Analysis of the clinical use of ABO Adsopak® 150 and 300 columns	
R–0719–02	
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1. SCOPE

To evaluate the clinical results of using ABO Adsopak® 150 and 300 columns in plastic housings based on post-marketing data.

2. CONTENT

3. INTRODUCTION

3.1. Description of medical device

ABO Adsopak® adsorption columns are medical devices designed to remove antibodies to blood groups antigens in patients in preparation for transplantation of ABO incompatible organs.

Columns differ in specificity: ABO Adsopak®-A columns are designed to remove antibodies to blood group A antigen, ABO Adsopak® -B - to remove antibodies to blood group B antigen, ABO Adsopak® -AB remove antibody-A and B simultaneously.

ABO Adsopak® - A and ABO Adsopak® - B columns in plastic housings have a volume of 150 ml and 300 ml, ABO Adsopak®-AB columns - 300 ml. Columns are used repeatedly individually. Column regeneration is achieved by passing regenerating solutions through the column after the procedure.

The columns are a plastic housing in which the sorbent is placed. Membrane filters inside the column pass liquid and hold the sorbent inside. Sorbent determines the specificity of the column, its properties and the declared action. The sorbent in the columns ABO Adsopak® - A and ABO Adsopak® - B is specific, respectively, to antigens A and B of the blood group. The sorbent in the column ABO Adsopak® - AB is a composition of anti A and anti B sorbents in equal proportions.

The binding of antibodies to sorbents is provided by ligands - synthetic analogues of antigens of blood groups A and B. These substances are oligosaccharides. During the synthesis of the sorbent, they are chemically immobilized on an inert insoluble matrix. The received sorbent is biocompatible, narrowly specific in relation to antibodies to antigens of blood groups, it does not absorb any other proteins and components of human blood plasma.

In the production of ABO Adsopak® columns, the company uses the same technology as in the production of its other products - LDL Lipopak®, Ig Adsopak®, Lp(a) Lipopak®, Toxipak® and Immuno-Adsopak® columns. The basis of the technology is aseptic production. Most of the incoming raw materials are identical to the raw materials from the new product line, including the column housing and matrix (Toxipak®, Immuno-Adsopak®), reagents.

The column does not contain medicinal substances, blood components and human tissues, as well as their derivatives.

The column is sterile, pyrogen-free, filled with a preservative solution, which is removed when preparing the column for use by washing with physiological solution according to the manufacturer's instructions.

The column housing is equipped with two plugs and fittings for connection to the plasma separator line during use. The column is packed in a sealed bag, then in a cardboard box.

ABO Adsopak® adsorption columns are used in the extracorporeal plasma adsorption procedure. During use, the column is integrated into the extracorporeal circulation circuit. When a patient's blood plasma passes through a adsorption column, synthetic antigens of blood groups specifically bind the antibodies circulating in the plasma. The column does not come in contact with whole blood.

The procedure is provided by a plasma separator, which is the primary device, separates the patient's blood into plasma and blood cells. Plasma enters the column, cells return to the bloodstream. The plasma flow rate through the column depends on how vascular access is provided and on the patient's condition, usually 30-60 ml/min. Functionally, the column acts as a filter. During the procedure, anticoagulation is necessary. The main precaution is the need for compliance aseptic rules when handling the column.

After passing 1.0-2.0 volumes of patient's blood plasma through the column, the procedure is stopped. Usually the procedure lasts 3-4 hours, during which up to 9 L of plasma passes through the column. After completion of the procedure, the column is disconnected from the extracorporeal circuit and regenerated. To do this, sterile pyrogen-free solutions are successively passed through it: physiological solution, glycine buffer pH 2.5, phosphate buffer. Between treatments, the column is stored in a preservation buffer containing 0.02% sodium azide. Before the next procedure, the preservation buffer is removed by washing with physiological solution.

ABO Adsopak® adsorption columns are intended for use in specialized medical institutions by specially trained personnel. The main purpose of the columns is to prepare the patient for transplantation in case of mismatch of blood groups in the patient and organ donor. Removal of antibodies in case of incompatibility by blood group allows expanding the possibilities of selecting a donor for transplantation, especially in cases of related kidney transplantation.

