

Positive Direct Antiglobulin Test With Unasyn - A Case Report

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Summary

A 56-year-old Chinese lady with valvular heart disease and atrial fibrillation was referred to us from a private hospital for further management of autoimmune haemolytic anaemia. Physical examination and laboratory investigations did not support the diagnosis of haemolytic anaemia. However, direct antiglobulin test (DAT) was strongly positive with anti-IgG and negative with anti-C3d. There was also mild anaemia and reticulocytosis, which was attributable to persistent haematuria. The DAT became positive after commencing Unasyn and cessation was associated with decreasing reactivity of the positive DAT. We believe that the positive DAT in this patient was most likely due to the Unasyn therapy.

Key Words: Unasyn, Positive direct antiglobulin test

Introduction

Many drugs have been reported to cause a positive direct antiglobulin test (DAT) and/or immune haemolytic anaemia. A positive DAT does not mean haemolysis¹. There are a number of mechanisms involved in the pathogenesis of the positive DAT and/or haemolytic anaemia. Methyldopa was previously a very common drug causing positive DAT with a rate of 10% - 40% after 3 - 6 months of therapy, but less than 1% of the patients developed evidence of haemolysis². Recently, Unasyn (ampicillin sodium plus sulbactam sodium (a beta-lactamase inhibitor)) has been suggested to be a far more common drug causing DAT². A high incidence of 39% has been reported². The pathogenetic mechanism of Unasyn-induced positive DAT without apparent haemolysis is thought to be due to the non-immunologic adsorption of proteins onto red blood cells². We report here a case of Unasyn-induced positive direct antiglobulin test without haemolysis.

Case Report

A 56 year old Chinese lady with valvular heart disease and atrial fibrillation, developed a cerebro-vascular accident involving the left cerebellar and right basal ganglia region in May 1997 which left her bed-ridden. In early 1998, she developed recurrent pneumonia and urinary tract infections due to her long-standing immobility. In October 1998, she was infected by *Enterococcus faecalis* and was treated at a private hospital with intravenous Unasyn 3g 12 hourly for 1 week and continued with oral Unasyn 2 tabs 12 hourly. A few days after the initiation of the Unasyn therapy, she was noted to have a positive direct antiglobulin test. Her haemoglobin then was 10.3g/dl, leukocytes were $3.3 \times 10^9/l$, ESR: 118mm/1st hour, blood urea: 3.4mmol/l, creatinine: 0.07mmol/l and serum albumin was 20g/l. She was referred to our hospital for further management of autoimmune haemolytic anaemia.

CASE REPORT

On admission, the patient was mildly anaemic but not jaundiced, tachypnoeic or tachycardic. She was oedematous generally. Examination of the cardiovascular system showed atrial fibrillation with a pansystolic murmur at the mitral area, and examination of the respiratory system showed scattered crepitations. There was no organomegaly but mild ascites was detected. Her urine was bloodstained. Haematology investigations showed: Hb: 8.5g/dl, MCV: 90.3fl, MCH: 30.9pg, leukocyte count: $5.9 \times 10^9/l$, platelet count: $158 \times 10^9/l$ and reticulocyte count: 7.7%. Blood film showed polychromasia with numerous burr cells but no microspherocytes or fragmented red cells. ESR was 116 mm/1st hour. Renal profile showed blood urea: 17 mmol/l, serum creatinine: 152 $\mu\text{mol/l}$, serum Na^+ : 123mmol/l, serum K^+ : 5.3mmol/l. Serum LDH (lactate dehydrogenase) was 358U/L. Urine examination showed urine bilirubin: 3+, urine RBC: 20 - 40/hpf, urine urobilinogen: trace, 24 hour urine protein: 5035mg/24 hour. Urine culture was negative. Liver profile showed hypoproteinaemia (total protein 40g/l), hypoalbuminaemia (albumin 13g/l), total bilirubin 6 $\mu\text{mol/l}$ and normal liver enzymes except for a mildly elevated alkaline phosphatase level (ALP137U/l). Serum iron and TIBC (total iron binding capacity) were low (5 $\mu\text{mol/l}$ and 19 $\mu\text{mol/l}$ respectively) and serum ferritin was high (2272.2 $\mu\text{g/L}$). Serum B_{12} was normal (602.4 pmol/l) but serum folate was low (3.30nmol/l). Direct antiglobulin test was 3+ (positive for anti-IgG and negative for anti-C3), indirect antiglobulin test was negative. Red cell eluate was shown to contain no antibody.

