Kandidatspeciale

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Polyauto-immunity in patients with Rheumatoid Arthritis

Resume

Formål: Formålet med dette studie var at bestemme prævalensen af polyautoimmunitet, dvs. tilstedeværelsen af mere end én autoimmun sygdom hos patienter med seropositiv reumatoid artritis (RA) sammenlignet med patienter med seronegativ reumatoid artritis på diagnosetidspunktet samt at estimere forekomsten af polyautoimmunitet i de første fem år efter diagnosetidspunktet ved igen at sammenligne seropositive med seronegative RA-patienter.

Metoder: Studiet var registerbaseret og benyttede DANBIO og Landspatientregisteret. Punktprævalsen blev bestemt som en odds ratio (OR) for patienter med seropositiv RA sammenlignet med seronegativ RA ved hjælp af logistisk regression. Ved cause-specific Cox-regression blev hazard ratio (HR) for diagnosticering af endnu en autoimmun sygdom i de følgende år bestemt, ligeledes for patienter med seropositiv RA sammenlignet med seronegativ RA.

Resultater: 12 517 patienter med nydiagnosticeret RA blev inkluderet i studiet, Grupperne var næsten identiske med hensyn til behandling, DAS28-CRP og HAQ-DI, men gruppen af patienter med seropositiv RA var yngre (medianalderen 59,4 år versus 63,0 år) og omfattede flere kvinder (68,6% mod 63,0%). På diagnosetidspunktet havde patienter med seropositiv RA en OR på 0,88 (95% CI 0,77-1,02) for polyautoimmunitet sammenlignet med patienter med seronegativ RA. På samme måde havde patienter med seropositiv sygdom en HR på 0,83 (95% CI 0,62-1,11) i de første fem år af opfølgning sammenlignet med seronegativ RA. Patienter med seropositiv RA havde dog en højere dødelighed end patienter med seronegativ RA (5,2% vs. 3,9%) under opfølgningen.

Konklusion: Undersøgelsen viste, at patienter med seronegativ RA havde højere risiko for polyautoimmunitet sammenlignet med patienter med seropositiv RA på diagnosetidspunktet samt i de følgende fem år uanset køn, alder, behandling og kliniske sygdomsaktivitet.

Abstract

Objectives: The aims of this study were to determine the prevalence of polyautoimmunity, i.e., the presence of more than one autoimmune disease in seropositive compared with seronegative patients with rheumatoid arthritis (RA) at the time of diagnosis; and to estimate the incidence of polyautoimmunity in the first five years of the disease, again comparing seropositive with seronegative RA patients.

Methods: The study was register-based and used the nationwide DANBIO rheumatology register and the national administrative registers including the Danish National Patient Registry. The prevalence of polyautoimmunity was determined as the proportion of patients with at least one autoimmune disease in addition to RA at the time of RA diagnosis, and the odds ratio of having another autoimmune disease in seropositive compared with seronegative patients was determined using logistic regression. To estimate the incidence and hazard ratio (HR) of being diagnosed with yet another autoimmune disease in the subsequent 5 years, cause-specific Cox regression models accounting for the competing risk of death was performed.

Results: The study analysed data from 12 517 patients recently diagnosed with RA. The groups were almost identical regarding treatment, DAS28-CRP and HAQ-DI but patients with seropositive RA were younger (median age 59.4 years vs. 63.0 years) and included more women (68.6 % vs. 63.0 %). At time of diagnosis, patients with seropositive RA had an OR of 0.88 (95% CI 0.77-1.02) for polyautoimmunity compared to patients with seronegative RA. Likewise, patients with seropositive disease had a HR of 0.83 (95% CI 0.62-1.11) in the first five years of follow-up compared with seronegative RA. However, patients with seropositive RA had a higher mortality rate than patients with seronegative RA (5.2 % vs. 3.9 %) during follow up.

Conclusion: The study showed that patients with seronegative RA had higher risk of polyautoimmunity compared to patients with seropositive RA at time of diagnosis as well as in the following five years regardless of sex, age, treatment and clinical disease activity measures.

