Acquired hemolytic anemia

10

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Introduction

As described earlier, hemolysis is a process characterized by accelerated red cell destruction, which can be compensated for if the body steps up production of new red blood cells. However, if red cell destruction surpasses production, hemolytic anemia could result. Hemolytic anemia is traditionally categorized by cause as either congenital or acquired. Congenital hemolytic anemia has previously been presented in detail; therefore, this chapter is restricted to a discussion of acquired hemolytic anemia.

The term 'acquired hemolytic anemia' was first coined in the early 1900s[1] and it is now commonly used to describe hemolytic anemia triggered by extrinsic factors such as immune disorders, drugs, infections, mechanical trauma to red cells, exposure to toxins, and other miscellaneous causes. Generally, acquired hemolytic anemia can be classified as immune hemolytic anemia (autoimmune, alloimmune or drug-induced) and non-immune hemolytic anemia (infection-induced, mechanical trauma, and other miscellaneous causes, including paroxysmal nocturnal hemoglobinuria). In this chapter, the various categories of acquired hemolytic anemia are reviewed including pathogenesis, clinical presentation, treatment and management.

Clinical presentation and laboratory features

Similar to other anemia, the symptoms of acquired hemolytic anemia generally include: fever, abdominal pain, back pain or pain in the limbs (which may mimic acute abdominal conditions or musculoskeletal diseases); cardiovascular symptoms such as dyspnea, angina and tachycardia; or non-specific complaints of generalized malaise or dizziness. In some cases, patients can be asymptomatic and their pallor is detected by family members or other individuals. Jaundice and brownish discolored urine are also typical of acute hemolysis. Sometimes, in massive acute hemolysis, shock and renal failure can occur.

Determining whether the hemolytic anemia has an intravascular or extravascular origin can be helpful when establishing the diagnosis. The differential diagnosis of extravascular hemolysis is more extensive than that of intravascular hemolysis with the five most common intravascular hemolytic disorders being: cold autoimmune hemolysis; malaria; drug-induced hemolysis; major ABO blood group incompatibility; and paroxysmal nocturnal hemoglobinuria. Table 10.1 provides a summary of the laboratory results typically seen in intravascular and extravascular hemolysis.

The diagnostic pathway and related laboratory tests used to determine whether a patient has a hemolytic anemia are presented in Chapter 6 of this book. Laboratory test information is discussed in this section as they pertain to specific acquired hemolytic anemia. The laboratory tests that are typically reviewed when diagnosing acquired hemolytic anemia are: peripheral blood film examination, reticulocyte count, bilirubin, haptoglobin, hemoglobinemia, methemalbumin and hemopexin, hemoglobinuria and hemosiderinuria, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), bone marrow examination, direct antiglobulin test (Coombs' test), and determination of red cell lifespan using radioactive isotope-labeled red cells.

Table 10.1 Typical features that distinguish between intravascular and extravascular hemolysis

	Intravascular hemolysis	Extravascular hemolysis
Mechanism	Red cell destruction in the intravascular compartment resulting in hemoglobin being released into the plasma	Red cells are recognized as foreign or become more rigid and are sequestered in the spleen with subsequent phagocytosis
Possible causes	Complement, toxins, membrane defects, enzyme deficiencies, drugs	Immunoglobulin, complement, membrane defects
Laboratory feature		
Hemoglobinemia	Present	Absent/present in severe cases
Hemoglobinuria	Present	Absent/present in severe cases
Haptoglobin	Reduced or absent	Normal or reduced
Methemalbumin	Present	Absent
Hemosidinuria	Present	Absent
LDH	Grossly elevated	Elevated
Jaundice	Present	Present
Splenomegaly	Absent	Present
Blood film	Schistocytes, helmet cells, fragmented red cells	Spherocytes, erythrophagocytosis

LDH, lactate dehydrogenase.

Pathogenesis of immune and non-immune hemolytic anemias

In this chapter, immune hemolytic anemia is classified as autoimmune, alloimmune and drug-induced; whereas, non-immune hemolytic anemia is presented under the headings of infection-induced, mechanical trauma and other miscellaneous causes. Note that different causes of hemolytic anemia can overlap. For example, drug-induced hemolysis often overlaps with immune hemolysis because drugs can cause hemolysis either by immune mechanism or by direct damage to the red cells.

Immune hemolytic anemia

Immune hemolytic anemia is the most common form of acquired hemolytic anemia and it can be classified as autoimmune, alloimmune or drug-induced. Irrespective of the etiology, the typical feature in the peripheral blood smear is microspherocytosis (Fig. 10.1).

Autoimmune hemolytic anemia

Autoimmune hemolytic anemia (AIHA) involves the premature destruction of red blood cells by autoantibodies. AIHA may be idiopathic (no underlying cause identified) or secondary to underlying malignancy or disorder such as lymphoid malignancy, connective tissue disorders, infection, medication, vaccinations, HIV-induced, myelodysplastic syndrome,[2] or graft-versus-host disease. In one study, neoplasia-related AIHA was the most common secondary cause (233/1834 patients; 2.7%) followed by drug-induced hemolysis (140/1834 patients; 7.6%).[2] For diagnostic evaluations, it is important to determine the autoantibody idiotype. Autoantibodies may readily be separated into warm antibody or cold antibody based on the thermal range of the antibody and this classification serves as the framework of the following discussion.

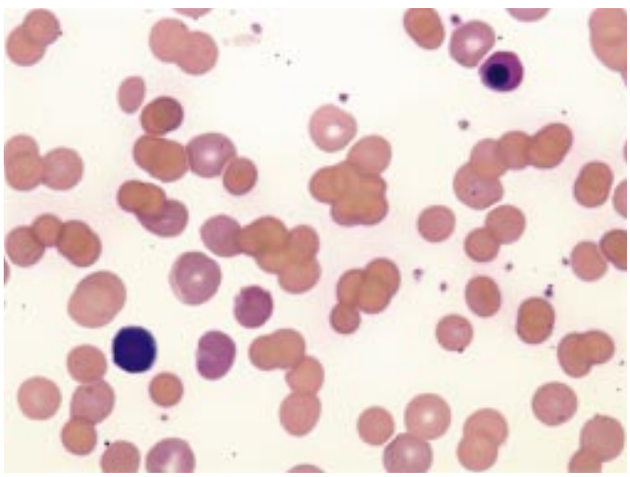

Fig. 10.1 Peripheral blood smear showing microspherocytosis, nucleated red cell, and red cell clumps in a patient with immune mediated hemolysis. The spherocytes lack central halo of normal red cells and they are smaller than the nucleus of a normal lymphocyte. Wright stain, × 1000, oil field.

Warm autoimmune hemolytic anemia (WAHA)

Warm autoimmune hemolytic anemia (WAHA) accounts for more than 70% of AIHAs[3] and it is caused by production of an autoantibody directed against the patient's red cells. Most frequently, the autoantibody is IgG (particularly IgG1 and IgG3 subclass),[4] although IgM and IgA can be detected in addition to IgG.[5] IgA warm autoantibody is rare, and most antiglobulin reagents are unable to detect this immunoglobulin. Warm autoantibody (IgG) may cause extravascular hemolysis through one of two mechanisms: (1) Fc receptor-mediated immune adherence; and (2) complement mediated hemolysis. Most warm autoantibodies do not cause autoagglutination or intravascular hemolysis.

