Survivin improves the early recognition of rheumatoid arthritis among patients with arthralgia: A population-based study within two university cities of Sweden

Malin C. Erlandsson, PhD⁵, Minna Turkkila⁴, Filip Siljehult⁵, Rille Pullerits, MD, PhD⁵,⁶, Catharina Eriksson, MD, PhD⁶,⁷, Solbritt Rantapää-Dahlqvist, MD, PhD⁶, Maria I. Bokarewa, MD, PhD⁵,⁎

Department of Rheumatology and Inflammation Research, Sahlgrenska University Hospital, University of Gothenburg, Box 480, SE 405 30 Gothenburg, Sweden
Department of Clinical Immunology and Transfusion Medicine, Sahlgrenska University Hospital, University of Gothenburg, Gothenburg, Sweden
Department of Public Health and Clinical Medicine, Rheumatology Unit, University of Umeå, Umeå, Sweden
Laboratory of Clinical Immunology University Hospital of Umeå, Umeå, Sweden

Keywords: Rheumatoid arthritis Arthralgia Survivin Population-based study Disease prediction Auto-antibodies

Abstract

Objectives: The aim of this study was to validate the use of survivin for preclinical recognition of rheumatoid arthritis (RA) among patients with unexplained arthralgia.

Methods: Serum levels of survivin and the arthritis-specific autoantibodies RF and ACPA were measured in total of 5046 patients with musculoskeletal complaints during 12 consecutive months in Gothenburg and in Umeå. Among them, 303 arthralgia patients were identified and prospectively followed.

Results: After 48 months, 12.2% of the arthralgia patients developed RA. Most of RA cases had high serum survivin, which increased the relative risk for RA (RR = 5.90, p = 3 × 10⁻⁷). Combination of survivin with autoantibodies was present in only 4.6% of the arthralgia patients and increased further the risk of RA and shortened time to RA development. Presence of any single autoantibody in the survivin-negative patients was associated with a minor risk for RA and had RA-free survival similar to the reference group.

Conclusion: This study shows that measurement of survivin in serum improves estimation of RA risk and prospectively predicts RA development in patients with arthralgia. Survivin may indicate a phase preceding autoantibody production.

© 2017 The Authors. Published by Elsevier HS Journals, Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Late referral of patients with rheumatoid arthritis (RA) to rheumatologist for diagnosis and initiation of anti-rheumatic treatment generates substantial healthcare costs due to increasing morbidity burden [1,2], loss of employment [3], and premature mortality of RA patients [4,5]. Early recognition of RA patients in general medical practice meets objective difficulties related to insidious increase of symptoms and subtle clinical signs at physical assessment [6]. Major weight in recognition of RA is put on serological findings.

Among the biologic markers that have been evaluated for recognition of RA disease, only a few have found implication in clinical practice. Biological nature of early events predisposing to RA is currently recognized by production of autoantibodies [7–9]. The immunologically relevant antigens targeted by antibodies of RA patients display a wide spectrum of cartilage components, stress proteins, enzymes, and nuclear proteins, while the clinical and diagnostic interest is focused on measurement of rheumatoid factors (RF), and anti-citrullinated peptide antibodies (ACPA) [10]. This attention is mainly explained by specificity of these autoantibodies for arthritis [11] and their predictive capacity ahead of clinical symptoms [12,13]. Relatively low prevalence of RF and ACPA in unselected RA cohorts [11] and the diversity of antigenic epitopes for ACPA reactivity hamper, however, their connection to pathogenic processes and sensitivity for preclinical diagnosis.

Abbreviations: RA, rheumatoid arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ELISA, enzyme-linked immunosorbent assay; DMARDs, disease-modifying antirheumatic drugs; ACPA, anti-citrullinated peptide antibodies; RF, rheumatoid factors; ROC, receiver operating characteristic; AUC, area under the curve; PPV, positive predictive value.

Role of the funding source: The funding sources have no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Corresponding author.

E-mail address: maria.bokarewa@rheuma.gu.se (M.I. Bokarewa).
In addition to autoantibodies, several prospective studies reported systemic elevation of inflammatory markers in preclinical phase of RA [14–17]. A pattern of cytokines produced by activated T helper cells has been revealed as an early sign of immunological aberrations in pre-RA patients followed by a broad-spectrum response of signal molecules representing stromal cells and angiogenetic factors [16,18]. Acute-phase reactants, C-reactive protein, and erythrocyte sedimentation rate are used for recognition of RA patients [19,20]. Insufficient specificity and liability of these inflammatory markers limit their diagnostic value in RA.

