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Review

Diagnosis and classification of autoimmune hemolytic anemia

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ABSTRACT

Uncompensated autoantibody-mediated red blood cell (RBC) consumption is the hallmark of autoimmune hemolytic anemia (AIHA). Classification of AIHA is pathophysiologically based and divides AIHA into warm, mixed or cold-reactive subtypes. This thermal-based classification is based on the optimal autoantibody-RBC reactivity temperatures. AIHA is further subcategorized into idiopathic and secondary with the later being associated with a number of underlying infectious, neoplastic and autoimmune disorders. In most cases AIHA is confirmed by a positive direct antiglobulin test (DAT). The standard therapeutic approaches to treatment of AIHA include corticosteroids, splenectomy, immunosuppressive agents and monoclonal antibodies.

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Contents

1. Introduction

Hematology has long fascinated mankind. As early as 400 BC Hippocrates toiled with the humoral theory that attempted to correlate blood components with health and disease. Early studies such as those by Andral in 1843 first described idiopathic anemia described as "without previous blood loss" and "typically associated with darkened urine" [1]. The first association of hemolytic anemia with jaundice specifically being distinguished from hepatic diseases was described by Hayem in 1898. Hayem is credited with the initial description of congenital and acquired hemolytic anemias [1,2]. A more complete understanding of human hematology led to what is considered to be the first description of autoantibody-mediated autoimmune disease by Donath and Landsteiner in the early 1900s. Further refinements in analytical methods

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led to a better understanding of the immune system, and based on this in 1951 autoimmune hemolytic anemia (AIHA) was first described as a specific disorder [1].

2. Epidemiology

AIHA is now known as a disease in which autoantibodies are produced that target RBC antigens, resulting in the premature destruction with inadequate compensation [3,4]. This group of diseases is relatively rare, affecting approximately 1–3 in 100,000 annually [5–7]. AIHA is primarily limited to adults although children with primary immunodeficiency disease or autoimmune lymphoproliferative syndrome (ALPS) are commonly affected [8]. Children often develop a more self-limited disease course often precipitated by viral illness. Adults tend to develop a more severe and relentless disease process that frequently requires treatment and can, on occasion, be life-threatening. While there is no age or familial predispositions, there are known associations that include malignant lymphoproliferative diseases, drugs and viral infections, however the majority of cases have an unknown cause and are considered idiopathic [9].

3. Pathophysiology and classification

Classification of AIHA is pathophysiologically based and divides AIHA into warm, mixed or cold-reactive subtypes, Table 1. This thermal-based classification is based on the optimal RBC-autoantibody reactivity temperatures. AIHA can be further classified into primary (idiopathic) or secondary in nature [5,6,10–12]. Further sub-classification of cold AIHA (cAIHA) includes primary and secondary cold agglutinin syndrome (CAS) and paroxysmal cAIHA [10–12].

While AIHA and cAIHA constitute much of the AIHA prevalence some less frequent types do arise, namely mixed-type AIHA (mAIHA) and drug-induced AIHA (diAIHA) Table 1. diAIHA is even more rare, afflicting an estimated 1 in 1 million. diAIHA can be classified into subcategories depending on if the drug is required to be present for hemolytic activity (drug-dependent AIHA), or if hemolytic activity is observed without the drug present (drug-independent AIHA) [13]. While each subtype of AIHA is innately part of the same family, pathogenesis, diagnostics, treatments, and prognosis vary greatly. Accurate diagnosis is therefore crucial to assess clinical manifestations, predisposing factors and treatment optimization.

Table 1
Classifications of autoimmune hemolytic anemia.

