Comment on "Applying molecular immunohaematology to regularly transfused thalassaemic patients in Thailand"

Silvia Manfroi, Pasqualepaolo Pagliaro

Immunohematology and Transfusion Service, Policlinico S. Orsola-Malpighi, Bologna, Italy

Dear Sir,

We thank you for the opportunity to comment on the article entitled "Applying molecular immunohaematology to regularly transfused thalassaemic patients in Thailand"[1], which led us to make some reflections.

As outlined by the authors, red blood cell (RBC) genotyping in chronically transfused patients (such as those affected by β-thalassaemia, sickle cell disease or myelodysplastic syndrome) is one of the major applications of RBC genotyping and, probably, the only way to define RBC antigens in these patients[2,3].

Once the genes encoding the major blood group antigens had been cloned, it was relatively easy to correlate differences in DNA sequences with surface antigen expression. It was also fairly simple to apply polymerase chain reaction and molecular technologies (widely used in other fields, such as immunogenetics) to immunohaematology.

Molecular typing was initially used on a small scale to resolve complex clinical cases. The subsequent introduction of high-throughput platforms and CE-marked kits had a great impact on immunohaematology, in extended genotyping of both patients' and donors' RBC antigens. The goal was to improve the safety and efficacy of RBC transfusion and it has been suggested that, in the not so distant future, RBC genotyping will change pre-transfusion testing in developed countries.

On this background, in the past decade the pivotal role of haemagglutination in RBC groups typing has been eroded by the molecular methods[4]. Nevertheless, serology is simple and quick to perform, it does not require a lot of equipment and it can easily be applied even in places with financial difficulties; in developed countries serology can be automated and enables high throughput, reducing subjectivity of obtaining, reading and interpreting results and improving traceability. Serology is still the "gold standard" method for RBC antigen typing and is sufficiently sensitive and specific for the majority of transfusions[5].

On the other hand, it is well known that serology has certain limitations and that genotype is not phenotype. In fact the phenotype, i.e. the molecular structure genetically determined on the RBC surface which could induce an immune response, is not only the expression of the coding sequence, but also depends on promoters, regulatory genes and splicing site interactions; moreover DNA mutations always occur. In fact false positive and negative results in genotyping are possible, because the test depends on the specificity of the primers for DNA polymorphisms. Thus in the literature we read that "genotype is not phenotype" but, better, it predicts RBC phenotype. Indeed, although RBC genotyping has a higher positive predictive value, it is always recommended that a predicted antigen type, particularly a negative one, should be confirmed by serology. In this way genotyping is neither alternative nor antithetical to serology, but can counterbalance the limitations of serology and vice versa. For example genotyping enables RBC typing in recently transfused patients, as described by the authors, and can contribute to resolving discrepancies concerning serological results.

Molecular techniques are more frequently applied in immunohaematology reference laboratories. In fact molecular methods are more expensive than haemagglutination and molecular genotyping of patients must be centralised to reduce costs; for the same reason, extended RBC genotyping of blood donors (for the management of rare blood registries) is also centralised. Apart from economic and logistic reasons, we think that genotyping should be performed in reference laboratories because the genotyping results should be interpreted in the context of serology by an immunohaematology specialist.

We, therefore, believe that a modern immunohaematology laboratory should maintain its serological and historical abilities and, at the same time, it should develop specific expertise in the molecular basis of blood group polymorphisms and in molecular technologies, both per se and applied to RBC antigen typing.

Molecular genotyping has emerged as an intriguing way to perform immunohaematology and it will be a potent tool to ensure better quality and effectiveness of transfusion therapy, but its full value can only be exploited in conjunction with serology.

The Authors declare no conflict of interest.

Blood Transfus 2015; 13: 164-5 DOI 10.2450/2014.0158-14
© SIMTI Servizi Srl

References

1) Rujirojindakul P, Flegel WA. Applying molecular immunohaematology to regularly transfused thalassaemic patients in Thailand. Blood Transfus 2014; **12**: 28-35.
2) Hillyer CD, Shaz BH, Winkler AM. Reid M. Integrating molecular technologies for red blood cell typing and compatibility testing into blood centers and transfusion services. Transfus Med Rev 2008; **22**: 117-32.
3) Westhoff CM Molecular DNA-based testing for blood group antigens: recipient-donor focus. ISBT Science Series 2013; **8**: 1-15.
4) Anstee DJ. Goodbye to agglutination and all that? Transfusion 2005; **45**: 652-3.
5) Reid ME. Applications of DNA-based assays in blood group antigen and antibody identification. Transfusion 2003; **43**: 1748-57.

Arrived: 30 June 2014 - Revision accepted: 7 October 2014
Correspondence: Silvia Manfroi
Servizio Immunoematologia e Trasfusionale
Policlinico S. Orsola-Malpighi
Via Massarenti 9
40138 Bologna, Italy
e-mail: silvia.manfroi@aosp.bo.it