REGULAR ARTICLE

blood advances

Association of ABO blood group with bleeding severity in patients with bleeding of unknown cause

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Key points

- Blood group O is more prevalent in patients with BUC than in the normal population and is associated with increased bleeding severity.
- Occurrence of oral mucosal bleeding is increased in patients with BUC with blood group O, independent of VWF.

Blood group O has been associated with an increased bleeding tendency due to lower von Willebrand factor (VWF) and factor VIII (FVIII) levels. We explored whether blood group O is independently associated with bleeding severity in patients with mild-to-moderate bleeding of unknown cause (BUC) in the Vienna Bleeding Biobank cohort. Bleeding severity was recorded with the Vicenza bleeding score (BS). Blood group O was overrepresented in 422 patients with BUC compared with its presence in 23 145 healthy blood donors (47.2% vs 37.6%; odds ratio, 1.48; 95% confidence interval [CI], 1.22-1.79). The BS and the number of bleeding symptoms were significantly higher in patients with blood group O than in patients with non-O after adjustment for VWF and FVIII levels and sex (least-square [LS] means of BSs: 6.2; 95% CI, 5.8-6.6 vs 5.3; 4.9-5.7; and of number of symptoms: LS, 3.5; 95% CI, 3.2-3.7 vs 3.0; 2.8-3.2, respectively). Oral mucosal bleeding was more frequent in those with blood group O than in those with other blood types (group non-O; 26.1% vs 14.3%), independent of sex and VWF and FVIII levels, whereas other bleeding symptoms did not differ. Patients with blood group O had increased clot density in comparison with those with blood group non-O, as determined by rotational thromboelastometry and turbidimetric measurement of plasma clot formation. There were no differences in thrombin generation, clot lysis, or platelet function. Our data indicate that blood group O is a risk factor for increased bleeding and bleeding severity in patients with BUC, independent of VWF and FVIII levels.

Introduction

Antigens of the ABO blood group system have an impact on the hemostatic balance. Blood group O is associated with lower VWF levels[1] and has been found to be overrepresented in patients with von Willebrand disease type I (VWD).[2-4] Still, the well-known association of blood types other than O (blood group non-O) and risk of thrombosis[5] is independent of von Willebrand factor (VWF) and factor VIII (FVIII).[6]

In patients with different bleeding symptoms, an overrepresentation of blood group O has been reported in studies summarized in a meta-analysis.[7] Most of the studies investigated patients with gastrointestinal bleeding (eg, duodenal ulcers), in whom blood group O was overrepresented compared with the controls,[8-12] whereas for other bleeding manifestations (eg, postpartum bleeding), results were conflicting.[13,14] Only 3 of the 22 studies, which investigated bleeding symptoms in females, in patients with skin and mucous membrane bleeding, and in patients with bleeding during anticoagulant treatment

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with vitamin K antagonists, reported VWF levels.[15-17] These studies described a strong dependence of VWF levels on the ABO blood type, but the role of blood group O as an independent risk factor for bleeding remains unclear.[15,16] Thus, the independent effect of the ABO blood group on bleeding tendencies, when considering VWF as a major contributing variable for bleeding, has not yet been thoroughly investigated.

Only in a small subgroup of patients with a mild-to-moderate bleeding tendency can an established bleeding disorder be identified, despite thorough investigation of plasmatic coagulation and platelet function. The remainder, patients without abnormalities in plasmatic coagulation or platelet function, are defined as patients with bleeding of unknown cause (BUC).[18,19] Patients with BUC have a bleeding phenotype similar to that of those with a diagnosis of an established bleeding disorder.[18] Yet, the underlying mechanisms of their bleeding tendency are currently unclear.[20]

To identify the novel mechanisms that influence bleeding tendency in patients with BUC and considering the widely reported impact of blood group on hemostasis, we sought to investigate the role of blood group O as an independent risk factor for increased bleeding severity in a large cohort of thoroughly characterized patients with BUC. We compared the prevalence of blood group O in patients with BUC with that in a large group of healthy blood donors. Furthermore, we analyzed bleeding severity according to the blood groups and its dependence on VWF and FVIII levels. To identify potential mechanisms underlying the effect of blood group O on the bleeding phenotype, we analyzed results from global hemostatic and platelet function tests.

