Title

Discrepant subtyping of blood type A2 living kidney donors: Missed opportunities in kidney transplantation

Journal

Clinical Transplantation, 35(10)

ISSN

0902-0063

Authors

Garg, Neetika
Warnke, Leza
Redfield, Robert R
et al.

Publication Date

2021-10-01

DOI

10.1111/ctr.14422

Peer reviewed

HHS Public Access

Author manuscript

Clin Transplant. Author manuscript; available in PMC 2023 March 15.

Published in final edited form as:

Clin Transplant. 2021 October ; 35(10): e14422. doi:10.1111/ctr.14422.

Discrepant subtyping of blood type A2 living kidney donors: Missed opportunities in kidney transplantation

Neetika Garg[1], **Leza Warnke**[2], **Robert R. Redfield**[3], **Karen M. Miller**[2], **Matthew Cooper**[4], **Garrett R. Roll**[5], **Valerie Chipman**[6], **Alvin Thomas**[7], **David Leeser**[8], **Amy D. Waterman**[9,10], **Didier A. Mandelbrot**[1]

[1]Division of Nephrology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[2]UW Health, Madison, Wisconsin, USA

[3]Transplant Surgery, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

[4]MedStar Georgetown Transplant Institute, Washington, District of Columbia, USA

[5]Transplant Surgery, University of California, San Francisco, California, USA

[6]Donor Network West, San Ramon, California, USA

[7]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[8]Department of Surgery, East Carolina University, Greenville, North California, USA

[9]Division of Nephrology, University of California, Los Angeles, California, USA

[10]Terasaki Institute of Biomedical Innovation, Los Angeles, California, USA

Abstract

Background: Despite the institution of a new Kidney Allocation System in 2014, A2/A2B to B transplantation has not increased as expected. The current Organ Procurement and Transplantation Network policy requires subtyping on two separate occasions, and in the setting of discrepant results, defaulting to the A1 subtype. However, there is significant inherent variability in the serologic assays used for blood group subtyping and genotyping is rarely done.

Methods: The National Kidney Registry, a kidney paired donation (KPD) program, performs serological typing on all A/AB donors, and in cases of non-A1/non-A1B donors, confirmatory genotyping is performed.

Correspondence: Neetika Garg MD, 1685 Highland Ave, 4171, Medical Foundation Centennial Building, Madison, WI 53705, USA. ngarg@medicine.wisc.edu.

AUTHOR CONTRIBUTIONS

Neetika Garg, Leza Warnke, and Didier A. Mandelbrot designed the study and drafted the manuscript. Neetika Garg, Leza Warnke, Alvin Thomas, and Didier A. Mandelbrot analyzed the data. All authors participated in interpretation of data and revised the manuscript. All authors approved the final draft of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Results: Between 2/18/2018 and 9/15/2020, 13.0% (145) of 1,111 type A donors registered with the NKR were ultimately subtyped as A2 via genotyping. Notably, 49.6% (72) of these were subtyped as A1 at their donor center, and in accordance with OPTN policy, ineligible for allocation as A2.

Conclusion: Inaccurate A2 subtyping represents a significant lost opportunity in transplantation, especially in KPD where A2 donors can not only facilitate living donor transplantation for O and highly sensitized candidates, but can also facilitate additional living donor transplants. This study highlights the need for improved accuracy of subtyping technique, and the need for policy changes encouraging optimal utilization of A2 donor kidneys.

Keywords

A2 subtyping; disparities; genotyping; living donor transplantation

1. | INTRODUCTION

In the United States, blood type B kidney transplant candidates have the longest wait-times and blood type O candidates have the second longest wait-times.[1] Additionally, more blood type B patients with end-stage kidney disease (ESKD) than any other blood type belong to ethnic minority groups, whose access to kidney transplantation, and in particular living donor kidney transplantation, is already limited.[2] As a consequence, access to kidney transplantation for these minority subpopulations is further exacerbated.

