Low TAFI levels increase the risk of hemorrhagic complications during long-term warfarin therapy

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Background

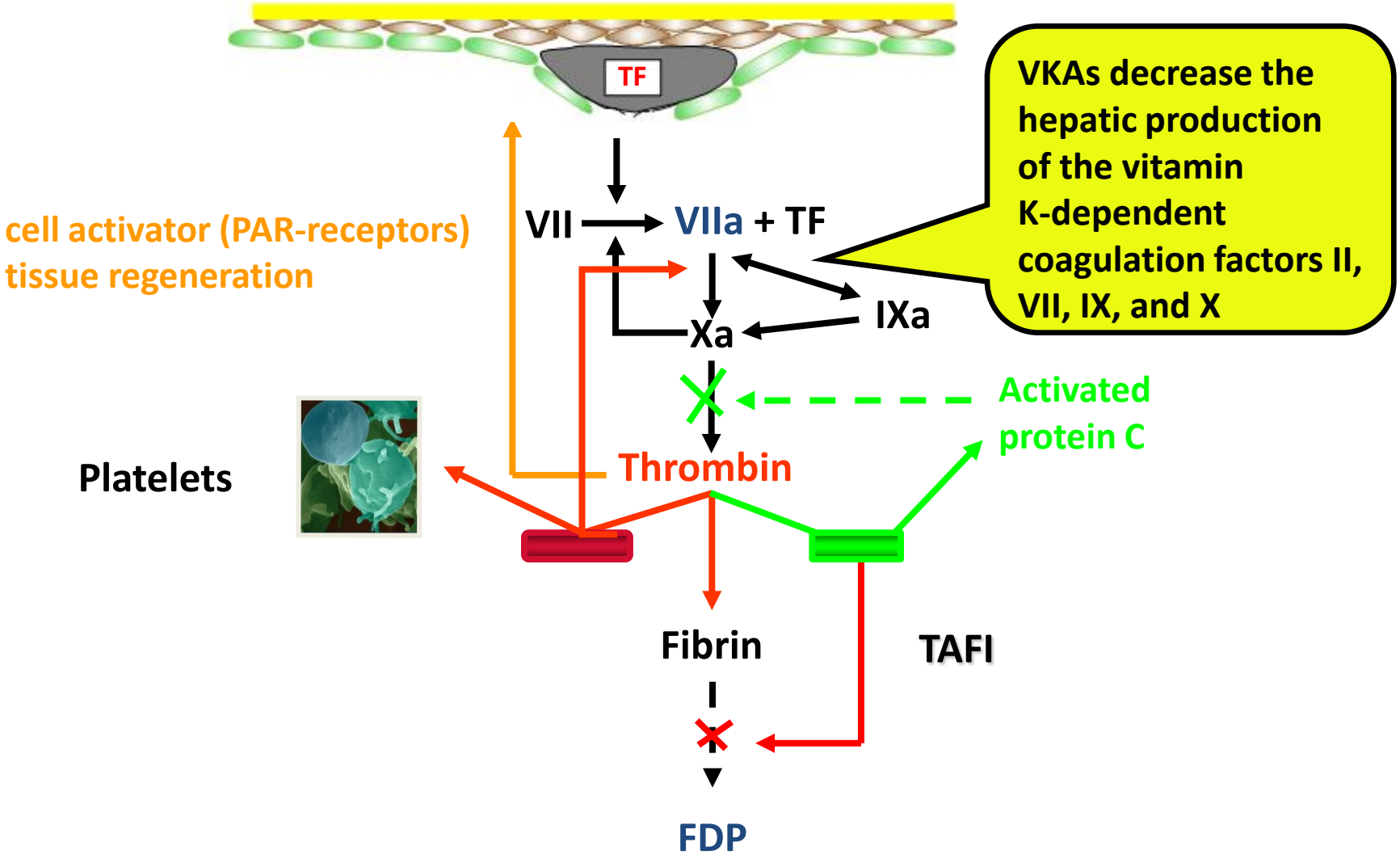
- **Vitamin K antagonists (VKAs) are highly effective for the primary and/or secondary prevention of thromboembolic complications in patients with atrial fibrillation, venous thromboembolism, prosthetic heart valves and after myocardial infarction**
- **The serious complication of VKA therapy is bleeding**
- **The annual incidence of VKA-associated major bleeding is estimated at 1% to 3%[1]**