Patients and methods

Study design and patients

Patients aged ≥16 years with a mild-to-moderate bleeding tendency and no previous diagnosis of an established bleeding disorder were included in the Vienna Bleeding Biobank, an observational, single-center cohort study.[18] Recruitment started in October 2009 and is ongoing. The detailed inclusion and exclusion criteria have been reported and are included in the supplemental Table 1.[18] The study was approved by the Ethics Committee of the Medical University of Vienna (EC No 603/2009). All authors had access to primary clinical data.

For this analysis, we selected patients consecutively recruited from October 2009 through April 2019 with normal results in assessments of plasmatic coagulation and platelet function, categorized as patients with BUC (Figure 1).

Laboratory testing and assessment of bleeding severity

After providing informed consent, all patients underwent a structured interview regarding their previous medical and bleeding history and a thorough hemostatic laboratory assessment (supplemental Table 2).[18] Bleeding severity was recorded by a standardized bleeding assessment tool (Vicenza BAT),[21,22] and biomaterial was stored by the biobank facility of the Medical University of Vienna (www.biobank.at).[23] Details on study procedures and the measurements of thrombin generation, plasma clot properties, and rotational thromboelastometry (ROTEM), as well as the platelet function test results are included in the supplemental Data (paragraphs 1-5).

Healthy blood donors

Data on blood group distribution in 23 145 healthy first-time blood donors (mean age ± standard deviation [SD], 25.8 ±9.5 years; 12 334 men and 10 811 women) in Eastern Austria in 2018 were provided by the Austrian Red Cross for comparison. Blood donors came from the same geographic region as the patients.

Statistical analysis

Continuous variables are expressed by mean (±SD) or median values and interquartile range (IQR) in case of nonnormally distributed data. The χ^2 test was used to calculate differences in the frequency of blood group O in patients with BUC compared with healthy blood donors, and the odds ratio (OR; 95% confidence interval [CI]) is given to describe the strength of the association. Continuous data were compared between patients with blood group O and those with non-O by the Student *t* test or the Wilcoxon rank sum test for nonnormal distribution. The χ^2 or Fisher's exact test was used for comparison of categorical variables between groups. To evaluate the adjusted difference between patients with blood group O and those with blood group non-O in the primary outcome variable bleeding score (BS) and in the secondary outcome variable number of bleeding manifestations, analysis of covariance (ANCOVA) models were calculated. Within these ANCOVA models, the continuous confounding factors VWF antigen (VWF:Ag), VWF:ristocetin cofactor (VWF:RCo), and FVIII levels and the categorical factor sex were considered for adjustment. The adjusted group differences were provided by least-square (LS) means with 95% CI. Furthermore, ANCOVA models were used for comparison between the individual blood groups (O, A, B, and AB). The Tukey-Kramer multiplicity correction was used for pairwise comparisons between the 4 blood groups. The same ANCOVA models were used to explore potential differences due to blood group (O vs non-O) in thrombin generation, plasma clot properties, ROTEM, and platelet function data. In case of right-skewed distributions, the data were log transformed, and in case of left-skewed distributions, data were squared before statistical analysis. A multivariable logistic regression model was used to evaluate whether oral mucosal bleeding was independently associated with blood group O (adjusted for sex and VWF:Ag, VWF:RCo, and FVIII levels). To account for the number of multiple comparisons performed within the individual secondary research questions, the Bonferroni-Holm correction (BHC) was applied. All *P*-values are results of 2-sided tests; $P < .05$ indicated statistical significance. SAS (version 9.5, 2012; SAS Institute Inc, Cary, NC) was used for statistical calculations.

Results

Blood group distribution and patients' characteristics

We analyzed 422 patients with BUC (Figure 1), of whom 199 (47.2%) had blood group O, 149 (35.3%) blood group A, 49 (11.6%) blood group B, and 25 (5.9%) blood group AB (Table 1). Blood group O was more prevalent in patients with BUC than in the control group of 23 145 healthy blood donors (37.6%), whereas blood type A was underrepresented, and there were no differences in the occurrence of blood types B and AB. Patients with BUC had a higher probability of having blood group O (OR, 1.48; 95% CI, 1.22-1.79; $P < .001$).

