An in Vitro Comparison of Intra-Operative Isohemagglutinin and Human Leukocyte Antigen Removal Techniques in Pediatric Heart Transplantation

Emily A. Hayes [1], Ashley Walczak [1], Erin Goodhue Meyer [2], Kathleen Nicol [3], Matthew Deitemyer [1], Vicky Duffy [1], Michelle Moore Padilla [4], Robert J. Gajarski [1], Deipanjan Nandi [1]

[1] The Heart Center, Nationwide Children's Hospital, Columbus OH; [2] Department of Hematology/Apheresis, Nationwide children's Hospital, Columbus OH; [3] Department of Pathology, Nationwide Children's Hospital, Columbus OH [4] Division of Cardiology, Department of Pediatrics, Emory University School of Medicine, Atlanta GA

Key words: Isohemagglutinin; Human Leukocyte Antigen; Plasmapheresis; Pediatric Heart Transplantation; Cardiopulmonary Bypass

Disclosure: No conflicts of interest

Corresponding author: Emily Hayes - emily.hayes@nationwidechildrens.org

Address: 700 Children's Drive, Columbus OH 43205

Phone Number: 614-722-6047

Article available at https://ject.edpsciences.org or https://doi.org/10.1051/ject/2023034

ABSTRACT:

Background: Highly sensitized pediatric patients awaiting heart transplantation experience longer wait times and higher waitlist mortality. To improve the likelihood of successful transplantation, various strategies have been utilized, including peri-operative plasmapheresis, to broaden potential donor pools.

Methods: This *in vitro* study utilized two membrane-based plasmapheresis (MP, Prismaflex) and two centrifuge-based plasmapheresis (CP, Spectra Optia, Terumo BCT) circuits incorporated into four separate extracorporeal (EC) circuits primed with high titer, highly sensitized type O donor whole blood. Assays were performed to determine baseline anti-A and B isohemagglutinin titers (IT) and anti-human leukocyte antigen (HLA) antibodies and then at 30-minute increments until completion of the run at two hours.

Results: There was a decrease in anti-A and anti-B IgM and IgG titers with both MP and CP. Mean anti-A and anti-B titer reduction was by 4.625 titers (93.7% change) and 4.375 titers (93.8% change) using MP and CP, respectively. At two hours of apheresis, CP reduced mean fluorescence intensity (MFI) by 2-3.5 fold and MP reduced MFI by 1.7-2.5 fold.

Conclusions: In this *in vitro* plasmapheresis model of IT and anti-HLA antibody reduction, both MP and CP can be used quickly and effectively to reduce circulating antibodies, and CP may have some greater degree of efficiency. Further *in vivo* research on incorporating CP or MP into EC circuits at the time of transplant is needed. However, with further clinical research there is potential to broaden potential donors and improve patient safety in sensitized recipients.

Introduction:

Heart transplantation remains the most effective treatment for patients with advanced heart failure refractory to medical management (1). Over the past several decades, the outcomes following pediatric heart transplantation have continued to improve with a 1-year survival of over 90% (2). However, the waitlist mortality in the United States remains high at 17%, ultimately limiting the use of this treatment in the pediatric patient population (3). Furthermore, waitlist times are prolonged for smaller and highly sensitized children, leading to increased mortality in these patients (4).

To decrease the waitlist mortality, strategies for transplanting highly sensitized patients and expanded use of ABO-Incompatible (ABO-I) transplantation have progressed. One strategy is the use of peri-operative plasmapheresis, including membrane-based plasmapheresis (MP) and centrifuge-based plasmapheresis (CP). (5) Despite the common use of these techniques for both blood group isohemagglutinin and HLA antibody removal, limited data exists comparing their effectiveness. This study's aim was to compare the *in vitro* magnitude of isohemagglutinin and HLA antibody removal between MP and CP incorporated into the extracorporeal (EC) circuit. While desensitization techniques incorporating MP and CP are utilized prior to transplant, the effects are variable in effect and duration and can be associated with serious complications. (6, 7) As transplant timing is difficult to predict, we focused on the feasibility of intra-operative plasmapheresis incorporated into the EC circuits.

