# Galectin-3-binding protein is a novel predictor of venous thromboembolism in systemic lupus erythematosus

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## Abstract

### Objective

*Venous (VTE) and arterial (AT) thrombosis in systemic lupus erythematosus (SLE) are poorly explained and difficult to predict. Leptin and tumour necrosis factor-like weak inducer of apoptosis (TWEAK) have been linked to subclinical atherosclerosis and galectin-3-binding protein (G3BP) to type I interferon activation and a pro-thrombotic environment. Thus, we explore serum G3BP, interferon gamma-induced protein 10 (IP-10), soluble CD163 (sCD163), TWEAK and leptin as predictors of VTE and AT, damage accrual, and all-cause mortality during follow-up in a Swedish SLE cohort.*

### Methods

*Baseline data were available from 162 SLE patients. VTE (deep vein thrombosis and/or pulmonary embolism), AT (myocardial infarction and/or stroke), damage accrual, and survival data were the main study outcomes and available at follow-up (median of five years). Baseline serum G3BP, IP-10, sCD163, TWEAK and leptin were measured and analysed by univariable and multivariable methods for association to the study outcomes.*

### Results

*During the follow-up, 10 (6%) VTE and 13 (8%) AT events occurred. The SLICC/ACR Damage Index increased in 78 (48%) patients, and 19 (12%) patients died. In the univariable regression analysis G3BP levels were significantly associated with an increased risk of VTE (hazard ratio (HR) 1.11, 95% confidence interval (CI): 1.01–1.22, p=0.03). This persisted in the adjusted multivariable analyses (HR 1.18, 95% CI: 1.05-1.33, p=0.007). The other biomarkers were not associated with AT/VTE, damage accrual, or all-cause mortality.*

### Conclusion

*Our study identifies serum G3BP as a novel predictor of VTE in SLE. Further studies are needed to understand the role of G3BP in VTE and translate this into clinical practice.*

**Key words**

systemic lupus erythematosus, venous thromboembolism, DVT, galectin-3-binding protein

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## Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with a wide spectrum of manifestations. The disease course is unpredictable and often complicated by accrual of damage, including arterial thrombosis (AT) and venous thromboembolism (VTE). Acquired organ damage has repeatedly been shown to associate with both poor quality-of-life and mortality in SLE (1-4). Despite the fact that all-cause mortality in SLE has declined over the past 20 years, the risk of death due to cardiovascular disease remains essentially unchanged (5). The presence of antiphospholipid antibodies (aPL), lupus nephritis (LN), persistent disease activity, damage accrual, high cumulative glucocorticoid exposure and/or the traditional Framingham cardiovascular risk factors can only partly explain the increased risk of thrombosis (1, 4, 6). Also, despite thromboprophylaxis, patients with SLE and antiphospholipid antibody syndrome (APS) still develop thrombotic events (3). Thus, there is a great need of improved pathogenic insight and identification of new biomarkers to identify patients at increased risk.

In SLE, type I interferons (IFN), circulating immune complexes, constant immune stimulation, dyslipidaemia, pro-inflammatory cytokines, chemokines and adipokines in combination with activated leukocytes, macrophages, platelets, microparticles (MPs) and the endothelium contribute to a pro-thrombotic environment (7). Biomarkers of these processes thus hold promise as future clinical biomarkers.

Ongoing type I IFN activation has been linked to platelet activation, endothelial dysfunction, premature vascular damage, development of venous thrombosis, and subclinical atherosclerosis in SLE, but not yet to cardiovascular events (8-16). Additionally, antimalarials, *e.g.* hydroxychloroquine (HQ), interfere with type I IFN signalling and seem to protect against thrombosis and infections (17-19). The productions of galectin-3-binding protein (G3BP) and IP-10 are IFN-inducible and they may serve as convenient measures of type I and II IFN activity (20-23). G3BP also seems to have IFN-independent functions directly involved in atherosclerosis and VTE in the general population as well as in SLE patients (14, 15, 24-27). Adipokines, *e.g.* leptin, have been linked to endothelial dysfunction, and soluble tumour necrosis factor-like weak inducer of apoptosis (sTWEAK) has been linked to increased rates of atherosclerosis, inflammation, angiogenesis, and apoptosis (28, 29). Plasma levels of leptin were elevated in SLE cases with atherosclerotic plaques when compared to healthy control subjects, and recently, McMahon *et al.* also showed that high leptin and sTWEAK levels were significantly associated with the presence of carotid plaque in SLE (30, 31).

Another contributor to SLE pathogenesis is dysregulation of macrophage phenotype and function, and macrophages are major components of atherosclerotic plaque (32). The soluble form of the haemoglobin-haptoglobin receptor CD163, sCD163, reflects macrophage activation (33). Soluble CD163 has been associated with carotid artery disease in the setting of chronic viral infection (human immunodeficiency virus (HIV) and hepatitis C virus (HCV)) with high type I IFN activity suggesting similar could be the case also in SLE (25).

Thus, these biomarkers have been linked to a pro-thrombotic environment and to subclinical atherosclerosis or VTE in cross-sectional studies, but they have not yet been investigated as predictors of clinical thrombotic events in SLE. Herein, we aimed to expand on these observations and explore if serum levels of G3BP, IP-10, TWEAK, leptin and/or sCD163 are predictive of occurrence of future venous or arterial thrombotic events, damage accrual, and all-cause mortality in a cohort of Scandinavian patients with SLE.