Fc receptor (FcR)-mediated immune adherence

Antibody-coated red cells can be removed from the circulation by two different mechanisms: phagocytosis and cell lysis. During the phagocytic process, macrophages engulf and lyse red cells by formation of oxygen radicals in the cytoplasm; whereas, for cytotoxic cell lysis, the target cell is destroyed by the lysosomal enzyme released by the phagocytic cells.[6]

The phagocytic process is facilitated by the deposition of opsonins, including antibody or C3b, on the antigen. The immune effector cells have receptors (FcR) on the cell surface for the Fc portion of antibodies. There are at least three different classes of IgG FcR on the macrophages (FcγRI, FcγRII and FcγRIII). FcγRII and FcγRIII bind to IgG oligomer[7,8] but FcγRI binds only to monomeric IgG.[9,10] FcγRII and FcγRIII binds IgG3 oligomer and IgG1 oligomer with the highest affinity.[9] The high affinity of IgG3 to FcRs might explain why IgG3 is the most efficient warm antibody in extravascular hemolysis *in vivo*.[3,11] The *in vivo* functions of FcγRIII include phagocytosis, endocytosis and antibody-dependent cell-mediated cytotoxicity.[12,13] FcγRII is an inhibitory receptor and acts as a negative regulator of B-cell and mast cell

activation.[14,15] On the other hand, FcγRI exclusively mediates *in vitro* cytotoxic activity.[16]

Apart from IgG, the presence of other classes of anti-red cell antibodies (IgM and IgA) is a major determining factor of hemolysis.[4] IgM, apart from complement activation, may synergistically enhance IgG-mediated hemolysis. It has been demonstrated that patients with red cells coated with IgG and IgM have more severe hemolysis than those with IgG alone as these patients have lower level of haptoglobin.[5] IgA autoantibodies, although rare, probably induce hemolysis via FcR in the same way as IgG.[2] However, the exact relationship between FcR and the different combinations of immunoglobulin classes remains unclear. Circulating monocytes, K-cells and granulocytes also have FcR; however, the role of these cells in the pathogenesis of hemolysis is uncertain, although it has been postulated that they may play a role in patients with immune hemolytic anemia refractory to splenectomy.[17]

The amount of antibody bounded on the red cell surface also determines the mechanism of FcR-mediated hemolysis. When the amount of antibody on the red cell membrane is low, the phagocytic process is predominant.[2] However, because IgG1 is a less efficient mediator of phagocytosis than IgG3, IgG1 coated red cells are not always rapidly destroyed.

Under normal conditions, a typical splenic macrophage has 30 000–40 000 FcR per cell.[18] Concomitant infection or immunization worsens the hemolysis by increasing the number and affinity of FcR, possibly through cytokines such as gamma interferon.[19] Circulating neutrophils probably have only a marginal role in autoimmune phagocytosis, however, they may become important during infection.[20]

In summary, the FcR-mediated cell destruction processes depend on a number of factors: the specific immune characteristics of the immunoglobulins; the amount of antibody bound to the red cells; and the overall activity of the macrophages in the reticuloendothelial system.

Complement-mediated hemolysis

The activation of the complement cascade, up to and including the terminal pathway, brings about the formation of a membrane attack complex and intravascular hemolysis. To activate the complement pathways, two FcRs must be spatially close together. IgM is the most efficient antibody class in the activation of complement; however, in warm autoimmune hemolysis, IgG on the red cell membrane occasionally may be of sufficient density to activate complement. Of the IgG subclass, IgG3 and IgG1 bind readily to C1q, but not IgG4.

Complement activation is controlled by a number of regulatory mechanisms. Some complement fragments can become enzymatically cleaved and the cleavage products, such as C3d or C4d, are incapable of further activation. The complement inhibitors on cell membrane include decay accelerating factor (DAF),[21] C8-binding protein,[22,23] membrane cofactor protein, CR1/CD35 protein and P18/CD59 protein.[24] Other proteins such as C1 inhibitors, C4 binding protein, factor H and factor I are circulating in the plasma. The complement cascade is arrested and early complement protein fragments are bound to the red cell membrane. The complement-coated red cells are then recognized by complement receptors on hepatic Kupffer's cells. It has been demonstrated that about 550–800 bound C3b molecules are required to trigger hepatic clearance of red cells. As these receptors are less efficient than the splenic macrophage FcRs, complement-sensitized red cells often escape the phagocytic or cytotoxic process and circulate in the peripheral blood with normal or slight reduction in red cell survival. Because C3d or C4d coated red cells have less binding sites for other complement proteins, these cells are in fact protected from further hemolysis. However, C3d and C4d coated cells are less deformable than normal erythrocytes; hence, the complement proteins may augment IgG-mediated phagocytosis.[25–27] Consequently, the extravascular hemolytic process may be enhanced by the presence of complement on the cell, whereby complement acts as an opsonin to facilitate the phagocytic process.

Immune specificity of WAHA

In initial serologic testing, most warm autoantibodies are panagglutinins reacting with all red cells. These panagglutinins can be classified into different categories based on the agglutinating pattern with certain Rh phenotypes: (1) the antibodies react with all red cells with a normal Rh phenotype but not with those having Rh_{null} phenotype, or with those having partially deleted Rh antigens; (2) the antibodies are specific to part of the Rh antigen system (i.e. react with normal Rh phenotype and cells with partially deleted antigens); and (3) the antibodies react to all cells including Rh_{null} cells.[28] The first two categories, which are specific to the Rh antigen system, account for 50–70% of warm autoantibodies[29] as most warm autoantibodies react with all red cells except those of the Rh_{null} phenotype. In one series of 150 persons with warm autoantibodies, only four were specific to only a single Rh antigen (i.e. anti-e or anti-c specificity).[30] Because Rh antigens have a relatively low density on the red cell membrane, autoantibodies against Rh antigens do not activate complement.

Autoantibodies not showing Rh specificity can sometimes show specificity for other red cell antigens. Using immunoprecipitation methods, up to 50% of warm autoantibodies precipitate band 3 and glycophorin A.[31,32] Band 3, an anion transport protein, requires a portion of glycophorin A to form Wr^b antigen. Interestingly, Di^b, an alloimmune antigen of Diego system, is also carried on protein band 3. Compared with antigen Wr^b, Di^b is a rare target for autoantibodies.[30] The glycosylation and the complexity of the Wr^b and Rh antigen system may implicate their immunogenicity.[30]

Autoantibody formation can also impact red cell antigenic expression.[33] Decreased expression of blood group antigens in the following systems have been described: Kell, Rh, MNS, Duffy, and the Kidd system.[33,34] The mechanism of this phenomenon is unknown, but it may be an effect to protect against hemolysis.

