DOAC score is associated with elevated growth differentiation factor 15 and 3-nitrotyrosine in atrial fibrillation: Prediction of bleeding at one-year follow-up

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INTRODUCTION

Atrial fibrillation (AF) patients on oral anticoagulation are at greater risk of major bleeding. Existing clinical decision-making aids for evaluating the risk of bleeding in AF patients were originally designed for individuals treated with warfarin [1]. The HAS-BLED score has shown limited accuracy in numerous studies and was validated in cohorts of AF patients with a relatively low bleeding risk [2]. In 2023, the DOAC score was developed and validated on AF patients receiving direct oral anticoagulants (DOACs) [3]. This score, assessed in randomized controlled trials with dabigatran and apixaban, exhibited a stronger predictive performance compared to the HAS-BLED score in predicting bleeding events [3]. Since 2016, there has been growing evidence for a predictive role of elevated growth differentiation factor 15 (GDF-15), a stress response protein whose levels increase in inflammation, oxidative stress, and tissue injury [4]. GDF–15 was found to be a valuable biomarker that can predict major bleeding in anticoagulated AF patients [4, 5]. To our knowledge, there have been no studies linking the DOAC score with bleeding risk biomarkers in AF. We investigated whether a high DOAC score is related to elevated GDF-15 or circulating biomarkers related to inflammation and prothrombotic state in AF patients and if the DOAC score is associated with bleeding in a 1-year follow-up while on DOAC.

MATERIAL AND METHODS

We studied 245 consecutive AF patients treated with DOACs enrolled during routine visits in the outpatient center between June 2020 and December 2021. The study population, exclusion criteria, and definitions of comorbidities were described previously [6]. AF classification was based on the 2020 European Society of Cardiology guidelines [5]. The study was approved by the ethics committee (1072.6120.186.2020), and all participants provided written informed consent.

The DOAC score was assigned as very low (score 0–3), low (score 4–5), moderate (score

6–7), high (score 8–9), and very high (score 10). Individuals with scores >10 were assigned a score of 10 [3].

Blood samples from antecubital veins were drawn before the intake of the morning dose of a DOAC. Routine laboratory investigations were conducted using standard laboratory techniques. Serum GDF-15 levels, matrix metalloproteinase 9, and plasma 3-nitrotyrosine were assayed using enzyme-linked immunosorbent assays (ELISAs). Endogenous thrombin potential and fibrin clot properties were assessed as previously described [6, 7]. For details, please see Supplementary material.

Patients were followed up by telephone or clinical visits at least twice during 12 months ±3 weeks. We recorded major, non-major clinically relevant, and minor bleedings [8].

Statistical analysis

Variables were presented as numbers (percentages) or medians (interquartile ranges). The normality of data distribution was assessed using the Shapiro–Wilk test. Differences between the two groups were compared using the Wilcoxon test. Categorical variables were compared using Pearson's χ^2 test or Fisher's exact test. Correlation analyses were conducted utilizing Spearman or Pearson's correlation coefficients, as appropriate. A *P*-value of <0.05 was considered statistically significant. For details, please see Supplementary material. Statistical analyses were performed with STATISTICA software (Version 13.3, TIBCO Software, Palo Alto, CA, US).

RESULTS AND DISCUSSION

Among 245 AF patients (40% women) paroxysmal AF was the most prevalent condition (45.7%; Supplementary material, *Table S1*). The median CHA_2DS_2-VASc score was 3 (2–4), with a maximum value of 9 and HAS-BLED score was 2 (1–2) with a maximum value of 4. The median DOAC score was 4 (interquartile range 2–6; range from 0 to 10; Supplementary material, *Table S2*). As many as 103 patients (42%) were at very low risk of bleeding, 68 (27.7%) at low risk of bleeding, 52 (21.2%) at moderate risk of bleeding, 9 (3.7%) at high risk of bleeding, and 13 (5.3%) at very high risk of bleeding. We categorized patients with the DOAC score above 3 points as a low-to-very high bleeding risk group for further analysis (see Supplementary material).

In the whole group, GDF-15 correlated with age (r = 0.36; *P* <0.001), CHA_2DS_2-VAS score (r = 0.3; *P* <0.001) and HAS-BLED (r = 0.21; *P* = 0.001). The DOAC score correlated positively with GDF-15 (Figure 1), but not with other markers. Patients with low-to-very high bleeding risk were characterized by a 45.5% higher GDF-15 level, also after adjustment for age and sex (*P* = 0.02), compared to patients with very low bleeding risk.

In terms of laboratory parameters, GDF-15 weakly correlated with CRP (r = 0.23; *P* = 0.001), 3-nitrotyrosine (r = 0.2; *P* = 0.002), and inversely with clot permeability (K_s; r = –0.34; *P* <0.001). Interestingly, patients at low-to-very high bleeding risk had 57% higher 3-nitrotyrosine concentration than others (Supplementary material, *Table S1*). Analysis of fibrin clot properties and thrombin generation showed no differences in relation to the DOAC score (Supplementary material, *Table S1*).

