Autoimmune Hematological Diseases

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Kenji YOKOYAMA* and Yasuo IKEDA**

*Assistant, **Professor, Department of Internal Medicine, Keio University School of Medicine

Abstract: Idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA), and pernicious anemia are autoimmune hematological diseases associated with autoantibodies against platelets, red blood cells, and gastric mural cells and intrinsic factor, respectively. The diagnosis of ITP requires thrombocytopenia with normal morphology of red blood cells and white blood cells (except for cases complicated with AIHA or iron-deficiency anemia). Bone marrow examination indicates a normal or increased number of megakaryocytes. The first-line treatment is the administration of glucocorticoids, and splenectomy is performed in patients who are unresponsive to initial glucocorticoid therapy. The diagnosis of AIHA requires findings indicating hemolysis and serological evidence of anti-red blood cell (RBC) autoantibodies. Like ITP, the first-line treatment is the administration of glucocorticoids. Splenectomy and the administration of immunosuppressive agents are considered in patients who are unresponsive to initial glucocorticoid therapy. Neurological symptoms in addition to anemic symptoms are common in patients with pernicious anemia. The diagnosis of pernicious anemia requires findings of dyserythropoiesis and low vitamin B12 value. Anti-intrinsic factor and/or anti-gastric mural cell antibodies are often positive. Pernicious anemia is treated with the intramuscular injection of vitamin B12.

Key words: Idiopathic thrombocytopenic purpura; Autoimmune hemolytic anemia; Pernicious anemia

Introduction

Idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA), and pernicious anemia are autoimmune hematological diseases associated with autoantibodies against platelets, RBCs, and gastric mural cells and intrinsic factor, respectively.

This paper discusses these three diseases.

Idiopathic Thrombocytopenic Purpura (ITP)

ITP is one of the hematological diseases associated with thrombocytopenia. ITP is an autoimmune thrombocytopenic disorder in
observed. ITP patients who are found to have thrombocytopenia at routine medical checkups are often asymptomatic.

Peripheral blood cell count and morphological examination of blood cells should first be performed to diagnose ITP. It is also necessary to exclude pseudothrombocytopenia. Pseudothrombocytopenia occurs when platelet count with an automatic hemocytometer mistakenly reads a low value due to platelet agglutination in a test tube containing ethylenediaminetetraacetic acid (EDTA), an anticoagulant. Pseudothrombocytopenia is reported to occur in about 0.1% of adults and should be suspected in patients with a low platelet value.

Table 1 Diagnostic Criteria of Idiopathic Thrombocytopenic Purpura (ITP)
(Investigation and research group of disease specified by the Ministry of Health and Welfare; revised in 1990)

1. Bleeding symptoms are observed.
   The main bleeding symptom is purpura (petechia and ecchymosis), and other symptoms include stomatorrhagia, rhinorrhagia, menela, hematuria, and hypermenorrhea. Usually, no intrajoint bleeding is found. ITP may be detected in asymptomatic patients found to have thrombocytopenia at a medical checkup.

2. The following laboratory findings are observed.
   1) Peripheral blood
      (1) Thrombocytopenia with platelet count of 100,000/μl or less. Attention should be paid to the possibility of pseudothrombocytopenia when the result is obtained with an automatic hemocytometer.
      (2) Erythrocytes and leucocytes are quantitatively and morphologically normal. Blood loss-induced or iron-deficiency anemia is sometimes observed. A slight fluctuation of leukocyte count may be observed.
   2) Bone marrow
      (1) Megakaryocyte count in the bone marrow is normal or increased. Many of megakaryocytes are lacking in platelet adhesion.
      (2) The cellularity and the morphology of both the erythroid and myeloid series are normal. The myeloid/erythroid ratio (M/E ratio) is normal.
      (3) Platelet-associated immunoglobulin G (PAIgG) is increased.
         The increase in PAIgG is sometimes not observed, while it can be observed in thrombocytopenia associated with other diseases.

3. Other diseases associated with thrombocytopenia can be excluded. Note)

4. Idiopathic thrombocytopenic purpura may be diagnosed when a patient meets the above characteristics of (1) and (2) and the above requirement of (3). The shortage of platelet life may be helpful to exclude ITP.

5. Disease type differentiation criteria
   1) Acute type: ITP resolved in 6 months of the estimated onset or diagnosis
   2) Chronic type: ITP that persists for more than 6 months of the estimated onset or diagnosis.
      However, ITP may be considered as the acute type in infants when it develops acutely with prior viral infection.

