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The association between autoantibodies and risk for venous thromboembolic events among patients with rheumatoid arthritis

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Abstract

Objectives: To assess the association between venous thromboembolic (VTE) events and autoantibodies, following patients from RA diagnosis, measuring occurrence, levels and collective load of different autoantibodies against post-translational protein modifications, in particular recognizing citrullination (e.g. citrullinated fibrinogen) and RF by isotype.

Methods: A cohort of 2814 patients with newly diagnosed RA were followed for incident VTE through register linkages. Sera from RA diagnosis were centrally analysed for antibodies to second generation cyclic citrullinated peptides (anti-CCP2), 20 anti-citrullinated protein antibody (ACPA) fine-specificities, antibodies to additional protein modifications (carbamylation and acetylation) and RF by isotype. Association between baseline serology status and future VTE was analysed using Cox regression adjusted for age, sex and calendar period of RA diagnosis, overall and stratified by anti-CCP2 and RF positivity.

Results: During a median 16 years of follow-up, 213 first-ever VTE events were registered (5.0/1000 person-years). IgG anti-CCP2 (present in 65% of cohort) associated with VTE (hazard ratio [HR] = 1.33, 95% CI: 1.00, 1.78), in a dose-response manner. The risk of VTE increased with number of ACPA fine-specificities. IgM RF, but no other RF isotypes, associated with VTE (HR = 1.38, 95% CI: 1.04, 1.82). The associations were independent from smoking and HLA-DRB1 shared epitope alleles. None of the carbamylated or acetylated antibody reactivities associated with VTE.

Conclusion: Anti-CCP2, load of ACPA fine-specificities and IgM RF at RA diagnosis are associated with an increased risk of future VTE in RA. Antibodies to citrullinated fibrinogen did not differ substantially from other ACPA fine-specificities. Autoreactivity to other post-translational modifications was not associated with VTE risk.

Keywords: RA, antibody, fibrinogen, venous thromboembolism, risk, cohort, Sweden

Rheumatology key messages

- Presence and level of IgG anti-CCP2, number of ACPA fine-specificities and IgM RF are associated to incident VTE in RA.
- These associations were not readily explained by other RA risk factors such as smoking or HLA-DRB1 shared epitope alleles.

Introduction

A wealth of studies have demonstrated a strong association between RA and cardiovascular diseases, a major driver behind the increased morbidity and preterm mortality in RA. While most of these reports have studied ischaemic heart disease or composite endpoints such as major adverse cardiovascular events, several studies have also documented a 50–100% increased risk of venous thromboembolic (VTE)

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events, both deep venous thrombosis and pulmonary embolism, in patients with RA [1–3]. During the last few years, the so far unresolved signals of increased VTE risks with the targeted DMARD family of Janus kinase inhibitors (JAKi), increasingly used in RA, have underscored the need to better understand and clinically predict VTE risks in RA.

The underlying reasons behind the increased VTE risks in patients with RA remain incompletely understood but may conceptually be due to (i) co-occurrence of other conditions that themselves increase VTE risk, (ii) RA disease activity and inflammation, (iii) RA treatments, and/or (iv) inherent features of the RA autoimmunity.

When considering the occurrence of other conditions, there may in RA exist an over-representation of confounding conditions such as physical inactivity, surgery and malignancy. However, in view of the prevalence of such conditions in RA *vs* the general population, they seem unlikely to account for the entire risk increase [4].

With respect to RA disease activity and levels of inflammation, we recently reported a strong association between several measures of RA disease activity and the ensuing 1-year risk of VTE, equally so in seropositive and seronegative RA [5]. Others have reported on transiently increased VTE risks in individuals switching or starting a new DMARD. As many patients switch/start DMARD due to insufficient RA disease control, this observation offers indirect support for a role of RA disease activity for VTE risk [4, 6, 7]. Indeed, studies on VTE in the general population have linked markers of inflammation, including fibrinogen, with increased VTE risk [8].

Regarding anti-rheumatic therapies as potential drivers of VTE risk in RA, no clear signals of increased risks have been observed for biologic DMARDs [9, 10]. For JAKis, results to date are conflicting but obviously cannot explain the increased VTE risks observed in RA prior to their market entry [11–13].