I. Warm reactive AIHA: Optimal reactivity of autoantibodies at 37 °C
A. Primary or idiopathic
B. Secondary
i. Associated with various lymphoproliferative disorders (e.g., non-Hodgkin's lymphoma, chronic lymphocytic leukemia)
ii. Associated with rheumatic disorders (e.g., systemic lupus erythematosis (SLE))
iii. Associated with non-lymphoid malignancies (e.g., ovarian cancer)
iv. Associated with chronic inflammatory disorders (e.g., ulcerative colitis)
v. Drug induced autoimmune hemolytic anemia
II. Cold reactive AIHA: Optimal reactivity of autoantibodies <37 °C
A. Cold agglutinin syndrome
i. Primary or idiopathic
ii. Secondary
a. Post-infectious (e.g., mycoplasma or infectious mononucleosis)
b. Associated with B cell lymphoproliferative disorder
B. Paroxysmal cold hemoglobinuria (Donath–Landsteiner syndrome)
III. Mixed type AIHA: Characterized by the presence of both warm and cold type autoantibodies
A. Primary or idiopathic
B. Secondary
i. Often associated with rheumatic disorders
IV. Drug induced AIHA: Associated with an estimated 150 drugs
A. Drug dependent AIHA
i. Hapten or drug absorption
ii. Immune (ternary) complex
B. Drug independent AIHA

3.1. Warm autoimmune hemolytic anemia (wAIHA)

3.1.1. Primary and secondary wAIHA

An estimated 1 in 80,000 are afflicted by wAIHA, constituting about 75% of all AIHA cases. Idiopathic AIHA accounts for approximately half of all wAIHA [10]. wAIHA is the subtype that most often affects children ages 2–12 [14]. Secondary wAIHA is associated with various conditions including infectious mononucleosis, systematic lupus erythematosus (SLE), autoimmune hepatitis, human immunodeficiency virus, and other lymphoproliferative or autoimmune disorders [6,11,12,15]. Of AIHA-associated lymphoproliferative disorders chronic lymphocytic lymphoma (CLL) is the most common cause [16]. In fact, roughly 11% of CLL patients develop secondary wAIHA, while an annual incidence of 2–3% is observed in patients with non-Hodgkin's and Hodgkin's lymphoma [14–17].

The polyclonal immunoglobulin (Ig) class IgG is typically involved in the autoantibody activity of warm AIHA (wAIHA), showing maximal reactivity with erythrocytes at 37 °C. Less frequently however, wAIHA can be associated with IgA and IgM. wAIHA exhibits a depleted immune tolerance of RBCs commonly due to the binding of self-antibodies to Rh proteins, causing Fc-gamma receptors to mediate removal of RBCs extravascularly within the spleen [10]. wAIHA has recently been linked to a number of immune system imbalances. Interleukin-12 (IL-12) and interleukin-10 (IL-10) imbalances are believed to mediate the altered immune response in some patients with AIHA [18,19]. The pattern of IL-10 and IL-12 production is generally thought to play a role in the pathogenesis of wAIHA and correlates with increased activity of the Type-2 Helper T Cell (Th2) pathway and the inhibition of Type-1 Helper T Cell (Th1) pathway [18,19]. The domination of the Th2 pathway leads to increased autoantibody production mediating AIHA [18–20].

3.2. Cold autoimmune hemolytic anemia (cAIHA)

3.2.1. Cold agglutinin syndrome (CAS)

CAS is much less prevalent than wAIHA, comprising about 15% of all AIHA cases, primarily occurring in the middle aged or elderly. CAS causes AIHA in a complement-dependent manner where autoantibody-dependent lysis is mediated primarily by C3 proteins, leading to intravascular hemolysis upon detachment of antibodies at 37 °C [21]. Targeted RBC phagocytosis is primarily mediated by liver Kupffer cells while the membrane attack complex (MAC) is a minor mechanism if the IgM titre is relatively low. The presence of cold stress increases autoantibody activity, facilitating RBC lysis particularly in the extremities. A notable feature of CAS is a high variability in hemolysis, and in turn the need for transfusions varies greatly from patient to patient [21]. The degree of hemolysis in CAS patients is primarily dependent on active autoantibody concentration, rather than the more abundant membrane bound C3 protein concentration [21,22].

