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The relationship between ABO blood group and the risk of bleeding during vitamin K antagonist treatment

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Major hemorrhage is the most important complication of vitamin K antagonists (VKAs) such as warfarin, acenocoumarol and phenprocoumon. This risk is substantial (1–3% per year), even when the International Normalized Ratio (INR) is in the therapeutic range [1]. Several genetic and acquired patient characteristics such as advanced age, previous gastrointestinal bleeding, arterial hypertension, malignancy, and cytochrome P450 CYP2C9 or VKORC1 DNA polymorphisms are considered risk factors for bleeding during VKA treatment [1–3].

One obvious risk factor candidate for bleeding is ABO blood group. ABO blood group is associated with the plasma levels of von Willebrand factor (VWF), which in turn is the major determinant of factor (F) VIII levels [4–6]; two crucial procoagulant proteins that are not taken into account by the INR. Therefore, we set out to establish the relationship between ABO blood group genotypes and bleeding risk during VKA treatment.

We used data from a case–control study (FACTORS: FACTors in ORal anticoagulation Safety) that was designed to search for risk factors for bleeding during anticoagulant treatment with acenocoumarol or phenprocoumon [2,3]. Patients from two Dutch anticoagulation clinics, who had experienced a non-traumatic non-fatal major bleeding complication during the period 1999–2001, were asked to participate in this study. Major bleeding was defined as bleeding leading to death or hospitalization, hemoglobin decrease ≥ 1.25 mmol L^{-1}, intracranial, intraocular, muscle and joint bleeding. The controls were selected from the same database, considering age, indication of anticoagulation (e.g. atrial fibrillation, venous thromboembolism, mechanical heart valve), sex, anticoagulation clinic, type of VKA (acenocoumarol or phenprocoumon) and whether the drug had been discontinued prior to time of blood collection. We enrolled 110 cases and 220

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controls. International Review Boards of Academic Medical Center in Amsterdam and Leiden University Medical Center approved the study and all participating subjects signed an informed consent form before inclusion in the project.

Blood was collected in 3.2% sodium citrate solution and kept at 4 °C until centrifugation for 20 min at 2250 × ***g***, 4 °C. Plasma was stored at −80 °C and used to measure FVIII activity (FVIII:C) and VWF antigen (VWF:Ag). FVIII:C was measured with a one-stage clotting assay on a Behring Coagulation System (Dade Behring, Marburg, Germany) with protocols and reagents from the manufacturer. VWF:Ag levels were determined with an in-house ELISA, using antibodies from Dako (Glostrup, Denmark). FVIII activity and VWF:Ag levels were determined relative to a plasma pool of > 170 healthy individuals that was calibrated against the 21st (NIBSC 00/586) and 9th British standard (NIBSC 01/168), respectively.

DNA, which was extracted from the remnant white blood cells and stored at −20 °C, was used for polymerase chain reactions (PCR) in order to establish blood group. The PCR protocols were designed to amplify exons 6 and 7 of the *ABO* gene in two separated reactions. The sequence of the primers was described previously by Olsson and Chester [7]. The PCR products that correspond to exons 6 and 7 were digested with the restriction enzymes *Kpn*I and *Msp*, respectively (New England BioLabs, Ipswich, MA, USA). The digestion products were analyzed by electrophoresis using a 4% agarose gel. It was possible to identify A^1, A^2, B, O^1 and O^2 alleles. The genetic analysis of blood groups was confirmed in 320 individuals by serological test using a commercial kit (DiaCellA1,B®; Dia-Med, Cressier s/Morat, Switzerland).

The statistical analysis was performed in SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA). Odds ratios (OR) were calculated by unconditional logistic regression, and 95% confidence intervals (95% CI) were based on the model.

The distribution of ABO blood groups in cases and controls is listed in Table 1. In total, fourteen different ABO genotypes were observed. For simplicity, the ABO genotypes were also categorized in an OO blood group (O^1O^1/O^1O^2) and a non-OO blood group (the other genotypes).

Odds ratio and 95% CI are shown in Table 2. The bleeding risk in non-OO blood group carriers was 30% lower than in carriers of OO blood group (OR 0.7; 95% CI: 0.4–1.1). In the extended analyses for each blood group, using the OO blood group as the reference category, the groups that remained protective against bleeding were A^1A^1, A^1A^2, BB, BO and A^1O combinations. The AB genotypes did not present a lower bleeding risk, although the number of patients in this group was very small. The A^2A^2 and A^2O combinations did not differ from OO genotype, which is in keeping with similar low efficacy of the enzymes that result from A^2 and O alleles [8–10]. This finding is also in agreement with the fact that A^2A^2 and A^2O combinations also did not modify thrombosis risk [4].