Regarding the role of features of RA autoimmunity, one study of blood donors in the general population has linked the occurrence and level of RF to VTE risk [14]. However, since RF positivity in these individuals may be linked to unknown factors such as autoimmunity, infections or smoking, which may themselves increase VTE risk [15], the implication of this finding for RA remains unclear. Moreover, we and others have demonstrated an association between ACPAs and the risk of acute myocardial infarction, major adverse cardiovascular events and stroke [16, 17], but whether or not ACPAs relate to an increased VTE risk is not known.

One potential mechanistic link between ACPAs and VTE risk could be related to fibrinogen. Citrullinated fibrinogen (Cit-Fib) is abundant in RA synovial tissue where it is recognized by ACPAs [18], and antibodies binding to different epitopes of Cit-Fib are prevalent in RA serum. Polymorphisms of fibrinogen have been shown to differ with respect to clot stability, and thus potentially influence VTE risk [19]. Furthermore, post-translational protein modifications, including citrullination and carbamylation (Carb), have recently been shown to alter the function of fibrinogen, and to alter clot stability [20–23]. In RA, circulating autoantibodies towards Cit, Carb and acetylated (Ac) proteins, collectively termed anti-modified protein antibodies (AMPAs), are common and may form immune complexes with modified fibrinogen and thus further influence clot stability [24]. Despite the obvious clinical relevance, it remains poorly understood whether these experimental data translate into clinical risks for VTE. Therefore, we aimed to examine the association between AMPAs (overall and fine-specificities, Cit-Fib in particular), RF isotypes and their association with VTE risk in RA.

Methods

Study design and setting

We conducted a cohort study using prospectively collected data for patients newly diagnosed with RA in Sweden, for whom stored serum samples from the time of RA diagnosis were analysed for CCP2 (IgA and IgG), RF by isotype and AMPAs, including ACPA fine-specificities and reactivity to additional post-translational modifications. Through register linkages, patients were followed for incident VTE events, and the association between the presence and pattern of RA-related antibodies at RA diagnosis and risk of incident VTE was assessed. The study population and methodological approach is conceptually similar to that in a previous study from our group [17].

Study population

RA patients in the current study were identified from the Swedish Epidemiological Investigation of RA (EIRA) study [25, 26], diagnosed with RA at 18–70 years of age any time from 1996 through 2009, fulfilling the RA 1987 ACR classification criteria, and had previously been screened for ACPA fine-specificities ($n = 2859$) [27]. At diagnosis, participants were asked to give informed consent, fill out a questionnaire with lifestyle information and contribute a blood sample. For this study, we included information on age, sex, date of RA diagnosis and self-reported smoking status at RA diagnosis. Patients were genotyped for human leucocyte antigen (HLA)-DRB1 alleles and classified as positive for shared epitope (SE) if carrying at least one copy of HLA-DRB1*01 (excluding *01:03), *04:01 or *10, as previously described [28]. Through the unique personal identifier issued to all permanent residents in Sweden, we linked this incident RA cohort to the following nationwide, virtually complete Swedish registers: the National Patient Register (NPR), the Prescribed Drug Register (PDR), the Cause of Death register and the Total Population Register (TPR) (see Supplementary Table S1, available at *Rheumatology* online for details about the data sources, available at *Rheumatology* online).

The study complied with the Declaration of Helsinki and was approved by the Stockholm ethics review board (DNR 2021-00125, DNR 2015/1844-31/2, 2010/810-32, 2007/889-31/2 and 2005/1387-31). Written informed consent was obtained for all participants in EIRA.

Serological data

We centrally screened baseline sera for IgG anti-CCP2 using the CCPlus ELISA (Euro-Diagnostica AB, Malmö, Sweden) according to manufacturer's instructions, IgA anti-CCP2 using an EliA immunoassay (Phadia AB, Uppsala, Sweden) as previously described [29], 20 different ACPA fine-specificities (Supplementary Table S2, available at *Rheumatology* online), using a custom-made multiplex solid phase microarray platform (Thermo Fisher Scientific, ImmunoDiagnostics, Sweden), as previously described [27], and IgA, IgG and IgM RF isotypes, using an EliA immunoassay (Phadia), as previously reported [30]. IgG and IgA reactivity to homocitrulline (Carb), acetyl-lysine (Lys[Ac]), and acetyl-ornithine (Orn[Ac]) was assessed using the Orgentec, Germany modified vimentin assay (Mod-Vim). The assay is based on the modified Cit-Vim

peptide GRVYAT(Cit)SSAVR where the original citrulline/arginine is also replaced with the other modifications [31–33]. IgG reactivity to full-length Carb-Fib and Carb-fetal calf serum (FCS) was measured by an in-house ELISA as previously reported [34] (see also Supplementary Table S2, available at *Rheumatology* online).

