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Survivin as a predictive biomarker for rheumatoid arthritis

Degree Project in Medicine

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Programme in Medicine

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Abstract

Degree Project in Medicine: Survivin as a predictive biomarker for rheumatoid arthritis – Elmira Sarreshtedari.

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Background: Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation and joint destruction. In patients with established RA, high levels of the onco-protein survivin are associated with an active and therapy-resistant disease. Results of previous studies suggest that survivin can be detected early in upcoming RA and may precede arthritis-specific antibody production.

Aim: To investigate if survivin measures in serum of patients with arthralgia help identifying individuals at risk of developing RA.

Material: Out of 2031 first-visit patients at the Rheumatology Clinic, Sahlgrenska University Hospital between July 2018 and June 2019, medical records of 201 arthralgia patients were reviewed. For patients who developed arthritis during the median follow-up duration of 15 months, the 2010 EULAR/ACR criteria for RA were applied. Survivin positive and negative patients were compared according to inflammatory markers, autoantibodies and Clinically Suspect Arthralgia (CSA) characteristics. Patients who developed arthritis and remaining patients were compared according to inflammatory markers, autoantibodies and CSA characteristics.

Results: 63 of 201 patients (31.3%) had high levels of survivin in serum. Survivin+ patients had significantly lower total CSA score than survivin- patients ($p = 0.0012$). 12 of 201 (6 %) developed arthritis, and half of these patients had indicated definitive RA according to the

EULAR RA criteria. No association was found between survivin levels and the later development of arthritis ($p = 0.83$). There was no significant gender difference in developing new arthritis ($p = 0.28$). In patients with new developed arthritis, clustering was found between number of affected small joints and ACPA, EULAR RA criteria, age and CSA score. Survivin and inflammatory markers (ESR, CRP, WBC, platelet count, IFN-γ) clustered with large joints.

Conclusions: Measurement of survivin in serum could not prospectively predict development of RA in arthralgia patients.

Key words: Rheumatoid arthritis, arthralgia, survivin

Abbreviations

ACPA: Anti-citrullinated protein antibodies

CI: Confidence interval

CRP: C-reactive protein

CSA: Clinically suspect arthralgia

ELISA: Enzyme-linked immunosorbent assay

ESR: Erythrocyte sedimentation rate

EULAR: European League Against Rheumatism

IFN-γ: Interferon-gamma

IQR: Interquartile range

OR: Odds ratio

RA: Rheumatoid arthritis

RF: Rheumatoid factor

WBC: White blood cell

Background

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation, cartilage and bone destruction. Typical disease presentation involves pain, swelling and morning stiffness in multiple joints, especially small hand joints. Seropositive patients have a presence of RA-specific autoantibodies; rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA). Presence of autoantibodies in combination with elevated C-reactive protein level (CRP) or erythrocyte sedimentation rate (ESR) increases the likelihood of a RA diagnosis. Both these parameters are used in the recent classification criteria of RA proposed by The European League Against Rheumatism (EULAR) in 2010. The lifetime prevalence of RA is up to 1 percent worldwide. RA is three times more common in women than in men. The highest prevalence is found in women older than 65 years. [1-3]

The underlying cause for developing RA is not known. Risk of developing RA can be attributed to both genetic factors and environmental risk factors. Genetic factors stand for 50 % of the risk, and are particularly associated with ACPA-positive RA. A family history of RA increases the risk of acquiring the disease. The heritability is estimated to be higher in seropositive patients (40 - 65 %) than in seronegative patients (20 %). Smoking is the main environmental risk factor for ACPA-positive RA. [2, 4]

It has been postulated that autoantibodies can be present years before disease onset in some patients [5]. Approximately 50 % of RA patients have detectable autoantibodies (RF, ACPA or both). RF was the first autoantibody considered to be characteristic for RA and is directed against the fragment crystallizable region (Fc-region) of the IgG antibody. ACPA was

discovered later and stands for antibodies directed against citrullinated peptides. ACPA is present in 50 % of RA patients and has a high specificity for RA. [1, 6]

RA involves an autoimmune response causing joint damage. Immune activation causes inflammation in the synovial membrane, resulting in chemotaxis and infiltration of leukocytes into the synovial compartment, leading to swelling of joints. The leukocytes in synovitis consist of both innate immune cells such as monocytes, dendritic cells, mast cells and adaptive immune cells, including T-helper cells, B cells and plasma cells. ACPAs can activate macrophages, or activate osteoclasts by creating immune complexes that involve Fc-receptors, causing bone loss. RF is more directly involved in activation of macrophages and cytokines. [4]

From an individual perspective, disease burden involves musculoskeletal deficits, decreased physical function and quality of life. Patients at risk for a severe RA disease; those with the presence of autoantibodies, persistently high disease activity and early joint damage at radiographic examination, benefit from an early diagnosis. By doing so, treatment can be initiated early, which reduces and controls inflammation and prevents damage progression. Moreover, the socio-economic costs for treatment of RA and its co-morbidities are high. Health care costs due to functional disability and reduced work capacity of RA patients are considerable [4]. Finding those individuals at high risk of developing RA would therefore mean significant advantages both for the individual patient and for society.

Survivin in cell pathology and pathophysiology of RA

Survivin is an intracellular protein that functions as an inhibitor of apoptosis. Survivin located in the nucleus regulates cell division and micro-RNA synthesis, while survivin in mitochondria and cytoplasm inhibits apoptosis. Survivin is associated with cell proliferation and tissue growth, particularly tumorigenesis. Inflammation, hypoxia and smoking enhances the expression of survivin. In inflammatory conditions, survivin boosts antigen presentation, supports formation and propagation of autoreactive cells and maintains production of autoantibodies [7, 8]. Although survivin is an intracellular protein, it can be measured in serum and synovial fluid of RA patients. Survivin is highly expressed in inflammatory synovial tissue, which may stand for its role in the pathology of RA [9]. Over 50 % of RA patients have high survivin levels in serum during their first visit to a rheumatologist. This is in turn associated with a therapy-resistant disease at prospective follow-up [10]. In addition, it has been shown that survivin acts as a prognostic marker for radiographic skeletal destruction and functional disability in patients with RA [11, 12]. Survivin was detected early in RA patients. According to previous studies its extracellular release could precede autoantibody production. Patients with the combined presence of survivin and autoantibodies had the shortest expected time to RA [13].

Survivin in serum has been tightly associated with other risk factors for RA, such as HLA-DRB1 gene carriage and the presence of RF and ACPA autoantibodies [14]. Patients who have circulating autoantibodies (RF or ACPA), are susceptible to more severe symptoms, persistence of active RA disease, poor response to antirheumatic therapy and increased mortality [4]. Therefore, survivin is an interesting target for further investigation of its predictive potential for RA, to aid early recognition of patients at risk of progress to RA.

Transition from Arthralgia to RA

Clinical arthritis is often preceded by arthralgia, which entails joint pain that cannot be explained by swelling of muscles and tendons around the joints. Therefore, characterization of musculoskeletal symptoms has been suggested to increase the likelihood of identifying individuals at risk of developing RA. [15, 16]

Patients with clinically suspect arthralgia (CSA) are considered to be at increased risk of developing RA. These patients only comprise a small portion of all arthralgia patients on a first visit to the rheumatology clinic (~7 %), and a larger percentage develop RA during follow-up (~20 %) [15]. This is the case for patients with both arthralgia and autoantibodies, not for antibody-negative arthralgia. Hence, the European League Against Rheumatism (EULAR); an expert committee of European rheumatologists, other health professionals and patients, has proposed a set of clinical characteristics to define arthralgia patients who are at risk for progression to RA, also known as clinically suspect arthralgia. These characteristics could be applied in a preclinical phase of RA. They are combined into 16 clinical and demographic parameters, and include both history taking and physical examination. Each parameter fulfilled generates one point. The presence of ≥ 3 parameters indicate arthralgia at risk of RA with high sensitivity, and the presence of ≥ 4 parameters provides a high specificity. It was expected that the clinical characteristics in combination with other factors such as autoantibodies or imaging results, are necessary to identify patients with upcoming RA. The 16 parameters are presented in Table 1. [17]

Table 1. 16 clinical parameters defining clinically suspect arthralgia (CSA). Each parameter fulfilled generates one point. [17]

History taking		Physical examination
1. Joint symptoms <1 year	7. Duration of morning stiffness ≥60 min	13. Local tenderness involved joints at physical examination
2. 4 - 10 joints with symptoms	8. Most severe symptoms in the early morning	14. Positive squeeze test of MCP joints
3. Symptoms in MCP joints	9. Improvement of symptoms during the day	15. Positive squeeze test of MTP joints
4. Symptoms in MTP joints	10. Increasing number of joints with symptoms over time	16. Difficulty making a fist
5. Symptoms in several small joint regions	11. Patient experience of swelling of small hand joints	
6. Symmetric symptoms or signs	12. Presence of a first-degree relative with RA	

MCP joints, metacarpophalangeal joints; MTP joints, metatarsophalangeal joints; Positive squeeze test, pain in compression of the joint area; RA, rheumatoid arthritis.

