CORRESPONDENCE

Autologous Blood Transfusion as a Life Saving Measure for a Trauma Patient with Fracture Femur and Drug Induced Hemolytic Anemia: A Case Report

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Abstract A positive direct antiglobulin test has been reported in 1:1000 to 1:14,000 blood donors and 1–15 % of hospital patients. Drugs may cause a positive direct antiglobulin test result and/or immune-mediated haemolysis with an incidence of approximately 1 in a 1 million population. Our aim is to highlight the importance of following strict transfusion protocols and management insight in a direct antiglobulin test positive patient showing incompatibility with multiple units possibly due to drug induced immune haemolytic anaemia (DIIHA). We also aim to highlight importance of autologous blood transfusion in an orthopaedic procedure in which homologous transfusion may be needed. We are presenting case of a 36 year old male with alleged h/o of road traffic accident with comminuted intra-articular fracture of the distal femur. He had h/o of ankylosing spondylitis since last 20 years on medication with indomethacin and methotrexate. A review of the literature was performed which showed use of drug methotrexate as an uncommon clinical entity for DIIHA; sporadic reports exist in the medical literature to support this view. The review of the literature in combination with our own data showed methotrexate can be a cause of DIIHA. We therefore advocate proper immunohaematological work up and use of autologous blood for management of at-risk-patients of DIIHA.

Keywords DIIHA · Methotrexate · Autologous blood transfusion

Introduction

Drug induced immune haemolytic anaemia (DIIHA) is not a common entity (1 in a million populations) [1]. DIIHA was first suspected in 1953 in a patient who developed pancytopenia with hemolytic anemia (HA) with a positive direct antiglobulin test (DAT), due to intake of a drug mephenytoin [2]. A review of literature by Dausset and count [3] found 34 published cases of drug induced immune hemolytic anemia (DIIHA) due to 15 drugs. In 2007, Garratty and Arndt reported 125 drugs showing evidence to suggest DIIHA [4]. They have found that earlier methyldopa and penicillin were the two common drugs associated with DIIHA. Nowadays, most cases of drug-induced AIHA are caused by second- and third-generation cephalosporin [5].

We aim to emphasize the role of immuno haematological work-up for assessment of a patient for suspected drug methotrexate induced immune haemolytic anaemia and utility of autologous transfusion in management of this patient showing DAT and ICT positivity and donor incompatibility.

Case History

A 36-year-old male presented to us with c/o pain and swelling in right knee with alleged h/o road traffic accident. His physical examination, clinical evaluation and radiological findings (X-ray) confirmed comminuted intra articular fracture of the distal femur. On further evaluation

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past history of ankylosing spondylitis since last 20 years and medication with methotrexate and non-steroidal anti-inflammatory drugs (NSAID) was found. No h/o of previous blood transfusion was found. During preoperative evaluation, in view of his clinical signs and symptoms two units of packed red blood cells (PRBC) was demanded but during cross match blood incompatibility was noted.

Immunohaematological Work-Up

Following this thorough immuno haematological work-up was done which showed; Blood group: B Rh (D) positive; DCT-Positive (2+ by gel cards); ICT-Positive (using gel cards); 3-cell antibody screening cell-Positive (pan reactive); and 11-cell-identification panel-Positive (pan reactive). Auto-control: positive (1+ by gel cards). For evaluation of DCT cold acid elution was performed and elutes was tested in parallel with last wash. Eluate showed same pattern as that of ICT.

Other Laboratory Investigations (Table 1)

CBC revealed decreased haemoglobin and haematocrit with mild increase in the reticulocyte count. Careful peripheral blood smears (PBS) were examined and bone marrow studies were planned to rule out any megaloblastic anaemia or haemolysis. PBS was showing mild microcytic hypochromic picture with occasional nucleated RBC (02/100 WBC). No specific features of megaloblastic changes or signs of haemolysis were noted on blood smears. As patient was receiving haematinics including iron, vitamin B12 and folic acid, it was difficult to conclude the exact cause. Therefore folate levels were not performed. Mild increase in the reticulocyte count was noted. Liver function tests including alanine aminotransferase, aspartate aminotransferase and direct and

indirect bilirubin were found to be within the normal range. Urinalysis was unremarkable.

Diagnosis

Absence of previous transfusion ruled out presence of any underlying alloantibody so following review of the patient's medications (methotrexate since last 20 years), a possibility of drug induced immune hemolytic anemia was raised, which could not be ruled out although the patient was found to be DCT positive.

Treatment and Outcome

As the patient required immediate RBC transfusion, 20 units of PRBC were randomly cross-matched, and fortunately two units had reaction less than auto control. This best matched unit was issued and transfused to the patient without any untoward transfusion reaction. As the patient was posted for surgical reduction of the fracture, two more units of PRBC were requested. The condition was then discussed with the concerned orthopaedic surgeon and it was decided that they would be performing autologous transfusion in order to avoid further transfusion of any incompatible blood.

