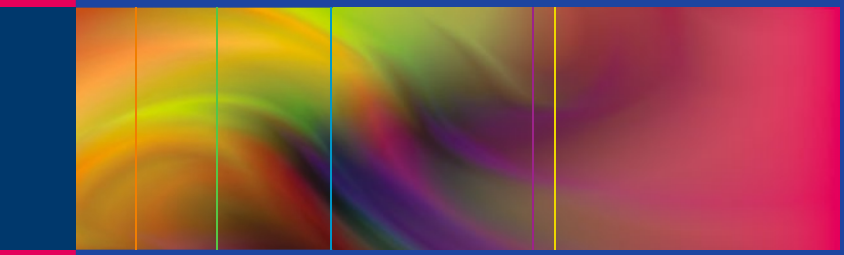
Giorgio Berlot · Gabriele Pozzato
Editors



Hematologic Problems in the Critically Ill

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Chapter 1
Introduction

Giorgio Berlot and Gabriele Pozzato

Three o'clock a.m. You just sit down and drink a cup of coffee when the phone rings. It is the ED: 10 min ago a man was admitted with hypotension, fever and leukopenia associated with low platelet count and abnormal coagulation tests. More or less an hour ago you visited another patient with ever-decreasing hemoglobin values in whom the most common sources of bleeding have been excluded. You are blaming yourself because you failed to buy a textbook of hematology you saw at a congress a couple of weeks ago and the hospital administration because a hematologist will be available only after 9.00 a.m. In the meanwhile, you are expected to keep these patients alive till someone with a more in-depth knowledge of hematological disease will arrive to help you and your colleagues.

Actually, the presence of hematological alterations is very common in critically ill patients just for the kind of diagnosis of

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admitted cases, that is, severe traumas, car crashes, septic shocks, severe respiratory distress and so on. In these patients, the finding of anemia or leukocytosis is an expected feature of the acute event and does not alert doctors and nurses. The requests of hematological counseling occur when there are discrepancies between the clinical situation and the main hematological parameters: for example, sepsis is improving and leukocyte level is still increasing or there is a worsening anemia without evidence of blood loss.

In these critical patients, the traditional tools for evaluating the nature of the hematological diseases are not feasible: the family and the personal history of the patients are often unavailable, and other anamnestic features like changes in stool habits or dietary history are irrelevant and useless. Even to perform the physical examination is often difficult, given the common presence of several medical devices (nasogastric tube, central vein catheters, endotracheal tube, invasive hemodynamic monitoring) and the absence of patient cooperation. Therefore, to identify the cause of the hematological alterations, there is the need of several key laboratory tests.

Obviously, a different approach is indicated in case of cytopenias (anemia, thrombocytopenia, leukopenia) and in the case of thrombocytosis, leukocytosis or, rarely, of erithrocytosis. These hematological alterations could be mixed in different ways with regard of the several acute and chronic pathological conditions present in the same critical patient. However, for didactic reasons, the main hematological conditions requiring counseling will be separately discussed. Since the most common hematological problem in the critically ill patient is anemia, the opening chapter will discuss this pathological condition.

Chapter 2
Anemia

Gabriele Pozzato

Anemia is not a disease by itself but a condition that is a consequence of acquired or genetic abnormalities. Functionally, anemia is defined as an insufficient red cell mass to deliver adequate amount of oxygen to organs and peripheral tissues, and, for practical reasons, an Hb concentration less than 14.0 g/dL for men and 12.0 g/dL for women. At present, Hb concentration, as well as other red cell parameters, is determined by electronic cell counters able to deliver the results in few minutes. In most patients, blood determination of Hb levels is useful for assessing anemia, but there are some limitations that must be recognized:

1. Hb changes may reflect altered plasma volume, not a change in red cell mass. In pregnancy, for example, the increased plasma volume decreases the Hb concentration and, in fact, total red cell mass is increased but to a lesser degree than plasma volume. Likewise, very often the critically ill patient is hyper-hydrated to avoid dangerous hypotension or shock;

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this common therapeutic approach determines an increase of plasma volume and reduces Hb concentration and the degree of anemia may appear severe. Conversely, burn patients, through the injured skin, lose plasma and not red cells; therefore, Hb concentration appears normal or even high while the red cell mass could be decreased.

2. Several abnormal Hb have altered ability to bind and to release the oxygen and this is associated with different Hb concentrations. The carriers of Hb with high affinity for oxygen show levels of Hb higher than normal, while the carriers of Hb with decreased oxygen affinity (and better oxygen delivering to tissues) have lower than normal Hb levels.
3. There are several pathological conditions that determine a compensatory increase of red cell mass, the most common are the emphysema (and similar pulmonary diseases) or the right-to-left cardiac shunt (often unknown). These patients have abnormally elevated Hb levels; therefore, a normal Hb level may represent an "anemia" since tissue oxygenation is impaired. Conversely, the patients with hypothyroidism (decreased oxygen needs) may have low Hb level with adequate oxygen delivery to tissues.
4. Acute blood loss is another example of the problem of evaluating anemia by the Hb concentration. In fact, immediately after blood loss, the Hb is normal because the compensatory response to acute hemorrhage is the vasoconstriction. Therefore, the decrease of the Hb concentration begins after 4–6 h. The recognition of this situation is generally easy for the patients recovered in intensive care units since they are monitored in a continuous fashion.

Once the diagnosis of anemia is defined, the cause of this condition must be identified. The classification of the anemia is not simple, but a useful approach could be to ask several questions stepwise (Fig. 2.1).

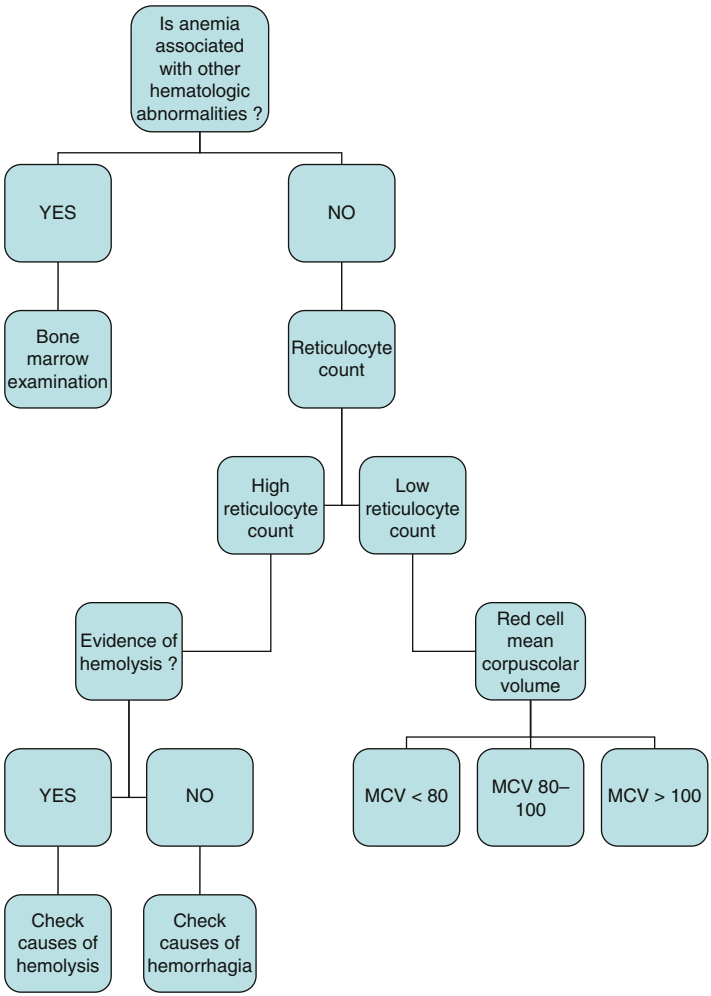

Fig. 2.1 Diagnostic algorythm for anemia

The first question is whether anemia is associated with other hematological abnormalities such as low platelet levels and/or low leukocyte counts and/or presence of abnormal leukocytes (blasts) on blood smear. If this is the case, the presence of bone marrow failure (aplastic anemia) or of malignant hematological disorders such as acute leukemias or myelodysplastic syndromes is likely. In these cases, the bone marrow biopsy and the appropriate cytometric studies of marrow and peripheral blood are mandatory.

The second question is whether anemia determined is associated with an appropriate reticulocyte response. The reticulocyte count is important to evaluate the new red cell production and is very helpful in determining the marrow response to anemia. Very often the reticulocyte count is lacking for the evaluation of the anemic conditions, while this test has a crucial role in the diagnostic process. Until a few years ago, the red blood cells were stained with brilliant cresyl blue, which allows the visualization of ribosomes and reticulin network, thereafter the blood smear was examined by microscope with manual count of stained cells. This method was time-consuming and often the responses were delayed, thus reducing the clinical impact of the test. Lately, automated reticulocyte analyzers are available; these counters have a higher degree of precision than can be achieved manually and, in addition, the responses are immediate. These automated reticulocyte counters may show errors in few rare conditions as the case of presence of Heinz or Howell-Jolly bodies inside red cells. Much more important than the percentage of reticulocytes is their absolute count, which can be easily determined starting from the red cell count: absolute reticulocytes count = % of reticulocytes × red cells count/L^3. The value over 100×10^9/L is indicative of a bone marrow responding normally to hemolysis or blood loss. If the anemia is associated with a poor reticulocyte count (less than 25×10^9/L), an impaired red cell production is likely.

2.1 Anemias with High Reticulocyte Count

In the case of high reticulocyte count, the subsequent question is: Is there evidence of hemolysis or not? The laboratory tests used to identify a hemolytic process are available easily in any hospital: *Serum unconjugated bilirubin*, *serum lactic dehydrogenase* (*LDH*), and *serum aptoglobin*. These tests are related to the red cell increased destruction rate and, in most patients, are indicative of a hemolytic process, but in critically ill patients may be misleading. An increased level of total and unconjugated bilirubin is a common finding in intensive care units for several reasons: prolonged fasting or artificial nutrition, hypotension or shock with reduced liver blood flow, heart failure or tamponade with secondary liver venous stasis, hepatosplenic blood flow modification by endotoxemia or peritonitis, portal thrombosis, preexisting chronic liver diseases, and other less common causes. LDH is an enzyme not specific to the red cells, and it can be found in any organ and tissue; therefore, any cytolitic process is able to increase LDH serum levels. In critically ill patients, high of very high level of serum LDH can be found very easily due to crush syndrome with muscle necrosis, lung inflammatory processes, chronic and acute viral liver diseases or acute cholestasis, fatty liver, sepsis, myocardial ischemia, bone fractures, and others. In addition, high LDH levels without evidence of disease can be found in about 3 % of normal people. The LDH isoenzymes could be useful for determining the involved tissue, but this test is not available in most hospitals and it is used for research purposes only. In conclusion, LDH is not trustworthy in the context of the critically ill patient. The haptoglobin is a protein synthesized by the liver, and it is able to bind to Hb when this molecule is released in the plasma (like occurs in hemolysis). The complex haptoglobin-Hb is removed by the hepatocytes. Despite the presence of haptoglobin in serum only, this protein decreases or becomes undetectable in

case of both intravascular and extravascular hemolysis. Serum haptoglobin determination is useful in the diagnostic path of the majority of patients, but in the intensive care units the interpretation of its levels is complicated and its diagnostic power is significantly reduced. In fact, haptoglobin is an acute-phase protein, therefore, its synthesis increases in response to inflammation, infections, or malignant diseases. Taking into account these characteristics, in critically ill patients, the increased synthesis of this protein due to sepsis, infections, inflammatory states of various etiologies, may overcome the decrease induced by hemolytic process. Conversely, abnormal low levels of haptoglobin can be found in the absence of hemolysis in the case of malnutrition or of the other clinical situations characterized by abnormal protein loss like occurs after extensive burns or for nephritic syndrome; by preexisting chronic liver disease; or by the impossibility of a normal aliment absorption like occurs in large intestine resections for vascular disease or for accident perforation, events not uncommon in the intensive care units. In conclusion, the usual laboratory tests used to identify a hemolytic process are have a limited diagnostic value in the intensive care setting and, often, additional tests and a careful follow-up of the patient are needed for a correct diagnosis. Even the diagnosis of posthemorrhagic anemia may be difficult in these patients. In fact, after an acute blood loss, the plasma volume and red cell mass are reduced in proportional amount; consequently, the Hb concentration does not change. Therefore, the amount of blood loss can be underestimated by the degree of anemia, especially early. In the days following the blood loss, the reticulocyte count is normal and increases only after 6–10 days; in this "window," even the iron stores are unmodified, and mean corpuscular volume is still normal. An external hemorrhage sufficient to determine anemia is usually evident, but internal bleeding may be less apparent. If the hemorrhage occurs in retroperitoneal space, into a body cavity or in a cyst, the decrease of Hb level may be a diagnostic problem. In

addition, the breakdown and the absorption of red cell in the tissues are able to increase indirect bilirubinemia, and this picture, along with high reticulocyte count, can be confused with a hemolytic anemia. Therefore, a careful follow-up of the patient and appropriate tests are mandatory for a correct diagnosis.

If repeated tests confirm high reticulocyte counts (in the absence of blood loss) and a possible hemolytic process is suspected, the main causes of hemolysis should be carefully checked. Since in the adult patients the most common acquired hemolytic disorders are the immune-mediated processes, the direct anti-globulin test (Coomb's test) should be determined. Thereafter, the diagnostic process can be separated for the patients with positive and negative direct anti-globulin test.

