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ABO blood group genotypes and the risk of venous thrombosis: effect of factor V Leiden

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ABO blood group and more recently high von Willebrand factor (VWF) and factor (F)VIII levels have been associated with thrombotic disease. An excess of non-O blood group has long been recognized in patients with ischemic heart disease [1] and venous thrombosis [2]. In 1995, we demonstrated that non-O blood group, high VWF levels and high FVIII levels all increased the risk of deep vein thrombosis [3]. In multivariate analysis only FVIII remained a risk factor, whereas the thrombosis risk associated with VWF and ABO blood group largely disappeared. Since then, several other studies have identified high FVIII levels as a risk factor for venous thrombosis [4–7].

Usually blood group phenotypes are used to study the association between blood group and venous thrombosis. Blood group genotypes may be more informative since genotypes can distinguish between heterozygous and homozygous carriers of A, B and O alleles and between A^1 and A^2 alleles. Therefore we studied the effect of ABO genotype on thrombosis risk in a large population-based case–control study of venous thrombosis (Leiden Thrombophilia Study, LETS). This study, which included 474 patients and 474 control subjects, has been previously described [3]. For the present study DNA was available for 471 patients and 471 control subjects.

Blood was collected into 0.1 volume 0.106 mol L^{-1} trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 × *g* at room temperature and stored at − 70 °C. FVIII coagulant activity (FVIII:C), FVIII:Ag, VWF:Ag and blood group phenotype were measured as previously reported [3–5].

High-molecular-weight DNA was isolated from leukocytes and stored at 4 °C. Polymerase chain reaction (PCR) was designed to amplify exons 6 and 7 of the ABO blood group gene in two separate reactions. The sequences of the primers have been described previously [8]. The amplified DNA fragments corresponding to exons 6 and 7 were digested with *Acc*65I (MBI Fermentas) or *Msp*I (MBI Fermentas), respectively, in two separate reactions and separated by electrophoresis on 3.5% agarose gels. With this method we discriminated A^1, A^2, B, O^1 and O^2 alleles. There was 99% agreement between ABO blood group phenotype and genotypes in all patients and controls.

Table 1 (upper part) shows the frequency of the ABO blood group genotypes in patients and controls. Odds ratios (OR) were calculated as estimates of the relative risk by an unmatched method. Ninety-five percent confidence intervals were assessed according to Woolf [9]. All non-OO genotypes except A^2 homozygotes or A^2–O combinations, i.e. $A^2O^1/A^2O^2/A^2A^2$, were associated with an increased thrombosis risk when compared with OO genotypes. This reinforces the concept that blood group exerts its thrombotic risk largely via FVIII levels, since A^2O/A^2A^2 genotypes correspond to the lowest FVIII levels among non-OO genotypes (data not shown). Adjustment for age and sex did not alter the risk estimates.

Because blood group is known to affect plasma levels of VWF and FVIII and because VWF and FVIII levels influence thrombosis risk, we adjusted the thrombosis risk associated

Table 1 Thrombosis risk for ABO blood group genotypes

Genotypes	No. of patients (%) $n = 471$	No. of controls (%) $n = 471$	OR	95% CI
O^1O^1/O^1O^2	137 (29.1)	202 (42.9)	1*	
Non-OO	334 (70.9)	269 (57.1)	1.8	(1.4-2.4)
A^1A^1	29 (6.2)	20 (4.2)	2.1	(1.2-3.9)
A^1A^2	19 (4.0)	15 (3.2)	1.9	(0.9-3.8)
A^1O^1/A^1O^2	177 (37.6)	130 (27.6)	2.0	(1.5-2.7)
$A^2O^1/A^2O^2/A^2A^2$	34 (7.2)	41 (8.7)	1.2	(0.7-2.0)
$BB/BO^1/BO^2$	52 (11.0)	47 (10.0)	1.6	(1.0-2.6)
A^1B/A^2B	23 (4.9)	16 (3.4)	2.1	(1.1-4.1)
OO and FVL (–)	113 (24.0)	193 (41.0)	1*	
OO and FVL (+)	24 (5.1)	9 (1.9)	4.6	(2.0-10.1)
Non-OO and FVL (–)	266 (56.5)	264 (56.0)	1.7	(1.3-2.3)
Non-OO and FVL (+)	68 (14.4)	5 (1.1)	23.2	(9.1-59.3)