Methodology:

As highly sensitized, high titer donors were needed for this study, blood was obtained from the Red Cross from female donors with blood type O and at least one prior pregnancy. Twelve donor units of whole blood were purchased and nine of the most sensitized units were pooled for a total volume of 3.5 liters in a common reservoir. Heparin Sodium (Sagent Pharmaceuticals, Schaumburg, IL) was added to achieve a heparin concentration of 4.0 units/mL, which is standard dosing for cardiopulmonary

bypass. Sodium Bicarbonate (Hospira, Inc., Lake Forest, IL) and Calcium Chloride (American Regent, Inc., Shirley, NY) were added to achieve physiologically normal blood gases. Once the common reservoir was homogenized (achieving a final hematocrit of 35-55%), aliquots of 450 mLs were utilized to prime each individual study circuit.

Four identical extracorporeal (EC) circuits consisted of a CAPIOX® BabyFX hard-shell reservoir (Terumo Cardiovascular, Ann Arbor, MI), a roller pump (Getinge, Goteborg, Sweden), and ¼ inch tubing (Terumo Cardiovascular, Ann Arbor, MI) coated with Xcoating™ (Terumo Cardiovascular Group, Ann Arbor, MI, USA) creating a closed loop circuit. A plasmapheresis circuit was then incorporated into each of the four EC circuit, two of the EC circuits incorporated a membrane-based plasmapheresis (Prismaflex, Baxter Healthcare Corporation, Deerfield, IL) circuit, and the other two study circuits incorporated a centrifuge-based plasmapheresis (Spectra Optia Apheresis System, Terumo Blood and Cell Technologies, Inc, Lakewood, CO) circuit (Figure 1). The two Prismaflex circuits for the membrane-based plasmapheresis utilized a TPE2000 (Baxter, GAMBRO Industries, Meyziu, France) filter for this study.

Blood flow through each of the EC circuits was maintained at 500 mL/min to simulate a 2.2 L/min/m^2 cardiac index for a 4-kilogram patient. The MP circuit flow rates were maintained between 100-400 mL/min and the CP circuit flow rates were maintained between 5-142 mL/min, according to manufacturer's instructions for use. Fresh Frozen Plasma (FFP) from AB+ donors were used for the plasma exchange volume. The study was set to complete once 1.5-2x plasma exchange occurred or two hours, whichever came first. Two hours was set as the average transplant time to simulate a true clinical scenario, particularly of an infant with limited access in whom prolonged pre- or post-transplant pheresis may not be as readily possible.

Baseline blood samples were analyzed for isohemagglutinin titers (IT) and Panel Reactive Antibody (PRA) panel as well as a complete blood count (CBC) and plasma hemoglobin to assess for cell damage. IT and a PRA panel were performed at 30-minute increments throughout the duration of the study. At the conclusion of the study, samples were again sent for final IT, PRA, CBC, and plasma hemoglobin. All samples were stored at four degrees Celsius until completion of the study, at which time they were delivered to the clinical laboratory for analysis.

Due to the few time points, statistical analysis cannot be undertaken. Mean change in IT and PRA strength are presented graphically. The study was reviewed by the Nationwide Children's Hospital Institutional Review Board (IRB) and determined not to involve human subjects under 45 CFR part 46.102(f) and evaluation was therefore waived.

Results:

Centrifuge-Based Plasmapheresis

The mean baseline IT were 1:256 for anti-A IgM and 1:512 for anti-A IgG. Anti-B titers were lower for both IgM and IgG starting at 1:128 and 1:192, respectively. All four antibodies showed a decrease in titers with the first two passes and stable or a continued decrease in titers with the subsequent two passes (Figure 2A). After the fourth pass, anti-A titers decreased to a mean of 1:20 for IgM and 1:48 for IgG, representing a reduction of 50% and 39%, respectively. Anti-B titers decreased to a mean of 1:2 for IgM and 1:12 for IgG, representing a reduction of 86% and 53%, respectively. The overall mean anti-A and anti-B titer reduction was 4.375 titers (93.8% change) with 62.5% of titers ≤ 1:4 at the end of the run at two hours.

There were also high baseline class II anti-HLA antibody levels with all samples demonstrating a mean fluorescence intensity (MFI) >3,000. Of note, the supplied multiparous blood samples did not have

significant class I antibodies present. The cumulative MFIs prior to the runs were 12,106 and 14,615. Both CP circuits demonstrated a rapid decrease in MFI with reduction to <3000 by the second pass at the 60-minute mark (Figure 2B). After the fourth pass, the cumulative MFI was reduced to 6,133 and 4,133 corresponding to a 2.0-3.5-fold decrease at the end of the two-hour run.