Two units of autologous blood units were collected 72 and 24 h before the surgery and were transfused when required. The postoperative period remained uneventful, without any adverse transfusion reaction.

Review of Literature

We performed a search of the literature on DIIHA using the Internet (Medline/PubMed). A lot has been published on the subject of DIIHA in the last decade, but most of the

Table 1 Laboratory investigations during hospital stay

Parameters	Day 1 (before transfusion)	Day 6 (after transfusion of 2 incompatible units)	Day 12 (after transfusion of 2 autologous units)
Hb (gm/dl)	8.12	9.7	9.0
RBC count (millions/mm ³)	2.85	2.95	3.12
TLC (per mm ³)	9110	10,600	10,000
Platelets (lakh/mm ³)	2.3	5.04	9.0
PT (s)-control/patient	13.7/14.4		12
APTT (s)-control/patient	29.5/18.9		13.7/12.0
INR	1.07		29.5/18.2
Urea (mg/dl)	27	24	21
Bilirubin (T/D) (mg/dl)	0.8/0.4	0.9/0.4	1.0/0.5
Protein (gm/dl)	6.4	6.6	6.6
Reticulocyte (%)		3.4	3.0

articles deal with DIIHA with different aetiology. Since we were interested in DIIHA with methotrexate only, a few articles were useful. A case of rheumatoid arthritis associated with autoimmune haemolytic anaemia (AIHA) due to weekly low-dose methotrexate therapy [6] and a case of methotrexate-induced AIHA in a patient of metastatic breast carcinoma [7] has been reported in literature.

Discussion

The standard approach to laboratory analysis for most cases of DIIHA is to perform a DAT to determine whether complement and/or IgG bind to the patient's RBCs. If the DAT is positive, then the patient's serum should be tested with drug-treated RBCs or untreated and enzyme-treated RBCs in addition to the drug of interest. If clinical signs and symptoms of haemolysis are present and the DAT is positive, an eluate prepared from the patient's RBCs should be tested against drug-treated RBCs with the drug present [8, 9]. If intravascular haemolytic anaemia develops with DAT positivity, the clinician needs to investigate and try to identify the cause of AIHA. The clinician should review the history of medication profile, any diagnostic work-up for neoplasm or lymphoproliferative disorders and whether the patient has received recent blood transfusions. Decision to transfuse a patient in AIHA should be taken on basis of the clinical condition rather than laboratory values [10]. Conventional criteria such as haemoglobin concentration, reticulocyte count, bilirubin (unconjugated) concentration, LDH and haptoglobin concentration may be valuable in assessing the severity of the disease process in AIHA [11]. Blood transfusions should be used with caution because the donor RBCs may also be haemolysed, leading to further anaemia [12].

Advanced immunohaematological work-up with tests like adsorption study and antigen phenotyping on eluted red cells can help to differentiate alloantibodies (in patients with history of transfusion, pregnancy or transplantation) from autoantibodies [13, 14].

Mechanism of drug induced antibody reaction proposed by George Garratty [15] hypothesizes that (a) drugs may covalently bind to proteins (e.g. RBC membrane) and a drug antibody (usually IgG) can be made to the drugs causing in vitro reactions and later clearance by macrophages, typical of a drug adsorption reaction (most commonly seen in penicillin type); (b) drugs may bind to RBC membrane components producing in vitro reactions usually seen in autoantibodies; (c) a part of drug and a part of RBC membrane component may produce an in vitro reaction typical seen in immune complex mechanism. The combined membrane plus drug can create an immunogen; the antibodies formed (which can be IgM or IgG) may activate

complement, which can lead to fatal reactions like acute intravascular haemolysis and sometimes renal failure. A study by Woolleys et al. [7] demonstrated that a macrophage assay showed increased phagocytic uptake of MTX-treated RBC cells in the presence of patient serum (mainly IgG-3 subclass antibodies) showing DCT and ICT positivity in a patient with h/o methotrexate intake. This phagocytosis was not enhanced with use of complement. The ⁵¹Cr treated red cell survival of autologous MTX cells was markedly shortened which indicated rapid in vivo destruction. So this antibody does not fix complement, but sensitizes red blood cells to phagocytosis by macrophages.

The nature of the antibody found in this patient appears to be directed against a high frequency antigen. The initial findings suggest the presence of an autoantibody since the DAT is positive and all donor cells are reactive. Further workup showed pan reactivity instead of a specific antibody with its reactivity with 3 cell panel and extended 11 cell panels having the corresponding antigen.

