Pain (0–100)	7 (2, 16)	0.017	7 (−1, 15)	0.09
Morning stiffness (0–100)	11 (3, 10)	**0.006**	0.48 (−10, 11)	0.93
Fatigue (0–100)	5 (−3, 14)	0.24	6 (−4, 17)	0.26
Functional disabilities (0–3)	0.2 (0.1, 0.4)	**0.005**	0.2 (−0.1, 0.4)	0.17
Presenteeism (0–100)	8 (−1, 17)	0.10	4 (−7, 15)	0.50

Mean differences in symptom/impairment scores throughout the course for ACPA-negative patients compared with ACPA-positive patients (scores ACPA negative – scores ACPA positive) towards (**A**) IA development or (**B**) CSA resolution. Thus, during the pre-RA period, ACPA-negative CSA patients have 7 points more pain than ACPA-positive patients. Differences in scores throughout the course are derived from LMM analyses including all measurements of a symptom/impairment as a dependent variable and ACPA status and the time of the measurement as covariates. After confirming no statistical differences in slopes between ACPA groups, the overall difference between ACPA groups was calculated.
Bold values are statistically significant.
[a] *P*-values were corrected for multiple testing by applying the Bonferroni correction, implying that the cut-off for statistical significance is $P < 0.01$ (corrected for five studied symptoms/impairments).

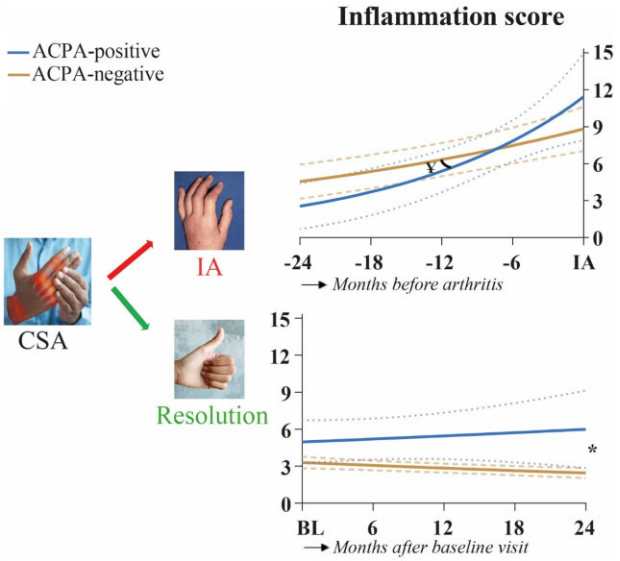

Figure 2. The course of subclinical joint inflammation over time in CSA patients during progression to IA and resolution of CSA, stratified for ACPA

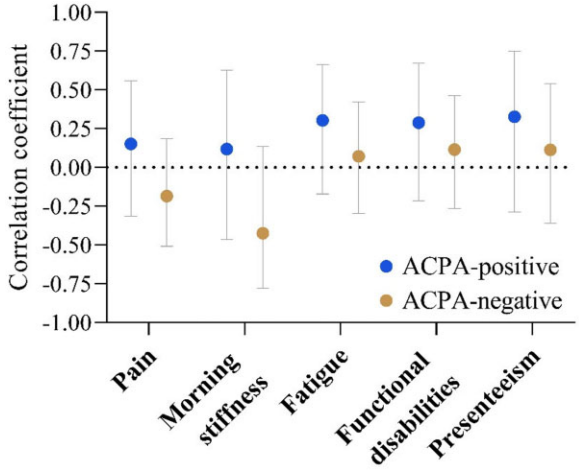

Figure 3. Correlation between change in patient burden with simultaneous change in subclinical joint inflammation during 12 months before IA development in ACPA-positive and ACPA-negative CSA

In ACPA-negative patients who achieved CSA resolution, the decrease in pain was weakly correlated with a decrease in subclinical joint inflammation while the decreases in morning stiffness, fatigue, functional disabilities and presenteeism were not correlated with a decrease in joint inflammation (Supplementary Table S10, available at *Rheumatology* online).

Sensitivity analyses

The analyses on the course of patient burden and subclinical joint inflammation were repeated in those with RA development ($n = 121$) instead of IA development ($n = 136$). Results were mostly similar: patient burden increased towards RA development in both ACPA groups but, similarly, scores over time were higher in ACPA-negative patients (Supplementary Table S11a, available at *Rheumatology* online). In line with the observation with IA as the outcome, the course of subclinical joint inflammation increased towards RA development, with a steeper increase towards ACPA-positive RA (Supplementary Table S11b, available at *Rheumatology* online).

