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A Review Article on Etiology, Clinical Presentation, Laboratory Evaluation and Treatment of Autoimmune Hemolytic Anemia

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ABSTRACT

Red blood cell (RBC) autoantibodies are a relatively uncommon cause of anemia. Nevertheless, autoimmune hemolytic anemia (AIHA) should consider in the differential diagnosis of hemolytic anemia whenever patient has an accompanying lymphoproliferative disorder, autoimmune disease, viral or mycoplasma infection. AIHA is further categorized as warm AIHA, cold agglutinin syndrome, paroxysmal cold hemoglobinuria, mixed-type and drug-induced AIHA. It is the characteristic of the autoantibodies which are accountable for various clinical entities. Diagnosis is based on clinical presentation and work up of AIHA. This review discusses epidemiology, causes, clinical presentation, and laboratory workup and treatment options available for AIHA.

Keywords: etiology; clinical presentation; laboratory evaluation; autoimmune hemolytic anemia

INTRODUCTION

Diagnosis of Autoimmune hemolytic anemia is a tiresome task because laboratory testing is difficult and often requires intensive serological workup particularly when blood transfusion is required. Often, treatments are needed as soon as possible. The purpose of this review is to provide a general overview of experimental and a brief treatment strategy for AIHA.

AIHA is defined as an increase hemolysis of RBC due to autoantibodies with and without complement stimulation. AIHA investigational characteristics include the convergence of clinical and laboratory indication of red blood cell hemolysis with autoantibody identification and /or complement on erythrocyte as demonstrated mainly by a positive direct antiglobulin (DAT) test. A negative DAT does not contravene the diagnosis of AIHA.[1-3] The prognosis of AIHA is related to a specific disorder (secondary AIHA) in more than 50% of patients, but can happen without any indication of an underlying illness (Idiopathic or primary AIHA, Table 1).[4, 5] Depending on the temperature required for RBC sensitization, AIHA is distributed by cold antibody (CA-AIHA), Warm antibody AIHA (WA-AIHA), or AIHA due to biphasic auto-AB (paroxysmal cold haemoglobinuria, PCH).

WA-AIHA is a very rare disease with a very low incidence of 1:100,000 when compared to CA-AIHA 1:1,000,000[2] In contrast, 10% of AIHA arises in background of SLE [6] Sometimes lymphoma is complicated by AIHA, but a lymphoma that has not yet been diagnosed will still be considered. This is proven by the fact that at a later stage, 18% of patients with primary AIHA develop lymphoma [7, 8]

TABLE 1: Risk factors of autoimmune hemolytic anemia

Autoantibody (incidence)
Warm autoimmune Hemolytic Anemia (1:100,000)
Primary (idiopathic)
Secondary
Lymphoproliferative disease (lymphoma)
Autoimmune diseases (SLE, Ulcerative colitis)
Acute leukemia
Solid malignancy (ovarian carcinoma)
HIV Infection
Cold autoimmune Hemolytic Anemia CAIHA **(1:1000000)**
Primary (idiopathic): frequently herald of occult lymphoma
Secondary
Lympho proliferative disease (Waldenstrom's macroglobulinemia, lymphoma) Infection (mycoplasma, EBV)
Biphasic haemolysins (rare)
Idiopathic
Secondary
Postviral,syphilis
Mixed forms with warm and cold antibodies
Idiopathic
Secondary
Autoimmune diseases (SLE)

Pathogenesis:
Autoantibodies directed against epitopes of RBC containing sugar and / or protein synthesis are critical to the pathogenesis of AIHA. This epitopes are essential for diagnosis of autoantibody. IgM isotype antibodies form a pentameric structure and are effective in the stimulation of complement. IgG3 and IgG1 are both capable complement activators, while IgA and IgG2 only have a poor complement activation power. The complement is not activated by IgG4. Typically, the complement mechanisms are not fully activated to cause hemolysis so complement degradation products (C3c, C3d) should be detected as bits as traces on RBC.

