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ORIGINAL ARTICLE

A high Gas6 level in plasma predicts venous thromboembolism recurrence, major bleeding and mortality in the elderly: a prospective multicenter cohort study

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Essentials

- Predictive ability of pro-hemostatic Gas6 for recurrent venous thromboembolism (VTE) is unknown.
- We measured Gas6 levels in 864 patients with VTE over 3 years.
- High Gas6 (> 157%) at diagnosis is associated with VTE recurrence, major bleeding and mortality.
- Gas6 plasma levels measured 12 months after the index VTE are discriminatory for VTE recurrence.

Summary. *Background:* Growth arrest-specific gene 6 (Gas6) is a prohemostatic protein with an unknown predictive ability for recurrent venous thromboembolism (VTE). In the elderly, VTE results in higher mortality but does not have a higher rate of recurrence than in younger patients. Consequently, anticoagulation management in the elderly is challenging. *Objective:* To prospectively investigate the performance of Gas6 in predicting VTE recurrence, major bleeding and mortality in the elderly. *Methods:* Consecutive patients aged ≥ 65 years with acute VTE were followed for a period of 3 years. Primary outcomes were symptomatic VTE recurrence, major bleeding, and mortality. Plasma Gas6 was measured with ELISA. *Results:* Gas6 levels were measured in 864 patients at the time of the index VTE (T1) and, in 70% of them, also 12 months later (T2). The Gas6 level at T1 was discriminatory for VTE recurrence (*C*-statistic, 0.56; 95% confidence interval [CI] 0.51–0.62), major bleeding (0.60, 95% CI 0.55–0.65) and mortality (0.69, 95% CI 0.65–0.73) up to 36 months. VTE recurrence up to 24 months after T2 was discriminated by the Gas6 level at T2 (0.62, 95% CI 0.54–0.71). High Gas6 levels (> 157%) and continuous Gas6 levels at T1 were associated with VTE recurrence up to 6 months and 12 months, respectively. *Conclusions:* In elderly patients, a high Gas6 level is associated with higher risks of VTE recurrence, major bleeding, and death. These findings support further studies to assess the performance of Gas6 in adjusting the length of anticoagulation.

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Introduction

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), represents a worldwide major health issue, and is a leading cause of cardiovascular death [1]. VTE incidence rises with age [2–5]. In the elderly population, VTE results in higher mortality but does not have a higher rate of recurrence than in younger patients [4]. Elderly patients more often present with comorbidities, and therefore a higher risk of bleeding [4]. Consequently, management of the anticoagulation in the elderly constitutes a challenge.

Because the risk of VTE recurrence is greatest in the first 6–12 months following the initial event and progressively decreases afterwards [6], the benefit of extended anticoagulation may be exceeded by the risk of clinically important bleeding [7–12].

Growth arrest-specific gene 6 (Gas6), the product of the gene *GAS6* [13], is a secreted vitamin K-dependent protein. The Gas6 plasma level is known to be elevated in a variety of clinical conditions, including inflammation or sepsis [14–18], obesity [19], chronic renal failure, and cancer [20]. Importantly, no change in the Gas6 plasma level with increasing age was previously observed [21]. Nevertheless, the Gas6 level progressively decreased with increasing International Normalized Ratio under warfarin therapy [21].

Gas6 has multiple functions, including regulation of cell growth [13] and inflammation [22]. It also has effects on platelet function and coagulation, enhancing platelet aggregation and tissue factor expression in endothelial cells, as well as promoting the recruitment of platelets and leukocytes to the endothelial cell membrane [23–28]. In mice, the absence of Gas6 is protective against thrombosis without causing excessive bleeding, pointing to Gas6 as an attractive target for antithrombotic therapy [23,25].

In a cross-sectional study including 279 patients and 79 controls, Blostein *et al*. [29] measured a higher Gas6 plasma level in patients 4 months after VTE than in healthy controls. In addition, they observed that subjects with elevated Gas6 levels in plasma had an increased risk of VTE as compared with those with lower Gas6 levels after adjustment for age, sex, medications, and comorbidities. However, elevated Gas6 plasma levels were not predictive of VTE recurrence [29]. Finally, most of the patients included in this study were aged < 65 years.

Here, in a cohort of 864 patients aged ≥ 65 years with VTE, we prospectively investigated the performance of Gas6 plasma levels at admission and 1 year after the index VTE in predicting the risk of VTE recurrence, major bleeding, and mortality.

Methods

Cohort sample

The study was conducted between September 2009 and December 2013 as part of the Swiss Cohort of Elderly Patients with VTE (SWITCO65+), which was a prospective multicenter cohort study to assess medical outcomes and quality of life in elderly patients with acute VTE from all five university hospitals and from four high-volume non-university hospitals in Switzerland [30].

Consecutive patients aged ≥ 65 years with acute VTE were identified in the inpatient and outpatient services of all participating study sites, and followed for a period of 3 years. We defined DVT as acute onset of leg pain or swelling plus incomplete compressibility of a venous segment on ultrasonography or an intraluminal filling defect on contrast venography [31].

Because iliac veins and the inferior vena cava may be technically difficult to compress, additional diagnostic criteria for iliac/caval DVT comprised abnormal duplex flow patterns compatible with thrombosis or an intraluminal filling defect on spiral computed tomography (CT) or magnetic resonance imaging venography [32–34].

Given that ultrasonography has reduced sensitivity and specificity for distal DVT [35], patients with isolated distal DVT were included only if the incompressible distal deep vein transverse diameter was at least 5 mm [36,37].

Symptomatic PE was defined as: acute onset of dyspnea, chest pain, or syncope, coupled with a new high-probability ventilation/perfusion lung scan; a new contrast filling defect on spiral CT or pulmonary angiography; or the new documentation of a proximal DVT either by venous ultrasound or by contrast venography [37,38]. Radiographic studies used to diagnose VTE were interpreted by on-site vascular specialists or radiologists.

