Factor H Autoantibodies in Patients with Antiphospholipid Syndrome and Thrombosis

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ABSTRACT. ***Objective.*** Autoantibodies to complement factor H (FH) are associated with atypical hemolytic uremic syndrome, but can also be detected in patients with rheumatoid arthritis and in patients positive for lupus anticoagulants and thus potentially antiphospholipid syndrome (APS). To our knowledge, no data are available on the association between the presence of FH autoantibodies in APS and clinical manifestations.

Methods. We determined FH autoantibody levels using ELISA in 2 cohorts of patients with primary (PAPS) and secondary APS (SAPS) from Serbia and Italy, and an additional cohort including patients with venous thromboembolism (VTE) from Sweden.

Results. FH autoantibodies were detected in 13.7% of patients (n = 73) with PAPS and 30.3% of patients (n = 33) with SAPS in the Serbian cohort. FH autoantibody frequency in the Italian cohort was 33.3% (n = 15) and 36% (n = 25) in PAPS and SAPS, respectively. Both FH autoantibody levels and frequencies observed in both APS cohorts were significantly higher than in matched healthy controls (5%). Further, patients with PAPS with venous thrombosis in the Serbian cohort had significantly higher levels of FH autoantibodies. Therefore, we analyzed a dedicated Swedish thrombosis cohort and found that patients with FH autoantibody positivity had higher risk of VTE recurrence (HR 2.0, 95% CI 1.2–3.3, p = 0.011) compared with the reference group of FH autoantibody–negative patients.

Conclusion. Overall, the data indicate that in patients with APS and recurrent venous thrombosis, there are increased levels of FH autoantibodies, a finding associated with poor clinical outcome. (First Release August 15 2015; J Rheumatol 2015;42:1786–93; doi:10.3899/jrheum.150185)

Key Indexing Terms:

FH AUTOANTIBODIES
SYSTEMIC LUPUS ERYTHEMATOSUS
ANTIPHOSPHOLIPID ANTIBODIES
ANTIPHOSPHOLIPID SYNDROME
THROMBOSIS
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Plasma protein factor H (FH) is a main soluble inhibitor of the human complement system. The complement system is a central innate defense system that promotes the inflammatory response and contributes to the destruction of pathogens. In addition, the complement system is involved in the instruction of the adaptive immune response and the clearance of dead cells and misfolded proteins[1]. Aside from its beneficial effects, the complement system is an aggressive, self-amplifying cascade that needs to be tightly regulated by both soluble and membrane-bound inhibitors to prevent

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damage of host tissues[2,3]. Therefore, insufficient functional activity of FH, which can be caused by heritable deficiencies or autoantibodies, is associated with pathology. Thus, both detrimental mutations in the *CFH* gene and the presence of autoantibodies against FH are associated with atypical hemolytic uremic syndrome (aHUS)[4,5,6,7]. Further, we observed that FH autoantibodies are also present at a significant frequency in rheumatic diseases such as rheumatoid arthritis (RA)[8]. It was also reported that the deletion of complement FH-related proteins 1 (CFHR1) and 3 is associated with a subtype of aHUS with the presence of FH autoantibodies[9]. This subtype of aHUS with unique characteristics was termed "DEAP-HUS" (Deficiency of CFHR plasma proteins and Autoantibody Positive form of HUS)[10].

In our previous study[8], we detected an increased frequency of FH autoantibody–positive individuals in a cohort of patients with suspected antiphospholipid syndrome (APS). APS can develop as a primary disease (PAPS) or secondary (SAPS) to systemic lupus erythematosus (SLE). Both forms of APS are characterized by arterial and/or venous thrombosis[11] and recurrent fetal morbidity often with thrombocytopenia and elevated levels of antiphospholipid antibodies (aPL) such as anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA)[12]. Autoantibodies may also be generated against cell surface proteins such as β2 glycoprotein 1 (β2-GP1)[13,14]. These antibodies are pathogenic and elicit thrombosis as well as complement activation. There is evidence that the complement system is tightly connected with hemostasis[15], and it is known that complement activation contributes to thrombosis and pregnancy complications in APS[16]. Because we have previously found FH autoantibodies in a small cohort of LA-positive patients with APS-like disease[8], and others have suggested that deficiencies of complement inhibitors may result in excessive complement activation and increased risk for thrombosis[17], we therefore analyzed FH autoantibodies in 2 independent cohorts of patients with APS. Detection of increased FH autoantibodies levels in both cohorts resulted in the extension of the study to patients with venous thromboembolism (VTE).