Membrane-Based Plasmapheresis

The mean baseline IT were 1:256 for anti-A IgM and 1:768 for anti-A IgG. Baseline anti-B titers were again lower for both IgM and IgG starting at 1:128 and 1:192, respectively. Like CP, all four antibodies showed a decrease in titers with the first two passes. The following two passes demonstrated continued decrease or stable IT (Figure 3A). After the fourth pass, anti-A titers decreased to a mean of 1:18 for IgM and 1:72 for IgG, representing a reduction of 56% and 42%, respectively. Anti-B titers decreased to a mean of 1:1.5 for IgM and 1:20 for IgG, representing a reduction of 93% and 47%, respectively. The overall mean anti-A and anti-B titer reduction was 4.625 titers (93.7% change) with 50% of the titers ≤ 1:4 at the end of the run at two hours.

High baseline class II anti-HLA antibody levels (MFI >3,000) were noted initially with all samples. The cumulative MFIs prior to the runs were 14,324 and 17,110. Both MP circuits demonstrated a rapid decrease in MFI with reduction to <3,000 by the third pass at the 90-minute mark as demonstrated in Figure 3B. After the fourth pass, the cumulative MFI was reduced to 8,176 and 6,756, corresponding to a 1.7-2.5-fold decrease at the end of the two-hour run.

Cell damage

There was no difference in degree of hemolysis or plasma hemoglobin between MP and CP from baseline to final assessment.

Discussion:

The presence of antibodies against HLA and ABO antigens and the historic association with rejection remains a barrier to successful transplantation for many patients (8). There are various treatment strategies for antibody removal available to mitigate these risks at the time of transplant, including plasma exchange transfusion, plasmapheresis, and immunoadsorption columns. There is variable use of these strategies among transplant centers with no clear evidence that one strategy is associated with improved outcomes post-transplant.

Plasma exchange transfusion performed in the operating room prior to transplantation remains the simplest and most cost-effective method to remove circulating antibodies. Prior to initiation of cardiopulmonary bypass, approximately 1.5-3 times the patient's total plasma volume is exchanged for type AB donor plasma (9, 10). However, there are several disadvantages to this strategy. Plasma exchange is not selective and therefore removes protective antibodies and clotting factors, increasing the risk of post-operative bleeding and infection. Additionally, the volume of blood products required limits use in larger patients and infers an increasing risk of transfusion related mortality (11).

Robertson *et al* recently published data utilizing immunoadsorption columns incorporated into the cardiopulmonary bypass circuit in ABO-I heart transplantation. With this method, a plasma separator and immunoadsorption column are incorporated into the EC circuit. After initiation of cardiopulmonary bypass, plasma is removed by the separator and filtered through the column with depleted plasma returned to the circulating volume of the EC circuit (12). However, immunoadsorption columns are not readily available in many institutions in the United States. Furthermore, as this is a new technology, there is limited data on long term outcomes.

Alternatively, plasmapheresis is commonly used in the peri-transplant period. MP utilizes a filter with pores that are large enough to remove plasma and the desired macromolecules, whereas CP relies on the rotational forces of centrifugation to separate blood components based on their density (13).

Plasmapheresis circuits can also be incorporated into the EC circuit to remove a portion of patient plasma that is then replaced with albumin or donor fresh frozen plasma, although in a much smaller volume than with plasma exchange. We found that both membrane-based and centrifuge-based plasmapheresis incorporated into the EC circuit quickly and effectively reduced circulating IT and HLA antibodies. Both membrane-based and centrifuge-based plasmapheresis reduced anti-A and anti-B titers, with similar efficacy.

The limitations of our study are related to the *in vitro* nature of the study. This study was performed on a small scale with four EC circuits. Larger-scale studies are needed to further compare the efficacy of the two methods of plasmapheresis. Furthermore, the model simulated that of a small infant and further research is needed to determine feasibility in larger patients. Nevertheless, this study demonstrates the feasibility of incorporating a plasmapheresis circuit into an EC circuit and provides useful data on antibody clearance.

Recognizing that both MP and CP can provide similar antibody clearance, with CP perhaps being slightly more efficient, either technology can be an important adjunct to transplant programs. Being able to effectively remove antibodies, can broaden the potential donor pool as well as decrease wait time, and in turn, decrease waitlist mortality. Assessment of these technologies against immunoadsorption is a necessary next step.