Diagnostic aid for RA

There are no diagnostic criteria for rheumatoid arthritis. While the typical patient presents with pain, swollen, morning stiff joints and abnormal inflammatory laboratory tests, this presentation is not specific to RA. Other forms of arthritis such as reactive arthritis, psoriatic arthritis and infectious arthritis cannot be precluded. A diagnosis of undifferentiated arthritis is therefore made in patients where no specific diagnosis can be made [4]. In 2010, the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) renewed the classification criteria for RA. The classification criteria aim to ease early diagnosis of RA, enabling therapeutic intervention at an early stage and further prevention of irreversible joint damage. The criteria can be applied on patients with at least one swollen joint, where other diseases cannot explain the clinical symptoms. The classification criteria take into consideration the extent of joint involvement, presence of serological markers (RF and ACPA), symptom duration and inflammatory markers. It is

important to note that the classification criteria can support a diagnosis of RA, but are not synonymous with a diagnosis. The 2010 EULAR/ACR criteria for RA are presented in Table 2. A maximum of 5 points can be generated for swollen or tender joints, 3 points for autoantibodies, 1 point for acute phase reactants and 1 point for duration ≥ 6 weeks. A score of ≥ 6/10 points indicates definitive RA. [3]

Table 2. The 2010 EULAR/ACR classification criteria for RA. A maximum of 5 points can be generated for swollen or tender joints, 3 points for autoantibodies, 1 point for acute phase reactants and 1 point for duration ≥ 6 weeks. A score of ≥ 6/10 points indicates definitive RA. [3]

2010 RA criteria	**Score**
1 large swollen/tender joint	0 p
2-10 large swollen/tender joints	1 p
1-3 small swollen/tender joints	2 p
4-10 small swollen/tender joints	3 p
> 10 swollen/tender joints, at least 1 small joint	5 p
Low positive RF or ACPA	2 p
High positive RF or ACPA	3 p
Abnormal level of CRP/ESR	1 p
Symptom duration ≥ 6 weeks	1 p

ACPA, antibodies to citrullinated peptides; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; EULAR/ACR RA criteria, European League Against Rheumatology/American College of Rheumatology Rheumatoid arthritis criteria; RA, rheumatoid arthritis; RF, rheumatoid factor.

Aim

The primary aim of this study is to investigate if survivin measures in patients with arthralgia help identifying individuals at risk of developing RA. Further, to compare clinical characteristics, symptomatology and laboratory markers of arthralgia patients with high and low levels of serum survivin. Also, to examine the adequacy of the CSA characteristics in detecting patients who are at risk of developing RA.

Material and methods

Selection of study participants

Initially, 2031 patients who had been on a first visit to a rheumatologist at the Rheumatology Clinic at Sahlgrenska University Hospital between July 1st 2018 and June 30th 2019 were identified. Patients with any form of arthritis including rheumatoid arthritis, psoriatic arthritis, unspecified arthritis, polyarthritis, monoarthritis and juvenile arthritis, or other rheumatic disease including giant cell arteritis, systemic lupus erythematosus and polymyalgia rheumatic were excluded. All patients with local musculoskeletal problems such as tendinitis, muscle rupture or degenerative disease such as gout or osteoarthritis were also excluded. To be included, patients with a diagnosis of arthralgia required a medical record of the first visit, a blood sample adjacent to that visit, as well as at least one measurement of RF and ACPA on the blood sample. Finally, the 201 remaining arthralgia patients were enrolled in the observational patient cohort and followed for 9-21 months to see if they developed arthritis (Figure 1).

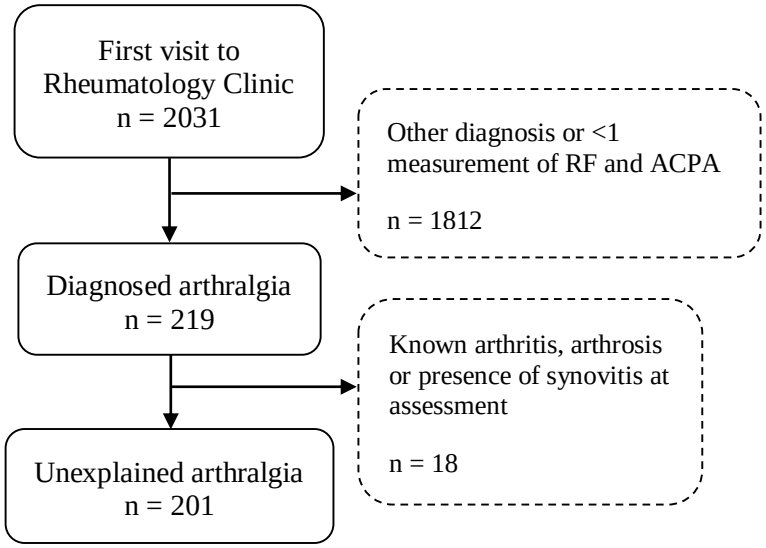

Figure 1. Flow chart diagram of the selection of patients into the study cohort. Out of 2031 first-visit patients, patients with diagnosed arthralgia and at least one measurement of RF and ACPA were included. Patients with other diagnoses were excluded. 201 arthralgia patients were chosen for an observational patient cohort. ACPA, antibodies to citrullinated peptides; RF, rheumatoid factor.

Collection of Clinical Data

Medical records of first visits were reviewed according to the EULAR defined characteristics describing arthralgia at risk for RA (Table 1). Subsequently, all clinical visits visits during a follow-up duration of 9-21 months were reviewed. If joint swelling (arthritis) appeared at a prospective visit, the RA Classification Criteria were applied on the records of the clinical visit with present arthritis (Table 2). Information regarding each patient's smoking history was collected from medical records and defined as current, previous or never-smoker at the time of assessment.

Laboratory measurements

ACPA and RF

ACPA and RF were measured at the accredited laboratories of Clinical Immunology at Sahlgrenska University Hospital. ACPA was detected using an automatic multiplex method (anti-CCP2, BioRad, Hercules, CA). The cut-off level for ACPA positivity was set to 3.0 U/ml. RF was measured by fluoroenzyme immunoassay using Phadia ImmunoCAP 250. The cut-off level for RF positivity was >5 kIU/L for RF-IgM, >20 kIU/L for RF-IgA and >40 kIU/L for RF-IgG. For analysis, RF was considered either positive (if at least one isotype was positive) or negative. For this reason, no quantitative measures were performed on RF data.

CRP and ESR

Serum levels of CRP were measured at the Laboratory of Clinical Chemistry at Sahlgrenska University Hospital, by nephelomety (Beckman Immage 800). The cut-off level for CRP positivity was set to 5 mg/L.

ESR was measured at the Laboratory of Clinical Chemistry at Sahlgrenska University Hospital, using the Westergren method in mm/hour. Pathological ESR levels for females < 50

years was set to ≥ 20 mm/h and for females ≥ 50 years to ≥ 28 mm/h. For males < 50 years pathological ESR levels was set to ≥ 13 mm/h and for males ≥ 50 years to ≥ 20 mm/h.