In the current study, we assess if presence of the onco-protein survivin in serum may identify patients at risk of developing RA. Survivin has recently emerged as a novel biomarker in RA. Serum levels of survivin measured in healthy RF and ACPA negative donors identified low prevalence of detectable survivin levels in healthy subjects [21]. This study was used to define pathologically high level of survivin. Specificity of high serum survivin levels for RA has been confirmed in an epidemiological study of MyEIRA [22]. In that study survivin was associated with RA specific genetic and environmental background, and with production of RF and/or ACPA. Indeed, survivin-positive patients are enriched among the carriers of HLA-DRB1 alleles, autoantibodies [22,23] and in smokers [24,25]. Survivin is highly expressed in the inflamed synovial tissue, an epicentre of rheumatoid pathology [26–28]. The experience of studies in the early RA patients suggests that serum levels of survivin are reduced and may convert negative with anti-rheumatic treatment [24,29]. Taken together, these reports indicate an essential role of survivin in RA pathology. High levels of survivin in serum are measured in > 50% of RA patients at the first visit to rheumatologist, and its prognostic value for future joint damage [30] and progressive functional disability [29] has been shown.

Survivin is a biomarker distinct by nature from autoantibodies and from acute phase reactants. It shows seldom liability with the clinical activity of RA [23,31]. Survivin is a multifunctional protein that belongs to the inhibitor of apoptosis family. Survivin with nuclear localization regulates cell division and micro-RNA biogenesis, whereas mitochondrial and cytoplasmic survivin inhibits apoptosis [32,33]. Survivin is an intracellular protein and its high levels in serum remain an enigma. Our recent translational investigations [32,33]. Survivin is an intracellular protein and its high levels in serum remain an enigma. Our recent translational investigations showed an essential role of survivin in RA pathology. High levels of survivin in serum are measured in > 50% of RA patients at the first visit to rheumatologist, and its prognostic value for future joint damage [30] and progressive functional disability [29] has been shown.

Methods

Participants and study design

The inception cohort for this prospective clinical study comprised the patients referred by general practitioners for analysis of RA and/or ACPA to the Laboratories of Clinical Immunology at the University Hospitals of Gothenburg (GU, November 5, 2012–November 4, 2013) and Umeå (UU, January 1, 2010–December 31, 2010) during the period of 12 consecutive months (Fig. 1). Since these laboratories function as accredited referral centres within the respective city of Sweden, we expect that the sample collection covers most of the target population with new cases of inflammatory musculoskeletal complaints. At the end of the sample collection period, all medical records of the first-visit patients were retrieved within the respective Rheumatology unit. The medical records were carefully sorted in two steps by experienced rheumatologists (S.R.D., R.P., and M.B.). During the first step, we excluded all patients with known diagnosis of inflammatory joint or spine disease, systemic rheumatic disease, gout and generalised pain conditions. During the second step, patients with arthralgia at the first-visit that could not be explained by swollen joints were identified (Fig. 1). Inclusion in the study required the presence of a blood sample of the predefined date and a medical record of the first visit to rheumatologist in adherence to the blood sample. We also excluded the individuals below 18 years of age and those who have been registered deceased for the time point of their medical records evaluation. This resulted in two independent study cohorts of patients with unexplained arthralgia that comprised 180 patients in Gothenburg and 123 patients in Umeå.

The study protocol was reviewed and approved by the Ethical Review Boards of Gothenburg and Umeå. All methods used in this study were performed in accordance with the relevant guidelines and regulations.
study were carried out in accordance with relevant Swedish guidelines and regulations and following the Good Clinical Practice. The study is based on the routine rheumatologic evaluation and the results of blood analysis after the referral from general practitioners, which does not require the informed consent. The informed consent was obtained from all subjects in case of additional clinical visits or blood sampling.

Data collection

Medical records of the first and all consecutive visits were reviewed for the period of 48 prospective months. The review of records was done by two assessors, and independently at each medical centre (at GU, SB, and SK; at UU, SRD, and FS). The collection of serological characteristics including the acute-phase reactants, autoantibodies and survivin were performed separately. The assessors were instructed and trained to keep a keen eye for clinical signs of swollen joints as identified by rheumatologists. Patients with signs of swollen joints at the first visit were excluded from further analysis. When signs of arthritis appeared at prospective visits, the EULAR/ACR 2010 Classification RA Criteria form [10] was completed starting from the first clinical visit. The family history of RA and smoking habits were retrieved from medical records based on patient self-reports.