IgG anti-CCP2 positivity was defined as ≥25 arbitrary units (AU)/ml according to the manufacturer's instructions, and anti-CCP2 load categorized into no (<25 AU/ml), low (25 to <75 AU/ml), high (≥75 to <1500 AU/ml) and extreme (≥1500 AU/ml) levels, and IgA anti-CCP2 positivity was defined ≥2 μg/l based on the 99th percentile among 101 blood donors [29]. We based the cut-off level for the ACPA fine-specificities and RF isotypes on the 98th percentile value among 538 general population control subjects from the EIRA study [27, 35]. IgG Mod-Vim cut-off was set based on the 97th percentile among 480 population controls from the EIRA study and IgA Mod-Vim cut-off was set to 2× the 97th percentile among the same controls, as previously reported [32]. Cut-off for positivity for Carb-Fib and Carb-FCS was set based on mean reactivity + (2 × S.D.) in 316 population controls from the EIRA study.

Follow-up and outcomes

We followed all individuals from inclusion in the EIRA study until any first event of VTE, migration from Sweden, death or end of study period (31 December 2020), whichever occurred first. Using previously devised and validated definitions [5], we defined incident VTE events based on information from the NPR. We defined a VTE event as the first ever main diagnosis with an international classification of diseases revision 10 (ICD-10) code of I26.0, I80.0, I80.1, I80.2, I80.3, I80.8, I80.9, I81, I82.0, I82.1, I82.2, I82.3, I82.8, I82.9 or I26 listed as cause of death or any of the three first contributory causes of death.

Statistical analysis

For the statistical analyses, we included all AMPAs and ACPA fine-specificities with a frequency of at least 10% in our study population. As previously described [17], we also defined the ACPA-antibody 'load' as the total number of ACPA fine-specificities expressed by an individual, and categorized these into tertiles based on the distribution in our cohort. We then estimated the hazard ratio (HR) with 95% CI for the association between anti-CCP2, load of anti-CCP2, all ACPA fine-specificities, and all RF isotypes (exposures) and incident VTE (outcome). The model was adjusted for age, sex and calendar period of RA diagnosis (1996–2000, 2001–2004, from 2005). We additionally adjusted for smoking status at diagnosis (never, former, current, non-regular and other tobacco use than cigarettes) and shared epitope (SE) (no *vs* any copy of SE) in separate models as well as jointly. We tested the Cox proportional hazards assumption using the *cox.zph* function in R (R Foundation for Statistical Computing, Vienna, Austria) and a significance threshold of 0.05.

To further explore the data, we investigated the association between the different RF isotypes in the anti-CCP2 positive and negative subsets, and IgG anti-CCP2 in the IgM RF positive and negative subsets.

Sensitivity analysis

We investigated the impact of alternative definitions of VTE by changing the outcome from first ever VTE to first VTE event during follow-up.

Results

Patient characteristics

The data extraction is presented in Supplementary Fig. S1, available at *Rheumatology* online. We included 2809 individuals. Of these, 27 had a history of a VTE event prior to the start of follow-up and were excluded from the main analysis, 72% were women, the median age at RA diagnosis was 54 (inter-quartile range [IQR] 18) years, and the median follow-up time was 15.5 (IQR 6.8) years. During follow-up, 213 incident VTE events were observed corresponding to an incidence of 5.0 per 1000 person-years (Table 1).

Anti-CCP2 and ACPA fine-specificities

In our cohort, 1797 (64.6%) were positive for IgG anti-CCP2. The HR for VTE with IgG anti-CCP2 positivity was 1.33 (95% CI: 1.00, 1.78). The risk of VTE increased with the level of anti-CCP2, with an HR of 1.49 (95% CI: 0.99, 2.22) for the group with extreme levels (*P*-value for trend 0.048). For IgA anti-CCP the HR was 1.35 (95% CI: 0.99, 1.84).

