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# ABO blood group is a determinant of von Willebrand factor protein levels in human pulmonary endothelial cells

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## ABSTRACT

ABO blood group antigens are expressed on von Willebrand factor (VWF) and glycosylation patterns influence circulating VWF levels. The aim of this study was to examine the effect of ABO blood type on tissue-associated VWF protein levels. We selected 35 formalin-fixed paraffin-embedded pulmonary tissue blocks obtained at autopsy from decedents who died from pulmonary embolism with known ABO blood groups (O, A, B and AB phenotypes), prepared tissue microarrays (TMAs) and stained TMAs with antibodies to VWF and platelet/endothelial cell adhesion marker-1 (PECAM-1) as a marker of endothelial cells. A pixel count scoring algorithm was used to quantify VWF and PECAM-1 staining intensity in pulmonary arterioles in digitised images. Compared with type O, non-O individuals have a significantly higher amount of endothelial cell-associated VWF protein expression. VWF protein levels associated with pulmonary vascular endothelial cells is influenced by ABO antigenic determinants.

## INTRODUCTION

von Willebrand factor (VWF) is a multimeric procoagulant glycoprotein synthesised and secreted by endothelial cells and megakaryocytes.<sup>1</sup> VWF function in haemostasis is twofold; VWF mediates platelet adhesion to the subendothelium of blood vessels and binds plasma clotting factor VIII (FVIII), protecting FVIII from proteolytic degradation.<sup>2</sup> The majority of endothelial cell-derived VWF is secreted constitutively; only 5% of VWF is expressed on the endothelial cell membrane or stored in intracellular Weibel-Palade bodies.<sup>2</sup> On stimulation with various agonists, such as thrombin and the vasopressin analogue 1-desamino-8-d-arginine vasopressin, mature, glycosylated VWF subunits are secreted from endothelial cells into the circulation with loss of Weibel-Palade bodies.<sup>2</sup>

ABO blood group antigens, A, B and H, are expressed on VWF<sup>3</sup> and mature subunits of VWF are glycosylated by ABO N-linked oligosaccharides.<sup>4</sup> The ABO gene encodes glycosyltransferases that add carbohydrates residues to the precursor structure or H antigen. Modified by addition of N-acetyl-D-galactosamine yields type A antigen, whereas the addition of D-galactose by  $\alpha$ -3-D-galactosyltransferase produces the type B antigen.<sup>5</sup> VWF in individuals with type AB blood group express both complex carbohydrate antigens, whereas individuals with blood type O lack both glycosyltransferases and express only the

H antigen. Numerous studies indicate that these N-linked oligosaccharides protect circulating VWF from proteolysis and decrease the rate of clearance from plasma.<sup>6-9</sup> Based on genotype, individuals homozygous for the O allele (O blood type or OO genotype) have the lowest levels of circulating VWF compared with those heterozygous for either A or B glycosyltransferase alleles (AO or BO); whereas, individuals with both alleles (AA, AB and BB) have the highest VWF plasma levels.<sup>7-10</sup> Compared with type O, non-O blood type individuals have higher VWF plasma levels and an increased risk for venous thromboembolism.<sup>11</sup> The effect of ABO blood group on endothelial cell associated VWF protein levels has not been reported.

The aim of this study was to use immunohistochemistry to investigate VWF protein levels in pulmonary vascular endothelial cells in individuals with known ABO phenotypes. Using platelet/endothelial cell adhesion marker-1 (PECAM-1) as marker of endothelial cells,<sup>12</sup> we compared the intensity of VWF and PECAM-1 protein expression between blood types O, A, B and AB. Similar to plasma VWF concentrations, we found that ABO blood group is a determinant of endothelial VWF protein levels.

