

**OPTN/UNOS Operations and Safety Committee ABO Workgroup
Meeting Minutes
May 2, 2019
Conference Call**

**Michael Marvin, MD, Chair
Christopher Curran, CPTC, CPTBS, CTOP, Vice Chair**

Introduction

The Operations and Safety Committee (OSC) ABO Workgroup met via teleconference on May 2, 2019 to discuss the following agenda items:

1. Recap of April 4th Meeting
2. Alternative Testing Methods and Availability
3. Takeaways from Membership and Professional Standards Committee (MPSC) Letter
4. Review of Established Goals
5. Next Steps

The following is a summary of the Operations and Safety Committee ABO Workgroup discussion.

1. Recap of April 4th Meeting

The Vice Chair provided an overview of the discussion from the April 4th workgroup meeting.

Summary of Discussion

The Vice Chair provided the workgroup with a review of the April 4th workgroup meeting. During the last workgroup's meeting, there was a review of the established goals and a debrief of the Membership and Professional Standards Committee (MPSC) letter to the Operations and Safety Committee (OSC) on considerations in moving forward with the ABO project.

A subject matter expert (SME) volunteered to review and provide a response to the MPSC letter from a blood banking perspective.

2. Alternative Testing Methods and Availability

An SME presented and discussed alternative testing methods with workgroup members.

Summary of Discussion:

An SME provided information on alternative testing methods for ABO. The SME reviewed ABO genotyping methods and discussed various scenarios.

Genotyping has often been from the transfusion perspective and not from the transplantation perspective. Genotyping is typically done when there is typing discrepancy between the forward and reverse reactions, which can be picked up by serology and is used when trying to resolve the discrepancy. This requires looking at other antigens after transfusions that were not done during serology of the first sample.

The SME continued by stating that in regards to buccal swabs, they are not needed. When red cells are genotyped, the process is done from white blood cells (WBCs). There have not been wide scale studies on genotyping that have been generally found acceptable even if transfused with red blood cell (RBC), platelets (PLT), and plasma. It was added that some of the assays do not work well with buccal swabs.

The SME discussed the main assays that are on the market:

- Low Density Single Neucleotide Polymorphism (SNP) Typing: The only FDA approved assays for labeling blood products in the United States; do not include ABO.
- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass (MALDI-TOF) SNP Typing: Beneficial and cheap to run. There are a growing number of assays to look at different molecular changes with this technology. These assays have a 99.2% accuracy.
- Single Specific Primer/Allele Specific Polymerase Chain Reaction (SSP/AS-PCR) Typing: Runs by gels. Has not been widely adopted with the transfusion community.
- Sanger Sequencing: Not typically done outside of reference labs for ABO as the technique is laborious. This method is often employed when looking at specific regions or allele specific PCR.
- Real Time Polymerase Chain Reaction (PCR): There are currently two assays that are on the market – SYBR and Dye Quencher. These assays are expensive but are not prohibitive in terms of the cost of a transplant. They are prohibitive in the terms of donor screening at mass scale. The typing has the same work flows as the human leukocyte antigen (HLA). There are some ambiguities which causes the inability to get to every ABO allele. From testing, there has been 100% concordance with the serology and works better also for subtyping. In regards to swabs, the SYBR assay does not work very well with the swabs; it is uncertain if the Dye Quencher assay can do swabs. This type of assay would be typical of what a transplantation lab would run to do ABO genotyping.

The SME continued by discussing work around High Density SNP typing, which is a low cost assay with higher coverage. There is currently a high density array blood typing pilot with 8,000 samples, which has shown 99.92% accuracy for 48 antigens and 99.85% accuracy for ABO. The error rate is very low (0.1%) and is expected to continue to decrease.

The SME continued with an explanation of Next Generation Sequencing (NGS Typing). Most of the changes that are being observed are identified with this testing. The phases can extend similar to HLA. From swabs, this type of typing can in general only reliably amplify exons 6 to 7. Most of the changes needed to be observed are within these exons, which makes typing available for this method. If there is a blood sample, the entire gene can be amplified.