One approach to an antibody directed against a high frequency antigen is to determine how the antibody reacts with RBCs treated with any of a variety of enzymes and with sulfhydryl-reducing agents; this help us to narrow down the possibilities based on the known effects of these agents, thus allowing conservation of some of the laboratories rare RBC samples. Many commonly found antibodies directed against high-frequency antigens exhibit "high titer, low avidity" behaviour, so many technologists perform a titration as well. Patient's ethnic background may also suggest certain possibilities like presence of anti-Jk³ or anti-Di^b in individuals of Pacific island origin or presence of anti-U in individual of African heritage. Absence of antibodies against high frequency antigens may be a probable reason why two blood units were compatible with this patient's blood.

The International Society for Blood Transfusion (ISBT) recognizes 12 antigens of very high frequency that have not been shown to belong to any blood group system or collection: Vel, Lan, Ata, Jra, Oka, JMH, Emm, AnWj, Duclos, PEL, ABTI, and MAM [16–18]. Many other high-frequency antigens, which do not fill the criteria necessary for recognition by the ISBT, are known by immunohematology reference laboratories. It is a fact that haemolytic transfusion reactions are expected to occur when incompatibility is due to clinically important alloantibodies [19]. But experience indicates if incompatibility is due to the presence of a RBC autoantibody, the survival of transfused RBC is generally as good as that of the patient's own RBCs, and transfusion can be expected to cause significant temporary benefit [20, 21]. The decision to transfuse does not depend on compatibility test results alone instead it depends on the patient's need for transfusion [20–22]. The process of using least incompatible blood for transfusion of

patients will not be reliable to detect alloantibodies and is unacceptable in modern day transfusion medicine [22–24]. This is the reason why we accepted two units of least incompatible blood initially when patient required immediate blood transfusion. Later we opted for autologous transfusions as it is supposed to be the safest blood product.

Autologous blood donation was used 20 years ago, but reduced risks of allogeneic blood induced transfusion transmitted infections have limited its use. Autologous blood is indicated in surgical patients to avoid allogeneic blood use and the proportion of autologous blood that is reinfused is used during the perioperative period [25–28].

Other advantages of autologous blood donation are (a) the blood donation occurs in the weeks prior to operation and (b) autologous donors will not require the larger volumes of fluid replacement to re-establish the normovolemia. The time gap between collections of autologous blood may effectively replace some of the autologous red cells removed preoperatively, but most autologous donors are unable to effectively restore a pre-donation haematocrit [29, 30]. An additional advantage of pre-deposit autologous blood is that the units can be reinfused at any time during the perioperative period. Contrary to acute normo volumic hemodilution or cell scavenging techniques that are primarily an intraoperative method to return autologous blood, pre-donated autologous blood can be stored until later in the postoperative period. A disadvantage of using autologous blood is that a blood bank exposes donors to the iatrogenic errors while collecting, labelling, storing and checking a patient's autologous blood.

Conclusion

As the mechanism by which methotrexate induces AIHA continues to be investigated, the concept of cross-reactivity and the possibility that metabolites of methotrexate may cause severe drug-induced haemolysis remain to be clarified. We recommend further methotrexate treated red cell testing with elute to confirm the possibility of DIIHA in patients giving history of drug intake for long duration and showing DAT and ICT positivity with donor incompatibility. In addition, increased reporting of these occurrences and further investigations including extended antigen panels are necessary to better understand the mechanism of these enigmatic reactions. This case brings to attention several aspects: the role for proper history taking specially history of drug intake for patients showing cross match incompatibility, knowing the likely complications, the need for proper Immunohaematological workup to confirming the diagnosis of DIIHA and use of autologous blood and acute normovolemic hemodilution for management of the patients.

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Compliance with Ethical Standards

Conflict of interest There were no conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

- Petz LD, Garratty G (2004) Immune haemolytic anaemia's, 2nd edn. Churchill Livingstone, Philadelphia, p 261e317
- Snapper I, Marks D, Schwartz L, Hollander L (1953) Hemolytic anemia secondary to mesantoin. *Ann Intern Med* 39:619–623
- Dausset J, Contu L (1967) Drug-induced haemolysis. *Ann Rev Med* 18:55–70
- Garratty G, Arndt PA (2007) An update on drug-induced immune haemolytic anemia. *Immunohematology* 23:105–119
- Garratty G, Arndt PA (2014) Drugs that have been shown to cause drug-induced immune hemolytic anemia or positive direct antiglobulin tests: some interesting findings since 2007. *Immunohematology* 30(2):66–79
- Yoshida K, Kurosaka D, Ozawa Y, Yokoyama T, Tajima N (2000) A case of rheumatoid arthritis associated with autoimmune hemolytic anemia due to weekly low-dose methotrexate therapy. *Ryumachi* 40(4):693–698
- Woolley PV, Sacher RA, Priego VM, Schanfield MS, Bonnem EM (1983) Methotrexate-induced immune haemolytic anaemia. *Br J Haematol* 54(4):543–552
- Arndt PA, Leger RM, Garratty G (1999) Serology of antibodies to second- and third-generation cephalosporin associated with immune hemolytic anemia and/or positive direct antiglobulin tests. *Transfusion* 39:1239–1246
- Johnson ST, Fueger JT, Gottschall JL (2007) One center's experience: the serology and drugs associated with drug-induced immune hemolytic anemia—a new paradigm. *Transfusion* 47:697–702
- Petz LD (2004) A physician's guide to transfusion in autoimmune haemolytic anaemia. *Br J Haematol* 124:712–716
- Chaplin H Jr (1990) Red cell-bound immunoglobulin as a predictor of severity of hemolysis in patients with autoimmune hemolytic anemia. *Transfusion* 30:576–578