Blood type A consists of two serologic subtypes: A1 and non-A1. Serologically, A1 is distinguished from non-A1 by the reactivity of anti-A1 lectin, which agglutinates A1 red blood cells (RBC) in a suspension. When blood type A RBCs are not agglutinated, they are labeled as non-A1. Similarly, AB blood type is classified as A1B and non-A1B. Nearly 20% of A and AB blood types are non-A1 and non-A1B.[3] Most non-A1 individuals are subtype A2, and A2 is often used as a shorthand for non-A1, but several other non-A1 subtypes exist, such as, Aint, Aend, and Ax. Most centers rely on serologic testing to differentiate between A1 and non-A1, however, the available assays are not standardized and there is significant inherent variability in test results.[4] Subtyping of A and AB donors is important in kidney transplantation because among non-A1 individuals, the A antigenic expression is both quantitatively and qualitatively lower. As a result, kidneys from non-A1 (or non-A1B) donors can be successfully transplanted into blood type B or O recipients (or B) using the same immunosuppression protocols as ABO-compatible transplantation, without desensitization, as long as anti-A titers are low in the recipient at the time of transplantation.[5]

With the goal of improving equity by increasing access to transplantation for blood type B and minority populations in the United States, the Kidney Allocation System (KAS) implemented in December 2014 preferentially allocates non-A1 and non-A1B kidneys to B candidates.[6] However, subsequent studies show the full potential of this policy has not been realized. The most recent available Organ Procurement and Transplantation Network (OPTN) report of 2018 commented that despite the KAS provision, transplant rates among B candidates had not increased markedly.[1] Another recent analysis showed that

compared to whites, there was no difference in the likelihood of non-A1 to B transplants in minority populations.[7] While the reasons for this are not fully understood, they are likely multifactorial.

While there is no provision for allocation of non-A1 kidneys to O candidates in deceased donor transplantation, the same set of principles apply to living donor kidney transplantation. This becomes especially relevant in kidney paired donation (KPD). Because O donors can donate to ESKD candidates of any blood type (as long as they are immunologically compatible), KPD donor pools are inherently imbalanced, and the number of A donors usually exceeds that of A candidates seeking transplantation. In the KPD context, appropriate identification of non-A1 donors can substantially improve living donor transplant options for O candidates, as well as highly sensitized candidates of any blood type. In addition, the use of non-A1 donors to start PKD chains with O recipients can significantly increase the number of patients who benefit from live donation.

The National Kidney Registry (NKR) is a nonprofit 501c organization and the largest kidney paired donation (KPD) program in the United States. All A and AB potential donors are serologically subtyped, and if identified as non-A1, confirmatory genotyping is done. In this study, we utilized NKR's data to explore the degree of discrepancy in subtyping obtained through the NKR versus the participating transplant center laboratories, and the impact it may have on utilization of non-A1 and non-A1B kidneys into type B and O recipients.

2. | METHODS

2.1. | OPTN policies on ABO subtyping for kidney donors

Clinical policies about how to perform transplants that are primary blood type incompatible but are done using the aforementioned subtyping results are determined by the transplant program. As per OPTN Policy 8.5.D, transplantation of non-A1 or non-A1B kidneys into B candidates or non-A1 into O candidates requires that, (1) the transplant program establish a written policy regarding its recipient titer threshold for transplantation of such kidneys, and (2) the transplant program obtain informed consent from each blood type B/O candidate regarding their willingness to accept a non-A1 kidney.[8]

OPTN mandates that all donors be blood typed on two separate occasions, and yield the same results.[9] When non-A1/non-A1B to B or non-A1 to O transplants are planned, OPTN similarly requires donor subtyping on two separate occasions, and proceeding as non-A1/non-A1B only if the two tests yield the same results. The policy notes "it is never acceptable to use two out of three results for a subtype determination. If there are any discrepant results, then only primary type can be used for allocation." Of note, this subtyping is mandatory for blood type A deceased donors, and optional for blood type AB deceased donors and all living donors. Importantly, there are no standards for how laboratories should report ABO subtypes, and many indeterminate A and AB subtyping results are by default reported as A1 and A1B, respectively. Additionally, the OPTN policies do not comment on use of genotyping to accurately determine the subtype.

