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**ABO blood group: old dogma, new perspectives**

**Abstract:** Human blood group antigens are glycoproteins and glycolipids expressed on the surface of red blood cells and a variety of human tissues, including the epithelium, sensory neurons, platelets and the vascular endothelium. Accumulating evidence indicates that ABO blood type is implicated in the development of a number of human diseases, including cardiovascular and neoplastic disorders. In this review, besides its physiologic role in immunohematology and transfusion medicine, we summarize the current knowledge on the association between the ABO blood group and the risk of developing thrombotic events and cancers.

**Keywords:** ABO blood group; cancer; cardiovascular disorders; thrombosis; von Willebrand factor.

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**Introduction**

The antigens of the ABO blood group system were first discovered and the knowledge of their structure and function has greatly improved thanks to more than a century of research [1]. ABO blood groups are defined by carbohydrate moieties on the extracellular surface of the red blood cell (RBC) membranes [1]. However, along with their expression on RBCs, ABO antigens are also highly expressed on the surface of a variety of human cells and tissues, including the epithelium, sensory neurons, platelets, and the vascular endothelium [2]. The clinical significance of the ABO blood group system extends beyond transfusion medicine as several reports have suggested an important involvement in the development of neoplastic and cardiovascular disorders [3, 4].

In this review, we summarize the current knowledge on the ABO blood group system, focusing in particular on its role in the pathogenesis of human diseases.
bacteria, such as Enterobacteriaceae, which have been shown to have ABO-like structures on their lipopolysaccharide coats [7, 8]. Therefore, endogenous synthesis of anti-A and anti-B can begin as early as 3–6 months and almost all children have the appropriate isohemagglutinins in their sera at 1 year [9]. The titers of anti-A and anti-B increase during early childhood and reach adult levels by 5–10 years. Furthermore, ABO antigens can be detected on RBCs of embryos as early as 5–6 weeks of gestation, but the amount of ABO antigens on cord RBCs is less than that on adult erythrocytes; with increasing age more A and B antigen is present on RBCs and a normal ABO expression is usually observed by 2–4 years.

The frequency of ABO blood groups varies greatly in different races and populations. In most populations, about 50% are Group O, followed closely by group A, with groups B and AB showing a much lower incidence. In many non-European populations, group B has higher frequencies (e.g., 26% of the Chinese population) [10] or is even the commonest group (38.2% of the Bengalese population) [11], while the incidence of group A shows a corresponding marked reduction. In some populations, such as the Blackfoot (North American Indians) the frequency of group A is 82% [12], and in the Lapp population there is a relatively high frequency of A_2. In the Australian Aboriginal population, only groups O and A, are found and South American Indians all belong to group O [11]. The frequencies of ABO phenotypes in a few selected populations are reported in Table 2 [9–11, 13]; further data on the racial and ethnic distribution of ABO blood types sorted by population groups can also be found at the Bloodbook.com website [12].

The ABO blood group system also has a key role in genetics, anthropology, and population studies. The concept of evolutionary selection based on pathogen-driven blood group changes is also supported by studies on the genetic characterization of the ABO blood group in the Neanderthals [14] and ancient Egyptian mummies [15]. These studies suggest a potential selective advantage of the O allele influencing the susceptibility to several different pathogens responsible for diseases, such as severe
malaria [16], Helicobacter pylori infections [17], and severe forms of cholera [18]. The positive selective pressure could have been caused by the absence of the A and B antigens (that can be used as receptors by infectious agents) and by the presence of anti-A and anti-B antibodies. Therefore, the study of the evolution of the ABO blood groups can contribute to the determination of when during human history the different alleles emerged, and can help to identify the selective forces that might have acted on the different alleles.