Exclusion criteria were inability to provide informed consent (i.e. severe dementia), conditions incompatible with follow-up (i.e. terminal illness or place of residence too far from the study center), insufficient German-speaking or French-speaking ability, thrombosis at a site other than a lower limb, and catheter-related thrombosis.

Treatment of VTE, e.g. the type of anticoagulant used (i.e. parenteral anticoagulant followed by vitamin K antagonists, parenteral anticoagulant alone, or direct oral anticoagulant), the duration of the anticoagulation, and the prescription of compression stocking, was entirely left to the discretion of the managing physicians.

Eligible patients were approached for informed consent to participate in the study. The ethics committees at each study site approved the study, and written informed consent was obtained from all participants. A detailed description of the study methods has previously been published [30].

Data collection

For all enrolled patients, trained study nurses prospectively collected baseline demographic information (age and sex), type, history and complication of VTE (distal DVT, proximal DVT, overt PE, presence of post-thrombotic syndrome, prior VTE, provoked index VTE, or cancer-related VTE), concomitant use of estrogen therapy during the past 3 months, immobilization during the last 3 months, major surgery during the last 3 months, comorbid conditions (history of major bleeding, chronic liver disease, renal disease, chronic or acute heart failure, cerebrovascular disease, diabetes mellitus, body mass index of > 30, acute rheumatic disease during the last 3 months, inflammatory bowel disease, or severe infection or sepsis during the last 3 months), a high risk of falling, laboratory findings (anemia or low platelet count), concomitant use of antiplatelet drugs, arterial hypertension, a heart rate of $\geq$ 110 beats min^{-1}, systolic blood pressure of < 100 mmHg, a respiratory rate of $\geq$ 30 min^{-1}, a temperature of < 36 °C, arterial oxygen saturation of < 90%, and VTE-related treatment, by the use of standardized data collection forms. Follow-up included one telephone interview and two face-to-face evaluations during the first year of study participation, and then semi-annual contacts, alternating between face-to-face evaluations (clinic visits or home visits in house-bound patients) and telephone calls, as well as periodic reviews of the patient's hospital chart. During each visit/contact, study nurses interviewed patients to obtain information about the date and type of clinical events (recurrent VTE, bleeding, or death). If a clinical event had occurred, this information was complemented by reviewing medical charts and interviewing patients' primary-care physicians and family members. Collected data were recorded on standardized forms.

Blood samples

Blood was collected after minimal venostasis into 1/9 of its volume of 0.0160 M trisodium citrate (Sarstedt; Thermo Fischer Scientific, Waltham, Massachusetts, USA) at the time of the index VTE diagnosis and 12 months later [39]. Citrated platelet-poor plasma (PPP) was prepared by centrifugation for 10 min at 2700 × *g* and room temperature, and recentrifugation of the supernatant plasma for 10 min at 2700 × *g* to remove remaining platelets [39]. The resulting citrated PPP was stored in aliquots of 2 mL at − 80 °C within 1 h of blood collection [39]. Citrated PPP was used for Gas6 ELISA.

Gas6 ELISA

To measure Gas6, we used the ELISA method developed by Clauser *et al*. [40], with some modifications [17]. Wells from 96-well plates (Maxisorp; Nunc, Nümbrecht, Germany) were coated with 100 μL per well of polyclonal goat anti-human Gas6 antibody (AB885; R&D Systems, Abington, UK) diluted in 0.1 M $NaHCO_3$ (pH 8.2), and incubated overnight at 4 °C. After two washes with phosphate-buffered saline (PBS)–Tween 0.05%, 100 μL of PBS–bovine serum albumin (BSA) 1%/5% was added to the wells, and plates were incubated for 2 h at room temperature. After three washes, samples diluted 50-fold and 100-fold and a normal plasma serial dilution with PBS–BSA 1% were added to the wells, and this was followed by overnight incubation at 4 °C. After three washes, 100 μL of biotinylated polyclonal goat antibody (BAF885; R&D Systems) was added to each well, and plates were left for 2 h at room temperature. Signals were amplified with avidin–horseradish peroxidase (BD Pharmingen, Oxford, UK), and plates were incubated for 20 min at 37 °C. Finally, *o*-phenylenediamine dihydrochloride (Sigma-Aldrich St. Louise, Missouri, USA) was added. Reactions were stopped by the addition of 50 μL of 3 M HCl. Absorbance was measured at 492 nm, and the results were expressed as percentages relative to normal plasma, with its serial dilution as standard curve [17,40]. This ELISA was specific for human Gas6, with no cross-reactivity with human protein S.

D-dimer

D-dimer was measured by ELISA (Vidas D-dimer exclusion test; bioMérieux, Marcy-l'Etoile, France).

Outcome variables

We defined objectively confirmed, symptomatic VTE recurrence, major bleeding and overall mortality up to 3 years as primary study outcomes.

VTE recurrence was defined as a fatal or new non-fatal PE or new DVT [41]. The diagnosis of recurrent VTE during follow-up was established according to the following criteria: for DVT, on the basis of abnormal results on ultrasonography; and for PE, on the basis of CT or angiography showing new intraluminal defects, or on the basis of a ventilation–perfusion lung scan showing a high-probability pattern with new perfusion defects. A new proximal DVT, based on abnormal results on ultrasonography, associated with new PE symptom(s) (shortness of breath, chest pain, and syncope) was also considered as recurrent PE.

Major bleeding was defined as fatal bleeding, symptomatic bleeding at critical sites (intracranial, intraspinal, intraocular, retroperitoneal, intra-articular, pericardial, or intramuscular with compartment syndrome), or clinically overt bleeding with a reduction in hemoglobin level of at least 20 g L^{-1}, or leading to transfusion of two or more units of packed red blood cells [42].

