The ORBIT bleeding score is associated with lysis and permeability of fibrin clots

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The ORBIT bleeding score is associated with lysis and permeability of fibrin clots

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\textbf{Short title:} The ORBIT bleeding score is associated with lysis and permeability of fibrin clots

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INTRODUCTION

Clinical schemas largely based on age and co-morbidities, in particular the CHA2DS2-VASc and HAS-BLED scores, have been reported to predict thromboembolism or bleeding, respectively. However, current European Society of Cardiology guidelines did not recommend any preferred scoring system to predict major bleeding but rather suggest to identify and correct modifiable bleeding risk factors.[1] Several biomarkers have been investigated,[2] and currently some of them may be incorporated into bleeding risk assessment.[3]

Fibrin constitutes a key protein component of thrombi causing ischemic stroke.[4] It has been shown that formation of compact clot networks, evidenced by low fibrin clot permeability, is an independent predictor of both thromboembolic events and major bleedings in AF patients on oral anticoagulation, and low permeability and HAS-BLED score ≥3 had a predictive value for major bleeds.[5] Similar observations have been made for patients on rivaroxaban.[6] Clot lysis has been found to be prolonged in AF patients without any impact of CHA2DS2-VASc and HAS-BLED scores.[7] It remains unclear whether fibrin-related prothrombotic biomarker(s) added to a scoring system based on clinical risk factors can result in more accurate risk assessment across different AF populations.[8]

In this preliminary report, we investigated whether clinical bleeding risk scores are associated with plasma fibrin clot properties and thrombin generation in AF patients.

METHODS

We studied 100 subjects with documented nonvalvular AF, derived from the cohort described in detail previously.[9] Briefly, the exclusion criteria were the current anticoagulation, myocardial infarction or venous thromboembolism within the preceding 3 months, malignancy, acute infection, kidney failure requiring dialysis, and liver cirrhosis. ORBIT.[10]
HEMORR2HAGES,[11] HAS-BLED[12] and modified HAS-BLED[13] scores were used to evaluate bleeding risk. The study protocol was approved by the University Ethical Committee.

Routine laboratory investigations were assayed by standard methods. N-terminal pro-B-type natriuretic peptide (NT-proBNP), were measured using an immunoassay (Roche Diagnostics). We determined in plasma tissue-type plasminogen activator antigen (tPA, Diagnostica Stago), plasminogen activator inhibitor-1 antigen (PAI-1, American Diagnostica), thrombin-activatable fibrinolysis inhibitor (TAFI, Chromogenix) and von Willebrand factor antigen (vWF, Diagnostica Stago). Chromogenic assays were used to measure α2-antiplasmin and plasminogen (Diagnostica Stago). Fibrinogen was determined using the Clauss assay. Calibrated automated thrombography (Thrombinoscope BV) was used to measure endogenous thrombin potential (ETP) as described.[9] Fibrin clot permeability was measured using a pressure-driven system as described previously.[9] A permeation coefficient (Ks), which indicates the pore size in the fiber network (higher values indicate looser fibrin structure) was calculated.

Clot lysis time (CLT) was defined as the time required for change from the midpoint of the clear-to-maximum-turbid transition, to the to the final plateau phase at 405 nm, induced by 32 ng/ml tPA (Boehringer Ingelheim).[5] All measurements were performed by technicians blinded to the sample status (interassay and intra-assay coefficients of variation <7%).

**Statistical analysis**

Data were presented as the mean (standard deviation) or median (IQR, interquartile range), as appropriate. Normality was checked using the Shapiro-Wilk test. The Student’s t-test or the Mann-Whitney test were used to test differences between 2 groups as appropriate. Means between the 3 groups were compared by one-way ANOVA followed by Tukey post-hoc test, medians were analyzed by Kruskal–Wallis test followed by test for multiple comparisons of
mean rank. Correlations were assessed by the Pearson or Spearman test, as appropriate. Categorical variables were analyzed using chi-square test or Fisher's exact tests. A value of P<0.05 was considered significant. Statistical analyses were performed using STATISTICA version 13 (Statsoft Inc, Tulsa, OK).

RESULTS

The AF patients were presented in Table 1 and supplemental table 1. The mean ORBIT, HEMORR²HAGES, HAS-BLED, and modified HAS-BLED scores were 1.78 (1.48), 1.43 (1.14), 2.07 (1.12), and 2.70 (1.16), respectively. A high bleeding risk defined as ORBIT ≥4, HEMORR²HAGES ≥4, HAS-BLED ≥3, and modified HAS-BLED ≥3 was found in 13%, 7%, 33% and 58% patients, respectively.