Measurements of survivin

Survivin levels were measured in the serum samples diluted 1:10 using a sandwich enzyme-linked immunoassay (ELISA) comprising a pair of matched antibodies and recombinant standard (DY647, R&D Systems, Minneapolis, MN); the detection limit was 100 pg/ml. The cut-off level for survivin positivity was set to 450 pg/ml corresponding to the mean + 3 SD of 104 healthy individuals [21].

Analyses of ACPA and RF

The measurements of ACPA and RF were performed at the accredited laboratories of Clinical Immunology at the Sahlgrenska University Hospital and at the Laboratory of Clinical Immunology at the Umeå University hospital. In GU, ACPA was detected using an automatic multiplex method (anti-CCP2, BioRad, Hercules, CA). In UU, ACPA was measured by fluorochrome immunoassay on ImmunCAP250 (ThermoFisher Diagnostics, Uppsala, Sweden). The cut-off levels above 3.0 and 10 U/mL, respectively, were set as covariates. The Cox regression was run stepwise with backward eliminations, excluding variables with p > 0.1. To analyse the predictive value of survivin in relation to autoantibodies, the material was grouped into five groups, S−AB−, S−AB+, S+AB−, and S+AB++; where AB+ indicates positivity of either RF or ACPA, and AB++ indicates positivity of both RF and ACPA. To visualise differences in time and frequency of RA cases, the Kaplan-Meier curves were constructed and the Cox proportion hazard analysis was performed pairwise.

Results

Characteristics of patients with arthralgia

The patients within the two parallel cohorts in Gothenburg and Umeå were predominantly women. The cohorts were similar with respect to age, smoking history and systemic inflammation at the first visit (Table 1). The Gothenburg cohort had higher prevalence of the survivin-positive (34% vs. 9%, p < 0.001) and of the autoantibody-positive (29.6% vs. 16.4%, p < 0.001) patients than the Umeå cohort (Table 1). In total, 72 (61 GU + 11 UU) patients were survivin-positive, 29 (21 GU + 8 UU) of them were also positive for RF and/or ACPA. Other 44 (32 GU + 12 UU) patients were positive for RF and/or ACPA and negative for survivin. A smaller group of patients (9 GU + 5 UU, 4.6%) was recognized by simultaneous presence of survivin, RF, and ACPA.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gothenburg, n = 180</th>
<th>Umeå, n = 123</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, n (%)</td>
<td>145 (80.6)</td>
<td>83 (67.5) p = 0.014</td>
</tr>
<tr>
<td>Age, y, median [IQR]</td>
<td>48 [36–57]</td>
<td>47 [18–81]</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>33/164 (20.1)</td>
<td>18/96 (18.7)</td>
</tr>
<tr>
<td>Former smoker, n (%)</td>
<td>35/164 (21.3)</td>
<td>24/96 (25.0)</td>
</tr>
<tr>
<td>Increased CRP or ESR, n (%)</td>
<td>52/176 (29.0)</td>
<td>27/123 (22.0)</td>
</tr>
<tr>
<td>Survivin-positive, n (%)</td>
<td>61 (34.0)</td>
<td>11 (9.0) p = 2.1 × 10⁻⁷</td>
</tr>
<tr>
<td>ACPA-positive OR RF-positive, n (%)</td>
<td>53 (29.5)</td>
<td>20 (16.4) p = 3.4 × 10⁻⁴</td>
</tr>
<tr>
<td>n (%)</td>
<td>RF-positive, n</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>ACPA-positive, n</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>ACPA AND RF positive, n</td>
<td>9 (5.0)</td>
</tr>
</tbody>
</table>

P values indicate differences between the cohorts. AB+, autoantibody positive; ACPA, antibodies to citrullinated peptides; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, inter-quartile range; RF, rheumatoid factor.
Incident cases of RA at prospective follow-up

During the follow-up period of 48 months, 32 (17.8%) patients from the Gothenburg cohort and 5 (4.1%) from the Umeå cohort developed arthritis and fulfilled the EULAR/ACR 2010 criteria for RA. The frequency of new RA cases was higher among the survivin-positive patients of the both cohorts. This was associated with 5.90 folds (IQR: 3.17–10.97) increase in the cumulative relative risk for RA \((p = 3 \times 10^{-7})\) (Fig. 2A). The survivin-positive arthralgia corresponded to PPV of 0.83 for RA and provided specificity for arthritis similar to the earlier reported [22]. The area under the ROC curve was fit to 0.76 \((p < 0.0001)\), indicating that serum levels of survivin in arthralgia patients were predictive for new RA (Fig. 2B).