We assessed the outcomes by using patient or proxy interviews, interview of the patient's primary-care physician, and/or hospital chart review [30]. A committee of three blinded clinical experts confirmed all outcomes and classified

all deaths as being definitely attributable to PE, possibly attributable to PE (e.g. sudden death without an obvious cause), attributable to major bleeding, or attributable to another cause [30]. The final classification was made on the basis of the full consensus of this committee [30].

Statistical analyses

We compared the baseline characteristics of patients in relation to elevated plasma Gas6 (above versus below the median) by using the chi-squared test and the non-parametric Wilcoxon rank-sum test as appropriate. We calculated incidence rates of a first VTE recurrence, a first major bleed or death up to 3 years after the index event in relation to the level of Gas6. Gas6 was categorized into low, medium and high levels on the basis of lower and upper quartiles. We estimated the cumulative incidence of these outcomes by using the Kaplan–Meier method, and compared survivor functions across groups by using the log-rank test.

The discriminative power of Gas6 for predicting VTE recurrence, major bleeding and mortality was assessed by the use of Harrell's *C* concordance statistic.

Associations between Gas6 and the time to a first VTE recurrence and major bleeding were assessed by the use of competing risk regression accounting for non-PE-related and non-bleeding-related death, respectively, as a competing event, according to the method of Fine and Gray [43]. The method yields subhazard ratios with corresponding 95% confidence intervals (CIs) and *P*-values for the failure event of primary interest. For mortality, an ordinary Cox regression with robust standard errors was calculated. We adjusted the model for previously published predictors of VTE recurrence or major bleeding [6,41,42,44–52]. For overall mortality, analyses were adjusted for age, gender, cancer, provoked VTE, prior VTE, overt PE, renal disease, history of major bleeding, heart failure, chronic lung disease, elevated heart rate, low blood pressure, low oxygen and periods of anticoagulation as a time-varying covariate [49,53].

All analyses were performed with STATA 14 (Stata Corporation, College Station, Texas, USA).

Results

Study sample

Of 1003 enrolled patients aged ≥ 65 years with acute VTE, we excluded 139 patients at the time of index VTE

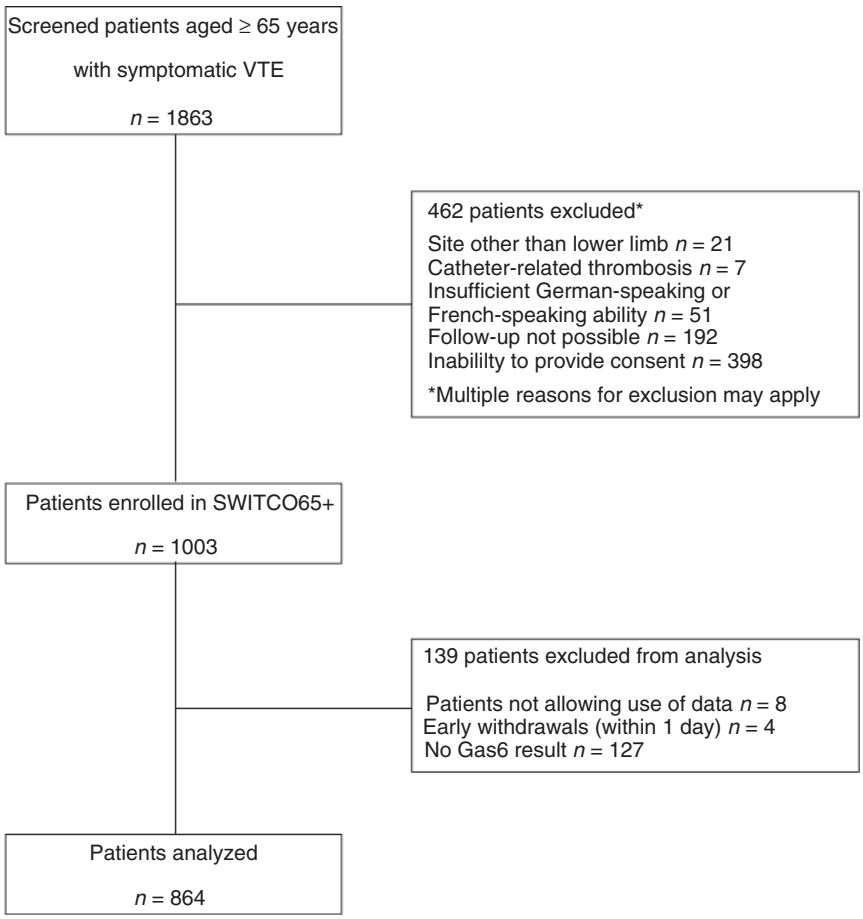

Fig. 1. Flow diagram of patients included in the study. Gas6, growth arrest-specific gene 6; VTE, venous thromboembolism.

diagnosis (eight patients did not allow the use of data, four withdrew their consent within 1 day, and 127 had no Gas6 measurement), leaving a study sample of 864 patients (Fig. 1). Of these patients, 601 (69.6%) had Gas6 measurement 12 months after the index VTE.

Characteristics at the time of the index VTE diagnosis are shown in Table 1. Overall, 476 patients (44.9%) were women, and the median age was 75.0 years (interquartile range [IQR] 69.0–81.0 years). Five hundred and ninety-nine patients (69.3%) presented with an index PE. Two hundred and fifty-one patients (29.1%) had experienced prior VTE. Five hundred and twenty-two patients (60.4%) had an unprovoked index VTE, 185 (21.4%) had provoked VTE, and 157 (18.2%) had cancer-related VTE. Patients with an unprovoked index VTE or with prior VTE were more likely to present with PE (70%) than with proximal (24%) or distal DVT (6%) only. PE was more frequent in patients with unprovoked VTE (72%)

Table 1 Patient characteristics at the time of the index venous thromboembolism (VTE) by growth arrest-specific gene 6 (Gas6) plasma level (above versus below or at the median)