References:

1. Canter CE, Shaddy RE, Bernstein D, et al. Indications for heart transplantation in pediatric heart disease: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young; the Councils on Clinical Cardiology, Cardiovascular Nursing, and Cardiovascular Surgery and Anesthesia; and the Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation. 2007;115(5):658-76. doi: 10.1161/CIRCULATIONAHA.106.180449. PubMed PMID: 17261651.

2. Colvin M, Smith JM, Hadley N, et al. OPTN/SRTR 2018 Annual Data Report: Heart. Am J Transplant. 2020;20 Suppl s1:340-426. Epub 2020/01/04. doi: 10.1111/ajt.15676. PubMed PMID: 31898418.

3. Almond CSD, Thiagarajan RR, Piercey GE, et al. Waiting list mortality among children listed for heart transplantation in the United States. Circulation. 2009;119(5):717-27. Epub 2009/01/28. doi: 10.1161/CIRCULATIONAHA.108.815712. PubMed PMID: 19171850; PubMed Central PMCID: PMCPMC4278666.

4. Mah D, Singh TP, Thiagarajan RR, et al. Incidence and risk factors for mortality in infants awaiting heart transplantation in the USA. J Heart Lung Transplant. 2009;28(12):1292-8. Epub 2009/09/29. doi: 10.1016/j.healun.2009.06.013. PubMed PMID: 19782580; PubMed Central PMCID: PMCPMC4269350.

5. Leech SH, Lopez-Cepero M, LeFor WM, et al. Management of the sensitized cardiac recipient: the use of plasmapheresis and intravenous immunoglobulin. Clin Transplant. 2006;20(4):476-84. Epub 2006/07/18. doi: 10.1111/j.1399-0012.2006.00509.x. PubMed PMID: 16842525.

6. Saadi TA, Lawrecki T, Narang N, et al. Outcomes of pre- heart transplantation desensitization in a series of highly sensitized patients bridged with left ventricular assist devices. J Heart Lung Transplant. 2021;40(10):1107-11. Epub 2021/07/21. doi: 10.1016/j.healun.2021.05.019. PubMed PMID: 34281777.

7. Byku M, Chang PP. Desensitization for sensitized patients awaiting heart transplant. Curr Opin Organ Transplant. 2019;24(3):233-8. Epub 2019/05/16. doi: 10.1097/MOT.0000000000000639. PubMed PMID: 31090629.

8. Tambur AR, Pamboukian SV, Costanzo MR, et al. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. Transplantation. 2005;80(8):1019-25. Epub 2005/11/10. doi: 10.1097/01.tp.0000180564.14050.49. PubMed PMID: 16278580.

9. West LJ, Pollock-Barziv SM, Dipchand AI, et al. ABO-incompatible heart transplantation in infants. N Engl J Med. 2001;344(11):793-800. Epub 2001/03/15. doi: 10.1056/NEJM200103153441102. PubMed PMID: 11248154.

10. Foreman C, Gruenwald C, West L. ABO-incompatible heart transplantation: a perfusion strategy. Perfusion. 2004;19(1):69-72. Epub 2004/04/10. doi: 10.1191/0267659104pf708oa. PubMed PMID: 15072258.

11. Murphy GJ, Reeves BC, Rogers CA, et al. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation. 2007;116(22):2544-52. Epub 2007/11/14. doi: 10.1161/CIRCULATIONAHA.107.698977. PubMed PMID: 17998460.

12. Robertson A, Issitt R, Crook R, et al. A novel method for ABO-incompatible heart transplantation. J Heart Lung Transplant. 2018;37(4):451-7. Epub 2017/05/31. doi: 10.1016/j.healun.2017.05.006. PubMed PMID: 28554587.

13. Grazioli A, Athale J, Tanaka K, et al. Perioperative Applications of Therapeutic Plasma Exchange in Cardiac Surgery: A Narrative Review. J Cardiothorac Vasc Anesth. 2020;34(12):3429-43. Epub 2020/03/10. doi: 10.1053/j.jvca.2020.01.054. PubMed PMID: 32147326.