Complement activation can continue until the formation of C6-C9 (MAC) which leads to erythrocyte lysis. The optimal temperature at which autoantibodies bind to RBC is also of clinical significance. Cold autoantibodies (CA-Ab) have optimum temperature for binding to RBC and such temperature below from 30 °C and are mainly IgM isotype. CA-Ab with optimal binding around 30°C is clinically relevant because it can induce complement activation in-vivo. Warm autoantibodies exhibit optimum binding at 37 °C and are usually IgG, less frequently IgM, and rarely IgA [(1).] Biphasic auto-Ab are IgG that displays optimal binding below 30°C and induced activation of the complement at 37°C [(7).] IgG coated RBC with/without C3c/C3d are preferentially eliminated by Fc- gamma receptor-mediated spleen phagocytosis whereas C3b receptor are present, not C3c/C3d coated RBC are killed by complementary receptor mediated phagocytosis in the liver and spleen (extravascular Hemolysis) in the absence of IgG. Complement activation continue before MAC is implanted in the presence of IgM that is reactive above 30 C, leading to intravascular RBC destruction (intravascular hemolysis) [(9)]

DIAGNOSIS
Clinical considerations:
The clinical picture of AIHA is no more different from the other cases of acute hemolytic anemia or acute crisis of chronic hemolytic anemia. The patient is frequently jaundiced and shows signs and symptoms of anemia. Haemoglobinuria; an indication of intravascular hemolysis is occasional but patients should be specifically examined about this symptom. In case of cold agglutination, cold exposure can lead to agglutination of RBC in the circulatory system which is reflected in a cyanotic discoloration of toes, fingers, ears and nose. Subsequently warming up, the cyanotic discoloration quickly disappears and unlike Raynaud phenomena reactive hyperemia does not occur. Table 1 offers a description of the various forms and etiologies of AIHA.

General laboratory findings:
Laboratory investigations play a vital role in diagnosis of AIHA because it detects both hemolysis and auto-Ab to the erythrocyte. Elevated indirect hyperbilirubinaemia, lactate dehydrogenase (LDH), reduced reticulocytosis and haptoglobulin reflect high RBC breakdown whether due to extra or intravascular hemolysis. Standard LDH levels cannot rule out the existence of hemolysis! Reticulocytosis may remain normal in the beginning of AIHA and/or in situations of the reduced efficient ability of bone marrow such as during chemotherapy. In peripheral blood smears, microspherocytes are usually detected. These are RBC coated with autoantibodies that loose bilateral structure because part of the membrane is lost as they pass through the spleen. In case of intravascular hemolysis, hemoglobin is extracted from the destroyed RBC and is removed by the kidneys, causing a brown discoloration of the urine (haemoglobinuria). Hemosiderin is detected in the urine even days after the hemolytic episode. [(10)]

Immunohaematological diagnostics:
The primary aim is to identify autoantibody against erythrocytes. The key tests are the direct and indirect antiglobulin tests DAT/IAT and the auto control. The DAT detects what is already attached to the RBC surface, i.e. complement fragments and/or immunoglobulins. The IAT incubates serum or plasma and RBC to allow antibodies present in the serum/ plasma to bind to the RBC and then a DAT is performed. The auto control is an IAT using the patients (instead of donor) RBCs against his own plasma/serum.

The DAT may be performed manually in the tube or by newer technologies using gel or glass beads. The manual test requires meticulous, repeated washing to remove any residual plasma around the RBC since that plasma will inactivated the antiglobulin reagent and cause a false negative result. The gel and/or glass bead technologies do not require the washing step since their reagent cards completely separate the RBCs from the plasma.

Whenever using the manual anti human globulin AHG reagent, a control of a sensitized cell coated with IgG and complement must be run. If the control is negative, the test is invalid, usually due to insufficient washing of the RBCs.Antiglobulin regents may detect immunoglobulins and/or complement fragments (e.g C3d, C3c, C5). Normally, a polyspecific regent is performed first; and if positive, then monospecific IgG and complement reagents are used. A polyspecific reagent detects IgG and complement, usually the C3d fragment. A monospecific reagent detects immunoglobulins or complement fragments, usually C3d. immunoglobulin reactivity may be whole molecule (both heavy and light chains) or heavy-chain specific (e.g. gamma heavy chain specific).