2.1.1 Patients Positive for Direct Anti-globulin Test

These cases have presumably an immune-hemolytic anemia and can undergo immediate glucocorticoids therapy, which remains the treatment of choice of this immune disorder. Intravenously administered doses of 1.0 mg/kg b.w. of methyl-prednisolone daily are efficacious in most cases. The response may not be evident for several days and an increase of Hb level can be noticeable only after 7 days of treatment. A further delay in the response is expected in critically ill patients since many acute factors may interfere in the red cell production like prolonged fasting or artificial nutrition, hypotension, reduced liver blood flow, acute renal failure with reduced erythropoietin production, endotoxemia or other acute stress situations. In the rare cases of lack of response or in the case of worsening of the hemolytic process, high-dose i.v. immunoglobulin administration (1 g/kg b.w.) can be useful in decreasing the clearance of the red cells by the monocyte macrophage system. This therapy can be repeated after 1 or 2 weeks if required.

2.1.2 Patients Negative for Direct Anti-globulin Test

In these cases, the clinical history (when available) is helpful to exclude the exposure to chemical or physical agents; thereafter, some infections (malaria, leishmaniasis, trypanosomiasis, bartonellosis) should be taken into consideration in white people back from recent adventure travels in the third world or in people shortly after arriving from Africa or from other underdeveloped countries. In critically ill patients, the septicemia of Clostridium perfrigens should be taken into consideration, in fact it may occur after traumatic wound infections, necrotizing enterocolitis, genitourinary or gastrointestinal surgery, and other acute severe conditions. In this case, a severe, often-fatal, hemolytic anemia occurs with a massive hemolysis, and hemoglobin concentration may fall to a very low level in a matter of hours. The diagnosis is suspected when high fever, jaundice, and anemia occur together in a patient of the intensive care unit. The clostridial infection responds well to antibiotics therapy but the treatment must be started as quickly as possible, even before the blood culture results are available.

After the exclusion of these infective causes with appropriate tests, the other causes of nonimmune hemolytic anemia should be considered. For the diagnosis of the most common diseases, a few laboratory investigations are needed:

1. Hb electrophoresis
2. Osmotic fragility test
3. Red cell enzyme determination
4. Blood smear examination

The Hb electrophoresis may indicate the presence of genetic diseases like sickle cell anemia, or thalassemia or of the rare conditions associated with abnormal Hb (Hb C, SC, D, SD, and

E). The osmotic fragility test is able to discover the spherocytic anemia and related disorders, and, finally, the enzyme determination is useful to detect the glucose-6-phosphate deficiency (G6PD), known as favism, or pyruvate kinase deficiency. All these conditions are inherited diseases; some of these are common in Italy like thalassemias or favism, while others are very rare in Europe, like sickle cell anemia or the unstable Hb diseases. All these diseases worsen the degree of anemia in patients in critical medical conditions and should be recognized to avoid unnecessary support treatments or delay in discharging the patient fearing covert bleeding.

The blood smear examination by microscope is a disregarded tool, which, on the contrary, is able to give important information on the etiology of many hematological disorders even in the setting of the intensive care units. In the case of patients with overt hemolysis and negative for the direct anti-globulin test, the blood smear is very important for the diagnosis of the so-called *fragmentation hemolysis*, a relatively common condition in the critically ill patient.

When the red blood cells are subjected to physical trauma, as occurs in the alterations of heart or for the appearance of microvascular thrombi in small vessels, they may undergo fragmentation, thereby resulting in hemolytic anemia. In these cases, the blood smear shows characteristic fragmented red blood cells named schistocytes; these cells have a crescent shape or take the form of triangles or helmets or other bizarre forms. The identification of the presence of schistocytes is very important since usually there are not other diagnostic tools to recognize the clinical condition characterized by the fragmentation hemolysis. The main causes of red cell fragmentation are indicated in Table 2.1. As shown, only a fraction of the pathological conditions indicated in the table are associated with acute diseases that can be found in the intensive care units; in the following paragraphs only these conditions will be discussed, since the others are outside the scope of this book.

Table 2.1 Clinical condition associated with fragmentation hemolysis

Heart and great vessels abnormalities
Synthetic valvular prostheses (especially aortic)
Unoperated valve diseases (especially aortic stenosis)
Teflon patch repair of atrio-ventricular defects
Ruptured chordae tendineae
Valve porcine xenografts or homografts or xenobioprostheses
Coarctation of aorta
Small vessel diseases (*microangiopathic hemolytic anemias*)
Thrombotic thrombocytopenic purpura (Moshkowitz's disease)
Hemolytic uremic syndrome
Disseminated malignant disease
Transplant-associated microangiopathy
Malignant hypertension
Disseminated intravascular coagulation
Giant hemangiomas and liver hemoangioendothelioma
March hemoglobinuria
Pregnancy-associated thrombotic microangiopathy
HELLP syndrome
Pregnancy-associated thrombotic thrombocytopenic purpura and hemolytic uremic syndrome
Autoimmune diseases
Lupus erythematosus
Wegener granulomatosis

2.2 Heart and Great Vessels Abnormalities

Many patients, after open-heart surgery, are recovered in the intensive care units; therefore, in the management of these patients, medical staff should be able to recognize the laboratory signs of fragmentation hemolysis. In fact, some patients, soon after surgery, develop anemia of different severity. The incidence of hemolysis is reported to be variable ranging from 5 to 25 %. This great variability depends on the method used for detecting hemolysis, lower if only haptoglobin level is determined, higher if more sophisticated methods, like red cell

survival, are available. Several mechanisms are involved in the hemolysis, but all are referable to high turbulence. When the lumen of the aortic prosthesis is small relatively to the stroke volume, a shearing stress higher than 3,000 dyn/cm^2 can easily be generated and this determines mechanical hemolysis. The presence of a severe fragmentation hemolysis with anemia requiring transfusions immediately after open-heart surgery often indicates malfunction of valvular prosthesis. Since this condition does not improve spontaneously, a prompt surgery and valve replacement is indicated. Awaiting the surgery, the patients must be kept at bed rest since hemolysis becomes worse after even slight physical activity.

2.3 Thrombotic Thrombocytopenic Purpura (TTP)

This disease is characterized by disseminated microvascular thrombi in small vessels and by a syndrome including hemolytic anemia, severe thrombocytopenia, neurological symptoms, renal dysfunction, and fever. At the time of presentation, the clinical conditions of the affected patients can be critical; therefore, they are often recovered in intensive care units. Excluding the very rare inherited forms (Upshaw-Shulman syndrome) that appear during childhood, TTP has a peak of incidence between 30 and 40 years. Like most autoimmune diseases, TTP is more common in women than in men (ratio of 2:1). The pathogenesis of the TTP has been clarified in the past years. The von Willebrand Factor (vWF) is a multimeric protein synthesized and stored as ultra-large multimers in endothelial cells, and released at constant rate in circulation. The ultra-large multimers of vWF are immediately cleaved by a metalloprotease present on surface of the endothelial cells and in plasma. This enzyme, known as ADAMTS13, is able to cut the ultra-large

vWF in small multimers necessary for normal platelet adhesion. If the ADAMTS13 does not work for either inherited disease or for antibodies, the ultra-large vWF multimers bind to platelets, promoting platelet agglutination and aggregation, and, at the end, coagulation activation and disseminated microthrombi formation. These microthrombi may be found throughout the body, but they are seen most commonly in brain (especially cortical grey matter), kidney, pancreas, spleen, heart, and cortical glands. The hemolytic anemia is related to the red cell damage for the interaction with fibrin networks and microthrombi in the small vessels, this interaction produces the schistocytes evident on blood smear. Schistocytes have a short life span since the spleen rapidly removes them.

At the presentation of TTP, the neurologic symptoms are the most common, while, despite severe thrombocytopenia, the hemorrhagic problems are not remarkable. The neurologic symptoms include headache, cranial nerve palsies, paresis, dysphasia, aphasia, and confusion; these symptoms are transient but recurrent and, if the disease is not recognized, may progress shortly to stupor, seizures, and coma. Fever and the symptoms of a rapid-onset anemia are present in 50 % of the cases. Less common symptoms are abdominal pain (due to pancreatitis), acute respiratory distress symptoms, cardiac conduction abnormalities, and infarcts.

In addition to anemia and thrombocytopenia, the main laboratory findings are those of a hemolytic process, that is, elevated unconjugated bilirubin, undetectable haptoglobin, and very high level of LDH, usually more than 1,000 U/L. The LDH increase may be the expression of not only red cells' destruction but even of disseminated tissue damage.

The diagnosis of TTP is clinically easy since the presentation, at the same time, of neurologic symptoms associated with hemolytic anemia and thrombocytopenia is uncommon in other diseases. However, the presence of some comorbidities like preexisting neurologic problems or liver cirrhosis or other

diseases able to lower platelet levels, may confound the clinical picture. In these cases, there are no diagnostic tools to confirm the diagnosis of TTP outside blood smear examination for detecting schistocytes. At present, commercial kits to determine ADAMTS13 activity as well as the presence of anti-ADAMTS13 antibodies are available, but these tests are troublesome and cannot be used in an emergency since responses are delayed for weeks. Conversely, we need to confirm the clinical suspect of TTP as soon as possible, since the treatment should start immediately to avoid fatal neurologic complications. Therefore, the detection of schistocytes at the microscopic examination of blood smear remains the stronghold of the diagnosis.

The treatment of TTP is based on aggressive plasma exchange. If the treatment starts shortly after the diagnosis, the survival rate is more than 80 %. Before the introduction of this procedure, TTP was fatal in over 80 % of the cases within 3 months and only less than 10 % of the patients survived more than 12 months. Plasma exchange determines a favorable outcome even in the presence of renal failure or advanced neurologic complications. The infusion of large amount of fresh plasma, containing intact ADAMTS13, can be considered only as a temporary therapy in the case of delay of the plasma exchange. In fact, in a controlled prospective therapeutic trial comparing plasma infusion and plasma exchange, the latter demonstrated significantly better outcomes. The extraordinary effect of plasma exchange is due to the removal of anti-ADAMTS13 antibodies and of ultra-large vWF multimers together with the replacement of the fresh enzyme, able to cleave residual abnormal vWF multimers. The response is often dramatic; the neurologic complications disappear within a few hours and main laboratory alterations improve in a short time. The procedure should be performed daily until the platelet count is normal and hemolysis is minimal. Since the disease is due to autoantibodies, traditionally patients receive, in addition to plasma exchange, high-dose corticosteroids. Immunosuppressive

treatment seems more useful to prevent early relapse than to reduce the specific antibodies levels, thus increasing significantly the serum ADAMTS13 activity.

2.4 Hemolytic Uremic Syndrome

Hemolytic uremic syndrome (HUS) is a rare disease characterized by three primary symptoms: hemolytic anemia (with schistocytes), low platelet count, and acute renal failure. HUS is classified into two primary types: (1) HUS due to infections, often associated with diarrhea; and (2) HUS related to complement abnormalities—such HUS is also known as "atypical HUS" and is not diarrhea associated. The HUS associated to infection is common in children aged 1–5 years, at least in Europe and North America. The disease is due to a toxin (Shiga toxin) produced by some bacteria: *Escherichia coli* is the most commonly involved species; *Shigella Dysenteriae* type I and *Citrobacter freundii* have been less frequently observed. The toxin, produced in the gut, is absorbed and, in target organs (e.g., kidney and gut) it binds to glycolipid receptors on the cell surface, then the toxin is endocytosed and transported to the Golgi apparatus and the endoplasmic reticulum, it is later translocated to the cytosol where it inactivates ribosomes and causes cell death. HUS is a pediatric disease and diagnosis is relatively easy in cases of typical presentation with watery diarrhea, followed by bloody diarrhea and abdominal cramps. In the following days, the symptoms are related to severe anemia, hemolysis, and renal failure. The diagnosis of the atypical HUS, or not-infectious HUS, is much more complicated since the diarrhea is absent and a trigger of the disease cannot be found. The atypical HUS is a very rare event (0.2 cases/100,000/year) and more than 70 % of cases are in pediatric age, since the disease is related to inherited abnormalities of some complement factors or of the

cobalamin metabolism. In children, the age of onset, family history, and clinical presentation are useful for a correct differential diagnosis, while in adults, autoimmune diseases, pregnancy, transplantation, and drugs are causes of atypical HUS. In adult patients with thrombocytopenia and hemolysis presenting renal involvement, the presence of atypical HUS should be suspected and the blood smear examination for schistocytes is mandatory. The diagnosis might be performed as soon as possible since a prompt treatment avoids the progression of the renal failure. The treatment is the fresh frozen plasma infusions, and, when disease activity is not controlled, the plasma exchange should be performed. Prophylactic antibiotics should be administered because infections can trigger relapse.

2.5 Disseminated Intravascular Coagulation (DIC)

This disease is a relatively common problem in the intensive care units, and DIC still remains a diagnostic and therapeutic challenge. The clinical features of DIC are bleeding manifestations, often very serious and of abrupt onset, therefore, the anemia is due more easily to hemorrhages than to hemolysis. However, clinicians should be aware of the possibility of the presence of a hemolytic process proportional to the severity of the coagulation abnormalities, this to avoid unnecessary tests and/or treatment delay. The prognosis of DIC depends on its etiology and on the possibility to remove or to treat the trigger of the process; the most common causes of DIC are reported in As shown, some hematological diseases can determine DIC, and, in rare cases the beginning of the disease could be a DIC.

Among these cases, the promyelocytic leukemia is the most common: a sudden and severe bleeding often of serious magnitude

can be the onset of the disease. To recognize immediately the presence of this leukemia is very important since the treatment must start immediately and, consequently, the prognosis is very good with a predicted long-term survival of more than 90 % even avoiding chemotherapy. In these cases, together with the clinical and laboratory signs of the DIC, abnormal leukocyte count with immature cells are present on peripheral blood, therefore the diagnosis is easy. The age-adjusted incidence rate of acute myeloid leukemia (AML) in adults is about 3.7 per 100,000/year for both sexes, and promyelocytic leukemia represents the 10–5 % of all AML, therefore promyelocytic leukemia has an incidence ranging from 0.4 to 0.2 cases/100,000/year.