Integrated utility of survivin and arthritis-specific autoantibodies enhances the RA risk

To identify characteristics, which could assist distinguishing patients at risk to develop RA, the multivariate Cox regression analysis was performed on the Gothenburg cohort. At the final step of the regression analysis, survivin positivity, presence of autoantibodies, current smoking and age > 50 years remained significantly associated with new RA. Stepwise results of the regression analysis are presented in the Supplemental Table S1.

Since both survivin and autoantibodies were independently predicting development of RA in arthralgia patients, we analysed if a combination of these parameters changed the probability of RA. The analysis was performed on the total (GU + UU) cohort; which was split into 5 groups based on the survivin positivity (S+) and the presence of any one (AB+) or both (AB++) autoantibodies. The absolute levels of survivin (ng/ml, median [IQR]) were similar between the S+AB− (2.35 [0.87–6.60]), S+AB+ (2.26 [0.66–19.68]) and S+AB++ (1.60 [0.81–3.36]) groups. The levels of antibodies were higher in the S+AB+ group compared to S−AB+ (RF: 53.5 [23–340] vs. 37 [21–64], not significant; ACPA: 225 [33.5–4968] vs. 6.75 [4.70–51.5], \(p = 0.012\)). The total frequency of RA cases within the groups is indicated in Fig. 3B.

The Kaplan-Meier plots were constructed for the total cohort (Fig. 3A) and independently for GU and UU cohorts (Fig. 3C). The Cox proportion hazard test revealed that the patients presenting any of these parameters separately (S−AB−, S−AB+) or in combination (S+AB+, S+AB++) had a significantly increased probability of developing RA compared to the group negative for the serological markers (S−AB−). The combination of survivin with both autoantibodies (S+AB++) had higher probability of RA compared to the presence of any of the parameters separately. Indeed, the combination of survivin with one of the autoantibodies (S+AB+) significantly increased the RA risk compared to one antibody alone (S−AB+) \(p = 0.004\). In contrast, the risk of RA in the S+AB+ and S+AB− groups was similar. Here, we observed no effect of the antibody specificity on the outcome. The presence of one autoantibody in the survivin-negative patients (which was RF in 75% case and ACPA in 25%) was associated with minor risk to develop RA (Fig. 3B).

Serological profile defined the time of RA onset in the patients with arthralgia

The analysis of RA-free survival pattern presented in Fig. 3A clearly showed that the time of RA onset differed between the groups. In total, 49% of RA cases developed arthritis within 12 months and 86% developed within 24 months of the follow-up period. The groups that combined survivin and antibodies, S+AB+ and S+AB++, had high frequency of RA onset within the first 12 months, when 78% (10/13) of RA cases were developed.

The Cox proportion hazard test confirmed that the estimated median RA-free survival time was shortest in the S+AB− group and equal to 12 months. The S+AB− group had a slightly delayed RA onset, with only 27% (3/11) cases developed within the first 12 months. This was followed by an intensive RA onset during the period between 12 and 18 months, when 55% (6/11) of the RA cases were developed. In the survivin-negative groups, including S−AB+ and S−AB−, the onset of RA occurred equally throughout
the follow-up period. No accumulation of the RA cases was observed within the early months after the baseline.

The probability of developing arthritis within the $S+\text{AB}^{++}$, $S+\text{AB}^{+}$, $S+\text{AB}^{-}$, and $S-\text{AB}^{+}$ groups during the period between 0 and 48 months is shown in Table 2. The comparison is done with the reference to the $S-\text{AB}^{-}$ group. These observations let us to suggest that the serological profile defines the time of RA onset, where the combination of survivin and antibodies predisposed to shortest RA-free survival for the arthralgia patients. With referral delay, these patients have highest probability to have arthritis at the first visit.

