

Mixed autoimmune haemolysis in a SLE patient due to aspecific and anti-Jk(a) autoantibodies; case report and review of the literature

In this report we describe a patient with mixed haemolysis, who was subsequently diagnosed with SLE. In addition to aspecific cold and warmth autoantibodies, rarely occurring specific complement binding warmth auto-antibodies to Jk(a) were found. The clinical presentation and the therapeutic options of mixed haemolysis are discussed.

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Case report

A nineteen year-old negroid patient was referred to the Emergency Department because of progressive dyspnoea. She reported cough, fever and abdominal pain in the days preceding admission. Physical examination revealed a tachycardic (150 bpm), tachypnoeic (20 breaths per minute), pale girl with a fever (39.5°C). Patches of darker skin on the cheeks and her arms (she reported a hypersensitivity to sunlight for the past 1.5 years) were noted, but otherwise, physical examination was unremarkable. Laboratory investigation showed a normal platelet count, slightly elevated leucocytes with a normal differentiation, however a Hb of 1.9 mmol/L was found. As MCV (109 fl), reticulocytes (72‰), bilirubin (38 µmol/L) and LDH (1981 U/L) were all elevated, haemolysis was suspected. Patients history revealed that irregular antibody screening, performed during her 12th week of pregnancy in 2001, had been positive, showing both aspecific cold IgM autoantibodies and warm IgG autoantibodies and anti-S alloantibodies. The phenotype of the erythrocytes of the patient was then determined as B-Rhesus D positive (ccDEe), together with S and additionally K negativity. No pregnancies had followed and patient had received no transfusion.

Further screening at presentation showed a positive direct antiglobulin test with IgG, IgM autoantibodies and complement. Excessive autoagglutination of patient erythrocytes due to the cold autoantibodies prevented determination of IgG subclasses, despite repeatedly washing the cells with warm saline. Investigation of free erythrocyte antibodies in serum showed that the cold complement binding autoantibodies were aspecific. Cardiac ischemia and impending coma necessitated direct transfusion of erythrocytes. Cross matching was impossible because of positive reactions, probably due to autoantibodies, therefore the patient was transfused with in total nine non-cross matched O-Rh negative erythrocytes, and based on earlier findings negative for C, K, and S.

Later, at the reference laboratory of Sanquin diagnostic services, in addition to the aspecific warm and cold autoantibodies and anti-S alloantibodies, anti-Jk(a) antibodies were found. We determined anti Jk(a) specificity after adsorption techniques with donor erythrocytes of different antigenic make up. The anti Jk(a) antibodies remained after adsorption of the aspecific autoantibodies with Jk(a) negative donor erythrocytes, and were demonstrated using an indirect antiglobulin testing after adding bovine serum albumin. As erythrocyte antigen genotyping showed the patient to be Jk(a) positive, Jk(b) negative, the anti Jk(a) antibodies therefore were presumed to be autoantibodies. Later, during remission of her disease and at least three months after her last transfusion this could be confirmed by erythrocyte phenotyping, showing expression of Jk(a) antigen and not of Jk(b) antigen. In re-

spect, all nine RBC units that our patient received were found to be Jk(a) negative.

Because of the observed skin abnormalities, it was investigated whether patients' mixed autoimmune haemolytic anaemia (AIHA) was secondary to another autoimmune disorder. Indeed laboratory investigations were compatible with the diagnosis of SLE: ANA, ENA, pANCA, anti-ssA, anti-Sm and anti-RNP were all found to be positive. Complement was found to be decreased; C4 <0.1 g/L (normal 0.16-0.47 g/L), C3 0.55 g/L (normal 0.88-2.01 g/L). A skin biopsy showed vacuolisation and a minimal perivascular infiltrate, indicative of LE. Therefore, the diagnosis of SLE was made, based on the photosensitivity, discoid plaques on the skin, haemolysis and the presence of Anti Nuclear Antibodies and anti-Sm antibodies. At admission treatment with prednisone 1 mg/kg was already started, because of auto immune haemolytic anemia. In addition, Clarithromycin was given because of her complaints prior to presentation and the cold antibodies, being compatible with a Mycoplasma Pneumoniae infection. This was confirmed by an increased titer (1:320) 14 days later.

Despite high dose prednisone, haemolysis persisted. From a Hb of 6.9 mmol/L after initial transfusion, it fell to 4 mmol/L after 30 days. As at that time warm IgG autoantibodies were still demonstrable (aspecific as well as anti Jk(a)), intravenous gammaglobulins were administered in order to prevent a splenectomy. After this, the Hb level stabilised, with decreasing reticulocytes, allowing tapering of prednisone dosage. At present there is a persistent clinical remission of haemolysis without prednisone therapy, as indicated by a stable Hb of 8.1 mmol/L and normal reticulocytes. Although the aspecific cold and warm antibodies persisted, treatment led to disappearance of the anti Jk(a) antibodies, indicating a causative role of anti Jk(a) antibodies in haemolysis.

In conclusion, our patient has a mixed autoimmune haemolytic anemia, with aspecific cold autoantibodies and warm autoantibodies, complicating SLE. In addition, warm autoantibodies to Jk(a) were detected. A slow response to high dose corticosteroids occurred, necessitating concomitant treatment with immunoglobulins, by which a splenectomy could be prevented. Treatment eventually led to disappearance of anti Jk(a) autoantibodies and clinical remission of haemolysis.