Even rarer is the paroxysmal nocturnal hemoglobinuria (PNH) whose incidence is still unknown, but the data collection from different sources has given quotes of about 0.5 cases/100,000/year. Since the disease is underdiagnosed, its incidence may be higher. This disease, due to the mutations of the gene PIG-A placed on X chromosome, shows a complex pathogenesis and it may present mild hemolytic anemia associated with recurrent hemoglobinuria, mainly during the night, or the features of an aplastic anemia, and finally a thrombotic syndrome. In rare cases, the onset of the disease is a severe hemolytic episode; these attacks are associated with general malaise, fever, headache, and abdominal and lumbar pains. Since in PNH the hemolysis is intravascular, a massive hemolytic episode is able to activate coagulation cascade and to initiate the DIC. In these very rare cases, the diagnosis is particularly difficult: only the more or less massive hemoglobinuria may suggest PNH, while the other laboratory features of the disease are not specific. In addition, the diagnosis underlies on the demonstration of the lack of CD59 and CD55 expression on red cells, granulocytes, and platelets on flow cytometry, not available in any hospital.

Sickle cell anemia is an inherited disease due to the substitution of a single mutation (GAG vs. GTB) in the sixth codon of

the β gene; this determines a substitution of valine instead of glutamine in the sixth position of the β chain in the Hb (HbS). This also determines a decrease of the Hb solubility of Hb when deoxygenated with formation of HbS polymers inside red cells and subsequent erythrocyte deformation. These "sickled" erythrocytes have poor deformability and patients develop a diffuse veno-occlusive disease and, consequently, with acute events like painful crisis; stroke; acute chest syndromes; priapism; and chronic organ damage, especially bones and joints, cardiovascular system, kidney, pulmonary system, liver, and eyes. The disease has its highest prevalence in tropical Africa; in several countries about 45 % of the population has sickle trait. In USA, about 8 % of Afro-Americans are carriers of the sickle gene. In Europe, sickle cell anemia is present only in the countries of the Mediterranean basin (Italy, Greek) with a very low incidence. In some cases, even in carriers of the trait only, life-threatening hyper-hemolytic crisis may occur with abrupt anemia; the massive hemolysis (like in PNH) is able to activate the coagulation and a DIC may appear. The hyper-hemolytic crisis may be triggered by infections or by exposition to cold temperature and by strenuous physical exercise. The diagnosis of sickle cell anemia should be suspected in any African or Afro-American patient, and, since there are an increasing number of immigrants in any European country, the doctors, especially those working in intensive care units, must be able to recognize the disease. The diagnosis is straightforward since a simple electrophoresis using cellulose acetate is rapid, inexpensive, and effective to separate the normal Hb from variants and the method is available in any hospital. The presence of other inherited Hb diseases, like β-thalassemia, may complicate the diagnostic path and additional tests are required like isoelectric focusing or HPLC. However, the presence of HbS on electrophoresis must be considered as the hallmark of the disease, also if other Hb electrophoretic abnormalities are found. Therefore, in addition to the therapy of the DIC, in patients with hyper-hemolytic crisis

other therapeutic measures are indicated such as red cell transfusion, hyper-oxygenation, and blood alkalization to avoid further HbS polymerization.

Suggested Reading

1. Eisenstaedt R, Pennix BWJH, Woodman RC (2006) Anemia in the elderly: current understanding and emerging concepts. Blood Rev 20:213–226
2. Hayden SJ, Albert TJ, Watkins TR, Swenson ER (2012) Anemia in critical illness. Insights into etiology, consequences, and management. Am J Respir Crit Care Med 185(10):1049–1057
3. McLellan SA, McClelland DBL, Walsh TS (2003) Anaemia and red blood cell transfusion in the critically ill patient. Blood Rev 17(4):195–208
4. Scharte M, Fink MP (2003) Red blood cell physiology in critical illness. Crit Care Med 31(12 Suppl):S651–S657
5. Wickramasinghe SN (2006) Diagnosis of megaloblastic anaemias. Blood Rev 20(6):299–318
6. Young NS, Calado RT, Scheinberg P (2006) Current concepts in the pathophysiology and treatment of aplastic anaemia. Blood 108(8): 2509–2519

Chapter 3
Anemia in the Critically Ill Patient

Giorgio Berlot and Perla Rossini

Clinical Vignette

Mr. T. was a 72-year-old man admitted to the ED with chest pain and initial pulmonary edema. Two years before he suffered a postero-inferior acute myocardial infarction treated with the positioning of two bare metal stents. From then on, the patient was treated with aspirin and clopidogrel and did not report any symptom attributable to the underlying coronary artery disease. Only in the last few days preceding the current admission, he complained of worsening fatigue associated with shortness of breath. The history revealed a cerebellar stroke occurred at the age of 48 years, a type 2 diabetes mellitus treated with metformin, a mild chronic renal failure (serum creatinine: 1.6 mg/dl), and a Hashimoto's thyroiditis treated with thyroxin. The symptoms resolved quickly with nitrates and diuretics and the patient was transferred to the coronary care unit. The blood chemistries were normal apart from transient mild elevation

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of troponin and BNP and a macrocytic anemia (Hb = 8.5 g/dl, MCV = 110 fL), which likely accounted for the cardiac symptoms; the reticulocytes were also abnormally elevated. In the following days, the anemia worsened despite multiple transfusions of packed red blood cells given in the following days aiming at achieving an Hb value of at least 10 g/dl. Initially, the cause of anemia was attributed to different factors, including (a) a gastroenteric chronic blood loss favored by the dual anti-aggregating agents; (b) a possible gastrointestinal neoplasm; and (c) the failed adsorption of the adsorption of Vitamin B12 and folate due to the presence of autoantibodies directed against the gastric ells, as often reported in patients with Hashimoto's thyroiditis. However, serum values of Vitamin B12, folate, and Fe were normal and the endoscopies of the digestive tract did not demonstrate any source of bleeding; also a biopsy of the gastric mucosa resulted normal. As either serum LDH was raised and haptoglobin was decreased, a hemolytic anemia was suspected, which was confirmed by the detection of cold hemoagglutinins belonging to the IgM class; a bone marrow biopsy was then obtained, which demonstrated a non-Hodgkin's lymphoma. The patient was subsequently transferred to the Dept of Hematology where an appropriate chemotherapy was carried on. Two years after the described episode, the patient is free of symptoms, his Hb values are normal, and is conducting a regular life.

3.1 Introduction

The patient just described represents a typical case of symptomatic and potentially dangerous anemia which had been initially attributed to a number of causes before arriving at a correct

diagnosis, with the subsequent potential risks for patients, which fortunately did not occur in his case. Actually, anemia, which is defined by the World Health Organization as a hemoglobin (Hb) concentration <10 g/dl and/or a hematocrit value (Ht) <30 % [1, 2], occurs frequently among critically ill patients admitted to the Intensive Care Unit (ICU). Indeed, roughly 70 % of them are anemic since the beginning and almost 100 % develop anemia during the first week after the ICU admission [3, 4]. In many circumstances, the source of anemia can be obvious (trauma, rupture of major vessels, etc.), but in other cases the underlying disorder(s) can be more elusive, thus requesting a diagnostic workup which could result unfamiliar even to an experienced intensivist. To further complicate the issue, both Hb and Ht can be influenced by factors other than the mere production and loss of red blood cells (RBC), including wide volume shifts between intravascular and extravascular compartments due to the alteration of the endothelial wall permeability, the administration of large amounts of fluids and the transfusion of blood and derivates [1].

The aims of this chapter are (a) to review the main causes leading to the occurrence of anemia in critically ill patients; (b) to provide some clues for the diagnosis, starting from some basic but fundamental variables related to the RBC (Table 3.1); and, perhaps more importantly, (c) to distinguish between forms amenable by the intensivists alone and others which require a more in-depth hematological competence.

3.2 The Kinetics of Red Blood Cells

RBC are generated in the bone marrow under the influence of erythropoietin (EPO) and their production requires a number of factors, including zinc, iron (Fe), vitamin B_{12}, folate, tyrosine, androgen hormones, and cortisol [4]; the basal release of RBC is

Table 3.1 Some biological variables used in the diagnosis of anemias. All values refer to adult patients

Variable (adults)	Normal values
Red blood cells count (/ml)	Male $4.5–5.9 \times 10^6$ Female $3.5–5.0 \times 10^6$
Hematocrit (%)	Male: 39–49 Female: 33–43
Blood hemoglobin level (Hb)	Male: 13–17 g/dl Female: 13–15 g/dl
Reticulocyte count (%)	0.8–2.5
Mean corpuscolar volume (MCV)	85–100 fL
Mean corpuscolar Hb concentration (MCHC)	31–35 g/dl
Mean corpuscolar Hb (MCH)	28–33 pg/cell
Serum transferrin	200–300 mg/dl
Serum Fe	75–160 mcg/ml (m) 60–150 mcg/ml (f)
Serum Ferritin	20–300 ng/ml (m) 20–120 ng/ml (f)
Serum haptoglobin	50–220 mg/dl
Vitamin B12	200–1,000 pg/ml
Folate	2–10 ng/ml

15–20 ml/day but this rate can decuplicate during acute anemia provided that the iron stores are repleted and in the presence of a normal renal function [5]. As the mature RBC are devoid of mitochondria as well as of intrinsic reparatory mechanisms, their ageing-related decrease of energy levels is associated with changes of the membrane properties, including the reduction of their fluidity and deformability and the increase of density and viscosity; all these changes ultimately lead to their removal from circulation and destruction in the spleen and in the reticuloendothelial system (RES) [6–8]. In normal conditions, the overall life span of RBC is 120 days. Other processes responsible for their anticipated elimination from the bloodstream include the premature death of mature RBC (eryptosis) and the removal of RBC

just released from the bone marrow (neocytolisis). Both mechanisms are responsible for the maintenance of an appropriate circulating mass of RBC and are inhibited by EPO [5].

3.3 The Physiological Consequence of Acute Anemia

Basically, Hb plays a dual role. First, as RBC carry O_2 from the lungs to the cells, according to the formula:

$$\text{Oxygen delivery}\left(DO_2\right)=\text{Arterial}\,O_2\,\text{content}\left(CaO_2\right)\times\text{Cardiac output}\left(CO\right)$$

it appears that a reduction of the CaO_2, which is mainly determined by the total Hb and its O_2 saturation (SaO_2), being negligible, the amount of O_2 dissolved in the plasma in normobaric conditions sets the stage for a reduced O_2 availability to the tissues with the subsequent onset of anaerobic metabolism [9, 10]. Second, since Hb scavenges CO_2 from the cells to the lungs, its drop is associated with the increase of the tissue CO_2 content. In a resting healthy organism, a number of mechanisms can counterbalance an acute isovolemic reduction of Hb to as low as 5 g/dl [11]; these include (a) the leftward shift of the Hb dissociation curve determining a facilitated download of O_2 toward the cells, leading to an increased extraction of O_2 (O_{2ER}); (b) a compensatory tachycardia and tachipnea; and (c) the concomitant increase of the heart rate (HR), stroke volume (SV), and CO driven by the hypoxia-induced increased production of catecholamines. However, if the anemia aggravates and/or in the presence of concomitant limited cardiac and respiratory reserves, all these adaptative mechanisms become exhausted and tissue respiratory and metabolic acidosis ensue due to

the contemporaneal increase of CO_2 and of the lactate produced under anaerobic conditions [4, 9, 12]. Moreover, it has become clear that the tissues are not equally vulnerable to a reduced O_2 availability and marked differences exist in terms of O_{2ER} capabilities among different organs and sometimes also within the same organ [12].

3.4 Causes of Anemia in the Critically Ill Patient

Similarly to other fields of medicine, either a reduced production of RBC and/or a decrease of their life span account for the occurrence of anemia in critically ill patients [4]; actually, both mechanisms can act simultaneously in many conditions commonly encountered in the ICU independently from the cause of admission, including advanced age, poor nutritional condition, recent surgical procedures, unresolved inflammatory conditions, etc. In these circumstances, a severe anemia can develop in 1 week from the onset of the disease, requiring the ICU admission [3].

3.4.1 Anemia Due to a Reduced Production of RBC

Albeit a reduced production of Hb can be caused by several factors, those more frequently encountered among patients admitted to the ICU include:

(a) Persisting inflammatory conditions, not only determined by chronic conditions such as neoplasms, vasculitides, and rheumatologic conditions but also by unresolving sepsis, post-operative states, etc. [13]. These conditions appear particularly relevant as more and more elderly subjects

survive the initial insult determining their admission to the ICU, only to become chronic critically ill patients who cannot be weaned from the mechanical ventilation [1]. In these circumstances, several inflammatory and counter-inflammatory mediators produced during either the initial or the more advanced phase of their disease, including Tumor Necrosis Factors-α (TNF- α) and Interleukin-1 (Il-1) and Il-6, negatively reduce Fe metabolism and impair the feedback existing between its enteric adsorption and the body stores [4]; this latter phenomenon is aggravated by an increased production of hepcidin, a protein synthetized in the liver, which also inhibits the release of the Fe stored into the RES. At the same time, since the production of EPO is inappropriately reduced even in the presence of abnormally low values of Hb and the number of its receptors on the target cells is decreased, the response of the bone marrow is blunted.