2.2. | NKR's policies on ABO subtyping for kidney donors

As of February 18, 2018, the NKR has been subtyping all A and AB donors using the VRL Eurofins Laboratory.[10] Samples determined to be non-A1 and non-A1B undergo sequence-based genotyping at the Histogenetics laboratory.[11] All known serological motifs are located in three exons (exon 2, exon 6, and exon 7). If any discrepancies between DNA sequence-based genotyping and serological phenotyping are observed, entire exons are sequenced.

2.3 | Data collection and analysis

Deidentified information was obtained on blood type subtyping on all blood type A and AB donors registered with the NKR between February 18, 2018 and September 15, 2020. To assess the percentage of A2 donors, as well as to assess the degree of discrepancy between various tests, the data included the blood type and subtype provided by the donor center, as well as the subtyping determined by VRL Eurofins' serologic testing and by Histogenetics' DNA sequencing.

The clinical and research activities of this study are consistent with the Declaration of Helsinki and Declaration of Istanbul. The Institutional Review Board at the University of Wisconsin-Madison determined this project to be exempt from formal review.

3. | RESULTS

3.1. | Proportions of A2 and A2B donors

Over the study period, 1111 blood type A donors were registered with the NKR. Of the A donors, 13.0% (145) were serologically typed as non-A1 and confirmed to be of the A2 subtype after genotyping. Additionally, 129 AB donors were also registered with the NKR. 22.7% (27) were serologically typed as non-A1B and confirmed to be of the A2B subtype by genotyping.

3.2. | Determination of A2 and A2B subtyping

Of the 145-blood type A2 donors, 49.6% (72) were subtyped as A1 at their donor center but determined to be non-A1 by the VRL Eurofins Laboratory and subsequently genotyped as A2 (Table 1). An additional 45.5% (66) of the A2 donors were either not ABO typed or A subtyped at their center. Only 4.8% (7) of A2 donors were subtyped as non-A1 at the donor center and confirmed to be A2 through NKR's protocol.

Of the 27-blood type A2B donors, 14.8% (4) were determined to be A1B at their donor center, but found to be non-A1B by the VRL Eurofins Laboratory testing and confirmed to be of the A2B genotype. An additional 25.9% (7) of the A2B donors did not have ABO typing or AB subtyping performed at their center. 59.2% (16) were subtyped as non-A1B at their center and subsequently confirmed to be A2B through NKR's protocol including genotyping.

3.3. | Donors with discrepant subtyping results

There were 79 donors with a discrepancy between the subtype reported by the donor center and that determined by the VRL Eurofins Laboratory. The most common scenario, in 91.1% (72) of these cases, was that the subtype was reported as A1 at the donor center but non-A1 using the VRL Eurofins serology; all of these were confirmed to be of the A2 subtype by genotyping. There were 6.3% (5) cases where the subtype was determined to be A1B by the donor center, but VRL Eurofins reported as A2B. Four of these were confirmed to be A2B by the sequencing methodology. Confirmatory testing was not performed in one individual.

In addition, there was one donor registered as non-A1, and ultimately determined to be A1. Upon review by the NKR, this was determined to be a data entry error by the donor center. There was one additional case where the donor was subtyped by the donor center as non-A1; while VRL Eurofins' testing found the donor to be of the A1 subtype, sequencing confirmed that the donor was indeed A2.