Polyautoimmunity in patients with Rheumatoid Arthritis

Rationale and background information

Rheumatoid arthritis (RA) is the most common chronic autoimmune arthritis with a prevalence of 0.52 % in Denmark, affecting women more often than men (Soussi et al., 2020). RA is categorized as either seropositive or seronegative disease referring to the presence or absence of IgM rheumatoid factor (IgM-RF) and/ or anti-citrullinated protein antibodies (ACPA). The specificity of ACPA for RA is up to 90 % (Kudo-Tanaka et al., 2007) and both IgM-RF and ACPA have been detected in patients several years before they developed RA (Nielen et al., 2004).

Also other antibodies such as anti-mutated citrullinated vimentin (anti-MCV), anti-carbamylated protein bodies (anti-CarP) and anti-acetylated protein bodies have been found to be associated with RA (Syversen et al., 2012; Derksen, Huizinga et van der Woude., 2017). There is a positive correlation between levels of both IgM-RF, ACPA and anti-MCV and joint damage (Syversen et al., 2012, Meyer et al., 2006). Other studies indicate that ACPA is involved in perpetuating inflammation as well as bone erosion and arthralgia (Derksen, Huizinga et van der Woude, 2017).

Despite the clinical overlap between seropositive and seronegative RA the two serotypes are quite different regarding genetic risk factors, cellular pathology and response to treatment, and it has been proposed that the two serotypes are more likely two different disease entities (Pratt and Isaacs, 2014). On the other hand, patients with seronegative RA occasionally go on to develop autoantibodies during the course of their disease. This phenomenon is called seroconversion and a study has found that it occurs in 1.3-8.9 % of patients with early inflammatory arthritis within the first 5 years of the disease (Barra et al., 2011), thus making the above-mentioned theory of two separate disease entities less probable.

It is well known that patients with an autoimmune disease more often develop other autoimmune diseases than individuals without an autoimmune disease. In a study from 2007, it was shown that in a group of patients with RA, the prevalence of several other autoimmune diseases was higher when compared to the general population, including autoimmune thyroid disease, type 1 diabetes mellitus and inflammatory bowel disease (IBD) (Eaton et al., 2007). There is a significant overlap in the clinical presentations of several autoimmune diseases. As an example, arthritis can be an extraintestinal symptom of IBD. Therefore, it can be difficult to distinguish between concomitant autoimmune diseases and extraintestinal

manifestations of one autoimmune disease (Wilson et al., 2015).

The cause of the association between different autoimmune diseases remains unknown but is proposed to include genetic as well as environmental factors such as nutritional factors, infections and intestinal dysbiosis (Lerner & Matthias, 2015). In addition studies have found that tobacco smoking is associated with formations of several different autoantibodies including IgM-RF, ACPA and anti-CarP (Derksen, Huizinga et van der Woude, 2017). Regarding genetic factors, a study investigating CTLA-4 and PTPN22 polymorphisms concluded that these polymorphisms are not solely responsible for neither RA nor autoimmune thyroid disease (Lazúrová et al., 2014).

The role, if any, of the drugs used in treatment of patients with RA in developing other autoimmune diseases is still unclear. A study of antinuclear antibodies (ANA) in patients with RA before and after starting treatment with infliximab found increased titers or new appearance in 12 of 26 patients 14 weeks after treatment initiation (Elkayam et al., 2005). On the other hand, a study among patients suffering from ankylosing spondylitis has shown that anti-thyroid peroxidase antibodies (TPO) were increased in a significantly smaller part of the patients receiving TNF-inhibitors (TNFi) compared to the non-TNFi treated patients (Tarhan et al., 2013). A Swedish study including 8090 patients with RA found that these patients had a higher prevalence of thyroxine-treated autoimmune thyroid (AIDT) disease at the time of RA diagnosis and in the 5 years prior to this, while on the other hand, 5 years after diagnosing RA the incidence of AIDT was lower for the RA-patients than for the general population, suggesting that antirheumatic therapies may prevent AIDT (Waldenlind et al., 2018).