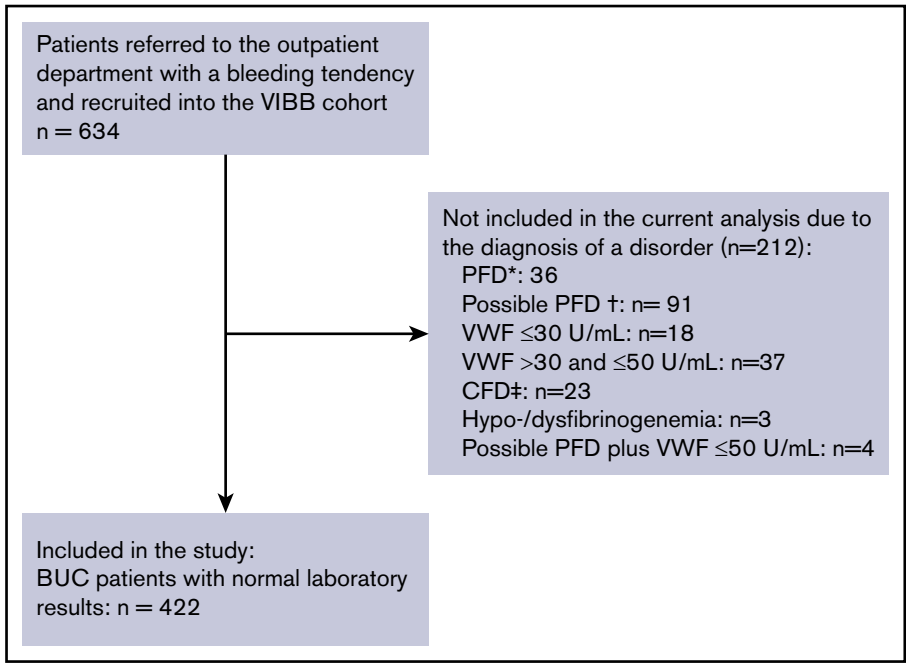

Figure 1. Patients included in the study. PFD, platelet function defect; CFD, coagulation factor deficiency; *Definite PFD in at least 2 different occasions, defined by abnormalities in LTA with 2 or more agonists. †Possible PFD defined by abnormal LTA curves upon stimulation with 1 or more agonists or when platelet function was investigated at only 1 occasion; includes 2 patients with additional mild FXI deficiency (≤60%). ‡CFDs include FVIII activity ≤50% (n = 14; 60.9%); FIX activity ≤50% (n = 5; 21.7%), FXI activity ≤60% (n = 3; 13.0%), and FXIII activity ≤10% (n = 1; 4.3%).

Characteristics for the total patient cohort and according to blood group O and non-O are shown in Table 2. Albeit within the normal range, the levels of VWF:Ag, VWF:RCo, and FVIII were lower, and the activated partial thromboplastin time was longer, in patients with blood group O. Characteristics of patients according to each blood group are given in supplemental Table 3.

Bleeding severity and bleeding symptoms

Among all blood groups, the BS was highest in patients with blood group O (supplemental Table 4). Between all blood groups, there was an overall difference in the BS, with adjustment for VWF:Ag, VWF:RCo, and FVIII levels and sex (adjusted ANCOVA, $P = .042$).

To further analyze blood group O as an independent risk factor for bleeding, we summarized the data for blood groups A, AB, and B in a non-O group (n = 223). The BS was increased in patients with blood group O vs non-O (Table 3). The differences between blood groups O and non-O persisted after adjustment for VWF:Ag, VWF:RCo, and FVIII levels and sex (LS mean, 6.2; 95% CI, 5.8-6.6 vs 5.3; 4.9-5.7; $P = .006$).

There was no overall significant difference in the number of bleeding manifestations between all blood groups after adjustment for VWF and FVIII levels and sex ($P = .078$; supplemental Table 4).

In the comparison of patients with blood group O with those with non-O, those with blood group O had a higher number of bleeding manifestations after adjustment for VWF:Ag, VWF:RCo, and FVIII levels and sex (LS mean, 3.5; 95% CI, 3.2-3.7 vs 3.0; 2.8-3.2; $P = .016$; Table 3).

The detailed analysis of the prevalence of the different bleeding symptoms, shown in Table 4 and supplemental Table 5, revealed that oral mucosal bleeding occurred in 26.1% of patients with blood group O, and thus more often than in those with blood group non-O (14.3%). This difference persisted after correction for multiple testing and after adjustment for VWF and FVIII levels and sex ($P = .013$). There was no difference in the distribution of the other bleeding manifestations between patients in blood group O and those in non-O.