3.2. Approaches to conducting AB0 - incompatible transplantation

When transplanting solid organs, the leading role is played by the compatibility of the donor and the recipient in the ABO blood group system. The significance of the ABO system is explained by the fact that antigens of the ABO system are expressed on the surface of almost all body cells. According to the law formulated by Landsteiner, antibodies to ABO system antigens that are absent in his own body are present in human serum. If antibodies are present in the serum of the recipient in high titer, then they launch over-acute rejection of the transplant, which expresses antigens on the surface of its cells that are absent in the A/B recipient. The intensity of expression of ABO antigens on the surface of different tissues is different. Thus, the density of ABO antigens on the surface of kidney tissues cells is approximately two times higher than on the surface of liver tissue cells. In most people, antibodies to allogeneic antigens of the ABO system are produced without prior sensitization. Antibodies appear as a result of contact of the human immune system with identical epitopes, which are randomly expressed on many microorganisms.

The mismatch between the blood type of the donor and the recipient has long been an insurmountable obstacle in organ transplantation. Currently, there are three methods for removing anti-group antibodies from the patient's bloodstream: surgical, pharmacological, and antibody removal using extracorporeal therapy.

The surgical approach involves performing splenectomy (removal of the spleen) before or during an organ transplant operation. However, removal of the spleen does not always guarantee complete cessation of antibody synthesis and does not always prevent the development of humoral

rejection in the post-transplant period. In addition, there is a high risk of developing serious septic complications in patients undergoing splenectomy, transplantation and receiving immunosuppressive therapy.

The pharmacological method involves drug immunosuppressive therapy. The most effective modern immunosuppressant is rituximab, a chimeric humanized mouse anti-CD20 monoclonal antibody that has the ability to selectively reduce the content of B-lymphocytes. Rituximab is widely used as an inducer of immunosuppression in ABO-incompatible transplantation of solid organs.

Methods of extracorporeal therapy include plasmapheresis, cascaded plasmapheresis, Ig apheresis, and specific sorption of anti-A and/or anti-B antibodies. These methods can effectively reduce the level of anti-group antibodies [1].

Plasma exchange is a non-selective method for removing the patient's blood plasma, in which all or part of the patient's plasma is replaced by donor plasma and plasma-replacing solutions. Usually, for successful multidimensional transplantation surgery, 5-7 plasma exchange procedures are necessary, as a result of which the patient receives up to 20 liters of donor plasma. The main disadvantage of this method is the high risk of infection and adverse reactions.

Cascade plasma filtration is the removal of high molecular weight plasma proteins of the patient. In the case of antibodies to blood groups, cascade plasma filtration requires the removal of a significant portion of plasma proteins. In this case, the use of replacement solutions is also necessary. This method has fewer risks than plasmapheresis, but is also not selective.

More selective are procedures using anti-IgG sorbents, such as sorbents based on antibodies to immunoglobulins G or staphylococcal protein A, which is specific for human IgG. These sorbents remove only immunoglobulins, which include antibodies against blood groups. The procedures are called Ig apheresis. However, the concentration of total immunoglobulins in blood plasma is 10-15 mg/ml, and antibodies to blood groups - not more than 1 μg/ml. Obviously, to effectively reduce the level of antibodies, it is necessary to remove about 50 g of immunoglobulins, of which only 1/10000 are the "target" antibodies. Therefore, this approach is also not optimal.

The most specific is the adsorption of target antibodies on the column with antigens of blood groups, which are obtained as a result of chemical synthesis. Specific adsorption of anti-A or anti-B antibodies occurs on A or B antigens attached to a biocompatible matrix. The plasma purified from antibodies is returned to the patient. Donor blood and/or plasma substituting solutions are not used for the procedure. Due to the specificity of the columns which binds only antibodies to A or B antigens, there is no loss of total protein.

As a rule, the preparation protocol for ABO-incompatible transplantation is a combination of the above approaches, with the most widely used methods of extracorporeal therapy in Europe.

Thanks to the use of adsorption columns, ABO-incompatible kidney transplantation has become an almost routine procedure in different European countries, especially in Sweden and Germany. Three products are used to remove antibodies: Ig Therasorb® columns (Miltenyi Biotec GmbH, Germany), Immunosorba® (Fresenius Medical Care, Germany) and Glycosorb®-ABO (Glycorex, Sweden) [2-4].