12. Gehrs BC, Friedberg RC (2002) Autoimmune hemolytic anemia. *Am J Hematol* 69:258–271
13. Bercher ME (2005) The positive direct antiglobulin test and immune mediated red cell destruction. In: American Association of Blood Banks (ed) Technical manual, 15th edn. Bethesda, MA, AABB, pp 453–482
14. Das SS, Chaudhary R (2009) Utility of adsorption techniques in serological evaluation of warm autoimmune haemolytic anaemia. *Blood Transfus* 7:300–304
15. Garratty George (2010) Immune hemolytic anemia associated with drug therapy. *Blood Rev* 24:143–150
16. Daniels GL, Anstee DJ, Cartron JP et al (1995) Blood group terminology. From the ISBT working party on terminology for red cell surface antigens. *Vox Sang* 1995(69):265–279
17. Daniels GL, Anstee DJ, Cartron JP et al (1996) Terminology for red cell surface antigens. Makuhari report. *Vox Sang* 71:246–248
18. Montgomery W, NanceS Kavitsky D et al (1997) First example of hemolytic disease of the newborn (HDN) due to an antibody to a high incidence antigen present on multiple cell lines (MCL) (abstract). *Transfusion* 37(Suppl):41S
19. Jenner PW, Holland PV (1996) Diagnosis and management of transfusion reactions. In: Petz LD, Swisher SN, Kleinman S, Spence RK, Strauss RG (eds) Clinical practice of transfusion medicine, 3rd edn. Churchill-Livingstone, New York, pp 905–929
20. Salama A, Berghofer H, Mueller-Eckhardt C (1992) Red blood cell transfusion in warm-type autoimmune haemolytic anaemia. *Lancet* 340:1515–1517
21. Garratty G, Petz LD (1993) Transfusing patients with autoimmune haemolytic anaemia. *Lancet* 341:1220
22. Petz LD, Garratty G (2004) Blood transfusion in autoimmune hemolytic anemias. Immune hemolytic anemias, 2nd edn. Churchill Livingstone, New York, pp 375–400
23. Garratty G, Telen MJ, Petz LD (2002) Red cell antigens as functional molecules and obstacles to transfusion. *Hematology Am Soc Hematol Educ Program* 1:445–462. doi:[10.1182/asheducation-2002.1.445](https://doi.org/10.1182/asheducation-2002.1.445)
24. Petz LD (2003) ‘Least incompatible’ units for transfusion in autoimmune hemolytic anemia: should we eliminate this meaningless term? a commentary for clinicians and transfusion medicine professionals. *Transfusion* 43:1503–1507
25. AuBuchon JP, Birkmeyer JD (2003) Controversies in transfusion medicine; Is autologous blood transfusion worth the cost? *Transfusion*. doi:[10.1046/j.1537-2995.1994.34194098612.x](https://doi.org/10.1046/j.1537-2995.1994.34194098612.x)
26. Birkmeyer JD, Goodnough LT, AuBuchon JP, Noordsu PG, Littenberg B (1993) The cost effectiveness of preoperative autologous blood donation for total hip and knee replacement. *Transfusion* 33:544–551
27. Etchason J, Petz L, Keeler E, Calhoun L, Kleinman S, Snider C, Fink A, Brook R (1995) The cost effectiveness of preoperative autologous blood donations. *N Engl J Med* 332:719–724
28. Murray DJ (1999) Point of view: hemodilution as a method to reduce transfusion requirements in adolescent spine fusion surgery. *Spine* 24(3):223–224
29. Cohen JA, Brecher ME (2003) Preoperative autologous blood donation: benefit or detriment? A mathematical analysis. doi:[10.1046/j.1537-2995.1995.35895357894.x](https://doi.org/10.1046/j.1537-2995.1995.35895357894.x)
30. Murray D (2004) Acute normovolemic hemodilution. *Eur Spine J* 13(Suppl 1):S72–S75. doi:[10.1007/s00586-004-0755-8](https://doi.org/10.1007/s00586-004-0755-8)