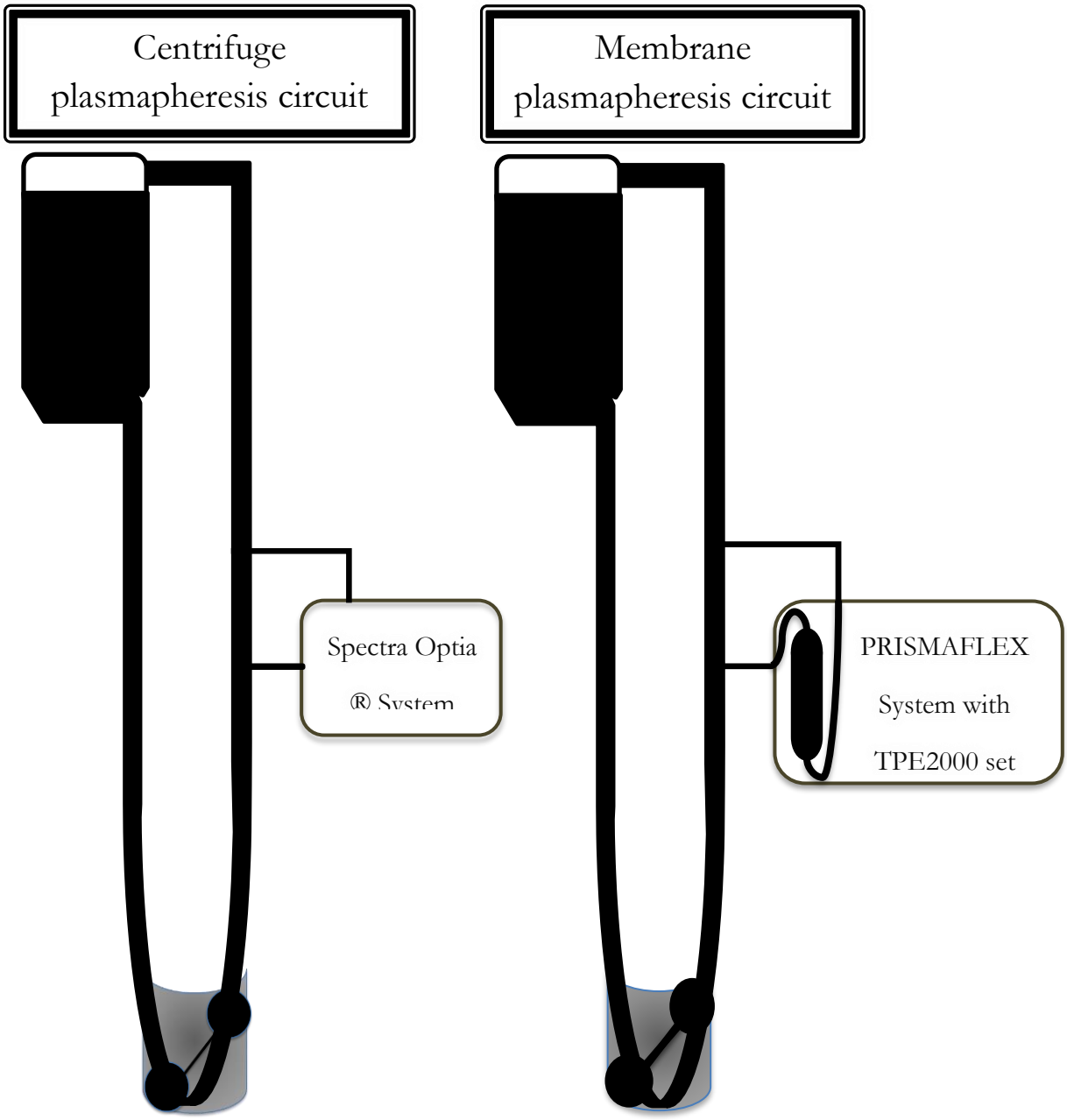
Figures

Figure 1. Centrifuge-based plasmapheresis (left) and membrane-based plasmapheresis (right) circuits incorporated into a cardiopulmonary bypass circuit.

Figure 2. Centrifuged-based plasmapheresis results. A – Reduction in titers of anti-A and anti-B IgG and IgM. B – Reduction in HLA antibodies by average mean fluorescence intensity (MFI) at the DQ locus.

Figure 3. Membrane-based plasmapheresis. A – Reduction in titers of anti-A and anti-B IgG and IgM. B – Reduction in HLA antibodies by average mean fluorescence intensity (MFI) at the DQ locus.

