Potential serological challenges caused by anti-IH antibody in the crossmatch laboratory

Sir,

Cold auto-agglutinins constitute the human sera and react optimally at lower temperatures ranging anywhere from 0°C to 4°C.[1] These antibodies are clinically benign, having low titers and demonstrate a very narrow thermal amplitude (TA).[2] Likewise, anti-IH is a complex cold agglutinin having a benign nature and preferentially act at lower temperatures. Its demonstration requires a co-expression of both I and H antigens on the erythrocyte membrane (EM). Further, the reactivity of anti-IH usually depends on the amount of H antigens present on the EM, which makes it react more with O and A_2 cells as against A_1 and A_1B cells.[2] Seldom, this may present as a clinically significant antibody resulting in an acute hemolytic transfusion event.[3] We describe herein, an instance of a 42-year-old male having aplastic anemia and past history of cerebrovascular accident who was admitted for the management of his anemia at our hospital. He experienced a recent history of blood transfusion almost 10 days back from an outside facility. His hematological investigations revealed a low hematocrit of 19.7% that necessitated a packed red blood cell (PRBC) transfusion. The pretransfusion work up was performed according to our departmental standard operating procedures. His blood grouping by conventional tube technique showed a discrepancy. Whereas, cell grouping suggested A_1 Rh (D) positive, his serum showed varying grades of agglutination with A_1, B, and O pooled erythrocytes. It is worthwhile noting that his serum showed a higher grade of agglutination especially with the O pooled erythrocytes as against A_1 cells, respectively [Table 1]. Repeat serum grouping after incubation at 4°C and 37°C [Table 2] for 30 min each, showed an irregular antibody having a preferential action at 4°C, hence bringing up the suspicion of a cold agglutinin. The irregular antibody did not resolve completely even at 37°C and showed a weaker grade of reaction (w+). This was hence, typed as type IV discrepancy with the presence of an unresolved irregular cold antibody. His direct *Coombs* test and auto-control both were negative. On performing the antibody screening and identification with the commercially available Diamed Gel cards (Biorad, Switzerland), pan-agglutination was observed. Cold antibodies such as anti-I and anti-H were ruled out since patients' serum showed weaker (w+ and 1+) grades of agglutination with O_h (Bombay) I+ adult cells and O_i (Cord) H+ cells, respectively. The reaction pattern, however, agreed with another cold agglutinin namely, anti-IH. On performing serial dilutions of patients' serum with O (I+ H+) adult erythrocytes at 4°C, 18°C–22°C and 37°C temperatures, resultant titers obtained were 32, 8, and 4, respectively. We also treated patients' serum with dithiothreitol (DTT) that yielded a zero grade in the first tube while, with phosphate buffer saline (PBS) it showed the titer of 16 and 4 both at saline as well as the anti-human globulin (AHG) phase, respectively. Broad TA rather than its titer was inferred to be a critical measure depicting the clinical significance of the underlying antibody. While cross-matching patients' serum with donor erythrocytes, A_1 cells showed a reduced grade of reactivity pattern typically ranging from 0 to 1+ as against higher grade of reactivity (4+) with O cells. The patient was eventually transfused with one pint of AHG cross-matched compatible A_1 Rh (D) positive PRBC using a blood warmer without any adverse consequence.

In general, cold auto-agglutinins are directed against the Ii blood group collection.[2] Therefore, because of the biochemical relationship of ABH and Ii antigens, it is not surprising that ABH–Ii complex specificities (e.g., anti-IH and anti-IA) that require the presence of

Table 1: Patient blood grouping performed by the conventional tube technique

Anti-A	Anti-B	Anti-D	Anti-A1 lectin	Auto control	A1 pooled cells	B pooled cells	O pooled cells	Interpretation
4+	0	4+	3+	0	2+	4+	4+	A_1 Rh (D) positive with? irregular antibody

The significance is towards the interpretation of varying grades of agglutination ranging from 0 to 4+