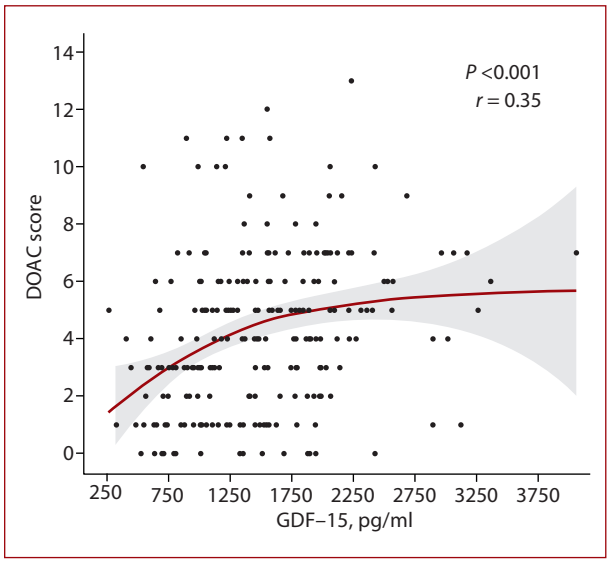

Figure 1. Association between the DOAC score and GDF-15

Abbreviations: DOAC, direct oral anticoagulants; GDF-15, growth differentiation factor-15

During follow-up, none of the patients was lost. Bleeding events were observed in 19 (7.8%) patients, including minor and non-major bleeding in 17 (6.9%) and major bleeding in 2 (0.8%) individuals (Supplementary material, *Table S1*). The median DOAC score for patients who experienced bleeding was 6 compared to 4 for non-bleeding subjects. No difference was observed for the HAS-BLED score (*P* >0.05). The DOAC score, with each additional point scored, was associated with 28% higher risk of all bleeding events (OR, 1.28 [95% CI, 1.09–1.53]; *P* = 0.004). With each point in this score, GDF-15 levels rose by 72.4 (95% CI, 43.3–101.5) pg/ml. However, there was no difference between patients with bleeding events during follow-up compared to others in terms of GDF-15 levels (*P* >0.05).

To our knowledge, this study is the first to show that a new 10-point DOAC score for prediction of bleeding in AF patients on DOACs is associated with increased GDF-15, a biomarker known to predict bleeding. This finding provides additional evidence for a predictive value of the DOAC score in a real-life setting in contrast to GDF-15 alone or the HAS-BLED score. Our findings indicate that this new system is worth further validation in large registries and could empower decisions about anticoagulation in AF patients [3]. GDF-15 was also included in biomarker-based bleeding risk scores, such as the ABC-bleeding and ABC-death risk scores [9, 10]. However, the calculation of these scores was beyond the scope of this study. Association between GDF-15 and higher bleeding risk might result from increased GDF--15 expression in cellular stress and vulnerability, potentially heightening the risk of bleeding across tissue injury and its inhibitory effects on platelet activation [4]. In our study,

we demonstrated that patients with very low bleeding risk exhibited lower GDF-15 concentrations compared to others, and further studies to confirm this observation are needed.

This study shows that 3-nitrotyrosine, reflecting oxidative stress and myocardial injury [11], is higher in patients with low-to-very high bleeding risk, which is a novel finding. It might be speculated that the association of 3-nitrotyrosine with bleeding risk in AF shares a similar mechanism to that of GDF-15 since both markers are related to cellular stress and vulnerability.

We failed to show associations between the DOAC score and fibrin clot properties. Drabik et al. [12] showed that low K_s in AF patients predicted major bleeds but was not associated with the HAS-BLED score. Similar findings were reported by Janion-Sadowska et al. [13] in AF patients on rivaroxaban. It seems that fibrin-related mechanisms of bleeding reach beyond clinical scoring systems available now, which still cannot predict a large proportion of bleeding events in AF patients.

Our study has several limitations. First, the group size was limited but represented typical real-life AF patients [14, 15]. Second, long-term follow-up was only 12 months long, and the bleeding rates were low. Third, we excluded patients with advanced renal failure, cancer, or acute thromboembolism; therefore, our findings could not be extrapolated to these patient subsets. In addition, all parameters were assessed only once, and changes over time cannot be excluded. We did not assess other biomarkers listed in the European Society of Cardiology guidelines in relation to bleeding risk [5].

Our hypothesis-generating study showed that GDF-15 and possibly 3-nitrotyrosine combined with the DOAC score may improve bleeding risk stratification in AF patients. However, larger long-term studies are needed to validate this observation.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/kardiologia_polska.

Article information

Conflict of interest: None declared.

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