The SME presented three cases with the workgroup to discuss the best testing method approach.

- ABO Case #1: Deceased Donor
 - Serology: several lectin tests were performed at various labs. There were a range of results of non-A1 and A1. A Real Time PCR test was done that revealed the patient as A2/O. There was further testing of targeted NGS, which resolved down to a full allele of ABO*A2.01/*O.01.01. The SME clarified that there are about 70 different forms of that O deletion and to be able to get to the full allele resolution requires NGS.
- ABO Case #2: Deceased Donor
 - Serology: there was a mixed field where the deceased donor was thought to be A, but was transfused with O. A Real Time PCR revealed the donor as A1/O. Further testing of targeted NGS showed the donor to being ABO*A1.01/*O.01.02.
- ABO Case #3: Massively transfused donor
 - Serology: The deceased donor was typed as B POSITIVE, then massively transfused with 12 units of red blood cells. A Real Time PCR revealed the deceased donor as B/O and further testing with targeted NGS showed the deceased donor as ABO*B.01/*O.01.01.

The hope would be that the Real Time PCR would be able to be performed alongside clinical work flows to resolve any doubts in typing.

The SME summarized that:

- Several RBC genotyping assays use technology similar to HLA assays
- Swab testing is generally not needed as WBC are used for DNA source
- ABO Realtime and SSP take hours
- ABO NGS is the gold standard, but takes a few days
- Although ABO genotyping will never be 100% accurate, it is a powerful adjunct in conjunction with serology

The Vice Chair asked that in the research and experience that forms the reliability of the various testing methods, how many of the patients in the testing experienced massive transfusions. The SME stated that the literature that has been reviewed involved sickle cell patients, where only one or two were massively transfused. There are no known studies where there is a large sample of people studied who have been massively transfused. If the DNA source is accurate for the HLA typing, there would be no thought of why it would be contaminated for red cell genotyping. The Vice Chair clarified that since the DNA source involves white cells, it would be more reliable. The SME confirmed that this is true as most of the white cells would be coming from the recipient.

Another SME stated that the targets are not the minority population and rather amplifies everything. The PCR step is the whole population of the alleles. Any contaminate is amplified out in the PCR reaction.

The Vice Chair continued by asking how long the Real Time PCR takes to determine an ABO. The SME stated that it takes approximately 90 minutes to determine results. The Vice Chair asked if the machines that the assays are run on routinely available within HLA labs. The SME confirmed that this was correct and stated that the assays would be well suited.

The Vice Chair asked if the tests have to be conducted one at a time on the real time PCR. The SME stated that it was believed that the assays are being sold in single use kits as well as kits that run about eight samples at one time.

There were no further comments or questions.

3. Takeaways from Membership and Professional Standards Committee (MPSC) Letter

An SME provided members with a summary of takeaways from a blood typing perspective in response to considerations made by the MPSC.

Summary of Discussion

The SME began with an explanation of the first question about the actual definition of mass transfusion, how it relates to patient size and when it would impact the patient's blood type. The SME explained that in determining when the massive transfusion impact blood type, there is no specific answer as it would depend on the patient's size, how many units, and which types of products they received. It is ideal to get a pre-transfusion sample, but if this is not possible, it is recommended to get a blood sample within the first 30 minutes of a massive transfusion. The SME added that if a blood type is known, it is best to have a transfusion that matches best with the patient's blood type to prevent using the universal donor group O red cells.

The Vice Chair commented that in regards to pre-transfusion samples and putting this in the context of deceased donation, there are times where there may be one sample available, but OPTN policy requires at least two samples. Although there is one sample, there is not another sample available to rely on.

The SME continued with the next question around what the other reasons were that there may be a discrepancy with reverse typing without massive transfusions. There are many reasons besides transfusion that could result in a discrepancy. Some of the reasons can be due to:

- ABO subgroups
- Previous transplantation
- Presence of non-ABO alloantibodies or autoantibodies
- Infusion of Intravenous Immunoglobulin (IVIg)
- Hypogammaglobulinemia, and age-related (either infants or elderly patients)

There are protocols, algorithms, and testing methods within the lab that can be used to resolve the discrepancy. It may be different within each lab on how the discrepancies are reported out, which would need to be further discussed among staff.