3.3. Paroxysmal cold hemoglobinuria (PCH)

PCH, also known as Donath–Landsteiner syndrome and is a form of cAIHA activated primarily by polyclonal IgG antibodies (Donath–Landsteiner antibody) [15]. Similarly to CAS, PCH activates complement at cold temperatures. Complement activation is via P-antigen binding on RBCs with subsequent intravascular hemolysis being initiated upon rewarming to normal body temperatures [15,23]. PCH is considered a form of secondary AIHA and typically develops within the first week after infection most often seen in children. The infections are primarily upper respiratory, and the causative agent is often not identified. Late-stage or congenital syphilis was historically linked to cases of PCH in adulthood but this is becoming less and less common [15,23].

3.4. Mixed-type AIHA (mAIHA)

mAIHA is characterized by the presence of both warm and cold type antibodies as well as both IgG and IgM antibody subtypes. mAIHA accounts for less than 5% of the total AIHA incidence, and is even less common in children [24]. mAIHA can be both idiopathic or arise secondarily from malignant or autoimmune disorders such as SLE or lymphoma. It can be difficult to determine which autoantibodies (IgG or IgM) and the required thermal range are causative. Patients with mAIHA can have both warm and cold components that can react with different antigens.

3.5. Drug-induced AIHA (diAIHA)

DiAIHA is relatively rare, may go undiagnosed in many cases, and the magnitude of hemolysis can vary widely. There are estimated to be 150 drugs that are known to be associated with diAIHA and are categorized by drug-independent (via auto-antibodies) and drug-dependent antibodies (Table 2) [25–27]. Drug-dependent AIHA can be categorized into two subtypes: 1) hapten type which is due to the noncovalent binding of the drug to the RBC which is then targeted by the autoantibody in a drug-dependent manner: 2) drug-autoantibody immune (ternary) complexes that are mediated by a complement-dependent hemolysis that is drug dependent.

A drug-dependent antibody (DDAB) activates a response only while the drug is present. This class is the most common case of diAIHA and can be mechanistically variable depending on the molecular nature of the drug and it's RBC interaction. DDAB may specifically attach to the drug, the drug's metabolites, and/or drug-RBC neoantigens. Antibiotics cefotetan and high doses of penicillin are the best understood mediators of diAIHA. The binding of the DDAB mediates RBC phagocytosis via Fc receptor-mediated mechanisms similar to wAIHA with the notable difference that the autoantibody binds directly to the RBC in wAIHA and to the drug-bound RBC in diAIHA.

Other drugs such as ceftriaxone and piperacillin interact with the membrane of RBCs but bind via RBC neoantigens. It is uncertain how drug binds to the membrane and if the complex formed is covalent or loosely bound. The drug and RBC membrane form an immune complex mediating DDAB binding to the drug, membrane, or equal proportions of the drug–membrane complex [27].

In contrast drug independent antibodies (DIABs), are capable of creating an autoimmune response in the absence of the offending drug. Various mechanisms exist by which drugs (i.e. fludarabine, cladribine, methyldopa) stimulate autoantibody formation via adsorption, immune dysregulation, or other mechanisms but none of which have been fully elucidated.

Table 2
Representative drugs and their associations with drug induced autoimmune hemolytic anemia.

I. Hapten and drug adsorption mechanisms
a. Drugs such as penicillins, cephalosporins, tetracycline, carbromal, hydrocortisone, oxaliplatin, and tolbutamide
II. Immune/ternary complex mechanisms
a. Drugs such as stibophen, metformin, quinine, quinidine, cephalosporins, amphotericin b, rifampicin, antazolinc, thiopental, tolmetin, probenecid, nomifensine, cephalosporins, diclofenac and doxepin
III. Autoantibody mechanism
a. Drugs such as cephalosporins, tolmetin, α-methyldopa, L-dopa, mefenamic acid, teniposide, pentostatin, cladribine, fludarabine, lenalidomide, procainamide and diclofenac
IV. Non-immunologic protein adsorption
a. Cephalosporins, carboplatin, cisplatin and oxaliplatin
V. Unknown methods of AIHA causation
a. Drugs such as mesantoin, phenacetin, insecticides, chlorpromazine, acetaminophen, ibuprofen, thiazides, omeprazole, carboplatin, nalidixic acid, erythromycin, and streptomycin

The most common drug usage linked to diAIHA is methyldopa, which can continue in a subject months after cessation of the drug. The common treatment practice for diAIHA is mediated via blood transfusion and discontinuation of the offending agent. Most drugs are cleared from the system quickly and the drug-dependent antibodies only persist in the case where there is a persistence of RBC membrane-bound [27,28].