Additionally, the course of patient burden and subclinical joint inflammation was analysed in autoantibody-positive and autoantibody-negative patients with IA development ($n = 80$ *vs* 56) instead of ACPA-positive and ACPA-negative patients (Supplementary Table S12, available at *Rheumatology* online). Comparable courses were observed.

Discussion

ACPA-positive and ACPA-negative RA differ in genetic and environmental risk factors and in long-term outcomes. In contrast, the clinical phenotype at the time of RA diagnosis is comparable for both ACPA groups. This could suggest two potential scenarios for the trajectory of CSA to RA. First, there may be a common pathway in the development of clinically apparent arthritis for both ACPA groups (in line with the similar phenotype at the time of occurrence of arthritis). Alternatively, the course from CSA to RA differs, in line with differences in risk factors. This study increased our understanding of the trajectory from CSA to RA for ACPA-positive and ACPA-negative disease. We observed that patient burden and subclinical joint inflammation increased towards RA development and decreased towards CSA resolution. However, ACPA-positive and ACPA-negative disease differed in severity. While ACPA-negative disease had more severe patient burden, ACPA-positive disease had more severe subclinical joint inflammation, and the increase in symptomatic burden towards RA development had stronger correlations with joint inflammation. Together, these data reveal ACPA-associated differences in the final stages of RA development. Thus this points more towards the hypothesis that the course from CSA to RA differs between ACPA-positive and ACPA-negative disease.

Our study revealed that ACPA-negative CSA patients have a higher disease burden than ACPA-positive CSA patients during progression from CSA to RA. This may seem surprising, as ACPA-positive disease, rather than ACPA-negative disease, is considered the more severe subtype when evaluating long-term outcomes such as joint destruction. However, our results are in line with previous research within classified RA, demonstrating that disease burden of ACPA-negative RA was at least as high in ACPA-positive RA [19, 20]. Although the definition of clinically relevant differences in the pre-arthritis stages is unknown, a 0.09 change in the HAQ score has been defined as clinically relevant in early RA [15, 21], and a 10-point reduction is considered relevant for visual analogue scale pain assessment [22]. In line with the results on symptoms and impairments, we also observed that ACPA-negative CSA patients had more tender joints than ACPA-positive patients. Hence the observations of patient-reported outcomes are in line with findings at physical joint examination.

Opposite findings were obtained regarding subclinical joint inflammation: ACPA-positive disease was accompanied by more severe subclinical joint inflammation during RA development. Although the interaction between time prior to IA development and ACPA status did not reach statistical significance ($P = 0.089$), the lines evidently crossed and subclinical joint inflammation increased more rapidly in the months before IA development in ACPA-positive disease. This is also in line with a previous study that revelated ACPA-positive CSA

developed RA faster than ACPA-negative CSA [12]. The statistical significance may be challenged by a lower number of MRI measurements long before IA development, especially in ACPA-positive patients.

The question arises on how to explain the higher burden but lower local joint inflammation in ACPA-negative disease. Importantly, when analysing CSA patients who developed IA, we studied patients who were, in retrospect, truly in a pre-RA phase at CSA presentation. Additionally, we performed sensitivity analyses with RA development as the outcome instead of IA development and observed comparable ACPA differences. Therefore it is not likely that the observed differences between ACPA groups during IA development are due to potential misdiagnosis. Furthermore, our results are in line with research in early classified RA showing that the Patient Global Assessment did not correlate with inflammatory outcomes as swollen joint count in autoantibody-negative RA, in contrast to autoantibody-positive RA [23]. An immunological explanation could be that the immune response differs between ACPA-positive and ACPA-negative RA: ACPA-negative RA is characterized by less lymphocyte infiltration in the synovium and a higher degree of synovial stromal cells producing pro-inflammatory cytokines and chemokines [1]. Possibly, differences in the humoral and innate immune response contribute to the observed discrepancy. Another explanation may include factors other than autoimmunity or autoinflammation. Patient burden is multifactorial in nature and includes not only inflammation, but also cognitive and behavioural factors (e.g. thoughts, feelings, behaviours) and personal factors (e.g. work/caregiving responsibilities, social support, environment) [24, 25]. Perhaps these factors have a greater contribution to the disease burden in ACPA-negative RA development. However, this remains speculative and is a subject for future research.