Effect of blood group O on global hemostatic tests

To analyze the effect of blood group O on hemostatic potential, we analyzed differences in thrombin generation, plasma clot formation and lysis, and ROTEM in patients with blood group O and those with non-O.

There was no difference in the thrombin generation parameters when comparing patients with BUC with blood group O with those with non-O (Table 5).

In the analysis of plasma clot properties, time to peak was significantly prolonged in patients with blood group O. Still, the difference did not persist after adjustment for VWF and FVIII levels and sex (Table 5). The maximum clot absorbance was increased in patients with blood group O after adjustment for VWF and FVIII

Table 1. Blood group distribution

	N	Blood group, n (%)				O vs non-O OR (95% CI)
		O	A	B	AB	
Healthy blood donors	23 145	8709 (37.6)	9744 (42.1)	3231 (14.0)	1461 (6.3)	
BUC	422	199 (47.2)	149 (35.3)	49 (11.6)	25 (5.9)	1.48 (1.22-1.79)

Table 2. Characteristics of patients with BUC

	All patients (n = 422)	Blood group O (n = 199)	Blood group non-O (n = 223)	P
Age, mean (SD), y	42.5 (15.7)	43.5 (15.8)	41.6 (15.5)	.223
Female, n (%)	363 (86.0)	171 (85.9)	192 (86.1)	.960
Positive family history, n (%)	153 (36.3)	75 (37.7)	78 (35.0)	.417
White, n (%)	417 (98.8)	196 (98.5)	221 (99.1)	.563
Diabetes, n (%)	5 (1.2)	4 (2.0)	1 (0.4)	.193
Arterial hypertension, n (%)	41 (9.7)	20 (10.1)	21 (9.4)	.870
Hypothyroidism, n (%)	10 (2.4)	6 (3.0)	4 (1.8)	.410
Hemoglobin, mean (SD), g/dL	13.6 (1.3)	13.6 (1.2)	13.5 (1.3)	.494
Platelet count, mean (SD), $\times 10^9$/L,	252.9 (57.8)	252.1 (55.0)	253.5 (60.3)	.804
APTT-STA, median (IQR), s	35.5 (33.4-37.7)	36.0 (34.0-38.6)	34.8 (32.7-37.3)	<.001
FVIII activity, median (IQR), %	126 (103-162)	110 (95-135)	146 (119-181)	<.001
VWF:Ag, median (IQR), U/mL	99 (80-124.3)	86 (73-103)	110 (94-143)	<.001
VWF:RCo, median (IQR), U/mL	86 (69-126)	72 (63-90)	110 (78.25-140)	<.001

APTT-STA, activated partial thromboplastin time, according to the STA coagulation analyzer.

levels and sex (LS mean OD, 0.77; 95% CI, 0.74-0.79 vs 0.71; 0.69-0.74; $P < .05$).

ROTEM showed that patients with blood group O had a longer clotting time and lower maximum lysis (Table 6). After adjustment for VWF and FVIII levels and sex and correction for multiple testing, the maximum clot firmness was higher (LS mean millimeters, 57.4; 95% CI, 56.5-58.3 vs 55.8; 55.0-56.6; $P < .05$) and the maximum lysis remained lower (LS mean percentage, 13.3; 95% CI, 12.5-14.1 vs 15.1; 14.4-15.9; $P < .05$) in patients with blood group O, whereas the difference in clotting time did not persist.

Effect of blood group O on platelet function

For patients with blood group O, prolonged closure time, when tested by the Platelet Function Analyzer-100 (PFA-100) with both epinephrine and adenosine diphosphate, did not persist after adjustment for of VWF and FVIII levels and sex (Table 7). In platelet function analysis with light transmission aggregometry (LTA) upon stimulation with different agonists, no difference was found between patients with blood group O and those with non-O (Table 7).

Discussion

In this study, we observed an overrepresentation of blood group O in patients with BUC compared with a large cohort of healthy blood donors. Blood group O was independently associated with increased bleeding severity. We found that the prevalence of oral mucosal bleeding was significantly higher in blood group O than in those with a non-O blood group. The association was also independent of VWF:Ag, VWF:RCo, and FVIII levels. When the influence of blood group O on tests of global hemostatic capacity was analyzed, the results indicated increased clot firmness and reduced lysis. We could not identify significant differences in platelet function between patients with blood group O and those with non-O.