The following 18 ACPA fine-specificities had a frequency ≥10% in our sample: cfc1-cyc (CCP1), Cit-Vim$_{60–75}$, Cit-Vim$_{2–17}$, Cit-Fib$\beta_{36–52}$, Cit-Fib$\alpha_{563–583}$, Cit-Fib$\alpha_{580–600}$, CEP-1, Cit-Fib$\alpha_{621–635}$, Cit-Fib$\alpha_{36–50}$, Cit-Fib$\beta_{60–74}$, Cit-P.PAD (CPP3), Cit-CII-C1, Cit-CII-F4$_{(cit–cit)}$, Cit-Peptide-Z1, Cit-Peptide-Z2, Cit-Peptide-1, Cit-Peptide-5 and Cit-Peptide Bla26 (see Supplementary Table S2, available at *Rheumatology* online for more information on antigen peptide and proteins). A detailed description is given in reference [27], except for Cit-CII-C1 and Cit-CII-F4, which are described in references [36, 37], and Cit-P.PAD (CPP3), described in [38]. The median number of ACPA fine-specificities recognized was 6 (IQR 11).

The risk of VTE increased with the number of ACPA fine-specificities expressed (*P*-value for trend 0.033). At the 0.05 significance level, two fine-specificities were each associated with VTE: the hnRNP derived Cit-Peptide-Z1 (HR = 1.40, 95% CI: 1.06, 84) and Cit-Peptide-1 (HR = 1.47, 95% CI: 1.12, 1.93) (Table 2). None of the six antibodies against Cit-Fib assessed were statistically significantly associated with VTE risk.

Rheumatoid factor isotypes

Among the three RF isotypes, only IgM RF was statistically associated with VTE (HR = 1.38, 95% CI: 1.04, 1.83) (Table 3).

Table 1. Characteristics of the 2809 incident cases with RA included in the study

	Main analysis	Subset with data on smoking and shared epitope
n (% overall)	2809 (100)	
Events before start of follow-up, *n*	27	
In analytical cohort, *n* (% women)	2782 (72)	2714 (72)
Age, median (IQR), years	54.0 (18.0)	54.0 (18.0)
VTE events, *n*	213	209
Follow-up, median (IQR), years	15.5 (6.8)	15.6 (7.0)
Incidence per 1000 person-years	5.0	5.0

IQR: interquartile range; VTE: venous thromboembolic.