## Materials and methods

### *Study design*

The study was designed as an analytical observational prospective SLE cohort study. During follow-up the four main outcomes: VTE, AT, damage and survival, was registered. The objective was to determine potential associations between five experimental serum biomarkers (G3BP, IP-10, TWEAK, leptin and sCD163) and the main outcomes.

*Patients*

Baseline clinical and serological data and serum from 162 Swedish prevalent and incident SLE cases consecutively recruited during the period 2008-2011 were available for study. All clinical data were obtained by a trained rheumatologist at a tertiary referral centre, the outpatient clinic at Linköping University Hospital, as previously described (2).

*Ethics approval*

Oral and written informed consent was obtained from all SLE subjects. The study protocol was approved by the Regional Ethics Review Board in Linköping, Sweden (decision number M75-08/2008).

*Clinical and para-clinical baseline assessment*

All patients with established SLE were regularly followed in the out-patient clinic whereas incident cases were continuously included. Clinical manifestations, disease activity (SLEDAI-2K), and SLICC/ACR Damage Index (SDI) were recorded by a trained rheumatology specialist (Christopher Sjöwall) (34, 35). A review of all previously available medical records was also done to obtain pertinent clinical information for the interval. Traditional risk factors at time of inclusion were registered. Body-mass index (BMI) was calculated (height (metre) divided by weight (kilogram)$^2$). Current and ever smokers (patient reported at inclusion interview) were noted. Ever smokers were used in the statistical analysis. Diabetes was defined according to the SDI, *i.e.* counted regardless of therapy(35). Presence of hypertension was defined as those who continuously received antihypertensive therapy. Routine biochemistry and serology were obtained. We did not have access to data on the lipid profile, homocysteine levels or subclinical atherosclerosis. IgG and IgM anti-cardiolipin (aCL) and anti-β2-glycoprotein I (anti-β2GPI) were detected using fluoroenzyme-immunoassays (Phadia-250 instrument; Thermo-Fisher Scientific Phadia AB, Uppsala, Sweden) and cut-offs for medium-to-high titre aPL isotypes were set above the 99$^{th}$ percentile of healthy referents as previously described (36). Levels above the cut-off for each isotype was considered positive (3). Lupus anticoagulans (LA) was measured by the dilute Russell's viper venom time (dRVVT) method (Siemens Healthcare Diagnostics, Erlangen, Germany). Duration of SLE was defined as the time elapsing from the date the patient met ≥4 of the ACR criteria for SLE (diagnosis date) to the first visit (baseline). Follow-up time was defined as the period between the first and last visit.

*Outcomes*

VTE (*i.e.* deep vein thrombosis and/or pulmonary embolism) and AT (*i.e.* myocardial infarction and/or cerebrovascular infarction) data were available at baseline and follow-up with a median follow-up period of 5 years (range 0–8). VTE and AT were defined according to SDI. Deep vein thrombosis (DVT) where documented by clinical presence of swelling, ulceration or evidence of venous stasis and/or by ultrasound. Pulmonary embolism (PE) where documented by CT-scan. Myocardial infarction (MI) where documented with electrocardiography, myocardial enzymes (troponin T) and coronary angiography. Cerebrovascular insult were documented by CT-scan of the cerebrum.

*Analysis of the five experimental biomarkers*

Serum were separated according to routine protocols, stored at -80°C, and thawed prior to the analyses.

Baseline serum concentrations of G3BP, IP-10, TWEAK, leptin, and sCD163 were quantified using ELISA according to the manufacturer's instructions. G3BP was analysed using the Human 90K/Mac-2BP Platinum ELISA kit (BMS234, Bender MedSystems, Vienna, Austria) with concentrations expressed in μg/ml. For sCD163 measurements thawed serum samples were diluted 1:200 and analysed in duplicate using Human sCD163 Ready-SET-Go! ELISA (Catalogue no. 88-50360, Bender MedSystems, Vienna, Austria). Concentrations were expressed in μg/ml. For IP-10 measurements thawed serum samples were diluted 1:10 and analysed in duplicate using Human IP-10 Instant ELISA (BMS284INST, Bender MedSystems, Vienna, Austria). Concentrations were expressed in pg/ml. For TWEAK measurements thawed serum samples were diluted 1:2 and analysed in duplicates using Human TWEAK INSTANT ELISA KIT (BMS2006INST, Bender MedSystems, Vienna, Austria). Concentrations were expressed in μg/ml. For leptin measurements thawed serum samples were diluted 1:50 and analysed in duplicates using Human Leptin INSTANT ELISA™ Kit (BMS2039INST, Bender MedSystems, Vienna, Austria). Concentrations were expressed in μg/ml.

*Statistics*

Cox proportional hazard (PH) regressions and binary logistic regression were applied for associative evaluation between baseline clinical/paraclinical parameters with thrombotic events, damage accrual, and all-cause mortality. We included the five biomarkers and a pre-defined set of variables (available traditional cardiovascular and non-classical risk factors, medication, and disease activity measures) in the univariable Cox regression analyses. All statistical analyses were performed in R software 3.4.3 (The R Foundation for Statistical Computing, Vienna, Austria). The R add-on package 'survival' was applied for Cox regressions. All five serum biomarkers and the variables with *p*-values less than 0.1 in the univariable analyses were subsequently included in the multivariable Cox regression analyses. Co-variates that violated the proportional hazard assumption were stratified (continuous variables were dichotomised prior to stratification). Data are presented as hazard ratios (HR), 95% confidence intervals (CI), and *p*-values. *p*-values <0.05 were considered statistically significant.