Although most AIHA will show positivity by using IgG or complement, there are cases that are DAT negative. These require special reagents to detect IgM and/or IgA or the use of more sensitive techniques such as Flowcytometry to detect antibodies that are present in very low amounts. You can suspect a DAT negative hemolytic anemia by examining the peripheral blood smear for sperocytes and polychromasia.

Positive DAT: what to do next?
In order to detect whether RBC is coated with IgM, IgG, IgA, and C3c or/ and C3d a monospecific reagents are required. If complement component (C3c/C3d) can be identified in the lack of an autoantibody, the existence of CA-Ab (IgM), WA-Ab (IgM, IgA) or biphasic antibodies should be measured. In such condition, additional laboratory diagnosis is compulsory to consider the existence of whether IgA or IgM. IgA auto-Ab is very rare without IgG auto-abs. Though they indicate an optimum binding at 37 °C and can lead to fulminant and fatal hemolysis.[(11-13)] It is difficult to detect IgM auto-Ab because it usually is removed by washing procedure due to its pentamer size when performing DAT and it may not always be detected with all DAT reagents if they do not detect light chains. In addition, it is important to observe optimum temperature requirements for IgM binding and the temperature at which the DAT is carried out.

In a further step, the characteristic of IgM to directly agglutinate RBC due to its size (pentamer) can be utilized (complete antibody). A CA-Ab IgM should be suspected if there is random agglutination followed by incubation of RBC with the patient's serum at 16 °C. If agglutination occurs at 30 °C, a possibly clinically associated cold antibody will have to be recognized.

Another useful test for identifying binding antibodies in patient serum is one that uses RBC pre-treated with enzymes (much more sensitive to complement mediated lysis compared to normal RBC) incubated with patient serum at both 16 and 37 °C. It is a hemolysis test. Serum with an acidic pH is added as a complement source and incubation is done. A clinically significant antibody that can possibly cause hemolysis or reduced the RBC life span should be considered if lysis occurs. Auto-Ab has the potential to induce lysis in non-pretreated RBC in vitro in cases of fulminant intravascular hemolysis. The pre-analytical handling of patient's sample is crucial when a CA-Ab is suspected. The blood sample must be immediately placed at 37 °C after venipuncture, this is because auto-Abs binds to RBC at room temperature and reduce the concentration of auto- Ab in the serum which risks causing false-negative results.

Using laborious elution methods, the warm auto-Abs is isolated for red blood cells to identify the specific antibody. Similar to IAT, the eluate (containing autoantibodies that bind to RBC) is tested in the standard panel of RBC. If the specificity of the eluted antibody can be confirmed, this is specified in the diagnostic report (for example, anti-C). However, in numerous cases, the specificity could not be determined. Specific WA-Ab's are recurrently directed to parts or to the entire Rhesus system, rarely to the Kell system.[2] CA-Ab are often directed to H-antigen or I-antigen, whereas biphasic auto-Ab's have anti-P specificity.[9]

Type and screen: remnants task in AIHA:

If blood transfusion is planned, then typing and screening must be completed In addition, identification of alloantibodies is of vital importance in the diagnosis of auto-ab. Literature indicates that in 15-43 % of patients with AIHA, alloantibodies can be detected after several transfusions.[12] In addition, the risk of producing multiple alloantibodies is substantially increased in patients with one alloantibody.[14] The type and screen procedure is complicated by the existence of auto-Ab in serum. Determining the blood group of the patient by serological methods remains difficult particularly in patients with CA-Ab, and needs time intense washing steps (wide washing to become free of the auto-Ab bound RBC). Occasionally, a serological blood group is not possible to determine. In such conditions, genotyping for the most significant blood groups (Rhesus, Kell, Duffy, Kidd, and SS's) should deal be considered. There is an opportunity to prevent genotyping in case of WA-Ab related to RBC typing using monoclonal reagents. It remains difficult to detect alloantibodies, because patient auto-Ab reacts with RBC tests. This is also demonstrated by the fact that cross matching is positive for all specified RBC concentrates in certain situations. Auto-Ab should be separated from the patient's serum with different absorption methods in order to achieve adequate screening for alloantibodies. These procedures however, take Considerable time and may require a reference immunohematology laboratory.