Genetic inheritance and biochemistry

Fundamentally, the ABO blood group system consists of three alleles: two co-dominant A and B alleles, and one silent and recessive O allele. Inheritance of the ABO genes follows Mendelian principles. The system is controlled by a single gene located on the terminal portion of the long arm of chromosome 9 (9q34.2); the ABO gene is quite large and consists of 7 exons spread over 18 kilobases at the chromosomal locus [19]. This gene encodes a glycosyltransferase enzyme that adds a sugar residue to a carbohydrate structure called the H antigen. In fact, the antigens of the ABO, H, and also those of Lewis I, and P blood group systems, are defined by small carbohydrate epitopes on glycoproteins and glycolipids. Therefore, ABO expression is also regulated by the H gene that is responsible for the synthesis of the H antigen substrate, the precursor of A and B antigens. The ABH antigens also occur as soluble glycoprotein antigens in secretion that include saliva, tears, breast milk, and seminal fluid.

The presence of ABH antigens in the secretion is controlled by a Se gene. This salivary secretor characteristic was found to be inherited as a dominant Mendelian trait [20]. Secreted antigens are absent when two se genes are inherited (20% of the population). The H antigen is present in the membrane of RBCs and also of most epithelial and endothelial cells. People lacking the H gene do not have H-bearing structure on the erythrocyte membrane and do not express A or B antigens; these individuals are homozygous for the Bombay gene (hh) and have the rare O, phenotype [21], the so-called Bombay phenotype, which was recognized in 1952 [22]. The A allele codes for an enzyme that adds a N-acetyl galactosamine to the subterminal galactose of H antigen, while the B allele, which differs from the former by four amino acid changes, codes for an enzyme that adds D-galactose to the same subterminal galactose. In group AB individuals, both A and B structures are synthesized. The O allele occurs most frequently in modern humans and carries a human-specific inactivating mutation, which produces a non-functional enzyme, thus the H antigen remains without further modification on the surface of the cells [23, 24].

ABO subgroups

ABO subgroups are distinguished by decreased amounts of antigens on RBCs and, in secretors, present in the saliva. A subgroups are more frequent than B subgroups [25]. The two principal A subgroups are A₁ and A₂. RBCs from A₁ and A₂ subjects both react strongly with anti-A reagents in direct agglutination tests. The serologic distinction between A₁ and A₂ is based on the agglutination of A₁ RBCs but not A₂ with anti-A₁ lectin from Dolichos biflorus seeds. Approximately 80% of blood type A or AB are classified as A₁ or A₁B. The remaining 20% are either A₂ or A₂B. Subgroups weaker than A₁ are not frequent, and are characterized by a decreasing number of A antigen sites on the RBCs and a reciprocal increase in H antigen activity. Subgroups A₁int, A₂p, A₁x, A₁nst, A₂nst, and A₁m are met only rarely in transfusion practice, and the last four cannot reliably be identified on the basis of blood typing tests alone.

B subgroups are even less common than A subgroups and, similarly, are classified by the amount of B antigen, which decreases in the order B, B₂, B₃, B₄, and B₅ [26].

ABO blood group testing and clinical importance

ABO testing

The ABO group system was the first of the 33 current blood group systems [27] to be identified, and is the most significant for transfusion medicine practice as accurate testing of the blood donor and recipient for ABO compatibility is fundamental for the prevention of hemolytic transfusion reactions (HTRs). The determination of the ABO type of transfusion therapy candidates and blood donors has a key role in guaranteeing the safety of blood transfusion. The ABO blood group test is performed in two steps. The forward type is commonly carried out through murine monoclonal reagents to establish whether A or B antigens are present on the RBCs. The reverse type is a complementary test based on the inverse reciprocal relationship between the presence of A and B antigens on erythrocytes and the presence of naturally occurring antibodies in their sera. Therefore, by knowing which ABO antigens are
present on the RBCs and which antibodies are contained in the serum, the ABO type of recipients can be determined, thus allowing compatible blood components to be supplied.

Routine grouping of donors and patients must include both RBC and serum tests, each serving as a check on the other. However, according to the Italian Standards of Transfusion Medicine, recently translated into English and freely available at the Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) website [28], the ABO blood group of donors who have not already been typed shall be determined on RBCs using the anti-A, anti-B, and anti-A, B reagents and “in serum/plasma using at least A1 and B erythrocytes”, while “unequivocally identified donors who have already been typed for the ABO and RhD systems” shall be typed through RBC testing “using at least anti-A and anti-B reagents”. In addition, “for ABO blood group determination in serum/plasma, it may also be advisable to use A and/or O erythrocytes, in order to identify weak phenotypes or antibodies against ABH specificities”. The same applies to patients. Only RBCs are tested when blood grouping is performed on samples from infants <4 months of age.