White blood cell and platelet count

White blood cell counts and platelet counts in blood were measured at the Laboratory of Clinical Chemistry at Sahlgrenska University Hospital using particle count with optical measurement. The reference range for white blood cell count was 3,5 - 8,8 10^9/L. The reference range for platelet count was 165 – 387 10^9/L for women, and 145 – 348 10^9/L for men.

Survivin and IFN-γ

Survivin levels were measured in serum of first-visit patients using a sandwich enzyme-linked immunoassay, ELISA (DY647, R&D Systems, Minneapolis, MN). Serum samples were diluted 1:10. The cut-off level for survivin positivity was set to 450 pg/ml, according to the mean + 3SD of 104 healthy individuals [18].

IFN-γ levels were measured in serum using a sandwich enzyme-linked immunoassay, ELISA (M1933, Sanquin, Amsterdam, The Netherlands). Serum samples were diluted 1:3. The cut-off level for IFN-γ positivity was set to 10.2 pg/ml, corresponding to the lowest value in the highest tertile of measured samples on included patients.

Statistical methods

Statistical analysis was performed using Graphpad Prism (version 8.4.2, San Diego, CA) and www.openepi.com software for Chi-square tests with two-by-two tables. The non-parametric Mann-Whitney U test was used for comparing continuous data. Chi-square tests were used for analysis of categorical data. A heat map was created in R using the non-parametric Spearman algorithm. A two-tailed p-value of < 0.05 was considered statistically significant. Information regarding RF was missing in 11 arthralgia patients, thus the missing data was replaced using imputation by SPSS (version 25, Chicago, IL) software to generate substitute values. Data is presented as median and minimum to maximum values.

Ethics

The study is approved by the Ethical Review Board of Gothenburg (registration number T446-18/257-13, exp 2018-06-27), and in line with the Declaration of Helsinki. Ethical permissions for reviewing medical records for research purposes was acquired from the head of operations at the Rheumatology Clinic at Sahlgrenska University Hospital through written approval. Patient data were handled according to current legislations in Sweden and Data Protection Authority, biobank law and privacy laws were strictly followed.

Results

Characteristics of the study cohort

Characteristics of the arthralgia patients included in the study cohort are presented in Table 3. The study cohort consisted of 201 patients, the majority (72.6 %) were women and the remaining 27.4 % were men. The median age for all patients was 48 years (inter-quartile range 36 - 57). The proportion of patients with autoantibodies (ACPA and/or RF) was 19.4 %. Sixty-three of 201 (31.3%) had high levels of survivin in serum and comprised the survivin+ group. 25.4 % of the survivin+ group were ACPA and/or RF positive. A smaller share (2.5 %) had the simultaneous presence of survivin, RF and ACPA.

Table 3. Characteristics of the arthralgia patients.

Characteristics	**All patients** ***n* = 201**	***Survivin* +** ***n = 63***	***Survivin* –** ***n = 138***
Women, n (%)	146 (72.6)	50 (79.4)	96 (69.6)
Men, n (%)	55 (27.4)	13 (20.6)	42 (30.4)
Age, years, Median [IQR]	48 [36 - 57]	50 [37.5 – 57.5]	46.5 [36 - 57]
Current smoker, n (%)	25/150 (16.7)	9/42 (21.4)	16/108 (14.8)
Former smoker, n (%)	34/150 (22.7)	12/42 (28.6)	22/108 (20.4)
Increased CRP or ESR, n (%)	64/188 (34)	22/55 (40)	42/133 (31.6)
Increased CRP, n (%)	46/189 (24.3)	15/56 (26.8)	31/133 (23.3)
Increased ESR, n (%)	44/190 (23.2)	17/57 (29.8)	27/133 (20.3)
ACPA-positive and/or RF-positive, n (%)	39 (19.4)	16 (25.4)	23 (16.7)
RF-positive, n	25	12	13
ACPA-positive, n	23	9	14
ACPA and RF positive, n (%)	9 (4.5)	5	4
IFN-γ positive, n	67	19	48

ACPA, antibodies to citrullinated peptides; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IFN-γ, interferon-gamma; IQR, inter-quartile range; RF, rheumatoid factor.

Survivin in relation to clinical features of arthralgia patients

Comparisons between survivin+ and survivin- groups were performed. The groups were similar by age, as shown in Figure 2. In the survivin+ group, the median age was 50 years (range 22 – 79), which was similar to the survivin- group where the median was 46.5 years (range 20 – 85).

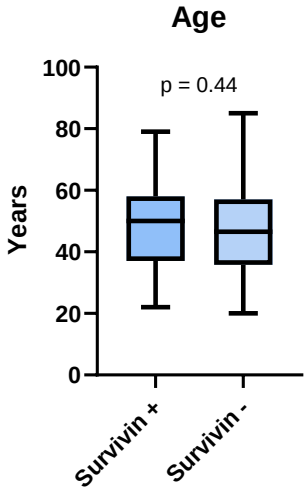

Figure 2. Age range of patients who were survivin positive and survivin negative.

There was no significant difference between levels of CRP and ESR in the survivin+ and survivin- groups (p = 0.79 respectively p = 0.22, Figures 3 and 4). In the survivin+ group, the median CRP levels were 2 mg/L (range 0 – 53) which was similar to CRP levels of the survivin- group (2 mg/L, range 0 – 72). The ESR levels in survivin+ patients range between 1 – 79 mm/h and in survivin- patients between 1 – 107 mm/h, both with a median of 10 mm/h.

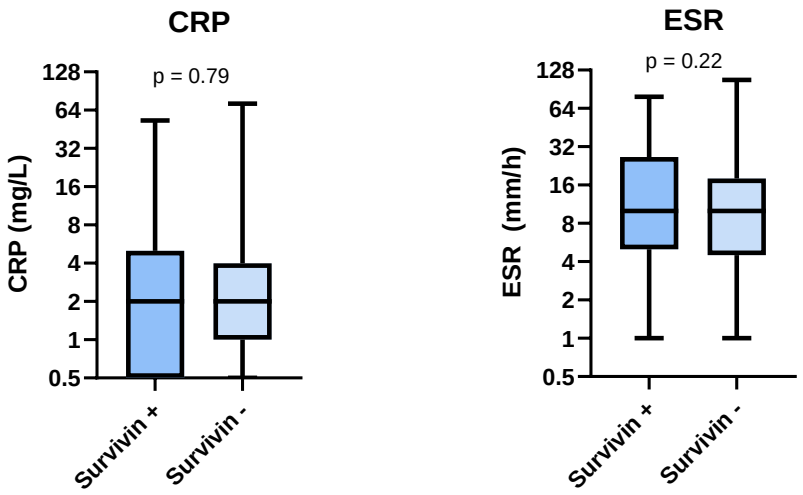

Figure 3, 4. Levels of CRP (Figure 3, left) and ESR (Figure 4, right) in survivin positive and negative patients. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

The odds ratio of being survivin positive in relation to selected clinical characteristics was calculated with a 95% confidence interval (Figure 5). Odds ratio above 1 indicates that the characteristic is reported more frequent in survivin+ patients. There was a tendency of higher odds to be RF positive in the survivin+ group (p = 0.067, OR 2.25 [95 % CI 0.95 - 5.35]). There was no significant difference in gender composition between the groups as there was no observed higher prevalence of female gender in the survivin+ group (p = 0.15, OR 1.68 [95 % CI 0.83 – 3.51]). No significant difference in serum levels of ESR, IFN-γ, ACPA, and CRP was found between the survivin+ and survivin- groups.

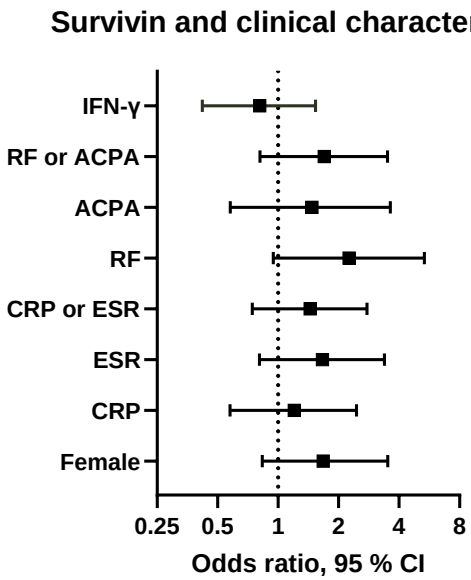

Figure 5. Clinical characteristics in survivin positive and survivin negative arthralgia patients. Odds ratio above 1 indicates that the characteristic is reported more frequent in survivin+ patients. ACPA, antibodies to citrullinated peptides; CI, Confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IFN-γ, interferon-gamma; RF, rheumatoid factor.