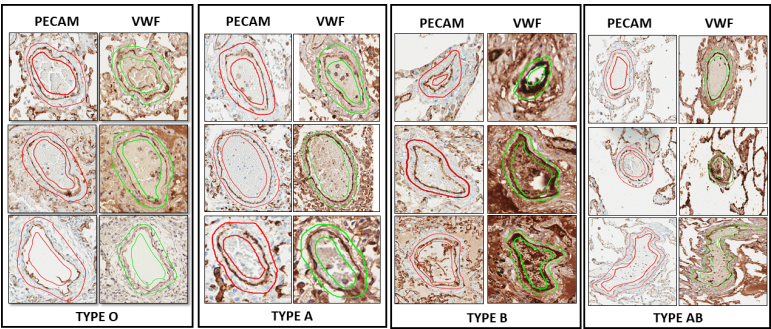
## RESULTS

This Institutional Review Board (IRB) at the University of Tennessee (UTHSC; Memphis, TN, USA) approved this study (IRB # 17-05683) with a HIPAA waiver of consent. The database at the UTHSC was searched for medical autopsies between 2004 and 2017 in which the primary cause of death was pulmonary embolism. Contributory causes of death included heart disease (atherosclerosis, hypertension or myocardial infarction), recent surgery, infection, malignancy, hemoglobinopathy or coagulopathy. Thirty-five formalin-fixed, paraffin-embedded lung tissue blocks from deceased patients with known blood types were procured (13 patients with group O blood type, 13 patients with group A blood type, 6 patients with group B blood type and 3 patients with blood type AB). For this study, pulmonary tissue was used because it is highly vascular and lung tissue blocks were readily available since the cause of death was pulmonary embolism. For each case, pulmonary tissue was selected from grossly normal pulmonary parenchyma to create a tissue microarray (TMA) for each blood group. The TMA blocks were cut at 4  $\mu$ m sections and stained with H&E, a monoclonal antibody to



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**Figure 1** PECAM-1 and VWF immunohistochemical staining in alveolar arterioles in the four blood types (O,A, B and AB) . Shown are digitised Aperio scans from representative pulmonary arterioles for each of the four blood groups. PECAM-1 highlights the endothelial cells in each arteriole. VWF in the concordant arteriole shows variable staining intensity in the endothelial cells. The red/green lines demarcate vascular areas used to quantify staining intensity. PECAM-1, platelet/endothelial cell adhesion marker-1; VWF, von Willebrand factor.

VWF (F8/86, Thermo Fisher Scientific) or a monoclonal antibody to PECAM-1 (PECAM-1/CD31; JC70, Roche). For VWF immunohistochemistry, antigen retrieval was performed using 10mM sodium citrate (pH 6.0) and microwaved for 10min. After antigen retrieval, the VWF tissues were blocked using 3% H<sub>2</sub>O<sub>2</sub>-methanol for 15 min at room temperature, washed with ddH<sub>2</sub>O, phosphate-buffered saline (PBS) and then incubated with the VWF antibody (F8/86) diluted in bovine serum albumin-PBS at 1:100 for 1 hour at 37°C. The VWF-stained TMAs were then washed with PBS-Tween20, incubated with a horse-radish peroxidase-conjugated secondary antibody followed by 3,3' diaminobenzidine chromogen. VWF-stained tissue sections were counterstained with haematoxylin. PECAM-1 (CD31) was stained on an automated stainer (Ventana).

Whole slide imagining with Aperio (Leica BioSystems) was performed to digitise the histology and quantify VWF:PECAM staining intensity in uniquely identifiable blood vessels in each blood group. A uniquely identifiable blood vessel was defined as a blood vessel with similar size, shape and spatial location that could be recognised on sequential VWF-stained and PECAM-stained TMA sections. To define endothelial cells, positive and negative annotations were created for each vessel; the inner boundary defined the intraluminal/intravascular space and the outer boundary defined the muscle layer (red and green lines, figure 1). A total of 148 pulmonary arterioles were analysed on VWF-stained and PECAM-stained TMAs. The number of vessels analysed in each blood group is shown in table 1.

A scoring algorithm was created to define strong positive and weak positive pixel thresholds for VWF and PECAM-1 within the annotated area for each blood vessel. The VWF:PECAM intensity ratio was defined as:

Table 1 Average staining intensity for VWF and PECAM according to ABO blood type			
Blood type	VWF average staining intensity	PECAM-1 average staining intensity	VWF:PECAM-1 intensity ratio
Group O (n=46 vessels)	0.366	0.623	0.595
Group A (n=57 vessels)	0.595	0.598	1.022
Group B (n=22 vessels)	0.697	0.567	1.320
Group AB (n=23 vessels)	0.666	0.494	1.521

PECAM-1, platelet/endothelial cell adhesion marker-1; VWF, von Willebrand factor.

