Applications of blood group genotyping
Aplicações da genotipagem de grupos sanguíneos

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ABSTRACT

Introduction: The determination of blood group polymorphism at the genomic level facilitates the resolution of clinical problems that cannot be addressed by hemagglutination. They are useful to (a) determine antigen types for which currently available antibodies are weakly reactive; (b) type patients who have been recently transfused; (c) identify fetuses at risk for hemolytic disease of the newborn; and (d) to increase the reliability of repositories of antigen negative RBCs for transfusion. Objectives: This review assessed the current applications of blood group genotyping in transfusion medicine and hemolytic disease of the newborn. Search strategy: Blood group genotyping studies and reviews were searched in general database (MEDLINE) and references were reviewed. Selection criteria: All published data and reviews were eligible for inclusion provided they reported results for molecular basis of blood group antigens, DNA analysis for blood group polymorphisms, determination of fetal group status and applications of blood group genotyping in blood transfusion. Data collection: All data were collected based on studies and reviews of blood group polymorphisms and their clinical applications.

Keywords: Genotype; Blood group antigens; Hemaglutination; Blood transfusion; Erythroblastosis, fetal; Blood grouping and crossmatching

INTRODUCTION

Blood group antigens are polymorphisms of proteins and carbohydrates on the outside surface of the red blood cell (RBC) and are defined by serum alloantibodies produced in response to an immunizing event such as transfusion or pregnancy. It is the antibody that causes clinical problems in transfusion incompatibility, maternal-fetal incompatibility, and autoimmune hemolytic anemia.

The major risks of transfusions are unexpected incompatibility reactions¹ and the transmission of infections agents. Iron overload and alloimmunization are also frequently observed among some categories

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of chronically transfused patients. Alloimmunization leads to an increased risk of transfusion reactions, reducing the available pool of compatible blood for transfusion in subsequent crises. Alloimmunization is the source of a variety of problems during long-term medical and transfusion management, with the main problem being the identification of appropriate antigen-negative RBCs for transfusion\(^2\).

Thus, in transfusion medicine, much time and effort are expended in detecting and identifying blood group antibodies. Next to ABO, the most clinically significant antibodies are those in Rh, Kell, Duffy, and Kidd blood group systems\(^3\).

Red blood cell phenotyping is essential to confirm the identity of suspected alloantibodies and to facilitate the identification of antibodies that may be formed in the future. Accurate antigen typing of transfused patients is often a difficult task due to the presence of donor RBCs in the patients' circulation. Thus, in these patients phenotyping can be time consuming and difficult to interpret. It is also complicated to type cells when a patient's RBCs have a positive direct antiglobulin test and no direct agglutinating antibody is available.

The DNA technology led to the understanding of the molecular basis of many blood group antigens. The genes encoding 28 of the 29 blood group systems (only P remains to be resolved) have been cloned and sequenced\(^4-7\), which has permitted the elucidation of the molecular basis of many common blood group antigens. There are many molecular events that give rise to blood group antigens and phenotypes (chart 1), however, the majority of genetically defined blood group antigens are the consequence of a single-nucleotide polymorphism (SNP). (For current information regarding blood group antigen SNPs, the following website is recommended: www.bioc.aecom.yu.edu/bgmut/index.htm). This knowledge allows to use DNA based-assays to detect specific blood group SNPs and that can be used to overcome the limitations of hemagglutination assays\(^8,9\).

Several assays for blood group genotyping of patients have recently been developed to predict the blood group antigen profile of an individual, with the goal of reducing risk or helping in the assessment of the risk of hemolytic disease of the newborn (HDN)\(^10-16\). They include PCR-RFLP, allele-specific PCR, sequence-specific PCR as single or multiplex assays, real-time quantitative PCR.

These assays can be applied for blood group antigens to type patients who have recently received transfusion; to type patients whose RBCs are coated with immunoglobulin; to identify a fetus at risk for HDN; to determine which phenotypically antigen-negative patients can receive antigen-positive RBCs; to type donors for antibody identification panels; to type patients who have an antigen that is expressed weakly on RBCs; to determine \(RHD\) zygosity; to mass screen for antigen-negative donors; to resolve A, B, and D discrepancies.

**OBJECTIVES**

This review summarized the most important concepts of DNA technology to determine blood group antigens as an alternative to hemagglutination and showed the potential application of this technology for transfusion medicine and maternal-fetal medicine.