Survivin and CSA characteristics

The analysis of clinical characteristics of arthralgia was started by calculating the percentage of data loss for each of the 16 CSA characteristics in all 201 arthralgia patients. Information regarding positive squeeze test in MCP and MTP (CSA 14, CSA 15) and difficulty making a fist (CSA 16), were absent in medical records of at least 50 % of the patients (Figure 6). Questions 14, 15 and 16 with data loss above 50 % were therefore excluded from the analysis. Scores derived from the CSA characteristics were compared among the survivin positive and negative patients. Survivin+ patients had significantly lower total CSA score than survivin- patients ($p = 0.0012$, Figure 7).

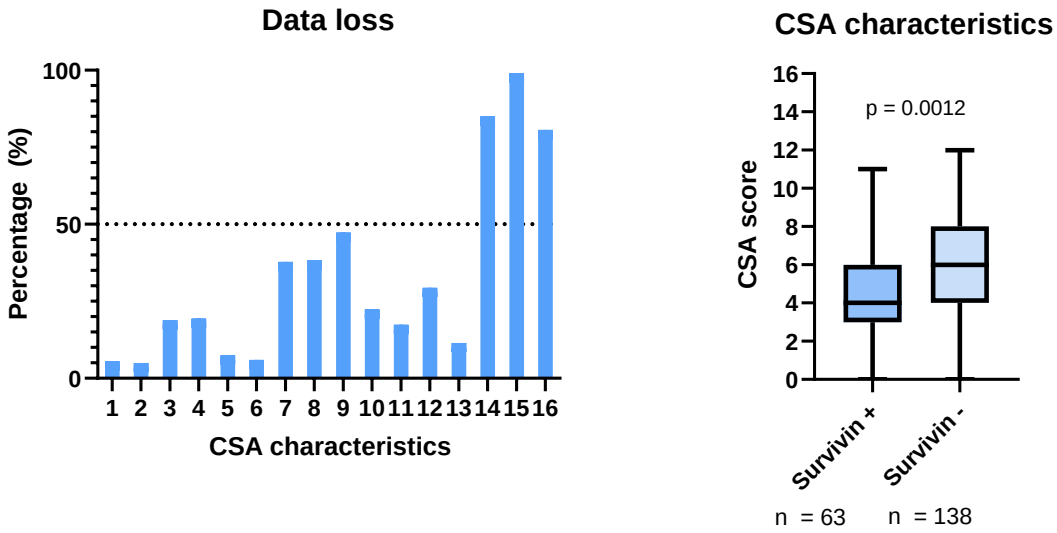

Figure 6, 7. The percentage of data loss regarding the 16 CSA characteristics is illustrated in Figure 6 (left). Figure 7 (right) shows a comparison of CSA scores among survivin positive and negative patients. (1) Joint symptoms onset <1 year, (2) 4–10 joints with symptoms, (3) Symptoms in MCP joints, (4) Symptoms in MTP joints, (5) Symptoms in several small joint regions, (6) Symmetric symptoms and signs, (7) Duration of morning stiffness >60min, (8) Most severe symptoms in the early morning, (9) Improvement of symptoms during the day, (10) Increasing number of joints with symptoms over time, (11) Patient experience of swelling of small hand joints, (12) Presence of a first-degree relative with RA, (13) Local tenderness involved joints at physical examination, (14) Positive squeeze test of MCP joints, (15) Positive squeeze test of MTP joints, (16) Difficulty making a fist. CSA, Clinically suspect arthralgia.

Individual CSA characteristics were compared between survivin positive and negative patients and an odds ratio (OR) with a 95% confidence interval (CI) was calculated for each characteristic. OR below 1 indicates that the characteristic is reported less frequent in survivin+ patients. It was significantly less common for survivin+ patients to have symptoms in several small joint regions (CSA 5), symmetric symptoms (CSA 6), morning symptoms (CSA 8), improvement of symptoms during the day (CSA 9) and increasing number of joints with symptoms (CSA 10) (Figure 8).

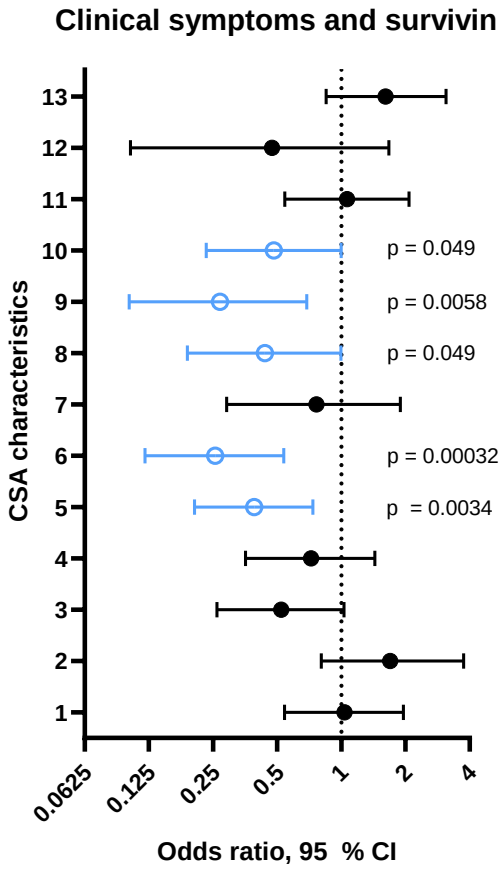

Figure 8. Prevalence of clinical symptoms of arthralgia in survivin positive patients. The prevalence is shown as odds ratio between the survivin positive (n = 63) and survivin negative (n = 138) groups. Odds ratio below 1 indicates that the characteristic is reported less frequent in survivin+ patients. Significant values have open circles. (1) Joint symptoms onset <1 year, (2) 4–10 joints with symptoms, (3) Symptoms in MCP joints, (4) Symptoms in MTP joints, (5) Symptoms in several small joint regions, (6) Symmetric symptoms and signs, (7) Duration of morning stiffness >60min, (8) Most severe symptoms in the early morning, (9) Improvement of symptoms during the day, (10) Increasing number of joints with symptoms over time, (11) Patient experience of swelling of small hand joints, (12) Presence of a first-degree relative with RA, (13) Local tenderness involved joints at physical examination. CI, Confidence interval; CSA, Clinically suspect arthralgia.

Characteristics of patients who developed new arthritis

The follow-up period had a median of 15 months (IQR 12-18 months). During the follow-up period, 12 of 201 patients (6 %) developed arthritis. All patients developed arthritis within 10 months, and 42 % did so within 3 months. 6 of 12 (50 %) patients received the RA score of ≥

6 points, indicating definitive RA according to the classification criteria of EULAR. Characteristics of patients who developed arthritis are presented in Table 4. The majority (58.3 %) of patients who developed arthritis were women. They were also often ACPA and/or RF positive (50 %). The prevalence of new developed arthritis was higher among men (5/55, 9 %) compared to women (7/146, 5 %), however there was no significant gender difference (Figure 12). A third of patients with new arthritis were survivin-positive.

Table 4. Characteristics of patients with new developed arthritis.

Characteristics	***n* = 12**
Women, n (%)	7 (58.3)
Men, n (%)	5 (41.7)
Age, years, Median [IQR]	50.5 [44.5 – 56.25]
Current or former smoker, n (%)	3/8 (37.5)
Increased CRP or ESR, n (%)	5 (41.7)
Increased CRP, n (%)	4 (33.3)
Increased ESR, n (%)	4 (33.3)
Survivin-positive, n (%)	4 (33.3)
ACPA-positive and/or RF-positive, n (%)	6 (50)
RF-positive, n	4 (33.3)
ACPA-positive, n	5 (41.7)
IFN-γ-positive, n	3 (25)
≥ 6 points in EULAR RA criteria	6 (50)

ACPA, antibodies to citrullinated peptides; CRP, C-reactive protein; EULAR RA criteria, European League Against Rheumatology Rheumatoid arthritis criteria; ESR, erythrocyte sedimentation rate; IFN-γ, interferon-gamma; IQR, inter-quartile range; RF, rheumatoid factor.