MATERIALS AND METHODS

Patients: APS Serbian cohort. Serum samples were obtained from patients included in the APS Serbian National Cohort Study[18,19]. The patients met the Sydney criteria for APS, depending not only on aPL for diagnosis, but also on additional criteria such as thrombosis[12]. The patients were either affected by PAPS (73 patients) or by SAPS, mainly attributable to SLE (33 patients). Regional ethics committees in Belgrade (Scientific Ethical Committee of Clinical Hospital Center "Bezanijska Kosa") approved the study and all patients gave informed consent to participate according to the Declaration of Helsinki. All samples were stored at –80°C, and the clinical manifestations and characteristics of the patients are summarized in Table 1.

Laboratory tests. Blood samples from all included patients were analyzed for levels of factors such as C-reactive protein (CRP) by routine biochemistry. LA was assessed according to the guidelines of the International Society on Thrombosis and Haemostasis[12]. Full blood cell counts were determined and thrombosis was evaluated as described[18]. Anticardiolipin, anti-β2-GP1, and anti-DNA antibodies were measured by ELISA (Binding Site). Further, antinuclear antibodies (ANA) were determined by indirect immunofluorescence on mouse liver and Hep-2 cell substrate.

Table 1. Characteristics and distribution of clinical manifestations in the Serbian cohort of patients with APS included in the study. Values are n (%) unless otherwise specified.

Patient Characteristics or Clinical Manifestations	PAPS	SAPS
Patients, n	73	33
Age, yrs, median (range)	42 (21–79)	52 (27–80)
Women	62 (85)	29 (88)
Fetal losses	59 (81)	21 (64)
Anti-CL IgG	27 (37)	23 (70)
Anti-CL IgM	51 (70)	24 (73)
Anti-β2-GP1 IgG	24 (33)	19 (58)
Anti-β2-GP1 IgM	35 (47)	23 (70)
ANA	13 (18)	29 (88)
LA	38 (53)	13 (39)
Thrombosis venous	15 (21)*	3 (9)
Thrombosis arterial	28 (63)*	17 (52)
C3 low level	0 (0)	2 (6)

* Information about arterial/venous thrombosis was not available for 1 patient. APS: antiphospholipid syndrome; PAPS: primary APS; SAPS: secondary APS; CL: cardiolipin; Ig: immunoglobulin; β2-GP1: β2 glycoprotein 1; ANA: antinuclear antibodies; LA: lupus anticoagulant; C3: complement factor 3.

Patients: APS Italian cohort. Plasma samples from 40 patients ≥ 18 years of age with APS according to the Sydney criteria[12] were enrolled at the Lupus Clinic, Sapienza University of Rome. Fifteen patients had PAPS and 25 had SAPS (and SLE) diagnosed according to the American College of Rheumatology revised criteria[20]. Written consent was obtained from each patient and the Ethical Committee of Policlinico Umberto I hospital approved the study design. Characteristics and distribution of clinical manifestations of the patients are summarized in Table 2.

Laboratory tests. Among the laboratory tests, the following variables were evaluated: complete blood count, erythrocyte sedimentation rate, CRP levels, serum levels of creatinine and complement C3 and C4, ANA [indirect

Table 2. Characteristics and distribution of clinical manifestations in the Italian cohort of patients with APS included in the study. Values are n (%) unless otherwise specified.

Patient Characteristics or Clinical Manifestations	PAPS	SAPS
Patients, n	15	25
Age, yrs, median (range)	32 (20–62)	35 (12–76)
Women	14 (93)	24 (96)
Fetal losses	3 (20)	5 (20)
Anti-CL IgG	10 (67)	15 (60)
Anti-CL IgM	9 (60)	16 (64)
Anti-β2-GP1 IgG	7 (88)	10 (40)
Anti-β2-GP1 IgM	7 (50)	9 (36)
ANA	8 (53)	25 (100)
LA	10 (71)	17 (68)
Thrombosis venous	8 (53)	17 (68)
Thrombosis arterial	7 (47)	6 (24)
C3 low level	0 (0)	11 (44)

APS: antiphospholipid syndrome; PAPS: primary APS; SAPS: secondary APS; CL: cardiolipin; Ig: immunoglobulin; β2-GP1: β2 glycoprotein 1; ANA: antinuclear antibodies; LA: lupus anticoagulant; C3: complement factor 3.

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immunofluorescence (IIF) on Hep-2], anti-dsDNA (ELISA or IIF on *Crithidia luciliae*), extractable nuclear antigens by ELISA (anti-Sm, anti-RNP, anti-Ro/SSA, anti-La/SSB), anticardiolipin IgG and IgM, anti-β2-GP1 IgG and IgM. LA was assessed according to the guidelines of the International Society on Thrombosis and Hemostasis[12]. To be considered as present, each of these tests had to be positive on at least 2 occasions 12 weeks apart throughout the patient's clinical history.