## Results

*Patients*

Patient characteristics and baseline demographic data are presented in Table I and grouped by VTE status (never/ever). All 162 patients fulfilled the 1997 American College of Rheumatology (ACR) (37). The study population in-

cluded 16 male and 146 female patients with a median age of 54 years (range 18-89 years) and a median disease duration at baseline of 10 years (range 0–46 years). 11 patients (8%) were included as incident cases. 152 patients (94%) were of Caucasian origin. 25 patients (15%) met the criteria for APS (3). 39 patients (24%) received warfarin, including 19 with APS. At baseline, 14 patients (9%) had previously been diagnosed with VTE and 38 (23%) with AT or have had angina pectoris/coronary bypass. 2 patients had experienced a myocardial infarction twice. Ninety-two patients (57%) received antimalarials (hydroxychloroquine), and 46 patients (28%) were prescribed immunosuppressant therapy (methotrexate, azathioprine, cyclosporine, or mycophenylate mofetil).

*Venous thromboembolic events*

At inclusion, 12 patients (7%) had a previous history of VTE. Two of these patients had experienced both DVT and PE, *i.e.* 14 VTE prior to inclusion in the study. In the follow-up period, a total of 10 VTE occurred in 10 patients, 5 DVT and 5 PE, respectively.

In the univariable Cox regression analysis (Table II), G3BP levels were significantly associated with an increased risk of VTE (hazard ratio (HR) 1.11, 95% confidence interval (CI): 1.01–1.22, $p$=0.03), while no associations were observed for any of the other four biomarkers. Being classified with APS was associated with an increased risk of VTE (HR 4.08, 95% CI: 1.15–14.5, $p$=0.03) but not presence of any aPL (HR 2.22, 95% CI: 0.45–11, $p$=0.33). Also, significantly increased HR's where not observed when testing for the individual aPL (aCL and anti-β2GPI of IgG or IgM class) or positive LA test (data not shown). Age was also significantly associated with VTE (HR 1.08, 95% CI: 1.03–1.13, $p$=0.003), while gender, BMI, smoking (ever), previous LN, or disease activity scores were not. Ongoing immunosuppressive therapy or antimalarials was not associated with VTE. In the multivariable Cox regression analysis (Table II), significant HR for VTE were found for G3BP (HR 1.18, 95% CI: 1.05–1.33, $p$=0.007)

**Table I**. Patient characteristics grouped by status of venous thromboembolism ever/never.

| | VTE (never) | | VTE (ever) | |
|---|---|---|---|---|
| | n=142 | % | n=20 | % |
| Female/male | 130/12 | 92/8 | 16/4 | 80/20 |
| Age in years, median (range) | 52 | (18-89) | 66 | (44-89) |
| Disease duration in years, median (range) | 9 | (0-46) | 16 | (1-40) |
| Caucasian | 132 | 93 | 20 | 100 |
| Hypertension | 70 | 49 | 14 | 70 |
| Body Mass Index, median (range) | 25 | (18-46) | 27 | (20-32) |
| Diabetes | 6 | 4 | 3 | 15 |
| Ever/current smoker | 62 / 17 | 45/12 | 10 (/) 1 | 53/5 |
| Fulfilling ACR 1997 SLE criteria | 142 | 100 | 20 | 100 |
| Number of fulfilled ACR criteria, median (range) | 4 | (3-9) | 5 | (3-7) |
| Malar rash | 66 | 46 | 6 | 30 |
| Discoid LE | 25 | 18 | 4 | 20 |
| Photosensitivity | 80 | 56 | 10 | 50 |
| Oral ulcers | 14 | 10 | 1 | 5 |
| Non-erosive arthritis | 114 | 80 | 14 | 70 |
| Serositis | 50 | 35 | 13 | 65 |
| Renal disorder | 28 | 20 | 5 | 25 |
| Neurological disorder | 8 | 6 | 1 | 5 |
| Haematological disorder | 81 | 57 | 14 | 70 |
| Immunological disorder | 71 | 50 | 12 | 60 |
| Anti-dsDNA | 65 | 46 | 12 | 60 |
| Anti-Sm | 12 | 8 | 2 | 10 |
| aCL, anti-β2GPI and/or LA test positive* | 69 | 53 | 14 | 78 |
| Antinuclear antibodies | 140 | 99 | 19 | 95 |
| SDI, median (range) | 0 | (0-7) | 2 | (0-6) |
| APS | 16 | 11 | 9 | 45 |
| Cerebrovascular incident** | 14 | 10 | 2 | 10 |
| Myocardial infarction** | 9 | 6 | 4 | 20 |
| Angina pectoris or bypass | 7 | 5 | 2 | 10 |
| Pulmonary embolism*** | 0 | 0 | 2 | 10 |
| Deep venous thrombosis*** | 0 | 0 | 12 | 60 |
| Disease activity and manifestations at inclusion | | | | |
| SLEDAI-2K, median (range) | 2 | (0-19) | 0 | (0-8) |
| Arthritis | 8 | 6 | 1 | 5 |
| Proteinuria | 3 | 2 | 1 | 5 |
| Cytopenia**** | 9 | 6 | 0 | 0 |
| Mucosal ulcers | 3 | 2 | 0 | 0 |
| Pleuritis | 1 | 1 | 2 | 10 |
| Rash | 11 | 8 | 3 | 15 |
| Alopecia | 4 | 3 | 1 | 5 |
| Medication at inclusion | | | | |
| Prednisolone any dose | 92 | 65 | 14 | 70 |
| Prednisolone 7.5 mg or higher | 29 | 20 | 4 | 20 |
| Hydroxychloroquine | 82 | 58 | 10 | 50 |
| Synthetic DMARDs***** | 37 | 26 | 6 | 30 |
| Statins | 33 | 23 | 5 | 25 |
| Aspirin | 62 | 44 | 9 | 45 |
| Warfarin | 26 | 18 | 13 | 65 |
| Serum G3BP in µg/mL, median (range) | 8.3 | (1.7-28) | 10 | (5.4-26) |
| Serum IP-10 in pg/mL, median (range) | 270 | (98-3304) | 257 | (143-861) |
| Serum sCD163 in µg/mL, median (range) | 1.8 | (0.05-8.8) | 1.9 | (0.6-5.1) |
| Serum leptin in µg/mL, median (range) | 12.3 | (1.4-171) | 18 | (3.5-48.6) |
| Serum TWEAK in µg/mL, median (range) | 2 | (1.1-9.1) | 2 | (1.1-4) |

