The relationship between ABO groups and subgroups, factor VIII and von Willebrand factor

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Funding: Grant sponsor FAPESP, Fundação de Amparo a Pesquisa do Estado de São Paulo. Grant number 03/01904-9.

Manuscript received June 26, 2006. Accepted October 16, 2006.

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ABSTRACT

The aim of this study was to correlate ABO groups with plasma levels of factor VIII (FVIII), von Willebrand factor (VWF:Ag), and ristocetin cofactor (VWF:RCo). Serological and molecular tests defined blood groups from 114 donors (10 AA, 10 BB, 10 AB, 10 AO^1, 10 BO^1,16 O^1O^1, 20 A^2O^1, 20 A^2B, 4 A^3O^1, 3 A^xO^1, and 1 $B^{el}O^1$). The levels of VWF:Ag, FVIII and VWF:RCo observed in rare subgroups (A^3O^1, A^xO^1, $B^{el}O^1$) were similar to the values found in the O^1O^1 group. However, levels of these factors were significantly higher in A^2O^1 donors than in O^1O^1 donors (VWF:Ag p=0.01; FVIII p=0.04; VWF:RCo p<0.001). Strong correlations were demonstrated between plasma levels of VWF:Ag and FVIII (R=0.77; p=0.001) and between VWF:Ag and VWF:RCo (R=0.75; p=0.001).

Key words: ABO, blood groups, factor VIII, von Willbrand factor

Haematologica 2007; 92:236-239

Von Willebrand factor (VWF) is a multimeric glycoprotein whose circulating plasma levels vary significantly within and between individuals. These variations have been associated with ABO blood type, estrogen levels, age and stress.[1-4] In particular, ABO blood type exerts a major effect on plasma VWF levels. Recent studies have demonstrated that individuals carrying one O allele (AO and BO) have significantly lower plasma levels of VWF and FVIII than those carrying no O allele (AA, AB and BB).[5] The antigens of the ABO system consist of complex carbohydrate molecules. The A and B alleles encode A and B glycosyltransferase, which convert H antigen into A or B determinants. Group O individuals lack transferase enzymes and, consequently, continue to express H antigen.[6] In addition to the common phenotypes A^1 and A^2, numerous phenotypes with weak expression of A or B antigens have been found. Most of these phenotypes can be fitted into the following categories: A^3, A^x, A^{el}, B^3 and B^{el}. All have enhanced expression of H antigen.[7] O'Donnel *et al.*,[8] described a direct relationship between ABO genotype, A transferase expression, and the amount of A antigen expressed on circulating VWF. The susceptibility of VWF of blood group O, A, B and AB to proteolysis by the ADAMTS 13 metalloprotease was investigated and multimeric analysis indicated that the rate of VWF proteolysis differed between blood groups and was greater for group O VWF than for non-O VWF.[9] The aim of this study was to correlate ABO groups and rare subgroups with plasma levels of factor VIII (FVIII), von Willebrand factor (VWF:Ag), and ristocetin cofactor (VWF:RCo).

Design and Methods

Samples from 114 blood donors with known blood groups and subgroups were submitted to ABO serology and molecular analysis, VWF:Ag and FVIII dosages and ristocetin cofactor assay. The donors were males with no history of taking drugs. The age of the donors ranged from 18-60 years

old (median age 32). The donors were instructed regarding the nature and non-compulsory character of the research, and signed informed consent was obtained prior to collecting samples. This study was approved by the Research Ethics Committee of the State University of Campinas and Brazilian Medical Research Committee. Blood was drawn by venipuncture into evacuated siliconized glass tubes containing 3.2% sodium citrate, in a ratio of 1:9 with blood, for ristocetin cofactor assay, VWF:Ag and factor VIII dosages. Blood processing was completed within 2 hours. Blood samples for ABO serology and molecular analysis were collected according to standard blood banking practice.[10]