Note: Diseases that can cause thrombocytopenia include drug or radiation disorder, aplastic anemia, myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria, systemic lupus erythematosus, leukemia, malignant lymphoma, bone marrow metastasis of cancer, disseminated intravascular coagulation syndrome, thrombotic thrombocytopenic purpura, hypersplenism, megaloblastic anemia, sepsis, tuberculosis, sarcoidosis, and hemangioma. For infection, thrombocytopenia after viral infection or viral live vaccine inoculation in infants may be included in ITP. Congenital types of thrombocytopenia include Bernard-Soulier syndrome, Wiskott-Aldrich syndrome, May-Hegglin syndrome, and Kasabach-Merritt syndrome.

without clinical symptoms, including bleeding tendency. In such cases, platelet count should be verified to eliminate the possibility of pseudothrombocytopenia by (1) visually checking peripheral blood smear specimens and (2) collecting blood with other anticoagulants such as citric acid and heparin.

Peripheral blood smears from typical ITP patients show thrombocytopenia and normal or slightly larger platelets, with no giant platelets being observed. Erythrocytes and leucocytes are morphologically normal.

When ITP is suspected from peripheral blood smears, further examinations should be performed. According to American Society of Hematology guidelines, suspected patients of less than 60 years of age can be diagnosed as ITP without bone marrow examination, unless findings inconsistent with ITP are observed in medical history, physical examinations, and peripheral blood count or morphology.1)

In Japan, however, bone marrow examination is usually performed to verify the normal or increased number of megakaryocytes and to confirm that erythroid and myeloid cells are quantitatively and morphologically normal according to the diagnostic criteria proposed by a research group of the former Ministry of Health and Welfare (currently, the Ministry of Health, Labour, and Welfare), as shown in Table 1. Further examinations may be performed as required for differentiating ITP from other possible diseases listed in the Note of Table 1.2)

2. Treatment of ITP

Treatment is not always necessary in patients diagnosed as having ITP. The need for treatment depends on platelet count and severity of bleeding tendency. Although no definite criteria have been established for the indication for treatment, asymptomatic patients or patients with mild purpura who have a platelet count of 50,000/μl or higher at the first presentation are often followed without specific treatment.

In contrast, treatment is necessary for patients with a platelet count less than 20,000/μl, irrespective of symptoms, or those with evident submucosal bleeding and a platelet count less than 50,000/μl. The need for treatment in patients with mild bleeding tendency and a platelet count between 20,000 and 50,000/μl is often examined on a case-by-case basis in consideration of other hemorrhagic risk factors, such as blood pressure and peptic ulcer.

Patients with fatal hemorrhage irrespective of platelet counts, or those with evident submucosal hemorrhage and a platelet count less than 20,000/μl have to be immediately hospitalized for treatment.

The following treatments are available for ITP.

(1) Standard treatment

(i) Glucocorticoids: Glucocorticoids are the first-line therapy for ITP. Usually, oral prednisolone (PSL) is used. It is started at 1 mg/kg/day and continued for at least 3 to 6 weeks. Subsequently, the dose is gradually reduced, depending on its effect.

(ii) Splenectomy: Splenectomy is indicated in patients who do not respond to glucocorticoids or those who require 0.1 mg/kg/day or higher of PSL to maintain a safe platelet count. Previous reports showed that platelet count returned to normal levels in 60–70% of patients who received a splenectomy.

(2) Emergency treatment

(i) Immunoglobulin: Earlier studies reported that administration of an intact type of immunoglobulin at 0.4 g/kg/day for 5 days increased platelets in about 75% of patients with ITP. However, this is usually a transient increase, and the platelet count often returns to the previous level in 3 to 4 weeks.

The administration of immunoglobulin is indicated in patients with a platelet count less than 50,000/μl and fatal hemorrhage, or those who require a transient increase of platelets for surgery.

(ii) Platelet transfusion: Platelet transfusion is performed as an emergency measure for patients complicated with fatal hemorrhage.
However, the effect is temporary because anti-platelet antibodies bind to transfused platelets and cause platelet destruction.

(3) Treatment for intractable cases

(i) Immunosuppressive agents: Immunosuppressive agents (cyclophosphamide, azathioprine and cyclosporine) have been administered in patients with refractory ITP. Vincristine and danazol are also used. However, their effect is variable.

(ii) H. pylori eradication therapy: Recent clinical trials in ITP patients positive for Helicobacter pylori reported that platelet counts increased after H. pylori eradication therapy.\(^3\)\(^{-5}\) Although it remains unclear how H. pylori is involved in the onset of ITP, the therapy deserves further investigation.