Ig Therasorb® columns contain antibodies to human G immunoglobulins, Immunosorba® columns are based on staphylococcal protein A. Both products are designed for Ig apheresis (removal of the total fraction of human immunoglobulins) and include two columns that are regenerative and work alternately during the procedure. Repeated use of columns can effectively reduce the levels of IgG (up to 90%) and IgM (up to 55%), while reducing the titer of antibodies to blood groups is 3 steps [2, 3].

However, specific anti-A and anti-B sorption columns Glycosorb®-ABO, manufactured by Glycorex, Sweden, are most widely used. This product is a single 75 ml column containing A or B, or a mixture of A + B synthetic blood group antigens immobilized onto a matrix. The column is specific only to antibodies of blood groups and does not adsorb other plasma components. The

use of these columns provides the safest and most effective reduction in antibody titer in preparation for transplantation.

List of sources used:

1. Bosch T., Therapeutic apheresis - State of the Art in the year 2005. Therapeutic Apheresis and Dialysis, 2005, 9 (6):459–468.
2. Hickstain H., Koball S., Lehmann R., Protzel C., Stein K., Mitzner S., Hakenberg O. AB0 incompatible kidney transplantation using unspecific immonoadsorption. Transfusion and Apheresis Science 2014; 50 : 263–266.
3. Gjorstrup P., Watt R.M. Therapeutic protein A immunoadsorption. A review. Transfusion Science, 1990; 11, 281 -302.
4. Genberg H., Wennberg L., Tydén G., Kidney Transplantation. European renal diseases, 2007, 55-60.

4. METHODS FOR ASSESSMENT

4.1. The evaluation of the clinical data on the use of the medical device consisted of a statistical analysis of the results of the effectiveness of the use of ABO Adsopak® columns in plastic houses in India in the period 2016-2019 depending on the volume of the column, the initial titer before transplantation and the number of procedures performed.

4.2. Analysis of the effectiveness of the procedures was carried out on the basis of data presented from 42 clinics in India (a general list of 72 clinics is attached).

4.3. For statistical analysis, the MedCalc statistical software package was used.

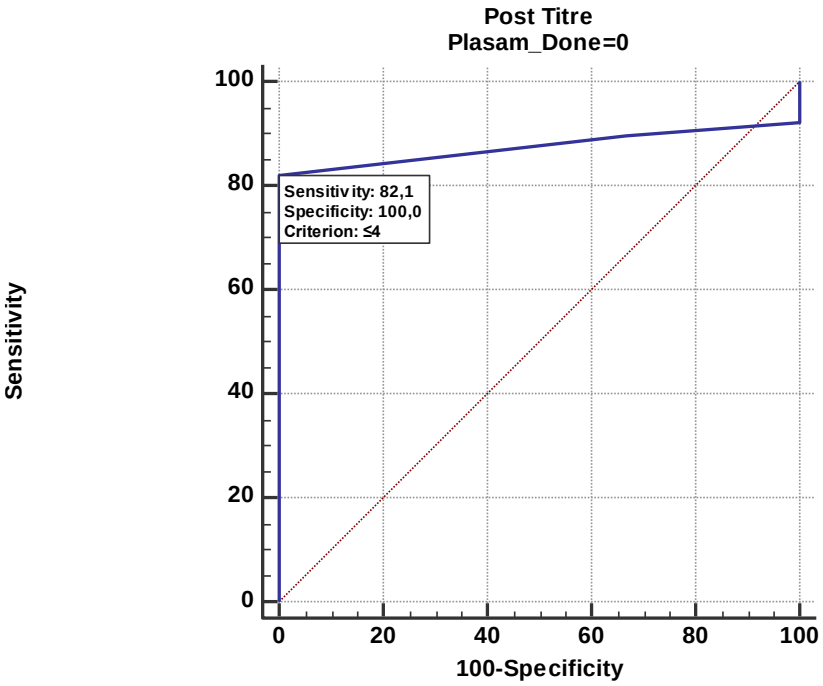
5. RESULTS

During the described period of time, 90 ABO Adsopak® 150 columns with a volume of 150 ml were delivered to 72 clinics in India: 42 ABO A150 and 48 ABO B150, and 21 columns ABO Adsopak® 300 with a volume of 300 ml, of which 11 ABO A300, 8 ABO B300 and 2 columns ABO AB300. In total, more than 360 procedures were performed on all types of columns.