ABO phenotypes were determined by agglutination and adsorption-elution tests using monoclonal and polyclonal anti-A, B and AB antibodies (Asem-NPBI, Itapecirica da Serra, São Paulo Brazil; DiaMed SA, Cressier s/Morat, Suisse; DiaMed Latino América, Lagoa Santa, Brazil). H antigen was determined using anti–H lectin from Ulex europaeus (DiaMed Latino América, Lagoa Santa, Brazil) and the agglutination reaction intensity was evaluated according to Marsh *et al.*[11] Serum screening for isoagglutinins and antibodies was performed by tube agglutination tests at 4°C and 22°C and by standardized serological procedures with a microtyping system (DiaMed SA, Cressier s/Morat, Suisse).[10] Saliva testing was performed using a technique described elsewhere.[10] Subgroup serologic status was defined according to Daniels.[7]

Genomic DNA was extracted from blood conserved with EDTA using the standard phenol-chloroform technique. ABO genotyping was performed by polymerase chain reaction (PCR) amplification of exons 6 and 7 of the *ABO* gene, followed by diagnostic restriction enzyme digestion. Four different primers were used to amplify two fragments, each spanning a different polymorphic site of the *ABO* gene. Primers, exon 6: P1-5´-TGCCAGCTCCATGTGACCGC 3´ (sense), P2-5´TCGCCACTGCCTGGGTCTCTAC 3´ (antisense); exon 7: P3-5´ CCGTCCGCCTGCCTTGCAG 3´ (sense), P4-5´ TGCCGGCAGCCCTCCCAGAG 3' (antisense). Primers P1/P2 in conjunction with the restriction enzyme *KpnI* and *BstE I* were used to differentiate the O^1 allele from the A, B and O^2 alleles.[12] Primers P3/P4 in conjunction with the restriction enzyme *AluI* were used to differentiate the A^2 from the A_1 allele. These same primers with the restriction enzyme *MboI* were used to discriminate the A_X from A^1, A^2 and A^3 alleles.[13] A_3(3) and B_{el}(1) serological and genetic molecular studies were previously described.[14,15] The *ABO* alleles are named according to the nomenclature used in the Blood Group Antigen Gene Mutation Database (*http://www.bioc.aecom.yu.edu/bgmut/abo.htm*).

Factor VIII coagulant was measured by a one stage clothing method using a factor-VIII deficient substrate.[16] The activity of vWF was measured by ristocetin cofactor assay – VWF:RCo – (Helena Laboratories, Beaumont, Texas, USA). VWF:Ag was measured by an enzyme-linked immunosorbent assay (ELISA) using polyclonal antiserum (Dako, Denmark). Lyophilised commercial reference preparations of VWF:Ag, FVIII and VWF:RCo, standardized against the World Health Organization standard, were used as the standards in this study.

Statistical analysis

Statistical analysis was performed using Wilcoxon's rank sum test and Spearman´s rank correlation. p values ≤0.05 were considered statistically significant.

Results and Discussion

The ABO serological and genotype distribution were AA (n=10), BB (n=10), AB (n=10), AO^1 (n=10), BO^1 (n=10), O^1O^1 (n=16), A^2O^1 (n=20), A^2B (n=20), A^3O^1 (n=4), A^xO^1 (n=3), and $B^{el}O^1$ (n=1). The antigen H investigation showed a score of 11 or 12 for O^1O^1, a score of 0 for AA, BB, AB, A^2B, AO^1 and BO^1; a score of 7 or 8 for A^2O^1; and a score of 10 or 11 for A^3O, A^xO and $B^{el}O$.[11] The rare subgroups A^3O, A^xO and $B^{el}O$, were considered as single group for the statistical analysis. Group O individuals and those carrying this allele had significantly lower levels of VWF:Ag, FVIII and VWF:RCo than did individuals of groups AA, AB and BB. The median levels of these factors were lower in subgroup A^2O^1 (VWF:Ag=89%; FVIII=96%; VWF:RCo=99%) than in subgroups AA, AB and BB (median: VWF:Ag=120%, p<0.001; FVIII=117%, p<0.001, VWF:RCo=19%, p<0.001) and A^2B (median: VWF:Ag=169%, p<0.001; FVIII=112%, p<0.001; VWF:RCo=132%, p=0.001) and higher than in subgroup O^1O^1 (median: VWF:Ag=69%, p=0.018; FVIII=75%, p=0.048; VWF:RCo=65%, p<0.001). The levels of the same factors in A^3O^1, A^XO^1 and $B^{el}O^1$ donors (median: VWF:Ag=75%; FVIII:C=88%; VWF:RCo=76%) were statistically significantly lower than those in groups AA, AB and BB (VWF:Ag, p<0.001; FVIII, p=0.004; VWF:RCo, p<0.001) and A2B (VWF:Ag, p<0.001; FVIII, p=0.001; VWF:RCo, p<0.001) (Figure 1). However, no statistically significant differences were observed when the results obtained in rare subgroups were compared with those in O^1O^1 (VWF:Ag, p=0.49; FVIII p=0.57; VWF:RCo, p=0.23), AO^1/BO^1 (median: VWF:Ag=80%, p=0.89; FVIII=89%, p=0.95; VWF:RCo=86%, p=0.95) and A^2O^1 for VWF and FVIII (VWF:Ag, p=0.66; FVIII, p=0.57). VWF:RCo in A^3O^1, A^XO^1 and $B^{el}O^1$ subgroups was statistically different from that in subgroup A^2O^1 (p=0.029). The results also showed higher VWF levels in A^2B individuals (median: VWF:Ag=169%) than in the AA, AB and BB groups (p=0.013) (Figure 1A).