Some warm autoantibodies appear to have specificity against certain red cell antigens on initial testing, but the antibodies can still be absorbed by red cells lacking the corresponding antigen. In addition, elutes made from the red cells used for this absorption procedure again demonstrate immune specificity against that particular antigen. The cause of pseudo-specificity of warm autoantibodies is unclear. Clinically, the pseudo-specificity of the autoantibodies is not directly associated with the severity of hemolysis, although the serologic results may lead to diagnostic confusion. Sometimes, autoantibodies of real specificity and of pseudo-specificity may coexist in the same patient.[33,35] This is of practical importance in selecting compatible blood for transfusing patients with warm autoimmune hemolytic anemia. Autoantibodies of pseudo-specificity have been described in blood group systems such as Kell, Duffy, Kidd and MNS systems.[33]

The diagnostic evaluation of a patient with suspected WAHA

With suspected WAHA, the most useful test to detect warm antibodies is the direct antiglobulin test (DAT) with polyclonal or monoclonal antibodies specific for IgG or C3d (also called the Coombs' test). However, the presence of antibodies and/or complement on the red cell membrane does not necessarily result in hemolysis and the strength of reactivity of the DAT is not directly related to the clinical severity of hemolysis. A positive DAT without evidence of hemolysis occurs in about one per 10 000 healthy blood donors.

IgG and C3 are detected on the red cells in 50% of the cases; IgG alone in 23% of cases and C3 in 27% of the cases. When IgG is detected on red cells of normal individuals, IgG1 appears to be the predominant IgG subclass detected. IgG1 has been shown to be less efficient than IgG3 for activating complement,[36] which may explain the lack of hemolysis. In about 2% of patients with WAHA, the DAT is negative (i.e. Coomb's negative autoimmune hemolytic anemia). There are at least two possible explanations: (1) the level of red cell antibody sensitization is below the sensitivity of the DAT; or (2) hemolytic anemia caused by IgA or other immunogloblulins[37–39] that would not be detected by conventional antiglobulin reagents. Variable expression of red cell antigen during the course of disease[40] may also play a role in Coomb's negative autoimmune hemolytic anemia.

The lower limit of immunoglobulin detection using the DAT is estimated to be about 100–300 antibody molecules per red cell.[41] Tests such as ^{125}I-radioimmune direct antiglobulin test,[42] enzyme-linked direct antiglobulin test,[43,44] and two-stage immunoradiometric assay with ^{125}I-staphylococcal protein A[45] may increase the sensitivity of detecting antibodies on the red cells of patients with DAT-negative autoimmune hemolytic anemia.[3] However, the significance of these findings is uncertain as it has been shown that clinical hemolysis is unlikely to occur if the number of IgG1 molecules is less than 1000 per cell.[46] Special techniques for detecting IgG, such as assays using staphylococcal protein A or functional assays (e.g. the monocyte-phagocytic test), are sometimes useful in these cases; however, these assays are difficult to perform and they are not routinely available. In some cases, concentrated elutes may demonstrate the presence of antibody even though the DAT is negative. Before the diagnosis of Coombs' negative autoimmune hemolytic anemia is made, other non-immune causes of hemolysis must be excluded.

The indirect antiglobulin test (IDAT) will detect autoantibody in the patient's serum in 57.4% of patients with a routine antibody screen, and up to 88.9% of cases when enzyme-treated red cells are used.[47] If the patient requires transfusion, the challenge for the laboratory is the detection of alloantibodies when warm autoantibodies are present. A warm autoabsorption technique will remove autoantibody and leave alloantibody to be detected. Alternatively, a titration technique can be useful if the alloantibody has a higher titer than the autoantibody.

Management of patients with WAHA

The fundamental therapy for WAHA is to suppress autoantibody production. However, it usually takes time for immunosuppressive therapy to exert its full therapeutic effect. Hence, acute supportive measures may be required in patients with acute hemolytic events. Treatment of

WAHA can be broadly divided into supportive and more definite treatments.

There should be no difficulty in finding compatible blood for transfusion if patients have a negative DAT. However, many patients with severe hemolysis have detectable plasma antibody (panagglutinins); hence, it may be impossible to find compatible blood for these patients. In this case, if clinically indicated, incompatible blood can be transfused if the laboratory takes special care to ensure it is ABO and Rh compatible. It has been reported that blood transfusion may adversely affect the patient by introducing more alloimmune antigens that further activate the immune system.[33] None the less, transfusion therapy is still an important supportive measure in patients whose anemia has put them at risk of serious complications or death.

Corticosteroid therapy, initiated with the blood transfusion, may suppress the immune destruction of the transfused red cells.[3] Rarely, other adjunct supportive measures such as oxygen, sedation, ventilator support, and hypothermia therapy may be needed.

High-dose intravenous gamma-globulin (IVIgG) has been investigated as an acute treatment of WAHA.[48–59] This therapy causes blockage of the reticuloendothelial system and reduces the clearance of the IgG-sensitized red blood cells. The use of IVIgG is well described in the treatment of hemolysis associated with Evan's syndrome and lymphoproliferative diseases, and during an acute phase of hemolytic crisis.[60] However, the use of IVIgG is consistently less effective for AIHA compared to immune thrombocytopenia. Consequently, a higher dose of gamma globulin may be needed because patients with autoimmune hemolytic anemia may have an expanded reticuloendothelial system.[60] The mechanism of action of IVIgG therapy is likely due to FcR blockade,[61–67] although other mechanisms such as the presence of anti-idiotype in the IVIgG,[61,68–72] interference with T-cell signals to B-cells,[73–76] activation of T-suppressor cells, and inhibition of B-cell maturation[77–82] have been described.[33] Because the therapeutic effect of IVIgG is usually short-lived, further immunosuppressive therapy is required to maintain clinical remission.

Treatment with high-dose steroids, usually in the form of prednisone at 1–1.5 mg/kg/day, is initiated once the diagnosis of WAHA has been confirmed. The median time of response is 7–10 days. The mechanism of action of steroid therapy include: suppression of red cell clearance by the reticuloendothelial system;[83] downregulation of the number of FcR;[84] inhibition of the release of lysosomal enzymes by macrophages; and suppression of autoantibody production.[85] Steroid therapy can reduce the concentration of autoantibody, but has no effect on alloantibody production.[33] When hematologic improvement is seen, the dose of steroid should be gradually tapered over the next several months to minimize the side-effects of long-term steroid therapy. In 60–70% of patients, complete remission can be achieved, but for some patients, maintenance therapy is required. Among these patients, 50% may relapse and further treatment with higher doses of steroid therapy may be beneficial. If there is a nonresponse to steroid by the end of the first 3 weeks, continued therapy with steroid alone is usually ineffective. Up to 40% of patients with WAHA become either steroid-dependent or steroid-resistant.[86,87]

Splenectomy is effective in about half of patients with WAHA.[88] Splenectomy removes the major site of antigen presentation and, in turn, reduces antibody production.[33,89,90] With the advance of laparoscopic splenectomy, the incidence of severe surgical complications has been reduced.[91,92] The major long-term complication of splenectomy is infection, particularly of encapsulated organisms. Therefore, all patients undergoing splenectomy should receive vaccine immunization against *Streptococcus pneumoniae, Meningococcus* and possibly *Hemophilus influenzae.*

Immunosuppressive therapies, including vinca alkaloids,[93] azathioprine and cyclophosphamide, have been reported to be beneficial in the treatment of WAHA, although the therapeutic effect may be delayed for 3–6 months after the initiation of treatments. In a small case series, the response rate was reported to be about 50%.[86] Patients who do experience a clinical response to this therapy may require maintenance therapy for up to 12 months to induce remission.