In the aforementioned circumstances and in the absence of other confounding factors, the anemia is mild to moderate, with Hb > than 8 g/dl, and with a normal mean cell volume (MCV) and mean cell hemoglobin (MCH) [4].

(b) Fe deficiency, caused by blood loss or inadequate dietary intake. Actually, Fe-deficiency anemia is rather common, having been reported in as many as 9 % of ICU patients [14]. The classic signs of iron deficiency anemia can be difficult to evaluate in ICU patients, and the diagnosis is based on the biochemical markers of the iron metabolism (Table 3.2). As in these patients factors other than iron deficiency can determine hyposideremia, other markers must be suited to confirm the diagnosis, including:

(i) Serum ferritin: Even if its value increases in the presence of sepsis and severe infections as it is an acute-phase reactant, low values are suggestive of iron deficiency.

Table 3.2 Differential features of anemia of chronic disease and iron deficiency anemia

Variable	Anemia of chronic disorders	Iron deficiency anemia
Hb	May be ≥8 g/dl	May be ≤8 g/dl
MCV	Normal/↓	↓
MCH	Normal/↓	↓
Serum Fe	↓	↓/↓↓
Serum Ferritin	Normal/↑	↓
Serum hepcidin	↑	↓
Reticulocytes	↓	↓

(ii) Serum transferrin and total serum binding capacity, which are increased during iron deficiency; in the same condition, the transferring saturation is reduced. However, it should be noted that other factors, including alcohol, neoplasm, and inflammatory conditions can decrease the sensibility and sensitivity of these markers.

(iii) RBC zinc protoporphyrin, whose values increase during iron deficiency and is not affected by a concomitant inflammatory state.

Hematological features of anemia associated with Fe-deficiency anemia include Hb values <8 g/dl and reduced MCV and MCH [14].

(c) Vitamin B12 and folate deficiencies have been reported in 2 % of ICU patients and are associated with a reversible failure of the bone marrow causing the disturbed synthesis of DNA and megaloblastic hematopoiesis, leading to the release of RBC larger than normal [15]. In case of isolated Vitamin B12 deficiency, a demyelinating disease of the nervous system can coexist or anticipate the onset of anemia [16]. Since the enteral adsorption of Vitamin B12 requires the action of the Intrinsic Factor (IF), which is synthetized by the gastric parietal cells and of another receptor located in the distal ileum, conditions associated with gastric or enteric mucosal disuse or atrophy, extensive gastric and/or

ileal resections can determine the occurrence of Vitamin B12 maladsorption and subsequent deficiency [16]. Moreover, H_2 receptor antagonists, proton pump inhibitors can impair the absorption of both Fe and Vitamin B12 [17–19]. The diagnosis of Vitamin B12 deficiency can be elusive, because (a) as many as 15–25 % of patients have normal Hb and MCV [20]; and (b) the affected patients admitted to the ICU cannot report the symptoms associated with the neuropathy. However, the diagnosis should be suspected in the presence of a progressive reduction of Hb and contemporaneal increase in MCV. The diagnostic workup of Vitamin B12 and folate deficiency-related anemia should include the following [16, 20]:

(i) The measurement of blood Vitamin B12 levels should be interpreted with caution, because, with the exclusion of extremely low values (<100 pg/ml) which are diagnostic, both false negative and false positive results have been reported, which have been ascribed to protein carrier other than cobalamin.
(ii) The measurement of serum methylmalonic acid and total homocysteine levels are useful in patients with suspected Vitamin B12 deficiency who have not been treated yet; the values of these markers are markedly elevated even before the appearance of anemia and sharply decrease after the initiation of Vitamin B12 supplementation.
(iii) The measurements of serum and RBC folate levels, which reflects the folate intake in the last 3 months and the measurement of serum methylmalonic acid and total homocysteine levels.

(d) Acquired Aplastic Anemia (AA) is characterized by pancytopenia and bone marrow aplasia determined by an autoimmunitary process mediated either by cytotoxic T lymphocytes directed against hematopoietic stem cells and/or by

antibodies directed against kinectin, which is a polypeptide expressed on the surface of human hemopoietic cells as well as of other organs, including the liver, the ovary, testis, and brain cells. The onset of AA can be abrupt and the presentation symptoms can resemble those commonly encountered in septic shock patients. In these circumstances, a thorough medical history is mandatory and a particular attention should directed on the possible assumption of drugs known to cause myelotoxicity [21–23]

3.4.2 Anemia Due to a Loss of RBC

This disorder occurs frequently in ICU patients. In some cases the source of hemorrhage can be rapidly identified and treated, but in other circumstances, and especially when the blood loss occurs subacutely, it can go unnoticed for a long time and the cause(s) can be difficult to recognize.

(a) Repeated blood samples represent a source of blood loss which is largely underrecognized. Actually, as many as 40–70 ml can be withdrawn each day from critically ill patients for various purposes, including blood chemistries, blood gas analysis and culture, etc. [3]. This amount exceeds the normal replacement rate in healthy individuals. Not surprisingly, it is likely that the amount of blood sent to the lab could be somewhat related to the severity of the underlying conditions, being larger in patients with more unstable conditions. Paradoxically, the volume of blood really processed for the required investigations is roughly 2 % of that sampled.

(b) Hemophagocytic Lymphohiatiocytosis (HLH) is characterized by fever, peripheral lymph nodes enlargement, pancytopenia, and splenomegaly possibly associated with the rapidly deteriorating function of multiple organs

ultimately leading to a multiple organ dysfunction syndrome (MODS). The cause of HLH is an inappropriate activation of macrophages which become engulfed with RBC, leukocytes and platelets occurring isolated, or in association with a number of pathologic conditions, including bacterial, viral, and fungal infections, hematological and solid malignancies, and systemic and rheumatologic diseases. The clinical picture of rapid deteriorating MODS is generally ascribed to a severe sepsis or septic shock and treated consequently. The diagnosis requires a wide array of clinical and laboratory investigations and must be confirmed by the microscopical examination of the bone marrow [6, 24].

(c) Autoimmune hemolytic anemia (AHA) is a relatively uncommon disorder due to the action of different categories of autoantibodies against mature circulating RBC. Albeit in many cases the clinical course is chronic, in some cases the onset of AHA can be abrupt and is associated with a dramatic drop of Hb levels, hyperbilirubinemia, and hemoglobinuria. Both, the site of hemolysis and the related symptoms can vary according to the class of autoantibodies involved: IgG-coated RBC are destroyed by the RES primarily in the spleen, liver, and bone marrow (extravascular hemolysis), whereas IgM-coated RBC are lysed into the bloodstream (intravascular hemolysis) after the activation of the complement [25]. Basically, although mixed forms exist, AHA can be subdivided (a) according to the body temperature level at which hemolysis occurs; and (b) by the absence (primary forms) or presence (secondary forms) of underlying disorders leading to the production of autoantibodies of the different classes. The secondary forms of AHA are mainly associated with lymphomas and systemic disease such as lupus erythematosus. According to their characteristics, the autoantibodies causing AHA can be subdivided as follows:

(i) Warm autoantibodies (WA), usually belonging to the IgG class, determine the RBC destruction after their binding on some receptors on the cell surface; the autoantibody-coated RBC are then eliminated mainly into the spleen and for a lesser extent by the Kupffer cells. Trapped RBC can partially escape the destruction and are released back in the bloodstream: however, this process is associated with the partial loss of the cell membrane and the subsequent change of the RBC shape which become spherical, more rigid, and less deformable than normal RBC. This cycle continues with the ongoing fragmentation and destruction at every passage through the liver and the spleen until their complete destruction occurs. Actually, although several types of WA can activate the complement, the occurrence of intravascular lysis is uncommon. The clinical suspicion arises in the presence of unexplained anemia, reticulocytosis of various degree and unconjugated hyperbilubinemia, and the final diagnosis requires the identification of antibodies and/or complement of the BRC surface, usually with the Coombs' direct test [25].

(ii) Cold autoantibodies (CA), belonging primarily to the IgM-class, although either IgG or IgA can be occasionally involved. The responsible autoantibody is detected in vitro as it causes hemolysis of circulating RBC with a double-step process, requiring an initial incubation in the cold, followed by another incubation at 37 °C. The symptoms are caused by the cooling of blood flowing through the acral parts of the circulation, allowing CA to bind the epitopes on the RBC surface, thus causing their agglutination and the activation of the complement via the classical pathway. Once returned in the warm core compartment, the CA detaches from the RBC, but the C3b fragment of the complement remains bound, allowing their uptake in the hepatic RES cells. Moreover, complement activation

can proceed until the production of the C5, which attacks the RBC membrane causing also an intravascular hemolysis. The laboratory diagnosis is based on the presence of anemia, by the autoagglutination of blood samples kept at room temperature for 30–60 min and on the positivity of the direct Coombs' test using an anti-C3 serum in association with its negativity when the patient's RBC are challenged with an anti-IgG serum. Albeit less frequent than the WA-AHA, the widespread use of therapeutic hypothermia after cardiac arrest and of norepinephrine in the treatment of septic shock could represent a trigger factor in predisposed patients [26].

3.5 Conclusions

The occurrence of anemia in the critically ill patient is so common to be considered almost normal, thus preventing a more in-depth analysis of its origin. In many cases, the cause(s) are evident and/or easy to detect, but in some circumstances an apparently mild and asymptomatic anemia can represent the tip of an iceberg constituted by severe systemic conditions, including AHA, leukemias, etc., that the intensivist could be not accustomed to deal with. A high index of suspicion is warranted especially in those patients who remain anemic despite multiple transfusions and in whom an appropriated diagnostic workup failed to demonstrate any accountable source of bleeding.

References

1. Eisenstaedt R, Pennix BW, Woodman RC (2006) Anemia in the elderly: current understanding and emerging concepts. Blood Rev 20:213–226

2. Nandigam V, Nandigam K, Badhe BA, Dutta TK (2004) Is adult definition of anemia applicable to a geriatric population? Study of erythrocyte parameters in Indian geriatric inpatients. J Am Geriatric Soc 52: 1589–1590
3. Hayden SJ, Albert TJ, Watkins TR, Swenson ER (2012) Anemia in critical illness. Insights into etiology, consequences, and management. Am J Respir Crit Care Med 185(10):1049–1057
4. Davis SL, Littlewood TJ (2012) The investigation and treatment of secondary anaemia. Blood Rev 26(2):65–71
5. Scharte M, Fink MP (2003) Red blood cell physiology in critical illness. Crit Care Med 31(12 Suppl):S651–S657
6. Creput C, Galicier L, Buyse S, Azoulay E (2008) Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. Intensive Care Med 34:1177–1187
7. Aouba A, Lambotte O, Vasiliu V et al (2004) Hemophagocytic syndrome as a presenting sign of transformation of smoldering to acute adult T-cell leukemia/lymphoma: efficacy of antiretroviral and interferon therapy. Am J Hematol 76:187–189
8. Srichaikul T, Punyagupta S, Mongkolsritrakul W, Jidpugdeebodin S (2004) EBV and the hemophagocytic syndrome: analysis of 3 cases, with speculation on clinical features, therapy and role of EBV. J Med Assoc Thai 87:974–983
9. McLellan SA, McClelland DBL, Walsh TS (2003) Anaemia and red blood cell transfusion in the critically ill patient. Blood Rev 17(4): 195–208
10. Hameed SM, Aird WC, Cohn SM (2003) Oxygen delivery. Crit Care Med 31(12 Suppl):S658–S667
11. Rizzo JD, Brouwers M, Hurley P, Seidenfeld J, Arcasoy MO, Spivak JL et al (2010) American Society of Clinical Oncology/American Society of Hematology clinical practice guideline update on the use of epoetin and darbepoetin in adult patients with cancer. J Clin Oncol 20:4083–4107
12. Vincent JL, De Backer D (2013) Circulatory shock. N Engl J Med 369(18):1726–1734
13. Nathan C, Ding A (2010) Nonresolving Inflammation. Cell 140:871–882
14. Pieracci FM, Barie PS (2006) Diagnosis and management of iron-related anemias in critical illness. Crit Care Med 34(7):1898–1905
15. Rodriguez RM, Corwin HL, Gettinger A, Corwin MJ, Gubler D, Pearl RG (2001) Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. J Crit Care 16:36–41
16. Stabler SP (2013) Vitamin B12 deficiency. N Engl J Med 368(2): 149–160

17. Ito T, Jensen TR (2010) Association of long-term proton pump inhibitor therapy with bone fractures and effects on absorption of calcium, vitamin B12, iron, and magnesium. Curr Gastroenterol Rep 12:448–457
18. McColl KEL (2009) Effect of proton pump inhibitors on vitamins and iron. Am J Gastroenterol 104:S5–S9
19. Valuck RJ, Ruscin JM (2004) A case-control study on adverse effects: H2 blocker or proton pump inhibitor use and risk of vitamin B12 deficiency in older adults. J Clin Epi 57:422–428
20. Wickramasinghe SN (2006) Diagnosis of megaloblastic anaemias. Blood Rev 20(6):299–318
21. Marsh JCW (2005) Management of acquired aplastic anaemia. Blood Rev 19:143–151
22. Brodsky RA, Jones RJ (2005) Aplastic anaemia. Lancet 365: 1647–1656
23. Young NS, Calado RT, Scheinberg P (2006) Current concepts in the pathophysiology and treatment of aplastic anaemia. Blood 108(8):2509–2519
24. Gauvin F, Toledano B, Champagne J, Lacroix J (2000) Reactive hemophagocytic syndrome presenting as a component of multiple organ dysfunction syndrome. Crit Care Med 28(9):3341–3345
25. Packman CH (2007) Hemolytic anaemia due to warm autoantibodies. Blood Rev 22(1):17–31
26. Petz LD (2007) Cold antibody autoimmune hemolytic anaemias. Blood Rev 22(1):1–15

Chapter 4
Leukopenia in the Critically Ill Patient

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4.1 Introduction

White blood cells (WBC) count includes neutrophils, basophils, eosinophils, macrophages, monocytes, and lymphocytes. White blood cells are the major components of the immune system.