Thus, despite theories of seropositive and seronegative RA being different diseases altogether, there is currently no evidence of differences in autoimmune disease patterns. Hence, the aim of this study was to investigate the prevalence and 5-year incidence of polyautoimmunity in seropositive compared with seronegative patients with RA.

Methods

Study Population

The study population was patients with RA registered with a first time-visit in DANBIO between 2006 and 2019 with an ICD-10 code of M05 or M06. The positive predictive value of an RA diagnosis in DANBIO has been estimated to 92 % (Ibfelt et al., 2016).

Only patients who according to their DANBIO information had been diagnosed within the past year prior to their first registered visit in DANBIO were included, thus limiting the population to incident or early RA patients.

Exposure

The primary exposure was seropositive RA defined as a DANBIO-registered patient with an M05 ICD-10 code compared with seronegative RA, i.e. M06 as the ICD-10 code.

Outcome

The primary outcome was polyautoimmunity defined by the presence of one or more of the diagnoses listed in Table 1. Presence of polyautoimmunity was estimated as the point prevalence at time of first registered visit in DANBIO, and as the incidence rate in the following five years.

The list does not include all autoimmune diseases. All diseases of the musculoskeletal system and connective tissue were excluded as there is a significant overlap between RA and other rheumatological conditions which will make it impossible to distinguish between extraarticular manifestations of RA and additional autoimmune diseases.

Table 1 ICD-10 codes for autoimmune diseases

III Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism

ICD10	
D51.0	Pernicious anaemia
D59.1	Autoimmune haemolytic anaemia
D69.3	Trombocytopenic purpura

IV Endocrine, nutritional and metabolic diseases

ICD10	
E05	
E05.0	Graves' disease
E06.3	Autoimmune thyroiditis
E06.3A	Hashimoto thyroiditis
E10	Diabetes type 1
E27.1	Addison disease

VI Diseases of the nervous system

ICD10	
G35	Multiple sclerosis
G36.0	Neuromyelitis optica
G61.0	Guillain Barré syndrome
G70	Myasthenia gravis

XI Diseases of the digestive system

ICD10	
K50	Crohn's Disease
K51	Ulcerative Colitis
K74.3	Primary Biliary Cirrhosis
K83.0	Primary sclerosing cholangit
K75.4	Autoimmune hepatitis
K90.0	Coeliac disease

XII Diseases of the skin and subcutaneous tissue

ICD10	
L10	Pemphigus
L10.0	Pemphigus vulgaris
L12.0	Pemphigoid
L13.0	Dermatitis herpetiformis
L63	Alopecia areata
L80	Vitiligo
L90.0	Lichen sclerosus

XIV Diseases of the genitourinary system

ICD10	
N02	IgA nephropathy
N30.1	Interstitial Cystitis

Names and codes for autoimmune diseases used in the study are from International Statistical Classification of Diseases and Related Health Problems, 10th Revision.

Confounders

A study has found that female gender and familial autoimmunity are factors correlated with polyautoimmunity (Rojas-Villarragas et al., 2012). All models are adjusted for sex while on the opposite we do not have data about familial autoimmunity.

Moreover, it has been shown that tobacco smoking is associated with polyautoimmunity in patients with Sjögren Syndrome (Anaya et al., 2016). Additional models adjusting for tobacco smoking have been performed.

Statistical analysis

Descriptive continuous data are presented as medians with interquartile ranges (IQR).

To determine the likelihood of having another autoimmune disease at time of RA diagnosis in seropositive compared with seronegative patients, logistic regression was used to estimate the odds ratio (OR) of

polyautoimmunity. A crude and a sex and age (restricted cubic spline with 6 degrees of freedom) adjusted OR were estimated.