A recent study has suggested that Danazol may induce long-lasting remission in patients with refractory WAHA.[94–97] The possible mechanisms include: reduction in red cell bound C3d;[98] immunomodulation by alteration of T-cell subsets;[99] and reduction of FcR in the reticuloendothelial (RE) system.[100] Side-effects from Danazol include virilization effects and dose-dependant hepatic toxicity.

Cold autoimmune hemolytic anemia

Cold autoimmune hemolytic anemia (CAHA), also known as cold hemagglutinin disease, is much less common than WAHA. In a large prospective series,[3] 391/2390 patients (16.4%) undergoing investigations for red cell autoantibodies presented with cold autoantibodies while another 10 patients (0.4%) had both warm and cold autoantibodies. In another series AIHA,[101] a combination of warm and cold autoantibodies was reported in 8% of patients with AIHA. These antibodies react best at cold

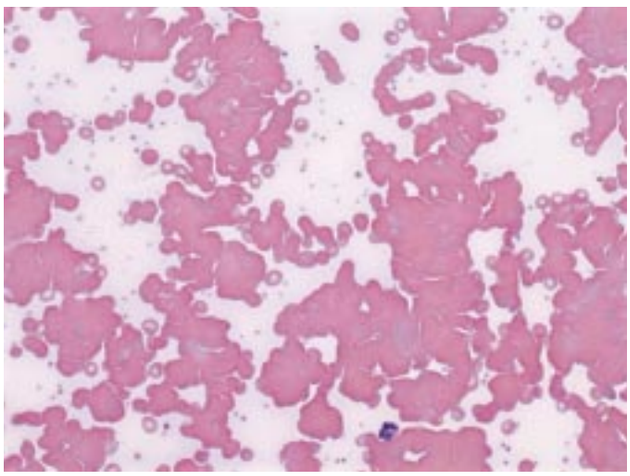

Fig. 10.2 Peripheral blood smear showing agglutination of red cells in a patient with Waldenström's macroglobulinemia. The background of the smear is bluish because of high protein content, which contains IgM hemagglutinin. Wright stain, × 400.

temperatures (below 30°C). When the peripheral blood of patients with cold hemagglutinin examined at room temperature, it typically shows agglutination of red cells (Fig. 10.2). This temperature-dependent reactivity of the cold autoantibodies is of clinical significance because hemolysis is unlikely to occur if the thermal reactivity is below 30°C. Cold autoantibodies are mainly IgM (85%);[3] however, the IgG biphasic Donath–Landsteiner antibody accounts for approximately 15% of the cases, especially in the pediatric age group.[3,102]

Red cell sensitization by cold IgM usually occurs in the body extremities (e.g. fingers, ears, nose) where temperatures may fall below 30°C allowing antibody binding to the red cells. The complement cascade is activated, and if the cascade proceeds to activation of the membrane attack complex, hemolysis will occur. This gives rise to the characteristic clinical features of Raynaud's phenomenon, acrocyanosis or gangrene. If the inhibitors of complement stop the cascade, the red cells (now coated with C3b) can be removed through extravascular phagocytosis. Eventually C3b is degraded to C3d on the cell surface. As macrophages do not have receptors for C3d, these red cells will not be cleared from the circulation. It is this component of complement that is detected by the DAT. The most common autoantibody specificity in CAHA is to the 'I' blood group antigen. Auto-anti-I typically occurs during or after *Mycoplasma pneumoniae* infection. In contrast, anti-I may be formed during or following infectious mononucleosis. Other less frequent autoantibody specificities include: P, Pr, A1, D, Vo, Gd (glycolipid dependent gangliosides),[103,104] -Lud,[105] F1,[106] Ju and IA[3,107] antigens. Specificity will be apparent at temperatures between 15 and 20°C. Clinically, the specificity of cold antibody is less important than the thermal amplitude.

The presence of a cold hemagglutinin is readily demonstrated using the cold agglutinin test. When determining the thermal range of the antibody using this procedure, albumin should be added as this improves the clinical correlation. The cold agglutinin often causes problems with ABO and Rh phenotype cross-match. To overcome this dilemma, the red cells should be washed with warm saline to remove the cold agglutinin. The cross-match and antibody screen should be done using a prewarmed sample and using monospecific anti-IgG to avoid false-positive reactions. The DAT, using monoclonal reagents, is usually negative for IgG, but positive for C3d when using an ethylene diamine tetra-acetic acid (EDTA) sample.

Most patients with CAHA have mild symptoms or none at all. Patients are warned to avoid exposure to cold. During a severe acute hemolytic episode, plasmapheresis can be used to reduce the level of IgM cold autoantibody. Plasmapheresis is especially effective in CAHA because IgM is confined to the intravascular space. This procedure is best performed in a warm environment and the tubing of the plasmapheresis machine may have to be prewarmed. Blood transfusion, when needed, is infused at room temperature with a controlled rate. It is still controversial whether the use of a blood warmer offers additional benefits. If CAHA is secondary to an underlying neoplastic disease, chemotherapy including alkylating agents may reduce the production of cold autoantibody.

Paroxysmal cold hemoglobinuria

Paroxysmal cold hemoglobinuria (PCH) was one of the first recognized anemias when described in the mid-1800s[107] and, for years, it was commonly believed to be a rare acquired AIHA associated with congenital syphilis. However, it is now recognized that PCH causes up to 40% of acute transient hemolytic anemia in young children[108] during viral infections such as measles, mumps, chickenpox and influenza; however, due to the transient nature of the disease, the diagnosis of PCH may be difficult after acute episodes. The cold biphasic IgG antibody (Donath–Landsteiner antibody) directs against globoside glycosphingolipid (P antigen) and causes hemolysis by a unique mechanism. The antibody binds to red cells at cooler temperatures in the peripheral circulation. The complement pathway is activated and intravascular hemolysis occurs when red cells return to the warmer core body temperature. The Donath–Landsteiner antibody is more potent than IgM cold agglutinin in its

ability to initiate intravascular hemolysis, mainly because antigen / antibody affinity of the cold IgM antibody decreases when red cells return to core temperatures.[2,109] It has been demonstrated that IgG3 is the major immunoglobulin subclass for Donath–Landsteiner antibody.[110]

The definitive test for PCH is the Donath–Landsteiner test, which can be performed as a direct or indirect procedure.[111] The direct test is conducted by incubating a test sample at 0°C for 1 h, and then at 37°C for an additional 30 min. If the Donath–Landsteiner antibody is present, it will bind to the red cells during the cold incubation phase and hemolyse the cells during the warm phase. A positive test result is not considered validated unless a control tube, maintained at 37°C throughout the test, shows no hemolysis. The indirect test is done by mixing the patient's serum with ABO compatible P-positive red cells of a normal person in the presence of fresh serum as a source of complements. The indirect test has a much higher sensitivity than the direct test for several reasons: (1) complement proteins are present in fresh normal serum; (2) the serum-to-cell ratio can be adjusted to increase sensitivity; and (3) the indicator cells from an allogenic donor are more susceptible to lysis than the patient's own red cells as the indicator cells are not coated with C3d. The sensitivity of the test can be increased further by using enzyme-treated red cells. False-positive results can occur due to IgM hemolytic antibody with a high thermal range. False negatives occur less frequently in the indirect Donath–Landsteiner test, although they can occur when the antibody titer is low or if soluble globoside (P antigen) present in the fresh normal serum causes inhibition of the antibody.[102]

The management of PCH may require urgent blood transfusions depending on the level of anemia and whether the patient is symptomatic. Theoretically, donor cells from P-negative patients may minimize *in vivo* hemolysis; however, P-negative blood is usually not available and in most cases, transfusion with P-positive blood can be beneficial. Patients with PCH can be successfully transfused if the transfusion rate is slow and the patient is kept warm and closely monitored.[112] The use of a blood warmer may be beneficial for some patients. It has been shown that the removal of complement proteins from donor plasma by washing red cells[113] and by using steroid therapy have not been beneficial.[102,108,112]

Alloimmune hemolytic anemia

Alloimmune hemolytic anemia occurs when the immune system is sensitized and antibodies form in response to red cell alloantigens. Typically, this occurs following a blood transfusion, during or after pregnancy, or following bone marrow or stem cell transplantation. Apart from another individual's red cells, the sources of alloantigen also can be from environmental antigens unrelated to erythrocytes.