*Total aPL positive patients included in the regression analysis. Anti-β2-glycoprotein I (n=32) was included here although it is not part of the criteria set. Lupus anticoagulant were not tested in 21 patients.

**The number represent total number of events. Two patients have had both a CVI and MI, *i.e.* 27 patients have had an AT. Thus percentages are not stated.

***The number represent total number of events. Two patients have had both a DVT and PE, *i.e.* 12 patients have had VTE.

****Anaemia, thrombocytopenia and/or, leukopenia according to SLEDAI-2K

*****Methotrexate, azathioprine, cyclosporine, mycophenolate mofetil

and age (HR 1.08, 95% CI: 1.03–1.13, $p$=0.003). Adjusting for age and disease duration did not alter this association.

By exclusion of the 12 cases with previous VTE, 8 patients had VTE during the follow-up period (2 patients had VTE before as well as during follow-up). Repeating the univariable Cox regression analysis for G3BP and VTE resulted in a HR 1.11, 95% confidence interval (CI): 1.00–1.22, $p$=0.05 (data not shown in Table II).

*Arterial thrombotic events*

At inclusion, 27 patients (17%) had a previous history of MI or CVI. In the follow-up period, a total of 13 patients developed an AT, whereof 5 were MI and 8 CVI, respectively.

In the univariable Cox regression analysis (Table III), no significant association was observed between AT and any of the five biomarkers. Age, APS, history of AT or diabetes, treatment with aspirin or statins were associated with significant HR's for AT (HR 1.07, 95% CI: 1.02–1.12, $p$=0.002; HR 5.15, 1.73–15.34, $p$=0.003; HR 8.83, 95% CI: 2.89–27.1, $p$<0.001; HR 6.48, 95% CI: 1.78–23.59, $p$=0.005; HR 7.47, 95% CI: 1.66–33.7, $p$=0.009; HR 3.88, 95% CI: 1.3–11.55, $p$=0.02). Ongoing immunosuppressive therapy or antimalarials did not seem to add to the risk or protect from AT. In the multivariable Cox regression analysis (Table III), APS and aspirin were significantly associated with AT (HR 11.52, 95% CI: 1.92–69.3; $p$=0.008 and HR 10.63, 95% CI: 1.61–70.1, $p$=0.01). No association was observed for any of the serum biomarkers.

By exclusion of the 27 cases with previous AT, only 5 patients had AT in the follow-up period (8 patients had AT before and during follow-up) which compromised further analysis.

*Damage accrual*

At inclusion, the median SDI was 0 (range 0–7). In the follow-up period, 78 patients (48%) increased their damage scores (median SDI score 1, range 0–10, for the whole cohort). In the univariable binary logistic regression analysis (Table IV), sCD163 showed a weak non-significant association with SDI scores (OR 1.25, 95% CI: 0.98–1.63, $p$=0.08). There were no associations between the other biomarkers and SDI. Age, disease duration, APS, a history of AT, warfarin, aspirin and statin treatment were significantly associated with a progression in SDI (OR 1.06, 95% CI: 1.03–1.08, $p$<0.001; OR 1.03, 95% CI: 1.001–1.07, $p$=0.04; OR 2.65, 95% CI: 1.1–6.87, $p$=0.04; OR 8.36, 95% CI: 3.02–29.7, $p$<0.001; OR 2.73, 95% CI: 1.3–5.96, $p$=0.009; OR 2.46, 95% CI: 1.31–4.69, $p$=0.006; OR 2.58, 95% CI: 1.22–5.64, $p$=0.02). In the multivariable binary regression analysis (Table IV), there were no significant associations between any of the biomarkers and increase in SDI scores. In contrast to the univariable analyses, age was the only variable associated with damage accrual (OR 1.05, 95% CI: 1.02–1.07, $p$<0.001).

*All-cause mortality*

During the follow up period, 19 (12%) patients died. In the univariable and multivariable logistic regression analysis (Table V) we did not observe any association between the studied biomarkers and mortality. Previous AT (OR 7.99, 95%CI: 2.92–22.43, $p$<0.001), diabetes (OR 7.31, 95% CI: 1.8–28.7, $p$=0.007), age (OR 1.09, 95% CI: 1.05–1.14, $p$<0.001), treatment with aspirin and statins (OR 5.33, 95% CI: 1.9–18.1, $p$=0.001 and OR 2.75, 95% CI: 1.01–7.25, $p$=0.05, respectively) were associated with all-cause mortality. The associations observed in the univariable analysis persisted for age and treatment with aspirin (OR 1.06, 95% CI: 1.02–1.12, $p$=0.002 and OR 3.80, 95% CI: 1.12–14.71, $p$=0.03, respectively).