4. | DISCUSSION

The 2014 KAS policy directed at improving transplantation rates for blood type B candidates was based on prior data showing that the outcomes achieved in non-A1/non-A1B to B transplants were similar compared to ABO-compatible transplants, as long as the recipients had low anti-A antibody levels.[5,12–14] Additionally, while there are no recent reports on what proportion of blood type B candidates have low anti-A levels, older reports from the pre-KAS Midwest Transplant Network that routinely performed such transplants reported a large majority of blood type B candidates (77% of whites and 69% of blacks) had consistently low anti-A titers,[14] and that 23–34% of their B candidates received non-A1 or non-A1B kidneys.[13,14] However, recent OPTN reports and other analyses show that following implementation of the new KAS, transplant rates for B candidates have not increased as expected.[1,7] While there are no published data on trends of non-A1 to O transplantation, since these transplants occur only in the living donor setting and there is no policy mandating subtyping of blood type A living donors, we anticipate non-A1 to O transplantation is even more underutilized. Factors for non-A1 underutilization potentially include the need to develop detailed protocols, barriers to testing anti-A titers regularly, limited availability of genotyping for group A subtypes, transplant center's experience with such transplants, and higher costs.[15] In this manuscript, we explored NKR's data and identified barriers to accurately subtyping blood type A and AB donors as a likely additional contributing factor.

During the study period, 13.0% of A donors were of the A2 subtype. Remarkably, of the 79 donors who had subtyping performed at their donor center and were confirmed to be of the A2 subtype through genotyping, 91.1% (72) were labeled as A1 by their center. Lack of a standardized assay is likely the biggest reason underlying this high level of discrepancy. The reagent used to define the non-A1 subtype is made by diluting the lectin Dolichos biflorus to a point where it loses reactivity against non-A1 but retains reactivity with A1 RBCs, and is variable depending on the phenotype of the panel of RBCs used to prepare it. This is because there is significant variability in the blood type A molecules present on the RBC surface of A1 (who genotypically can be A1/A1, A1/A2 or A1/O) and A2 individuals

(who genotypically can be A2/A2 or A2/O).[4] Additionally, the genotyping of the donor candidate also likely has a bearing on the antibody titer. As a consequence of the inherent variability in the test, blood banks relied upon by many transplant centers for subtyping are likely underreporting non-A1 subtypes when indeterminate results are obtained. For blood banks to err on the side of reporting borderline subtyping results as A1 is to be expected for blood transfusions, given the significant additional time and expense that would be required to genotype blood donors, and the risk of hemolysis. This is corroborated by our own data on deceased donors at the University of Wisconsin: only 6.3% (22/347) of A donors processed through our Organ and Tissue Donation service since the KAS went into effect on December 4, 2014 were determined to be of the non-A1 subtype. There is also variability in how each individual laboratory determines whether the subtype is A1 versus non-A1, often yielding indeterminate results. In the context of transplantation, our data highlight that the lack of standardized assays for subtype assessment represent one barrier to non-A1/non-A1B utilization.

Addressing the limitations of the current subtyping technique is important in reducing disparities in kidney transplantation. Based on OPTN data as of April 9, 2021, in 2020, 480 (10.3%) of 4663 deceased donors and 51 (3.1%) of 1625 living donors were registered as non-A1. Based on our study data, which shows that current subtyping methodologies identify only half the non-A1s as such, correct subtyping might have yielded an additional 1011 non-A1 kidneys (two per each deceased donor and one per each living donor). Similarly, if we assume 20% of blood type A donors are of the non-A1 subtype, subtyping all candidates accurately would have yielded 1180 additional non-A1 kidneys. In deceased donor transplantation, increased use of non-A1 kidneys for B recipients would be expected to reduce disparities for blood type B and minority candidates. In directed living donation, this may allow certain transplants for B and O candidates that would have otherwise been considered incompatible. And in KPD, strategic use of non-A1 kidneys can promote *living* donor transplantation for (1) O candidates (who tend to wait the longest in KPD), (2) highly sensitized candidates of any blood type (as long as their anti-A titers are low), and (3) importantly, facilitate additional transplants by starting chains and increasing options for exchanges. In the NKR, as in any KPD program, the number of O transplant candidates far exceeds the number of O donors. For example, over the study period included in our study, only 39% of donor candidates, as opposed to 57% of recipient candidates were of blood type O. A pair with a blood type O donor and a non-blood type O recipient is considered a “favorable pair.” Compatible pairs (where directed donation is possible, but the pair decides to enter KPD to obtain a more optimal kidney for the recipient) greatly help alleviate the imbalance, by making the KPD pool bigger and by infusing favorable pairs into the system. A recent analysis of 151 compatible pairs entered in the NKR between February 2008 and November 2018, half of whom where favorable pairs, documented that each compatible pair facilitated two additional transplants.[16] non-A1 donors, although not universal donors, if used strategically would be expected to yield a similar increase in transplantation through transplantation of sensitized candidates of any blood type, and increasing options for exchanges and starting chains in KPD.