Discussion

Anti Jk(a) autoantibodies have been rarely described. Review of the literature revealed only case reports. In most of these patients clinical relevant haemolysis was present.¹⁻⁷ Furthermore, similar to our patient, in the majority of patients auto-immune diseases, like autoimmune thrombocytopenia and ulcerative colitis, were diagnosed.^{3,4,5} Moreover, methyl dopa⁶ and Chlorpropamide,⁷ both known to induce autoimmune antibody formation were found to be responsible. Anti Jk(a) autoantibodies without clinical significance have been found during routine type and screen procedures, using specific commercial preparations containing parabens.^{8,9} In these cases the antibodies were only detectable in a test containing additives with paraben, not being used in our laboratory. In conclusion, in the majority of cases of auto anti-Jk(a) with clinical significance an underlying auto-immune disease or known antibody triggering agent is responsible for anti-Jk(a) induced clinical relevant haemolysis.

As in our patient, most anti Jk(a) autoantibodies are complement binding warm IgG antibodies. We cannot

state with certainty that her anti-Jk(a) autoantibody was complement binding, since additional aspecific complement binding autoantibodies prevented separate investigation of the complement binding properties of the anti Jk(a) antibodies. The simultaneous disappearance of the anti-Jk(a) antibodies and haemolysis, despite the persistence of aspecific autoantibodies, indicates that indeed the anti-Jk(a) antibody was responsible for the observed haemolysis.

In addition to specific anti Jk(a) autoantibodies, aspecific warm and cold autoantibodies were detected, therefore our patient has been diagnosed with mixed type autoimmune haemolysis. Mixed type autoimmune haemolysis, defined as both warm antibody AIHA and cold agglutination syndrome, has been described as a separate entity comprising 7-8% of all haemolytic anemias.¹⁰⁻¹⁴ This should be discerned from patients with warm AIHA, showing up to 35% of low-titer cold agglutinins, causing agglutination of red blood cells at 20°C or lower, however showing no activity from the cold antibody at 30°C or higher, therefore not playing a role in red blood cell destruction.¹⁵ In mixed AIHA however, a cold active IgM autoantibody in high titer with a wide thermal amplitude with activity up to 37°C is present, leading to red cell destruction in vivo. Therefore, these cold antibodies contribute to clinical relevant haemolysis. The high thermal amplitude might explain, why most patients with mixed type AIHA, despite generally severe haemolysis, respond well to initial treatment with steroids, in contrast to patients with primary cold agglutinin disease.

In contrast to AIHA due to warm antibodies or cold antibodies only, a much higher incidence of SLE has been described in mixed AIHA (25-44% versus 2-3% in non-mixed type AIHA). Therefore, patients with mixed AIHA should be thoroughly screened for the presence of autoimmune diseases and in our opinion it is advisable to follow these patients for the development of such diseases in case no abnormalities are found at presentation.

Similar to AIHA due to warm IgG autoantibodies, first line treatment of mixed hemolysis consists of high dose (1 mg/kg prednisone) corticosteroids. In our patient, however, a slow response was observed. In order to prevent a splenectomy, being only effective in 60-75% of patients with warm IgG autoantibodies, treatment with IVIG was started. Although less is known about the effectivity of IVIG in AIHA as compared to its value in auto-immune thrombocytopenia, in several selected cases,¹⁶⁻¹⁹ a therapeutic effect has been described. In mixed AIHA the effect of IVIG might be mediated by containing specific anti-idiotypic antibodies, by blocking Fc-receptor mediated destruction of IgG coated red blood cells and interfering with activation of complement. As a splenectomy is mostly unsuccessful in persistent control of haemolysis in mixed type AIHA, we propose IVIG as a valuable tool to await the effects of steroids in slow responders.¹¹ Cyclophosphamide and/or azathioprine have been described to be effective for both haemolysis and the underlying systemic diseases. Rituximab has been described as salvage therapy for AIHA²⁰⁻²³ with responses up to 85%. There is sparse information on rituximab effectiveness in mixed AIHA.²⁴ However, as rituximab has been described to inhibit complement mediated lysis, antibody dependent cytotoxicity, B-cell proliferation and induction of apoptosis, thereby interfering with both antibody production and hemolysis, it is tempting to hypothesize a positive effect.

In order to elucidate the factors contributing to hemolysis in SLE, the role of the proteins that protect erythro-

cytes against complement-mediated hemolysis has been investigated. These proteins, CD55 and CD59, are glycosylphosphatidyl-inositol (GPI) linked and their deficiency in patients with paroxysmal nocturnal hemoglobinuria (PNH) indeed results in intravascular hemolysis. Likewise, in 11 patients with AIHA associated with SLE, diminished expression of CD59 (n=6) and CD55 (n=10) was found as compared to patients with SLE without AIHA and normal subjects (25). We also found a diminished expression of CD59 on the erythrocytes of our patient when compared to a healthy donor (mean fluorescence intensity 96 versus 55). No PNH clone characterized by a complete deficiency of GPI anchored proteins on monocytes or neutrophils could be detected. It seems plausible that diminished CD55 and CD59 expression contribute to the anti-erythrocytic antibody induced hemolysis, but whether this diminished expression has a molecular basis (like PIG-A mutations in PNH), or is the result of antiphospholipid antibodies, remains to be explored. In our patient no antiphospholipid antibodies could be detected.

AIHA due to anti-Jk(a) complement binding warm autoantibodies is extremely rare. There might be an association with autoimmune diseases, as in our patient, or drug-induced antibody formation and these associations should be searched for, as they can influence the treatment. Due to the rareness of the disease, specific treatment options can not be defined. First line treatment should therefore consist of corticosteroids like in warm AIHA. In order to prevent splenectomy in slow responders IVIG is a valuable option.

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