Therapy:

Transfusion should be avoided if possible whenever there is a substantial chance of alloantibody development upon transfusion. In addition, transfusion will exacerbate continuous hemolysis, as auto-Abs also reacts with transfused RBC. Anemia should only be corrected when there is onset of clinical symptoms. Transfusion should be done after cardiac function (ECG), diuresis and renal function testing. It is best to wait for results of immunohaematological testing and the corresponding transfusion guidance based on this, if there is no vital sign for a transfusion. In the second strategy, the hemolysis mechanism must be ceased or at least attenuated by inhibition of development of autoantibodies and/ or inhibition of the premature degradation of RBC.

It is only possible to successfully cure secondary AIHA while the underlying disease is treated. Figure 2 summarizes the different treatments of AIHA. The importance of splenectomy is a matter of controversy because of the existence of treatment that effectively targets autoantibody producing B- cells (anti CD20 antibody therapy). Since AIHA is a genetic disorder that affects a heterogeneous patient group, randomized prospective studies assessing the effectiveness of different treatment modalities are not widely available. In addition, it is difficult to interpret the effectiveness of the therapeutic outcomes in these trials as there are no uniform descriptions of therapy reaction. Therapeutic procedures for WA-AIHA and CA-AIHA will be explored in the following section. Partial and full response concepts are adapted from the publications cited in the document.

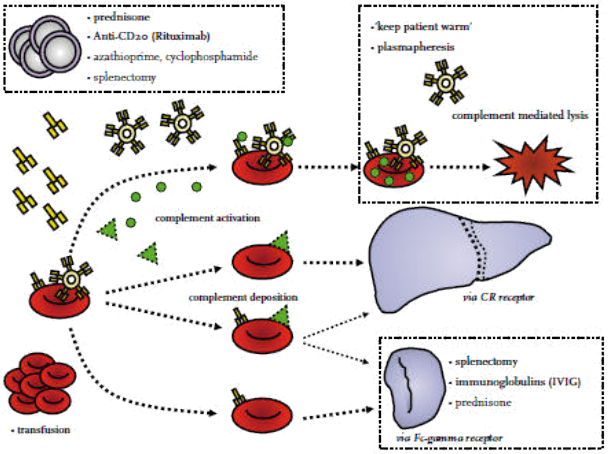

FIGURE 2: Mechanisms of red blood cell removal in autoimmune haemolytic anaemia

Erythrocytes coated with IgG autoantibodies are mainly removed via Fc-gamma receptors on macrophages in the spleen. Complement deposition on erythrocytes in the absence of IgG leads to red blood cell removal in the liver via complement receptors on Kupffer cells. In case of fulminant haemolysis, red blood cells are destructed in the circulation.

TREATMENT OF WA-AIHA

Transfusion:

The selected blood component should be antigen negative for the detected clinically significant antibodies. Extended phenotyping across RH, Kell, Duffy, Kidd, and MNSs system should be performed. Prophylactic antigen matching across these groups should be considered for chronically transfused patients. It may not be possible to find completely compatible RBCs by full AHG cross matching. In such cases, least incompatible blood may be released with the permission of the transfusion medicine physician.

Steroids:

Steroids decrease autoantibodies produced by immunosuppression of B cells [15] Furthermore, steroids decrease the concentration of Fc-gamma receptors on splenic phagocytes. [16, 17]. In 60- 70% of patients' partial remission was seen while 10-15% complete remission was observed [18, 19] Usually, the starting dose of prednisolone is 1 mg/Kg per day and it is gradually reduced depending upon the clinical response. After hemoglobin is stabilized prednisolone is reduced to a daily dose of 20 mg within 2 weeks. If the level of haemoglobin remnants stable, the dosage may be further reduced after one month to 10 mg/day. The steroid dosage can be further reduced and discontinued afterward 2 weeks. In order to diagnose steroid induced diabetes, glucose levels should be checked regularly.