The data used for statistical analysis were obtained from 72 patients who underwent a total of 300 procedures.

Statistical analysis showed that the effectiveness of the procedures, expressed as the success of the transplant, was significantly inversely related to the titer after the apheresis procedure ($p < 0.01$).

A titer of less than 1:4 with 82% sensitivity and 100% specificity was associated with successful transplantation for patients who did not undergo additional plasma exchange procedures (area under the curve (AUC) 0.876 (95% CI 0.738 - 0.957) $p < 0.0001$.



Picture.1. Analysis of operational performance curves (ROC analysis)

The percentage of achievement of target titer of 1:4 was 80% for both types of columns with a volume of 150 and 300 ml (Figure 2).

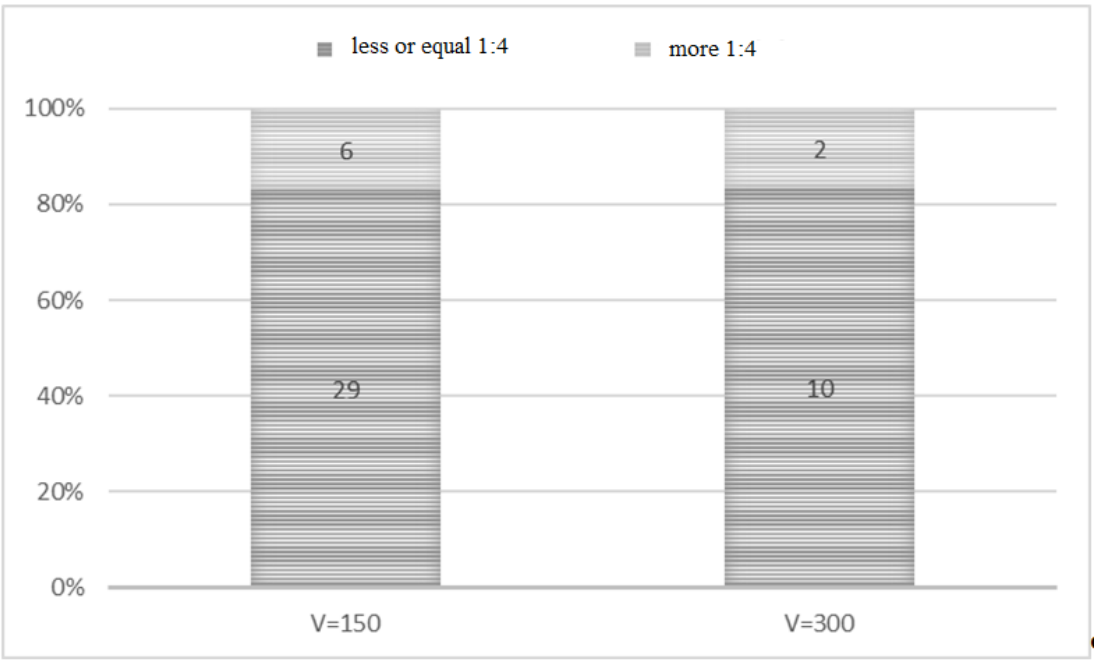


Figure 2. The percentage of achievement of the target titer of anti-erythrocyte antibodies during apheresis procedures in patients who did not undergo additional plasma exchange procedures.

However, the antibody titer after adsorption procedures did not depend on the number of procedures performed, which indicates a high binding capacity of the columns and a more complex mechanism for the removal and formation of anti-erythrocyte antibodies in patients in preparation for transplantation.

The titer of anti-erythrocyte antibodies varied over a wide range. Moreover, according to the analysis of the curves of operational characteristics, a successful transplantation operation with a sensitivity of 100% and a specificity of 67% was reliably associated with a titer reduction of more than two times (one step) - the area under the curve AUC = 0.879 (95% CI 0.744 - 0.958), p = 0.0020 (Figure 3).