Patients who developed new arthritis in relation to clinical features

The age range of patients who developed arthritis compared to patients who did not develop arthritis are presented in Figure 9. There was no significant association between development of arthritis and age ($p = 0.41$).

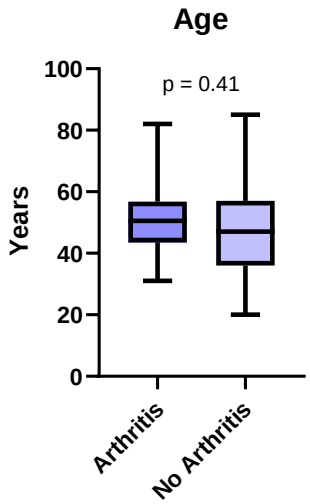

Figure 9. Age range of patients who developed arthritis and patients who did not develop arthritis.

ACPA levels and survivin levels of patients who developed arthritis were compared with the remaining arthralgia group (Figure 10, 11). The results showed that there was a significant difference in ACPA (p = 0.0014), but not in survivin levels (p = 0.83) between those groups.

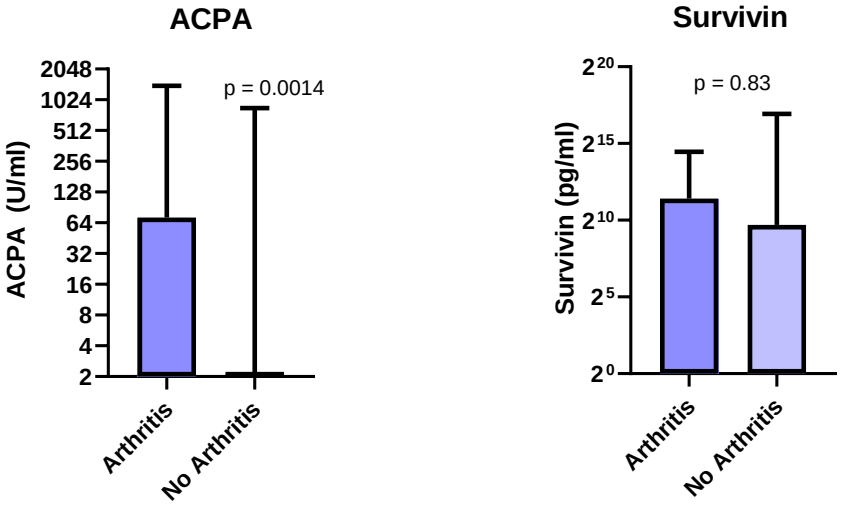

Figure 10, 11. Levels of ACPA (Figure 10, left) respectively survivin (Figure 11, right) in patients who developed and did not develop arthritis. ACPA, antibodies to citrullinated peptides.

The odds of developing arthritis in relation to different clinical characteristics are presented in Figure 12. The results showed that ACPA positivity was associated with significantly higher risk of developing arthritis (p = 0.0065, OR 6.67 [95 % CI 1.77 - 23.87). The same association was also found for the arthralgia patients with any arthritis specific antibody (RF and/or ACPA positivity) (p = 0.016, OR 4.68 [95 % CI 1.35 - 16.25). The results also revealed a tendency toward significant risk of arthritis development in RF positive patients (p = 0.055, OR 3.96 [95 % CI 0.97 - 14.31). There was no significant increase in risk of developing arthritis for females, and patients with high levels of survivin, IFN-γ, ESR and/or CRP.

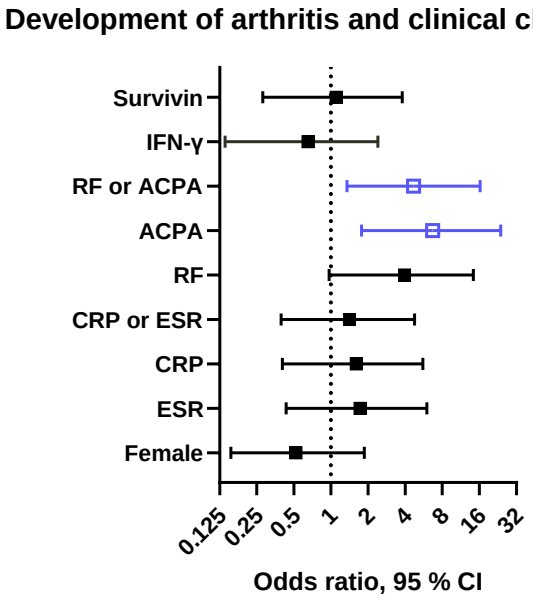

Figure 12. Clinical characteristics in relation to odds of developing arthritis. Odds ratio above 1 indicates that the characteristic is reported more frequent in patients who developed arthritis. Significant values have open squares. ACPA, antibodies to citrullinated peptides; CI, Confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IFN-γ, interferon-gamma; RF, rheumatoid factor.

The total CSA score was compared between patients who developed arthritis and the remaining arthralgia group. No significant difference was found in CSA scores between these groups (p = 0.88, Figure 13). Individual CSA characteristics were compared between patients

who developed arthritis and remaining patients who did not develop arthritis (Figure 14). An odds ratio (OR) with a 95% confidence interval (CI) was calculated for each characteristic. There was a significant association between having 4-10 joints with symptoms (CSA 2) and development of arthritis (p = 0.027). There was no significant difference between patients who developed and did not develop arthritis in remaining characteristics.