Characteristic*	All	Gas6 level above median (> 129%) *n* (%) or median (IQR)	Gas6 level below or at median (≤ 129%) *n* (%) or median (IQR)	*P*-value
Total number of patients	864	435	429	
Patient age (years)	75.0 (69.0–81.0)	76.0 (70.0–82.0)	74.0 (69.0–80.0)	0.001
Female sex	388 (44.9)	207 (47.6)	181 (42.2)	0.111
VTE location				
Distal DVT only	70 (8.1)	29 (6.7)	41 (9.6)	0.053
Proximal DVT	195 (22.6)	111 (25.5)	84 (19.6)	
Pulmonary embolism	599 (69.3)	295 (67.8)	304 (70.9)	
Type of VTE†				
Unprovoked	522 (60.4)	242 (55.6)	280 (65.3)	< 0.001
Provoked	185 (21.4)	92 (21.1)	93 (21.7)	
Cancer-related*	157 (18.2)	101 (23.2)	56 (13.1)	
Estrogen therapy during the last 3 months*	27 (3.1)	9 (2.1)	18 (4.2)	0.073
Immobilization during the last 3 months	190 (22.0)	115 (26.4)	75 (17.5)	0.001
Major surgery during the last 3 months	131 (15.2)	72 (16.6)	59 (13.8)	0.251
Prior VTE	251 (29.1)	125 (28.7)	126 (29.4)	0.837
Presence of PTS*,‡	453 (52.4)	251 (57.7)	202 (47.1)	0.003
History of major bleeding*	89 (10.3)	54 (12.4)	35 (8.2)	0.039
Chronic liver disease	13 (1.5)	10 (2.3)	3 (0.7)	0.053
Renal disease§	170 (19.7)	97 (22.3)	73 (17.0)	0.051
Chronic or acute heart failure	103 (11.9)	57 (13.1)	46 (10.7)	0.280
Cerebrovascular disease (stroke, TIA)	84 (9.7)	44 (10.1)	40 (9.3)	0.695
Diabetes mellitus	137 (15.9)	79 (18.2)	58 (13.5)	0.062
BMI > 30*	201 (23.3)	107 (24.6)	94 (21.9)	0.360
High risk of falling*,¶	406 (47.0)	233 (53.6)	173 (40.3)	< 0.001
Acute rheumatic disease during the last 3 months	29 (3.4)	17 (3.9)	12 (2.8)	0.365
Inflammatory bowel disease	31 (3.6)	9 (2.1)	22 (5.1)	0.016
Severe infection or sepsis during the last 3 months	71 (8.2)	42 (9.7)	29 (6.8)	0.121
Anemia*,**	335 (38.8)	206 (47.4)	129 (30.1)	< 0.001
Platelet count of < 150 G L^{-1}*	132 (15.3)	78 (17.9)	54 (12.6)	0.039
Antiplatelet therapy††	275 (31.8)	147 (33.8)	128 (29.8)	0.212
Arterial hypertension	552 (63.9)	289 (66.4)	263 (61.3)	0.116
Heart rate of ≥ 110 beats min^{-1}*	79 (9.1)	49 (11.3)	30 (7.0)	0.031
Systolic BP of < 100 mmHg*	28 (3.2)	13 (3.0)	15 (3.5)	0.664
Respiratory rate of ≥ 30 min^{-1}*	28 (3.2)	16 (3.7)	12 (2.8)	0.469
Temperature of < 36 °C*	65 (7.5)	27 (6.2)	38 (8.9)	0.119
Arterial oxygen saturation of < 90%*	93 (10.8)	62 (14.3)	31 (7.2)	0.001

BP, blood pressure; BMI, body mass index; DVT, deep vein thrombosis; IQR, interquartile range, PTS, post-thrombotic syndrome; TIA, transient ischemic attack. *Values were missing for estrogen therapy during the last 3 months (0.1%), presence of PTS (1.9%), history of major bleeding (0.1%), BMI > 30 (0.6%), high risk of falling (0.1%), anemia (5.8%), platelet count (5.8%), heart rate of ≥ 110 beats min−1 (2.1%), systolic BP of < 100 mmHg (1.6%), respiratory rate of ≥ 30 min−1 (21.1%), temperature of < 36°C (7.8%), and arterial oxygen saturation of < 90% (21.3%). †Provoked VTE is defined as immobilization, surgery or estrogen therapy during the last 3 months. Cancer is defined as any solid or hematological cancer that required chemotherapy, radiation therapy, surgical treatment or palliative treatment during the last 3 months. ‡Defined as a Villalta score of > 5 or the presence of an ulcer on the left or right side. §Chronic renal disease or creatinine clearance of < 30 mL min−1. ¶Defined as answering yes to at least one screening question: (i) Did you fall during the last year? (ii) Did you notice any problem with gait, balance, or mobility? **Anemia: a hemoglobin level of < 12 g dL−1 for females or of < 13 g dL−1 for males. ††Defined as antiplatelet therapy such as aspirin 100–300 mg daily, clopidogrel, prasugrel or aspirin/dipyridamole at the time of the index VTE.

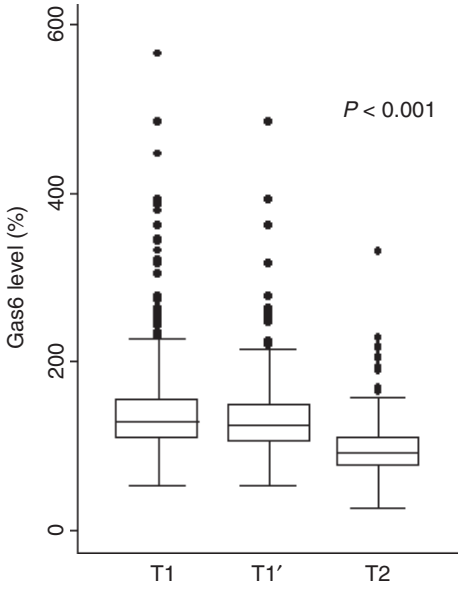

Fig. 2. Growth arrest-specific gene 6 (Gas6) plasma levels at the time of venous thromboembolism (VTE) diagnosis and 12 months later. Box-plot of Gas6 levels presented as median with interquartile range (IQR) and whiskers with a maximum length of 1.5 IQR. T1, Gas6 level at the time of the index VTE of all patients (Gas6: $n = 864$). T1′, Gas6 level at the time of the index VTE of patients who also had the Gas6 level measured at T2 (Gas6: $n = 601$). T1′ and T were compared using the Wilcoxon matched pairs signed-ranks test. The *P*-values indicate that the differences were significant. The Spearman correlation between T1′ and T2 was $r_s = 0.33$ for Gas6.

than in patients with provoked (66%) or cancer-related (64%) VTE ($P = 0.01$). Twelve months after the index VTE, 432 patients (50%) were still receiving anticoagulation, most of them with vitamin K antagonists.