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### Table 2 Diagnostic Criteria of Autoimmune Hemolytic Anemia (AIHA)

(Investigation and research group of disease specified by the Ministry of Health and Welfare; revised in 1990)

1. The diagnostic criteria of hemolytic anemia are met.
2. The direct Coombs’ test using wide spectrum antiserum is positive.
3. Alloimmune hemolytic anemia (incompatible blood transfusion and hemolytic disease of the newborn) and drug-induced immune hemolytic anemia are excluded.
4. A diagnosis of AIHA is made with the above 3 criteria. AIHA is then further classified into the following 3 disease types by the optimal temperature for the reaction of anti-erythrocyte autoantibodies.

   (1) Warm AIHA

   Warm AIHA clinically varies among patients. In principle, IgG alone, or the combination of IgG and a complement factor is detected in the direct Coombs’ test at 37°C using specific antiserum. However, there may be cases positive only for anti-complement or wide spectrum antiserum. This type of AIHA may be diagnosed by excluding the following (2) and (3).

   (2) Cold agglutinin syndrome (CAS)

   Serum cold agglutinin is increased, and exacerbated hemolysis by the exposure to cold or chronic hemolysis is observed. Complement components are detected at the direct Coombs’ test at 4°C.

   (3) Paroxysmal cold hemoglobinuria

   Hemoglobinuria and detection of serum biphasic hemolysin (Donath-Landsteiner antibody) characterize this disease.

5. The clinical course and cause of AIHA are classified as follows:

   - Acute type: Resolved within 6 months of the estimated onset or diagnosis.
   - Chronic type: Persisted for more than 6 months of the estimated onset or diagnosis.
   - Idiopathic type: No underlying disease is observed.
   - Secondary: A prior or concomitant underlying disease is observed.

6. Remarks

   1) Morphological findings of erythrocytes (spherocytes and hemagglutination) are helpful for making a diagnosis of AIHA.
   2) Occasionally, the direct Coombs’ test by the common method is negative in some cases with warm AIHA.
   3) Idiopathic warm AIHA may be complicated with idiopathic thrombocytopenic purpura (ITP).
   4) Hemolysis of cold agglutinin syndrome does not always occur in parallel with cold agglutinin titer; hemolytic symptoms may occur even at a low titer.
   5) Antibody emigration technique is used to determine the properties of autoantibodies. Further examination may be performed, as required, because some autoantibodies may show special properties, depending on the type of immunoglobulin and immunobiological activity.
   6) Underlying diseases include autoimmune diseases, rheumatic diseases, lymphoproliferative disorders, immunodeficiency syndrome, tumors, and infections (mycoplasma and virus). These diseases may become evident during the clinical course of patients with idiopathic AIHA. AIHA associated with prior viral infection in infants shall be considered idiopathic. Cold agglutinin syndrome associated with mycoplasma or viral infection shall be considered secondary.
   7) Attention should be paid to the fact that the direct Coombs’ test with wide spectrum antiserum is positive in drug-induced immune hemolytic anemia. It is helpful for making a diagnosis of the drug-induced anemia to examine the clinical course and effect of the discontinuation of the potentially responsible drug. Except for the autoimmune type (α-methyldopa), drug specificity of antibodies can be proved with appropriate techniques. For the autoimmune type, the direct Coombs’ test may become positive after the long-term administration of the responsible drug, and warm AIHA may occur in some patients.

Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AIHA) develops because anti-RBC autoantibodies (IgG or IgM) bind to RBCs and cause accelerated RBC destruction by the reticuloendothelial system, including the spleen. AIHA is classified by the characteristics of autoantibodies into warm AIHA, which has warm-type IgG antibodies, cold agglutinin syndrome (CAS), which has cold-type IgM antibodies, and paroxysmal cold hemoglobinuria (PCH), which has biphasic IgG antibodies (Donath-Landsteiner (D-L) antibodies).

1. Diagnosis of AIHA

Patients with AIHA often complain of anemic symptoms, including palpitations and dypnea on exertion, although their severity differs by patient. Physical examination of the AIHA patient reveals anemia, jaundice, and splenomegaly. To make a diagnosis of AIHA, it is necessary to (1) detect clinical or laboratory finding(s) suspected to indicate hemolysis, and (2) serologically demonstrate anti-RBC antibodies. The diagnostic criteria of the research group of the former Ministry of Health and Welfare (currently the Ministry of Health, Labour, and Welfare), revised in 1990, are used in Japan. See Table 2.

Laboratory findings suggesting hemolysis include anemia, increased reticulocytes, increased bilirubin with the predominant increase of indirect bilirubin, increased LDH, and reduced haptoglobin. Anti-RBC antibodies can be demonstrated by detecting immunoglobulins or complement components combined with RBC membrane using direct Coombs’ test.