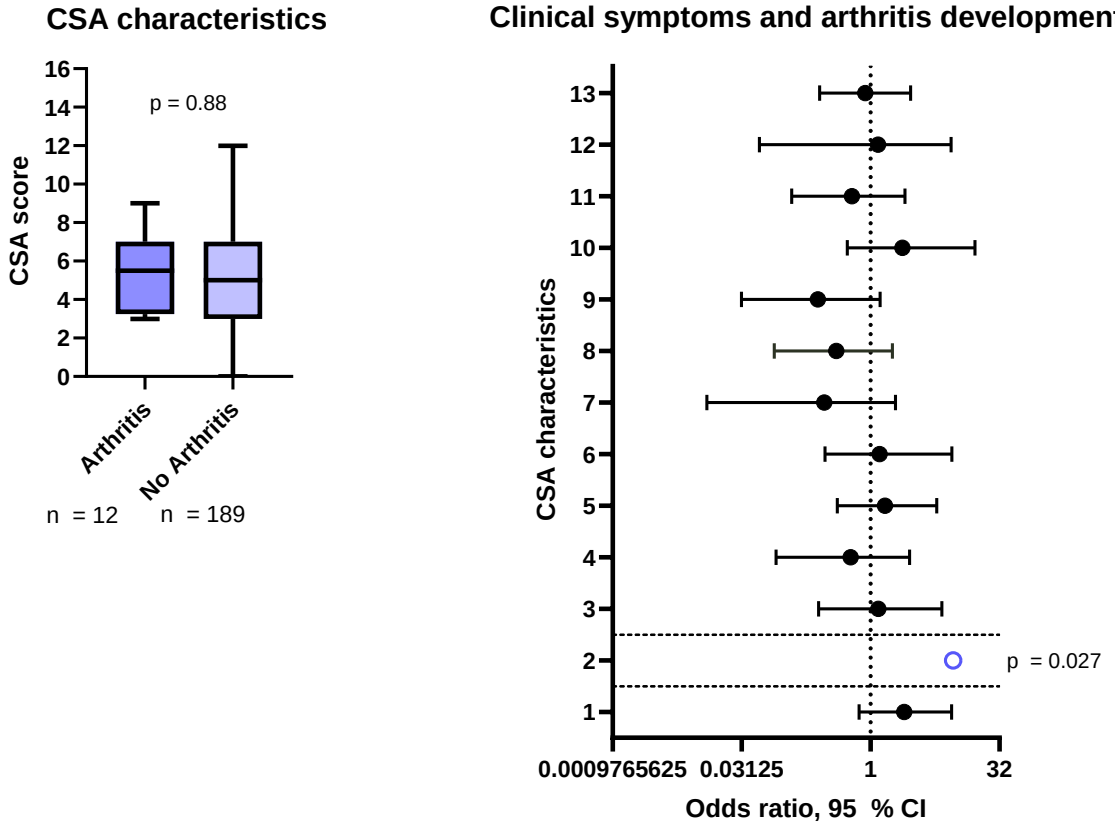

Figure 13, 14. A comparison of CSA scores among patients who developed arthritis and did not develop arthritis are illustrated in Figure 13 (left). Figure 14 (right) presents odds ratio between patients who developed arthritis (n = 12) and did not develop arthritis (n = 189) for the CSA characteristics 1-13. Odds ratio above 1 indicates that the characteristic is reported more frequent in patients who developed arthritis. CSA 2 has an open circle representing a significant odds ratio, however no confidence interval was calculated since a cell value was equal to zero. (1) Joint symptoms onset <1 year, (2) 4–10 joints with symptoms, (3) Symptoms in MCP joints, (4) Symptoms in MTP joints, (5) Symptoms in several small joint regions, (6) Symmetric symptoms and signs, (7) Duration of morning stiffness >60min, (8) Most severe symptoms in the early morning, (9) Improvement of symptoms during the day, (10) Increasing number of joints with symptoms over time, (11) Patient experience of swelling of small hand joints, (12) Presence of a first-degree relative with RA, (13) Local tenderness involved joints at physical examination. CI, Confidence interval; CSA, Clinically suspect arthralgia.

The frequency of RA classification according to the EULAR/ACR RA criteria among patients who developed arthritis is illustrated in Figure 15. All patients fulfilled the criterion considering symptom duration of > 6 weeks (RA criteria 8). Collectively, symptoms in small joints (RA criteria 3, 4) were present in nearly 75 % of patients, thus being more frequent than symptoms in large joints (RA criteria 1, 2). At least 50 % of patients had high RF or ACPA levels (RA criteria 7) as well as abnormal CRP or ESR levels (RA criteria 9). All seropositive patients (RF or ACPA) had high levels of autoantibodies (RA criteria 7), as opposed to low levels of RF or ACPA (RA criteria 6). Having symptoms in > 10 joints was not typical (RA criteria 5).

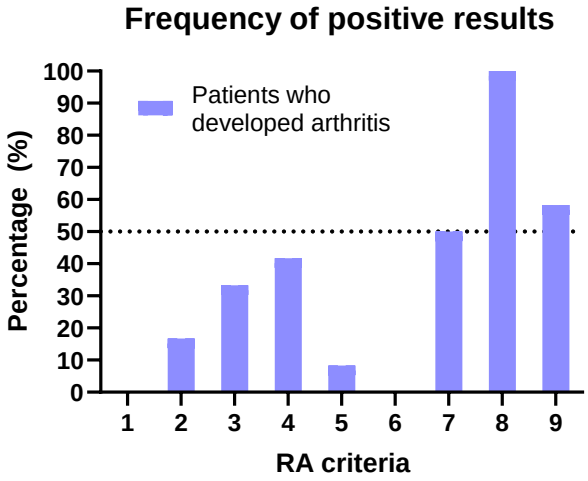

Figure 15. Percentage of positive results according to the EULAR/ACR RA criteria in patients with new developed arthritis. (1) 1 large swollen/tender joint, (2) 2-10 large swollen/tender joints, (3) 1-3 small swollen/tender joints, (4) 4-10 small swollen/tender joints, (5) > 10 swollen/tender joints, at least 1 small joint, (6) Low positive RF or ACPA, (7) High positive RF or ACPA, (8) Symptom duration ≥ 6 weeks, (9) Abnormal level of CRP/ESR. ACPA, antibodies to citrullinated peptides; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; EULAR/ACR RA criteria, European League Against Rheumatology/American College of Rheumatology Rheumatoid arthritis criteria; RF, rheumatoid factor.

Figure 16 illustrates the localization of arthritis among patients who developed arthritis. The number of affected large respectively small joints were counted in each patient. Large joints are defined as shoulder, elbow, hip, knee and ankles. Small joints include MCP, PIP, MTP 2-5, thumb IP, wrist and sternoclavicular joint. Affected joints indicate either swollen or tender joints. The prevalence of arthritis was significantly higher in small joints compared to large joints ($p = 0.0006$).

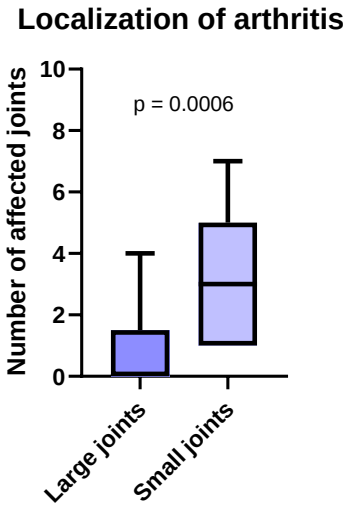

Figure 16. Localization of arthritis in patients who developed arthritis. Large joints are defined as shoulder, elbow, hip, knee and ankles and small joints as MCP, PIP, MTP 2-5, thumb IP, wrist and sternoclavicular joint. Affected joints indicate either swollen or tender joints.

Heat map of patients who developed new arthritis

The group of arthralgia patients who developed arthritis represented a heterogeneous group. To get an overall view of clinical and serological parameters in this group a clustering analysis of the core parameters was performed. Figure 17 demonstrates a heat map of the parameters based on the comparison of Z-scores for each parameter in the 12 patients who developed arthritis. According to the dendrogram, two separate clusters were obtained. One cluster consisted of number of small joints with arthritis in combination with ACPA, EULAR RA criteria, age and CSA score, and represented true RA patients. The second cluster

consisted of large joints combined with survivin and inflammatory markers (ESR, CRP, WBC, platelet count, IFN-γ), which represented patients with undifferentiated arthritis.