Gas6 plasma levels in study samples

At the time of the index VTE diagnosis, the median Gas6 level was 129.3% (IQR 108.9–156.6) (T1 in Fig. 2). Patients with elevated Gas6 levels (> 129%) at the time of the index VTE were slightly older (median age of 76 years versus 74 years, $P = 0.001$). However, the correlation between Gas6 level and age was weak both at the time of the index VTE (Spearman correlation, $r_s = 0.12$) and 12 months later ($r_s = 0.09$). Patients with elevated Gas6 levels at the time of the index VTE diagnosis were more likely to have cancer-related VTE. They were also more immobilized during the last 3 months and showed higher prevalences of post-thrombotic syndrome, history of major bleeding, anemia, thrombocytopenia, heart rate of ≥ 110 beats min^{-1}, and oxygen saturation of < 90% (Table 1). In contrast, these patients were less likely to be still receiving oral anticoagulation 12 months after the index VTE (180 [41.4%] patients with Gas6 levels above the median versus 230 [53.6%] patients with Gas6 levels below or at the median, $P < 0.001$). Interestingly, patients with inflammatory bowel disease were more likely to have a lower Gas6 level ($P = 0.016$). Twelve months after the index VTE, the median Gas6 level was 93% (IQR 77.1–111.7) (T2 on Fig. 2). However, at this time point, the median Gas6 level was lower in patients receiving anticoagulation than in patients not receiving anticoagulation (86.1% [IQR 70.4–107.3] versus 100.2% [IQR 85.3–116.8], $P < 0.001$).

Gas6 plasma levels were generally lower 12 months after the index VTE (T2) than at the time of the index VTE (T1′ versus T2, $P < 0.001$; Fig. 2). In a minority of patients ($n = 97$, 11%), the Gas6 level increased from the time of VTE diagnosis to 12 months later.

The correlation between Gas6 and D-dimer was weak, both at the time of the index VTE (Spearman correlation, $r_s = 0.06$) and 12 months later ($r_s = 0.24$).

Incidence rates of VTE recurrence, major bleeding, and mortality

After a follow-up of 3 years, 100 patients had developed recurrent VTE, resulting in an incidence rate of 5.6 per 100 person-years (95% CI 4.6–6.8). During the same period, 170 of 864 patients had died (mortality rate of 9.0 per 100 person-years; 95% CI 7.8–10.5). The mortality rate was higher during the initial 6 months, whereas the VTE recurrence rate remained stable over the observation period (Table S1). During the whole follow-up, the incidence rates of VTE recurrence and major bleeding were higher in patients with high Gas6 levels than in patients with medium or low Gas6 levels measured at the time of the index VTE (Table S1). Likewise, the 2-year cumulative incidence of VTE recurrence was higher for patients with high (> 157%) than for patients with medium (109–157%) and low (< 109%) Gas6 levels measured at the time of the index VTE, although not significantly ($P = 0.087$) (Fig. 3A). The 2-year cumulative incidence of major bleeding was higher for patients with high (> 157%) than for patients with medium (109–157%) and low (< 109%) Gas6 levels measured at the time of the index VTE ($P = 0.0004$) (Fig. 3B).

The 2-year cumulative incidence rates of overall mortality were 7%, 15% and 35% ($P < 0.001$) for patients with low, medium and high Gas6 levels, respectively (Fig. 3C).

Discriminative power of Gas6 levels for outcomes

In order to evaluate the discriminative power of Gas6 levels, *C*-statistic (95% CI) values were calculated (Table 2). Gas6 levels measured at the time of the index VTE were discriminatory for VTE recurrence, major bleeding and mortality up to 36 months.

The Gas6 level measured 12 months after the index VTE was discriminatory for VTE recurrence up 24 months. In contrast, when measured 12 months later, the Gas6 level was not discriminatory for major bleeding and mortality up to 24 months (Table 2).