A higher occurrence of blood group O in patients with increased bleeding has been reported previously, and data have recently been summarized in a systematic meta-analysis by Dentali et al.[7] This meta-analysis included various patient groups, with most of the patients having upper gastrointestinal bleeding caused by ulcers or bleeding related to anticoagulant treatment.[7,8-12] In that meta-analysis, 52.0% of patients with hemorrhage and only 44.2% of controls had blood group O.[7] The resulting odds for blood group O were slightly lower than in our study (OR 1.33; 95% CI, 1.25-1.42; $P < .001$ vs 1.48; 1.22-1.79; $P < .001$).

The association between blood group O and bleeding has frequently been attributed to VWF levels, which are known to be lower in individuals with blood group O.[1] Contrary to our study, the contribution of VWF levels to bleeding severity was not analyzed in most of the studies reviewed.[7] Also a recent publication, which reported an association of blood group O with bleeding severity in patients with rare bleeding disorders, did not account for VWF levels.[24]

We investigated only patients with BUC who had normal results in the assessment of plasmatic coagulation and platelet function. Along with that, we took into account that, in patients with a definite

Table 3. Bleeding outcomes in patients with BUCs

	All patients	Blood group O	Blood group non-O	P	P*
Vicenza bleeding score, mean (SD)	5.73 (2.98)	6.10 (3.00)	5.40 (2.93)	.016	.006
Bleeding manifestations, mean (SD)	3.22 (1.69)	3.39 (1.70)	3.07 (1.67)	.048	.016

*ANCOVA, adjusted for sex and VWF:Ag, VWF:RCo, and FVIII levels.

Table 4. Bleeding symptoms in patients with BUCs

	N*	All patients	Blood group O	Blood group non-O	P†	BHC
Epistaxis	422	133 (31.5)	60 (30.2)	73 (37.2)	.568	NS
Hematoma/easy bruising	422	279 (66.1)	134 (67.3)	145 (65.0)	.616	NS
Small-wound bleeding	422	145 (34.4)	67 (33.7)	78 (35.0)	.777	NS
Oral mucosal bleeding	422	84 (20.0)	52 (26.1)	32 (14.3)	.003	<.05
Gastrointestinal bleeding	422	56 (13.3)	30 (15.1)	26 (11.7)	.302	NS
Bleeding after tooth extractions‡	370	140 (37.8)	75 (43.9)	65 (32.6)	.051	NS
Postsurgical bleeding§	383	232 (60.6)	115 (61.8)	117 (59.3)	.171	NS
Menorrhagia‖	363	215 (59.2)	104 (60.8)	111 (57.8)	.843	NS
Postpartum bleeding¶	229	67 (29.3)	31 (31.3)	36 (27.7)	.224	NS
Muscle bleeding	422	5 (1.4)	4 (2)	2 (0.9)	.427	NS
Joint bleeding	422	7 (1.6)	3 (1.5)	4 (1.8)	.999	NS

Data are number of patients (percentage of total group analyzed).
*N, the number of patients with the respective characteristic.
†Comparison of categorical variables between groups (blood group O vs non-O) was performed by χ^2 test or Fisher's exact test.
‡Includes all patients who had tooth extractions.
§Includes all patients who underwent surgery.
‖Includes all women.
¶Includes all women who gave birth.

diagnosis of a bleeding diathesis, the underlying defect is the main prohemorrhagic driver, which may mask an effect of blood group O on bleeding severity. Only 1 other study separately analyzed patients with BUC: Antón et al, who investigated functional hemostatic polymorphisms in 269 patients with mucocutaneous bleeding and reported a higher occurrence of blood group O. Also in their subgroup of 160 patients with BUC, 66.2% had blood group O, which was more frequent than in the control group, but lacking in statistical significance.[25] In our study, we found normal, but lower levels of VWF in patients with BUC with blood group O than in those with blood group non-O. This result indicates that, in BUC cohorts that do not include patients with VWD and/or other coagulation defects, adjustment for VWF level also has to be measured. Our data revealed an effect of ABO blood group on bleeding severity that was independent of VWF or FVIII levels.