Table 2. Hazard ratio with 95% CIs for CCP2 and the ACPA fine-specificities

	n (% positive)	Hazard ratio for VTE (95% CI)[a]	Hazard ratio for VTE adjusted for smoking and shared epitope (95% CI)[b]
Anti-CCP2 (IgG)	1797 (64.6)	1.33 (1.00, 1.78)	1.31 (0.96, 1.79)
Anti-CCP2 (IgA)	897 (46.5)	1.35 (0.99, 1.84)	1.36 (0.99, 1.86)
Anti-CCP2 levels (IgG)			
None (<25 AU/ml)	985 (35.4)	Ref	Ref
Level 'low' (25 to <75 AU/ml)	190 (6.8)	1.41 (0.81, 2.48)	1.22 (0.66, 2.24)
Level 'high' (75 to < 1500 AU/ml)	1203 (43.2)	1.27 (0.93, 1.74)	1.28 (0.91, 1.78)
'Extreme' (≥1500 AU/ml)	404 (14.5)	1.49 (0.99, 2.22)	1.45 (0.95, 2.22)
P-value for trend		0.048	0.067
Load of ACPA fine-specificities			
Ab load (0–17), median (IQR)	6 (11)	1.02 (1, 1.05)	1.02 (1.00, 1.05)
None 0	554 (20.0)	Ref	Ref
Low (1–4)	668 (24.0)	1.39 (0.89, 2.17)	1.49 (0.94, 2.36)
Medium (5–10)	805 (28.9)	1.68 (1.10, 2.55)	1.78 (1.14, 2.78)
High (11–17)	755 (27.1)	1.56 (1.02, 2.39)	1.66 (1.05, 2.62)
P-value for trend		0.028	0.033
Individual ACPA fine-specificities			
Cfc1-cyc (CCP1)	1210 (43.5)	1.19 (0.91, 1.56)	1.19 (0.90, 1.57)
Cit-Vim$_{60–75}$	1279 (46.0)	1.06 (0.81, 1.39)	1.03 (0.77, 1.37)
Cit-Vim$_{2–17}$	911 (32.7)	1.07 (0.81, 1.43)	1.07 (0.80, 1.44)
Cit-Fib$\beta_{36–52}$	1319 (47.4)	1.24 (0.95, 1.62)	1.24 (0.93, 1.64)
Cit-Fib$\alpha_{563–583}$	1138 (40.9)	1.18 (0.90, 1.55)	1.17 (0.88, 1.55)
Cit-Fib$\alpha_{580–600}$	701 (25.2)	1.10 (0.82, 1.49)	1.07 (0.78, 1.45)
Cit-Fib$\alpha_{621–635}$	964 (34.7)	1.31 (1.00, 1.73)	1.30 (0.98, 1.73)
Cit-Fib$\alpha_{36–50}$	485 (17.4)	0.96 (0.67, 1.38)	0.99 (0.69, 1.42)
Cit-Fib$\beta_{60–74}$	1623 (58.3)	1.24 (0.94, 1.64)	1.23 (0.92, 1.66)
CEP-1	1315 (47.3)	1.20 (0.92, 1.58)	1.19 (0.89, 1.58)
Cit-CII-C1	312 (11.2)	0.82 (0.52, 1.29)	0.81 (0.52, 1.28)
Cit-P.PAD (CPP3)	609 (21.9)	1.20 (0.88, 1.65)	1.19 (0.86, 1.64)
Cit-CII-F4$_{(cit–cit)}$	777 (27.9)	1.18 (0.88, 1.58)	1.19 (0.88, 1.60)
Cit-Peptide Z1	1476 (53.1)	1.40 (1.06, 1.84)	1.42 (1.06, 1.90)
Cit-Peptide Z2	1133 (40.7)	1.25 (0.96, 1.64)	1.24 (0.93, 1.65)
Cit-Peptide 1	955 (34.3)	1.47 (1.12, 1.93)	1.49 (1.12, 1.97)
Cit-Peptide 5	1495 (53.7)	1.15 (0.87, 1.51)	1.13 (0.85, 1.51)
Cit-Peptide Bla26	879 (31.6)	1.31 (0.99, 1.74)	1.33 (1.00, 1.78)

[a] Reference: negative for serological marker in question. Hazard ratio adjusted for age, sex and calendar period of RA diagnosis.
[b] Analysis performed in the subcohort of 2714 with data on smoking and share epitope. IQR: interquartile range; VTE: venous thromboembolic.

Table 3. Hazard ratio with 95% CIs for the RF isotypes

	n (% positive)	Hazard ratio for VTE (95% CI)[a]	Hazard ratio for VTE adjusted for smoking and shared epitope (95% CI)[b]
IgA RF	1177 (42.3)	1.25 (0.95, 1.64)	1.27 (0.95, 1.68)
IgG RF	889 (32.0)	1.26 (0.95, 1.67)	1.27 (0.96, 1.69)
IgM RF	1599 (57.5)	1.38 (1.04, 1.82)	1.39 (1.04, 1.87)

[a] Reference: negative for serological marker in question. Hazard ratio adjusted for age, sex and calendar period of RA diagnosis.
[b] Analysis performed in the subcohort of 2714 with data on smoking and share epitope. VTE: venous thromboembolic.

Antibodies to carbamylated proteins and Mod-Vim peptides

A subset of 1928 individuals had data on autoantibodies to carbamylated Fib/FCS as well as the Mod-Vim assays, covering citrullinated, acetylated and carbamylated peptides with the same backbone. Characteristics and demographics were the same as in the full group of patients (data not shown). Neither of the autoantibodies to carbamylated fibrinogen or FCS were statistically significantly associated to VTE, nor was being positive for any of them (HR = 1.27, 95% CI: 0.93, 1.73) (Table 4). We did not find any statistically significant associations among the anti-Mod-Vim antibodies (Table 5).

Impact of smoking and shared epitope

A total of 2714 individuals had data on smoking and SE. Of these, 864 (31%) patients were never smokers, 734 (27%) were current smokers, 808 (30%) were former smokers, 159 (6%) were non-regular smokers, 167 (6%) smoked other tobacco than cigarettes and 2001 (74%) individuals had at least one copy of SE. Adjusting for smoking and SE had little impact on any of the HRs (Tables 2–5).

