Commentary

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Technological Advancement in Blood Group Genotyping

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About the Study

The antigen typing of Red Blood Cells (RBCs) in both recipients and donors is critical for safe and effective transfusion. Despite the existence of over 330 RBC antigens, current routine pre-transfusion testing only includes matching the patient and donor for ABO and D using methods that have not changed significantly in over 60 years, despite clinically significant limitations. Approximately 3% of Caucasian recipients and 30-50% of African-American patients receiving chronic transfusion therapy will develop an anti-RBC antigen alloantibody. Once sensitized, a patient is more likely to develop additional alloantibodies and must receive donor units that have been tested and found to be negative for those antigens in order to avoid transfusion reactions. However, in the absence of an efficient, affordable, and scalable extended antigen typing strategy, alloantibody complications following transfusion have been accepted as a level of risk.

Traditional antibody-based serologic typing methods are time-consuming and difficult to scale for non-ABO/D antigen typing (i.e., extended antigen typing). Furthermore, serologic reagents for all clinically significant antigens are not commercially available. These constraints, particularly reagent availability, can be overcome with DNA-based genotyping by predicting the RBC antigen phenotype using genetic information. Currently available genotyping assays rely heavily on PCR amplification to detect Single Nucleotide Variants (SNVs) either directly (e.g., allele specific primers) or indirectly (e.g., probe hybridization or Sanger sequencing). While PCR-based genotyping can be used to broadly genotype the majority of antigens, current assay formats limit the number of SNVs and samples that can be tested at the same time (50 SNVs and 100 samples per run).

Furthermore, PCR-based assays detect Structural Variations (SVs) by using PCR primers that span the breakpoint region, which necessitates that the breakpoints be well characterized, which many are not. As a result, current PCR-based genotyping assays are unable to fully characterize all genetically understood antigens, particularly the highly polymorphic blood group systems ABO, Rh, and MNS. Furthermore, PCR-based genotyping assays frequently represent a significant cost and time investment per sample, limiting their use to a subset of all blood donors.

The development of RBC antigen genotyping using Next-Generation Sequencing (NGS) and high-density DNA arrays has the potential to overcome many of the limitations listed above. However, many technical challenges had to be overcome before this potential could be fully realized: (I) a lack of array and NGS data with paired conventional serology or PCR-based results; (II) the need for fully annotated and complete electronic databases of RBC antigen allele genotypes to phenotypes; (III) previously published RBC genetic changes were defined using cDNA sequences without mapping to human reference genome coordinates; and (IV) a lack of software capable of automatically interpreting the large amount of data. Many organizations have addressed these issues over the last decade.

The limitations of current serologic and PCR-based genotyping assays prevent them from being scaled to type all blood donors for all genetically understood antigens (i.e.,>330 RBC antigens). NGS enables unprecedented evaluations of genetic changes, including novel genetic changes and complex SVs, and is the new gold standard for RBC antigen genotyping. With the ability to run 1,000s of samples at a time, high-density DNA arrays enable low-cost evaluation of all known genetic changes, including SVs. As a result, NGS will almost certainly replace conventional PCR genotyping for discordant and complex serologic workups, providing an unprecedented new level of discovery for new RBC antigens. High-density DNA arrays have the potential to make routine genotyping of all blood donors possible for all genetically understood diseases. Antigens will fundamentally alter transfusion medicine practice.

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