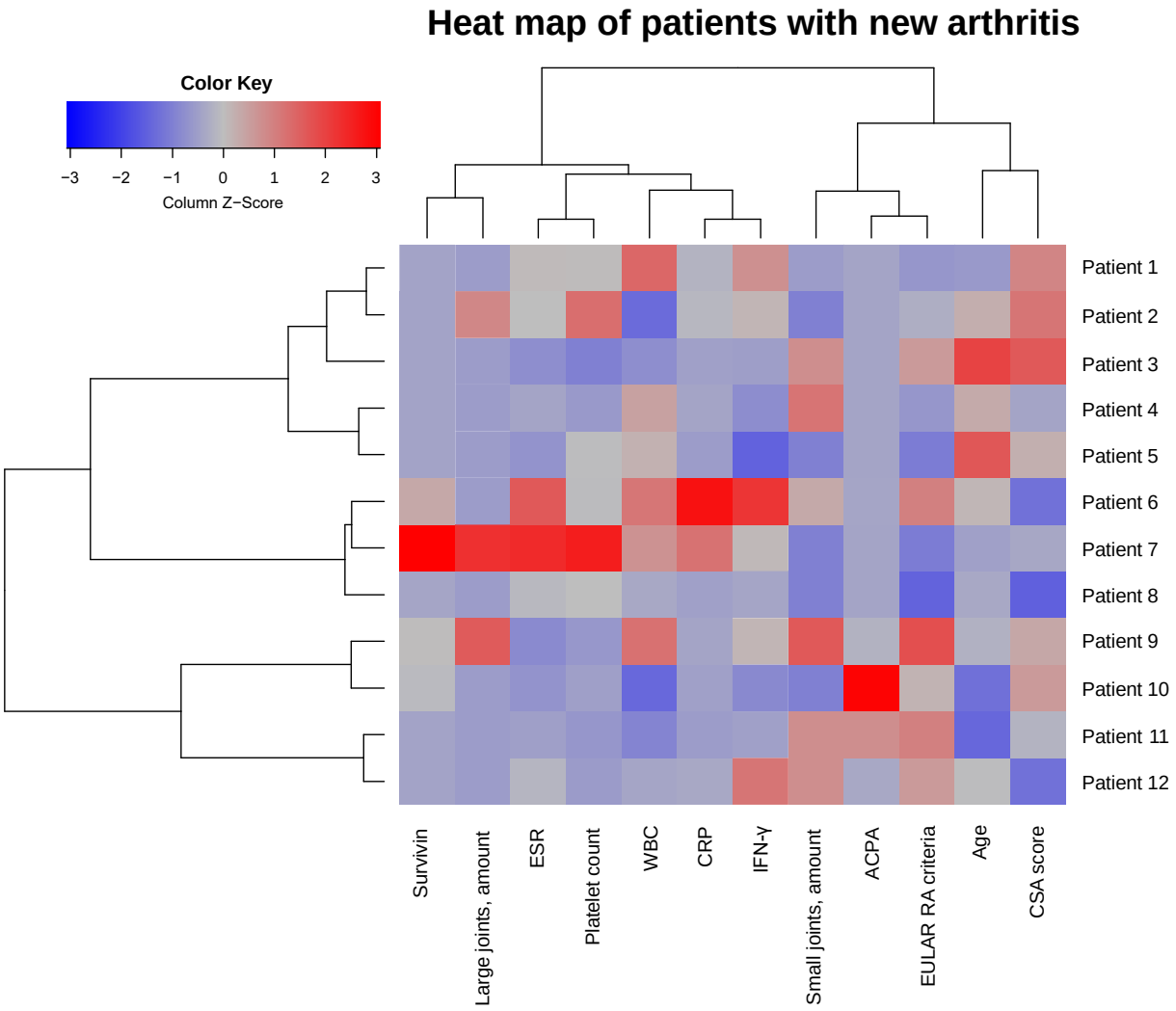

Figure 17. Clustered heat map of patients with new developed arthritis. One cluster consists of number of small joints with arthritis in combination with ACPA, EULAR RA criteria, age and CSA score. The second cluster consists of large joints combined with survivin and inflammatory markers (ESR, CRP, WBC, platelet count, IFN-γ). ACPA, antibodies to citrullinated peptides; CSA, Clinically suspect arthralgia, CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; EULAR RA criteria, European League Against Rheumatology Rheumatoid arthritis criteria, IFN-γ, interferon-gamma; WBC, white blood cell count.

Discussion

In this observational cohort study the aim was to investigate if high serum levels of survivin could identify a group of patients at high risk of developing arthritis. Patients were prospectively followed during a period of 9-21 months to see if they developed arthritis. In this study cohort, there was no observed association between high survivin levels and risk of developing arthritis. Importantly, neither clinical characteristics of arthralgia nor the presence of other known parameters of RA risk, including female gender and presence autoantibodies or inflammation were enriched in the survivin positive group. These results differ from those reported previously in the material collected during the period 2012-2013 by Erlandsson et al. [13, 16]. To better understand the reason for such difference, the cohorts of patients from 2013 and 2019 were compared.

Out of 2031 first visits patients at the Rheumatology Clinic, 201 patients with unexplained arthralgia were identified, corresponding to 9.9 % of all first-visit rheumatology patients. The proportion of seropositivity (ACPA or RF) was 19,4 % and approximately one third of arthralgia patients were survivin positive. Similar characteristics were found in the previous patient cohort collected in 2013. In the same study, ~40 % of survivin positive patients were also positive for RF and/or ACPA [13]. In an early RA cohort by Chun-Lai, Too et al. (2015), a high proportion of ACPA-positive cases was present in survivin positive patients [14]. In contrast, results in this study from 2019 found that only 25 % of the survivin positive group were RF and/or ACPA positive. Hence, a smaller number of patients combining survivin and autoantibodies was recognized in this arthralgia cohort. A reason for such difference in biomarker profile could be due to cases of spondyloarthritis or psoriatic arthritis among the arthralgia patients. Patients with a combination of psoriasis and arthralgia who in fact are at

risk of psoriatic arthritis could have received the ICD code of arthralgia, thereby not representing clinically suspect arthralgia patients at risk of RA. Patient's delay in seeking healthcare, due to patient dependent factors, has been identified as a reason for delay in RA patients seeing a rheumatologist [19]. Alternatively, patient's delay could have led to enrichment of arthralgia patients by late referral from primary care to the rheumatology clinic. It is possible that those patients developed RA before their first visit, consequently depriving the study of arthralgia patients at high risk of developing RA, such as survivin and autoantibody positive individuals.

A relatively large proportion of arthralgia patients had increased levels of CRP and/or ESR (34 %) particularly in relation to the smaller group of seropositive patients, compared to the 2013 cohort [13]. Additionally, the survivin-positive group did not have increased levels of CRP and/or ESR. The presence of increased acute phase proteins in arthralgia may indicate an early phase of RA. In a cohort on early RA patients, seronegative patients had higher inflammatory activity than seropositive patients [20]. This may indicate that presence of autoantibodies does not necessarily imply increased inflammation. The same connection could possibly exist between inflammatory parameters and survivin. On the other hand, a considerable portion with increased acute phase proteins may be caused by late referrals of seronegative arthralgia patients to rheumatologists, coinciding with the result that all patients who developed arthritis did so within 10 months and half of them were seronegative. The relationship between elevated acute phase proteins and development of arthritis in seronegative patients was not analyzed in this study, which makes this an interesting point for future studies.

6 % of the arthralgia patients developed arthritis, and only half of these patients fulfilled the classification criteria for RA. The prevalence of arthritis and RA cases in this study is lower than in previous prospective studies, where up to 20 % of patients with CSA respectively 35 % of seropositive arthralgia patients developed arthritis, and the overwhelming majority classified as RA [15, 21]. This could be explained partly by the non-selective inclusion criteria where the presence of autoantibodies was not obligatory, which resulted in an extensive group of arthralgia patients. Since presence of autoantibodies is associated with an increased risk of developing arthritis, which was also observed in this study, it is conceivable that a larger study cohort and/or more selective inclusion criteria with the presence of autoantibodies would result in a larger number of RA cases. The study also had a slightly short follow-up period (median of 15 months), especially for first-visit patients in early summer of 2019. Whether a longer follow-up would change the results remains unclear when taking into consideration that all patients developed arthritis within 10 months, and 42 % of these did so within 3 months. In previous studies on seropositive arthralgia and patients with CSA, most RA cases developed within 24 months [15, 21]. It is therefore possible that a longer follow-up would result in more RA cases. As previously mentioned, a delay in seeking healthcare among RA patients also strengthens the theory that a longer follow-up period could generate more cases of RA [19].

As expected, females were overrepresented in the arthralgia group (72.6 %). While the majority of patients with new developed arthritis were females, the prevalence of new developed arthritis was higher among males (9 %) compared to females (5 %). On the other hand, there was no significant difference in risk of progress to arthritis in females compared to males. Based on the statistics that 75 % of RA cases are prevalent in females, a stronger

association between females and the risk of developing arthritis would have been expected [2]. Findings in this study are in line with a previous cohort by van de Stadt et al., where no association was found for females and risk of developing arthritis in seropositive arthralgia patients (74 % females) [21]. These findings could indicate that female arthralgia patients constitute an unspecific group in terms of risk of developing arthritis. Larger studies on clinical characteristics of female arthralgia patients who prospectively develop RA would be interesting, to identify clinically suspect females with musculoskeletal complains or arthralgia.

Surprisingly, the frequency of several reported CSA characteristics and overall CSA score was significantly less common in survivin+ arthralgia patients. Additionally, CSA was neither predictive nor associated with new arthritis cases. Along with this observation, survivin was not associated with an increased risk of developing arthritis in this study. In the previous prospective study of 2013, survivin positivity was significantly associated with most of the CSA characteristics, supporting the hypothesis that survivin measurement can identify arthralgia patients that eventually will develop RA [16]. This association was not observed in this study. The proportion of data loss on the CSA questions in medical records could have contributed to the unexpected results. When comparing survivin positive and negative patients according to data loss on the CSA characteristics, survivin+ patients had at least 50 % data loss on CSA 7 and CSA 9. This could have contributed to bias for survivin+ patients that affected the results. A larger group of cases with new developed arthritis, possibly subsequent to a longer follow-up duration, could also have resulted in a stronger association between survivin, CSA and the risk of developing arthritis.