**METHODS**

Criteria for considering studies for this review:

**Types of studies:** All data were collected based on studies and reviews on molecular insights into blood groups, blood group polymorphisms in different populations, blood group genotyping assays and their clinical applications in transfusion medicine and maternal-fetal medicine. For this review only studies published in the medical literature were considered (MEDLINE).

**Search strategy for identification of studies:** A comprehensive literature search was performed to identify all relevant publications. The search process used MEDLINE and focused on studies as of 1998 that included any one of the following terms in the title, abstract or in their keyword list: DNA analysis for blood groups, molecular basis of blood groups, applications of blood group genotyping, fetal blood group status.

The literature search and selection were initially performed with the terms blood group genotyping and noninvasive determination of fetal RhD status, yielding 117 studies. With the objective of filtering search results we restricted only to papers and reviews focusing the clinical applications of blood group genotyping. All studies were selected taking account the methodology employed and preference was given to recent studies.
RESULTS

APPLICATIONS TO TRANSFUSION MEDICINE

For multiply – transfused patients: The ability to determine a patient antigen profile by DNA analysis when hemagglutination tests cannot be used is a useful adjunct to a serologic investigation. Blood group genotyping in the transfusion setting is recommended for multiple transfused patients, as part of antibody identification process.

Determination of a patient’s blood type by analysis of DNA is particularly useful when a patient, who is transfusion-dependent, has produced alloantibodies. This is because identification of the patient’s probable phenotype allows the laboratory to determine to which antigens the patient can and cannot respond to make alloantibodies.

It has been demonstrated the relevance of genotype determination of blood groups for the management of multiply transfused patients with diseases such as Sickle Cell Disease (SCD) and b-Thalassemia by allowing the determination of the true blood group genotype, and by assisting in the identification of suspected alloantibodies and the selection of antigen-negative RBCs for transfusion. Furthermore, we have observed that taking genotype into account allowed better selection of compatible units for patients with discrepancies between genotype and phenotype, leading to increased cell survival and a reduction of the transfusion frequency.

For patients whose RBCs are coated with IgG: Patients with autoimmune hemolytic anemia (WAIHA), whose RBCs are coated with IgG cannot be accurately typed for RBC antigens, particularly when directly agglutinating antibodies are not available, or IgG removal by chemical treatment of RBCs is insufficient. It has been shown that blood group genotyping, is very important for determination of the true blood group antigens of these patients. After genotyping, the patients received antigen-matched RBCs that had better in vivo survival, as assessed by raises in hemoglobin levels and diminished frequency of transfusions.

The possibility to have an alternative to hemagglutination tests to determine the patient’s antigen profile should be considered for multiply transfused patients and for patients with WAIHA by allowing the determination of the true blood group genotype and by assisting in the identification of suspected alloantibodies and in selection of antigen-negative RBCs for transfusion. This ensures more accurate selection of compatible donor units and is likely to prevent alloimmunization and reduce the potential for hemolytic reactions.

For blood donors: DNA-based typing can also be used to antigen-type blood donor both for transfusion and for antibody identification reagent panels. This is particularly useful when antibodies are not available or are weakly reactive. A good example is the Dombrock blood group polymorphism where DNA-based assays are used to type patients and donors for Do and Do to overcome the dearth of reliable typing reagents. Furthermore, the newer technologies have the potential to screening pools of DNA for rare blood types. The molecular analysis of a variant gene can also assist in resolving a serologic investigation.

As automated procedures attain higher and faster throughput at lower cost, blood group genotyping is likely to become more widespread. We believe that the PCR technology may be used in a transfusion service in the next few years to overcome the limitations of hemagglutination.

Resolution of weak A, B, and D typing discrepancies: A proportion of blood donors and patients who historically have been typed as group O are now being recognized as group A or group B with the use of monoclonal antibodies capable of detecting small amounts of the immunodominant carbohydrate responsible for A or B specificity. A typing result that differs from the historical record often results in time-consuming analyses. Since the bases of many of weak subgroups of A and B are associated with altered transferase genes, PCR-based assays can be used to define the transferase gene and thus the ABO group.

Similarly with the D antigen of the Rh blood group system, a proportion of blood donors that historically have been typed as D-negative are now reclassified as D-positive, due to monoclonal reagents that detect small and specific parts of the D antigen. The molecular basis of numerous D variants can be used to identify the genes encoding altered RhD protein in these individuals.