At present, ABO typing reagents are produced from monoclonal antibodies derived from cultured cell lines and they agglutinate most antigen-positive erythrocytes even without centrifugation. On the contrary, anti-A and anti-B antibodies in the sera of most patients and donors are usually too weak to agglutinate RBCs without centrifugation or incubation. Both tests can be carried out through slide, tube, microplate or column agglutination techniques.

**ABO discrepancies**

Technical errors and several clinical conditions or diseases can lead to discrepancies between erythrocyte and serum results in ABO grouping [9]. Technical errors include: specimen mix-up, too heavy or too light RBC suspension, failure to add reagents, missed observation of hemolysis, failure to comply with the manufacturer’s instructions, under- or over-centrifugation of test samples, incorrect interpretation or recording of the test results, contaminated reagents or dirty glassware, and expired or otherwise inactive reagents. ABO typing discrepancies may be caused by intrinsic problems with either RBCs or serum and can be classified in five categories: i) weak/missing RBC reactivity, resulting from weak ABO subgroup inheritance, leukemia/malignancy, transfusion, transplantation, and excessive soluble blood group substance; ii) extra RBC reactivity, caused by autoagglutinins/excess proteins coating RBCs, non-specific RBC aggregation due to abnormal concentrations of serum proteins or infused macromolecular solutions, pH- or reagent-dependent antibody, or rouleaux, transplantation, acquired A antigen [group O or group B people with activation of the Tn cryptantigen (namely N-acetyl galactosamine) usually associated with bacterial or viral infections], acquired B-like antigen (usually found in A1 individuals with diseases of the lower intestinal tract, carcinoma of the colon or rectum, intestinal obstruction, gram-negative septicemia, or gangrene of the lower extremities and more commonly due to deacetylation of the A antigen by microbial enzymes) [29], and out-of-group transfusion; iii) mixed-field RBC reactivity for recent transfusion, transplantation, foeto-maternal hemorrhage, and chimerism; iv) weak/missing serum reactivity, due to age (<4–6 months old or elderly persons), ABO subgroups, hypogammaglobulinemia, and transplantation; v) extra serum reactivity, caused by cold auto- or allo-antibodies, antibodies to reagent constituent, excess serum protein, transfusion of plasma, transplantation, or infusion of intravenous immunoglobulin.

**ABO clinical importance**

ABO incompatibility is also the cause of other severe diseases, such as hemolytic disease of the fetus and newborn (HDFN), and HTRs, and plays an important role in solid organ and hematopoietic transplantation.

HDFN as a result of ABO incompatibility between mother and baby is a relatively common event in group O mothers carrying a group A or B fetus. It may occur in the first or in any subsequent pregnancy and in the usual scenario, is caused by naturally occurring A, B IgG antibodies crossing the placenta [1]. However, despite the prevalence of ABO, HDFN is nearly always mild and hemolysis of fetal RBCs only rarely causes severe anemia. Several reasons can be listed for the paradoxical mildness of this disease: i) there are a smaller number of A and B antigenic sites on the fetal RBC membrane; ii) anti-A and anti-B are mostly IgM, which does not cross the placenta; iii) the small amount of antibody is neutralized by a myriad of tissue and soluble antigens.

The interaction between preformed antibodies and RBC antigens is the immunologic basis for acute HTRs. Life-threatening HTRs are associated with transfusion of ABO-incompatible RBCs or ABO-incompatible plasma that result in acute intravascular hemolysis. Although rare, the most common circumstance is when group O platelets from donors with high titers of anti-A are
transfused to group A patients [30, 31]. ABO incompatibility because of clerical errors are still one of the main risks for major morbidity and mortality associated with transfusion therapy [32].