**Table II.** Uni- and multivariable Cox proportional hazards regression models for Venous Thromboembolism (VTE) and the clinical/experimental variables.

| Venous thrombosis (DVT and/or PE) | | | | | | |
|---|---|---|---|---|---|---|
| | Univariable | | | Multivariable * | | |
| Independent variable | Hazard ratio | 95% CI | $p$-value | Hazard ratio | 95% CI | $p$-value |
| Serum G3BP (µg/mL) | 1.11 | 1.01-1.22 | **0.03** | 1.18 | 1.05-1.33 | **0.007** |
| Serum IP-10 (pg/mL) | 1.00 | 1.00-1.002 | 0.75 | 1.00 | 1.00-1.003 | 0.67 |
| Serum sCD163 (µg/mL) | 1.13 | 0.75-1.7 | 0.55 | 1.13 | 0.71-1.79 | 0.59 |
| Serum leptin (µg/mL) | 0.97 | 0.92-1.03 | 0.33 | 0.95 | 0.88-1.03 | 0.21 |
| Serum TWEAK (µg/mL) | 1.01 | 0.45-2.25 | 0.99 | 1.35 | 0.69-2.64 | 0.38 |
| Age (years) | 1.08 | 1.03-1.13 | **0.003** | 1.08 | 1.03-1.13 | **0.003** |
| Sex (male) | 3.97 | 1.03-15.4 | **0.046** | 4.73 | 0.83-26.9 | 0.08 |
| BMI | 0.95 | 0.82-1.09 | 0.43 | | | |
| Smoking (ever) | 1.25 | 0.36-4.31 | 0.73 | | | |
| Diabetes | 4.09 | 0.87-19.3 | 0.08 | 3.04 | 0.53-17.33 | 0.21 |
| APS | 4.08 | 1.15-14.5 | **0.03** | 3.66 | 0.92-14.63 | 0.07 |
| aPL ** | 2.22 | 0.45-11 | 0.33 | | | |
| Previous VTE | 3.15 | 0.67-14.8 | 0.15 | | | |
| Lupus nephritis (ever) | 1.36 | 0.17-10.74 | 0.77 | | | |
| SLEDAI-2K | 0.82 | 0.59-1.14 | 0.24 | | | |
| Disease duration (years) | 1.01 | 0.96-1.07 | 0.63 | | | |
| Hydroxychloroquine | 0.76 | 0.22-2.63 | 0.67 | | | |
| Aspirin | 1.27 | 0.37-4.39 | 0.71 | | | |
| Warfarin | 2.38 | 0.67-8.45 | 0.18 | | | |
| Statins | 2.11 | 0.59-7.47 | 0.25 | | | |
| DMARDs | 0.82 | 0.21-3.17 | 0.77 | | | |

Sample size (n) =162 / Events=10. Significant $p$-values are in bold. *Covariates with $p$-values <0.1 were included in the multivariable analysis. **Sample size (n) = 142 / Events=8.
aPL; antiphospholipid antibodies; CI: confidence interval; DVT: deep vein thrombosis; G3BP: galectin-3 binding protein; IP-10: interferon gamma-induced protein 10: PE: pulmonary embolism; sCD163: soluble CD163; TWEAK: Tumour necrosis factor-like weak inducer of apoptosis; VTE: venous thromboembolism.

## Discussion

This study investigated the associations between five serum biomarkers and development of VTE, AT, damage accrual, and overall mortality in a long-term follow-up of a large Swedish SLE cohort. These biomarkers were selected based on different important pathophysiological aberrations known to

**Table III.** Uni- and multivariable Cox proportional hazards regression models for arterial thrombosis and the clinical/experimental variables.

| Arterial thrombosis (CVI and/or MI) | | | | | | |
|---|---|---|---|---|---|---|
| | Univariable | | | Multivariable* | | |
| Independent variable | Hazard ratio | 95% CI | *p*-value | Hazard ratio | 95% CI | *p*-value |
| Serum G3BP (μg/mL) | 0.98 | 0.88-1.1 | 0.72 | 0.91 | 0.8-1.03 | 0.14 |
| Serum IP-10 (pg/mL) | 1.00 | 1-1.001 | 0.70 | 1.00 | 1-1.002 | 0.83 |
| Serum sCD163 (μg/mL) | 1.25 | 0.91-1.7 | 0.17 | 1.09 | 0.67-1.76 | 0.74 |
| Serum leptin (μg/mL) | 1.01 | 1-1.03 | 0.07 | 0.99 | 0.98-1.02 | 0.92 |
| Serum TWEAK (μg/mL) | 0.44 | 0.12-1.59 | 0.21 | 0.30 | 0.06-1.46 | 0.14 |
| Age (years) | 1.07 | 1.02-1.12 | **0.002** | 1.05 | 0.99-1.1 | 0.06 |
| Sex (male) | 1.77 | 0.39-7.98 | 0.46 | | | |
| BMI | 0.96 | 0.85-1.08 | 0.46 | | | |
| Smoking (ever) | 0.54 | 0.17-1.74 | 0.30 | | | |
| Diabetes | 6.48 | 1.78-23.59 | **0.005** | 4.22 | 0.8-22.34 | 0.09 |
| APS | 5.15 | 1.73-15.34 | **0.003** | 11.52 | 1.92-69.3 | **0.008** |
| aPL** | 3.05 | 0.65-14.4 | 0.16 | | | |
| Previous AT | 8.83 | 2.89-27.1 | **<0.001** | 1.44 | 0.29-7.18 | 0.65 |
| Lupus nephritis (ever) | 0.96 | 0.13-7.4 | 0.97 | | | |
| SLEDAI-2K | 0.83 | 0.62-1.1 | 0.18 | | | |
| Disease duration (years) | 1.03 | 0.98-1.07 | 0.28 | | | |
| Hydroxychloroquine | 1.17 | 0.38-3.58 | 0.78 | | | |
| Aspirin | 7.47 | 1.66-33.7 | **0.009** | 10.63 | 1.61-70.1 | **0.01** |
| Warfarin | 2.98 | 1.001-8.87 | **0.049** | 0.69 | 0.14-3.46 | 0.65 |
| Statins | 3.88 | 1.3-11.55 | **0.02** | 1.52 | 0.35-6.57 | 0.58 |
| DMARDs | 1.18 | 0.38-3.6 | 0.78 | | | |