[1]*Schulman S. et al. Chest 2008; 133 (suppl. 6): 257S-298S*

Risk factors for VKA-related bleeding

- Intensity of anticoagulant effect (INR > 3,0)
- Patient characteristics (age, history of bleeding, malignancy, ischemic stroke, hypertension, cerebrovascular disease, diabetes, alcoholism, renal insufficiency, liver disease, serious heart disease)
- Pharmacogenetic factors (CYP2C9 and VKORC1 polymorphisms)
- Concomitant use of antiplatelet drugs, acetaminophen, nonsteroidal antiinflammatory drugs, or cyclooxygenase type 2 inhibitors
- Drug interactions with VKAs (antibiotics, amiodarone and others)
- Duration of therapy

Thrombin is a multifunctional enzyme



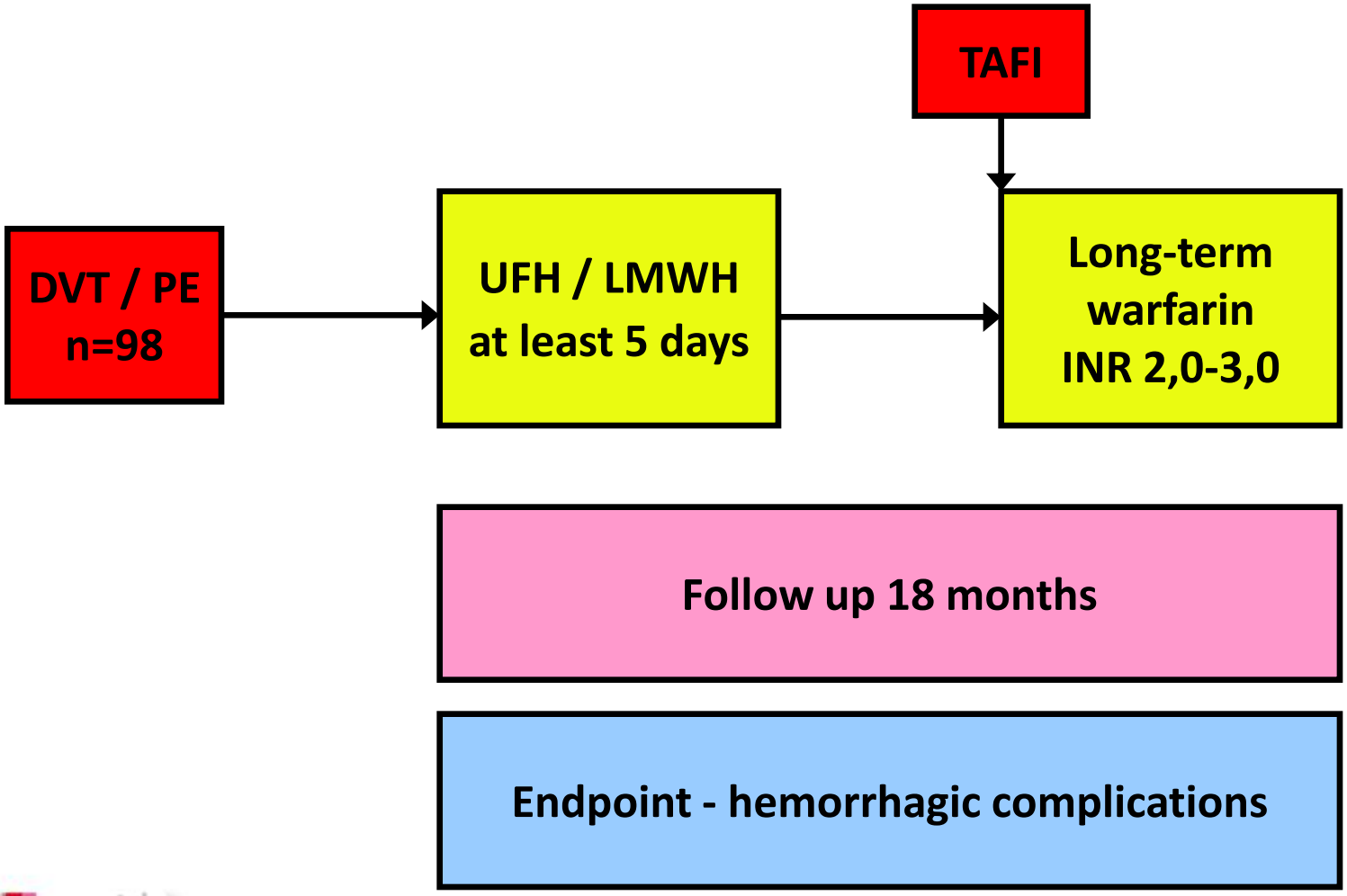
What is TAFI?