To estimate the risk of developing a concomitant autoimmune disease in seropositive compared with seronegative patients during the first 5 years after their RA diagnosis, a cause-specific Cox regression was performed accounting for the competing risk of death. In this analysis, all patients were included regardless if they already had another autoimmune diagnosis at start of follow-up, but these patients were only followed up for the development of yet another type of autoimmune disease. Attained age was the time scale. Two models were outlined, one adjusting for sex and age and another adjusting for sex, DAS28-CRP, HAQ-DI, and treatment with biological and conventional synthetic DMARDs. An additional model also adjusted for tobacco smoking status. Follow-up started at time of diagnosis and ended at the date of being diagnosed with another autoimmune disease, death, emigration, at 5 years of follow-up, or end of 2019, whichever occurred first. To illustrate the time-to-event relations in the two groups, we plotted the cumulative incidence of polyautoimmunity from start of follow-up and up to 5 years using the Aalen-Johansen estimator.

All statistical analyses were performed in R.

Sensitivity analyses

To further ensure that the ICD-10 defined exposure variable of serostatus was correct, 3 alternative definitions of serostatus were tested. These were based on 1) ICD-10 codes registered in The Danish National Patient Register (DNPR) corresponding to the time of diagnosis, 2) patients lab results for ACPA and IgM-RF entered by the physicians in DANBIO, and 3) for geographically restricted sample of our population (Capital Region of Denmark, Region Zealand, Region of Southern Denmark, The North Region of Denmark), lab results were directly available.

Furthermore, data on smoking habits and history corresponding to the time of diagnosis was available for 41 % of the study population. The logistic and cause-specific Cox regression models were reperformed while adjusting for smoking status categorized as current, previous, or never smokers.

In order to make sure that the outcome was correct alternative definitions for two of the most frequent outcomes, type 1 diabetes mellitus and autoimmune thyroid disease, were tested. In this sensitivity analysis, in addition to an ICD-10 diagnosis code registered in DNPR, a redeemed prescription of insulin or thyroid specific drugs, respectively, was also required.

Results

A total of 12 517 patients with RA were included in the study, hereof 7983 (63.8 %) with seropositive RA and 4534 (36.2 %) with seronegative RA (Table 2). In the group of patients with seropositive RA, there was a higher percentage of women (68.6 % vs 63.0 %). Moreover, patients with seropositive RA were slightly younger than the patients with seronegative RA (median age 59.4 vs. 63.0 years). Lastly there were more smokers, current as well as previous, in the group with seropositive RA (31.7 % vs. 21.0 % and 25.5 % vs. 22.5 % respectively) for the 5135 patients (41%) with available data on this. Besides these factors, the two groups were similar as seen in Table 2.

Prevalence at time of RA diagnosis

At the time of diagnosis or first visit in DANBIO, 6.9 % of patients with seropositive RA and 7.7 % of patients with seronegative RA

Table 2 Basic characteristics of patients included in the study

	Seropositive	Seronegative
Total number	7983	4534
Age	59.4 (48.6 to 69.1)	63 (51.5 to 72.3)
Sex (women)	5476 (68.6 %)	2856 (63 %)
Clinical factors		
HAQ-DI	0.857 (0.375 to 1.375)	0.875 (0.375 to 1.375)
DAS-28-CRP	4.3 (3.3 to 5.2)	4.6 (3.6 to 5.5)
CRP	10 (4 to 23)	10 (3 to 26)
Tender joint count (tjc)	5 (2 to 9)	6 (2 to 12)
Swollen joint count (sjc)	3 (1 to 7)	4 (2 to 8)
VAS global (patient)	54 (30 to 77)	57 (33 to 78)
VAS pain (patient)	47 (24 to 69)	49 (27 to 70)
VAS fatique (patient)	50 (23 to 71)	52 (25 to 72)
VAS (doctor)	27 (15 to 45)	30 (16 to 47)
Treatment		
Methotrexat	6838 (85.7 %)	3815 (84.1 %)
Conventional synthetic DMARD	1880 (23.6 %)	1068 (23.6 %)
Biologic	338 (4.2 %)	200 (4.4 %)
Tobacco smoking status		
Current smoker	31.7 %	21.0 %
Previous smoker	25.5 %	22.5 %
Non-smoker	42.7 %	56.4 %