Figure 1.


Centrifuge plasmapheresis circuit
Membrane plasmapheresis circuit
Spectra Optia ® System
PRISMAFLEX System with TPE2000 set

Centrifuge-based Plasmapheresis

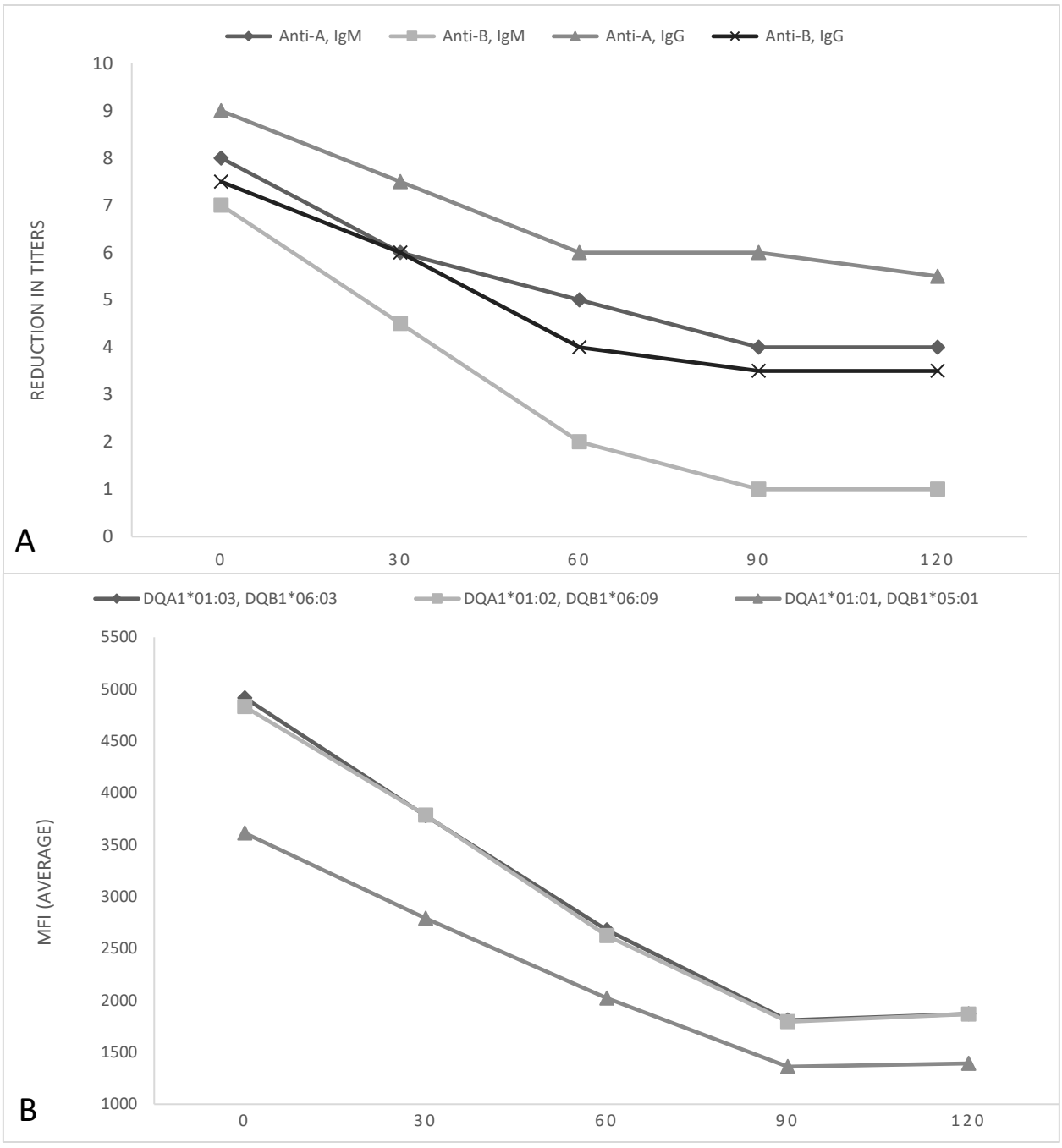


Figure 2.