Patients: Swedish VTE cohort. Plasma samples were taken from patients selected from the Malmö Thrombophilia Study (MATS), a prospective population-based study conducted at Skåne University Hospital in Sweden from 1998 to 2008 that included 1465 consecutive patients diagnosed with VTE[21]. The primary endpoint of the study was the diagnosis of deep vein thrombosis (DVT) and/or pulmonary emboli (PE) during the followup period. Diagnosis of DVT and PE was objectively confirmed by phlebography, duplex ultrasonography, computed tomography, lung scintigraphy, or magnetic resonance imaging[21]. All patients were initially treated with low molecular weight heparin or unfractionated heparin, and then with warfarin as an oral anticoagulant for 3–6 months. A selection of 132 patients with nonrecurrent and 134 patients with recurrent VTE matched in age and sex was used for our current study. In the recurrent subgroup, 39% of patients had acquired risk factors for VTE recurrence (malignancy, trauma, major surgery, immobilization, female hormone therapy, and pregnancy) whereas 38% of the nonrecurrent subgroup had acquired risk factors for VTE recurrence. Data regarding aCL were available for 48% of patients (n = 127). For each patient, 2 blood samples were analyzed: 1 in the acute stage (inclusion sample) and 1 at followup (posttreatment). Because of the availability of samples in the acute stage, FH autoantibodies were analyzed in 128 nonrecurrent and 107 recurrent VTE samples whereas in posttreatment samples, 130 nonrecurrent and 128 recurrent VTE samples were analyzed. The baseline characteristics of study population and distribution of FH autoantibodies in patients with nonrecurrent and recurrent VTE are presented in Table 3. All participants provided written informed consent according to the Declaration of Helsinki, and the study was approved by the ethics committee of Lund University (LU 237/2007).

Healthy controls. All serum healthy controls used in our study were collected from unrelated healthy, volunteers from Sweden. For analysis, these were matched in age and sex to the Serbian (n = 115) and Italian cohorts (n = 40). For the thrombosis cohort, all 163 controls were used (74% women, mean age 42 yrs). Healthy controls were not screened for aPL.

Table 3. Baseline characteristics of study population and distribution of FH autoantibodies in patients with nonrecurrent and recurrent VTE. Values are mean ± SD or % (n) unless otherwise specified.

Variables	Nonrecurrent VTE, n = 132	Recurrent VTE, n = 134	p
Age at inclusion, yrs	68.4 ± 16.0	68.3 ± 15.1	0.9*
Sex			0.90†
Men	51 (67)	51.5 (69)	
Women	49 (65)	48.5 (65)	
BMI, kg/m^2	26.2 ± 4.2	27.5 ± 5.1	0.025*
Malignancy			0.032†
Yes	16 (21)	7.5 (10)	
No	84 (111)	92.5 (124)	
Anti-FH§			0.046†
Positive	8 (10)	16 (20)	
Negative	92 (120)	84 (108)	
Duration of anticoagulant, days	171 ± 89	176 ± 77	0.51

* Mann-Whitney U test. † Chi-square test. § Eight samples were missing and therefore could not be analyzed for FH autoantibodies (nonrecurrent, n = 2 and recurrent VTE, n = 6). FH: complement factor H; VTE: venous thromboembolism; BMI: body mass index.

Determination of FH autoantibodies levels by ELISA. The assay was performed using FH immobilized in microtiter plates, incubated with serum or plasma with samples diluted 1:50 followed by the detection of bound autoantibodies with rabbit anti-human IgG Abs (DakoCytomation) and then by swine anti-rabbit horseradish peroxidase (HRP)-Abs (DakoCytomation) as described[22]. Concentrations of FH autoantibodies were calculated relative to a standard, polyclonal rabbit antibodies against FH (H-3000; Santa Cruz Biotechnology) set at 100 AU/ml. The samples with levels above the mean ± 2 SD of those in the control group were considered positive. The cutoffs varied slightly because these were calculated separately for each control group matched in age and sex to each of the 3 cohorts. The cutoffs for FH autoantibodies were > 95.4 AU/ml (APS, Serbia), > 73.6 AU/ml (APS, Italy), and > 98.6 AU/ml (thrombosis).