Sample size (n) =162 / Events =13. Significant *p*-values are in bold. *Covariates with *p*-values <0.1 were included in the multivariable analysis. **Sample size (n) =142 / Events=10.
aPL; antiphospholipid antibodies; AT: arterial thrombosis; CI: confidence interval; CVI: cerebrovascular incident; G3BP: galectin-3 binding protein; IP-10: interferon gamma-induced protein 10; MI: myocardial infarction; sCD163: soluble CD163; TWEAK: tumour necrosis factor-like weak inducer of apoptosis.

be involved in thrombogenesis and/or have been linked to thrombotic events or subclinical atherosclerosis in SLE. Other biomarkers have shown ability to identify which individuals that are at risk of acquiring early damage in SLE (38, 39). The main and novel discovery herein was the association between serum G3BP and increased risk of development of VTE. This is the first study to explore serum G3BP and risk of VTE in any disease group and it has not yet been studied in the general population. Thus, G3BP seems to constitute a novel and promising biomarker of the risk of VTE. Additionally, this is also the first identification of a predictive biomarker besides aPL for VTE in SLE. Also, our observation suggests a potential role for G3BP in venous thrombogenesis and further exploration of this seems warranted.

The regulation and roles of G3BP in thrombogenesis remain to be determined in order to fully explain the connection between G3BP and VTE. Firstly, human and animal studies indicate that G3BP have key functions in thrombogenesis. Secondly, both our and other studies oppose that the association is merely a reflection of excessive IFN activation in *e.g.* SLE. It has been proposed that G3BP may be crucial to venous thrombus formation and propagation by modulating galectin-mediated cell-cell adhesion and pro-inflammatory signalling and by interacting with the endothelium, platelets, MPs and leukocytes (40). In a murine model the size of the induced thrombus after vein occlusion was significantly reduced after antibody inhibition of G3BP or when using galectin-3 knock-out mice (15, 41). Interestingly, G3BP and galectin-3 were abundant on the thrombus-vein wall surface (15). G3BP is a heavily glycosylated scavenger protein, and galectin-1 and -3 bind to its sugar residues. Binding of galectin-1 to G3BP may activate the endothelium and cause upregulation of P-selectin and von Willebrand factor on the endothelial cells (40, 42). This may also increase the release of tissue factor and procoagulant MPs. Ultimately this favours adhesion of leukocytes and platelets and activation of the coagulation cascade promoting thrombus formation and amplification. We have previously observed that patients with SLE exhibit high levels of plasma G3BP that correlated to serum type I IFN activity (22). In two independent studies of circulating pro-thrombotic MPs, a high expression of G3BP distinguished MPs from SLE from autoimmune controls, and patients with ongoing DVT from relevant control groups (14, 43). Other IFN-inducible proteins were not increased in SLE or DVT MPs. The lack of association in this study between VTE and IP-10, which is also IFN-inducible, also indicate an IFN-independent function of G3BP in VTE. Unfortunately, we did not have direct measures of type I IFNs in our cohort to further explore this. A recent study suggests a link between type I IFNs, G3BP and atherosclerosis by demonstrating an association between plasma G3BP levels and subclinical atherosclerosis in patients with chronic HCV (25). Additionally, in patients with known coronary artery disease high G3BP plasma levels were associated with all-cause and cardiovascular mortality (26, 44). However, we did not find an association between G3BP and AT in our cohort, possibly due to a limited follow-up time.

IP-10 is another IFN-inducible protein biomarker and has been strongly associated with current and future disease activity in SLE (21, 23). A small study from 2015 showed that patients with coronary artery disease tended to have higher plasma concentrations of IP-10 in the aorta, as well as significantly higher transcoronary concentration gradients of circulating IP-10 compared to healthy controls (45). We did not observe associations between IP-10 and AT, damage accrual, or death. However, the numbers of patients and AT events may be too low to detect this. Also, while G3BP seems storage stable, IP-10 is more prone to pre-analytical handling and storage, and this could be another explanation for the lack of association between IP-10 and VTE, AT or any of the other outcomes of this study (22).

