Supplementary material

Mołek-Dziadosz P, Natorska J, Malinowski KP, et al. The DOAC score is associated with elevated growth differentiation factor 15 and 3-nitrotyrosine in atrial fibrillation: Prediction of bleeding at one-year follow-up. Pol Heart J. 2024.

MATERIAL AND METHODS

All AF patients were treated with DOACs at recommended doses. For hemostatic parameters, plasma samples from patients with plasma DOAC levels exceeding 30 ng/ml, measured using the Biophen DiXaI (for apixaban and rivaroxaban) or the Hemoclot thrombin inhibitor assay (for dabigatran; both Hyphen BioMed, Neuville-sur-Oise, France), were subjected to *in vitro* treatment with the DOAC-Stop (Haematex Research, Sydney, Australia) before coagulation assessment to eliminate potential residual anticoagulant effects [6].

Serum GDF-15 levels (R&D Systems, Minneapolis, MN, US), serum matrix metalloproteinase 9 (MMP-9; R&D), and plasma 3-nitrotyrosine (OxiSelect, Cell Biolabs Inc., San Diego, CA, US) were assayed using ELISAs.

Endogenous thrombin potential (ETP) was evaluated using calibrated automated thrombography (Thrombinoscope BV, Maastricht, the Netherlands) [7]. Fibrin clot properties were assessed in citrated plasma as described [7]. Fibrin clot permeability (K_s), indicative of fibrin network density, was determined using the pressure-driven system and calculated based on the equation: $K_s=Q\times L\times \eta/t\times A\times \Delta p$. Q is the flow rate in percolating time (t), L is the length of the fibrin gel, h is the viscosity of liquid, A is the cross-sectional area (cm^2), and Δp is the differential pressure (in $dyne/cm^2$). Clot lysis time (CLT), reflecting clot susceptibility to recombinant tissue plasminogen activator (rtPA)-driven lysis, was assessed turbidimetrically in citrated plasma mixed with 20 mM $CaCl_2$, 0.5 U/ml thrombin (Merck), 15 μM phospholipid vesicles (Rossix, Mölndal, Sweden), and 18 ng/ml rtPA (Boehringer Ingelheim, Ingelheim, Germany). Intra-assay and inter-assay coefficients of variation for fibrin variables ranged from 5% to 7%.

Statistical analysis

The study was designed to have an 80% chance to detect a 350 pg/ml difference in GDF-15 levels between the groups at the 0.05 significance level, and a minimum of 83 subjects were required in each group.

Nonlinear relationship between DOAC score and GDF-15 level was examined using locally estimated scatterplot smoothing (LOESS) with two-sided 95% confidence interval (CI). Simple logistic regression model was used to examine the relationship between independent variables of DOAC score and GDF-15 and occurrence of bleeding. Results were presented as odds ratio (OR) per unit increase in dependent variable with two-sided 95% CI. Multiple linear regression model was used to examine the age- and sex-adjusted differences in GDF-15 levels between bleeding risk groups.

RESULTS

Patients with low-to-very-high bleeding risk were older and more frequently female (*Table S1*). They also had higher CHA_2DS_2-VASc and HAS-BLED scores (*Table S1*). Moreover, this subgroup more frequently had hypertension, diabetes mellitus, hyperlipidemia, and prior myocardial infarction, and were more frequently treated with statin, aspirin, and clopidogrel in comparison to the remaining individuals (*Table S1*). Analysis of laboratory parameters showed that patients at low-to-very high bleeding risk had 9% higher creatinine than the remainder and 7% lower hemoglobin (*Table S1*), without any other differences.

Table S1. Baseline characteristics of atrial fibrillation patients on direct oral anticoagulants

Variable	**All (n = 245)**	**Very low bleeding risk (score 0–3) (n = 103)**	**Low-to-very high bleeding risk (score ≥4) (n = 142)**	***P*-value**
Age, years	69.5 (62–75.25)	60 (54–65)	75 (70–79)	<0.001
Female, n (%)	98 (40)	32 (31.1)	66 (46.5)	0.01
Obesity, n (%)	83 (33.9)	44 (42.7)	39 (27.5)	0.2
CHA_2DS_2-VASc score	3 (2–4)	2 (1–3)	4 (3–5)	<0.001
HAS-BLED score	2 (1–2)	1 (0–1)	2 (1–3)	<0.001
Paroxysmal AF, n (%)	112 (45.7)	55 (53.4)	57 (40.1)	0.38
Persistent AF, n (%)	48 (19.6)	26 (25.2)	21 (14.8)	0.36
Permanent AF, n (%)	85 (34.7)	22 (21.3)	63 (44.4)	0.20
Comorbidities and CVD risk factors				

