A case of rare anti-Hro alloantibody in a tertiary care center in India

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Abstract:
Anti-Hro is an alloantibody produced in individuals with -D- phenotype after a sensitizing event. Owing to the rarity of this antigen negative unit, registration in rare donor registries helps in procuring blood components at the earliest. We had a patient of -D- with anti-Hro antibody who required 7 units of red cells which was unavailable at our center. The patients near relatives were typed in search of a similar phenotype blood. Search was made for the rare units and Japanese Red Cross Society, American Red Cross Society, and International Blood Group Reference Laboratory, United Kingdom was contacted. Patient's brother and mother were typed as -D- and one unit from each of them was collected, irradiated, and transfused to the patient. Five units were imported from the Japanese Red Cross Society, Japan. Accessibility for identification and confirmation of rare blood groups and provision of the same can be centralized and liaison with the international registries can go a long way in the provision of blood components at the earliest.

Keywords:
Anti-Hro antibody, international rare donor registry, rare blood group

Introduction
Rh proteins are encoded by two genes: RHD and RHCE on chromosome 6. Due to various mutations, Rh is considered one of the most complex blood group systems. The additional RHD sequences in RHCE with a normal RHD explain the elevated D (-D-). Such individuals produce a rare antibody called as anti-Rh17 antibody (anti-Hro) after the immune stimulus.[1]

We report one such rare case and the challenges faced in resolving and providing transfusion support.

Case Report
A 36-year-old Indian male suffering from chronic liver disease was admitted for liver transplant at our institution. Owing to a transfusion requirement (Hemoglobin 4.7 g/dl), preliminary investigations pertaining to blood bank, that is, blood grouping and antibody screening were sent.

On testing, the blood group was found to be O RhD positive. The patient had a history of blood transfusion 2 years back. The antibody screen (4 cell panel, Immucor, Norcross, USA) was positive and the antibody identification was done. The sample reacted with all cells in the 16 cell panel (Capture-R Ready-ID, Immucor Inc., Norcross, USA) and 11 cell panel (ID-DiaPanel, Biorad). The auto control and direct antiglobulin test were negative. On phenotyping (Immucor, Norcross) for Rh and Kell antigens, it was found that the patient was serologically -D- K- [Table 1]. To confirm the absence of CE antigens, absorption and elution using antisera anti-C, anti-c, anti-E, and anti-e (Immucor, Norcross, USA) were done using the patient cells as per the procedure guidelines in American Association of Blood Banks (AABB).[2] The eluate was then tested for the presence of any antibody. The eluate was negative in all
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Table 1: Initial serological tests

| Test                                | Method                                      | Result                                      |
|-------------------------------------|---------------------------------------------|---------------------------------------------|
| ABO grouping                        | Tube, IS                                    | “O”                                         |
| Rh “D” grouping                     | Tube, IS using monoclonal IgM and IgG blend | D positive                                  |
| Antibody screening with 3 cell panel| SPRCA, Neo, Immucor, USA                    | 4+ agglutination with all three cells       |
| Auto control                        | RT, 37°C, IAT                                | Negative                                    |
| Direct antiglobulin test             | SPRCA, Neo, Immucor, USA                    | Negative                                    |
| Antibody identification 16 cell panel| SPRCA, Neo, Immucor, USA                    | 4+ agglutination with all cells of the panel|
| Antibody identification 11 cell panel| Tube technique (ID-DiaPanel, Biorad) RT, 37°C, LISS additive IAT | 1+ at 37°C with all cells and 4+ agglutination with all cells in IAT |
| Rh CE phenotype                     | SPRCA, Neo, Immucor, USA                    | No agglutination with anti C, c, E, D–phenotype |

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iS=immediate spin, SPRCA=Solid phase red cell adherence assay, RT=Room temperature, IAT=Indirect antiglobulin test, LISS=Low-ionic salt solution, IgM=Immunoglobulin M, IgG=Immunoglobulin G

the cases. It was confirmed that the patient was a case of elevated D with anti-Rh 17 antibody (anti-Hro) which is clinically significant. Only Rh null or -D- phenotype red cells will be compatible with the patient.

None of the units in our inventory was -D-. Search for compatible blood among first-degree relatives was made. The patient has two siblings, one of which was -D-. The youngest sibling was CCee phenotypically. Patient’s mother had -D- phenotype with the “O” blood group. One unit of group O -D- was collected from each of them, irradiated and transfused. Meanwhile, search for this rare unit was ongoing and Japanese Red Cross Society, American Red Cross Society, and International Blood Group Reference Laboratory (IBGRL), United Kingdom was contacted. IBGRL suggested that they could do the confirmatory testing again. Due to financial constraints, we did not send the sample to IBGRL. However, all the supplementary tests to confirm the phenotype were done in-house. The formalities for the shipment were done and 5 Hro negative units were imported from the Japanese Red Cross Society. They were transfused to the patient after due processing of the units as per the departmental protocol for the same. The units were compatible at anti-human globulin phase on cross-matching. No acute or delayed hemolytic transfusion reaction was noted.

**Discussion**

Rare blood groups are those which have a prevalence of <1 in 1000 of random population. Rarity may vary from country to country depending on the prevalent phenotypes in a given geographical and ethnic population. Lack of the high-frequency antigen Rh17 is considered among one of the rare phenotypes in India. As per a report by Joshi and Vasantha, four such cases were found in India. Internationally, there have been few reported cases of hemolytic disease of fetus and newborn (HDFN) due to anti-Rh17 in the literature, most of them being from Japan.

We encountered a case where anti-Rh 17 was developed after an immunizing event. Negative direct antiglobulin and auto control test excluded the possibility of autoantibodies.

The youngest sibling of the patient was CCee phenotypically, which explains why the mother had repeated abortions after the third childbirth. She might have developed the anti-Hro antibody which might have led to HDFN in her subsequent pregnancies which went undiagnosed.

Prevalence of -D- in the Japanese population is estimated to be in <1 in 100,000. The Japanese Red Cross maintains an inventory of the rare blood groups and is actively providing many rare phenotype units around the world. Although the components are available and imported from another country, in our case Japan, it consumes time and requires high financial and legal documentary support to arrange the same which might often be limiting. It imposes further psychological, physical, and mental stress amidst the already existing combat with life. Here comes the role of establishing a rare donor registry in India and international collaborations which prove fruitful in such adverse needs. Maintaining an accessible indigenous rare donor program can go a long way in helping out such cases at the earliest. Although Immunohematology Reference Laboratory at the National Institute of Immunohematology, Mumbai, under the aegis of the Indian Council of Medical Research, Government of India has been earmarked for the same and has rare donor phenotypes identified and registered as delineated by Kaur and Jain, we required more number of units so the international registries were contacted. Such close collaboration between international societies meant availability of such rare units making the world a global village. India, being the second largest population in the world, the provision for identification and confirmation of the rare phenotypes should be made accessible to most of the blood banks or at least few reference centers need to be earmarked...
for the same so that the high potential in the country can be well utilized.

**Conclusion**

Anti-Hro is an alloantibody produced in individuals with –D- phenotype after a sensitizing event. Owing to the rarity of this antigen negative unit, registration in rare donor registries helps in procuring blood components at the earliest. We had a patient with anti-Hro antibody who required many units of red cells which were imported from the Japanese Red Cross Society. Accessibility for identification and confirmation of rare blood groups and provision of the same can be centralized and liaison with the international registries can go a long way in the provision of blood components at the earliest.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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