Western blot analysis for CFHR1 deletions. Samples from 106 patients with APS (Serbia), 40 patients with APS (Italy), and 163 healthy controls were investigated for the presence of CFHR1. Serum or plasma samples were separated under nonreducing conditions using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred onto PVDF membrane and incubated with mouse monoclonal anti-FH (C18/3; Santa Cruz Biotechnology) that identified the conserved C-terminus of FH (150 kDa) and the 2 differentially glycosylated forms of CFHR1α and CFHR1β (37 kDa and 42 kDa). Bound antibodies were detected with a polyclonal anti-mouse IgG antibody conjugated with HRP (DakoCytomation). Finally, the blots were developed using 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich).

Statistical analyses. Statistical significance of differences was tested for multiple groups with continuous data using the Kruskal-Wallis test with Dunn multiple comparisons posttest. For comparison of 2 groups, Mann-Whitney U test was used. For nominal data, Pearson chi-square or Fisher's exact test was used for 2 groups. For the MATS cohort, the time to event analysis was performed by Kaplan-Meier analysis. The log-rank test was used to compare recurrence-free survival in patients with VTE positive and negative for FH autoantibodies. Univariate and multivariate regression analyses were performed using the Cox proportional hazards model. Statistical analyses were performed using Prism5 (GraphPad), JMP 11 (SAS), or IBM SPSS 21 (IBM). Two-sided p values of < 0.05 were considered significant.

RESULTS

FH autoantibodies were present in sera/plasma of patients with APS. The Serbian cohort has been characterized in previous studies[18] while the Italian cohort was published here for the first time. Despite the limited size, the clinical manifestation of patients included in the Italian cohort corresponded well to the manifestations presented in a large cohort of 1000 patients with APS in which the most common reported manifestations were DVT (31.7%), thrombocytopenia (21.9%), aCL IgG (43.6%), aCL IgM (12.2%), ANA (59.7%), and LA (53.6%). Fetal loss was the presenting manifestation in 14% of female patients with early fetal losses (35.4%)[23].

The presence of FH autoantibodies was analyzed in sera or plasma samples from 2 independent cohorts of patients with APS as well as healthy controls using ELISA (Figure 1A–B). The samples with levels above the cutoff, defined as the mean ± 2 SD of the control group, were considered positive, as indicated by the dotted line. In the 2 control groups, matched individually by age and sex to their respective cohorts, only 6 individuals (5.2%) in the Serbian cohort and 2 (5.0%) in the Italian cohort were positive for FH

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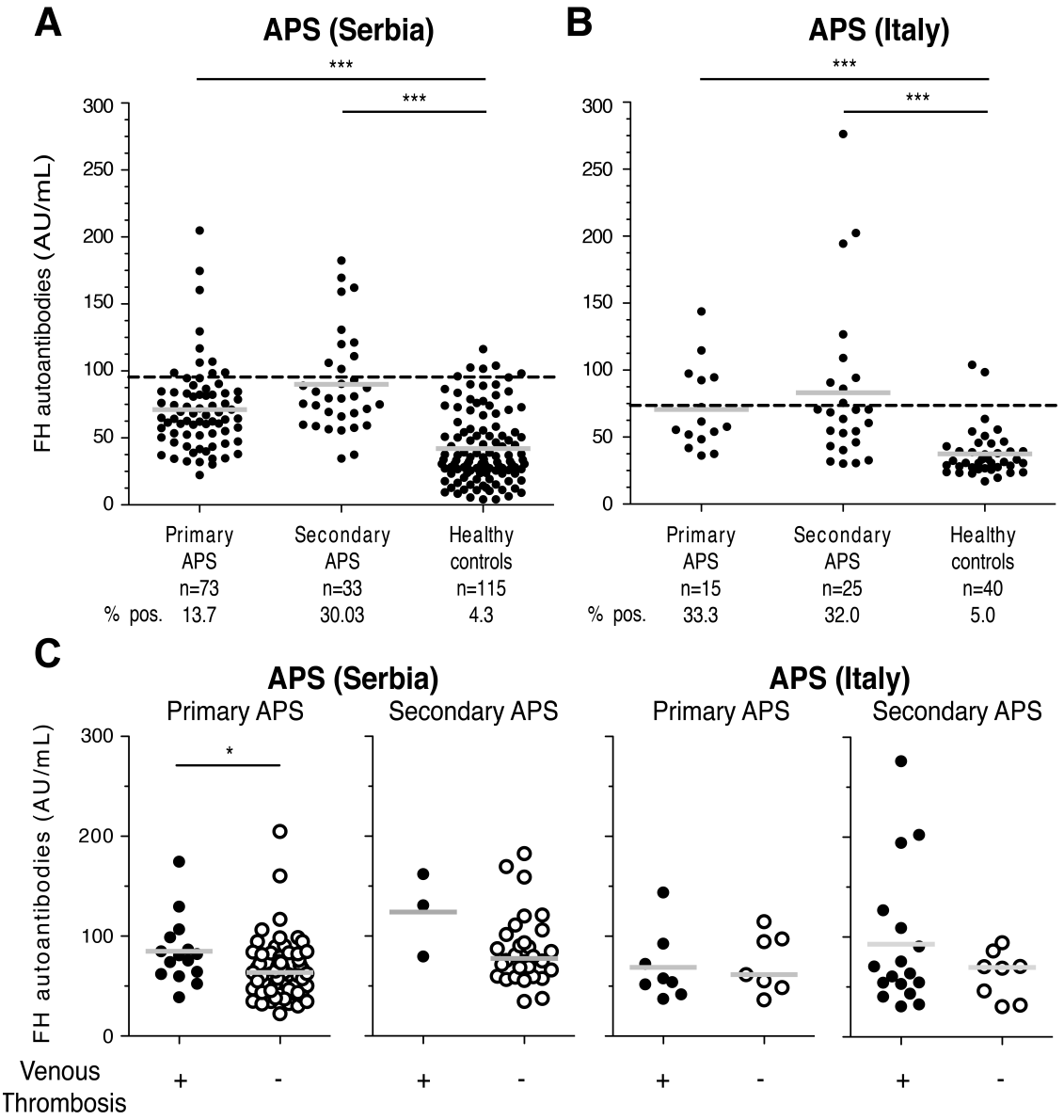