Number of subjects composing the genotypes with lower frequency: patients, A^1O^2 ($n = 3$), A^2A^2 ($n = 2$), BB ($n = 4$), BO^2 ($n = 3$), A^2B ($n = 4$), O^1O^2 ($n = 3$). A^2O^2 genotype was not observed among patients; controls, A^1O^2 ($n = 4$), A^2O^2 ($n = 1$), A^2A^2 ($n = 1$), BB ($n = 1$), BO^2 ($n = 1$), A^2B ($n = 3$), O^1O^2 ($n = 11$). FVL, Heterozygous (1691AG) and homozygous (1691AA). *Reference category.

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with blood group non-OO genotypes for VWF and FVIII in a logistic regression model (see also Koster *et al*. [3]). The aim of this analysis was to assess whether a blood group effect on risk was present that did not act via levels of VWF and FVIII. VWF:Ag and FVIII:C had been measured in 301 patients and 299 controls in whom ABO genotypes were also determined. The crude thrombosis risk for non-OO carriers compared with OO carriers [OR 2.0; 95% confidence interval (CI) 1.4-2.8] decreased after adjustment for FVIII:C only (OR 1.5; 95% CI 1.0-2.1), for VWF:Ag only (OR 1.6; 95% CI 1.1-2.3), or for both FVIII:C and VWF:Ag (OR 1.4; 95% CI 1.0-2.1). For this analysis we stratified FVIII:C and VWF:Ag levels into approximate quartiles. Similar results were obtained when FVIII:Ag was used instead of FVIII:C and when FVIII:C and VWF:Ag were entered into the model as continuous variables. So, even after extensive adjustment for VWF and FVIII levels, some risk-enhancing effect of blood group remained present. One explanation is that, due to measurement error, we were not able to adjust completely for VWF and FVIII levels. The other explanation is that there is an additional effect of blood group on thrombosis risk. It is unlikely that this effect also acts via VWF (e.g. via its effect on platelet adhesion and aggregation), since adjustment for FVIII only and for FVIII and VWF together reduced the risk associated with non-OO blood group to the same extent.

Among the 471 patients, 92 (19.5%) carried factor (F)V Leiden compared with 14 (3.0%) of the 471 controls, yielding an OR for venous thrombosis of 7.9 (95% CI 4.4, 14.1) [10]. In carriers of FV Leiden, non-OO genotypes were present in 68/92 (74%) patients vs. 5/14 (36%) controls. Table 1 (lower part) shows separate and combined effects of ABO blood group and FV Leiden on thrombosis risk. The risk of the combination of non-OO blood group genotypes and FV Leiden, compared with subjects with OO genotypes and without FV Leiden was 23-fold increased. This is higher than expected on the basis of the effects of non-OO genotype (OR 1.7) and FV Leiden (OR 4.6) separately. Adjustment for age and sex did not influence these risk estimates. Similar results were obtained when we limited the analysis to carriers of the risk-enhancing A^1 and B alleles (because A^2O/A^2A^2 genotypes were not associated with risk). This finding extends previous observations [11,12]. In one study, among 28 subjects with FV Leiden and venous thrombosis, 96% possessed non-O blood group, while in a second study [12] among carriers of FV Leiden the thrombosis risk for subjects with non-O blood group was increased 4-fold compared with those with O blood group. Also, our previous observation that in selected thrombophilic families with FV Leiden elevated FVIII levels ($= 150$ IU dL^{-1}) contribute substantially to the incidence rate of thrombosis in FV Leiden carriers, explains the higher frequency of non-O blood group in these families [13].