4. Clinical manifestations

Presenting complaints of AIHA are usually referable to the anemia itself, although occasionally jaundice is the primary manifestation. The onset of symptoms is typically slow or insidious over several months, but occasionally a patient may manifest with acute severe life threatening symptoms. In secondary AIHA the symptoms of the underlying or precipitating disease may overshadow the manifestations of AIHA. The physical examination may be normal with splenomegaly being present in approximately 20% of patients. However in very severe cases that are often of rapid onset patients may present with fever, pallor, jaundice, hepatosplenomegaly, tachycardia, angina and heart failure. Many patients with cAIHA have a more insidious chronic hemolytic anemia. In other patients the typical feature is episodic, acute hemolysis with hemoglobinuria mediated by chilling. Combinations of these clinical features may also occur. Acrocyanosis and other cold-mediated vasoocclusive phenomenon may affect the fingers, toes, nose and ears [5,26].

In PCH constitutional symptoms are prominent during a paroxysm. Within minutes to hours of cold exposure the patient typically develops aching pains, abdominal cramps, headache, often followed by chills and fever. The first passed urine after the onset of symptoms typically contains hemoglobin.

Evaluation for diAIHA should be assessed by a careful history of drug exposure in every patient with AIHA and/or a positive DAT. As with idiopathic AIHA the clinical manifestations of diAIHA can be quite variable. In general patients with hapten/drug absorption (i.e. penicillin) and autoimmune (i.e. methyldopa) types of diAIHA exhibit mild to moderate hemolysis, with insidious onset over a period of days to weeks. In contrast, the immune or ternary complex-mediated diAIHA (i.e. cephalosporins or quinidine) typically is associated with a sudden onset of severe hemolysis and hemoglobinuria [25–27].

5. Diagnosis

5.1. wAIHA

The first and most important criterion for the diagnosis of AIHA is the recognition of hemolysis and anemia. The appearance of jaundice and abnormally dark urine is usually suggestive of hemolysis but is only present in approximately 60% of patients. When diagnosing AIHA it is important to first rule out other causes of hemolysis such as microagiopathy, hereditary conditions (i.e. spherocytosis or G6PD) or sickle cell anemia. The predominant laboratory features are a positive direct antiglobulin test (DAT) and elevated reticulocyte count [10]. The DAT, also known as Coombs' test, is a common laboratory diagnostic tool used to detect bound immunoglobulins or fragments of complement proteins in a sample of RBCs [29]. This method of detecting immunoglobulin- and complement-bound RBCs is the primary serologic test available in diagnosing and differentiating forms of hemolytic anemia. Anti-human globulins (AHGs) are used to asses the presence of antibody coated RBCs via agglutination. If a polyspecific AHG test is positive, a monospecific test is done to determine if specific Ig or complement is present. Because wAIHA is most commonly caused by IgG classes, an IgG^{+} DAT is typically indicative of wAIHA. Confirmation of wAIHA is by negative complement fixation via the Coombs' test. While the DAT remains the most prominent test in diagnosing AIHA, there are some drawbacks, namely that it is labor intensive, costly, and subject to some degree of operator error.

Between 2% and 10% of all AIHA patients are Coombs' negative, which is likely due to varying sensitivity of the test and other factors. Recently, flow cytometry has demonstrated increased sensitivity for the detection of RBC-bound Ig. The use of flow cytometry can also be employed to confirm clinical suspicions that indicate AIHA in DAT negative cases [30,31].