ABO compatibility is also important for platelet transfusion [33]. In fact, platelets express ABO antigens on a large number of platelet glycoproteins and glycolipids and routine transfusion of ABO-incompatible platelets can be associated with cumulative adverse effects, including decreased post-transfusion recovery, increased platelet utilization, incompatible platelet crossmatches, HLA alloimmunization, and ABH-specific refractoriness [34]. The criteria for blood-group compatibility for the transfusion of RBCs, plasma, and platelets are reported in Table 3 [33, 35].

The clinical significance of anti-A and anti-B is also important in both solid organ and hematopoietic transplantation. ABO compatibility is not critical in the selection of potential hematopoietic stem cell transplantation donors because pluripotent and early-committed hematopoietic progenitor cells (HPCs) lack ABO antigens. Therefore, engraftment of HPCs is uninhibited even in the presence of circulating ABO antibodies. However, ABO incompatibility, which is present in 20%–40% of donor/recipient pairs, influences transfusion decisions and can fall into one of three categories: i) incompatible in the major crossmatch [the (group O) recipient has antibodies against blood group antigen(s) present on the donor’s (group A) RBCs]; ii) incompatible in the minor crossmatch [the (group O) donor has antibodies against blood group antigen(s) present on the recipient’s (group A) RBCs]; iii) incompatible bidirectionally (donor is group A and recipient is group B). Some evidence suggests that ABO-incompatible transplants may be associated with increased complications, but only in a selected group of patients [36]. Immune hemolysis remains a major complication in transplantation of ABO-incompatible solid organs [37], a relatively recent strategy that has met with moderate success. However, the results can also be improved by aphaeretic treatment together with immunosuppressive therapy and reach outcomes similar to those of normal ABO-compatible transplants [38].

Table 3 Selection criteria of the ABO phenotype of red cell units to transfuse.

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<th>ABO phenotype of the recipient</th>
<th>ABO phenotype of the units to select for transfusion (in order of preference)</th>
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<td>AB</td>
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*This could conversely be negative for high titer anti-A/A,B.

The role of the ABO blood group in human diseases

There is a high amount of clinical data documenting the involvement of ABO blood groups in the development of various human disorders. However, the more consistent data come from studies analyzing the association with malignancies and cardiovascular diseases.

The ABO blood group and cancer

A number of epidemiological studies have reported an association between ABO blood groups and risk of developing certain malignancies, especially gastric and pancreatic cancers.

In a combined analysis published more than fifty years ago on gastric cancer cases in 15 study locations in the USA, Europe and Australia, a significant positive association was reported between non-O blood group and risk of gastric cancer with an odds ratio (OR) of 1.24 [95% confidence interval (CI): 1.18–1.30] for patients with blood group A compared to those with blood group O [39]. More recently, the large Scandinavian Donations and Transfusions study, involving more than one million donors, who were followed for up to 35 years, demonstrated a similar magnitude of association with blood group A (OR 1.20, 95% CI: 1.02–1.42) [40]. In a case-control study published last year by Wang et al. [41], the risk of gastric cancer in group A was significantly higher than that in non-A groups (OR 1.34, 95% CI: 1.25–1.42) [40]. In a case-control study published last year by Wang et al. [41], the risk of gastric cancer in group A was significantly higher than that in non-A groups (OR 1.34, 95% CI: 1.25–1.44). Conversely, individuals with blood group O showed a reduced risk of gastric cancer compared with non-O groups (OR 0.80, 95% CI: 0.72–0.88). These findings were replicated in a meta-analysis made by the same authors combining their data with those from the PubMed database [41]. Interestingly, the authors also found that subjects with blood type A are
more prone to *H. pylori* infection than other ABO blood type individuals.