HAQ-DI, Health Assessment Questionnaire Disability Index; DAS-28-CRP, Disease Activity Score for Rheumatoid Arthritis with C-reactive Protein; VAS, Visual Analogue Scale; DMARD, Disease Modifying anti-rheumatic drugs.

had a concomitant autoimmune disease. The distribution of these additional autoimmune diseases is shown in Table 3. For patients with seropositive RA, autoimmune thyroid diseases accounted for the largest proportion (2.2 %) followed by type 1 diabetes (1.8 %) and IBD (1.4 %). For patients with seronegative RA, type 1 diabetes was the most frequent diagnosis (2.1 %) followed by autoimmune thyroid disease (1.9%) and IBD (1.5 %). None of the remaining diagnoses represented more than 0.4 % and the distribution of these remaining diseases was almost identical in the two groups.

OR for polyautoimmunity in patients with seropositive compared with seronegative RA was 0.88 (95% CI 0.77 to 1.02) when adjusting

Table 3 Point prevalence of autoimmune diseases at time of diagnosing RA

III **Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism**	Seropositive	Seronegative
Anaemia D51 + D59.1	9 (0.1 %)	8 (0.2 %)
Trombocytopenic Purpura D69.3	16 (0.2 %)	6 (0.1 %)
IV **Endocrine, nutritional and metabolic diseases**		
Autoimmune thyroid disease DE05.0 + DE06.3 + DE06.3A	176 (2.2 %)	86 (1.9%)
Type 1 diabetes DE10	143 (1.8 %)	94 (2.1 %)
Addison disease E27.1	3 (0.0 %)	7 (0.2 %)
VI **Diseases of the nervous system**		
Multiple sclerosis G35	22 (0.3 %)	20 (0.4 %)
Neuromyelitis optica G36	0 (0.0 %)	0 (0.0 %)
Guillan Barré syndrome G61.0	<3 (0.0%)	4 (0.1 %)
Myasthenia Gravis G70	13 (0.2 %)	9 (0.2 %)
XI **Diseases of the digestive system**		
IBD DK50 + DK51	110 (1.4 %)	69 (1.5 %)
Primary Biliary Cirrhosis K74.3	4 (0.1 %)	3 (0.1 %)
Primary sclerosing cholangit K83.0	7 (0.1 %)	3 (0.1 %)
Autoimmune Hepatitis K75.4	0 (0.0 %)	0 (0.0 %)
Coeliac disease K90.0	12 (0.2 %)	13 (0.3 %)
XII **Diseases of the skin and subcutaneous tissue**		
Pemphigus L10 + L10.0	0 (0.0 %)	< 3 (0.3 %)
Pemphigoid L12.0	< 3 (0.0 %)	<3 (0.0 %)
Dermatitis herpetiformis L13.0	0 (0.0 %)	<3 (0.0 %)
Alopecia Areata L63	7 (0.1 %)	7 (0.2 %)
Vitiligo L80	7 (0.1 %)	3 (0.1 %)
Lichen Sclerosus L90.0	25 (0.3 %)	13 (0.3 %)
XIV **Diseases of the genitourinary system**		
IgA nephropathy N02	3 (0.0 %)	4 (0.1 %)
Interstitial cystitis N30.1	16 (0.2 %)	18 (0.4 %)
Total	549 (6.9 %)	349 (7.7 %)
Odds Ratio	0.89 (0.77 to 1.02)	1 (ref.)
Odds Ratio (adjusted for sex and age)	0.88 (0.77 to 1.02)	1 (ref.)
Odds Ratio (adjusted for sex, age and tobacco smoking status)	0.79 (0.64 to 0.98)	1 (ref.)

for sex and age; and 0.79 (95% CI 0.64 to 0.98) when further adjusting for tobacco smoking status. In the sensitivity analysis of the outcome definition with the additional requirement of a redeemed prescription of insulin for type 1 diabetes or thyroid medication for those with autoimmune thyroid disease, the OR was 0.76 (95% CI 0.62 to 0.92).