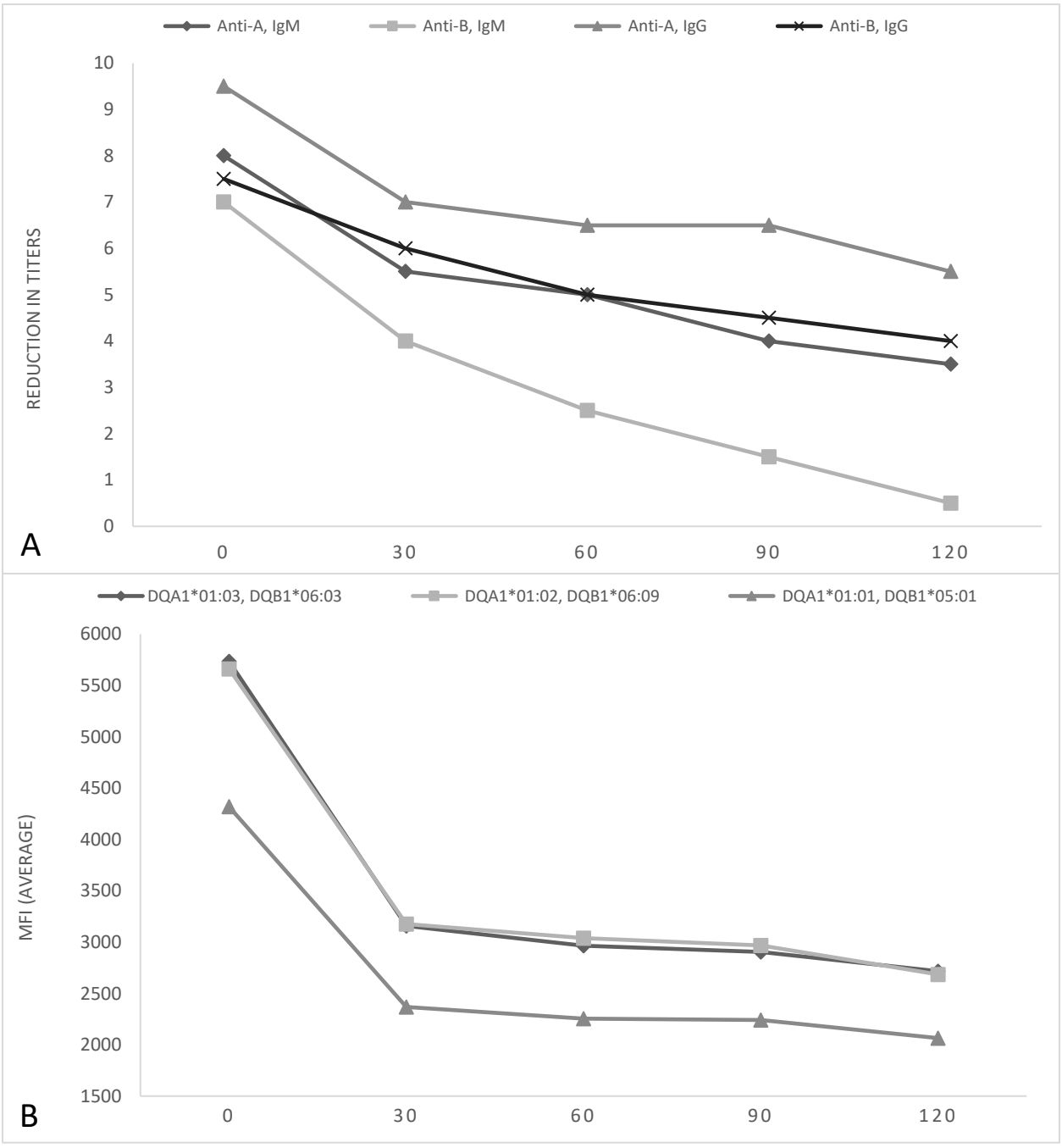

Membrane-based Plasmapheresis
Anti-A, IgM
Anti-B, IgM
Anti-A, IgG
Anti-B, IgG
REDUCTION IN TITERS
10
9
8
7
6
5
4
3
2
1
0
A
0
30
60
90
120
DQA1*01:03, DQB1*06:03
DQA1*01:02, DQB1*06:09
DQA1*01:01, DQB1*05:01
MFI (AVERAGE)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
B
0
30
60
90
120

Figure 3.