When the direct Coombs’ test is negative in patients with typical AIHA findings, it is necessary to quantitatively determine immunoglobulin combined with RBCs. It is also necessary to perform the direct Coombs’ test at different temperatures because AIHA is classified into subtypes by the temperature at which antibodies become active.

AIHA is classified into warm AIHA when antibodies (IgG) become active around 37°C; into CAS when cold-type IgM antibodies show strong activity at as low as 0 to 4°C with almost no activity at physiological temperature; and into PCH when D-L antibodies (IgG) that bind to RBC at 0 to 4°C and dissociate from RBC at 37°C. However, prior to dissociation, D-L antibodies initiate complement activation and cause hemolysis.

It is important to ask patients about their medical history because AIHA may develop in patients with an underlying disease (systemic lupus erythematosus, malignant lymphoma, or chronic lymphocytic leukemia) or infection (such as mycoplasma) or those who are taking drugs (such as α-methyldopa).

2. Treatment for AIHA

(1) Treatment for warm-type AIHA

Usually, folic acid is administered to increase hematopoiesis. The need for further treatment depends on the severity of hemolysis. The first-line therapy is the administration of glucocorticoids: usually 1 mg/kg/day of oral PSL is administered. Hemolysis is usually ameliorated within 3 weeks, and PSL is gradually reduced after 4 to 6 weeks of treatment. Although PSL can be discontinued in some patients, a maintenance dose of 10 to 15 mg/day is often required. Splenectomy and/or administration of an immunosuppressive agent is performed on patients who do not respond to PSL or those who require a high dose of PSL to maintain RBCs, as in the treatment for ITP.

(2) Treatment for CAS

CAS develops both primarily and secondarily. Primary CAS is often associated with mild anemia, and is therefore treated merely by instructing patients to avoid cold stimuli. Severe patients may be treated with an immunosuppressive agent or plasmapheresis, while the administration of glucocorticoids or splenectomy are not so effective. Patients with secondary CAS associated with advanced
hemolysis require the treatment of their underlying diseases.

(3) Treatment for PCH

Usually, PCH does not require any specific treatment except for avoiding cold stimuli. However, plasmapheresis is indicated in serious cases with fatal risk. Underlying syphilis should be suspected in patients with chronic PCH.

Pernicious Anemia

Pernicious anemia results from vitamin B\textsubscript{12} deficiency. Although vitamin B\textsubscript{12} deficiency can occur from insufficient intake, gastrectomy, or small intestine disease, only the anemia associated with vitamin B\textsubscript{12} deficiency by chronic atrophic gastritis is called pernicious anemia.

1. Diagnosis of pernicious anemia

Pernicious anemia develops and progresses slowly. The diagnosis of pernicious anemia is often made in patients around 60 years of age. In addition to anemic symptoms, this disease is associated with neurological symptoms (abnormal sense of perception, vibration, and position as well as positive Romberg’s sign, reduced muscle strength, and spastic gait) and lingual papilla atrophy (Hunter’s glossitis). Peripheral blood testing indicates macrocytic anemia and hypersegmented neutrophils often associated with leukopenia and thrombocytopenia.

Since hematopoiesis is ineffective, biochemical tests show increased LDH, increased indirect bilirubin, and reduced haptoglobin. Vitamin B\textsubscript{12} is low. The diagnosis of pernicious anemia can be confirmed in the Schilling test indicating that the absorption of vitamin B\textsubscript{12} is low and recovered with the addition of the gastric intrinsic factor.

Furthermore, patients with pernicious anemia show immunological abnormalities, such as the development of anti-intrinsic factor antibody (highly specific because it is positive in about 70% of patients) and anti-gastric mural cell antibody (positive in about 90% of patients).

Bone marrow examination indicates megaloblastic cells, giant metamyelocytes, and giant rod nuclear cells, although diagnostic bone marrow examination is not necessarily required in typical cases.

2. Treatment for pernicious anemia

The first-line therapy for pernicious anemia is the intramuscular injection of vitamin B\textsubscript{12}; usually, 1,000\(\mu\)g/day of hydroxocobalamin or methylcobalamin is administered for about 2 weeks continuously, which improves anemia and replaces a sufficient amount of vitamin B\textsubscript{12}. Subsequently, maintenance therapy is necessary every 2 to 3 months. An earlier study reported that there is another vitamin B\textsubscript{12} absorption route that does not require the gastric intrinsic factor, and that the oral administration at 2,000\(\mu\)g every day was comparable to, or more effective than, the intramuscular injection of 1,000\(\mu\)g once a month.\textsuperscript{7)}

Conclusion

This paper explains the latest view of the diagnosis and treatment of ITP, AIHA, and pernicious anemia. We hope that this paper will be helpful for those who encounter clinical cases suspected to have the diseases.

REFERENCES

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