Figure 1. Patients with APS have increased FH autoantibody levels. (A) Serum and (B) plasma samples of patients with APS and age- and sex-matched healthy controls were analyzed for the binding of autoantibodies to immobilized purified FH using ELISA. The samples with levels above the mean ± 2 SD of those in the control group were considered positive. The dotted lines represent the cutoffs calculated separately for each control group. (C) Association of FH autoantibody levels with venous thrombosis in patients with PAPS or SAPS from the 2 cohorts. Statistical significance of differences between autoantibody levels in disease groups compared with controls was calculated using a Kruskal-Wallis test and Dunn multiple comparisons posttest (for A and B) and Mann-Whitney U test (for C). P values lower than 0.05 were considered statistically significant. * $p < 0.05$. *** $p < 0.001$. APS: antiphospholipid syndrome; FH: complement factor H; PAPS: primary APS; SAPS: secondary APS.

autoantibodies. This frequency is within the range observed previously in the healthy controls in several studies[6,8].

In both cohorts, significantly increased FH autoantibody levels were observed in patients with APS compared with the age- and sex-matched healthy controls (Kruskal-Wallis test; Figure 1A–B). The frequencies of FH autoantibodies were also significantly different between PAPS and controls ($p = 0.02$) and SAPS and controls ($p < 0.001$) in the Serbian cohort, as evaluated with Pearson chi-square test. For the Italian cohort, the differences in frequencies were also significant for PAPS versus controls ($p = 0.005$) and SAPS versus controls ($p = 0.001$).

To investigate whether patients who were positive for FH autoantibodies were affected more often from any particular APS manifestation, we performed an association analysis. Because the 2 cohorts had different proportions of patients

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with PAPS and SAPS, we analyzed these groups separately. We observed that patients in the Serbian cohort with PAPS and FH autoantibodies were affected significantly more often from heart disorders (cardiac vegetations, acute myopathy, valve thickening, and intracardiac thrombosis) compared with those without FH autoantibodies (Table 4). This variable was not available in the Italian cohort. Further, in the Serbian cohort, patients with SAPS and FH autoantibodies were less affected by arthritis, and although this association did not reach statistical significance in the smaller Italian cohort, a similar trend was observed. Curiously, FH autoantibodies appeared to be less frequent in patients with SAPS with cutaneous disorder in the Serbian cohort and more frequent in the Italian cohort. One of the main manifestations of both forms of APS is thrombosis. We therefore investigated whether arterial or venous thrombosis were associated with elevated levels of FH autoantibodies. We did not observe any association with FH autoantibody levels and arterial thrombosis (data not shown). However, in the Serbian cohort, we observed that patients with SAPS with venous thrombosis displayed elevated levels of FH autoantibodies (Figure 1C). This association was also observed when analyzing PAPS and SAPS together in the Serbian cohort (data not shown). However, the association could not be observed in the smaller Italian cohort, although a trend was also present (Figure 1C). Because a subgroup of patients with APS with venous thrombosis displayed elevated levels of FH autoantibodies, we next investigated FH autoantibody levels in patients with VTE.