This is the first study to report use of genotyping for subtyping A/AB donors.[17] In deceased donors, use of genotyping would obviate the requirement for blood type testing prior to the

administration of blood products. However, the 14–21 day turnaround time for genotyping (as opposed to 8–12 h for serological testing) make its use impractical. In living donors, it calls to attention the need to consider revision of the OPTN policy. In its current form, in order to allocate an organ as non-A1/non-A1B, two concordant results are required, and a third tie-breaker test, regardless of the type of test, is not allowed. Given the risk of hyperacute rejection in the setting of an inadvertent ABO-incompatible transplant, we agree with the policy to not allow use of just a discordant serologic test to determine the subtype. However, given the inherent inconsistencies in serologic typing, if the donor subtype is confirmed to be A2/A2B based on genotyping, in our opinion, those kidneys should be allowed by allocated as non-A1. Until such rule changes are made, one specific approach to avoiding this situation in the context of the NKR is to not subtype A donors locally. By relying on the serological typing from VRL Eurofins and genotyping from Histogenetics, A2 donors can be identified accurately without the risk of having been previously been mistyped as A1, which prevents proceeding with the transplant as an A2. We are aware of at least one program who had a donor subtyped as A1 at their center but subsequently was serotyped as non-A1 and genotyped as A2. They proceeded with transplantation to a B recipient, and the transplant was uncomplicated, but led to a notice of noncompliance from the United Network for Organ Sharing because of the seeming discrepancy in the subtyping in the records.

One limitation of our study is that we did not have details of the subtyping performed at the donor centers regarding number and types of tests performed, and whether any discrepancies were found. In addition, the pool of A and AB donors entered into KPD may not be representative of the overall donor population, as certain donors determined to be non-A1/non-A1B may have been able to donate directly to their B or O recipients. Lastly, if the Eurofins serologic testing yields an A1 result, genotyping is not pursued, and there still remains a possibility that non-A1 donors are missed.

To conclude, our study shows that the limitations of the current blood type A and AB subtyping technique represent one barrier to identifying eligible donors for non-A1/non-A1B to B or non-A1 to O transplantation. This underscores the importance of standardizing serological subtyping. In living donor transplantations and in KPD where usual timelines allow genotyping, refining OPTN policies to allow use of genotype results for subtype assessment in the face of discrepant serological results would be expected to enhance utilization of these organs. Further studies are needed to address other barriers to pursuing these transplants, including lack of standardized assays for assessment of anti-A titers, which is needed for candidate eligibility determination, as well as further understand practices pertaining to these transplants at individual center level.