The result of clustering analysis of patients with new arthritis in this arthralgia cohort identified two independent clusters of patients (Figure 17). This argues for at least one of them representing patients with undifferentiated arthritis, and the other one reasonably representing RA patients. Findings in the clustering analysis suggest that there is a relation between number of affected small joints and ACPA, EULAR RA criteria, age and CSA score in patients that developed new arthritis. This is in line with previous knowledge about the involvement of small joints in RA. ACPA is an established predictive biomarker for RA. Similarly, CSA score and EULAR RA criteria are both understandably associated with RA. Survivin and inflammatory markers (ESR, CRP, WBC, platelet count, IFN-γ) were on the other hand related to large joints. It has been reported that IFN-γ regulates T-cell proliferation and apoptosis through upregulation of survivin expression in tumor-specific cytotoxic T-cells, resulting in survival and proliferation of said cells [22]. It is therefore reasonable that survivin and IFN-γ are related with each other, as survivin expression is regulated by IFN-γ. No association was however found between high levels of IFN-γ and survivin in this study. On the contrary, it was not expected that survivin levels were associated with arthritis in large joints. In a study published in 2010 by Svensson et al., high survivin levels were linked with the development of erosive joint destruction in small joints of hands and feet [11]. Again, the limited size of the patient group that developed arthritis, and the small share of survivin-positivity in this group (33.3 %), could explain this finding. Further studies on survivin and its connection to arthritis in large joints and IFN-γ are needed to clarify these findings.

Methodological considerations and future research

The strengths of this study include the broad patient inclusion and numerous patient parameters available for analysis, enabling a systematic comparison of symptomatology and

clinical markers in patients with arthralgia. Clinical records and biomarkers were available for all patients, which further strengthens the study. This relatively large group of arthralgia patients in addition to study cohort characteristics, is well comparable to previous similar studies, which is also a strength. This study also evaluated the localization of arthritis in arthralgia patients who prospectively developed arthritis, and its relation to survivin and inflammatory markers such as IFN-γ, which currently is a relatively unexplored research field. For future prospective studies it would be interesting to further explore survivin and its connection to localization of arthritis and other inflammatory markers in RA patients.

The initial study selection was based on the diagnoses patients received on the first visit at the Rheumatology Clinic. Diagnoses were based on ICD codes determined by each rheumatologist, however the basis for choice of ICD code is not standardized. Therefore, there is a possibility that clinical cases of unexplained arthralgia that did not receive the specific ICD code for arthralgia were excluded from the study. A suggestion for improvement would be a meticulous review of medical records of patients with unspecific diagnoses similar to arthralgia, that could contain cases of unspecific general pain.

Another limitation of this study is the short follow-up duration, as mentioned previously. A longer follow-up duration of at least 24 months for all patients would result in a more just evaluation of the prospective risk of developing arthritis or RA among arthralgia patients. In this study, a larger patient material based on a year of first-visits was chosen instead of a smaller group of arthralgia patients and longer median follow-up duration. Future prospective research based on patient material in this study is therefore desirable.

The considerable amount of data loss regarding the CSA characteristics in medical records is a further weakness of the study. The rheumatologists were not requested to take history of or examine the patients in accordance to the CSA characteristics, which explains the loss of information. It is probable that different rheumatologists had diverse evaluations of the patients, thus inter-individual variability may play an important role in weight of clinical symptoms. In a future study, it would be advantageous if the rheumatologists were trained to examine the patients according to the CSA characteristics, consequently avoiding substantial amounts of data loss in medical records.

Another concern might be that the development of arthritis was chosen as the clinical outcome, rather than RA. Arthritis is an unspecific diagnosis not necessarily equaling to RA. Comparing clinical characteristics in patients who developed arthritis may therefore be somewhat misleading when it comes to assessing the predictive potential of survivin for RA. Since a limited number of patients developed RA in this study, it deemed appropriate to use arthritis as the clinical outcome. For that reason, a heat map of the heterogeneous group that developed arthritis was created to provide clarification of the results.

Conclusions and Implications

In this study cohort, measurement of survivin in serum could not prospectively predict development of RA in arthralgia patients. Gender specificity, patient's delay in help seeking and a short follow-up period could provide some explanation to this unexpected fact. Further research on the role of survivin as a predictive biomarker for RA and its clinical associations is necessary. Validation of findings in this study is needed in future cohorts of arthralgia patients suspicious for progression to RA.

Populärvetenskaplig sammanfattning

Survivin som förutsägande tecken till ledgångsreumatism

Ledgångsreumatism (reumatoid artrit, RA) är en kronisk autoimmun sjukdom som innebär att man får smärta, svullnad och morgonstelhet i främst händernas småleder. Upp till 1 % av befolkningen drabbas av RA under sin livstid, och de flesta drabbade utgörs av medelålders kvinnor. Studier har visat att ledgångsreumatism brukar föregås av ledvärk. En lista av 16 karakteristiska symptom har därför framtagits för att underlätta för läkare att identifiera patienter med ledvärk som riskerar att övergå i RA, så kallad kliniskt suspekt ledvärk. Det finns även klassifikationskriterier som används på ledinflammationspatienter för att identifiera vilka individer som med störst sannolikhet har RA. Där ingår förekomst av specifika antikroppar, vilket ökar sannolikheten för diagnos.

Survivin är ett protein som finns inuti celler i alla levande vävnader som är under tillväxt eller där reparation av skador utförs. Studier har visat att patienter med RA och höga survivin-nivåer i blodet har ökad risk för svår ledförstörande sjukdom, som dessutom svarar sämre på behandling. Eftersom survivin kan föregå produktion av antikroppar, som idag används för att identifiera tidig risk för RA, är detta protein ett intressant forskningsämne för att undersöka dess potential att förutsäga RA i ett tidigt skede.

Syftet med denna studie var att undersöka om uppmätta nivåer av survivin hos ledvärkspatienter kan identifiera individer som har risk att utveckla RA. Dessutom att jämföra kliniska och laborativa fynd hos ledvärkspatienter med höga och låga nivåer av survivin i blodet (survivin-positiva respektive survivin-negativa).

Studien inkluderade 201 ledvärkspatienter som varit på ett första besök hos Reumatologkliniken vid Sahlgrenska Universitetssjukhuset. Patienter med höga och låga halter av survivin jämfördes med avseende på inflammatoriska prover, antikroppar och de karakteristiska symptomen för kliniskt suspekt ledvärk. Samtliga patienter följdes upp under 9 till 21 månader. Antalet patienter som utvecklade ledinflammation jämfördes därefter med övriga patienter angående inflammatoriska prover, antikroppar och de karakteristiska symptomen för kliniskt suspekt ledvärk.

De flesta ledvärkspatienter var kvinnor (73 %). 31 % av den totala populationen hade höga halter av survivin i blodet och utgjorde den survivin-positiva gruppen. Survivin-positiva patienter hade färre kliniska symtom för ledvärk som kan övergå till ledgångsreumatism, jämfört med survivin-negativa. Andra kända faktorer som brukar ökar risken för att drabbas av RA, såsom kvinnligt kön, förekomst av antikroppar och inflammatoriska prover var inte heller associerade med survivin. Under uppföljningstiden på 9 till 21 månader utvecklade 12 av 201 (6 %) ledinflammation, varav hälften (6 patienter) hade RA. Vi fann ingen skillnad i risk att utveckla ledinflammation vid jämförelse av kvinnor och män. Det fanns ingen koppling mellan höga survivin-nivåer och senare utveckling av ledinflammation.

Slutsatsen som kan dras från denna studie är att survivin inte kan förutsäga vilka ledvärkspatienter som kommer utveckla RA. Fler studier behövs för att verifiera vilka kliniska fynd hos ledvärkspatienter som kan tyda på utveckling till ledgångsreumatism, samt undersöka huruvida survivin kan användas för att förutspå utveckling från ledvärk till RA.

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