An SME commented that this section is very important and that there have been other cases where other variables come into play besides just massive transfusions. The Vice Chair clarified that the focus of this project will not just be massive transfusions but instead education and guidance on what to do when there are challenges with determination of ABO. The Vice Chair added that the policy revisions that will be proposed will address what should be done for any results that are in question, not just conflicting results.

Another SME also agreed that this would be a good focus of the workgroup. From experience of drawing healthy blood donors at the blood center, the most common causes of ABO discrepancies among these individuals are those who are weak subgroups and group O donors who have low titer

There were no additional questions or comments.

4. Review of Established Goals

The Vice Chair provided an overview of the established goals of the workgroup.

Summary of Discussion:

The Vice Chair reviewed the workgroup's established goals. The first goal of identifying alternative methods of ABO typing will be an important section of the guidance document. The Vice Chair suggested that there should be language included in policy that suggesting that if there is a concern about the reliability of testing with conflicting or indeterminate results, Organ Procurement Organizations (OPOs) employ testing that can be in a timely and more reliable way such as the Real Time PCR.

The Vice Chair continued by stating that the impact of massive transfusion on blood type determination is a topic that the workgroup should get more education on and should be discussed further during an upcoming call. When discussing protocols and definitions, this the focus not only be on defining what massive transfusions protocols are, but also protocols for OPOs providing steps that can be taken to mitigate risk when it comes to ABO.

The Vice Chair asked the SMEs their prospective on the established goal of identifying the time between blood samples – from the time that a donor is massively transfused, what timeframe has to pass? An SME stated that this is a challenging question to answer as the size of a patient can be a factor. Having a patient being flown in or transported in can be challenging to determine what transfusions may have occurred. Due to this, it can create a challenge to determine timing. Another SME agreed with this and added that what may help a bit in addressing this point would be in having lab techs more familiar with how to read mixed reactions.

The Vice Chair stated that there was an instance where there was a result from the tissue typing lab and donor hospital that did not raise any questions. The infectious disease lab delivered a discrepant result due to receiving mixed fields.

An SME stated that infectious disease testing is done on an automated platform so the cutoffs are very different. Some guidance on some mixed fields and training on how to deal with these samples would be important. The Vice Chair stated that most of the source documentation is from outside organizations. The Vice Chair asked if the most common methods of ABO typing by blood banks are the gel card or tube methods. The SMEs stated that both methods are common.

The Vice Chair continued by stating that the goal of this project is to educate the community and it was agreed by the workgroup that in addition to the guidance document, there would also be policy language changes.

There has been some discussion on the types of change within trauma hospital protocols related to massive blood transfusions and it was suggested that there could be a summary of what some of those protocols are within the guidance document.

There were no further questions or comments.

5. Next Steps

The Vice Chair discussed next steps of the ABO project with the workgroup.

Summary of Discussion:

The Vice Chair stated that there will be a more in depth discussion to better understand when a patient or donor might go from indeterminate results based on their size or what blood products they received and how to detect this. Now that the workgroup has a course of action, the project form could now be updated for submission to the Policy Oversight Committee (POC).

The Vice Chair will reach out to the blood bank SMEs to discuss more about defining massive transfusion as well as determining if there is a way to develop an algorithm or prediction of when a donor ABO may go from unreliable to reliable

The Vice Chair continued by stated there will be discussion during an upcoming call on the protocols that different OPOs have to addressing discrepant results. A member stated that this would be a good topic to discuss and to share best practices. There is a variation of issues that come up and it is unknown of whether this is documented. The Vice Chair suggested to having this topic added on the procurement council agenda at the AOPO meeting and get anecdotes or case studies to share with the workgroup.

There were no additional questions or comments. The meeting was adjourned.

Upcoming Meetings

- June 6, 2019 (Teleconference)
- July 9, 2019 (Teleconference)