In addition, since patients suffering from AIHA take steroids over a long period of time, osteoporosis prophylaxis must be given. Steroid treatment psychological side effects are often underestimated (e.g. lack of self-control, agitation, psychosis) and may become an incriminating issue for the patient and social environment. Thus, steroid doses often have to be reduced or the treatment has to be stopped. [20, 21]

Cytotoxic drugs:

Cyclophosphamide and azathioprine are immunosuppressive agents which cause reduction in the production of autoantibodies. If steroid therapy does not bring sufficient effects, these drug scan be added when a conservation dose of > 20 mg of steroid per day is required or the steroid does should be reduced due to adverse effects. [22, 23] It is possible to use cyclophosphamide (100mg/d) or azathioprine (100-150 mg/d) as a single therapy or in combination with steroids. Because of their myelosuppressive effects, peripheral blood cell counts should be regularly controlled and dosage maybe adjusted if necessary. Plus therapy with cyclophosphamide (50 mg/kg over 4 days) in combination with Mesna and G-CSF could be successful in refractory AIHA.[24] Vincristine could be a valuable alternative in desperate cases, with the benefit of being less myelotoxic than cyclophosphamide.[25] In some cases, immunosuppressive drugs such as cyclosporine or mycophenolate, mofetil, appear to be effective.[26, 27]

Splenectomy:

Splenectomy can reduce the destruction of red blood cells and reduce the production of autoantibodies. In more than 50% of patients, anemia is normalized two weeks after splenectomy.[28,29] Mortality in laparotomy splenectomy is about 1 percent while in cases of laparoscopy, it is about 0.5%.[30,31] Post Splenectomy these patients are at high risk of infections.[32,33] Vaccination against N. menigitidis, Str. pneumoniae, and H. influenzae, can significantly reduce the risk of infection in these patients if administered pre Splenectomy.[34]

Anti-C20 antibody:

Rituximab is a chimeric, specific antibody targeting CD20 expressed on all B-cells except plasma cells. It can reduce the production of autoantibodies by targeting B cell destruction. Retrospective studies have reported complete remission in 20 to 70% of the patients. In prospective studies, more than 60% of the patients achieved complete remission, however most patients relapse sooner or later (>24 months). Rituximab is very well tolerated with allergy, chills and hypotension as rare side effects. Progressive multifocal leucoencephalopathy after rituximab therapy in patients with systemic lupus erythematous has been reported. [35-40] Despite the lack of controlled prospective studies, the use of rituximab instead of Splenectomy must be considered as a treatment of choice for anti-steroid resistant WA-AIHA. If splenectomy is reconsidered after rituximab treatment fails, it should be remembered that vaccination with embedded bacteria may not be effective after rituximab.

Immunoglobulins:

Administration of immunoglobulin can temporarily improve anemia due to decrease RBC destruction in spleen. Moreover, gamma globulin immunomodulatory responses could also contribute to the beneficial impact. In cases of acute life-threatening crisis, immunoglobulin therapy may be considered to reduce patient or donor erythrocyte breakdown. [41, 42]

Treatment of CA-AIHA:

Usually there is a mild anemia in CA-AIHA which does not need any treatment. The mainstay is to “keep warm” by proper clothing in order to protect from cold and wearing gloves, hat and warm shoes.

If required, transfusion may be carried out at a temperature of 37 C under stable condition by a heating system. [35] The body temperature must be held at 37 C during the surgery. The requirement criteria for choosing blood product are similar to that in WA-AIHA. However, the treatment of CA-AIHA is still challenging. Only few controlled studies are available. Steroids are obviously less effective than in WA-AIHA. [43] Same goes for cyclophosphamide and azathioprine [9] In CA-AIHA, there is no role of splenectomy. Several studies have reported some beneficial effects of gamma globulins while rituximab has shown to induce a 40-50% response in two controlled studies. But the possibility of achieving remission again is very small with frequent relapses.[44, 45] As IgM is primarily located in blood vessels plasma exchange will cause a rapid decrease in IgM level which may contribute to the short-term stability of AIHA [46] Since plasmapheresis must be performed at 37 C, technical procedures are still a challenge. Treatment options for fulminant intravascular hemolysis are limited. Supportive therapy, close monitoring of vital functions, renal function and hemolysis parameters remains the mainstay of treatment. Gamma globulin and plasmapheresis have been reported as therapeutic alternatives in literature. An activation inhibitor of complement component C5 was administered in selected cases attenuating the development of a membrane attack complex. [47]

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