In view of her worsening renal function and acute pulmonary oedema, immediate peritoneal dialysis was performed. She was also treated with diuretics and anticoagulant in the form of low molecular weight heparin, but Unasyn was discontinued in our hospital. Because of her anaemia and persistent haematuria, she was transfused two pints of packed red cells without any complication. No problem of cross matching was encountered. Her haemoglobin remained relatively stable at about 9g/dl during hospitalization. At about 1 week and 2 weeks post cessation of Unasyn therapy, repeat direct antiglobulin tests were performed and showed results of 1+ and negative respectively. The patient was discharged from our hospital after her renal condition stabilised.

Discussion

A direct antiglobulin test (DAT) is used to demonstrate in-vivo coating of red blood cells with globulin particularly Immunoglobulin G (IgG) and complement 3d (C3d)¹. It is commonly used in investigating autoimmune haemolytic anaemia (AIHA), drug-induced haemolysis, haemolytic disease of newborn and alloimmune reactions to recently transfused red cells. Thus a positive DAT may result in a referral as in this case.

A positive direct antiglobulin test does not necessarily mean that the red blood cells have a shortened survival. Small amounts of Ig and complement appear to be present on all red cells. As many as 15% of hospital patients and between 1 in 1,000 to 1 in 14,000 blood donors, have a positive DAT without clinical manifestations of immune mediated haemolysis¹. In certain conditions such as sickle cell disease, β -thalassaemia, renal disease, multiple myeloma and other diseases with elevated globulin or blood urea nitrogen, elevated levels of IgG or complement have been noted on the red cells but there was no clear correlation between positive DAT and anaemia. Thus, a positive DAT must be interpreted in the context of the patient's clinical presentation and other laboratory results in order to avoid unnecessary steroid therapy.

This patient did not have autoimmune haemolytic anaemia because no autoantibody was detected in the serum as well as in the red cell eluate. Besides, this patient also did not show features of haemolysis: She was not jaundiced; no microspherocyte was observed in her blood film; her total bilirubin and LDH were normal; and her urine urobilinogen was trace. The anaemia and reticulocytosis was probably a response to the persistent haematuria. There was also no problem in the cross-matching of blood for her. This indicates that her serum did not contain any autoantibody, which react with the donor red cells. The positive DAT was probably due to a drug induced non-immunological protein adsorption as reported by other authors^{2,3}.

Many drugs have been reported to cause a positive DAT and/or haemolytic anaemia. Until now, methyl dopa was the most common drug causing a positive DAT with a reported rate of 15% - 20%¹ or 10% - 36%² after 3 - 6 months of therapy, and less than 1%^{1,2} of these patients

developed evidence of haemolysis. Some other drugs like phenacetin, guanidine, penicillins, cephalosporin and cisplatin have also been documented to cause a positive DAT. Recently, Garratty et al² has suggested that Unasyn causes a positive DAT more commonly than any other drug in present use.

Unasyn is commonly used for urinary tract and respiratory tract infections. Nausea, diarrhoea and rarely pseudomembranous colitis are the reported side effects. Unasyn with the β -lactamase inhibitor component i.e. sulbactam was demonstrated to react strongly with anti-human globulin especially anti-IgG. Lutz et al³ has found that 23.8% of the positive DAT in a 18 month period were associated with the use of Unasyn, none of them had a haemolytic anemia. They had also demonstrated an incidence of a positive DAT of 39% in patients receiving Unasyn, all with anti-IgG and without clinical haemolysis. Timentin is another drug that contains β -lactamase inhibitor (ticarcillin disodium plus clavulanate potassium) that has also been reported to cause a positive DAT. Garratty et al² have studied 4 patients taking Unasyn and Timentin who had a positive DAT with anti-IgG and a non-reactive eluate.

These recent reports suggest that Unasyn and Timentin induce a positive DAT by non-immunological protein adsorption. The β -lactamase inhibitors, sulbactam and clavulanate seem to affect the red blood cell membrane resulting in non-specific protein absorption, causing a positive antiglobulin test. This phenomenon was also shown when testing red blood cells treated in-vitro with the drugs.

In this case, a positive DAT was reported after intravenous Unasyn therapy. The serologic findings showed that the patient's red blood cells (presumably Unasyn-coated) reacted strongly with the anti-human globulin particularly anti-IgG, negatively for anti-C3d. Her serum and the red cell eluate were shown to contain no antibody. With the serologic results and also the decreasing reactivity of the positive DAT after discontinuation of the Unasyn, we believe that the positive DAT in this patient is Unasyn-induced and the mechanism involved may be due to the non-immunologic protein absorption onto the red cells.

This case report highlights a drug-induced phenomenon resulting in a positive DAT without haemolysis and causing unnecessary alarm in patient care.

References

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