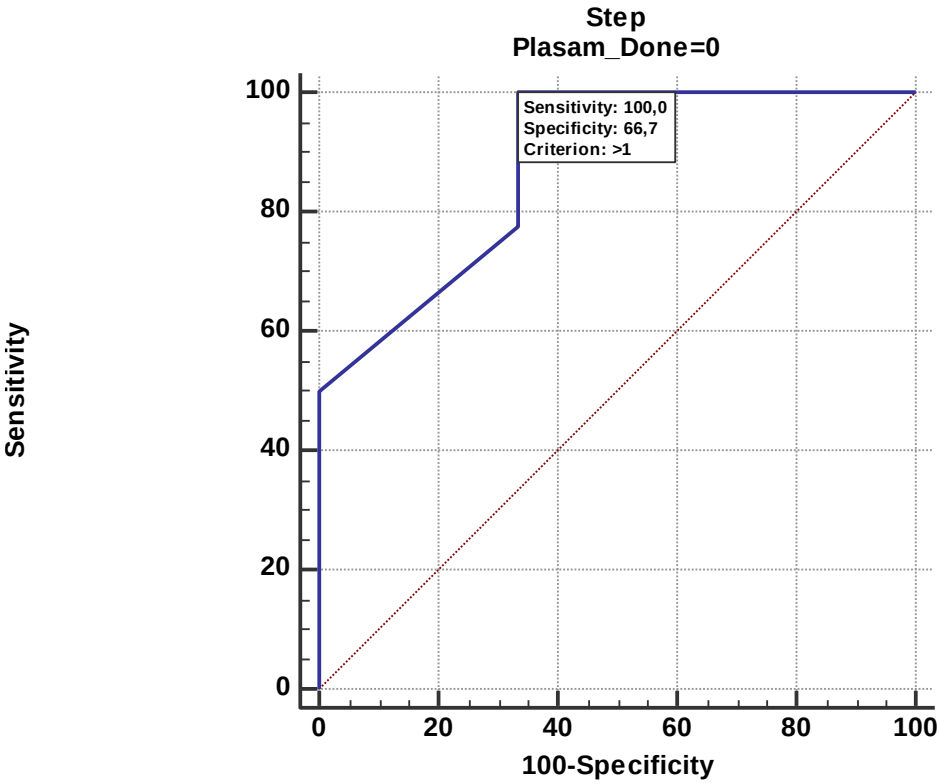


Figure 3. Analysis of the curves of operational characteristics (ROC analysis) of the success of the transplantation, depending on the decrease in titer of anti-erythrocyte antibodies (number of steps, step 2)

ABO Adsopak® A150 columns were used to treat 23 patients. The antibody titer ranged from 1:8 to 1:1024, the average number of procedures was 5 ± 2 procedures. The efficiency of reduction the titers of antiA antibodies was 97% (Fig. 3). ABO Adsopak® A300 columns were used to treat 11 patients. The antibody titer for such patients was significantly higher and varied from 1:64 to 1:2048, the average number of procedures was comparable and amounted to 5 ± 4 procedures. The efficiency of reducing the titers of antiA antibodies was also high 98% (Fig. 4).

The dynamics of titer changes, expressed in the number of steps, was higher when using 300 ml columns of 6.2 ± 2.4 compared to 150 ml columns of 4.8 ± 1.4 steps (Figure 5), but the differences did not reach statistical significance.