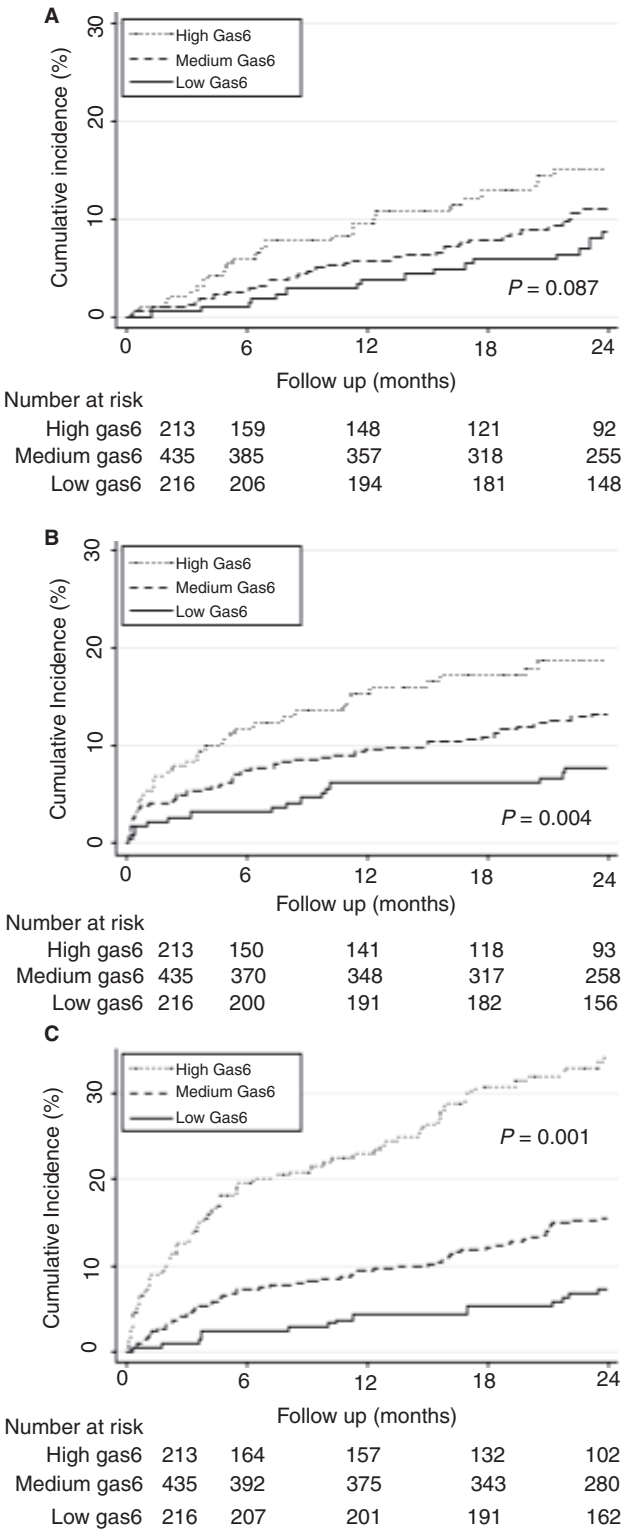

Fig. 3. Cumulative incidence rates of venous thromboembolism (VTE), major bleeding and mortality for strata of growth arrest-specific gene 6 (Gas6). The cumulative incidence rates of VTE (A), major bleeding (B) and mortality (C) for strata of Gas6 levels were estimated with the Kaplan–Meier method, and survivor functions across groups were compared by use of the log-rank test. Gas6 levels were categorized on the basis of the lower and upper quartiles as low (< 109%), medium (109–157%), and high (> 157%).

Association between Gas6 plasma levels and outcomes

High Gas6 levels (> 157%) measured at the time of the index VTE were associated with an increased risk of VTE recurrence up to 6 months (Table 3), and an increased risk of major bleeding up to 36 months (crude analysis) (Table 4). In continuous analysis (log-transformed Gas6 levels), the risk of VTE recurrence was increased up to 12 months (Table 3), and the risk of major bleeding was increased up to 36 months (crude analysis) (Table 4).

In addition, medium (109–157%) and high Gas6 levels were associated with increased overall mortality up to 36 months (Table 5).

These associations also remained after adjustment for potential confounding factors for the risk of VTE recurrence and overall mortality (Tables 3 and 5).

Regarding the risk of major bleeding, only the association with high Gas6 levels measured at the time of the index VTE remained up to 6 months after adjustment for potential confounding factors (Table 4).

We assessed the relationship between continuous log-transformed Gas6 values and risks of VTE recurrence

Table 2 Discriminative power of growth arrest-specific gene 6 (Gas6) plasma level for outcomes

	No. of events/no. of patients	*C*-statistics (95% confidence interval)	*P*-value*
From the time of the index VTE (T1) onwards using measurements performed at the time of VTE diagnosis (T1)			
Gas6 at the time of VTE diagnosis			
VTE recurrence			
Up to 6 months	24/864	0.67 (0.57–0.78)	0.001
Up to 12 months	48/864	0.61 (0.52–0.69)	0.010
Up to 24 months	83/864	0.58 (0.52–0.64)	0.010
Up to 36 months	100/864	0.56 (0.51–0.62)	0.031
Major bleeding			
Up to 6 months	62/864	0.62 (0.55–0.69)	< 0.001
Up to 12 months	82/864	0.60 (0.54–0.66)	0.001
Up to 24 months	103/864	0.60 (0.55–0.65)	< 0.001
Up to 36 months	118/864	0.60 (0.55–0.65)	< 0.001
Overall mortality			
Up to 6 months	77/864	0.73 (0.67–0.78)	< 0.001
Up to 12 months	97/864	0.71 (0.65–0.76)	< 0.001
Up to 24 months	149/864	0.70 (0.66–0.74)	< 0.001
Up to 36 months	170/864	0.69 (0.65–0.73)	< 0.001
From 12 months after the index VTE (T2) onwards using measurements performed 12 months after the index VTE (T2)			
Gas6 12 months after the index VTE			
VTE recurrence			
Up to 12 months	32/601	0.66 (0.56–0.75)	0.002
Up to 24 months	49/601	0.62 (0.54–0.71)	0.003
Major bleeding			
Up to 12 months	18/601	0.58 (0.43–0.72)	0.294
Up to 24 months	32/601	0.57 (0.47–0.68)	0.173
Overall mortality			
Up to 12 months	33/601	0.57 (0.47–0.68)	0.181
Up to 24 months	48/601	0.56 (0.48–0.65)	0.159

VTE, venous thromboembolism. *The *P*-value is from a test of the null hypothesis of no discrimination (i.e. a *C*-statistics of 0.5).

and overall mortality by using fractional polynomial competing risk and Cox proportional hazards models, which showed that (sub)-hazards and Gas6 levels increased linearly (Fig. S1).