5.2. cAIHA

While wAIHA cases are almost exclusively caused by IgG autoantibodies, CAS is dominated in 90% of cases by IgM thus an IgM^+ DAT typically indicates CAS; however, a small number of IgA and IgG-mediated cases do arise [21]. DAT testing for anti-C3D is also highly indicative of CAS and considered necessary in most diagnoses of CAS, as opposed to wAIHA that is indicated by negative complement fixation. A patient showing a positive Coombs' test for anti-C3D and negative for IgG antiglobulins is typically then assessed for thermal reactivity, and CAS is further suggested if thermal reactivity levels peak at 4 °C and are present until 30 °C [21]. While the manifestations of PCH are similar to CAS, it is typically diagnosed both serologically as well as clinically. A major feature distinguishing PCH from CAS is the fact that PCH is far more common in children with infection, while CAS is typically limited to adults [21]. A DAT^+ result is almost always seen in PCH, thus leading to the necessity for a Donath–Landsteiner antibody test to be performed as well [23].

5.3. diAIHA

Hemolysis facilitated by drug dependent antibodies can typically be detected after two weeks of starting the offending drug. diAIHA often goes unnoticed because regular red blood cell transfusions and presurgical screens are the only things that would initiate an evaluation. If suspicions do arise a DAT should be done and if positive should precipitate a screen of the patients' medical record for potential medications known to be associated with diAIHA. In appropriate patients additional testing may be needed to assess for the presence of drug dependent or independent antibodies.

5.4. mAIHA

Symptoms of mAIHA are often consistent with both wAIHA and cAIHA; having autoantibodies optimally reactive at both 37 °C and in the 0–10 °C range. Commonly, RBC agglutination on the peripheral blood smear is found along with a positive DAT for both IgG and C3 [24].

6. Treatment

6.1. wAIHA

Unfortunately there are few prospective clinical trials that have established treatment standards for patients with AIHA. The majority of the data is from retrospective case studies and recommendations are often experience-based. There is no accepted consensus on the definition of complete (CR) or partial remission (PR) or refractoriness. Many of these patients have an insidious course and transfusions are not required. However occasionally when there is rapid hemolysis and/or the patient has co-morbidity-mediated symptoms (i.e. cardiac, pulmonary) transfusions are indicated and may be lifesaving. Despite crossmatching transfused RBCs are often consumed as fast as or faster than host RBCs and are only considered a temporizing measure in critically ill patients. Glucocorticoids are the mainstay of treatment for wAIHA with two-thirds responding with 20% achieving a complete response. Responding patients are tapered slowly over 2–3 months. However relapses are common and the patient must be monitored carefully [5,26,32]. If the patient does not respond (approximately one-third), cannot be tapered (approximately one-third) or relapses, alternate therapies must be considered. Second line treatment includes splenectomy and the anti-CD20 mAb, rituximab. While there are no randomized trails and evidence is primarily historical, most recommend splenectomy in surgical candidates. Two-thirds of splenectomized patients will respond and approximately 60% will have a hematocrit greater than 30% [33]. In addition most patients that recur after splenectomy do need as high of a dose of steroids to maintain acceptable hematocrits. In patients that fail or are not splenectomy candidates rituximab is a reasonable option with overall response rates at approximately 80%. While long term follow-up is not available recent studies have reported that approximately 80% of responding patients are relapse free two years after treatment initiation [34–36]. For patients that have failed splenectomy rituximab salvage therapy options include retreatment with glucocorticoids, danazol, cyclophosphamide and azathioprine with response rates in the 40–60% range [33]. High dose immunoglobulin and plasmapheresis have unproven efficacy and should only be used when all other approaches have failed.

6.2. cAIHA

cAIHA is generally more insidious and mild with few patients needing treatment. Keeping the patient warm is important and may be the only maneuver necessary for patients with mild cases. Rituximab is useful for acute symptomatic cases and chlorambucil and cyclophosphamide for more severe chronic cases [37]. Splenectomy and glucocorticoids are less effective for cAIHA. Plasmapheresis may provide some temporary relief in critically ill patients. Post-infectious cAIHA is often self-limited.

6.3. diAIHA

Discontinuation of the offending drug is often the only treatment necessary and in severe cases may be life-saving. Glucocorticoids are typically not necessary and of questionable efficacy. Rituximab and splenectomy are unproven and usually not considered.

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