FH autoantibodies were present in plasma of patients with thrombosis. The presence of FH autoantibodies was analyzed in plasma samples obtained from patients with VTE at inclusion and posttreatment as well as healthy controls using ELISA (Figure 2A). The cohort of patients with VTE was composed of patients with VTE who were divided into 2 groups, depending on the recurrence of the thrombosis during the followup period (recurrent vs nonrecurrent). For each patient, 2 blood samples were analyzed for the presence of FH autoantibodies: 1 at the acute thrombosis stage (inclusion sample) and 1 posttreatment sample. In all 4 groups of patients with thrombosis, there was a tendency toward increased levels of FH autoantibodies compared with healthy controls, although these were not statistically significantly different among groups ($p > 0.05$, Kruskal-Wallis test; Figure 2A). However, when a chi-square test was used to compare frequencies of FH autoantibodies, we found in posttreatment samples a significantly higher frequency of positivity in patients with recurrent VTE compared with nonrecurrent patients and healthy controls ($p = 0.0053$). A similar trend was found in inclusion samples; however, results were not statistically significant ($p = 0.058$). In 48% of patients, aCL were analyzed and were present in 4% of patients in both recurrent and nonrecurrent groups.

Further analysis was performed using the Kaplan-Meier method. FH autoantibody–negative patients had significantly longer recurrent-free survival compared with FH autoantibody-positive patients when posttreatment samples were

Table 4. Correlation analysis between presence of FH autoantibodies and clinical manifestations. Table indicates % of FH autoantibody–positive or –negative patients in the 2 cohorts (Serbian and Italian) and disease classifications (PAPS and SAPS) depending on the presence of the indicated manifestations. Disorders are displayed in collective terms and include the following: obstetric disorder including fetal losses; coagulation disorder including arterial or venous thrombosis and cerebral, pulmonary, or renal thrombosis; hematological disorder including hemolytic anemia, leukopenia, lymphopenia, or thrombocytopenia; heart disorder including infarction, unstable angina, vegetations, cardiomyopathy, and valve dysfunctions; neurological disorder including migraine, dementia, and epilepsy; pulmonary disorder including pulmonary hypertension, intraalveolar hemorrhage, and pulmonary microthrombosis; renal disorder including glomerular capillary thrombosis and renal vein thrombosis; cutaneous disorder including skin ulcerations, superficial necrosis, and digital gangrene.

Cohort	Serbian		Italian	
Patient classification	PAPS	SAPS	PAPS	SAPS
a-FH^{pos}/a-FH^{neg}, n	10/63	10/23	5/10	9/16
Manifestation	n (% a-FH^{pos}/% a-FH^{neg})		n (% a-FH^{pos}/% a-FH^{neg})	
Obstetric disorder	59 (90/79)	21 (50/70)	3 (20/20)	5 (22/19)
Coagulation disorder	41 (44/60)	22 (70/65)		
Hematological disorder	—	—	6 (40/ 40)	16 (67/63)
Heart disorder	13 (40/14)*	17 (50/55)		
Neurological disorder	33 (40/46)	25 (60/83)	4 (40/20)	7 (33/25)
Pulmonary disorder	9 (10/13)	3 (20/4)	—	—
Renal disorder	3 (0/5)	0 (—/—)	1 (20/0)	9 (56/25)
Cutaneous disorder	17 (30/23)	30 (70/100)*	1 (0/10)	18 (100/56)*
Arthritis	5 (10/6)	25 (50/87)*	0 (—/—)	14 (44/63)

Significant difference in ratio between manifestation-positive patients in the 2 antibody groups was calculated using Pearson chi-square test and indicated as * $p < 0.05$. FH: complement factor H; APS: antiphospholipid syndrome; PAPS: primary APS; SAPS: secondary APS; a-FH^{pos}: FH autoantibody–positive; a-FH^{neg}: FH autoantibody–negative.