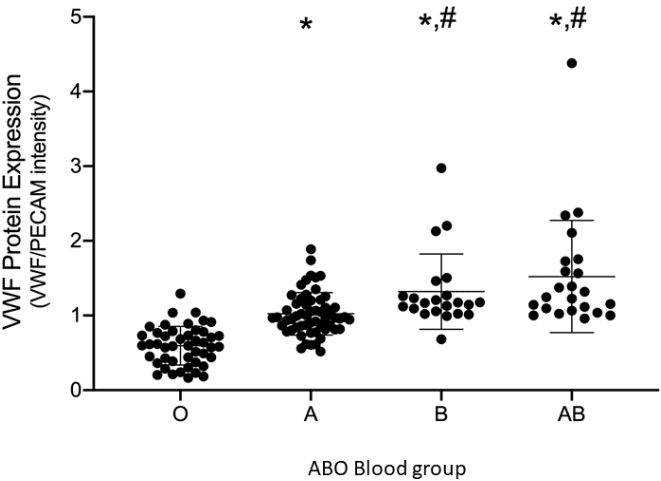
$$\text{VWF : PECAM} = \left( \frac{\frac{SP_{VWF}}{(WF_{VWF} + SP_{VWF})}}{\frac{SP_{PECAM}}{(WF_{PECAM} + SP_{PECAM})}} \right)$$

Intensity Ratio

Average PECAM-1 endothelial staining intensity remained relatively constant for each blood type (table 1). Average VWF staining intensity and VWF:PECAM-1 staining intensity ratio were lowest in type O blood type and highest in type AB (figure 2 and table 1). Groups were compared by one-way ANOVA followed by Turkey's post hoc test for multiple comparison (figure 2).

DISCUSSION

VWF protein is a multimeric glycoprotein synthesised and stored in endothelial cells and megakaryocytes. Plasma levels of VWF are influenced by genetic (eg, von Willebrand disease), hormonal (eg, oestrogen) and environmental factors (eg, smoking, acute phase reactant); however, population studies indicate that inherited ABO blood loci are a major determinant, accounting for ~30% of total plasma VWF levels.<sup>13</sup> Plasma levels are lowest in group O and highest in group AB individuals.<sup>5 14</sup> Beyond ABO phenotype, individuals with one O gene (genotypes AO



**Figure 2** VWF:PECAM staining intensity determined for each blood vessel according to ABO blood type. Each dot represents a uniquely identified blood vessel. Shown are the mean and SEM for each blood group. \*Significantly different from type O (p<0.001); # Significantly different from type A (for type B p=0.03, for AB p<0.001). PECAM, platelet/endothelial cell adhesion marker; VWF, von Willebrand factor.

and BO) had lower levels of circulating VWF antigen compared with individuals with no O gene (genotypes AA, AB and BB).<sup>10</sup> Lower circulating levels of VWF in blood group O individuals could be secondary to decreased VWF production/secretion or increased plasma clearance.<sup>15 16</sup> Previous studies indicate that ABO antigens influence the rate of VWF proteolysis and hepatic clearance of VWF multimers.<sup>6–9</sup> Group A and B carbohydrate residues with terminal sialic acid residues,<sup>17</sup> absent in type O individuals, protect circulating VWF from proteolytic cleavage by proteases<sup>6</sup> such as a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13).<sup>16 18</sup>