Transfusion reactions due to immune-mediated hemolysis

The first transfusion reaction was recorded in 1667 by Denis.[114] Today, the incidence of clinically relevant immune-mediated hemolytic reactions is estimated to be around one reaction per 20 000 units of blood transfused.[115–119] The spectrum of clinical presentations can range from life-threatening ABO incompatibility to subclinical hemolysis depending on the magnitude of antigen–antibody interactions, the degree of complement activation, and the activity of the reticuloendothelial system.

There are two major types of immune-mediated hemolysis following blood transfusion: acute and delayed. More than 80% of acute hemolytic transfusion reactions are due to ABO incompatibility.[116] The mortality rates of up to 40% seen in the past[115] have declined to 10%, probably reflecting improved management and support. Transfusion reactions due to intravascular hemolysis are generally more severe than with extravascular hemolysis because of the side-effects associated with complement activation which may be triggered by alloantibodies to Kell, Kidd and Duffy antigen systems.[114–116] Patients may experience fever, chills, joint pain, shock, renal failure and / or disseminated intravascular coagulation.

Delayed hemolytic transfusion reactions may be caused by primary alloimmunization but, more frequently, the transfused red cells trigger a delayed (anamnestic) IgG-mediated transfusion reaction 7–14 days after the blood transfusion. Although most patients have been previously alloimmunized, over time, their antibody level falls below detectability and it can be difficult to prevent this form of transfusion reaction.[33] Sometimes, the only clinical evidence of this reaction is a positive DAT.

One of the major dilemmas for clinicians in the management of post-transfusion reactions is how to differentiate immune-mediated transfusion reactions from pseudo-hemolytic transfusion reactions. Non-immune hemolytic anemia can occur if transfused red cells are subject to excessive thermal, osmotic, mechanical or chemical insults. Clinically, febrile symptoms secondary to hemolysis may have other causes including: (1) febrile reactions due to non-hemolytic transfusion reactions; (2) bacterially contaminated blood products; (3) transfusion-related acute lung injury; or (4) severe allergic symptoms

(hypotension and shock) that mimic acute hemolytic transfusion reactions. However, the presence of cutaneous manifestations suggest an allergic pathophysiology.

Hemolytic disease of the newborn

Hemolytic disease of the newborn (HDN), also called erythroblastosis fetalis, is hemolysis in the fetus caused by transplacental transfer of maternal IgG. During pregnancy, maternal IgG is transferred via specific FcRs on the placental cells.[120–123] Alloimmune hemolytic anemia may occur in the fetus if there is a blood group incompatibility between the mother and the fetus. Generally, by 12 weeks, maternal IgG is detectable in the fetal circulation[33] and the rate of IgG transfer progressively increases across the pregnancy so that, at term, the fetal IgG level may be equal to[124] or higher[33,120] than that of the mother's. The antibodies against the Rh and the ABO antigen systems are the two major causes of hemolytic disease of the newborn, with anti-D causing the most severe cases.[33] With the routine use of Rh immune globulin, HDN is now seen more often with antibodies of other blood group antigens.

During pregnancy, most women are exposed to less than 0.1 ml of fetal blood, and this small volume does not cause alloimmunization. However, in a subsequent pregnancy, the secondary immune response may trigger the production of large amounts of IgG antibody and this could cause severe hemolysis of the fetal red cells. Hence, Rh HDN seldom affects the first pregnancy. With Rh antibodies, the severity of the disease is progressive in each subsequent pregnancy. The risk of stillbirth in a woman with a previous history of mild Rh HDN is about 2% compared to a 70% risk in a woman with a previous history of Rh alloimmunization.[125] Conversely, ABO HDN may affect a firstborn infant as the antibodies are already preformed due to environmental stimulation. The severity of ABO HDN in a previously born fetus does not predict the severity in the next infant.[33]

Stillbirth and hydrops occur in the severest cases of HDN. Severe fetal anemia can result in extramedullary erythropoiesis, gross hepatosplenomegaly, portal hypertension and hepatic failure. More commonly, the affected fetus may present with anemia and hyperbilirubinemia within the first 24 h of life. Without proper treatment, kernicterus may develop. On the other end of the spectrum, positive serologic tests with no clinical findings may be the only indication of antibody transfer to the fetus. Since HDN cannot be diagnosed solely by serologic tests, it has been suggested that laboratory evidence of disease without clinical findings should be described simply as maternal–fetal blood group incompatibility.[33]

With the advance of modern medicine, hydrops fetalis due to Rh HDN is rarely seen. Primary prevention is the cornerstone of the management of Rh HDN. First, Rh-negative women who can bear children should receive Rh-negative blood. Second, Rh-negative pregnant woman should receive passive immunization with Rh immune globulin at 28-weeks' gestation and within 72 h of exposure to fetal D-positive red cells due to either delivery or an invasive procedure such as an abortion. Although the dose regimens are slightly different between North America and Europe, the rule of thumb is that 10 µg of anti-D should be given for each ml of Rh D positive whole blood. This dose can be decreased if intravenous anti-D is used. Kleihauer–Betke tests or flow cytometry can be useful in determining the amount of fetal cells in the maternal circulation so that the dose of Rh immune globulin can be adjusted. The successful rate associated with Rh immune globulin prophylaxis is estimated to be 98–99%.

The prenatal management of women at risk of HDN varies. All pregnant women should be typed for ABO and Rh D and have an antibody screen performed during the first trimester, usually at the first visit to the obstetrician. For D-negative women without detectable antibody, an antibody screening should be repeated at the 28th–30th week at the time that Rh immune globulin is given. If alloantibody is detected, titration studies should be performed at regular intervals. The antibodies titer may be used to guide the timing of fetal monitoring and further interventions;[126] however, it must be emphasized that the titer is only a semi-quantitative indicator. Once the titer reaches a certain level (1:16–1:32), other measurements should be considered to estimate the risk of severe HDN. The severity of HDN may be predicted by various methods including: (1) amniocentesis with measurement of total bile pigment in the amniotic fluid;[127] (2) ultrasonography to detect the evidence of extramedullary hematopoiesis;[128,129] and (3) percutaneous umbilical blood sampling.[130] However, there is no available test to identify women whose infants are at risk of ABO HDN. ABO alloimmunization does not affect the fetus *in utero;* however, symptoms of hemolysis occur 12–24 hours after birth.