Neutrophils, monocytes, and macrophages act directly to contrast infection by phagocytosis and other mechanisms, including the production and release of substance with antibacterial properties, free oxygen radicals, etc. On the other hand, lymphocytes act against microbial agents pathogens through production of cytokines and antibodies. The normal white cells count in adults is 4,000–11,000 cells/ml; neutrophils are from 45 to 75 %, band cells (immature neutrophils) are from 0 to 4 %, lymphocytes are from 16 to 45 %, eosinophils are from 0 to 4 %, and basophils are from 0 to 2 %. Leukopenia is defined as a concentration of WBC below 4,000 per ml. Leukopenia

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usually refers to neutropenia and/or lymphopenia [1, 2]. Other terms used to describe decreased number of WBC are granulocytopenia, referring to reduced number of neutrophils, eosinophils, and basophils; and agranulocytosis, referring to complete absence of neutrophils, eosinophils, and basophils.

4.2 Neutropenia

4.2.1 Pathophysiologic Mechanism of Neutropenia

Neutropenia is an abnormally low number of circulating neutrophils, which is based on a calculated number from a complete blood count. Neutropenia can be classified as mild (1,000–1,500 cells/ml), moderate (500–1,000 cells/ml), and severe (<500 cells/ml). Some racial and ethnic groups, such as Africans, African-Americans, and Yemenite Jews, have lower mean neutrophil counts than people of Asian or European ancestry. The mean differences in neutrophils are modest and have no recognized health consequences.

The bone marrow is the principal production center for WBCs, red blood cells, and platelets. Initially, a multipotent hematopoietic stem cell undergoes differentiation and proliferation. Under the influence of interleukin-3 and granulocytes-macrophage colony stimulating factor, the blast cell differentiates through the following stages: promyelocyte, myelocyte, metamyelocyte, band cell, and finally into the polymorphonuclear neutrophil. After being produced by the bone marrow, neutrophils are distributed into a circulating (only ~3 % of total neutrophils) and marginating granulocytic pool. In the peripheral circulation, neutrophils are active and survive for approximately 6 h. When an infection arises, neutrophils travel to and act at the site of infection, destroying bacteria principally by endocytosis.

Severe neutropenia is a predisposing factor for infections. The risk of infections is inversely related to the severity of neutropenia. Patients with neutrophil count less than 500/ml are at substantially greater risk, but the actual rate of infections varies considerably, depending on the cause and duration of neutropenia. Severe acute neutropenia (i.e., developing over a few hours or days) usually is associated with greater risk of infection than severe chronic neutropenia (usually present for months or years). Neutropenia resulting from disorder of production that affects early hematopoietic precursor cells (aplastic anemia, severe congenital neutropenia) leads to greater susceptibility to infections than do conditions with adequate neutrophil precursors in the marrow and neutropenia attributed to accelerated turnover in the blood (i.e., rheumatoid arthritis, Felty Syndrome, autoimmune neutropenia). For severely neutropenic patients after cancer chemotherapy, the risk is greater when the neutrophils are decreasing than, at similar count, when they are increasing. Neutropenia combined with monocytopenia, lymphocytopenia, or hypogammaglobulinemia is more harmful than isolated neutropenia. Other factors, including the integrity of the skin and mucous membranes, the vascular supply to tissues, and the nutritional state of the patient, influence the risk of infection. In critically ill patients with neutropenia, special attention should be paid to indwelling devices (Foley catheter, central venous catheters, etc.) as colonizing bacteria may easily cross mucosal surfaces and invade the bloodstream.

4.2.2 Causes of Neutropenia in the Critical Ill Patient

In adult patients admitted to the Intensive Care Unit (ICU), neutropenia has several etiologies that can be classified into primary (i.e., congenital) or secondary neutropenia, the latter largely exceeding the former.

(a) *Ineffective granulopoiesis* may be due to suppression of myeloid stem cells or committed granulocytic precursors. Common etiologies are chemotherapy and immunosuppressive agents, megaloblatic anemia, aplastic anemia, leukemias, and lymphomas. ICU admission of cancer patient was considered futile until the 1990s when studies showed very low survival rates in cancer patients requiring life-sustaining therapies. Since then, survival is increased in critically ill cancer patients overall. The impact of neutropenia is still a matter of debate since some studies found unacceptably high mortality rate, whereas others found no evidence that neutropenia affected mortality in cancer patients in the ICU [3].

(b) *Accelerated destruction or increased utilization* may result from infections or immunologically mediated injury (Felty Syndrome, rheumatoid arthritis, and systemic lupus erytematosus). Neutropenia can result from acute or chronic bacterial, parasitic, and viral diseases. Certain viral infections, such as infectious mononucleosis, infectious hepatitis, Parvovirus B19, and HIV infection may cause severe and protracted neutropenia and pancytopenia resulting from infection of hematopoietic precursor cells. Other agents, such as Rickettsia and Bartonella, can infect endothelial cells. These agents may cause leukopenia, neutropenia, thrombocytopenia, and anemia as part of a generalized vasculitic process. Increased neutrophil adherence to altered endothelial cell may occur in dengue, measles, and other viral infections. With severe gram-negative bacterial infections, neutropenia probably results from increased adhesion to the endothelial cells and increased consumption at the side of infection. Some chronic infections causing splenomegaly, such as tubercolosis, brucellosis, thyphoid fever, malaria, and kala azar, probably cause neutropenia because of splenic sequestration and marrow suppression.

The most frequent scenario in ICU is that of leukopenia occurring in patient with sepsis or sepsis-associated

conditions; indeed the Surviving Sepsis Campaign Guidelines consider leukopenia as a marker of these conditions [4].

(c) *Autoimmune neutropenia* is caused by the action of autoantibodies directed against some epitopes located on their cell surface leading to their destruction. Primary autoimmune neutropenia occur as isolated clinical entities especially in infancy. Secondary autoimmune neutropenia is more common and set against a background of connective tissue disease, such as primary Sjogren's Syndrome, Systemic Lupus Erythematosus, Primary Biliary Cirrhosis, and Rheumatoid Arthritis, or complicating hematological neoplasm, such as large granular lymphocytic leukemia [5].

(d) *Drug-induced neutropenia* has an estimated annual frequency of 2.4–15.4 case per million population [6, 7] and many classes of drugs are responsible for its occurrence (Table 4.1). Drug-related neutropenia can be subdivided into two different classes according to the underlying mechanisms. The first is dose-related toxicity resulting from the interference of the drug with the cell protein synthesis and/or replication. It can involve the pluripotent hematopoietic stem cells and highly proliferative cells in other organs. Prototype drugs for this type of reaction include phenothiazine, antithyroid drugs, chloramphenicol, and clozapine [8]. The second type is not dose-related and is caused by an immunologic reaction against some epitopes located in the stem-cells of more mature cells. Neutropenia may occur at any time but tends to occur relatively early in the course of treatment with drugs to which the patient has been previously exposed. Many drugs can trigger this form of neutropenia. The management of drug-induced neutropenia starts with the immediate withdrawal of any potential causative drug. The patient medical history must be carefully obtained in order to identify the suspected agent. Measures to be undertaken concomitantly include the aggressive treatment of possible concomitant infections as well as the prevention of secondary infections.

Table 4.1 Drugs that can cause neutropenia

Heavy metals	Arsenic compounds, gold, mercurial diuretics
Analgesics and NSAID	Acid acetylsalicylic, aminopyrine, benoxaprofen, diclofenac, diflunisal, dipyrone, fenoprofen, indo methacin,ibuprofen,phenylbutazone, piroxicam, sulindac, tenoxicam, tolmetin
Antipsychotics, sedatives, antidepressants	Amoxapine, chlordiazepoxide, clozapine, diazepam, haloperidol, imipramines, meprobamate, phenothiazines, risperidone, tiapridal, upstene
Anticonvulsants	Carbamazepine, ethosuximide, phenytoins, trimethadione, valproic acid
Antithyroid drugs	Carbimazole, methimazole, potassium perchlorate, thiocyanate, thiouracils
Cardiovascular drugs	Acid acetylsalicylic, aprindine, captopril, flurbiprofen, furosemid, hydralazine, methyldopa, nifedipine, phenindione, procainamides, propafenone, propanolol, quinidine, spironolactone, thiazide diuretics, ticlopidine, zestril
Antimicrobials	Cephalosporins, chloramphenicol, ciprofloxacin, clindamycin, cyclines, ethambutol, fasigyne, gentamicin, isoniazid, lincomycin, metronidazole, nitrofurantoin, novobiocin, penicillins, rifampicin,sulfamethoxazole, streptomycin, thiacetazone, vancomycin; Chloroquine, flucytosine, dapsone, hydroxychloroquine, levamizole, mebendazole, pyrimethamine, quinine, quinacrine; Acyclovir, zidovudine, terbinafine
Antihistamines	Brompheniramine, chloropheniramine, cimetidine, methaphenilene, mianserin ranitidine, tripelennamine, thenalidine
Miscellaneous drugs	Acetazolamide, allopurinol, aminoglutethimide, benzafibrate, colchicine, famotidine, flutamide, methazolamide, metoclopramide, levodopa, oral hypoglycemic agents (glibenclamid), penicillamine, retinoic acid, most sulfamides, tamoxifen, deferiprone

Symptomatic patient with drug-induced neutropenia usually present with fever, systemic symptoms, and sore throat but usually without skin rash or other evidences of allergy elsewhere. Blood count shows few or absent neutrophils.

(e) *Redistribution* caused by splenic sequestration and excessive margination. Diseases associated with splenomegaly and neutropenia include sarcoidosis, lymphomas, tuberculosis, malaria, kala azar, and Gaucher's disease.

(f) *Deficiencies of dietary vitamins and minerals* (*vitamin B12*, *folate*, *and copper*) typically cause neutropenia along with other cytopenias, but isolated and predominant neutropenia is possible.

(g) *Congenital disorders*, including Kostmann Syndrome, cyclic neutropenia, Chediak Higashi Syndrome and related congenital disorders [9]. They are diagnosed generally in childhood because of associated infections and/or concomitant lymphoctyte defects.

4.3 Lymphocytopenia

4.3.1 Causes

Lymphocytopenia is defined as a total lymphocyte count less than 1,500/ml. Approximately 80 % of normal adult blood lymphocytes are T lymphocytes and nearly two-thirds of blood T lymphocytes are CD4+ T lymphocytes. Lymphocytopenia can be primary or secondary to other conditions. Primary causes are uncommon and include a wide range of diseases characterized by a quantitative or qualitative stem cell abnormality. Furthermore, primary lymphopenia can be due to defect of lymphocytic cytoskeleton that causes premature destruction of lymphocytes, as observed in Wiskott–Albrich syndrome. Particularly elevated rates of inborn lymphocytopenia occur in some ethnic groups like as Ethiopians and Ckukotka natives.

Acquired lymphocytopenia can be associated to:

(a) Infectious diseases: The most common infectious disease associated with lymphopenia is the acquired immunodeficiency syndrome. The lymphocytopenia result in part from destruction and/or clearance of CD4+ T cells infected with HIV1–HIV2 [10, 11]. Other viral and bacterial diseases may be associated with lymphopenia. Patient with tuberculosis often have lymphocytopenia that usually resolves 2 weeks after initiating appropriate antimicrobial therapy. A 68 % of adult patients and 92 % of pediatric patients had reduced lymphocytic counts during the 2009 Influenza A pandemic.

Iatrogenic factors, including radiotherapy, chemotherapy, glucocorticoids, or administration of anti-lymphocyte globulin and monoclonal antibodies anti-lymphocytic antigens can lead to the destruction of circulating lymphocytes.

(b) *Malignancies and hematologic disorders: including* Hodgkin lymphoma and aplastic anemia.

(c) *Autoimmune, inflammatory disorders, and connective tissue disease*: including SLE, inflammatory bowel disease and inflammatory arthritis.

(d) *Systemic disease*: including chronic renal failure.

(e) *Poor nutritional conditions*: including zinc deficiency and excessive alcohol intake.

4.4 Clinical Approach to the Patient Presenting with Leucopenia

The initial approach to a leukopenic patient can be subdivided into different phases:

- *Recognition of the condition*: Leukopenia (WBC <4,000/ml) may derive from neutropenia (neutrophils <1,500/ml), from

lymphocytopenia (lymphocytes <1,500/ml), or from a combination of these two entities. Is important to investigate previous cell blood counts, if they are available, because these will clarify whether the leukopenia is new or long-standing. Long-standing leucopenia without a history of infection suggests a benign constitutional cause or a congenital cause without previous clinical manifestation. In case of severe leukopenia, the administration of antibiotics should be considered even in the absence of signs of over infection [12].

- *Identification of the possible cause*(*s*) must be sought by considering the history, which should be focused on the medications taken in the last few weeks, the presence of solid or hematologic malignancies, the physical examination (lymphoadenophathy, splenomegaly, joint swelling, bone pain, fever, etc.) and the nutritional status. In case of isolated lymphocytopenia an HIV-1 must be obtained even in subjects considered at low risk [13, 14]. If the patient has leukopenia plus other hematologic abnormalities, bone marrow evaluation is helpful.
- *Withdrawal of all drugs with potential effects on WBC* and follow-up blood counts. Leukopenia that has not corrected 8 weeks after the discontinuation of the suspected responsible agent therapy warrants a bone marrow evaluation, and immunologic workup which can be discussed with the hematologist.

