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Case Report

Drug-induced immune hemolytic anemia (Direct Antiglobulin Test positive)

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Case report

A 7-year-old male child was detected to have positive Direct Antiglobulin Test (DAT) with Hb of 6.9 g/dl. Demand for two units of PRBC was received to alleviate the anemia. His blood group was B Rh (D)+ve. On cross matching with four donor units of the same group, the patient's blood group was found incompatible (minor match). A DAT performed by conventional tube as well as gel micro column techniques (Liss/Coomb's ID-CARD from BIO-RAD, Switzerland) was found positive (4+) by both.

Detailed past history of the patient revealed him to be a case of blunt abdominal trauma with perforation peritonitis 4 months ago for which he was operated and treated with antibiotics (Piperacillin, Tazobactam, flagyl, etc.) for 1 month. At that time he was transfused 2 units PRBCs, 6 units FFP and RDP each, post operatively with no issues of cross match compatibility (both major & minor). His DAT was negative with Hb 10.5 g/dl a week after transfusion. Two months after the surgery he developed sepsis and was readmitted to hospital. His Hb fell to 8.5 g/dl and during cross matching his blood was found incompatible (minor match) with same group donor units (Table 2). His DAT was positive (1+). He was once again transfused with two units of PRBCs with no adverse reaction and his Hb recovered to 10 g/dl within 8 days. During this stint of hospitalization he received the same antibiotics as before. Six weeks later he was admitted for the third time in Oct 2011 when he came to notice; due to low Hb 6.9 g/dl, minor match incompatibility and positive DAT (4+).

Introduction

Drug-induced immune hemolytic anemia occurs rarely (1 in 1 million population).¹ It is an uncommon finding characterized by a sudden decrease in hemoglobin after treatment with the putative drug. To date, about 100 drugs have been implicated in causing a positive Direct Antiglobulin Test (DAT) and/or hemolytic anemia. The most common drugs associated with this, are penicillin and its derivatives, cephalosporins (cefotetan, ceftriaxone etc.), methyl dopa, β -lactamase inhibitors and quinidine. Drug antibodies fall into two types: drug-independent (autoantibodies) and drug-dependent ("penicillin type" or "immune complex type"). Some drugs cause non-immunologic protein adsorption onto drug-treated red blood cells (RBCs).^{1–3} All these mechanism are associated with positive DAT which may lead to hemolytic anemia.

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Table 1 – ABO and Rh typing.

Specimen	Anti-		Test cells		Anti-		Interpretation	
	A	B	AB	A1	B	D	ABO	Rh
Initial	0	4+	4+	4+	0	4+	B	+VE
Post-Piperacillin	0	4+	4+	4+	0	4+	B	+VE

At this time he was thoroughly investigated including detailed immunohaematology work up including repeat ABO & Rh grouping forward and reverse by both tube & gel techniques (Table 1). Patients autocontrol was negative. DAT was repeated using both techniques (Table 3). The Indirect Antiglobulin Test (IAT) on the patient's serum was negative. To further evaluate DAT positivity, elution was done to uncoat the red cells by ZZAP (mixture of 0.1 m dithiothreitol (DTT) plus 0.1% cysteine activated papain); heat elution technique (for removing IgM type antibody) & acid + chloroquine diphosphate elution method (for removing IgG type antibody).

Adsorption of the drug Piptaz (combination of Piperacillin & Tazobactam) was performed on 'O' pooled red cells. **Both eluates reacted with the drug adsorbed on 'O' pooled cells** (Table 4). Acid + chloroquine diphosphate treated red cells eluate reacted strongly with the drug adsorbed 'O' pooled cells showing the presence of drug-dependent (Piptaz in this case) IgG type antibody over the patients red cells. This was responsible for the immune mediated destruction of red cells and strong DAT (4+). This information was provided to the treating physician and the incriminating drug Piptaz was withdrawn. Antibiotics belonging to other families were substituted. After 15 days the patient became DAT negative and his Hb rose to 12 g/dl.

Discussion

Intake of drugs can result in hematologic abnormalities, including positive DAT. Drug-induced immune hemolytic anemia is considered to be rare but is likely under-recognized. In the previously reported cases of penicillin-induced

Table 3 – Direct Antiglobulin Test.