The findings of the sensitivity analyses revealed that these associations also remained after the exclusion of patients with cancer (Table S2) or with cancer and provoked VTE (Table S3). Moreover, in the subgroup of patients not receiving oral anticoagulation 12 months after the index VTE, continuous (log-transformed) Gas6 levels were associated with VTE recurrence up to 12 months (Table S4). This association also remained after adjustment for potential confounding factors (Table S5). Finally, medium, high and continuous (log-transformed) Gas6 levels were associated with increased mortality up to 36 months (Table 5).

Discussion

We prospectively followed 864 elderly patients with VTE for a period of 3 years, and observed that patients with higher Gas6 levels were more likely to have cancer-related VTE and comorbidities. Our findings are consistent with previous publications reporting high Gas6 levels in a number of clinical conditions, most of them associated with inflammation and organ damage [14,17,54,55].

Our data showed that an elevated Gas6 level was independently associated with recurrent VTE up to 12 months, with major bleeding up to 6 months and with mortality up to 36 months after the index VTE. Considering that patients with more comorbidities were more likely to have higher Gas6 levels, neither the association with VTE recurrence, the association with major bleeding nor the association with overall mortality was surprising. However, the observed association remained significant after adjustment for a large number of comorbidities (Table 3–5). The Gas6 level was also still associated with VTE recurrence and mortality after the exclusion of patients with cancer (Table S2) or with cancer and provoked VTE (Table S3). Because Gas6 is a prohemostatic protein [23–25], we may assume that the association

Table 3 Association between growth arrest-specific gene 6 (Gas6) plasma level and venous thromboembolism (VTE) recurrence – from the time of the index VTE (T1) onwards using Gas6 measured at the time of VTE diagnosis (T1)

	n/*N* (%)	Crude subhazard ratio (95% confidence interval)	*P*-value	Adjusted subhazard ratio (95% confidence interval)	*P*-value
Up to 6 months					
Gas6 at the time of the index VTE (categorized)					
Low (< 109%)	2/216 (0.9)	Reference		Reference	
Medium (109–157%)	11/435 (2.5)	2.77 (0.61–12.51)	0.185	2.95 (0.62–13.95)	0.172
High (> 157%)	11/213 (5.2)	5.74 (1.27–25.95)	0.023	6.65 (1.44–30.80)	0.015
Log-transformed Gas6 at the time of the index VTE					
Continuous (per log unit)	24/864 (2.8)	4.71 (1.98–11.19)	< 0.001	5.04 (2.14–11.88)	< 0.001
Up to 12 months					
Gas6 at the time of the index VTE (categorized)					
Low (< 109%)	8/216 (3.7)	Reference		Reference	
Medium (109–157%)	23/435 (5.3)	1.46 (0.65–3.25)	0.355	1.50 (0.66–3.40)	0.335
High (> 157%)	17/213 (8.0)	2.26 (0.98–5.23)	0.056	2.42 (1.00–5.89)	0.051
Log-transformed Gas6 at the time of the index VTE					
Continuous (per log unit)	48/864 (5.6)	2.42 (1.12–5.24)	0.025	2.47 (1.08–5.64)	0.032

Adjustments: VTE recurrence was adjusted for age, cancer, provoked VTE, prior VTE, overt pulmonary embolism, renal disease and periods of anticoagulation (oral or parenteral anticoagulation) as a time-varying covariate [6,41,44–52].

Table 4 Association between growth arrest-specific gene 6 (Gas6) plasma level and major bleeding up to 6 months

	n/*N* (%)	Crude SHR (95% CI)	*P*-value	Adjusted SHR (95% CI)	*P*-value
From the time of the index VTE (T1) onwards using Gas6 measured at the time of VTE diagnosis (T1)					
Gas6 at the time of the index VTE (categorized)					
Low (< 109%)	7/216 (3.2)	Reference		Reference	
Medium (109–157%)	32/435 (7.4)	2.33 (1.03–5.28)	0.043	2.07 (0.89–4.82)	0.093
High (> 157%)	23/213 (10.8)	3.47 (1.49–8.10)	0.004	2.58 (1.04–6.37)	0.040
Log-transformed Gas6 at the time of the index VTE					
Continuous (per log unit)	62/864 (7.2)	2.79 (1.42–5.46)	0.003	2.05 (0.95–4.41)	0.067

CI, confidence interval; SHR, subhazard ratio; VTE, venous thromboembolism. Adjustments: major bleeding was adjusted for age, cancer, provoked VTE, prior VTE, overt pulmonary embolism, renal disease, history of major bleeding, anemia, antiplatelet therapy and periods of anticoagulation as a time-varying covariate [51,59–73].

between high Gas6 levels and VTE recurrence might be at least partly causal.

Another important finding of this study is that Gas6 levels measured at the time of diagnosis were discriminatory for VTE recurrence and mortality. In addition, Gas6 levels measured 12 months after the index VTE were discriminatory only for VTE recurrence. A previous study comprising a lower number of patients than this study did not demonstrate the predictive ability of Gas6 levels for VTE recurrence [29].Thus, the data of the present study point to an elevated Gas6 level as an independent predictor for VTE recurrence, major bleeding and mortality up to 36 months in the elderly. The Gas6 level might therefore be useful in adjusting the intensity of surveillance in this group of high-risk patients. However, before considering the Gas6 level as an additional marker with which to predict recurrence and guide therapy, the Gas6 level would need to be compared with or integrated into established risk scores such as the DASH [56], HERDOO-2 [57] and Vienna [58] scores.