Table 2: Repeat serum grouping at different temperature ranges

Temperature (°C)	A1 pooled cells	B pooled cells	O pooled cells	Interpretation
4	3+	4+	4+	? Irregular cold antibody with a broad TA
37	1+	4+	3+	

The significance is towards the interpretation of varying grades of agglutination ranging from 0 to 4+. TA=Thermal amplitude

more than one antigen to react, is widely described in the literature.[4] Just like other cold agglutinins (with narrow TA), anti-IH also does not usually interfere during the pretransfusion testing; in fact these can often be circumvented on avoiding room temperature testing phases. Albeit, sometimes, anti-IH can behave as a clinically significant entity showing a broad TA. In this case, several factors including, its serologic presentation, potency, and specificity helped us clinch the diagnosis bench-side. Even the reactivity pattern in serum typing with A_1 pooled erythrocytes (having reduced H antigen expression) appeared weaker when compared to the O pooled cells. Because H antigen is the substrate for A and B antigens, the expression of A and/or B antigens is always reciprocal to its expression. While group O erythrocytes have the maximum expression of H antigen, group A_1B erythrocytes have the least ($O>A_2>B>A_2B>A_1>A_1B$).[3] Mohanan *et al*. have described a similar instance of a clinically significant anti-IH antibody showing a broad TA.[5] In our example, the anti-IH antibody demonstrated a higher grade of reactivity pattern and broader TA with group O adult erythrocytes as against group A_1 adult cells. Further, there was reduced reactivity with group Oi (cord) cells as well as Oh (Bombay) cells, respectively. Reduced reactivity of DTT-treated serum and TA studies pointed toward an IgM characteristic of the antibody. Broader TA was deduced from the fact that the reactivity pattern with group O adult (I+ H+) erythrocytes matched at both room temperature as well as 37°C. At the time of discharge, the patient's hematocrit was 24.7% and his overall condition was clinically stable.

To conclude, we reiterate the fact that a cold agglutinin of anti-IH specificity having a broader TA, although rare, can be potentially catastrophic, and a timely identification of the same can help circumvent any probable adverse event in the recipient.

Acknowledgment

The authors gratefully acknowledge the support of Mrs. Savina Prasad (Blood Bank technician) for her assistance in performing the patient's serological workup.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Manish Raturi, Shamee Shastry[1], Ganesh Mohan[1]

Department of Immunohematology and Blood Transfusion, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, [1]Department of Immunohematology and Blood Transfusion, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Udupi, Karnataka, 576104, India

Address for correspondence:
Dr. Manish Raturi,
Department of Immunohematology and Blood Transfusion, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Swami Ram Nagar, Jolly Grant, Dehradun - 248 016, Uttarakhand, India.
E-mail: manishraturi@srhu.edu.in

Submitted: 05-08-2019
Revised: 27-01-2020
Accepted: 07-06-2020
Published: 12-06-2021

References

1. Raturi M, Shastry S. Laboratory work-up and its translational significance in cold agglutinin syndrome. Int J Res Med Sci 2016;4:339-42.
2. Beck ML. The I blood group collection. In: Moulds JM, Woods LI, editors. Blood Groups: P, I, Sda and Pr. Arlington: American Association of Blood Banks; 1991. p. 23-47.
3. Irani MS, Richards C. Hemolytic transfusion reaction due to anti-IH. Transfusion 2011;51:2676-8.
4. Rosenfield RE, Schroeder R, Ballard R, Van Der Hart M, Moes M, Van Loghem J. erythrocytic antigenic determinants characteristic of H, I in the presence of H (IH), or H in the absence of I (H(-I)). Vox Sang 1964;9:415-9.
5. Mohanan N, Henry N, Rafi AM, Innah SJ. Anti IH: An antibody worth mention. Asian J Transfus Sci 2016;10:152-4.

Access this article online	
Website: www.ajts.org	**Quick Response Code:**
DOI: 10.4103/ajts.AJTS_71_19	

How to cite this article: Raturi M, Shastry S, Mohan G. Potential serological challenges caused by anti-IH antibody in the crossmatch laboratory. Asian J Transfus Sci 2021;15:115-6.