The influence of ABO blood groups on VWF protein levels in endothelial cells has not been reported. Using an immunohistochemical approach, we systematically investigated VWF protein levels in 148 uniquely identifiable vessels in lung tissue samples obtained from patients who died from pulmonary embolism with known ABO phenotypes. A limitation of this study is the use of pulmonary tissue collected at autopsy for this analysis. Although the primary cause of death was pulmonary embolism, premortem environmental factors that influence plasma VWF were not identical. In addition, while lung tissue sections used in this study were macroscopically unremarkable, histological sections showed variable postmortem changes, including endothelial cell injury and vascular congestion in a subset of vessels. In addition, histological tissue sectioning can limit the uniformity/thickness of the imaged vascular wall. To address these issues, we created positive and negative annotations for sequential slides to be as similar in area as possible to minimise secreted/intraluminal VWF and selected vessels with minimal endothelial cell injury as determined by PECAM-1 immunohistochemistry (see figure 1). When normalised to PECAM-1 levels in pulmonary vascular endothelium, VWF protein levels were significantly lower in individuals with type O blood group compared with non-O blood groups (type A, type B and type AB). The observed VWF protein levels in pulmonary arterioles parallels the relationship observed between ABO blood groups and circulating plasma VWF levels.<sup>14</sup> These studies showing that pulmonary endothelial-cell associated VWF protein is lower in individuals with type O compared with non-O blood groups, suggests that in addition to plasma clearance, ABO antigens contribute to VWF synthesis and/or secretion in pulmonary arterial endothelium. This finding may account for the lower incidence of thromboembolism among individuals with type O blood.<sup>11</sup>

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#### REFERENCES

- 1 Ruggeri ZM, Zimmerman TS. The complex multimeric composition of factor VIII/von Willebrand factor. *Blood* 1981;57:1140–3.
- 2 Sadler JE. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem* 1998;67:395–424.
- 3 Sodetz JM, Paulson JC, McKee PA. Carbohydrate composition and identification of blood group A, B, and H oligosaccharide structures on human factor VIII/von Willebrand factor. *J Biol Chem* 1979;254:10754–60.
- 4 Matsui T, Titani K, Mizuochi T. Structures of the asparagine-linked oligosaccharide chains of human von Willebrand factor. Occurrence of blood group A, B, and H(O) structures. *J Biol Chem* 1992;267:8723–31.
- 5 Franchini M, Capra F, Targher G, et al. Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. *Thromb J* 2007;5:14.
- 6 Federici AB, Elder JH, De Marco L, et al. Carbohydrate moiety of von Willebrand factor is not necessary for maintaining multimeric structure and ristocetin cofactor activity but protects from proteolytic degradation. *J Clin Invest* 1984;74:2049–55.
- 7 O'Donnell J, Boulton FE, Manning RA, et al. Amount of H antigen expressed on circulating von Willebrand factor is modified by ABO blood group genotype and is a major determinant of plasma von Willebrand factor antigen levels. *Arterioscler Thromb Vasc Biol* 2002;22:335–41.
- 8 Gallinaro L, Cattini MG, Sztukowska M, et al. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. *Blood* 2008;111:3540–5.
- 9 Eikenboom J, Federici AB, Dirven RJ, et al. VWF propeptide and ratios between VWF, VWF propeptide, and FVIII in the characterization of type 1 von Willebrand disease. *Blood* 2013;121:2336–9.
- 10 Shima M, Fujimura Y, Nishiyama T, et al. ABO blood group genotype and plasma von Willebrand factor in normal individuals. *Vox Sang* 1995;68:236–40.
- 11 Ohira T, Cushman M, Tsai MY, et al. ABO blood group, other risk factors and incidence of venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). *J Thromb Haemost* 2007;5:1455–61.
- 12 Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem* 2006;54:385–95.
- 13 Swystun LL, Lillcrap D. Genetic regulation of plasma von Willebrand factor levels in health and disease. *J Thromb Haemost* 2018;16:2375–90.
- 14 Gill JC, Endres-Brooks J, Bauer PJ, et al. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood* 1987;69:1691–5.
- 15 Lenting PJ, VAN Schooten CJM, Denis CV. Clearance mechanisms of von Willebrand factor and factor VIII. *J Thromb Haemost* 2007;5:1353–60.
- 16 Bowen DJ. An influence of ABO blood group on the rate of proteolysis of von Willebrand factor by ADAMTS13. *J Thromb Haemost* 2003;1:33–40.
- 17 Ward S, O'Sullivan JM, O'Donnell JS. Von Willebrand factor sialylation-A critical regulator of biological function. *J Thromb Haemost* 2019;17:1018–29.
- 18 Hayakawa M, Kato S, Matsui T, et al. Blood group antigen A on von Willebrand factor is more protective against ADAMTS13 cleavage than antigens B and H. *J Thromb Haemost* 2019;17:975–83.