**Table IV.** Uni- and multivariable logistic regression models for SLICC/ACR damage index (SDI) and the clinical and experimental variables.

| | SDI increase during follow-up | | | | | |
|---|---|---|---|---|---|---|
| | Univariable | | | Multivariable* | | |
| Independent variable | Odds ratio | 95% CI | *p*-value | Odds ratio | 95% CI | *p*-value |
| Serum G3BP (μg/mL) | 0.97 | 0.91-1.03 | 0.29 | 0.94 | 0.87-1.01 | 0.12 |
| Serum IP-10 (pg/mL) | 1.00 | 1-1.001 | 0.91 | 1.00 | 1-1.001 | 0.76 |
| Serum sCD163 (μg/mL) | 1.25 | 0.98-1.63 | 0.08 | 1.18 | 0.85-1.65 | 0.32 |
| Serum leptin (μg/mL) | 1.01 | 0.99-1.02 | 0.38 | 1.00 | 0.98-1.02 | 0.60 |
| Serum TWEAK (μg/mL) | 1.11 | 0.74-1.76 | 0.61 | 1.24 | 0.75-2.38 | 0.47 |
| Age (years) | 1.06 | 1.03-1.08 | **<0.001** | 1.05 | 1.02-1.07 | **<0.001** |
| Sex (male) | 1.09 | 0.4-3.1 | 0.88 | | | |
| BMI | 1.04 | 0.97-1.11 | 0.28 | | | |
| Smoking (ever) | 1.26 | 0.68-2.36 | 0.46 | | | |
| Diabetes | 4.04 | 0.94-27.72 | 0.09 | 0.79 | 0.13-6.58 | 0.81 |
| APS | 2.65 | 1.1-6.87 | **0.04** | 1.09 | 0.31-3.86 | 0.89 |
| aPL** | 1.11 | 0.57-2.17 | 0.76 | | | |
| Previous VTE | 3.52 | 1.01-16.3 | 0.07 | 2.93 | 0.56-18.9 | 0.22 |
| Previous AT | 8.36 | 3.02-29.7 | **<0.001** | 3.42 | 0.87-16.3 | 0.09 |
| Lupus nephritis (ever) | 2.29 | 0.69-8.86 | 0.19 | | | |
| SLEDAI-2K | 1.05 | 0.95-1.16 | 0.35 | | | |
| Disease duration (years) | 1.03 | 1.002-1.07 | **0.04** | 0.99 | 0.95-1.03 | 0.68 |
| Hydroxychloroquine | 0.59 | 0.31-1.09 | 0.09 | 0.82 | 0.38-1.74 | 0.59 |
| Aspirin | 2.46 | 1.31-4.69 | **0.006** | 1.86 | 0.86-4.1 | 0.12 |
| Warfarin | 2.73 | 1.3-5.96 | **0.009** | 1.29 | 0.41-4.08 | 0.66 |
| Statins | 2.58 | 1.22-5.64 | **0.02** | 1.07 | 0.38-2.97 | 0.90 |
| DMARDs | 1.00 | 0.52-1.93 | 1.00 | | | |

Sample size (n) =162 / Events=78. Significant *p*-values are in bold. *Covariates with *p*-values <0.1 were included in the multivariable analysis. **Sample size (n) =142 / Events=66.
aPL; antiphospholipid antibodies; AT: arterial thrombosis; CI: confidence interval; DVT: deep vein thrombosis; G3BP: galectin-3 binding protein; IP-10: interferon gamma-induced protein 10; PE: pulmonary embolism; sCD163; sCD163: soluble CD163; TWEAK: tumour necrosis factor-like weak inducer of apoptosis; VTE: venous thromboembolism.

Accordingly, IP-10 does not seem like a promising biomarker for AT events.

Our study did not show any significant association between AT and TWEAK, leptin, or sCD163. Recently, McMahon *et al*. found that high leptin and TWEAK levels were significantly associated with the presence of carotid plaques in patients with SLE (30). The presence of TWEAK is linked to increased rates of atherosclerosis, inflammation, angiogenesis, and apoptosis (28). Hyperleptinaemia in the general population is associated with endothelial dysfunction and plasma levels are also significantly higher among SLE cases with plaque when compared to control subjects (29, 31). Similarly, sCD163, as a marker of macrophage activation, has been linked to coronary artery disease in HIV and HCV patients (25, 46). Patients with inflammatory conditions such as rheumatoid arthritis, Gaucher's disease, haemophagocytosis, sepsis, and myelomonocytic leukaemia have increased sCD163 levels in plasma relative to healthy controls. Also, sCD163 in patients undergoing non-emergency coronary artery angiography have been shown to increase with the extent of coronary atherosclerosis and this correlation was independent of traditional risk factors and independent of C-reactive protein in plasma (47). These links to subclinical atherosclerosis indicate that sTWEAK, leptin, and/or sCD163 could be markers of future AT events, however, our study does not substantiate such notions and further studies are needed.

In line with expectations, our data confirmed the association between APS and VTE but surprisingly there was no association between aPL and VTE. The frequency of aPL-positive patients is in line with other reports, however, the low numbers may comprise any signal in the statistical analysis. In addition, data for LA test was unavailable for 32 of the included patients which is important since LA have been shown to be a more specific marker for VTE than the other aPL (48, 49).

Previous studies have shown that the risk of VTE is highest within the first year of SLE diagnosis (12). The association between higher age and VTE was significant, which is not surprising given that VTE are seen more frequently in the elderly compared to young individuals in the general population (50).The association with age and VTE in SLE is in line with the observations reported in the LUMINA XXV study (49).