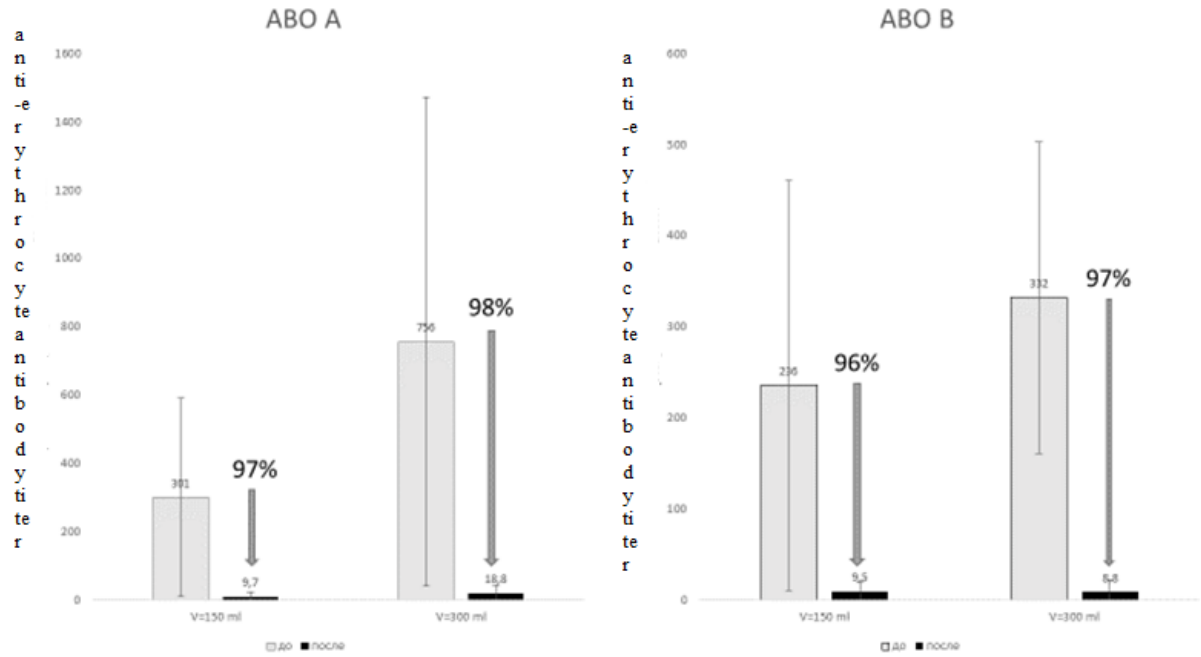


Figure 4. The effectiveness of reducing the titer of anti-erythrocyte antibodies in the process of apheresis in the preparation of patients for transplantation.

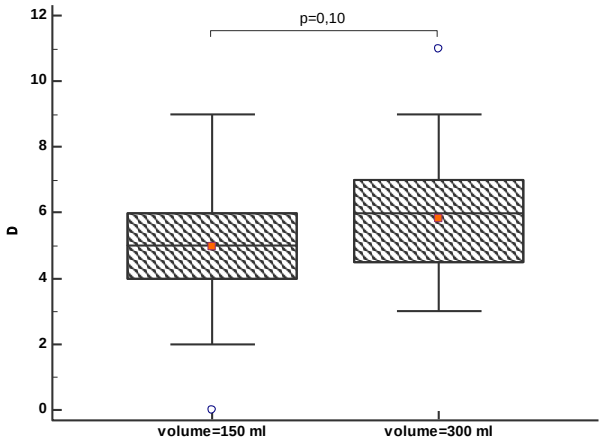


Figure 5. The effectiveness of reducing the titer of anti-erythrocyte antibodies in the process of apheresis during the preparation of patients for transplantation.

The number of successful transplantations was comparable in the case of 150 and 300 ml columns. Considering the significant difference in the initial antibody titers, we can talk about the advisability of using 300 ml columns for patients with high initial anti-erythrocyte antibody titers.

ABO Adsopak® B150 columns were used to treat 24 patients. The antibody titer ranged from 1:16 to 1: 1024, the average number of procedures was 4 ± 1 procedures. The efficiency of reducing the titers of anti B antibodies was 96% (Fig. 4).