Stratified analyses

In the analyses stratified by IgG anti-CCP2 status and by IgM RF status, none of the statistically significant associations

Table 4. Hazard ratio with 95% CIs for the anti-carbamylated antibodies

	n (% positive)	Hazard ratio for VTE (95% CI)[a]	Hazard ratio for VTE adjusted for smoking and shared epitope (95% CI)[b]
IgG anti-Carb-Fib pos	823 (42.7)	1.31 (0.97, 1.79)	1.31 (0.95, 1.79)
IgG anti-Carb-FCS pos	686 (35.6)	1.10 (0.80, 1.51)	1.07 (0.77, 1.48)
Any anti-Carb positivity	1021 (53.0)	1.27 (0.93, 1.73)	1.24 (0.90, 1.71)

[a] Reference: negative for serological marker in question. Hazard ratio adjusted for age, sex and calendar period of RA diagnosis.
[b] Analysis performed in the subcohort of 2714 with data on smoking and share epitope. VTE: venous thromboembolic.

Table 5. Hazard ratio with 95% CIs for the Mod-Vim antibodies

	n (% positive)	Hazard ratio for VTE (95% CI)[a]	Hazard ratio for VTE adjusted for smoking and shared epitope (95% CI)[b]
IgG Mod-Vim Cit	1156 (60.0)	1.00 (0.73, 1.36)	0.98 (0.70, 1.36)
IgA Mod-Vim Cit	323 (16.8)	1.10 (0.74, 1.65)	1.10 (0.73, 1.65)
IgG Mod-Vim Orn(Ac)	980 (50.8)	1.20 (0.88, 1.64)	1.20 (0.87, 1.67)
IgA Mod-Vim Orn(Ac)	192 (10.0)	1.38 (0.87, 2.19)	1.38 (0.87, 2.19)
IgG Mod-Vim Carb	785 (40.7)	0.96 (0.70, 1.32)	0.94 (0.68, 1.31)
IgA Mod-Vim Carb	113 (5.9)	1.08 (0.58, 1.99)	1.09 (0.59, 2.02)
IgG Mod-Vim Lys(Ac)	550 (28.5)	0.93 (0.65, 1.32)	0.92 (0.64, 1.31)
IgA Mod-Vim Lys(Ac)	145 (7.5)	1.31 (0.77, 2.23)	1.30 (0.76, 2.21)

[a] Reference: negative for serological marker in question. Hazard ratio adjusted for age, sex and calendar period of RA diagnosis.
[b] Analysis performed in the subcohort of 2714 with data on smoking and share epitope. VTE: venous thromboembolic.

observed in our main analyses remained (Supplementary Table S3, available at *Rheumatology* online).

Sensitivity analysis

In the sensitivity analysis including also individuals with a history of VTE event before RA diagnosis (Supplementary Table S4, available at *Rheumatology* online), both IgG anti-CCP2 (HR = 1.38, 95% CI: 1.04, 1.84) and IgA anti-CCP2 (HR = 1.39, 95% CI: 1.02, 1.88) were statistically significantly associated with VTE, (Supplementary Table S5, available at *Rheumatology* online). The association with anti-CCP2 load also remained (*P*-value for trend 0.027, Supplementary Table S5, available at *Rheumatology* online). For the fine-specificities, the association to Cit-Peptide-Z1 and Cit-Peptide-1 remained, but the associations with Cit-Fib$\alpha_{621–635}$ (HR = 1.34, 95% CI: 1.02, 1.75) and Cit-Peptide Bla26 (HR = 1.36, 95% CI: 1.04, 1.79) also became significant, although the association to Cit-Fib$\alpha_{621–635}$ was lost when adjusting for smoking and SE (Supplementary Table S5, available at *Rheumatology* online). The association to RF, carbamylated antigens and Mod-Vim remained similar to the main analysis (Supplementary Tables S6–S8, available at *Rheumatology* online).

The global *P*-value for the Cox proportional hazard assumption was above the significance threshold of 0.05 in all regression models.

Discussion

In this study, we prospectively followed a cohort of close to 3000 individuals with newly diagnosed RA and centrally analysed autoantibody serology in cryopreserved sera for incident VTE events during a median of 16 years from RA diagnosis. We observed (i) an association between anti-CCP2 positivity at RA diagnosis and VTE risk, including a clear trend towards higher VTE risks with higher IgG CCP2 levels, (ii) an (at least) equally strong association between the number of ACPA fine-specificities and VTE risk, (iii) no specific association with anti-Cit-Fib antibodies or with AMPA targeting carbamylated or acetylated antigens, (iv) an association between IgM RF and VTE risk, and (v) that none of the associations were readily explained by smoking or the HLA-DRB1 shared epitope.