- Thrombin activatable fibrinolysis inhibitor (TAFI) is a zymogen which can be activated by several proteases, but the most potent activator is the complex thrombin/thrombomodulin
- Mechanism of inhibition of fibrinolysis by TAFIa consists in the removal of carboxyterminal lysine residues from partially degraded fibrin that decreases binding and subsequent activation of plasminogen on fibrin surface
- Using this mechanism, TAFI stabilizes the fibrin clot and makes the clot more resistant to lysis
- Since activation of TAFI depends on generation of thrombin one may speculate that TAFI may be one of factors determining the efficiency and safety of anticoagulant therapy

Purpose

- To determine associations between TAFI levels and hemorrhagic complications during long-term warfarin therapy in patients with venous thromboembolism

Study design



Characteristics of VTE patients (n=98)

Sex (male/female)	70/28
Age, years [mean (range)]	54 (18-76)
Body mass index, kg/m^2 [median (IQR)]	28,4 (25,7; 32,9)
Isolated DVT, n (%)	71 (72%)
Isolated PE, n (%)	1 (1%)
DVT + PE, n (%)	26 (27%)
Lower extremity DVT, n (%)	92 (93,9%)
• Proximal DVT, n (%)	81 (88%)
• Distal DVT, n (%)	11 (12%)
Upper extremity DVT, n (%)	5 (5,1%)
Previous VTE, n (%)	34 (35%)
Family history of VTE, n (%)	11 (11%)
Idiopathic VTE, n (%)	35 (36%)

Measurement of TAFI levels

- **TAFI levels were measured by a chromogenic assay with reagent kits «STA STACHROM TAFI» (Diagnostica Stago)**
- **Reference values of TAFI levels are 108 ± 25% (according to the manufacturer)**

Genetic analysis

We investigated the following genetic polymorphisms:

- **CYP2C9 430C/T (allele 2)**
- **CYP2C9 1075A/C (allele 3)**
- **VKORC1 3673G/A**

- **Genotyping for the CYP2C9 mutations was performed by PCR amplification; PCR products were sequenced with the Biotage terminator kits and analyzed on an PSQ96MA automated sequencer (Pyrosequencing AB)**

- **Genotyping for the VKORC1 mutation was performed by PCR - restriction fragment length polymorphism**

Characteristic of endpoints

We analyzed all major and minor bleedings during 18 months of warfarin therapy.

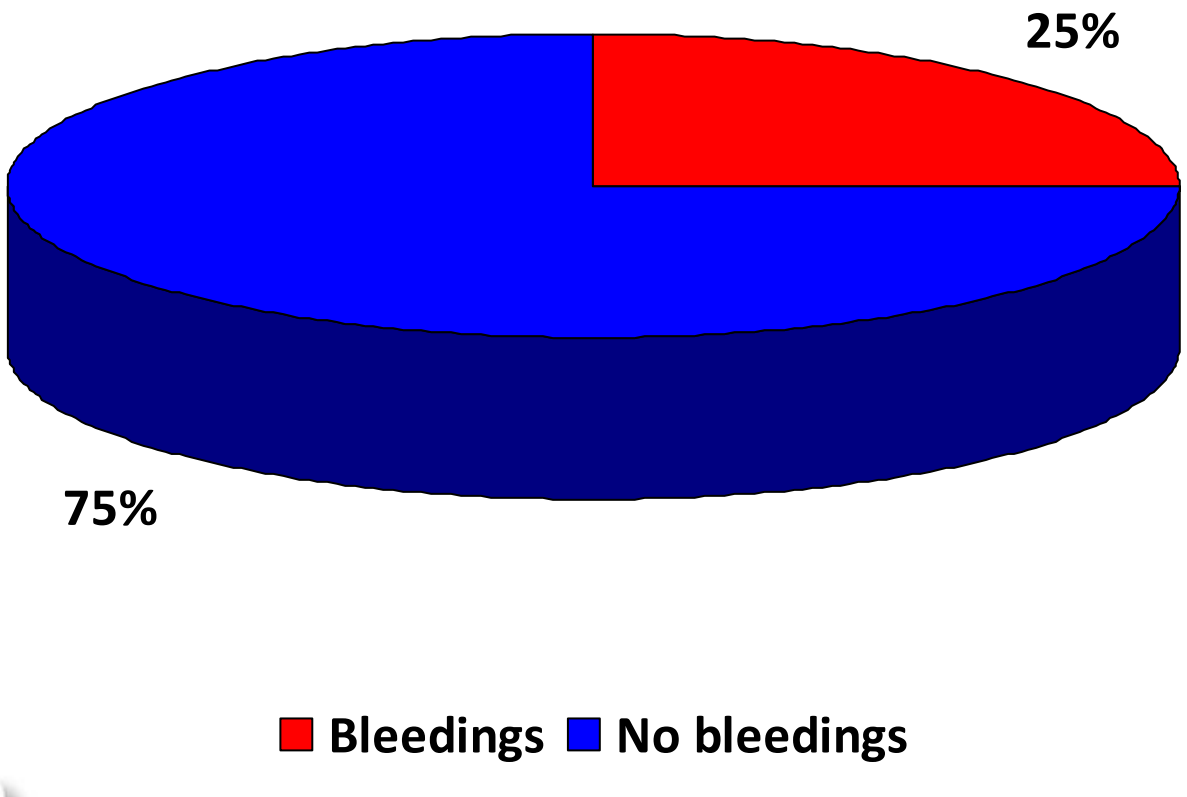
We assessed the following types of bleedings:

- Provoked bleedings occurred after provoking factors such as alcohol consumption, trauma, and drugs increasing the INR; most of them occurred at the INR > 3,0
- Spontaneous bleedings had no provoking factors; most of them occurred in a target INR (2,0-3,0) or at the INR < 2,0
- Bleedings in a target INR (2,0-3,0); they could be both provoked, and spontaneous

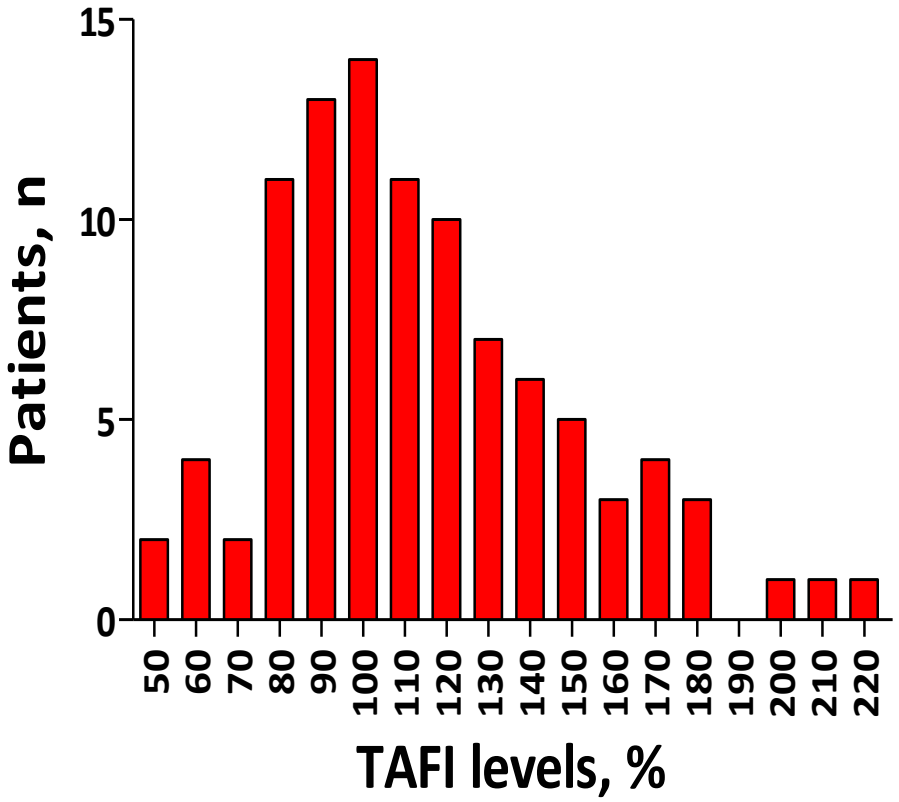
Statistical analysis

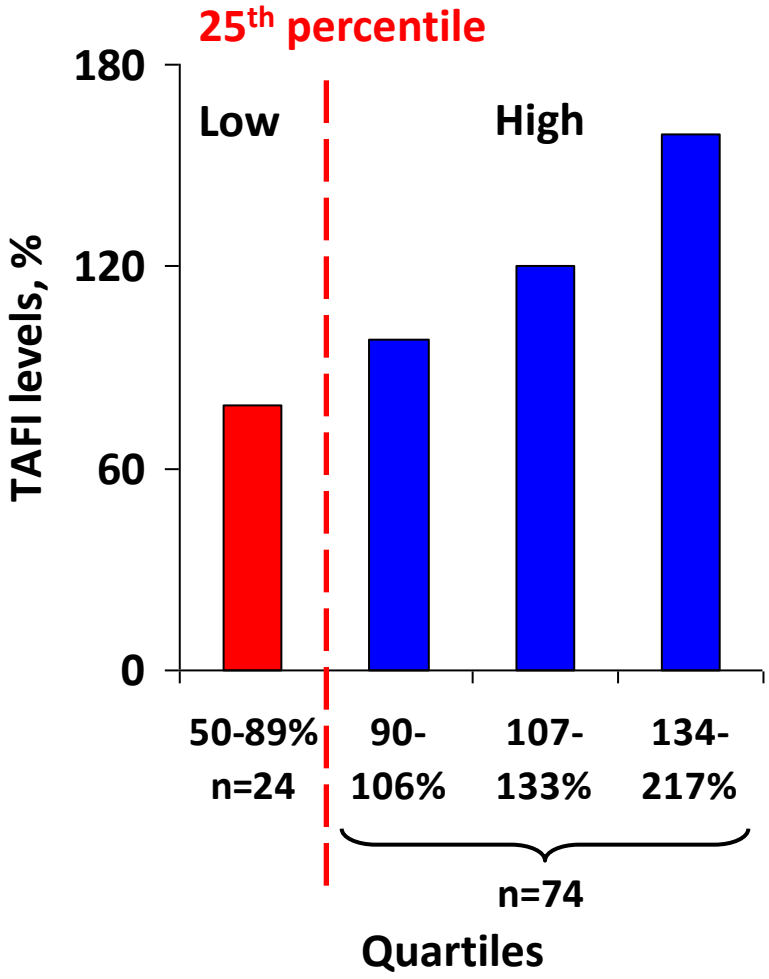
- Statistical analysis was performed using a commercially available statistical package (SPSS 11.5 for Windows, SPSS Inc., USA)
- Differences between groups were tested using two-sided Fisher's exact test
- Univariate Cox regression was used to calculate the hazard ratio and 95% confidence interval for the risk of hemorrhagic complications

The frequency of hemorrhagic complications during 18 months of warfarin therapy (n=98)