Overall, there was a strong correlation between VWF:Ag and FVIII levels (R=0.77; p=0.001) and between VWF:Ag and VWF:RCo levels (R=0.75; p=0.001) (Spearman's rank correlation).

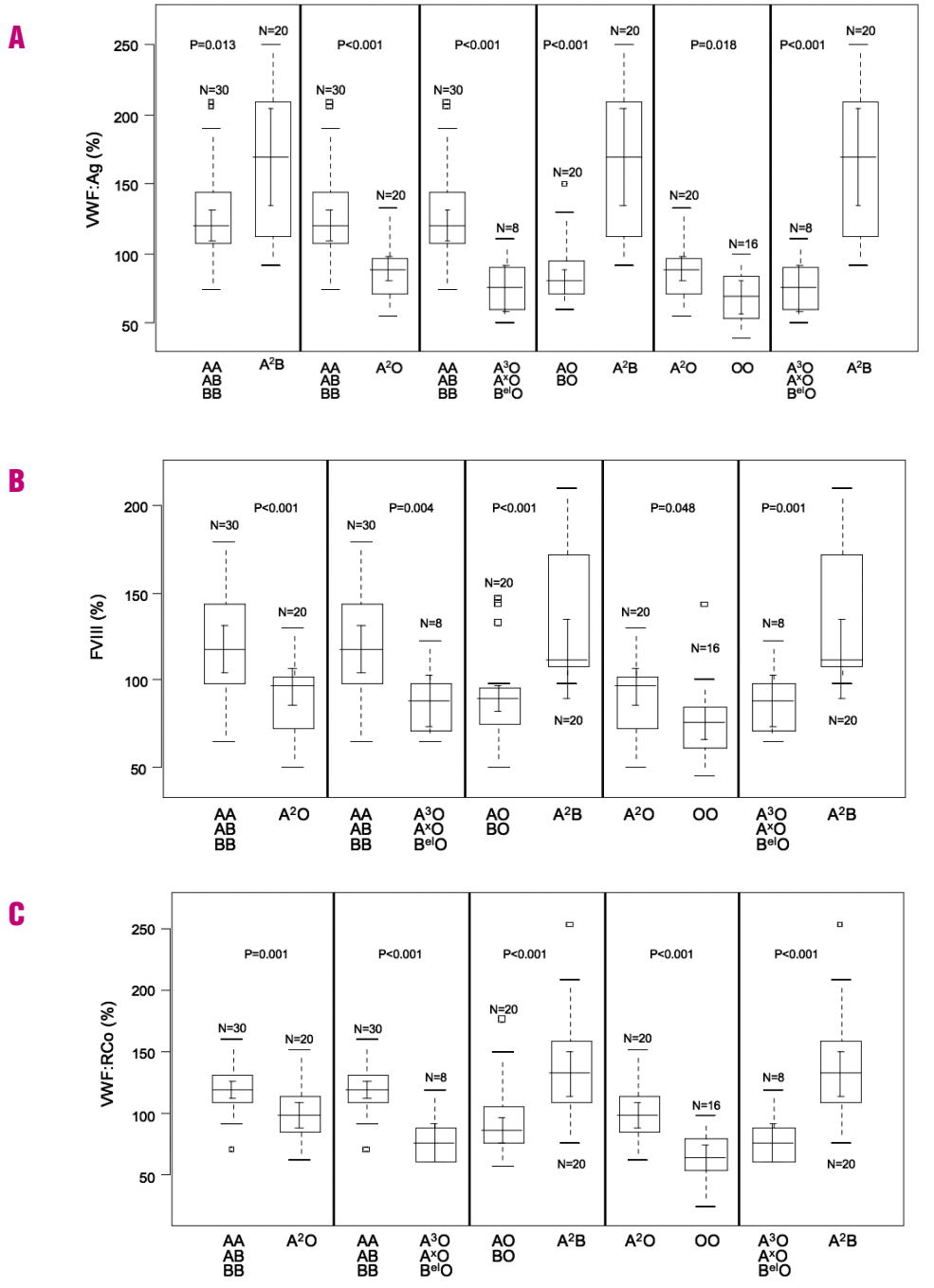

Figure 1 (A,B,C). Box-and-whisker plots representing the circulating levels of von Willebrand factor (VWF:Ag), factor VIII (FVIII) and ristocetin cofactor activity (VWF:RCo) in individuals with different blood groups. The graphics present the groups with statistically different results. The plots show a box defined by the lower and upper quartiles with the median factor dosages represented by a subdivision of the box. **A.** VWF:Ag median (%). **B.** FVIII (%). **C.** VWF:RCo.

This study was carried out to assess FVIII, VWF:Ag and VWF:RCo in 114 healthy male blood donors. It was restricted to males to avoid possible estrogen-related effects, although gender differences in FVIII and VWF:Ag were not seen in two previous studies of younger populations.[3,17] Our data demonstrated a strong correlation between VWF:Ag plasma levels and FVIII and also between VWF:Ag and VWF:RCo. The donors were ABO genotyped and the results clearly showed a significant linkage between the ABO locus and VWF antigen (p=0.001). Subjects with H-antigen-rich blood groups had significantly lower levels of FVIII and VWF:Ag antigen than did individuals with H-antigen-poor groups.[18] A^2O^1 subjects had lower levels of VWF, FVIII, and VWF:RCo

than did AA, AB, BB and A^2B subjects and higher levels than O^1O^1 subjects. These data corroborate a case-control study in which group O and A^2 individuals, presenting low levels of FVIII, were considered to have a low risk of thrombosis, in contrast to group A^1, A^1B and B^1 individuals.[19] The observation of high VWF:Ag levels in A_2B individuals was unexpected. The antigen H investigation showed score 0 for A^2B, the same value for AA, AB, BB and also for heterozygous AO and BO groups. The biological and clinical implications of this observation are unclear.