There are several therapeutic interventions to reduce fetal death due to severe HDN. IVIG, started at the 10th–12th gestational week at 1 g/kg every 1–3 weeks until delivery, is moderately effective with multifactorial effects including blockage of FcRs on the placenta and fetal RE system. Anecdotal reports describe that intense maternal plasmapheresis with IVIG replacement and/or plasma replacement from a D-negative donor may be a useful treatment between the 10th and 24th weeks, after

which intrauterine transfusion can be performed. As intrauterine transfusion requires specialized trained personnel and some risk of maternal/fetal hemorrhage, it should only be used in the case where fetal survival is at risk. Donor blood should be fresh, irradiated, cytomegalovirus (CMV)-negative, and lacking the antigen specific for the mother's antibody.[33] Some blood centers use group O blood whereas others use ABO-specific blood. Preterm delivery usually occurs around the 35th week gestation given the risk associated with HDN. After delivery, phototherapy and/or exchange transfusion may be used to reduce hyperbilirubinemia and the risk of kernicterus. Intravenous immunoglobulin (IVIg), directly infused to the newborn, has been shown to be beneficial in reducing the need of exchange transfusion.[131] If the infant is severely anemic, small volume transfusion may be required during the first few months of life.

Drug-induced hemolytic anemia

Drug-induced hemolysis can be immune mediated or non-immune mediated. The former is categorized into three major groups based on different mechanisms of action. Drugs, such as α-methyldopa, may induce true autoantibody production similar to warm autoimmune hemolytic anemia. Penicillin (hapten) binds to the red cell membrane and stimulates antibodies that are directed at the drug bound to the red cell. Drugs, such as quinine and quinidine, bind to plasma proteins, antibody, and then to the red cell. The last two categories illustrate the immunologic principle that a small chemical with a molecular weight under 500–1000 is unable to induce an immunologic response unless it is tightly bound to a macromolecule such as a protein. As the antibodies cannot bind to red cells in the absence of a drug, they are also called drug-dependent antibodies. On the other hand, some drugs may directly damage the red cells or induce oxidative changes in the cells. The former mainly occurs with industrial toxins such as copper and arsine, and do not involve antibodies (non-immune).

Drug-induced autoantibody

True autoantibody induction by α-methyldopa provides a human model for studying the mechanism underlying autoimmune disorders. It is one of the most extensively studied drug-induced autoimmune hemolytic anemia. None the less, the exact mechanism of autoantibody formation is still unclear. Up to 20% of patients on methyldopa therapy develop a positive direct antiglobulin test;[132] however, only 0.3–0.8% of patients develop hemolytic anemia.[133,134] Kelton[135] showed that patients with a positive DAT, but without hemolysis, have significant impaired RE function. The development of a positive DAT in patients on α-methyldopa depends on the dose[132] and the duration of the therapy. It typically occurs in patients after 3 or more years of continuous treatment.[136] Hemolysis resolves after discontinuation of the drug although serologic abnormalities usually resolve more gradually and this can last for up to 24 months.[137] The autoantibody induced by α-methyldopa is IgG,[138] and is directed against Rh antigens in many patients.[139,140] The antibody does not activate complement; hence, the DAT is positive only with IgG detected on the cells. The pattern of antibody specificities is similar to that in idiopathic autoimmune hemolytic anemia. Besides a positive direct antiglobulin test, patients may have positive antinuclear factor,[136,141,142] lupus erythematosis (LE) cells,[136,142–145] rheumatoid factor[142,145] and factor III inhibitor.[146] The underlying pathogenesis is still unknown but α-methyldopa is demonstrated to produce an aberration in lymphocyte proliferation by increasing lymphocyte c-AMP that inhibits suppressor T-cell function.[147] Other drugs including levodopa, procainamide and NSAIDs (e.g. mefenamic acid and diclofenac) may also induce autoantibody similar to α-methyldopa.[148]

Drug (hapten) dependent antibody

A number of drugs bind firmly to red cell membranes, possibly via covalent bonds[149] and they elicit the production of IgG specific for that drug. As a rule, the antibody binds to red cells in the presence of the drug and this causes a positive DAT result. Usually the positive DAT is a coincidental finding and only rarely will these antibodies cause overt hemolysis, which is usually extravascular and, very infrequently, intravascular.[150] Penicillin and cephalosporin are the most common drugs that stimulate antibody production by this mechanism. They share a common benzyl-penicillol group that acts as a hapten.[151] It should be noted that up to 97% of normal healthy adults have antipenicillin antibody; however, this is an IgM antibody that can be present even without prior treatment with penicillin or cephalosporin.[152] The occurrence of hemolytic anemia depends on the appropriate ratio of antigen and antibody. Therefore, penicillin-induced hemolytic anemia typically occurs in patients treated with high-dose penicillin for 7–14 days via intravenous route, or in patients with renal failure resulting in reduced drug clearance.[33,152] Hemolysis stops promptly when the drug is stopped.

Innocent bystander

Instead of binding to red cell membranes, this group of drugs (haptens) binds to plasma proteins, and the drug–protein complex becomes sufficiently large to trigger an immune response.[153] The drug–protein complex binds to the surface of red cells and is further stabilized by the drug-dependent antibodies that also have immune specificities against alloantigens on the red cell membrane. The antibody can be IgM, IgG or both. The antibody–drug–protein complex activates the complement pathway and triggers red cell clearance. Sometimes, other cell lines in the blood are also involved producing neutropenia or thrombocytopenia.[33,152] Examples of drugs in this category include quinine, quinidine, sulfonamides, sulphonylurea and thiazide.

It is likely that the pathophysiology of drug-induced hemolysis is more complex than noted previously. For example, Phenacetin, Streptomycin, and non-steroidal anti-inflammatory drugs can cause hemolysis by both autoantibodies (drug-independent antibodies) and drug-dependent antibodies.[138,154–156]

Oxidative injury to red cells

Hemoglobin binds oxygen and, consequently, it is prone to oxidation and denaturation by oxidative agents, particularly if the anti-oxidative protective mechanisms in the red cell are overwhelmed. The amount of oxidative hemolysis is determined by the strength and the blood level of the oxidant, and congenital deficiency of the G6PD or glutathione-dependant pathways. Older red cells are more prone to oxidative injury by oxidants than are young red cells. The characteristic features of oxidative hemolysis include the formation of methemoglobin, sulfhemoglobin and Heinz bodies. Clinically, methemoglobin and sulfhemoglobin may present as bluish discoloration indistinguishable from cyanosis. Heinz bodies are the microscopic appearance of denatured hemoglobin. Moreover, examination of a peripheral blood smear may also reveal 'bite cells',[118,119] blister cells,[114] and eccentrocytes[119] (Fig. 10.3). 'Bite cells' are semicircular remnants of red cells after being partially phagocytosed or after extrusion of the Heinz body from the red cells.[117] If the denatured hemoglobin shifts to one side of the red cell, the red cell may appear as a blister cell and this is usually an indicator of brisk hemolysis.