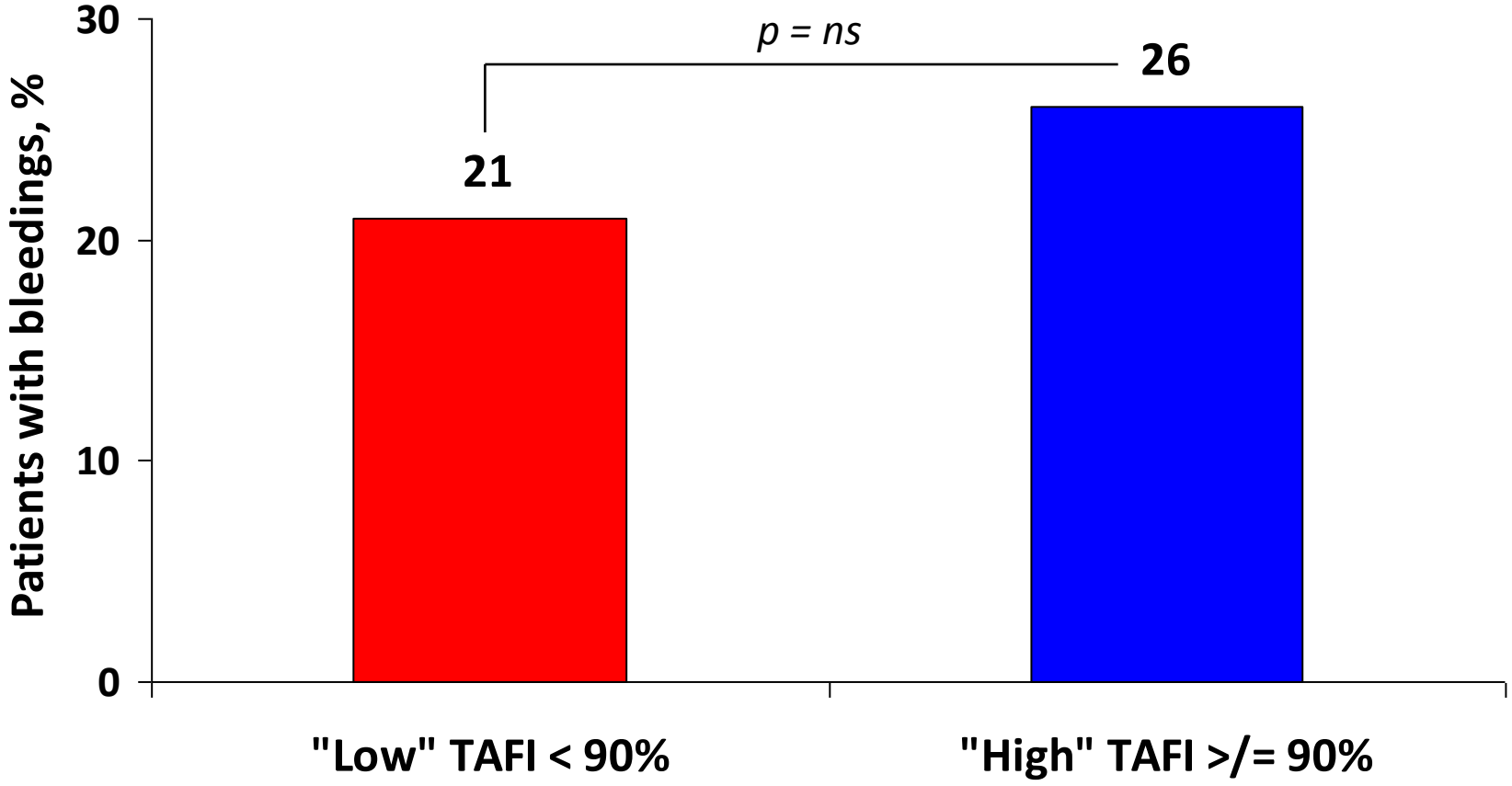


Distribution of TAFI levels in VTE patients (n=98)





The frequency of hemorrhagic complications