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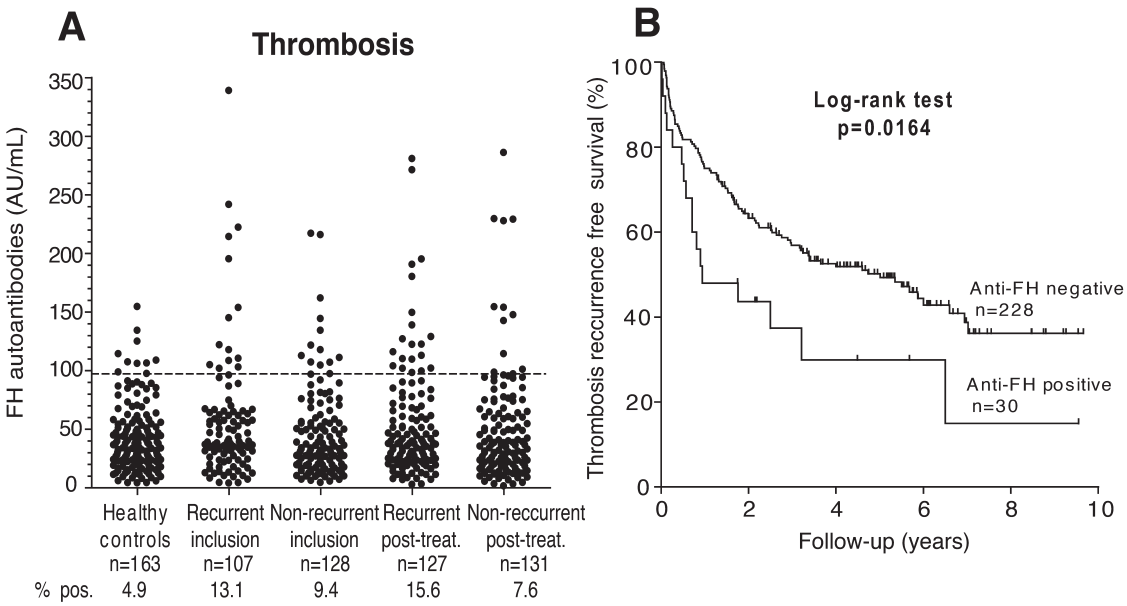

Figure 2. FH autoantibody positivity is associated with thrombosis recurrence. A. Plasma samples of Swedish patients with thrombosis and healthy controls were analyzed for the binding of autoantibodies on immobilized purified FH using ELISA. The samples with levels above the mean ± 2 SD of those in the control group were considered positive. The dotted line represents the cutoff calculated for the control group. Statistical significance of differences between autoantibody levels in diseases groups compared with controls was calculated using a Kruskal-Wallis test and Dunn multiple comparisons posttest. P values < 0.05 were considered statistically significant. B. Kaplan-Meier analysis of VTE recurrence according to FH autoantibodies in the posttreatment samples. Patients were divided according to positive (n = 30) and negative (n = 228) for FH autoantibodies. P = log-rank test, used to compare the recurrence-free survival between 2 groups. FH: complement factor H; VTE: venous thromboembolism.

analyzed using log-rank test (p = 0.0164, chi-square = 5.76; Figure 2B). Univariate Cox regression analysis on posttreatment (warfarin) levels of FH autoantibodies showed that patients positive for FH autoantibodies had higher risk of VTE recurrence compared with those negative for FH autoantibodies (HR 2.0, 95% CI 1.2–3.3, p = 0.011). Multivariate analysis showed that this association was independent of body mass index and duration of warfarin treatment (HR 1.9, 95% CI 1.1–3.3, p = 0.033). A similar trend was found for levels of FH autoantibodies and risk of VTE recurrence in inclusion samples; however, results did not reach statistical significance on univariate (HR 1.4, 95% CI 0.8–2.4, p = 0.30) or multivariate Cox regression analysis (HR 1.4, 95% CI 0.7–2.7, p = 0.31).

CFHR1 deficiency in patients with APS. Because in aHUS an association was reported between FH autoantibodies and homozygous deletions of CFHR1[9], we also analyzed whether there was the same association in APS. Genetic CFHR1 deficiency can be accurately measured by quantifying CFHR serum levels using Western blotting[24], and previously we observed a 100% match between Western blot results and Multiplex Ligation-dependent Probe Amplification[8]. Using Western blotting, we detected 7 CFHR1-deficient patients with APS in the Serbian cohort and 4 CFHR1-deficient patients with APS in the Italian cohort. We also identified 2 CFHR1-deficient healthy controls. None of the CFHR1-deficient patients with APS in the Serbian cohort were positive for FH autoantibodies, but 3 patients with APS (75%) in the Italian cohort were positive for FH autoantibodies. These frequencies were too low for meaningful statistical analysis.

DISCUSSION

Autoantibodies targeting complement components have been described for a number of complement proteins, and participate in several, especially renal, diseases[25]. Presence of FH autoantibodies has been reported in several aHUS cohorts[4,5,6,7], and was also observed in SLE, RA, and LA+/APS[8]. However, the latter cohort of LA+ patients was small and not fully diagnosed with APS. Therefore, we have now performed a study evaluating the frequency and levels of FH autoantibodies in 2 well-characterized and independent cohorts of patients with APS. Because we identified an association between FH autoantibodies and venous thrombosis in the Serbian APS cohort, we also analyzed a Swedish thrombosis cohort (MATS).