Our findings regarding IgG anti-CCP2 and 'load' of ACPA fine-specificities, and risk of VTE blend well with those from our previous study on other CV outcomes in this RA cohort. The association with high levels of IgG anti-CCP2 is also in keeping with other studies on CV risks (other than VTE) [16, 39]. The mechanism(s) underlying this association remains unclear. ACPAs and RF are associated with smoking, which is also associated with RA and with CV risks, VTE included. Nonetheless, adjustment for smoking status did not change the observed associations. However, we did not have longitudinal information on RA disease activity, and therefore could not explore whether the association between RA-related autoantibodies and VTE risk was mediated by, or independent of, accumulated RA disease activity. Independent of the mechanism, these RA-related antibodies serve as clinical risk factors for VTE risk.

Given the obvious importance of fibrinogen in the coagulation system, we were particularly interested in autoantibody responses to post-translationally modified fibrinogen-derived peptides/proteins. We did not find evidence of increased VTE risks specifically associated with reactivity towards post-translationally modified (citrullinated or carbamylated) fibrinogen-derived antigens. However, the cross-reactivity between different ACPA fine-specificities, as well as between ACPAs and anti-carbamylated protein antibodies, which has been demonstrated at both the polyclonal and the monoclonal levels, needs to be kept in mind when interpreting these data [32, 40, 41]. Two individual ACPA fine specificities, Cit-Peptide Z1 and Cit-Peptide 1, reached statistical significance in relation VTE risk, although risk estimates were not substantially higher than for IgG anti-CCP2 and IgM-RF,

respectively. Further work needs to address whether these particular associations are mechanistically relevant. It should also be noted that *in vivo* autoreactivity to post-translational modifications of other proteins, not covered by the antigen assays used in our study, could be of importance concerning VTE risks. Nevertheless, elevated IgG anti-CCP2 and large numbers of recognized autoantigens, in combination with IgM RF, suggest that increased formation of pro-inflammatory immune complexes is of importance.

Our study has certain limitations, and strengths. We did not have information on all traditional VTE risk factors, nor on their temporal distribution over the median 16 years of follow-up, and could not fully investigate the impact of such non-RA factors on our results. The incidence of VTE in our RA cohort, and the proportion of patients positive for anti-CCP2 and for RF, are all on a par with those reported from typical RA inception cohorts and from other studies of VTE in RA, and add to the generalizability of our results. The initial upper age limit in the EIRA study (initially 70 years) restricted the ability to study VTE risks among the elderly, among whom the underlying VTE incidence in the general population is the highest.

The centrally performed autoantibody analyses, where all sera were analysed simultaneously (per autoantibody type), using cryopreserved baseline samples, minimized lab-related technical variations. Notably, the EIRA serum samples have been stored at −80°C, which is considered sufficient to prevent significant degradation of antibodies, even if samples are stored for years.

By using baseline serum and a prospective register-linkage, we could determine exposure (RA-related antibodies, fine-specificities in particular), independently of outcome (VTE, for which we used validated algorithms with very high positive predictive values [5]), with near-complete coverage during follow-up.

The autoantibodies analysed are strongly correlated and correction for multiple testing, e.g. using the Bonferroni correction, would therefore not necessarily be appropriate. We therefore chose to present all results in full and let the readers interpret the findings themselves.

To conclude, RA-related antibodies analysed in clinical practice (IgG anti-CCP2, the spreading of ACPA fine specificities, and IgM RF) are associated not only with risk of myocardial infarction, stroke and cardiovascular death, as previously demonstrated, but also with VTE events, and these associations did not change when adjusting for smoking or HLA-DRB1 shared epitope positivity. By contrast, AMPAs and IgA RA-related antibodies were not associated to VTE risk in RA.

Supplementary data

Supplementary data are available at *Rheumatology* online.

Data availability statement

Due to the content of the ethical approval and consents, data on the individual level from EIRA cannot be publicly shared. For access to data on additional subsets of EIRA, please contact the principal investigators for data requests for applicable studies. For further information go to: http://www.eiraswe den.se/Kontakt_EIRA.htm.

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