Consequences of referral delay

To model the effect of potential referral delay on the probability of RA-free survival, the baseline was moved forward with the intervals of 6 months reducing the follow-up period. The probability of arthritis within the reduced time interval was calculated separately for each group. In agreement with high frequency of RA within the first 12 month, the Mantel-Cox hazard ratios were highest when the baseline was set at 6 and 12 month (Table 2). The risk for arthritis fell down after the baseline had been moved forward to 18 months or later. Comparison of the RA risk between

<table>
<thead>
<tr>
<th>Group</th>
<th>New RA</th>
<th>Total, n</th>
<th>48 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. S-AB-</td>
<td>7</td>
<td>186</td>
<td>0 0 1 1</td>
</tr>
<tr>
<td>2. S-AB+</td>
<td>6</td>
<td>44</td>
<td>33 11 3.60 [1.28-9.5] 0.025</td>
</tr>
<tr>
<td>3. S+AB-</td>
<td>11</td>
<td>43</td>
<td>0 0 6.80 [2.80-16] 4x10^-5</td>
</tr>
<tr>
<td>4. S+AB+</td>
<td>4</td>
<td>15</td>
<td>10 5 7.09 [2.33-21] 0.0053</td>
</tr>
<tr>
<td>5. S+AB++</td>
<td>9</td>
<td>14</td>
<td>14 14 17.1 [7.76-40] 10^-7</td>
</tr>
</tbody>
</table>

The Mantel-Cox hazard ratios are calculated in relation to $S-\text{AB}^{-}$ group.

Table 2 Probability of arthritis in the referral delay model

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>$S+\text{AB}^{++}$</th>
<th>$S+\text{AB}^{+}$</th>
<th>$S+\text{AB}^{-}$</th>
<th>$S-\text{AB}^{+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$ value</td>
<td>$93.7 \ p &lt; 10^{-5}$</td>
<td>$15.77 \ p &lt; 10^{-5}$</td>
<td>$24.22 \ p &lt; 10^{-5}$</td>
<td>$6.81 \ p = 0.009$</td>
</tr>
<tr>
<td>6-48 m</td>
<td>$74.45 \ p &lt; 10^{-5}$</td>
<td>$13.07 \ p &lt; 10^{-5}$</td>
<td>$26.31 \ p &lt; 10^{-5}$</td>
<td>$4.28 \ p = 0.038$</td>
</tr>
<tr>
<td>12-48 m</td>
<td>$18.22 \ p &lt; 10^{-5}$</td>
<td>$1.73 \ p = 0.11$</td>
<td>$21.63 \ p &lt; 10^{-5}$</td>
<td>$2.99 \ p = 0.08$</td>
</tr>
<tr>
<td>18-48 m</td>
<td>0</td>
<td>$2.58 \ p = 0.11$</td>
<td>$5.22 \ p = 0.022$</td>
<td>$1.72 \ p = 0.19$</td>
</tr>
<tr>
<td>24-48 m</td>
<td>0</td>
<td>0</td>
<td>$3.48 \ p = 0.062$</td>
<td>0</td>
</tr>
<tr>
<td>30-48 m</td>
<td>0</td>
<td>0</td>
<td>$10.6 \ p = 0.001$</td>
<td>0</td>
</tr>
</tbody>
</table>
the groups showed that the S+AB− and S+AB++ groups had significantly increased probability of arthritis at referral delay of 12 months. Additionally, only the S+AB− group continued to have a significantly increased risk for arthritis after 18 months of follow-up.

Discussion

In this prospective population-based follow-up of patients with unexplained arthralgia we show that the risk to proceed into RA is tightly connected with enrichment of oncoprotein survivin in serum of these patients. Indeed, survivin was present in 65% of the new RA cases. On the one side, survivin enlarges the group of patients where the risk of RA may be measured in blood sample. On the other side, it extends awareness of RA risk outside the autoantibody positive subjects. Lastly, it showed that the presence of survivin is essential for the development of arthritis, since neither RF nor ACPA alone increase the risk of RA when present in the survivin-negative context.

The combined presence of survivin and autoantibodies was found in a surprisingly small group of the arthralgia patients (4.6%). The presence of both RF and ACPA was required to reach the highest odds for RA. Here, the patients with combination of survivin and presence of both RF and ACPA had shortest estimated time to RA. This was the only group with the frequency of new RA cases above 50%. Most of RA cases developed within 24 months after the first assessment by rheumatologist, which is in agreement with observations done on ACPA+ patients and reported by the Dutch groups [36,39]. Interestingly, the probability risk in this group was limited over time, since none of the patients from this group developed RA after 18 months. In difference, the survivin-group was limited over time, since none of the patients from this group continued to develop RA after 18 months. In difference, the survivin-group was limited over time, since none of the patients from this group continued to have a significantly increased risk for arthritis after 18 months of follow-up.

The observation that the patients with survivin and autoantibodies continue to have a significantly increased risk for arthritis after 18 months of follow-up may be explained by the change in serological profile of those patients supporting the notion of self-limiting, often reversible nature of molecular events preceding the clinical flare of autoimmunity.