A significant increase in levels and frequencies of FH autoantibodies was found in both cohorts of patients with APS compared with matched healthy controls, in PAPS and SAPS. This finding reflects the fact that these patients generate a large variety of autoantibodies that are, in particular, directed to molecules present on cellular surfaces. Although FH is a soluble plasma protein mainly produced in the liver, it readily interacts with various types of cells, particularly when these are damaged[26,27] or contain

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deposits of C3b resulting from activation of the complement cascade.

Further, we analyzed whether the positivity for FH autoantibodies in APS was related to clinical characteristics. We observed that the presence of FH autoantibodies in APS was significantly associated with an increased prevalence of heart disease in PAPS in the Serbian cohort, with an increased frequency of cardiac vegetations, acute myopathy, valve thickening, and intracardiac thrombosis. We further revealed a contradictory association of cutaneous disorder and FH autoantibodies in the 2 cohorts that will require further study before a conclusion can be drawn. FH autoantibodies are convincingly associated with the pathology of aHUS and one could hypothesize that some cases of catastrophic APS may have some features resembling aHUS, thus suggesting that complement targeting could be an interesting therapeutic option. Support for such a hypothesis is also provided by others[28]. It is also important to note that FH autoantibodies are found not only in aHUS, but also in RA and now also APS, implying that impairment of FH function by autoantibodies may be involved in the pathology of several autoimmune diseases. Because FH autoantibodies are not correlated with aPL, we may expect that complement activation observed in APS[29,30] may in some cases be directly mediated by the presence of FH autoantibodies and not necessarily by anti-β2-GP1. However, one would expect that the molecular mechanism behind the generation of FH autoantibodies will vary between aHUS and autoimmune diseases that is in part supported by our observation that FH autoantibodies are not correlated with CFHR1 deletions in APS, as is the case in aHUS[5,6]. However, one should also bear in mind the very low frequency of CFHR1 deletions in our cohort, making statistical analysis impossible.

Perhaps the most interesting association identified in our study in patients with APS was that of the presence of FH autoantibodies in patients with venous thrombosis. Therefore, we analyzed a selection of samples from a large VTE cohort. Importantly, Cox regression analysis showed that patients who were positive for FH autoantibodies had a significantly higher risk of VTE recurrence compared with the patients who were negative for FH autoantibodies. This is particularly interesting since there is increasing evidence that the complement system is tightly connected with hemostasis[15]. Both complement and coagulation are cascades of proteolytic cleavages, and activation of one system often leads to some degree of activation of the other. FH has to date been mainly known as a complement inhibitor, but it is interesting to note its structural similarity (complement control protein domains) to the main target of autoantibodies in APS, β2-GP1[31,32]. A recent study has demonstrated that FH, such as β2-GP1, inhibits the anionic phospholipid–activated Hageman factor VII contact coagulation pathway[33]. However, we did not observe any association between the presence of FH autoantibodies and LA or specific autoantibodies against β2-GP1 or cardiolipin (data not shown), implying that the FH autoantibodies are specific and that the signal obtained in ELISA does not result from cross-reactivity of antibodies against β2-GP1. Another observation is that FH may interact with the von Willebrand factor (vWF), which results in inhibition of ADAMTS-13–mediated proteolysis of vWF multimers[34]. Importantly, vWF is released from endothelial cells upon inflammation and injury, and the same cells can secrete FH[35]. Thus, one may speculate that FH could participate in blood clotting at the site of endothelial injury by strengthening platelet aggregation and protecting vWF from excessive proteolysis. One could further hypothesize that this activity of FH may potentially be enhanced by the presence of autoantibodies leading to predisposition to VTE. Further, FH is readily taken up by platelets from plasma, and the exposure of platelets to the plasma of patients with aHUS with FH autoantibodies causes platelet aggregation, preventable by preincubation of the platelets with FH[36]. One common denominator of aHUS, APS, and thrombosis is vascular engagement, with activation of endothelium leading to platelet adherence, activation of coagulation, and formation of thrombus. There may be several mechanisms by which FH is engaged in these processes, which may be disturbed by the presence of autoantibodies.

One potential limitation of our study is the different ethnic backgrounds of the patients (from Serbia, Italy, and Sweden). However, all included patients were white and a large epidemiologic study showed little difference in the APS prevalence and phenotype among different cohorts in Europe[37].

We report an increased frequency of FH autoantibodies in 2 independent cohorts of patients with APS and identify potential correlation of such antibodies with recurrent VTE. This observation warrants further studies of the involvement of complement inhibitor FH in coagulation and hemostasis.

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