References

1. Munshi HG, Montgomery RB (2000) Severe neutropenia: a diagnostic approach. West J Med 172(4):248–252
2. Newburger PE, Dale DC (2013) Evaluation and Management of patients with isolated neutropenia. Semin Hematol 50(3):198–206
3. Darmon M, Azoulay E, Alberti C (2002) Impact of neutropenia duration of short term mortality in neutropenic critical ill cancer patients. Intensive Care Med 28(12):1775–1780

4. Dellinger RP, Levy MM, Rhodes A et al (2013) Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012. Crit Care Med 41(2):580–637
5. Youinou P, Jamin C, Le Pottier L (2014) Diagnostic criteria for autoimmune neutropenia. Autoimmun Rev 13(4–5):547–576
6. Andrès E (2008) Idiosyncratic drug-induced agranulocytosis or acute neutropenia. Curr Opin Hematol 15(1):15–21
7. Andrès E, Krutz JE (2002) Nonchemotherapy drug-induced agranulocytosis : experience of the Strasbourg teaching hospital (1985–2000) and review of the literature. Clin Lab Hematolog 24(2):99–106
8. Cohen D, Monden M (2013) White blood cell monitoring during long term clozapine treatment. Am J Psychiatry 170(4):366–369
9. Sokolic R (2013) Neutropenia in primary immunodeficiency. Curr Opin Hematol 20(1):55–65
10. Douek DC, Picker LJ, Koup RA (2003) T cell dynamics in HIV infection. Annu Rev Immunol 21:265–304
11. Okoye AA, Picker LJ (2013) CD 4(+) T cell depletion in HIV infection: mechanism of immunological failure. Immunol Rev 254(1):56–64
12. Legrand M et al (2012) Survival in neutropenic patients with severe sepsis or septic shock. Crit Care Med 40(1):43–49
13. Dawn B (2014) Investigating an incidental finding of lymphopenia. BMJ 348:172
14. Portman MD (2014) Routinely test for HIV in everyone presenting with unexplained lymphopenia. BMJ 348:g2433

Chapter 5
Leukocytosis in the Critically Ill Patient

Giorgio Berlot, Antoinette Agbedyro, and Barbara Presello

5.1 Introduction

Leukocytosis (for definitions see below) is a very common laboratory finding with a broad assortment of possible clinical interpretations since it is a physiological response to many stimuli and it may be observed in a wide variety of diseases.

Patients admitted in Intensive Care Units (ICU) usually are prone to infections due to their critical condition that may impair their immune system and the presence of invasive devices, including central and peripheral venous catheters, arterial lines, urinary bladder catheters, etc., and invasive monitoring. Moreover, they can have several comorbidities and sometimes it may be difficult to recognize the underlying cause of leukocytosis even if the infection is the most likely one. The aim of this chapter is to address the most common causes of leukocytosis in order to indicate the potential differential diagnoses in critically ill patients.

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5.2 The Biology of the White Blood Cells (WBC)

The peripheral count of the different classes of WBC reflects the equilibrium of several compartments or pools. The bone marrow contains a mitotic pool, a maturation pool, and a storage pool. Once released into the bloodstream, the WBC can be subdivided in the circulating pool and marginated pool, which primarily consists of neutrophils adherent to the vascular endothelium; other WBC are located in the tissues. Then, the absolute and relative WBC count reflects only the circulating pool. A complex interplay of factors regulates production of granulocytes and their movement from one pool to another. After maturation into the bone marrow, mature neutrophils are released into the bloodstream, and this process can be accelerated in response to inflammation, leading to the appearance of immature cells. A number of substances induce neutrophil movement from the bone marrow into the blood, including endotoxin, glucocorticoids, a leukocyte-mobilizing factor derived from the third component of complement (C3e), chemoattractants such as C5a, cytokines such as tumor necrosis factor-α (TNF-α), etc. Increased neutrophil production can be stimulated by myeloid growth factors and inflammatory stimuli. The maturation of WBCs is influenced by Granulocyte-colony stimulating factors (G-CSFs), several interleukins (ILs), TNF-α, and different complement factors. In normal subjects with a functioning bone marrow, the myeloid growth factors induce neutrophilia by at least two mechanisms acting in different time frames: (a) a rapid response, occurring in few hours after the administration, which induces a release of neutrophils; and (b) a slower response, occurring after few days, which is characterized by the production of new neutrophils along with a rise of RBC, megakaryocytes, eosinophils, basophils, and monocytes. Proliferation of the common progenitor is stimulated by several growth factors,

including IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), while later differentiation is regulated by G-CSF. The release of neutrophils from the marrow storage pool can result in a two-to-threefold increase in the neutrophil count within 4–5 h. At any given time, more than one-half of the neutrophils in the peripheral circulation adhere to the vascular endothelium. These marginated neutrophils can be released almost immediately (within minutes) under stressful conditions and this effect is mediated in part by the epinephrine released due to the activation of the hypothalamic–adrenal axis [1].

5.3 Definitions

The WBC count in adults varies from 4,400 to 11,000 cells/mL (4.4–11.0 × 10^9/L), the majority of which (~60 %) are mature neutrophils. Leukocytosis is defined as a total WBC count greater than 11,000/mL in adults.

By convention, leukocytosis values in excess of 50,000 cells/mL, when due to causes other than leukemia, is termed a leukemoid reaction or hyperleukocytosis.

Leukocytosis is most commonly due to an increase in the absolute number of mature neutrophils, but it can also reflect a marked increase in the absolute numbers of lymphocytes, eosinophils, monocytes, or, more rarely, basophils.

To guide the differential diagnosis, leukocytosis should be divided into the following classes:

- *Neutrophilia*, when the absolute neutrophil count exceeds 7,700 cells/mL in adults. Neutrophil leukocytosis is commonly seen in infection, stress, smoking, pregnancy, and following exercise. It can also occur in the chronic myeloproliferative disorders, such as polycythemia vera and chronic myeloid leukemia (CML).

- *Lymphocytosis*, indicating a lymphocyte count greater than 4,000 cells/mL. Lymphocytic leukocytosis can be seen following infections such as infectious mononucleosis and pertussis or in lymphoproliferative disorders such as the acute and chronic lymphocytic leukemia (ALC and CLL, respectively).
- *Monocytosis*, when the monocyte count is greater than 800 cells/mL. A monocytic leukocytosis can occur either in hematologic malignancies including the acute and chronic monocytic variants of leukemia and in acute bacterial infection or tuberculosis, chronic infections, autoimmune disorders, and after a splenectomy.
- *Eosinophilia and basophilia* indicate an eosinophil or basophil count exceeding 500/mL or 200/mL, respectively. Eosinophilic leukocytosis can occur in variant forms of chronic leukemia, solid tumors, infection with helminthic parasites, allergic reactions, and following treatment with Interleukin-2. The most common causes of basophilia include myeloproliferative disorders (myelodysplastic syndromes), other hematologic malignancies (basophilic leukemia, mastocytosis, hypereosinophilic syndrome, and atypical acute and chronic leukemias), allergic or inflammatory reactions, endocrinopathies, administration of estrogens, and infections (including viral infections, tuberculosis, and helminthes-associated infections). Basophilic leukocytosis is a distinctly unusual condition, and is most often associated with basophilic or mast cell variants of acute or chronic leukemia.
- *Hyperleukocytosis or leukemoid reaction* indicates a total white blood cell (WBC) count greater than 50,000–100,000/mL, which is often characterized by a significant increase in early neutrophil precursors along with increased numbers of band forms. In the bone marrow, a proliferation of all the normal myeloid elements is observed in contrast to acute leukemia, in which the most immature elements (e.g., promyelocytes, myeloblasts) predominate. Neutrophilic leukemoid

reactions can occur during infections but any strong stimulus to the bone marrow can trigger this reaction. In the presence of leukemoid reaction with a strong prevalence of neutrophils, a biopsy of the bone marrow is warranted in order to exclude acute and chronic myeloid leukemias (AML and CML, respectively) and other myelodysplastic/myeloproliferative neoplasms, which can present some morphologic overlap. Immature granulocytes (i.e., promyelocytes, myelocytes, and metamyelocytes) may be seen with either reactive neutrophilia or myeloid neoplasms such as CML [1–5].

- *Leukostasis* represents a condition of hyperleukocytosis characterized by an extremely elevated blast cell count and symptoms of decreased tissue perfusion due to the plugging of the microvascular network caused by aggregates of immature cells. This circumstance represents a medical emergency most commonly in patients AML or CML in blast crisis. Clinically, leukostasis is diagnosed empirically when a patient with leukemia and hyperleukocytosis presents with respiratory or neurological symptoms. Prompt treatment is indicated since, if left untreated, the 1-week mortality rate is approximately 20–40 %. In general, symptoms of leukostasis are more common in leukemias with large, poorly deformable blasts, as occurring in ALM [1, 6].

5.4 Major Causes of Leukocytosis

Leukocytosis may reflect either a primary disorder of bone-marrow production, which can be congenital or acquired (such as leukemias), or a secondary one in response to a pathologic process or the expositions to drugs or toxins. In critically ill patients, neutrophilia is far more common than increases in the absolute numbers of lymphocytes, eosinophils, monocytes, or basophils.

We will focus on the two following main lineages: neutrophils and lymphocytes.

5.4.1 Neutrophilia

As explained above, different mechanisms can account for the increase of circulating neutrophils, including (a) their increased production, whose effects appear after some days even with intense stimulation; (b) the accelerated release of mature cells from the marrow into the blood, which occurs within a few hours; (c) the shift between the marginated and circulating, which occurs in few minutes; (d) the reduced egress of neutrophils from the blood to tissues (primary disorder); and, finally, (e) a combination of all the mechanisms mentioned above.

The increase of the number of neutrophils occurring only in few minutes after the application of a stimulus is termed pseudoneutrophilia and is caused both by the detachment of neutrophils adhering to the endothelial walls mechanism, and on the redistribution from other vascular beds, including the pulmonary and splenic capillaries.

The increase in lymphocytes, monocytes, and neutrophils may be helpful in distinguishing pseudoneutrophilia from the neutrophilia in response to infections, protracted stress, or glucocorticoid administration. Actually, in this last condition, neutrophil counts are elevated, but lymphocyte and monocyte counts are generally depressed.

Acute neutrophilia is determined by the release of neutrophils from the marrow storage pool in response to inflammation and infections. In this circumstance, immature forms including metamyelocytes are not released into the bloodstream except under extreme circumstances.

Exposure of blood to foreign surfaces, such as hemodialysis membranes, activates the complement system and causes

Table 5.1 Causes of acute neutrophilia

Causes	Examples
Physical stimuli	Cold, heat, exercise, convulsions, pain
Emotional stimuli	Panic, rage, depression
Germs	Localized and systemic acute bacterial, rickettsial, and spirochetal infections
Tissue inflammation/necrosis	Trauma, burns, acute pancreatitis, electric shock, vasculitis, gout
Drugs and hormones	Epinephrine, glucocorticoids, tobacco, vaccines

transient neutropenia, followed by neutrophilia resulting from release of marrow neutrophils.

Colony stimulating factors (G-CSF and GM-CSF) cause acute and chronic neutrophilia by mobilizing cells from the marrow reserve and stimulate neutrophil production.

Chronic neutrophilia follows a prolonged stimulus to proliferation of neutrophil precursors. Mechanisms are not fully understood.

Many chronic noninfectious conditions cause neutrophilia, including many nonhematologic malignancies (lung, gastrointestinal, particularly when they metastasized to the liver and lung). In some cases, tumor cells produce colony stimulating factors. Neutrophilia as a manifestation of a hematologic disorder can be encountered in myeloproliferative syndromes including chronic neutrophilic leukemia and neutrophilic chronic myelogenous leukemia.

Neutrophilia in response to drugs is uncommon except for the well-known effects of epinephrine, other catecholamines, and glucocorticoids. Lithium salts cause sustained neutrophilia. The counts return to normal when the drug is discontinued [1]. The main causes of acute and chronic neutrophilia are summarized in Tables 5.1 and 5.2.

Table 5.2 Causes of chronic neutrophilia

Causes	Examples
Germs	Noneradicated infections causing acute neutrophilia
Tumors	Solid tumors, AML, CML
Drugs	Continued exposure to agents causing acute neutrophilia, lithium
Non-leukemic hematologic conditions	Rebound from agranulocytosis, therapy of megaloblastic anemia, asplenia
Hormones	Thyroid storm, pre-eclampisa and eclampisa, Cushing's Syndrome

5.4.2 Lymphocytosis

Circulating blood lymphocytes include populations of T cells, B cells, and natural killer cells. Levels of blood lymphocytes are higher in neonates and young children (within 12 years) with an absolute lymphocyte count as high as 8,000 cells/μL. In subjects older than 12 years, lymphocytosis is defined as an absolute count greater than 4,000 cells/μL.

Lymphocytosis can be due to a reactive proliferation or to a clonal expansion. The most common cause is infection. Reactive lymphocytosis is a physiologic or pathophysiologic response to infection, toxins, cytokines, or unknown factors. Normally it is characterized by polyclonal populations of lymphocytes with a pleomorphic morphology. Infectious mononucleosis (EBV) is the most common reactive cause. In this case, infected B cells stimulate the proliferation of atypical polyclonal T or NK cells which are observed peripherally. Pertussis infection, which is most often seen in pediatric populations, is an important exception. In fact, it is characterized by monomorphic lymphocytes. Nonclonal lymphocytes proliferation rarely exceeds 30,000 cell/ μL.