Incidence during first five years after RA diagnosis

During the 5-year follow-up, 180 (2.3 %) of the patients with seropositive RA and 115 (2.5 %) of the patients with seronegative RA developed a new autoimmune disease (Table 4). In both groups, type 1 diabetes was the most frequent diagnosis (1.1 % in both groups) followed by IBD (0.6 % for seropositive RA and 0.9 % for seronegative RA) and autoimmune thyroid disease (0.6 % for seropositive RA and 0.5 % for seronegative RA). The cause-specific Cox regression resulted in a hazard ratio of 0.86 for developing polyautoimmunity in patients with seropositive RA compared with seronegative RA patients when adjusting for sex and age. An additional model estimated the hazard ratio to 0.83 when further adjusting for HAQ-DI, DAS-28-CRP and RA-treatment (methotrexate, conventional synthetic DMARDs and biological treatment). When adjusting for tobacco smoking status the difference was almost evened out with HR 0.97.

While there was a higher percentage of patients with seronegative RA who developed polyautoimmunity, the opposite was the case regarding mortality. Overall, 413 (5.2 %) of the patients with seropositive RA died during the follow-up compared to 177 (3.9 %) of the patients with seronegative RA. The results are shown in Table 4.

The analyses based on the alternative exposure definitions were in accordance with the results of the primary analyses in this study as it showed cause-specific HR adjusted 0.86 (95% CI 0.66 to 1.12) using the DNPR definition, 1.02 (95% CI 0.59 to 1.75) using the DANBIO derived LAB-data and 0.33 (95% CI 0.07 to 1.54) using the LAB-derived data. Based on the alternative outcome definition, the HR was 0.72 (95% CI 0.53 to 0.98) in model 1 and 0.77 (95% CI 0.52 to 1.13) in model 2.

To illustrate the absolute 5-year risk of developing (another) autoimmune disease in seropositive and seronegative patients, we

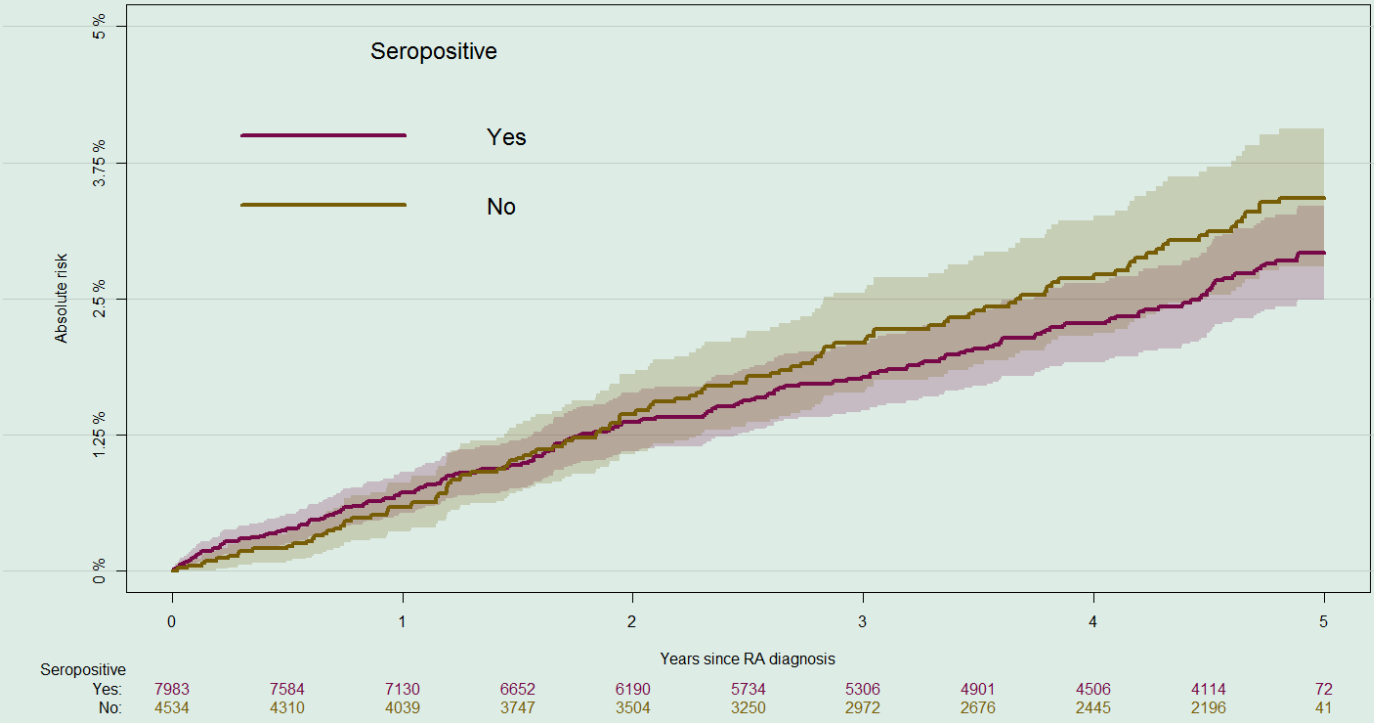
Table 4 Hazard rate for developing polyautoimmunity during the follow up