depending of TAFI levels (n=98)



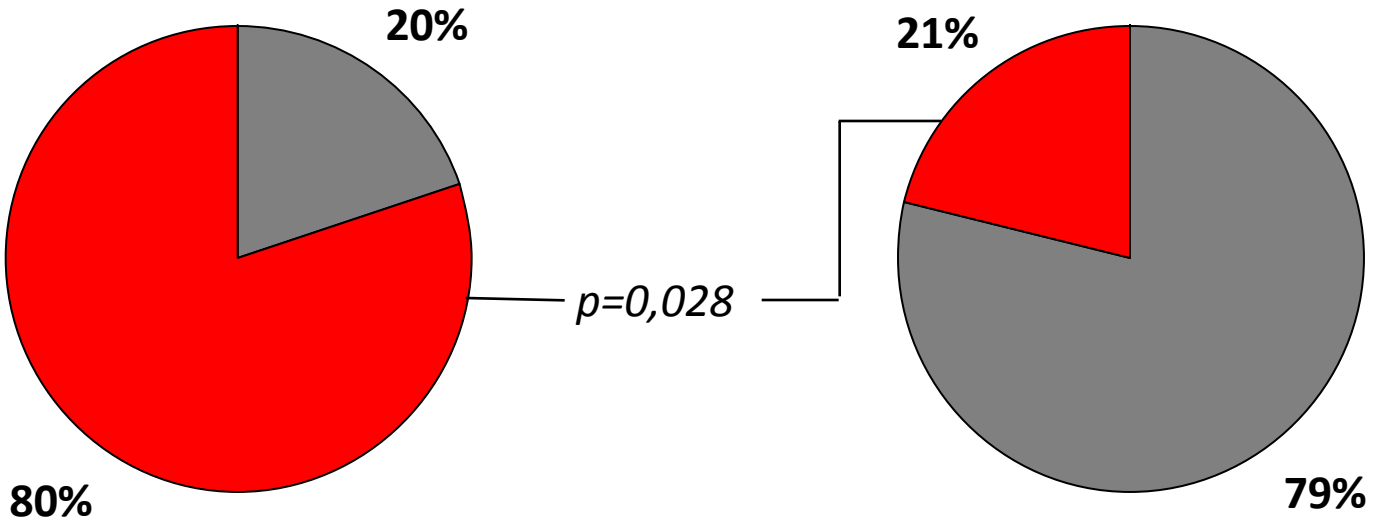
The analysis of 24 patients with hemorrhagic complications