Specimen	Poly sp.	Anti-IgG	Anti-C3d
Initial	–VE	–VE	–VE
Post-Piperacillin	+VE	+VE	–VE

hemolytic anemia the time to onset of symptoms ranged from 7 to 14 days after starting penicillin.² But in this case, the signs and symptoms of hemolysis developed after 30–45 days of the initiation of Piperacillin therapy. The course was slow and progressive, leading to severe hemolysis. Subsequently these signs and symptoms subsided after the withdrawal of drug (Piperacillin), which support the diagnosis of drug-induced hemolytic anemia. It has been explained that penicillin (the antigen) may bind loosely to RBCs in vivo, thus becoming immunogenic and stimulating the production of antibodies. After antibody production is initiated, immune complexes form (consisting of antibody and drug) and these complexes in turn bind non-specifically to other RBCs and ultimately leads to the activation of complement system. This "immune complex" mechanism usually generates a severe intravascular reaction, with a positive DAT.^{2–4} The consulting serologist plays a critical role in integrating serologic findings with the clinical history. There are various other mechanism by which a drug can cause positive DAT including autoimmune-like phenomenon (seen in drugs like alpha-methyldopa, mefenamic acid and procainamide) & membrane adsorption (seen in penicillins and cephalosporins) in which the drugs bind to red cells in vivo and if the patient produces antibodies to the drug, the antibody will bind to the drug adsorbed on the red cells, and cause a positive DAT which may lead to hemolysis.^{5–7}

In conclusion, although drug-induced hemolytic anemia is rare, it should be kept in mind, as it may be associated with various degrees of morbidities. Penicillin-induced immune hemolytic anemia should be suspected in patients treated with penicillin who develop hemoglobinuria, hemolyzed blood specimens, or severe anemia. Confirmation of the diagnosis requires a consultation for a DAT. In addition, increased reporting of these occurrences and further

Table 2 – Cross match.

Donor blood unit	Initial specimen		Post-Piperacillin			
	Test tube tech. saline (30 &370c) & AHG	ID-gel column tech. (LISS Coomb)	Test tube tech. saline (30 &370c) & AHG		ID-gel column tech. (LISS Coomb)	
			Major cross match	Minor cross match	Major cross match	Minor cross match
	1	Compatible		Compatible	Incompatible	Compatible
2	Compatible		Compatible	Incompatible	Compatible	Incompatible
3	Compatible		Compatible	Incompatible	Compatible	Incompatible
4	Compatible		Compatible	Incompatible	Compatible	Incompatible

Table 4 – Results of the reaction of eluates with the drug adsorbed on O pooled cells.

Eluates + drug adsorbed 'O' pooled cells	Result
ZZAP eluate	Agglutination 2(+)
Heat eluate	Agglutination 2(+)
Acid eluate	Agglutination 4(+)

investigation of compiled data are necessary to better understand the mechanism of these hemolytic reactions.

Conflicts of interest

All authors have none to declare.

Book Review

Sharma M, John BM, Sondhi VS. *The Acutely Ill Child: A Ready Reckoner*, JayPee Publications, New Delhi, Publication-2013, Pages-349, Price- Rs 495/-

Editors:

Acute care of sick children plays a pivotal role in the field of Pediatrics. Provision of the appropriate treatment based on evidence based guidelines is the key to satisfactory management. Pediatric emergencies come in various forms with differing manifestations and immediate/early interventions are extremely important. This handbook provides relevant information and an algorithmic approach to most of the common pediatric emergencies and would be extremely useful as a quick reference at the first point of contact. The book is likely to be a useful bridge between the child care provider and standard textbooks in pediatric emergency/intensive care. The handbook has been divided into various sections such as supporting the sick child followed by sections covering conditions under different systems which includes respiratory, cardiovascular, hemato-oncology, endocrine, renal, GIT & liver, neurology, IEM, and toxicology. This is followed by chapters on common procedures and medications including a list of IV drug compatibility. The

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well compiled set of appendices covers almost most of the charts and scales routinely looked at during the care of a sick child. These charts and scales are seldom available in a single book and hence is a very important and unique asset. The chapter contributors are mostly residents from the department of Pediatrics at AFMC, Pune and these have been co-authored by the experienced faculty from the same department. This approach leaves an indelible mark on the book for its immense practicability. The writing style of this concise handbook makes it very useful for the medical or nursing officer, post graduates and the pediatrician alike. This easy to read book has been reasonably priced making it a must have 'Ready reckoner'.

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