Table 5.3 Causes of reactive lymphocytosis

Causes	Examples
Acute and chronic infections	EBV and other mononucleosis syndromes
Hypersensitivity reactions	Insects bites, drugs
Tumors	Malignant thymoma
Others	Stress, autoimmune disorders, trauma, vaccines, postsplenectomy, smoking

Table 5.4 Causes of primary lymphocytosis

Causes	Examples
Malignancies, premalignancies	Acute lymphocytic leukemia, chronic lymphocytic leukemia, essential monoclonal B-cell lymphocytosis

Lymphoproliferative disorders are also associated with peripheral lymphocytosis and in early phases it may be difficult to distinguish them from a reactive lymphocytosis. The morphologic appearance of lymphocytes may help in this way. In fact, a monomorphic lymphocytosis favors a neoplastic proliferation. Major causes of lymphocytosis are summarized in Tables 5.3 and 5.4 [1–5].

5.5 Diagnostic Approach to Leukocytosis in the Critically Ill Patients

The differential diagnosis of leukocytosis includes physiologic responses to a broad range of infectious and inflammatory processes, as well as numerous primary hematologic disorders such as leukemias, lymphomas, and myeloproliferative neoplasms. Especially in critically ill patients with abnormally elevated WBC count with sepsis and sepsis-related conditions, the

presence of an infection as well must be primarily assessed; at the same time, other less common causes of leukocytosis must be looked for. Sampling errors, spurious leukocytosis, and all other possible confounding factors must be excluded. Spurious leukocytosis occurs in the presence of platelet clumping, fibrin clumping or cryoglobulinemia. In these cases, leukocytes count can be overestimated as clumps of platelets or agglutinated cryoglobulines can be counted as leukocytes. A peripheral blood smear is necessary either to confirm the elevated WBC count and to identify immature cells of the different lineages [7, 8].

Leukocytosis in response to drugs is commonly observed after the administration of corticosteroids and cathecolamines; in this case, leukocytes should not rise above 20,000–30,000/mL (mild-to-moderate leukocytosis) [1]. Many inflammatory stress conditions observed in intensive care unit (trauma, surgery, burns) can cause leukocytosis. Also, blood exposure to hemodialysis membranes can cause a secondary leukocytosis.

Leukocytosis associated with hypotension or shock may be related to a severe infection process or may be due to an important blood loss causing the release of neutrophils from the endothelial wall into the bloodstream; in these conditions, or in case of doubt, the measurement of C-reactive protein and procalcitonin may be of help to determine the presence of an infection. Actually, it should be noted that other clinical signs associated with leukocytosis like fever, anemia, thrombocytopenia, or thrombocytosis can be commonly observed both in infective and neoplastic diseases.

The diagnosis of a myeloproliferative disease should be considered if leukocytosis is caused by myelocytes and promyelocytes, increased basophils, and unexplained splenomegaly. In the presence of hyperleukocytosis, a leukemoid reaction must be excluded. Distinguishing myeloid–leukemoid reactions from myeloid malignancies can be challenging The presence of dysplasia, basophilia, WBC count greater than 50,000/mL, with a pronounced left shift (predominance of immature granulocytes), and increased blast count in the peripheral blood greater than

20 % address the diagnosis toward a myeloid malignancy. In this case, a bone marrow examination is recommended along with an appropriate ancillary testing.

Lymphocytosis differential diagnosis depends on patient age, clinical history, and morphologic findings. In adults, the first step will be excluding an infectious mononucleosis. The diagnosis can be made by a rapid test for heterophil antibodies. If these are negative, but the clinical suspicion of infectious mononucleosis is high, the serum sample should be tested for specific EBV antibodies. Furthermore, there may be a clinical overlap with acute lymphoblastic leukemia as the two disorders are distinguished on the basis of bone-marrow examination, lymphocyte immunophenotyping, and serological findings. Lymphocytosis is rarely seen in bacterial infections, with the exception of Bordetella pertussis infection [1–5].

References

1. Lichtman MA, Beutler E, Kipps TJ et al (2006) Williams Hematology, 7th edn. McGraw-Hill Medical, New York
2. George TI (2012) Malignant or benign leukocytosis. Hematology 2012:475–484
3. Stock W, Hoffman R (2000) White blood cells 1: non-malignant disorders. Lancet 355:1351–1357
4. Tefferi A, Hanson C, Inwards DJ (2005) How to interpret and pursue an abnormal complete blood cell count in adults. Mayo Clin Proc 80(7):923–936
5. Chabot-Richards DS, George TI (2014) Leukocytosis. Int J Lab Hematol 36:279–288
6. Porcu P, Cripe LD, Ng EW et al (2000) Hyperleukocytic leukemias and leukostasis: a review of pathophysiology, clinical presentation and management. Leuk Lymphoma 39:1
7. Savage RA (1984) Pseudoleukocytosis due to EDTA-induced platelet clumping. Am J Clin Pathol 81:317
8. Patel KJ, Hughes CG, Parapia LA (1987) Pseudoleucocytosis and pseudothrombocytosis due to cryoglobulinaemia. J Clin Pathol 40:120

Chapter 6
The Critically Ill Patient with Abnormal Platelet Count

Luca G. Mascaretti and Paola Pradella

Abnormal platelet counts are a common finding in critically ill patients. Whereas thrombocytopenia, defined as a platelet count less than $150*10^9$/L, affects 13–60 % of Intensive Care Unit (ICU) patients [1] and has been extensively studied, the occurrence of thrombocytosis (platelet counts $>400*10^9$/L) is observed less frequently and has not been studied to the same extent.

In this chapter, the main causes of thrombocytopenia and thrombocytosis in critically ill patients will be illustrated, and their implications on morbidity and mortality will be discussed. Due to its importance in the ICU setting, a section in this chapter will be dedicated to heparin-induced thrombocytopenia (HIT).

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6.1 Thrombocytopenia: A Classification

Before addressing the issues related to "true" thrombocytopenia, pseudo (or spurious) thrombocytopenia must be defined. In some conditions such as liver diseases, neoplasia, autoimmune disease, or in healthy subjects, antibodies mediated by anticoagulants such as EDTA are responsible for platelet clumping, which, not being detected by cell counters, will lead to falsely low platelet counts [2]. Pseudothrombocytopenia is not clinically significant and is diagnosed by microscopic examination of the blood smear (Fig. 6.1) and by repeating the whole blood count in tubes with a different anticoagulant (heparin- or citrate-based solutions).

"True" thrombocytopenia, to a variable degree, affects all types of ICU patients in all parts of the world; adult medical ICU patients are mostly affected, but it is also observed in surgical and pediatric patients. These observations underlie the comment made by R.I. Parker in his recent review [1] that thrombocytopenia in ICU patients is "a truly universal occurrence."

Although a threshold value of $150*10^9$/L is generally accepted to indicate thrombocytopenia, stable platelet counts between 150 and $100*10^9$/L are not necessarily considered pathological. Moreover, it is now recognized that the risk of clinically spontaneous bleeding is significantly high when platelet counts fall below $20–10*10^9$/L [3].

The two main mechanisms responsible for thrombocytopenia are reduced production and increased destruction of platelets; less frequently, a reduced platelet count may also be due to sequestration and hemodilution [1, 2].

Table 6.1 summarizes the main classification criteria for thrombocytopenia, the most frequent pathological mechanisms and the associated clinical conditions. The table does not include causes of thrombocytopenia in pregnancy and postpartum, since these conditions go beyond the scope of this chapter.

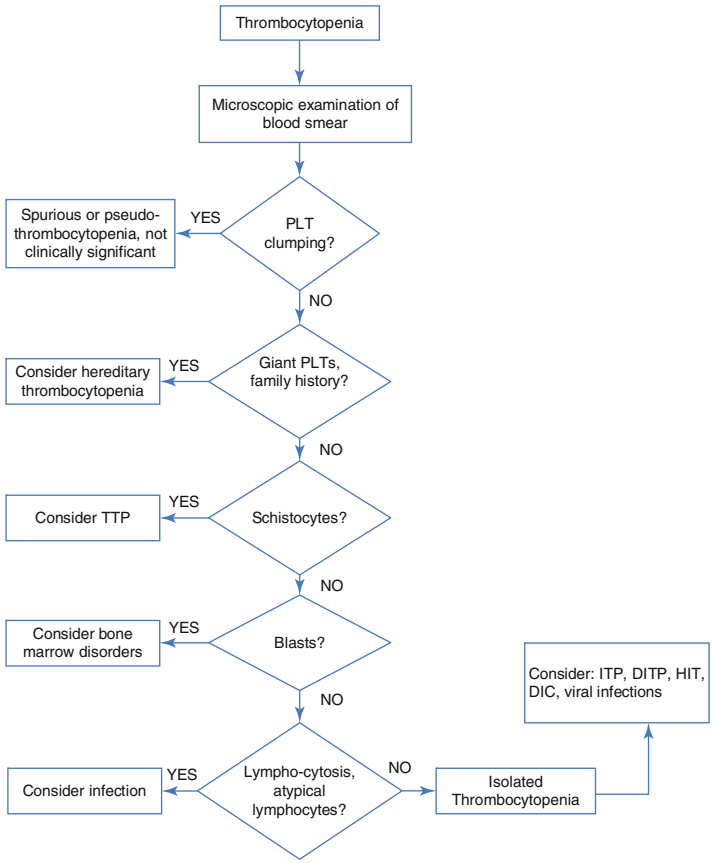

Fig. 6.1 Diagnostic algorithm based on blood smear (Adapted from Stasi [3])

It should always be remembered that in a significant number of cases, thrombocytopenia is due to multiple factors, such as for example in sepsis.

The diagnostic workup for thrombocytopenia must include, in addition to laboratory tests discussed in this chapter, a family

Table 6.1 Causes of thrombocytopenia

Main classification criteria	Pathological mechanism	Examples of clinical conditions	References
Decreased production	Primary bone-marrow failure	Myelodisplastic disorders, Fanconi's anemia, congenital amegakaryocytic thrombocytopenia	[4–7]
	Secondary bone-marrow failure	Sepsis, severe idiopathic aplastic anemia, severe malnutrition	[8]
	Infiltration of bone marrow due to neoplastic diseases	Acute leukemia, widespread marrow metastases	[9]
	Infiltration of bone marrow due to storage disorders	Gaucher's disease	[10]
	Drug-related marrow suppression	Chemotherapy, other drugs	[11]
	Marrow failure due to radiation therapy	Internal radiation, external radiation	[12]
Enhanced destruction	Nonimmune: mechanical	Intravascular devices such as central venous catheters, intraaortic balloon pump	[13]
	Nonimmune: microangiopathic	Thrombotic thrombocytopenic purpura (TTP), disseminated intravascular coagulation (DIC), subacute bacterial endocarditis (SBE), vasculitis	[14, 15]
	Nonimmune: platelet aggregation	Drugs	[11]
	Immune: platelet specific auto-antibodies	Immune thrombocytopenic purpura (ITP)	[16]
	Immune: immune complexes	Autoimmune disorders	[17]

	Immune: cell-mediated	Hypersplenism, hemophagocytic lymphohistiocytosis	[18]
	Immune: platelet-specific allo-antibodies	Posttransfusion purpura	[19]
	Immune: Drugs	Drug related immune thrombocytopenia: antiepileptics, gold compunds, vancomycin, thiazides, quinine/quinidine	[11]
	Immune: Heparin-induced	Heparin-Induced Thrombocytopenia (HIT)	[20]
	Immune: sepsis	Sepsis	[8]
Sequestration	Congestive splenomegaly	Portal hypertension leads to the redistribution of platelets from the circulating pool to the splenic pool	[21]
Hemodilution	Secondary to fluid infusion in case of massive hemorrhage	Transfusion of platelet-poor blood products, infusion of colloids and crystalloids	[22]

history for thrombocytopenia, the evaluation of its "dynamics," meaning if it is a new finding, if it is chronic or whether it has a relapsing presentation. Information on bleeding episodes is also very important, as is the history of concomitant diseases such as infections, tumors, or autoimmune diseases. Finally, it is of paramount importance to collect the history related to recent medication (heparin, antibiotics) and blood transfusion since especially for hospitalized patients, drug-induced thrombocytopenia (DITP) is among the most common causes of low platelet counts. Since the aim of this chapter is to discuss thrombocytopenia in critically ill patients, it goes without saying that it is challenging to understand this condition in these patients also because a complete history may be difficult to obtain.

Whereas by definition, the Whole Blood Count is the basic laboratory test for diagnosing thrombocytopenia, the microscopic examination of the blood smear gives additional, important information on the pathogenetic mechanism involved [3]. Figure 6.1 illustrates an algorithm that guides the hematologist in the diagnosis of isolated thrombocytopenia. Other tests employed in the diagnosis of the causes of thrombocytopenia are liver and renal function tests, coagulation tests including D-dimers, lactate dehydrogenase, and bone marrow aspirate and biopsy.

Platelet antibody assays and other tests such as reticulated platelets have a limited specificity and therefore their use is debatable [16].

Before describing the clinical conditions associated with thrombocytopenia, the importance of the rate of decline in platelet counts must be pointed out. When a constant, slow reduction in platelet number is observed with minimum (nadir) counts falling below $20*10^9$/L, a DITP due to marrow inhibition is the probable cause. On the other hand, when there is a fast rate of decline (24–48 h) in platelet numbers, an immune mechanism is suspected. A variable rate in platelet reduction is suggestive of consumptive coagulopathy [1].