According to our data, oral mucosal bleeding was more frequent in patients with blood group O than in those with non-O, whereas no significant difference in other bleeding manifestations was found. This result is in line with those in previous studies that reported an association between mucocutaneous bleeding and blood group O.[25] Nevertheless, we are the first to show that this effect is also independent of VWF and FVIII levels.

The influence of blood group on VWF and, consequently, on FVIII levels, which is most probably induced by posttranslational glycosylation stimulated by A and B antigens, is widely known.[26] However, it remains unclear how ABO antigens could have a VWF-independent impact on the hemostatic balance. ABO antigens are found on both red blood cells and platelets,[27,28] and both cell types could be involved in blood group–related impairment of hemostasis.

Table 5. Thrombin generation and plasma clot properties in patients with blood group O vs non-O

	Blood group O (n = 169)	Blood group non-O (n = 195)	P	BHC	P*	BHC*
Thrombin generation						
Lag time, mean (SD), min	11.2 (3.0)	11.0 (2.8)	.606	NS	.870	NS
Velocity index, median (IQR), nmol/L per min	30.6 (18.3-51.8)	34.8 (18.0-59.8)	.272	NS	.102	NS
Peak thrombin, mean (SD), nmol/L	244.4 (113.4)	258.8 (125.2)	.254	NS	.086	NS
TTP, median (IQR), min	18.1 (15.6-21.6)	17.6 (14.6-21.6)	.174	NS	.347	NS
AUC, mean (SD), nmol/L × min	3234.2 (737.5)	3287.0 (702.7)	.485	NS	.320	NS
Plasma clot properties						
Lag time, median (IQR), min	11.1 (8.2-14.4)	9.5 (6.9-13.6)	.028	NS	.145	NS
Vmax, mean (SD), OD/min	0.13 (0.05)	0.14 (0.05)	.078	NS	.575	NS
ΔAbs, OD 405 nm, mean (SD)	0.74 (0.17)	0.74 (0.17)	.792	NS	.004	<.05
TTP, mean (SD), min	20.5 (7.1)	18.7 (6.4)	.009	<.05	.069	NS
CLT, median (IQR), min	16.0 (13.5-19.7)	16.3 (13.5-19.7)	.989	NS	.145	NS

AUC, area under the curve; CLT, clot lysis time; OD, optical density; TTP, time to peak.
*ANCOVA, adjusted for sex and VWF:Ag, VWF:RCo, and FVIII levels.

Table 6. ROTEM in patients with blood group O vs non-O

	Blood group O (n = 136)	Blood group non-O (n = 150)	*P*	BHC	*P**	BHC*
Clotting time, mean (SD), s	671.5 (126.1)	637.4 (111.8)	.016	<.05	.492	NS
Clot formation time, median (IQR), s	177.0 (149.5-216.5)	162.0(137.0-206.0)	.043	NS	.777	NS
Maximum clot firmness, mean (SD), mm	56.7 (5.0)	56.5 (5.6)	.799	NS	.014	<.05
Maximal lysis, mean (SD), %	13.3 (4.0)	15.0 (4.5)	.001	<.05	.002	<.05

*ANCOVA, adjusted for sex and VWF:Ag, VWF:RCo, and FVIII levels.

To address this question, we analyzed data from global hemostatic tests and platelet function in patients with blood group O and those with non-O.

Thrombin generation is known to be impaired in plasma with low VWF and FVIII levels.[29] In accordance with those data, thrombin generation has been shown to be decreased in individuals with blood group O, although this seems to be more strongly determined by FVIII than VWF levels.[29,30] Our results did not show a difference in thrombin generation parameters between patients with blood group O vs non-O.

There is evidence that red blood cells are essential players in hemostasis, because many mechanisms such as deformability, aggregation, expression of adhesive proteins, and release of extracellular microvesicles by red blood cells may influence clot stability.[26,31] In our ROTEM of whole-blood samples, results indicated a denser, firmer clot with reduced lysis in patients with BUC with blood group O. Interestingly, similar results were found in the turbidimetric assessment of plasma clot characteristics from citrated plasma, which showed increased plasma clot density in patients with blood group O. These results do not directly suggest a role of red blood cells, but rather an effect of plasma components. We can only speculate on the underlying mechanisms of increased clot density in patients with BUC with blood group O. A higher density of clots could, among other possibilities, result from altered dynamics of clot formation, given that slower fibrin assembly results in denser, tighter clots, or altered plasma protein components.[32,33] On the other hand, these alterations in clot structure may represent counter-regulatory mechanisms, activated to balance low VWF and FVIII levels in individuals with blood group O.