Our study has some limitations. First, the scope of the study was limited to elderly patients, and 18.2% of them had cancer; the mortality resulting from comorbid diseases is naturally higher than the VTE recurrence rate, as persons with limited life-expectancy often do not have the time to develop recurrent VTE. Thus, it is indeed unclear whether the results can be extrapolated to younger persons with VTE. In addition, although the Gas6 plasma level was previously reported not to be influenced by age [21], both its predictive ability for VTE recurrence and its association with VTE recurrence would need to be studied in younger patients. Second, the Gas6 level was previously reported to be elevated in several other medical conditions. Nevertheless, in this study, we were able to demonstrate that the association between the Gas6 level and VTE recurrence and mortality remained after adjustment for these conditions. However, this needs to be verified in younger patients. Third, VTE treatment has changed since this cohort was constituted; that is, direct oral anticoagulants have replaced vitamin K antagonists for most patients. Therefore, it is unclear whether the results can be extrapolated to patients treated with direct oral anticoagulants. Fourth, as we enrolled patients with VTE in inpatient and outpatient hospital services, the proportion of patients with PE was relatively high, and represented 69% of our study sample. Fifth, Gas6 testing was performed only at the time of the index VTE and 12 months later, when 50% of the patients were still receiving oral anticoagulation. Because we and others [21] have demonstrated that Gas6 levels are affected by oral anticoagulation with vitamin K antagonists, we can assume that the significantly lower Gas6 level 12 months after the index VTE was at least partly attributable to the anti-vitamin K effect. Interestingly, Gas6 levels in the subgroup of patients not receiving oral anticoagulation at this time point were significantly lower than those in patients receiving anticoagulation. Thus, the correct interpretation of Gas6 levels would require patients to interrupt anticoagulation, exposing those with increased risk to the possibility of a VTE recurrence. Finally, even though we adjusted our analyses for many covariates, we might have missed important predictor variables.

In conclusion, in the elderly, a high Gas6 level is associated with higher risks of VTE recurrence and major bleeding, but only up to 6 months, a period of time during which most patients were still anticoagulated, and death. Our data suggest that a clinical decision to avoid prolonged anticoagulation could be attempted on the basis of Gas6 plasma levels in the elderly. Further studies are required to confirm whether the use of Gas6 levels for adjusting the length of anticoagulation leads to better outcomes, especially in younger patients.

Table 5 Association between growth arrest-specific gene 6 (Gas6) plasma level and overall mortality up to 36 months

	n/*N* (%)	Crude hazard ratio (95% confidence interval)	*P*-value	Adjusted hazard ratio (95% confidence interval)	*P*-value
From the time of the index VTE (T1) onwards using Gas6 measured at the time of VTE diagnosis (T1)					
Gas6 at the time of the index VTE (categorized)					
Low (< 109%)	20/216 (9.3)	Reference		Reference	
Medium (109–157%)	73/435 (16.8)	1.96 (1.20–3.19)	0.007	1.69 (1.00–2.84)	0.048
High (> 157%)	77/213 (36.2)	4.95 (3.04–8.05)	< 0.001	3.44 (2.03–5.82)	< 0.001
Log-transformed Gas6 at the time of the index VTE					
Continuous (per log unit)	170/864 (19.7)	7.21 (4.48–11.60)	< 0.001	5.00 (3.16–7.92)	< 0.001
From the time of the index VTE onwards using Gas6 as a time-varying covariate (at the time of the index VTE and 12 months later)					
Gas6 time-varying covariate (categorized)					
Low (< 109%)		Reference		Reference	
Medium (109–157%)		1.88 (1.26–2.80)	0.002	1.68 (1.09–2.57)	0.017
High (> 157%)		5.55 (3.63–8.47)	< 0.001	3.55 (2.21–5.71)	< 0.001
Log-transformed Gas6 time-varying covariate					
Continuous (per log unit)		8.50 (5.51–13.11)	< 0.001	5.18 (3.17–8.46)	< 0.001

VTE, venous thromboembolism. Adjustments: mortality was adjusted for age, gender, cancer, provoked VTE, prior VTE, overt pulmonary embolism, renal disease, history of major bleeding, heart failure, chronic lung disease, high pulse, low blood pressure, low oxygen, and periods of anticoagulation as a time-varying covariate [49,53].

Addendum

A. Schnegg-Kaufmann, S. Calzavarini, and A. Angelillo-Scherrer designed the protocol and the analysis plan, conducted the analyses, and drafted the manuscript. S. Calzavarini performed Gas6 measurements. A. Limacher performed the statistical analysis. A. Schnegg-Kaufmann, S. Calzavarini, and A. Angelillo-Scherrer interpreted the data. M. Méan, M. Righini, B. Frauchiger, J. Osterwalder, N. Kucher, and N. Rodondi organized data collection, intellectually reviewed the manuscript, and participated in funding procedures. A. Schnegg-Kaufmann, S. Calzavarini, A. Limacher, D. Staub, J. H. Beer, C. M. Matter, M. Husmann, M. Banyai, M. Aschwanden, L. Mazzolai, O. Hugli, M. Nagler, and M. Daskalakis organized data collection and intellectually reviewed the manuscript. D. Aujesky was principal investigator of the SWITCO65+ cohort, and was responsible for planning of the study, data collection, drafting of the manuscript, and obtaining funding. A. Angelillo-Scherrer was in charge of the Gas6 nested study, and was responsible for planning of the study, data collection, drafting of the manuscript, and obtaining funding. All authors approved the final version of the manuscript.

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Disclosure of Conflict of Interests

C. M. Matter reports receiving: grants from the Swiss National Science Foundation, during the conduct of the study; and grants from MSD, Bayer, AstraZeneca, Eli-Lilly, and Sanofi, and personal fees from MSD, AstraZeneca, Roche, Sanofi, Amgen, and Novartis, outside the submitted work. A. Limacher reports receiving grants from the Swiss National Science Foundation, during the conduct of the study. The other authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Incidence rates of VTE recurrence, major bleeding and mortality rates by level of Gas6 measured at the time of the index VTE.
Table S2. Sensitivity analyses: from baseline onwards using baseline Gas6, excluding patients with cancer.
Table S3. Sensitivity analyses: from baseline onwards using baseline Gas6, excluding patients with cancer and provoked VTE.
Table S4. Association between Gas6 measured 12 months after the index VTE in patients not receiving oral anticoagulation and VTE recurrence from 12 months after the index VTE onwards.
Fig. S1. Relative subhazards for VTE recurrence and relative hazards for overall mortality.

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