The mechanism by which non-O blood group contributes to the thrombosis risk in carriers of the FV Leiden mutation is mainly explained by its effect on FVIII levels. High FVIII levels are associated with a decreased responsiveness to activated protein C (APC) in the absence of FV Leiden [14]. In FV Leiden carriers this small additional effect on the APC sensitivity might result in an exponential increase in thrombosis risk. In a similar way the small additional effect of oral contraceptive use on the APC sensitivity ratio in FV Leiden carriers results in a more than additive effect on thrombosis risk [15]. There is also evidence that FV Leiden is a defective cofactor in the inactivation of FVIIIa by APC [16], in which case the combination of poor inactivation of FVIIIa and high FVIII levels associated with non-O blood group might result in a more pronounced reduction in the sensitivity for APC.

Our data indicate that carriers of blood group alleles A^1 and B have a 2-fold increased risk of a first deep vein thrombosis and that the non-OO genotypes strongly influence the risk of thrombosis in FV Leiden carriers. Therefore information on blood group genotype may play a role in the management of thrombophilia patients, especially when they are carriers of FV Leiden.

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Symptomatic deep vein thrombosis and immobilization after day-care arthroscopy of the knee

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Deep vein thrombosis (DVT) and pulmonary embolism (PE) are common and clinically important complications of major surgery. Without thromboprophylaxis, the risk of venographically detected DVT ranges from 50 to 58% of patients following total hip and 57 to 74% following total knee replacement surgery [1]. At present it is standard practise to use perioperative thromboprophylaxis. In the last decades, there has been a major shift from the full clinical approaches to day-care surgery. In North America two-thirds of all surgery is performed in day-care practice [2] compared with about 30% in the Netherlands [3]. There are few studies describing the incidence of postoperative venous thromboembolism (VTE) after day-care surgery. Arthroscopy of the knee seems to carry a relatively high risk of VTE in this setting [4,5]. In contrast to full clinical surgical approaches, in many institutions patients in day-care surgery do not receive perioperative thromboprophylaxis [6]. Until now it seems there has been no consensus regarding a thromboprophylactic regime. In the Netherlands, about 60% of all patients undergoing knee arthroscopy in a day-care setting receive a single injection of low-molecular-weight heparin (LMWH) postoperatively, while the remainder do not receive any form of thromboprophylaxis [6]. The potential for VTE increases with the duration and extent of the surgery performed [7]. Hence, the main rationale not to use thromboprophylaxis in day-care surgery is the idea that the operations performed in this setting are less invasive, leading to an earlier resumption of normal physical activities. At present, however, surprisingly little is known about postoperative mobilization after day-care surgery. The aim of this study was to assess the incidence of symptomatic VTE and the duration of immobilization after arthroscopy of the knee in a day-care setting.

A questionnaire was developed focusing on postoperative symptomatic VTE and duration of immobilization, within a follow-up of 6 weeks, after arthroscopy of the knee in a day-care setting. All consecutive patients who underwent an arthroscopy of the knee in day-care in the Academic Medical Center (AMC) in Amsterdam during 1 year were sent a questionnaire. Surgeons at the AMC operated a large part of their patients also in the Jan van Gooyen clinic (JvG) in Amsterdam. If no reply was received, a second package was mailed. To the remaining non-responders the general practitioner (GP) was sent a questionnaire. All data following arthroscopy in day-care were analyzed for the AMC and the JvG separately.

Categorical data and dichotomous variables were summarized as percentages when applicable. Continuous variables were compared using Student's *t*-test or in case of an abnormal distribution the Mann–Whitney *U*-test. Categorical data were compared with cross tabulation (χ^2 or Fischer's exact test).

In the year 2000, 270 patients underwent a unilateral arthroscopy of the knee, 88 in the AMC and 182 in the JvG. Replies were received from 173 patients (64%). Of the remaining 97, the GP returned 74 questionnaires (76%). The overall response rate was therefore 91% (247 out of 270), 93% and 91%, respectively, for the AMC and JvG. The patients'

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