	Seropositive	Seronegative
Type 1 diabetes	88 (1.1 %)	52 (1.1 %)
Autoimmune thyroid disease	46 (0.6 %)	23 (0.5 %)
IBD	45 (0.6 %)	41 (0.9 %)
Total	180 (2.3 %)	115 (2.5 %)
Hazard rate model 1*	0.86 (0.68 to 1.08)	1 (ref.)
Hazard rate model 2**	0.83 (0.62 to 1.11)	1 (ref.)
Hazard ratio model 3***	0.97 (0.57 to 1.65)	1 (ref.)
Died during the follow-up	413 (5.2 %)	177 (3.9 %)

*Adjusted for sex and age
** Adjusted for sex, age, HAQ-DI, DAS-28-CRP and RA-treatment (methotrexate, conventional synthetic DMARDs and biological treatment
***Adjusted for sex, age, HAQ-DI, DAS-28-CRP and RA-treatment (methotrexate, conventional synthetic DMARDs, biological treatment and tobacco smoking status

plotted the 5-year cumulative incidence based on the Aalen-Johansen estimator. Figure 1 shows that patients with seronegative RA have an increased overall risk compared to patients with seropositive RA, though the results are not statistically significant as the estimator was only adjusted for sex and age.

Figure 1



Discussion

The study compared the prevalence and 5-year incidence of polyautoimmunity in 7983 patients with seropositive RA compared with 4534 patients with seronegative RA. We found that 7.7 % of the patients with seronegative RA and 6.9 % of the patients with seropositive RA had a concomitant autoimmune disease at time of diagnosis amounting to an OR of 0.88 when adjusting for sex and age. The patients were followed in 5 years after time of diagnosing RA. During this follow-up, 2.3 % of the patients with seropositive RA and 2.5 % of the patients with seronegative RA developed a new autoimmune disease leading to a HR of 0.83 for patients with seropositive RA.

As it is seen in Table 2 the two groups were similar regarding clinical status and treatment at baseline. This contrasts with a recent study investigating this matter where patients with seronegative RA had more active disease at baseline compared to patients with seropositive RA while they had a better response to conventional DMARDs and lower disease activity after 1 and 2 years (Choi & Lee, 2018).