We also analyzed data generated by the PFA-100. Because of the known VWF dependency of this method[34] and lower VWF levels in our group of patients with blood group O, the significant differences in closure times were not unexpected. After adjustment for VWF and FVIII levels and sex, this significance did not persist. Interestingly, in a genome-wide association study, it was reported that genetic determinants in the ABO locus, which influenced the closure time of collagen-adenosine diphosphate in PFA-100 tests, were only partly mediated by VWF and FVIII.[35] This finding was not confirmed by our data, which did not show a VWF-independent effect of blood group O on PFA closure times in patients with BUC.

In a recent study, Dunne et al revealed an ABO-dependent effect on platelet interaction, with VWF showing a lower binding rate for GPIb/VWF because of glycosylation patterns on GPIb in blood donors with blood group O.[36] However, in our patient cohort, there was no difference in LTA, especially when using ristocetin, between patients with blood group O vs non-O, also with adjustment for sex and levels of VWF and FVIII.

Our reference population represented 23 145 healthy, first-time blood donors, including 20 317 donors from the general population and 2 828 donors in military service who did not differ in their blood group distributions. General blood donor communications and campaigning for first-time blood donors by the Austrian Red Cross do not focus on blood group O donors or contain any blood group–specific content. To exclude a bias in blood group distribution of healthy blood donors, which could arise from an invited group O donor recruiting a group O first-time donor, additional cohorts of 18 334 transplant recipients and 147 healthy controls were analyzed and revealed similar results (supplemental Table 6).

Table 7. PFA and LTA measurements in patients with blood group O vs non-O

	Blood group O (n = 188)	Blood group non-O (n = 211)	*P*	BHC	*P**	BHC*
PFA-100						
PFA-100 epinephrine, median (IQR), s	135.5 (122.0-164.5)	131 (110.0-147.0)	0.003	<.05	.164	NS
PFA-100 adenosine diphosphate, mean (SD), s	107.2 (34.5)	88.1 (89.7)	0.018	<.05	.891	NS
LTA						
ADP, median (IQR), %	94.0 (82.0-104.0)	93.0 (80.0-104.0)	0.425	NS	.296	NS
Arachidonic acid, median (IQR), %	89.0 (83.0-96.0)	89.0 (82.0-97.0)	0.831	NS	.788	NS
Collagen, mean (SD), %	91.5 (12.7)	93.2 (12.9)	0.184	NS	.310	NS
Epinephrine, median (IQR), %	95.0 (85.0-103.0)	95.0 (86.5-103.0)	0.783	NS	.795	NS
Ristocetin (1.2 mg/mL), median (IQR), %	94.0 (88.0-99.0)	94.0 (87.0-101.0)	0.786	NS	.644	NS

ADP, adenosine diphosphate.
*ANCOVA, adjusted for sex and VWF:Ag, VWF:RCo, and FVIII levels.

The strength of our study lies in the well-characterized, large cohort of patients with BUC and the available coagulation parameters, global hemostatic tests, and platelet function assessments. Nevertheless, our study also had limitations. One is the lack of an explanation for the increased bleeding severity in patients with BUC with blood group O in comparison with those with non-O. However, we are convinced that our data encourage studies with the purpose of further elucidating the mechanisms behind these observations.

In patients with BUC, the mechanisms underlying the bleeding phenotype are currently unclear and most probably multifactorial. We provide evidence that blood group O is associated with a more severe bleeding phenotype and oral mucosal bleeding in patients with BUC. This association was independent of VWF and FVIII. Our finding is important for a better, more holistic understanding of the underlying mechanisms of bleeding in patients with BUC.

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Authorship

Contribution: J.G., I.P., C.A., and D.M. designed the study; J.G., I.P., C.A., D.M., S.H., and J.R. recruited the patients; H.H. processed and stored the samples; E.E. supported data storage; C.J. provided the data from healthy blood donors; B.J. provided the data from ROTEM analyses; D.S. provided data from platelet function diagnostics; A.K. and D.M. performed statistical analyses; J.G., I.P., and D.M. analyzed the data; I.P., J.G., and D.M. interpreted the data; and D.M. and J.G. wrote the manuscript; and all authors reviewed, edited, and approved the manuscript in final form.

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