Supplement:

Material and Methods

Study population (Table 2S)

The Scripps Venous Thrombosis Registry is an ongoing case-control study of risk factors for VTE as previously described (16-19). Inclusion criteria for this study included age at thrombosis < 55 years, > 3 months since diagnosis of acute thrombosis, a life expectancy of at least three years and no lipid lowering medications or cancer. Age matched (±2 years) healthy controls were recruited through the General Clinical Research Center's (GCRC) blood donation program. Controls were from the community but most were employees or former employees of Scripps. The protocol was approved by the Institutional Review Board and subjects provided written informed consent. Participants in the blood donation program had normal complete blood count and negative HIV, hepatitis B and C testing. In this study, VTE patients (49 male and 64 female) and age matched controls were analyzed.

Clinical characteristics and the frequency of identified risk factors are shown in Table 2S. Fifty seven of VTE patients (50.4%) presented with idiopathic VTE, defined as events that did not occur within 90 days after surgery, trauma, or major immobilization. Of the VTE patients, 36 (31.9%) had experienced more than one episode of thrombosis and 68 (60.2%) had documented pulmonary embolism. Diabetes was present in 3 VTE patients. Hypertension was present in 14 VTE patients and in 2 controls. Current smoking was more frequent in male VTE patients compared with male controls (15, vs. 6, p= 0.06). Anti-phospholipid antibody syndrome was present in 9 VTE patients. Eighty four percent of patients were taking warfarin when blood was donated. Blood was collected after a 12 hour fast. EDTA-plasma was stored at -80ºC.

The conditional logistic regression analyses were used to determine the P values for categorical parameters.

Statistic Analysis

Statistical analyses including Mann Whitney test and two-tailed Pearson test with 95% confidence interval were performed using Prism^{TM} 4.0 software (Graph Pad Software Inc., San Diego, CA). To correlate coagulabilty with plasma SAA level, SAA values that were not normally distributed were log10 transformed to reduce the influence of outlying

observation. P values on categorized parameters in **Table 2S** were determined by Fisher's exact test using Prism™ 4.0 software. The odds ratio for the VTE (**in Table 3S)** were determined with a conditional logistic regression model using STATA 8.2 software (Stata Corporation, College Station, TX). The difference was considered significant when p was < 0.05.

Coagulation assays

Three clotting time assays (N=39) and ETP assays (N=19) were performed using plasma samples from healthy normal subjects. Activated partial thromboplastin time (APTT) assays using the APTT reagent Platelin LS (Trinity Biotec USA, Berkeley Heights, NJ) were performed following the manufacturer's instruction. Clotting of diluted plasma samples supplemented with fibrinogen were induced by dilute TF (1:40 dilution of Innovin, 350 pM final tissue factor) or similarly by addition of purified factor XIa (1.2 μg/ml, final) to test the coagulability of individual samples. To determine the potential thrombin generation of individual plasma, plasma samples were preincubated for 10 min at 37ºC. Then, TF (Innovin, final 4 pM) containing 30 mM $CaCl_2$ and fluorogenic substrate solution (I-1140) were added to the plasma to initiate coagulation activation. Thrombin generation was followed continuously in time using a microtiterplate SPECTRAmax GEMINI XS fluorometer (Molecular Devices, Sunnyvale, CA) with excitation and emission wavelengths set at 360 nm and 460 nm, respectively. Each thrombin generation measurement was calibrated against the fluorescence curve obtained in the same plasma with a fixed amount of thrombin-α_2-macroglobulin complex (thrombin calibrator, Thrombinoscope BV, Maastricht, the Netherlands).

Table 1S. Correlations between blood coagulability determined by plasma coagulation assays and plasma log-transformed SAA levels.

Variables	*r*	*p-value*
APTT	0.13	0.42
FXIa –induced clotting time	- 0.17	0.29
Dilute TF-induced clotting time	- 0.16	0.33
ETP (Endogenous Thrombin Potential)		
lag time	- 0.29	0.22
maximum peak height	0.60	**0.007**
AUC	0.47	**0.04**

Correlations between these coagulation parameters and $\log_{10}$-transformed SAA plasma levels were determined and Pearson correlation coefficient (*r*) and p values are indicated. Significant correlation p-values are shown in bold font.

Table 2S. Study Population Characteristics

Variables	*Controls N=113*	*VTE N=113*	*p-value*
Age, yr (SD)	45.3 (9.3)	45.0 (9.8)	
Range	22-66	22-65	
Female sex n (%)	64 (57)	64 (57)	1.0
Ethnic Group,			
Non-Hispanic White	102 (90.1)	102 (90.1)	1.0
Hispanic	5 (4.4)	6 (5.3)	
African ancestry	5 (4.4)	5 (4.4	
Asian	1 (1.0)	0 (0)	
Idiopathic VTE	-	57 (50.4)	
pulmonary embolism	-	68 (60.2)	
Risk factors,			
Factor V Leiden	6 (5.3)	28 (24.8)	0.001
Prothrombin 20210A	4 (3.5)	9 (8.0)	0.25
Anti-phospholipid syndrome	0 (0)	9 (8.0)	0.003
Personal History of VTE	-	36 (31.9)	
Family History of VTE	8 (7.0)	42 (37.2)	<0.001
Comorbidities, number of subjects			
Obesity (BMI>30)	32 (28.3)	38 (33.6)	0.47
Type-II diabetes	0 (0)	3 (2.7)	0.25
Hypertension	2 (1.8)	14 (12.4)	0.003
Current smoking	6 (5.3)	15 (13.2)	0.06
Other factors			
Estrogen user	32 (28.3)	38 (33.6)	0.47
Warfarin user	-	85 (75.2)	
Acute-phase proteins			
CRP mg/L (IQRs)	1.7 (0.86-4.4)	2.2 (1.1-5.1)	0.24
Fibrinogen, mg/ml (SD)	1.89(1.10)	1.97(0.99)	0.43

Table 3S.Odds ratio for VTE based on various levels of plasma SAA

Model / adjustment	cut-off values of SAA levels		
	90th percentile	*75th percentile*	*67th percentile*
I. unadjusted	**8.4 (3.3-21) p>0.001**	**5.4 (2.7-11)) p>0.001**	**4.0 (2.1-7.7) p>0.001**
II. CRP	**8.4 (3.3-21) p>0.001**	**5.5(2.7-11) p>0.001**	**4.1 (2.1-8.2) p>0.001**
III. obesity, diabetes, estrogen treatment, APS	**6.8 (2.7-17) p>0.001**	**4.5 (2.2-9.4) p>0.001**	**3.4 (1.7-6.6) p>0.001**
IV. FVL & prothrombin mutations, smoking, hypertension, FH	**9.3 (2.9-30) p>0.001**	**11 (3.5-35) p>0.001**	**6.3 (2.3-18) p>0.001**
V. model II plus III plus IV	**15 (3.0-72) P=0.001**	**14 (3.6-53) p>0.001**	**6.7 (2.3-20) p=0.001**

The odds ratios (**OR**) (95% CI) for VTE with the various cut-off levels of SAA are shown. Model **II** was adjusted for CRP. Model **III** included adjustments for parameters which may be associated with both SAA level and VTE risk including estrogen treatment, obesity, type II diabetes and anti-phospholipid syndrome (APS). Additional adjustments for model **IV** included other known VTE risk factors or condition/diseases that influence the development of VTE including factor V Leiden (FVL) and prothrombin nt G20210A polymorphisms, smoking, hypertension and family history of VTE (FH). Model **V** includes all adjustments made for models **II, III,** and **IV**.