Drugs that can cause oxidative hemolysis include nitrofurantoin, amniosalicylic acid, dapsone and pyridium (phenazopyridine). Very rarely, high-dose oxygen therapy can result in oxidative injury to the red cells, particularly in patients with vitamin E deficiency.[115,116,149,157]

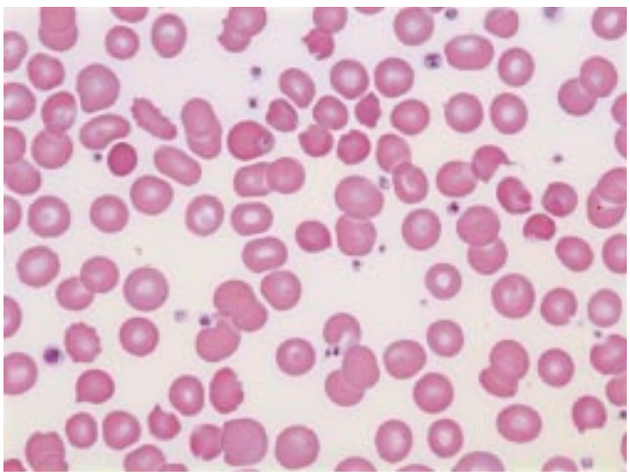

Fig. 10.3 Peripheral blood smear showing spherocytosis, bite cells and blister cells (ghost cells) in a patient with drug-induced oxidative hemolysis. Wright stain, × 1000, oil field.

Diagnosis of drug-induced hemolytic anemia depends on a detailed drug history. Demonstration of drug-dependent hemagglutination in the indirect antiglobulin test is confirmatory. However, sometimes, the diagnosis may only be established by resolution of the hemolysis on removal of the offending drug.

Non-immune hemolytic anemia

Infection-induced hemolytic anemia

Microorganisms may cause injury to red cells through different mechanisms such as: (1) physical invasion of red cells (e.g. malaria); (2) hemolysin secretions to damage the red cells directly (e.g. *Clostridium perfringen*) (Fig. 10.4); (3) infection that triggers formation of antibody (anti-I) against red cells (e.g. mycoplasma); (4) microangiopathic hemolysis caused by disseminated intravascular coagulation associated with infection; or (5) antibiotic therapy may cause hemolysis. In some cases, multiple mechanisms of hemolysis coexist, which often poses a diagnostic challenge to the clinician.

Mechanical trauma to red cells

Mechanical trauma to red cells can occur in three conditions: excessive shearing forces due to high-pressure gradient in the circulation; direct external impact; and microangiopathic thrombotic hemolysis.[158] On examination of a peripheral blood smear, burr cells

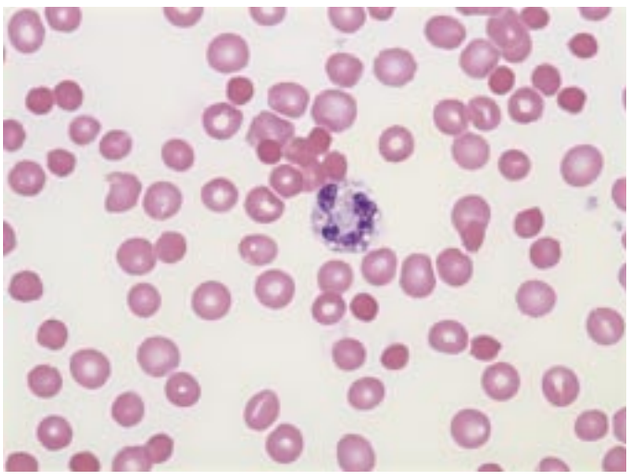

Fig. 10.4 Peripheral blood smear showing micro-spherocytosis and cytoplasmic vacuolation in neutrophils in a patient with clostridia infection. Wright stain, × 1000, oil field.

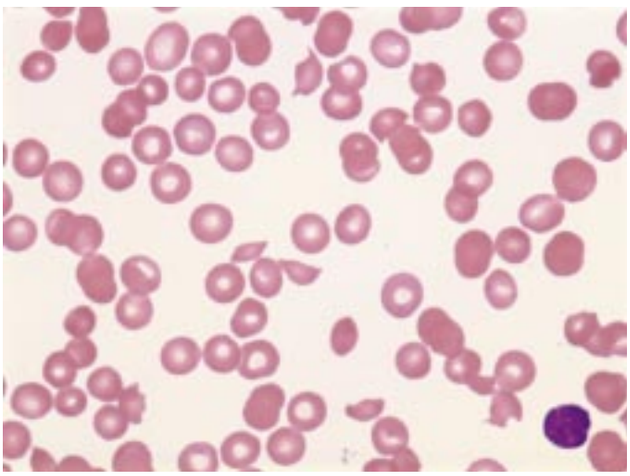

Fig. 10.5 Peripheral blood smear showing fragments of red cells and thrombocytopenia in a patient with thrombotic thrombocytopenic purpura. The size of red cells is approximately equal to the size of the nucleus in a non-reactive lymphocyte. Similar features may be present in patients with hemolytic uremic syndrome or microangiopathic hemolysis. Wright stain, × 1000, oil field.

and schistocytes of variable shapes such as crescents, helmets, micro-spherocytes and fragments are apparent. Therefore, this is commonly called schistocytic hemolytic anemia. Other non-specific features include aniso-poikilocytosis, polychromatic macrocytosis, thrombocytopenia and / or procoagulants activation. Extravascular hemolysis is the predominant feature, although intravascular hemolysis occurs in severe cases.

Schistocytic hemolytic anemia (SHA) is classified according to the size of the blood vessels where hemolysis occurs. Large-vessel SHA includes hemolysis in malignant hypertension and in patients with prosthetic heart valves. Small-vessel SHA occurs in march hemoglobinuria, autoimmune vasculitis, disseminated intravascular hemolysis, and thrombotic thrombocytopenic purpura – hemolytic uremic syndrome (TTP / HUS).

Thrombotic thrombocytopenic purpura – hemolytic uremic syndrome

In 1924, Moschowitz identified thrombotic thrombocytopenic purpura (TTP) as observed in a 16-year-old girl, while hemolytic uremic syndrome (HUS) was first described by Gasser in 1955. They are characterized by the triad of thrombocytopenia, anemia and renal dysfunction. Fever and neurological symptoms such as hemiparesis, aphasia, seizure, fluctuating mental function and coma are less common manifestations. Only about half of the patients present with full pentad symptoms (anemia, thrombocytopenia, renal dysfunction, fever and neurological dysfunction).[159] Because of the overlap in the clinical and pathologic features, TTP and HUS may actually be a different spectrum of the same disorder. Most adult cases of TTP / HUS syndrome are idiopathic. However, TTP / HUS syndrome may be triggered or associated with other disorders or conditions such as: (1) vaccination; (2) infection such as enterotoxin-producing *Escherichia coli* and *Shigella dysenteriae;* (3) human immunodeficiency virus (HIV); (4) drugs (e.g. quinine, quinidine, ticlopidine and mithramycin); (5) malignancy such as adenocarcinoma; (6) bone marrow transplantation; (7) pregnancy; and (8) collagen vascular disease.