CYP2C9 and VKORC1 polymorphisms and TAFI levels

Polymorphism	"Low" TAFI < 90% (n=5)	"High" TAFI ≥ 90% (n=19)	p
CYP2C9*1, %	80%	79%	0,999
CYP2C9*2, %	0%	10,5%	0,999
CYP2C9*3, %	20%	10,5%	0,521
VKORC1 3673GG, %	20%	47,4%	0,358
VKORC1 3673GA, %	60%	47,4%	0,999
VKORC1 3673AA, %	20%	5,3%	0,380

TAFI levels and the risk of spontaneous bleedings

In patients with TAFI levels < 90%, the risk of spontaneous bleedings is higher HR 4,16 (95% CI 1,04-16,65; p=0,044)



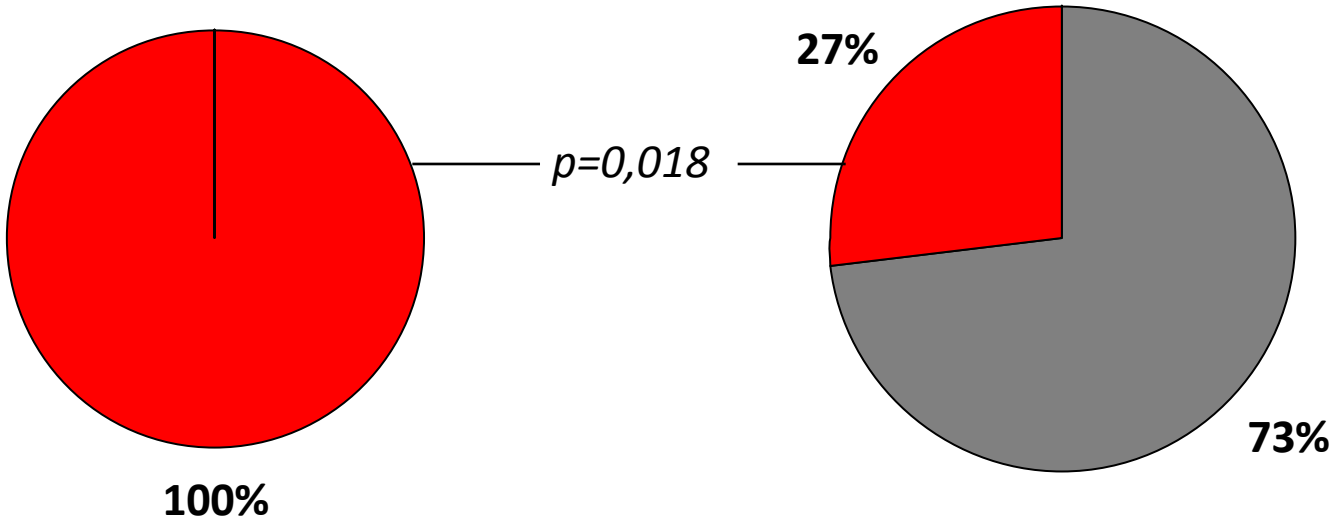
"Low" TAFI < 90% (n=5)

"High" TAFI ≥ 90% (n=19)

Spontaneous bleeding **Provoked bleeding**

TAFI levels and the risk of bleedings in a target INR (2,0-3,0)

In patients with TAFI levels < 90%, the risk of bleedings in a target INR is higher HR 6,06 (95% CI 1,35-27,24; p=0,019)



"Low" TAFI < 90% (n=5)

"High" TAFI ≥ 90% (n=19)

INR 2,0-3,0

INR < 2,0 or > 3,0

Conclusions

- **In VTE patients with hemorrhagic complications during long-term warfarin therapy, TAFI levels < 90% are associated with increase of the risk of spontaneous bleedings and bleedings in a target INR (2,0-3,0)**
- **Our pilot study showed that low TAFI levels may be one of the causes of hemorrhagic complications; therefore we suggest that further large prospective studies are needed**