Patients with ORBIT bleeding score ≥4 were older, had higher prevalence of chronic kidney disease stage 3, anemia, previous serious bleeding and use of antiplatelets. This high-risk group had 20.8% shorter CLT (80 [78-92] vs 101 [89-118] min; p=0.027), compared with those with ORBIT bleeding score of 0-2. There was no difference between CLT in the group with ORBIT bleeding score 3 and ≥4 (p=0.28). Kₜ was 7.7% and 12.3% higher (7.0 [0.8] vs 7.3 [0.9] vs 6.5 [0.7] cm²×10⁻⁹; p=0.002 and p=0.048, respectively) in patients with ORBIT bleeding score ≥4 and 3 compared with ORBIT bleeding score of 0-2.

No associations of CLT and Kₜ with HEMORR²HAGES and HAS-BLED and modified HAS-BLED scores were observed in the AF patients.

Multiple linear regression adjusted for age, BMI and fibrinogen showed that ORBIT bleeding score (β=−0.17), PAI-1 (β=0.31), tPA (β=0.18) and NT-proBNP (β=0.42) were the independent predictors of CLT (R²=0.40) in the whole group. In the multiple regression model ORBIT bleeding score (β=0.22), PAI-1 (β=−0.35) and plasminogen (β=0.22) were the independent predictors of Kₜ (R²=0.29) in the study group.

DISCUSSION
This study demonstrates that the five-element ORBIT bleeding score which has been reported to have better ability to predict major bleeding in AF patients as compared with HAS-BLED and ATRIA risk scores,[10,14] is associated with enhanced fibrinolysis and looser clot structure, which renders it prone to fragmentation. This finding extends our previous studies regarding plasma fibrin clot density and lysability in AF.[4,5] We did not observe any association between bleeding risk assessed by ORBIT, HEMORR2HAGES, HAS-BLED, modified HAS-BLED scores and thrombin generation, fibrinolytic proteins, and NT-proBNP. We observed that the highest ORBIT bleeding score was associated with impaired kidney function and use of aspirin or clopidogrel that have been reported to unfavorably alter fibrin structure and function and also predispose to bleeding.[4,9] The exact mechanisms underlying these observations remain to be established. Our study supports the concept of a complex clinical and biomarker-based approach in the bleeding risk assessment in patients with AF regarding moderate prediction using schemas based only on age and co-morbidities.[5,15] Our study has several limitations. A size of the study was limited especially in subgroup analyses. All laboratory measurements were determined once and changes with time cannot be excluded. Two biomarkers GDF-15 and troponin were not measured in the whole group and were not analyzed. Finally, statistical associations reported here do not necessarily mean direct cause-effect relationship. We did not collect follow-up data therefore the actual bleeding rate in this group is unknown.

This hypothesis-generating report suggests that tendency to form looser and more lysable fibrin clots can be observed in patients with high bleeding risk assessed by ORBIT score. Further studies are needed to corroborate these results.

**ACKNOWLEDGEMENTS:** None

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References


14. Natale A, Mohanty S, Gianni C. Comparison of different oral anticoagulant regimens in patients with atrial fibrillation: is the risk of left atrial appendage thrombus formation

<table>
<thead>
<tr>
<th>Variables</th>
<th>ORBIT Low Risk (0-2) (n=73)</th>
<th>Moderate Risk (3) (n=14)</th>
<th>High Risk (≥4) (n=13)</th>
<th>p-value</th>
<th>HEMORRHAGES Low Risk (0-1) (n=58)</th>
<th>Moderate Risk (2-3) (n=35)</th>
<th>High Risk (≥4) (n=7)</th>
<th>p-value</th>
<th>HAS-BLED Low Risk (0-2) (n=67)</th>
<th>High Risk (≥3) (n=33)</th>
<th>p-value</th>
<th>Modified HAS-BLED Low Risk (0-2) (n=42)</th>
<th>High Risk (≥3) (n=58)</th>
<th>p-value</th>
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<td>74 (68-77)</td>
<td>73 (73-75)*</td>
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<td>74 (70-77)*</td>
<td>74 (73-76)*</td>
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<td><strong>Women</strong></td>
<td>27 (37.0)</td>
<td>8 (57.2)</td>
<td>6 (46.2)</td>
<td>0.34</td>
<td>20 (34.5)</td>
<td>18 (51.4)</td>
<td>3 (42.9)</td>
<td>0.27</td>
<td>24 (35.8)</td>
<td>17 (51.5)</td>
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<td><strong>BMI (kg/m²)</strong></td>
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<td>29 (27-30)</td>
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<td>11 (84.6)</td>
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<td>44 (75.9)</td>
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<td><strong>Previous stroke</strong></td>
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<td>14 (24.1)</td>
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*P<0.05 vs low risk. Values are n (%), mean (standard deviation), or median and interquartile range.

For abbreviations see methods.