Will Genotyping Replace Serology in Future Routine Blood Grouping? – Opinion 5

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The answer to this question is in our opinion: *Yes*, genotyping will in future replace serology almost completely. Only to avoid fatal transfusion reactions because of unrecognized null alleles of the ABO system in the recipient, either serologic typing to confirm the ABO blood group of the recipient or a short serologic cross-match will be done. Genotyping will lead to revolutionary changes in transfusion medicine. At present, transfusion policy is aiming to prevent haemolytic transfusion reactions in patients alloimmunised by previous transfusion or pregnancy. The genotyping approach will make prevention of alloimmunisation possible. We believe that this approach has the future because it is safer for the patient. Fully matched donor blood will be selected by ‘in silico’ matching. This will lead to reduction or even elimination of transfusion-induced alloimmunisation and, therefore, to prevention of immediate or delayed haemolytic transfusion reactions. Moreover, there will be less delay in donor blood selection. Although the costs for genotyping assays will inevitably exceed the costs of agglutination assays, many other costs will be saved. For instance, reduction in alloimmunisation will largely reduce the numbers of advanced serological investigations required. An economic analysis is needed to estimate the net effects. The genotyping approach fits perfectly well in the general trend in medicine where medical care will become more and more personalised and SNP genotyping will become part of the diagnostic work-up of patients to identify their disease susceptibility, treatment response, or risk for adverse reactions to certain drugs.

The questions *when* genotyping will replace serology and *to which extent* genotyping will be done, remains to be answered. In our opinion the implementation of genotyping will occur in several steps.

Firstly, routine genotyping will be introduced for donor typing in blood banks. The application of NAT technology for viral screening shows already that large-scale molecular methods can meet the demands regarding automation and sample

tracking in a blood bank setting. In fact, the high-throughput methods as described in this issue [1–5] are already operational in different blood banks, not only for research but also for routine purposes [6]. The main goal of these genotyping efforts is to increase the number of donors tested for multiple clinically relevant blood group antigens. Moreover, it makes typing possible for antigens for which no suitable anti-sera are available (e.g. Scianna and Dombrock alleles). In this way donor blood negative for high-frequency antigens becomes widely available, as well as donor blood with rare antigen combinations. This makes the need for ‘rare donor programs’ less urgent. Far more easily blood can be found for patients with multiple antibodies and for patients where serological investigation is hampered, for example due to the presence of red cell autoantibodies. In this regard, it is to be expected that especially countries in which the donors are currently less extensively phenotyped will benefit more from the implementation of genotyping than countries in which the serological phenotyping of donors is already more comprehensive, like in the Netherlands. In our country already 30% of the donors are phenotyped for RhCcDEe, K, Fya/b, Jka/b, and Ss.

The introduction of genotyping in blood banks has logistic advantages in comparison with recipient testing. Moreover, the inherent risk of genotyping assays is the false prediction of an antigen-positive phenotype because of the presence of silent or so-called null alleles. This will cause no problems in case of blood donor typing, whereas it can be harmful in patient testing.

A major advantage of most genotyping platforms above classical serology is that the donor can be genotyped for multiple antigens in a single assay. Most of the costs of genotyping assays are related to DNA isolation and PCR reagents, and less by the number of SNPs tested. We expect therefore that platforms which can test for all clinically relevant antigens will be preferred and that therefore donor typing will be done

for all these antigens. For the same reason, a single assay can meet the requirements for all ethnic populations.

It should be realized that at present applied genotyping approach is still not replacing serology, but is only an addition to ABO and Rh serology. Most, if not all blood banks will be reluctant to abandon serology. However, when hundreds of thousands of donors have been genotyped in the near future, it might become clear that serology can be safely omitted. Moreover, the recognition of *RHD* genes in donors that are falsely typed as D-negative by serology already shows that *RHD* genotyping of donors is superior to RhD serology [7].

At present, only a limited number of blood donors are being genotyped. However, once the investment in equipment and automation has been done, the extension of genotyping to larger groups of donors will become relatively easy. This will be dependent on the demand on matched blood units, which will be influenced by the extent of patient typing.

Therefore, we expect that the next step in the implementation of genotyping for routine blood grouping will be the extensive genotyping of patients who will be chronically transfused or who are at high risk for alloimmunisation. Schonewille et al. [8] have shown that patients who are already immunized have high risk for making additional antibodies, suggesting that these patients represent the group of responders to red cell antigens [9]. Preventive matching will start therefore for these patient groups. The genotyping assays developed for donor typing will be transferred to the hospitals. To be widely applicable for patient testing, technical developments are foreseen to make genotyping assays faster. Moreover, the knowledge gathered with blood donor typing will make the genotyping assays more accurate since a growing number of null or variant alleles will be recognized. Next-generation sequencing will be instrumental for the elucidation of these alleles, but we

do not expect that this will become the technology of choice for blood grouping. The recognition of those alleles is more relevant for patient typing than for donor typing; it might therefore be envisaged that different assays will be applied for donor and patient typing. Since preventive matching for multiple antigens will become feasible for many but not all patients, much research efforts have to be put into the unravelling of which genetic and/or disease-related factors predict a high alloimmunisation rate [9, 10].

The growing awareness of clinicians that it has become possible to prevent alloimmunisation, will increase the demand on extensively typed donor blood, not only for the groups of patients described above but also for others. This will be paralleled by an increase in genotyping efforts in blood banks. In the present situation it will be impossible to have for all patients fully matched blood available. It is obvious that priority should be given for the most immunogenic antigen systems (RhD, K, Rh c/C, Rh E/e, Jka/Jkb, Fya/Fyb and S/s), and next for systems involved in severe haemolytic transfusion reactions. At this moment it is difficult to predict for how many blood group antigens preventive matching will finally become feasible since electronic matching will alter the inventory management of red cell units and transfusion policy. Moreover, matching will become possible for more antigens when blood banks are scaled up, and the ethnic background of donor populations better reflects the ethnicity of patients. In our opinion one should aim for matching for all antigens which can be involved in haemolytic transfusion reactions.

More than 100 years ago transfusion medicine has been the first to apply immunological methods at a large scale. It may very well be that transfusion medicine will be among the first disciplines in which high throughput SNP typing is used to personalise therapy.

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