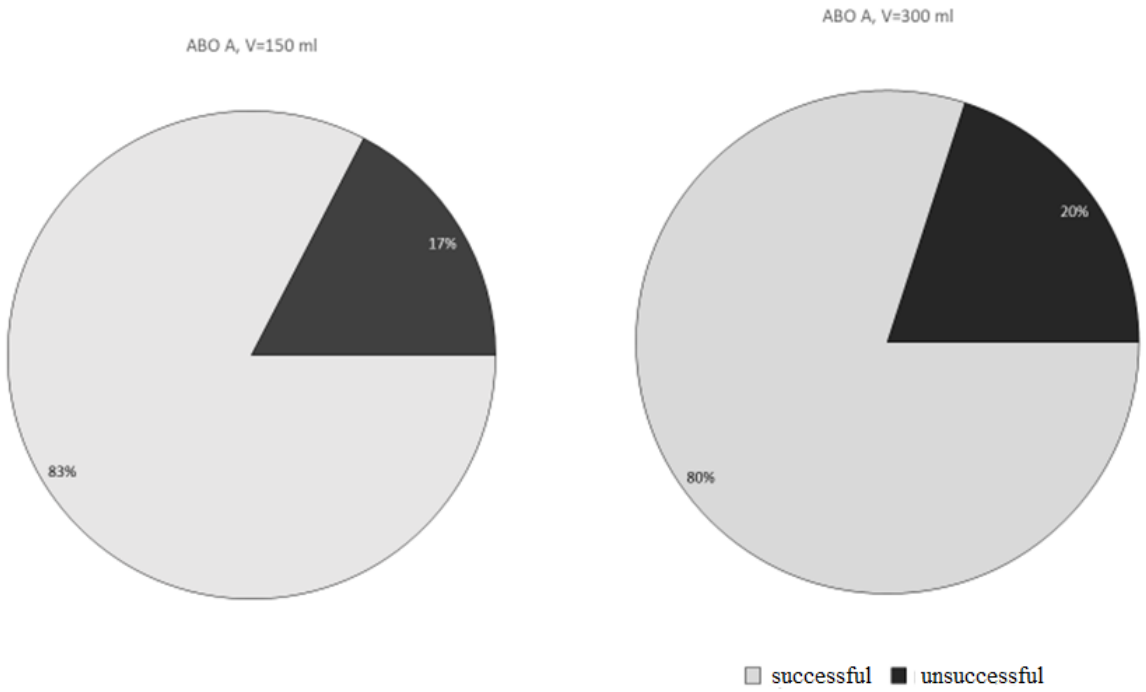


Figure 6. Successfully performed transplantation after the procedures on the ABO Adsopak® A columns, with a volume of 150 and 300 ml.

ABO Adsopak® A300 columns were used to treat 5 patients. The antibody titer for such patients was significantly higher and varied from 1:128 to 1:512, the average number of procedures was comparable - 5 ± 1 procedures. The efficiency of reducing the titers of anti B antibodies was also high 97% (Fig. 3).

The percentage of successful transplants when using 300 ml columns reached 100% (Fig. 7).