It is probable that ABO blood group acts as a risk factor for bleeding by modifying the levels of FVIII:C and VWF:Ag [5,6],

Table 1 The distribution of ABO blood group genotypes with the respective factor VIII activity (FVIII:C) and von willebrand factor antigen (VWF:Ag) means ± SEM in cases and controls

			Cases			Controls	
Genotypes	Total (%) $n = 330$	Cases (%) $n = 110$	FVIII mean ± SEM %	VWF mean ± SEM %	Controls (%) $n = 220$	FVIII mean ± SEM %	VWF mean ± SEM %
O^1O^1/O^1O^2	128 (39)	50 (46)	114 ± 4	150 ± 8	78 (35)	107 ± 2	131 ± 5
Non-OO	192 (58)	59 (53)	126 ± 4*	191 ± 9*	133 (61)	122 ± 2†	168 ± 4†
A^1A^1/A^1A^2	19 (6)	4 (4)	136 ± 7*	237 ± 14*	15 (7)	118 ± 3†	163 ± 10†
A^1O^1/A^1O^2	92 (28)	28 (25)	126 ± 5	190 ± 12*	64 (29)	120 ± 3†	166 ± 5†
$A^2O^1/A^2O^2/A^2A^2$	38 (11)	15 (14)	118 ± 7	171 ± 21	23 (11)	116 ± 5	164 ± 18
$BB/BO^1/BO^2$	33 (10)	7 (6)	130 ± 19	189 ± 31	26 (12)	134 ± 6†	175 ± 8†
A^1B/A^2B	10 (3)	5 (4)	132 ± 11	219 ± 28*	5 (2)	127 ± 8†	177 ± 22†
Missing samples	10 (3)	1 (1)			9 (4)		

*Statistically significant difference comparing with the OO blood group in cases.
†Statistically significant difference comparing with the OO blood group in controls.

Table 2 The bleeding risk for ABO blood group genotypes

Analysed groups	OR (95% CI)	Adjusted OR for FVIII (95% CI)	Adjusted OR for VWF (95% CI)	Adjusted OR for FVIII/VWF (95% CI)
OO	1	1	1	1
Non-OO	0.7 (0.4–1.1)	0.6 (0.4–1.0)	0.5 (0.3–0.9)	0.5 (0.3–0.8)
A^1A^1/A^1A^2	0.4 (0.1–1.3)	0.3 (0.1–1.1)	0.3 (0.1–0.9)	0.3 (0.1–0.9)
A^1O^1/A^1O^2	0.7 (0.4–1.2)	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.7 (0.5–0.9)
$A^2O^1/A^2O^2/A^2A^2$	1.0 (0.5–2.1)	1.0 (0.7–1.2)	0.9 (0.7–1.2)	0.9 (0.7–1.2)
$BB/BO^1/BO^2$	0.4 (0.2–1.0)	0.8 (0.6–1.0)	0.7 (0.6–0.9)	0.7 (0.6–1.0)
A^1B/A^2B	1.6 (0.4–5.7)	1.0 (0.8–1.3)	1.0 (0.7–1.3)	1.0 (0.7–1.3)

FVIII, factor VIII; OR, odds ratio; VWF, von Willebrand factor.

and therefore these levels were measured. As expected, the means of FVIII:C in the controls were lower (107%) for OO blood group carriers than for non-OO blood group carriers (122%, $P < 0.0001$). Similarly, VWF:Ag levels were 131% in OO individuals and 168% in non-OO individuals ($P < 0.0001$). When categorized according to blood group, levels of FVIII:C were 114% for OO blood group and 126% for non-OO blood group ($P = 0.035$), while levels of VWF:Ag were 150% and 190% ($P = 0.002$), respectively (Table 1). We interpret these data as indicating that blood group-mediated increases in the levels of FVIII:C and VWF:Ag, as observed in non-OO carriers, protect against bleeding. Such a conclusion is indirectly supported by the opposite findings in venous thrombosis, where non-OO blood group seems to increase the risk of a thrombotic event [4].

We then evaluated whether the bleeding risks were affected by adjustment for FVIII:C levels or VWF:Ag levels. The bleeding risk for non-OO carriers compared with OO carriers decreased somewhat further after adjustment for FVIII:C only (OR 0.6; 95% CI: 0.4–1.0), for VWF:Ag only (OR 0.5; 95% CI: 0.3–0.9) and for both FVIII:C and VWF:Ag (OR 0.5; 95% CI: 0.3–0.8).

One possible explanation for this effect of adjustment for FVIII:C levels and VWF:Ag levels is the finding that, paradoxically, FVIII:C and VWF:Ag levels (irrespective of blood group) were higher in cases than in controls. In isolation, this finding would suggest that higher levels of FVIII:C and VWF:Ag increase the risk for bleeding under VKA therapy, which is opposite to the conclusion based on the blood group analysis. This finding suggested that another causal pathway, possibly involving endothelial damage, increased both levels of FVIII:C and VWF:Ag and bleeding risk during VKA treatment. Such a scenario finds support in the notion that both FVIII and VWF are known to respond to endothelial activation and their high levels can be maintained if the stimulation continues, like vascular inflammation [11].

In conclusion, this study suggests a decreased bleeding risk in non-OO genotype carriers during treatment with acenocoumarol and phenprocoumon, and the way that FVIII and VWF levels decrease this risk needs to be elucidated.

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