In the multinational Pancreatic Cancer Cohort Consortium (PanScan) I genome-wide association study, 1896 individuals with pancreatic cancer and 1939 controls were genotyped, and a significant association was reported for rs505922, a single-nucleotide polymorphism (SNP) which maps to the first intron of the ABO gene [42]. This association was also replicated in an independent sample of 2457 affected individuals and 2654 controls from the PanScan II study [42]. A combined analysis of these groups yielded a multiplicative per-allele OR of 1.20 (95% CI: 1.12–1.28), thus supporting earlier epidemiologic evidences that people with blood group O may have a lower risk of pancreatic cancer than those with groups A, B or AB. Using SNP genotype data to determine serotype status, Wolpin and colleagues reported that, compared to individuals with blood group O, participants with blood groups A, AB or B were more likely to develop pancreatic cancer [adjusted hazard ratios for incident pancreatic cancer were 1.32 (95% CI: 1.02–1.72), 1.51 (95% CI: 1.02–2.23), and 1.72 (95% CI: 1.25–2.38), respectively] [43]. The age-adjusted incidence rates for pancreatic cancer per 100,000 person-years were 27 for participants with blood type O, 36 for those with blood type A, 41 for those with blood type AB, and 46 for those with blood type B, respectively [43]. The relevance of the interaction of the ABO blood group and *H. pylori* infection for the development of pancreatic cancer was recently analyzed by Risch et al. in a case-control study involving 373 case patients and 690 gender- and age-matched control subjects [44]. Interestingly, they reported that the increased risk of pancreatic cancer among the individuals with non-O blood group was even higher if they were also seropositive for CagA-negative *H. pylori* (OR 2.78, 95% CI: 1.49–5.20).

Finally, data from large prospective cohort studies indicate that the ABO blood group is associated with the risk of developing skin, ovarian and lung cancers [45–47], while no association was found with colorectal and breast cancers [48, 49].

### The ABO blood group and cardiovascular disorders

It has been known for approximately 50 years that the ABO blood group has a profound influence on hemostasis, as it has been a major determinant of von Willebrand factor (VWF) and, consequently, of factor VIII (FVIII) plasma levels [50–52]. In particular, VWF levels are approximately 25% higher in individuals who are a blood group other than O [53]. The presence of ABH structures in VWF N-linked oligosaccharides provides the molecular basis for the ABO regulation of VWF levels [54]. The active ABO A and B glycosyltransferase enzymes, found in the Golgi of endothelial cells, generate terminal carbohydrate modifications, that is, A and B antigens, on the existing VWF “H” oligosaccharides, whereas the enzymatically inactive ABO O protein cannot modify these VWF H antigens [55]. The addition of A or B terminal carbohydrate antigens to VWF in endothelial cells might influence circulating VWF levels and function by several mechanisms, including the alteration of the rate of VWF synthesis and/or secretion, the regulation of VWF proteolysis by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), the modulation of VWF clearance, the modification of VWF biological activity or perhaps a combination of such events [56].

Based on these experimental findings, in the last three decades several clinical studies have assessed whether the ABO blood group could influence the risk of developing arterial or venous thrombotic events [4]. In 2008, Wu et al. performed a systematic review and meta-analysis of studies reporting the association of non-O blood groups with a variety of vascular disorders [57]. Among these pathologies, significant risk could be calculated for peripheral vascular disease (OR 1.45; 95% CI, 1.35–1.56), myocardial infarction (OR 1.25; 95% CI: 1.14–1.36), ischemic stroke (OR 1.14; 95% CI: 1.01–1.27) and venous thromboembolism (VTE) (OR 1.79; 95% CI: 1.56–2.05). These latter findings were replicated in a more recent meta-analysis performed by our group on a larger number of studies and VTE cases (38 studies with 10,305 VTE cases) [58]. Indeed, we found a prevalence of non-O blood group significantly higher in VTE patients compared with controls with a resulting pooled OR of 2.08 (95% CI: 1.83, 2.37; p<0.00001). Finally, another recent publication including two prospective cohort studies and a meta-analysis confirmed the positive association between non-O blood group and the risk of coronary heart disease (RR 1.11; 95% CI: 1.05–1.18; p<0.001) [59]. The same results emerged in a retrospective case-control study conducted by our group [60].

### Conclusions

As well as its essential role in immunohematology and transfusion medicine, a consistent amount of clinical data have documented that the ABO blood group plays a key role in the risk of developing thrombosis, especially VTE. Further studies are needed to better clarify the pathogenic mechanisms of the interaction between the ABO blood group and hemostasis and their clinical and
therapeutic implications. Additional studies will also help us to clarify the link between the ABO blood group and the risk of cancer.

Conflict of interest statement

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