Hypertension, n (%)	195 (79.6)	66 (64.1)	129 (90.8)	<0.001
Diabetes mellitus, n (%)	59 (24.1)	12 (11.6)	47 (33.1)	0.001
Prior myocardial infarction, n (%)	28 (11.4)	5 (4.8)	23 (16.2)	0.006
Prior stroke, n (%)	25 (10.2)	10 (9.7)	15 (10.6)	0.83
Prior major bleeding, n (%)	8 (3.3)	3 (2.9)	5 (3.5)	0.79
Medications, n (%)				
Rivaroxaban	105 (42.6)	42 (40.8)	64 (45.1)	0.52
Dabigatran	94 (38.4)	39 (37.8)	55 (38.7)	0.91
Apixaban	46 (18.7)	22 (21.3)	24 (16.9)	0.37
Statin	172 (70.2)	60 (58.2)	112 (78.9)	0.001
Aspirin	27 (11.0)	1 (1.0)	26 (18.3)	<0.001
Clopidogrel	18 (7.3)	3 (2.9)	15 (10.6)	0.02
Laboratory investigations				
Platelet count, 10^3/µl	204 (174.5–251.7)	204 (175.7–256.7)	205 (173–250)	0.86
Hemoglobin, g/dl	14.2 (13.3–15.1)	14.7 (13.6–15.4)	13.8 (13.1–14.8)	<0.001
Creatinine, µM	89.0 (75.8–103.0)	83.8 (73.3–97.2)	91 (78–106)	0.005
C-reactive protein, mg/l	1.7 (1–4.4)	1.4 (1–4.6)	1.9 (1.1–4.1)	0.86
Fibrinogen, g/l	3.2 (2.5–3.8)	3.1 (2.5–3.4)	3.3 (2.7–3.8)	0.32
GDF-15, pg/ml	1455 (1010.1–1891.9)	1085.2 (758.9–1550.9)	1578.8 (1254.6–2020.9)	<0.001
MMP-9, ng/ml	410.5 (303.2–605.4)	414.8 (325.5–620.7)	397.1 (295.8–588.5)	0.27
3-nitrotyrosine, nM	86.9 (42.1–128.2)	61.6 (38.6–120.8)	96.9 (41.3–131.8)	0.03
ETP, nM × min	2027 (1757–2209)	1988.7 (1762.1–2202.1)	2120.8 (1778.1–2268.4)	0.67
K_s, $\times 10^{-9}$ cm^2	4.6 (3.7–5.5)	4.7 (4.0–5.7)	4.5 (3.6–5.3)	0.09
CLT, min	108 (98–129)	107 (99.2–126)	108 (98–126)	0.94
Follow-up				
All bleeding, n (%)	19 (7.8)	4 (3.9)	15 (10.6)	0.057
Minor or non-major bleeding, n (%)	17 (6.9)	4 (38.8)	13 (9.1)	0.13
Major bleeding, n (%)	2 (0.8)	0 (0)	2 (1.4)	0.51

Abbreviations: AF, atrial fibrillation; CLT, clot lysis time; GDF-15, growth differentiation factor 15; K_s, clot permeability; MMP-9, matrix metalloproteinase-9, ETP, endogenous thrombin potential

Table S2. DOAC scoring in AF patients

Clinical risk prediction tool	**Points**	**All (n = 245)**
Age, years		
65–69, n (%)	2	45 (18.4)
70–74, n (%)	3	46 (18.8)
75–79, n (%)	4	36 (14.7)
≥80, n (%)	5	35 (14.3)
Creatinine clearance/estimated glomerular filtration rate (ml/min)		
30–60, n (%)	1	73 (29.8)
<30, n (%)	2	3 (1.2)
Underweight (body mass index <18.5 kg/m^2) , n (%)	1	1 (0.4)
Stroke/transient ischemic attack/embolism history, n (%)	1	25 (10.2)
Diabetes, n (%)	1	59 (24.1)
Hypertension, n (%)	1	195 (79.6)
Antiplatelet use		
Aspirin, n (%)	2	27 (11)
Dual-antiplatelet, n (%)	3	12 (4.9)
Nonsteroidal anti-inflammatory (NSAID) use, n (%)	1	4 (1.6)
Bleeding history, n (%)	3	8 (3.3)
Liver disease, n (%)	2	7 (2.8)
Total score range: 0–10 (Maximum 10 points — individuals with scores ≥10 are assigned a score of 10)		