As patients with autoimmune diseases have a higher risk of contracting an additional autoimmune disease than the population in general it would be reasonable to suspect autoantibodies to be involved in this (Eaton et al., 2007, Dilas et al., 2011). However, in our study we

found that patients with seropositive RA had lower prevalence of other autoimmune diseases in general than patients with seronegative RA (6.9 % vs. 7.7%), suggesting that either 1) patients with seronegative RA also have autoantibodies which are yet to be identified, 2) autoantibodies are not an important part of the explanation to polyautoimmunity, or 3) the seronegative patients suffering from poly-autoimmunity will in time develop RA-related autoantibodies, i.e. seroconvert to seropositive disease. Furthermore, it could be postulated that polyautoimmunity in patients with seronegative RA is correlated with incidence of arthritis related to the autoimmune disease; e.g. extraintestinal manifestation. Previous evidence indicates that a family history of RA is a strong risk factor for other autoimmune diseases, especially for seropositive RA. Families with RA patients are reported to be enriched with cases of autoimmune diseases such as SLE, SSc and SS (Rojas-Villarraga et al., 2012). These findings suggest that these autoimmune diseases share part of the pathogenesis of RA. In the present study we may have investigated autoimmune diseases with other pathogenesis and heritability than those close to RA thereby finding a lower prevalence and incidence of polyautoimmunity among patients with seropositive RA than what is the actual case. In addition, it is worth mentioning that patients in this study are diagnosed as seropositive only in case of present IgM-RM and/or ACPA. New research suggests that RA can also be categorised as seropositive if there is presence of anti-carbamylated protein bodies (anti-CarP) or anti-acetylated protein bodies (Derksen et al., 2017).

Furthermore, it is worth noticing that there was a significantly higher mortality among the patients with seropositive RA compared to seronegative RA during the follow-up. By using cause-specific Cox regression we ensured that the competing risk of death was accounted for in our HR estimate of polyautoimmunity. A previous study has found that the most significant determinants of prognosis regarding mortality in RA-patients are the severity of presentation and management of the disease (Carmona et al., 2010). As mentioned above, there were no notable differences in clinical disease activity nor medical treatment between the two groups, while radiologic status at baseline was not available. Previous research found that seropositivity is a significant risk factor for cardiovascular disease (Sen, González-Mayda & Brasington, 2014). Prevalence and incidence of cardiovascular disease was not included in the present study and could be a part of the explanation on the difference in mortality rates. Also, we have found a notable difference in the proportion of tobacco smokers between the two groups which might also be of influence on the diverging mortality.

In the following some limitations in this study will be mentioned. Firstly, all the diagnoses used in analyses were extracted from DNPR in which only hospital-recorded diagnoses are included. Thereby, patients with an autoimmune disease followed and treated by their general practitioner or in primary care in general are not included and we have no data on the validity of the respective autoimmune diseases in DNPR. This is likely to have influenced on our total number of outcomes while it should have no influence on the observed associations between seropositive and seronegative RA and polyautoimmunity as we expect it to be of even extent for the two groups, i.e. non-differential outcome misclassification bias.

Another limitation is our exposure definition. Some patients will seroconvert over time, and there is a risk that some of the seropositive categorised patients were actually seronegative in the beginning of the follow-up. In case of an event in a seronegative patient who later seroconvert, the event will now count in the seropositive group which could have resulted in a falsely low incidence rate in the seronegative group. A systematic review has found seroconversion rates at 1.3-8.9 % within 5 years for patients with early inflammatory arthritis (Barra et al., 2011) though in our sensitivity analyses there was no indication that exposure misclassification bias was a major issue as the results did not deviate remarkably when using lab data to define the exposure.

On the other hand, the study also has strengths. By using DANBIO as the primary register we have ensured a great extent of precision regarding the RA diagnoses which is further supported by comparing it to DNPR. The study was nationwide and with complete follow-up.

In conclusion the study showed increased prevalence and incidence of polyautoimmunity for patients with seronegative RA compared to seropositive RA at time of diagnosis as well as in the following five years regardless of sex, age, treatment and clinical status.

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