ACKNOWLEDGMENTS

We thank the NKR for providing data for this project. Didier A. Mandelbrot is the recipient of an unrestricted research grant from the Virginia Lee Cook Foundation, which supported this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2018 annual data report: kidney. Am J Transplant. 2020;20(Suppl s1):20–130. [PubMed: 31898417]
2. Young CJ, Gaston RS. Renal transplantation in black Americans. N Engl J Med. 2000;343(21):1545–1552. [PubMed: 11087885]
3. Breimer ME, Samuelsson BE. The specific distribution of glycolipid-based blood group A antigens in human kidney related to A1/A2, Lewis, and secretor status of single individuals. A possible molecular explanation for the successful transplantation of A2 kidneys into O recipients. Transplantation. 1986;42(1):88–91. [PubMed: 3523889]
4. Bryan CF, Polesky H, Eisenbrey AB, et al. Implications of ABO error rates in proficiency testing for solid organ transplantation. Transplantation. 2006;82(6):733–736. [PubMed: 17006316]
5. Bryan CF, Cherikh WS, Sesok-Pizzini DA. A2 /A2 B to B renal transplantation: past, present, and future directions. Am J Transplant. 2016;16(1):11–20.
6. OPTN (Organ Procurement and Transplantation Network)/UNOS (United Network for Organ Sharing). OPTN Policies, Policy 14: Living Donation. http://optn.transplant.hrsa.gov/ContentDocuments/OPTN_Policies.pdf. Accessed March 19th, 2021, 2020.
7. Martins PN, Mustian MN, Maclennan PA, et al. Impact of the new kidney allocation system A2/A2B→B policy on access to transplantation among minority candidates. Am J Transplant. 2018;18(8):1947–1953. [PubMed: 29509285]
8. OPTN Policies. https://optn.transplant.hrsa.gov/media/1200/optn_policies.pdf. Accessed September 6, 2020, 2-2-.
9. Guidance for ABO subtyping organ donors for blood types A and AB. https://optn.transplant.hrsa.gov/media/2505/osc_guidance_abo_201806.pdf. Accessed September 6, 2020, 2020.
10. VRL Eurofins ABO subtyping. https://labs-inc.org/tests/directory/71. Accessed September 15th, 2020.
11. Histogenetics. https://www.histogenetics.com/#services Accessed September 15th, 2020.
12. Bryan CF, Winklhofer FT, Murillo D, et al. Improving access to kidney transplantation without decreasing graft survival: long-term outcomes of blood group A2/A2B deceased donor kidneys in B recipients. Transplantation. 2005;80(1):75–80. [PubMed: 16003236]
13. Bryan CF, Shield CF, Nelson PW, et al. Transplantation rate of the blood group B waiting list is increased by using A2 and A2B kidneys. Transplantation. 1998;66(12):1714–1717. [PubMed: 9884265]
14. Nelson PW, Shield CF, Muruve NA, et al. Increased access to transplantation for blood group B cadaveric waiting list candidates by using A2 kidneys: time for a new national system?. Am J Transplant. 2002;2(1):94–99. [PubMed: 12095063]
15. Shaffer D, Feurer ID, Rega SA, Forbes RC. A2 to B kidney transplantation in the post-kidney allocation system era: a 3-year experience with anti-A titers, outcomes, and cost. J Am Coll Surg. 2019;228(4):635–641. [PubMed: 30710615]
16. Chipman VLB, Cooper M, Cuffy MC, et al. Abstract: compatible pairs in paired kidney exchange—are there winners and loosers?. Am J Transplant;19(Supplement 3).
17. Westhoff CM. Blood group genotyping. Blood. 2019;133(17):1814–1820. [PubMed: 30808639]

TABLE 1

Determination of A2 and A2B subtyping results

	Non-A1 by serologic testing and confirmed as A2 by DNA sequencing	Non-A1B by serologic testing and confirmed as A2B by DNA sequencing
Typed as non-A1 or non-A1B at donor center, confirmed as A2 or A2B by NKR's testing	7	16
Typed as A or AB, subtype unknown at donor center, and subsequently typed as A2 or A2B by NKR's testing	50	5[a]
ABO unknown at donor center, and subsequently typed as A2 or A2B by NKR's testing	16[b]	2[c]
Typed as A1 or A1B at donor center, and subsequently typed as A2 or A2B by NKR's testing	72	4[d]
Total number of A2 or A2B	145	27

[a]One additional donor was resulted as non-A1B by serologic testing, but was A1B by molecular testing.

[b]One additional donor resulted as non-A1 by serologic testing, but confirmatory molecular testing was not performed.

[c]One additional donor resulted as A2B by serologic testing, but was A1B by molecular testing.

[d]One additional donor resulted as non-A1B by serologic testing, but confirmatory molecular testing was not performed.