An interesting and almost unexplored outcome in CSA is its resolution. We revealed that 42% of the total group of patients with CSA that were at risk of RA had a spontaneous resolution of this at-risk phenotype (defined as disappearance of CSA-specific symptoms according to the expertise of the rheumatologist). This resolution was paralleled by a decrease in patient burden and subclinical joint inflammation. To the best of our knowledge, this study is the first reporting about CSA resolution. Interestingly, CSA resolution occurred rarely in ACPA-positive patients. Moreover, we also observed the paradox that ACPA-positive CSA patients who achieved resolution had a lower patient burden over time but had higher scores for subclinical joint inflammation than ACPA-negative CSA patients. The median follow-up of the patients with CSA in the resolution group was 25 months (IQR 24–27). Longer follow-up is required to evaluate whether resolution remains persistent in these ACPA-positive patients or whether CSA might recur after several years. Furthermore, pathophysiological studies are also very interesting to investigate whether there are differences in autoantibodies, autoreactive B cells or other components of the humoral immune response that are different in ACPA-positive CSA patients without and with CSA resolution.

Patients with CSA resolution might, in retrospect, not be considered as pre-RA. However, at presentation with complaints they were considered to be at risk of RA based on a symptom complex that was recognized by treating rheumatologists. The presence of abnormal laboratory results (autoantibodies, increased acute phase reactants) and subclinical joint inflammation provides additional risks for RA development but are not elements of CSA inclusion. Misdiagnosis of patients with CSA resolution cannot completely be ruled out and this may be a semantic discussion. However, our observations on decreasing patient burden and decreasing MRI-detected joint inflammation in these patients suggest that an inflammatory response was presumably present at presentation with arthralgia but had a favourable outcome over time. Future studies are required to identify processes that are crucially related to progression from this at-risk stage to RA or to spontaneous resolution of this at-risk stage. A deeper pathophysiological understanding could potentially provide targets for future targeted interventions in this stage of RA development.

A limitation of our study, despite the presence of MRI data in 82% of patients, is that repeated MRIs were not present in all patients (present in 47% of studied patients). The main reasons for this missingness are that repeated MRIs were made only between 2012 and 2020 and scored MRI data were available until 2021. This indicates that repeated MRIs are largely missing completely at random. Nonetheless, some participants were not willing to undergo follow-up MRIs or had a contra-indication for MRI. This could have led to selection bias. Importantly, the missingness in repeated MRIs was not ACPA related, signifying that the proportion of ACPA positivity in patients with repeated MRIs was comparable to that in the total study population (Fig. 2). Thus the observed differences between ACPA groups over time were most probably not influenced by missingness in repeated MRIs. Missingness in patient burden was low (Table 1) and mixed model analyses were used to overcome missingness. Another potential limitation is that the focus of this study was ACPA differences, but the anti-modified protein antibodies response is wider and more autoantibodies have been described [26, 27]. Future research could include other autoantibodies (anti-acetylated peptide and anti-carbamylated protein antibodies) or autoantibody characteristics (levels, isotypes, Fab-glycosylation) and the relation with disease burden and inflammation in CSA [8, 28, 29].

Strengths of this study are the longitudinal and extended data collection on cardinal symptoms and impairments and subclinical joint inflammation in both ACPA-positive and ACPA-negative patients who are clinically suspect for RA development. As ACPA status was unknown before study entry and inclusion was thus not based on ACPA status, this study is the first investigating the trajectory from CSA to RA development and to CSA resolution for ACPA-positive and ACPA-negative disease.

In conclusion, we showed that the course of disease burden and subclinical joint inflammation differed between ACPA-positive and ACPA-negative patients with CSA. As such, ACPA-positive patients reported lower disease burden but had more local joint inflammation on MRI. In addition, the correlation between these outcomes seemed better in ACPA-positive disease. Together, the findings suggest that processes underlying disease burden differ between developing ACPA-positive and ACPA-negative RA. These results emphasize ACPA-associated differences that are also present during the pre-arthritis stage of arthralgia. This could encourage future translational research to study the processes underlying these differences. A deeper understanding of the underlying mechanisms could promote stratified or personalized treatment of

ACPA-positive and ACPA-negative CSA and RA in the future.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

Requests for data (such as de-identified participant data) can be made to the corresponding author and requests will be considered on an individual basis.

Authors' contributions

S.J.H.K., Ev.M. and Avd.H. designed the study. S.J.H.K., D.I.K. and H.K.G. collected the data. S.J.H.K. accessed and verified the data. S.J.H.K. analysed the data and acted as guarantor. All authors interpreted the data and wrote the report. Avd.H. was the principal investigator. All authors approved the final version of the manuscript and were responsible for the decision to submit the manuscript for publication.

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