One interesting finding was that A^3O^1, A^xO^1 and $B^{el}O^1$ subgroups had significantly lower VWF:Ag and FVIII levels when compared to the A^2B group, but no difference when compared to O^1O^1 and A^2O^1. These subgroups are, however, rare and thus the study numbers were small (eight cases). Previous reports have demonstrated that the normal range of VWF:Ag found in group O individuals reaches below 50 IU/dL.[8] Unless ABO group-specific VWF:Ag references ranges are used, normal group O and also ABO subgroup individuals could be identifying as having a level of VWF below the normal reference range and therefore at potential risk of bleeding. These results favor the hypothesis that the expression of H antigen on VWF is one of the most important determinants of circulating levels of VWF:Ag.[8] In conclusion, we demonstrated that the circulating levels of VWF:Ag and FVIII in subjects with rare subgroups were similar to those found in group O individuals. These data could contribute to the diagnosis of von Willebrand's disease and to the evaluation of thrombophilia in individuals with different ABO subgroups.

Authors' Contributions

NCS: acquisition of data, analysis and interpretation of data; drafting the article; final approval of submitted version; JM A-B: hemostasis laboratory head; conception; analysis and interpretion of data; critical review and final approval; MFL: immunohematology laboratory biologist (head); acquisition of data, analysis and interpretation of data; final approval of submitted version; VC: analysis and interpretion of data; critical review; final approval of submitted version; MLB-C: senior author (tutor) and grant recipient; study conception and design; analysis and interpretion of data; critical review and final approval of submitted version.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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