In SLE, the risk is increased 5–8 fold for myocardial infarction and 1.5–2 fold for ischaemic stroke compared to the general population (51, 52). Development of AT in SLE have been found in association with increased age, smoking, hypertension, diabetes mellitus, dyslipidaemia, presence of at least 2 traditional risk factors, aPL, positive LA test, nephrotic syndrome, LN, acquired organ damage, and a high cumulative dose of prednisone (53). This study could not confirm the associations between hypertension, smoking, nephritis, prednisolone dose and AT. We suspect that this may be due to the low numbers of patients with the specific manifestations/comorbidities combined with few events and could also reflect a well-controlled study population.

All-cause and cause-specific mortality is increased in patients with SLE in the range of 2- to 5-fold when compared to the general population (1, 54). In the first years of the disease major causes of death are severe infections or death from complications of active disease, while causes of late death include acquired damage, including treatment complications, and cardiovascular disease (5, 53, 55). As expected, we found a significant association between AT, age, and mortality, the latter being present in both the univariable and multivariate analyses. An association between nephritis and mortality in SLE has repeatedly been shown (54, 56, 57). One third of patients in this study met the ACR criterion for renal involvement. However, our study failed to confirm an association between LN and mortality.

Our study has several limitations. Inclusion of a validation cohort would strengthen our findings. 162 patients

**Table V.** Uni- and multivariable logistic regression models for All-cause mortality and the clinical and experimental variables.

| All-cause mortality | | | | | | |
|---|---|---|---|---|---|---|
| | Univariable | | | Multivariable* | | |
| Independent variable | Odds ratio | 95% CI | *p*-value | Odds ratio | 95% CI | *p*-value |
| Serum G3BP (μg/mL) | 1.03 | 0.94-1.12 | 0.51 | 1.01 | 0.89-1.14 | 0.85 |
| Serum IP-10 (pg/mL) | 1.00 | 1-1.001 | 0.66 | 1.00 | 0.99-1.001 | 0.99 |
| Serum sCD163 (μg/mL) | 1.06 | 0.72-1.46 | 0.75 | 1.02 | 0.59-1.6 | 0.95 |
| Serum leptin (μg/mL) | 0.99 | 0.96-1.02 | 0.67 | 0.99 | 0.94-1.01 | 0.30 |
| Serum TWEAK (μg/mL) | 0.59 | 0.18-1.4 | 0.37 | 0.87 | 0.14-1.44 | 0.66 |
| Age (years) | 1.09 | 1.05-1.14 | **<0.001** | 1.06 | 1.01-1.13 | **0.009** |
| Sex (male) | 1.28 | 0.24-4.67 | 0.74 | | | |
| BMI | 0.93 | 0.82-1.03 | 0.17 | | | |
| Smoking (ever) | 0.91 | 0.34-2.33 | 0.85 | | | |
| Diabetes | 7.31 | 1.8-28.7 | **0.007** | 3.45 | 0.5-29.9 | 0.21 |
| APS | 2.29 | 0.72-6.53 | 0.15 | | | |
| aPL** | 3.39 | 0.93-18.1 | 0.06 | 1.47 | 0.3-9.1 | 0.64 |
| Previous VTE | 0.93 | 0.1-4.3 | 0.94 | | | |
| Previous AT | 7.99 | 2.92-22.43 | **<0.001** | 1.30 | 0.2-9.1 | 0.78 |
| Lupus nephritis (ever) | 0.93 | 0.09-4.29 | 0.94 | | | |
| SLEDAI-2K | 0.88 | 0.68-1.06 | 0.20 | | | |
| Disease duration (years) | 1.00 | 0.96-1.05 | 0.80 | | | |
| Hydroxychloroquine | 0.82 | 0.32-2.14 | 0.69 | | | |
| Aspirin | 5.33 | 1.9-18.1 | **0.001** | 1.81 | 0.41-7.7 | 0.42 |
| Warfarin | 2.64 | 0.97-6.95 | 0.06 | 1.93 | 0.48-7.6 | 0.34 |
| Statins | 2.75 | 1.01-7.25 | **0.047** | 0.85 | 0.2-5.1 | 0.87 |
| DMARDs | 1.55 | 0.58-4 | 0.37 | | | |

Sample size (n) =162 / Events=19. Significant *p*-values are in bold. *Covariates with *p*-values <0.1 were included in the multivariable analysis. **Sample size (n) =142 / Events=13.
aPL; antiphospholipid antibodies; AT: arterial thrombosis; CI: confidence interval; DVT: deep vein thrombosis; G3BP: galectin-3 binding protein; IP-10: interferon gamma-induced protein 10; PE: pulmonary embolism; sCD163; sCD163: soluble CD163; TWEAK: tumour necrosis factor-like weak inducer of apoptosis; VTE: venous thromboembolism.

were included with a relatively low number of events which increases the uncertainty of our results and compromise the detection of weak associations. Moreover, the majority of patients had a long disease duration, this and higher age are well-known predictors of global damage in SLE (including VTE). The fact that the majority of patients had established disease, whereof some also had preexisting damage, constitute a limitation since damage is a well-known predictor of further damage (58). Information regarding LA test and aPLs were not available for all patients, neither was information on other causes of thrombophilia (genetic or acquired). This affects the analysis and conclusions regarding the observed associations (or lack of) between LA, aPL and the outcomes.

## Conclusion

Our findings support circulating G3BP as a potential predictor of VTE in SLE that may aid in future VTE risk stratification and thromboprophylaxis. Furthermore, the results corroborate previous indications of a pathogenic link between type I IFNs, G3BP, VTE and SLE. Larger studies are needed to replicate and translate these findings into clinical practice.

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