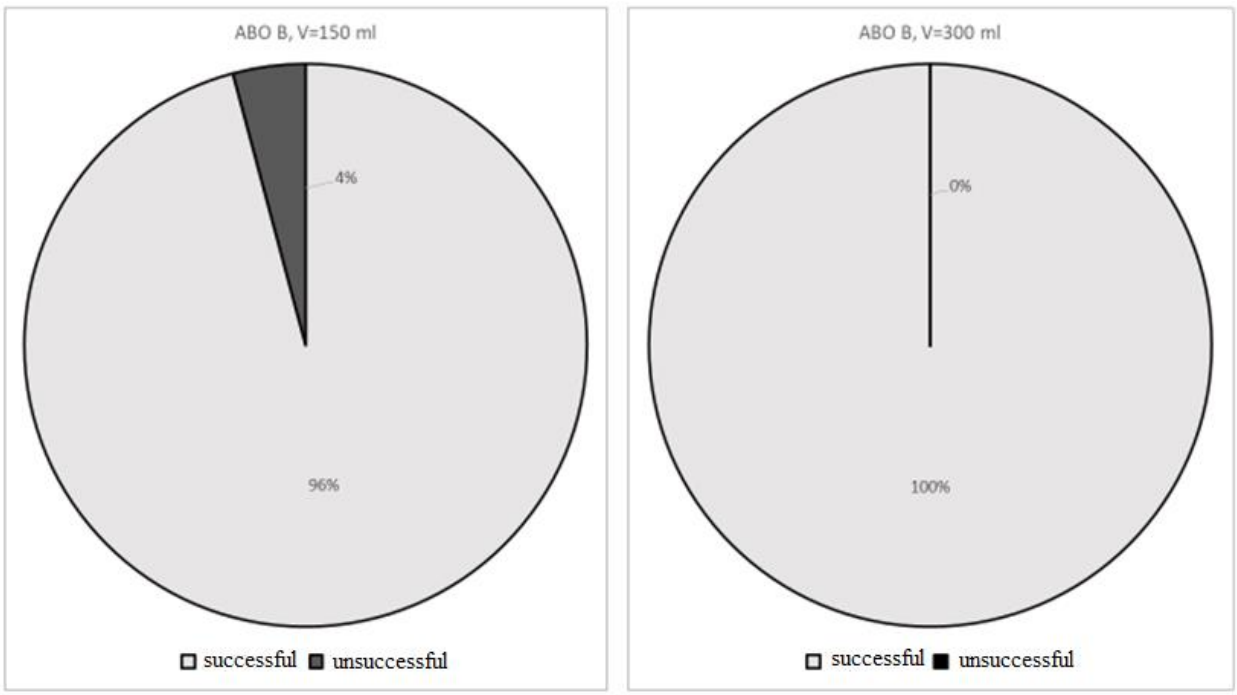


Figure 7. Successfully performed transplantation after the procedures on the ABO Adsopak® B columns, with a volume of 150 and 300 ml.

6. CONCLUSIONS.

6.1. For patients undergoing the preparation for transplantation in India, very high titers of anti-erythrocytic antibodies are characteristic.
6.2. A titer of 1: 4 or less with 100% sensitivity is associated with a successful transplant.
6.3. The effectiveness of the procedures calculated as a decreases in the titer of anti-erythrocyte antibodies increased or remained stable with a significant increase in the initial antibody titer regardless the volume of column used (150 or 300 ml).
The effectiveness of the procedures calculated as a number of successfully performed transplantation decreases in the titer of anti-erythrocyte antibodies increased or remained stable regardless the volume of column used (150 or 300 ml).
6.4. The use of 300 ml columns can be recommended for patient with an initially high titer of anti-erythrocyte antibodies.

Attachment1.

List of clinics in India using ABO Adsopak® columns, regardless of volume:

Item No.	Clinic Name
1	Abhishek
2	Alben Mathew Dsouza
3	All India Institute of Medical Sciences
4	Amit Kumar
5	Ananda A
6	Ankush Sharma
7	Apollo Hospitals
8	Army Hospital
9	Arvind Kumar
10	Ashish Dara
12	Ashok Kumar Singh
13	Ayush Kumar
14	Batra Hospital & Medical Research Centre
15	Bhupesh Pandey
16	Brunda
17	Command Hospital Air Force
18	Dandapani Padhy
19	Devki Devi Foundation
20	Dr. B.L. Kapur Memorial Hospital
21	Escorts Heart Institute & Research Centre Limited
22	Fortis Hospitals Limited
23	Gaurav Kumar Srivastava
24	Global Health Pvt. Ltd.
25	Global Hospital, Mumbai
26	Hometrail Estate PrivateLimited
27	Indraprastha Apollo Hospital
28	Ivy Health & Life Sciences (P) Ltd.
29	Jaslok Hospital & Research Centre

30	Jaypee Healthcare Ltd.
31	Kokilaben Dhirubhai Ambani
32	Kusum Lata Pal
33	Mahesh Chandra Rai
34	Manoj Tanwar
35	Max Healthcare Institute Ltd.
36	Medeor Hospital Limited (Rockland Hospitals Ltd)
37	Narayan Goutam
38	Narayana Hrudayalaya Limited
39	Pankaj Tiwari
40	Paras Hospital Gurgaon
41	Pradeep Kumar
42	Pushplata
43	Rajat Mittal
44	Rajender Singh
45	Rajesh Kumar
46	Rajinder Pal Singh
47	Ranjan Kr. Gupta
48	Rishikesh
49	Ritu Juyal
50	S G PHARMA
51	S.P. Drugs
52	Sami Ahmed
53	Sanjay Gandhi Post Graduate Inst. of Med. Sciences,
54	Sanjay Kumar
55	Satbir
56	SATTAR
57	Shashi Bhusan
58	Sheela Khandelwal
59	Sidheek
60	Sir Ganga Ram Hospital
61	Sudesh Rani
62	Sunil Kumar Sharma
63	Sushma A. Kanojia
64	Tara Chand Guleria
65	Unor Exim Pvt. Ltd. - NOIDA
66	Vedanta Pharmacy
67	Venkateshwar Hospital
68	Vijaya Super Speciality Hospital
69	Vijendra Pal
70	Vikas Mishra
71	Zafar Ali
72	Zydus Hospitals & Healthcare Research Pvt. Ltd.