It has been postulated that RA starts years before clinical awareness and may be identified by autoantibody measurement [40–43]. Prospective follow-up of patients with autoantibodies indicated that general risk for RA in those patients is low. The results of the present study advocate survivin to provide first insight in the pre-antibody processes. The arthralgia cohorts in this study were recognized by a high number of patients combining survivin and autoantibodies, while previously reported early RA cohorts have higher proportion of the antibody positive cases among survivin-negative patients [22,30]. The antibody production in RA is less prone to reverse [44,45] and may result in accumulation of the antibody-producing patients. From the prospective presented above we suggest that survivin occurs at the earlier phase of disease development followed by autoantibody production (Fig. 4). The observations done in the present study suggest that persistence of high serum survivin levels is critical for onset of RA. If consistently high, survivin-positive patients acquire antibodies and this increases probability for the development of RA. If survivin is lost and patients turn S−AB− or S−AB+, the risk for RA development decreases. This is in agreement with the view of antibodies as a catalysing factor for RA [40,42]. We further developed this concept and show that the catalytic effect of antibodies works under the conditions of high serum survivin. If to assume that survivin originates from the autoantibody producing cells of the bone marrow, which have been attacked and killed by CD8+ T-cells [25], a simultaneous measure of survivin and autoantibodies indicates a phase when the protective elimination of the auto-reactive cells fails and leaves a free path for RA flare. This hypothetical course of events associated with serum survivin is built on observations retrieved from a cohort study where only a single-time survivin measurement is available. Serial measurement of survivin in patients with arthralgia is essential to confirm this hypothesis.

The prevalence of RA cases in Gothenburg is comparable with other prospective studies on unexplained arthralgia [36,37,39]. This was unexpected due to the fact that the inclusion strategy for the study was not selective by the presence of autoantibodies and permitted inclusion of a broader group of arthralgia patients. Despite a comparable number of patients, Gothenburg and Umeå differed with respect to frequency of serological risk factors, and consequently with respect to frequency of RA cases. Patient-dependent factors as well as the distance to the rheumatology centre have been identified among the common reasons contributing to a delayed healthcare seeking at onset of the symptoms in RA patients [46–48]. A delay in help seeking could have lead to the natural pre-selection of arthralgia patients by late referral from primary care physician to rheumatologist. In this case, the group with high probability could have developed clinical RA before their first visit. The model of late referral has been tested in the Gothenburg cohort by moving the hypothetical first visit forward. This reproduced the RA development pattern of Umeå by draining the cohort of RA cases. Alternative reason for the distinct serological profile of the Umeå cohort could be the enrichment with spondyloarthritis including psoriatic arthritis among the individuals with arthralgia.

One would ask if the obtained results are sufficient to justify screening of survivin in individuals with arthralgia. The RA risk in
the survivin-positive patients are not inferior compared to those in the antibody-positive patients. Additionally, survivin covers a broader risk group and it increases precision for RA in the antibody-positive individuals. Being a step ahead of antibody detection, survivin measurement provides time for potential reversion of aberrant immunity, which has been shown of no success at the later antibody-producing step of the disease. Targeting preclinical cellular events could in theory prevent development of RA. Recent preventive treatment attempts applied to arthralgia patients have been focused on the antibody-producing group. A single centre study of intramuscular corticosteroids in antibody-positive arthralgia patients showed a modest reduction in autoantibody levels and could not delay development of arthritis [49]. A different study with B-cell depleting strategy using rituximab caused a delay but did not stop flare of clinical arthritis in arthralgia patients [50]. The achieved delay of arthritis onset in preclinical RA is similar to the arthritis improvement period achieved by rituximab in the established RA [51,52]. The experience of these studies fosters disbelief that B-cell depletion is sufficient to abrogate onset of RA once the process has advanced to the autoantibody production. Pathological events preceding autoantibody production have not been previously addressed in the clinical setting. In experimental setting, we have shown that inhibition of survivin at first signs of arthritis reduces the formation of antigen-specific and autoantibodies in parallel with alleviation of arthritis [53,54]. Therefore, measuring survivin adds new dimension to early recognition and monitoring of patients at risk of developing RA. It also increases diagnostic precision favourable for preventive care strategies potentially reducing medical costs.

**Declarations**

**Ethics approval:** The study was reviewed and approved by the Ethics Committees of Gothenburg and Umeå.

**Consent for publication:** Not applicable

**Availability for data and materials:** The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.