The pathogenesis of TTP / HUS syndrome is unknown. Disseminated platelet-rich thrombi are the key pathology and endothelial cell apoptosis induced by plasma from TTP patients,[160] disseminated platelet activation triggered by protein p37[161] or calpain,[162] and abnormally large multimers of von Willebrand factor (vWF)[163] have been shown. Recently, mutations in zinc metalloproteinase genes (ADAMTS13) have been found in familial TTP patients.[164]

Laboratory abnormalities of a TTP patient may include thrombocytopenia, fragmented red cells (Fig. 10.5), elevated LDH and renal impairment. Serial measurements of platelet counts using a phase contrast microscope are important because platelet count and LDH are the markers of TTP activity. In contrast, hemolysis indicates neither the severity nor the activity of TTP.

The primary treatment of TTP or HUS is plasma exchange with vWF-poor plasma, and 1–1.5 times the plasma volume should be exchanged with fresh-frozen plasma (FFP).[165] On-going trials comparing cryosupernatant or

detergent-treated FFP may provide evidence on the choice of replacement fluids. Plasma infusion is less effective than plasma exchange[166] because less plasma volume is being replaced.[167] However, plasma infusion has been used in familial TTP patients. Neurological symptoms may recover within hours after plasmapheresis. LDH and thrombocytopenia usually normalize within days. If possible, platelet transfusions should be avoided in patients with TTP or HUS. Antiplatelet therapy such as aspirin, ticlopidine or dipyridamole may be considered when the platelet count is above 50×10^9/l. Evidence of the value of corticosteroid therapy exists only from anecdotal studies and one retrospective study.[168] Intravenous IgG, splenectomy and vincristine should be reserved for refractory or resistant TTP patients. Without definitive treatment, the mortality of TTP exceeds 90%. However, if treated promptly, TTP has a relatively low mortality.

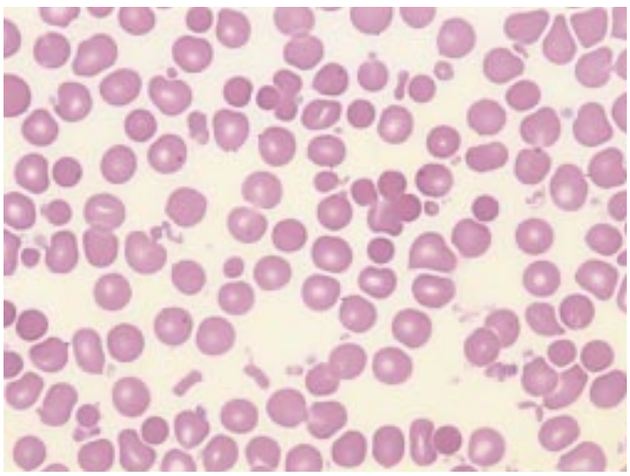

Fig. 10.6 Peripheral blood smear showing spherocytes of variable sizes in a patient with burn injury. The red cells have also formed micro-vesicles and fine filamentous structures. Wright stain, × 1000, oil field.

Cardiac hemolysis

Almost any intracardiac lesion that alters the hemodynamics and generates excessive shear force to the red cells can cause intravascular hemolysis. Traumatic hemolysis may occur after cardiac surgeries such as heart valve replacement or heart valve repair. Synthetic material, small valvular area, and complications such as thrombotic valve and perivalvular leaks are particularly at the risk of significant hemolysis. Hemolysis also occurs in patients with native valvular lesion including severe aortic stenosis, coarctation of aorta and ruptured aneurysm of the sinus of Valsalva. In addition, aortofemoral bypass has been described as being associated with traumatic hemolysis because of the same pathophysiology.[158]

External impact on the red cells

When red cells flow through small vessels over the surface of bony prominences, they are prone to external impact. March hemoglobinuria, a well-described but uncommon condition of intravascular hemolysis, typically occurs after strenuous marching or running on a hard surface in susceptible individuals who wear thin-soled shoes. There is usually no underlying intrinsic erythrocyte abnormality. This condition can be prevented by insertion of a soft inner sole.

Thermal damage of red cells

In patients with extensive burn injury, red cell denaturation and fragmentation occur (Fig. 10.6). Less commonly, a similar mechanism of hemolysis occurs in patients with heat stroke.

Osmotic damage of red cells

Fresh water or salt water drowning can cause hemolysis because of abrupt osmotic changes in the pulmonary circulation.

Miscellaneous causes of hemolytic anemia

Paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare type of intravascular hemolysis, caused by an abnormal membrane protein. The disease arises from abnormal clones of red cells with increased sensitivity to complements, particularly in an acidic environment. It is a disease of pluripotent stem cells and, therefore, other hematopoietic cells are affected. Patients with PNH can progress to aplastic anemia and acute myeloid leukemia. For uncertain reasons, it is sometimes complicated by thrombotic episodes at unusual sites.

More than 20 different proteins, including complement proteins, enzymes and various other receptors, are missing from the surface of PNH red cells.[169] Ferguson *et al*[170] identified glycosyl-phosphatidyl-inositol (GPI anchor) as the key glycolipid structure to hold the proteins on to the red cell membrane. The defect of GPI synthesis has been mapped to the mutation of *pig-a* gene on the X

chromosome (Xp22.1). However, it is still unclear why the abnormal PNH clone proliferates preferentially as compared with other normal hematopoietic stem cells.

The screening test of PNH is the Ham's test. This test demonstrates that the abnormal red cells are abnormally sensitive to be hemolysed by the complements in acidified serum. Although the Ham's test was the gold standard for PNH, it is semi-quantitative and not sensitive enough to detect small numbers of PNH clones. In addition, the sensitivity of the test is affected by recent hemolysis or blood transfusion. Flow cytometry with a combination of monoclonal antibodies has contributed significantly to the diagnosis of PNH. CD55 and CD59 are the best-studied monoclonal antibodies to detect GPI-linked proteins on the red cells from peripheral blood sample. Based on the extent of GPI deficiency, it is possible to classify the abnormal clones into partially deficient cells and completely deficient cells.[169] Characteristically, patients with PNH have a hypoplastic bone marrow in spite of significant hemolysis.

Immunosuppressive therapy using corticosteroids, antilymphocyte globulin or cyclosporin A has been used to treat patients with progressive pancytopenia.[171] Supportive treatment, such as blood transfusion with washed red cells, not only relieves the symptoms of anemia, but also suppresses the production of abnormal clones. Anticoagulation with Warfarin is required to prevent thrombosis. Bone marrow transplantation is considered in young patients and in patients with bone marrow failure. Up to 15% of the patients with PNH may have a spontaneous recovery.[172]

Venom-induced hemolytic anemia

Cobras, pit vipers, spiders such as *Loxosceles* (also known as violin spider), and black widow spiders (belonging to *Latrodectus* genus), produce a hemolytic venom that activates coagulation and causes disseminated intravascular hemolysis, although the cases are rarely fatal.[173]

Toxin-induced hemolytic anemia

A condition such as Wilson's disease causes an accumulation of copper in body organs and this can damage red cells by interference with glucose metabolism. The diagnosis of Wilson's disease should be considered when a patient presents with neurological symptoms and severe acute hemolysis. A pathognomonic physical sign is a Kayser–Fleischer ring in the eye. Treatment with Penicillamine can halt the hemolysis

Hemolytic anemia in organ dysfunction

Hemolysis may occur in patients with renal failure or hepatic failure. Hemolytic anemia in these conditions is of less importance than other causes of anemia.

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