APPLICATIONS TO MATERNAL-FETAL MEDICINE

Alloimmunization against the RhD antigen during pregnancy is the most frequent cause of hemolytic disease of the newborn (HDN). Immunization occurs when fetal cells, carrying antigens inherited from the father, enter the mother’s circulation following fetal-maternal bleeding. The mother, when not expressing the same antigen(s), may produce IgG antibodies towards the fetal antigen and these antibodies can pass through the placenta causing a diversity of symptoms, ranging from mild anaemia to death of the fetus. Apart from antibodies to the RhD blood group antigen, other specificities within the Rh system and several other
blood group antigens can give rise to HDN, but RhD is by far the most immunogenic(25).

Prenatal determination of fetal RhD status is desirable in pregnancies to prevent sensitization and possible hydrops foetalis in fetuses of RhD negative mothers with RhD positive fathers. Fetal DNA has been detected in amniotic cells, chorionic villus samples, and as recently reported, in maternal plasma(26). It is now well accepted that a minute number of copies (as low as 35 copies/ml) of cell-free fetal RHD DNA in the maternal plasma(27) can be utilized as a target for non-invasive genotyping of the fetus. Unlike fetal DNA isolated from the cellular fraction of maternal blood samples (where microchimerism has been shown to persist for decades(28), free fetal DNA isolated from maternal plasma has been shown to be specific for the current fetus and is completely cleared from the mother's circulation by post partum(29).

It has been reported that fetal RHD can be determined by PCR in DNA extracted from maternal plasma of pregnant women with RhD positive fetuses, in a non-invasive procedure(30-35). PCR amplification of RHD in maternal plasma may be useful for the management of RhD negative mothers of RhD positive fetuses and for the study of fetus-maternal cell trafficking.

There are, however, several challenges in fetal RHD genotyping due to the high complexity of the Rh system(36). Adding to the difficulty to be certain whether the detected genotype is representative for the phenotype is the need for a fetal-specific DNA control to confirm the presence of fetal DNA when the fetus is RHD negative. The amount of free DNA is obviously a limiting factor for fetal RHD genotyping in maternal blood. For the fetus, the consequence of a false-negative RHD determination may be more severe than a false positive result, as the latter would only result in unnecessary prophylaxis.

Before interpreting the results of DNA analysis, it is important to obtain an accurate medical history and to establish if the study subject is a surrogate mother, if she has been impregnated with no spousal sperm.

The discovery of fetal DNA in maternal plasma has opened up new and exciting possibilities for the non-invasive prenatal determination of fetal blood group status. However, a number of technical issues still need to be addressed and large scale multi center clinical trials need to be carried out. When these issues are resolved, it is likely that the prenatal testing of fetal blood group type will be carried out routinely and safely.

The determination of blood group polymorphism at the genomic level facilitates the resolution of clinical problems that cannot be addressed by hemagglutination. They are useful to (a) determine antigen types for which currently available antibodies are weakly reactive; (b) type patients who have been recently transfused; (c) identify fetuses at risk for hemolytic disease of the newborn; and (d) to increase the reliability of repositories of antigen negative RBCs for transfusion.

It is important to note that PCR based assays are prone to different types of errors that those observed with hemagglutination assays. For instance, contamination with amplified products may lead to false positive test results. In addition, the identification of a particular genotype does not necessarily mean that the antigen will be expressed on the RBC membrane.

A large number of people from a variety of ethnic backgrounds need to be studied to determine the occurrence of particular genotypes and to establish more firmly the correlation between blood group genotype and phenotype(33).

Advances are ongoing in the automation of SNP and DNA sequence analysis and the success of sequencing the human genome has shown that the potential for phenotyping large numbers of samples has already been realized(37). It should soon be possible to analyze major and many minor blood group alleles on a single synthetic chip. Indeed, it will be possible to test for many genetically defined conditions.

CONCLUSIONS

The identification of the molecular basis of blood group antigens provides an insight into the generation of gene diversity in humans. A molecular understanding of blood groups has enabled the design of simple assays that may be used to facilitate the provision of blood to patients who require antigen-matched red cells, both by phenotyping the patient to determine his/her requirements and by phenotyping red cell units.

Transfusion-dependent patients have sickle cell disease, thalassemias and aplastic anemias and frequently become alloimmunized. Blood group genotyping contributes substantially to the safety of blood transfusion in these recipients. Although it is unlikely that molecular genotyping will replace hemagglutination any time in the near future, together these techniques have substantial value in the resolution of clinical laboratory problems, and consequently in the quality of patient care.

REFERENCES