6.1.1 *Thrombocytopenia Due to Reduced Production*

Thrombocytopenia caused by bone marrow suppression may be due to acquired or congenital conditions. In the latter category are comprised Fanconi's anemia, congenital amegakaryocytic thrombocytopenia, thrombocytopenia, and absent radii syndrome; a comprehensive review of these clinical conditions has been recently published by Parikh and Bessler [4]. The inherited bone marrow failure syndromes are genetic disorders affecting blood cell lineages. They are characterized by a wide spectrum of symptoms ranging from aplastic anemia to symptoms related to the suppression of one or two cell lines. Congenital amegakaryocytic thrombocytopenia is an inherited bone marrow failure syndrome usually diagnosed at birth, and characterized by insufficient production of megakaryocytes due to a defect in the thrombopoietin receptor [5].

Acquired bone marrow failure is often due to myelodysplastic syndromes, a heterogeneous group of clonal bone marrow disorders characterized by ineffective hematopoiesis, morphological and functional abnormalities of hematopoietic cells, and increased risk of malignant transformation. The prevalence of thrombocytopenia in these diseases varies from 40 to 65 % [6], and together with platelet dysfunction, is responsible for the increased hemorrhagic risk in these patients.

Sepsis is a condition affecting a significant number of patients admitted to hospitals; a recent review reports that in the USA, 2 % of patients corresponding to 750,000 per year are septic, half of which are admitted to ICUs [8]. Clinical signs of sepsis are diverse and depend on the microorganism, site of original infection, and health condition of the patient. Thrombocytopenia in sepsis is a common finding and severe forms of sepsis are associated with coagulation disorders that can lead to disseminated intravascular coagulation (DIC).

Thrombocytopenia can also be caused by drugs that suppress the bone marrow, and in particular megakaryocyte proliferation and maturation. Whereas antimetabolytes, cytotoxic drugs, and alkylating agents exert a toxic effect on all bone marrow cell lines, some antibiotics such as linezolid, may cause a selective suppression of platelet cell lines [11].

Other causes of thrombocytopenia due to decreased production (Table 6.1) are storage disorders [10], infiltration of bone marrow due to neoplastic diseases [9] and radiation therapy [12].

Thrombocytopenia due to reduced production is not a frequent cause of admission to the ICUs, since it is more often preexistent.

6.1.2 Thrombocytopenia Due to Enhanced Destruction or Consumption

6.1.2.1 Thrombocytopenia Due to Enhanced Destruction: Nonimmune Mechanisms

Medical devices such as mechanical heart valves, left-ventricular assistance devices, and aortic balloon pumps may be responsible for the destruction of platelets. In a study on 1,302 patients who underwent percutaneous coronary intervention (PCI) with baseline normal platelet counts ($\geq 150*10^9$/L), 3.1 % developed post-PCI thrombocytopenia. Multivariate analysis showed that the use of intra-aortic balloon pump was an independent predictor of thrombocytopenia, with an odds ratio of 2.8, confidence intervals 1.1–6.8, $p=0.024$. Post-PCI thrombocytopenia was significantly associated with major adverse cardiovascular events at 6 months (hazard ratio 2.7, CI 1.3–5.5, $p=0.0069$) [13].

Microangiopathic processes such as thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and disseminated intravascular coagulation (DIC) may be

responsible for thrombocytopenia due to enhanced platelet destruction.

TTP is characterized by microvascular platelet clumping, which leads to thrombocytopenia and microangiopathic hemolytic anemia. Common findings are "broken" erythrocytes or schistocytes (see algorithm reported in Fig. 6.1), neurological disorders, renal failure, and fever [14]. The disease is due to a congenital or acquired deficiency in ADAMTS13, a metalloprotease which cleaves von Willebrand factor. ADAMTS13 deficiency is responsible for microvascular thrombosis and thrombocytopenia. Plasma exchange is the optimal therapy, and its effectiveness is probably due to the removal of anti-ADAMTS13 autoantibodies and large von Willebrand factor multimers.

HUS is similar to TTP in that microvascular thrombosis, thrombocytopenia, microangiopathic hemolytic anemia, renal insufficiency, and altered mental status are common features. However, ADAMTS13 is normal and the disease is generally due to endothelial cell damage caused by a toxin produced by pathogenic strains of *Escherichia* or *Shigella*. In HUS, thrombocytopenia is usually not severe but dialysis may be required to treat renal insufficiency [23].

DIC does not occur as an isolated event but is practically always associated with an underlying condition such as tissue damage (trauma, burns, hemolytic transfusion reaction, acute transplant rejection), neoplasia, systemic infection, obstetric conditions (abruption placentae, placenta previa, amniotic fluid embolism), and other clinical conditions such as shock, cardiac arrest, and aortic aneurysm. DIC is the result of an overstimulation of the coagulation system and its clinical presentation varies from severe hemorrhage to thrombosis (or both simultaneously). Thrombocytopenia, abnormal prothrombin time and activated partial thromboplastin time (PT and aPTT), decreased fibrinogen and elevated fibrinogen degradation products are common laboratory features of DIC. DIC-associated mortality is mostly due to

the original disease, which is complicated by hemorrhage or thrombosis. Multiorgan dysfunction syndrome is a frequent consequence of DIC and is due to hemorrhagic or thrombotic events in liver, heart, kidneys, central nervous system, and lungs [15].

The main therapeutic goal in DIC is that of treating the underlying condition. As far as transfusion of blood products is concerned, there has been a lot of debate on its benefit and potential harm; generally, platelet counts should be kept more than $20*10^9$/L in presence of mild bleeding and more than $50*10^9$/L when there is active bleeding. Plasma or cryoprecipitate should be considered when bleeding is associated with low fibrinogen levels. The aim of fibrinogen replacement is to maintain levels more than 100 mg/dl to prevent or treat bleeding [24].

6.1.2.2 Thrombocytopenia Due to Enhanced Destruction: Immune Mechanisms (Except HIT)

In addition to Heparin-Induced Thrombocytopenia (HIT) which will be discussed in the following section, primary Immune Thrombocytopenia (ITP), post-transfusion purpura (PTP), and drugs may lead to immune platelet destruction.

ITP is an acquired disorder mediated by immunological mechanism, characterized by low platelet counts in the absence of any possible known cause of thrombocytopenia. It affects children and adults (with a slight prevalence in women) and symptoms range from massive bleeding (gastrointestinal, skin–mucosal, and intracranial) to minimal bruising or only alterations in whole blood count. Evaluation of the blood smear is important in the diagnosis of ITP (Fig. 6.1) and antiplatelet antibody assays are not routinely performed due to the low specificity of this test. Adult ITP is treated with corticosteroids or IVIg and platelet transfusions are recommended only for emergency cases in presence of active bleeding [16].

PTP is a rare complication of transfusion occurring 7–10 days after a red blood cell or platelet transfusion and is characterized by a dramatic fall in platelet count reaching a nadir less than $10*10^9$/L. Thrombocytopenia is caused by platelet alloantibodies in the recipient which at first destroy the transfused platelets, but successively also react with self-platelets. PTP is managed by administering IVIg or if available, compatible platelets (usually HPA-1a negative) [19].

Drug-induced thrombocytopenia (DITP) may either be caused by drugs suppressing bone marrow (see previous section) or by drugs eliciting diverse types of antibodies. Table 6.2 summarizes the main types of antibodies implicated in DITP [11]. DITP may be hard to diagnose in critically ill patients, since thrombocytopenia may become evident several days after the beginning of therapy, and has to be distinguished from other causes of thrombocytopenia.

Other causes of thrombocytopenia include platelet sequestration and hemodilution. Thrombocytopenia is a common feature of liver cirrhosis and is attributable to portal hypertension with sequestration of platelets in the enlarged spleen [21].

In massive transfusion, defined as the transfusion of one blood volume in 24 h, coagulation abnormalities are almost always present and are in part due to hemodilutional thrombocytopenia. However, coagulopathy associated with massive transfusion has many additional components, among which are coagulation factor dilution, hypothermia, type of solutions used for volume replacement, and DIC [22].

6.1.2.3 Clinical Significance of Thrombocytopenia in Critically Ill Patients

Hui and coworkers published a review in 2011 [25] aimed at better understanding the clinical role of thrombocytopenia in critically ill patients. It analyzed 24 studies for a total of 6,894

Table 6.2 Antibodies and mechanisms implicated immune drug-induced thrombocytopenia

Type of antibody (Ab)	Mechanism and features	Main drugs
Drug-dependent Ab	"Classic" mechanism: severe thrombocytopenia occurs 5–10 days after beginning of new drug, which binds to platelet glycoproteins (IIb/IIIa, or IbIX). Abs bind to glycoproteins and sensitized platelets are eliminated by the reticuloendothelial system, resulting in severe thrombocytopenia. Abs react with glycoproteins only when the drug is present.	Quinine
Hapten-induced Ab	Drug acts like a hapten, i.e., small molecules that elicit an immune response when linked to larger carrier molecule. Abs will bind to the platelet membrane glycoproteins only when the hapten (drug) is covalently linked to them.	Penicillin
Fiban-dependent Ab	Drugs bind to glycoprotein IIb/IIIa and may form a neo-epitope: autoantibodies are either preexistent and therefore naturally occurring, or may be stimulated by exposure. Thrombocytopenia occurs after a few hours from beginning of therapy.	Tirofiban and eptifibatide
Fab-binding monoclonal Ab	Abciximab is a Fab fragment monoclonal antibody which reacts with glycoprotein IIIa, preventing fibrinogen binding. Thrombocytopenia associated with this drug is due to naturally occurring antibodies which recognize the murine portions of the monoclonal Ab.	Abciximab

Drug-induced autoantibody formation	Some drugs may elicit autoantibodies which do not require the presence of the drug to bind to platelet antigens. Thrombocytopenia can therefore persist after the drug is no longer administered.	Gold, L-dopa, procainamide, sulphonamides, alemtuzumab
Immune complexes	Heparin-induced Thrombocytopenia (see following chapter)	Heparin
Anti-megakaryocyte Abs	Some platelet Abs in addition to binding to circulating platelets may also react with megakaryocytes, thus inhibiting platelet production.	Eptifibatide, quinine

Based on data from Arnold et al. [11]

patients; whereas 8.3–67.4 % of patients had low platelet counts at admission, the proportion of patients which developed thrombocytopenia during their stay in the ICU ranged from 13 to 44 %. Major risk factors for the development of thrombocytopenia were high illness severity, organ dysfunction, sepsis, and renal failure. The review was unable to show convincing evidence for an association between thrombocytopenia and bleeding, but multivariate analysis conducted by six studies indicated that thrombocytopenia was an independent predictor of mortality. This finding is confirmed by Stansbury and coworkers [26] in their study on the prognostic significance of platelet counts in the first 24 h after severe injury.

6.2 Heparin-Induced Thrombocytopenia (HIT)

Critically ill patients are often suspected of having HIT, because both thrombocytopenia and heparin treatment are common in the ICU setting. Nevertheless, a recent study demonstrated that the diagnosis of HIT was confirmed in only 0.5 % of these patients [27].

Heparin-Induced Thrombocytopenia (HIT) is a particular type of drug-induced thrombocytopenia that is associated with a prothrombotic condition, despite a low circulating platelet count. Although this disorder may occur with any molecular-weight heparin, the incidence of HIT is higher with unfractionated heparin compared to low-molecular-weight heparin [28, 29]. Other risk factors are host-related, with the female sex more affected than the male [30] and the surgical population more affected than the medical [31].

Two types of HIT are described with different clinical features. Type 1 HIT is likely induced by a nonimmune mechanism, with circulating platelet clumping in the presence of heparin and their sequestration in the spleen. The consequent thrombocytopenia develops usually in 2–3 days after starting

heparin, is mild and resolves spontaneously with no thrombotic or hemorrhagic complications. Unlike the former, Type 2 HIT is an immunomediated disorder, in which the anticoagulant binds to Platelet Factor 4 (PF4), a protein released from activated platelets, and triggers the development of specific antibodies [32]. The macromolecular complex constituted by the antibody and heparin-PF4 binds a specific receptor on the platelet surface leading to further platelet activation [33] and to thrombin generation [34]. Activated platelets are cleared from circulation with consequent thrombocytopenia and a paradoxical enhanced risk for arterial and especially venous thrombosis.

Different laboratory methods are available to identify the presence of HIT antibodies:

- Functional assays with the HIT patient serum activating normal platelets in the presence of heparin
- Antigen assays to detect the binding of HIT antibodies to their target heparin/PF4

Functional assays are more specific for clinically relevant antibodies, but require specialized personnel, so antigen assays are the most widely used [35].

A typical feature of Type 2 HIT is the reduction of more than 50 % in the platelet count, leading to a moderate thrombocytopenia with a median platelet nadir of 50–60*10^9/L; unlike other drug-mediated thrombocytopenias, a platelet number less than 20*10^9/L is very uncommon. In naïve patients, the typical onset of thrombocytopenia is 5–14 days after the beginning of heparin exposure; in patients treated in the past 3 months, it may occur early within 24 h (early onset). Seldom platelet counts begin to fall after more than 15 days from the beginning of heparin treatment, sometimes after heparin discontinuation with a delay onset [36].

When HIT is strongly suspected, any heparin treatment (even exposure to heparin flushes or lines washing procedure) must be discontinued and replaced with another anticoagulant, for example, direct thrombin or activated FX inhibitors [37]. In a

few days, platelet count returns to normal values or to pretreatment values.

Actual guidelines suggest a clinical evaluation with a scoring system to test the likelihood of the disorder [38, 39]. The most widely used is the 4 T's score, based on clinical traits of HIT such as the degree of thrombocytopenia, the timing of the onset, the presence of a new or enlarged thrombosis, and an eventual different cause of platelet count decrease, as is shown in Table 6.3 [38].

With a low score (≤3), HIT can be excluded without any laboratory assay and the heparin treatment may be continued; if the score is moderate or high (4–6), all heparin exposure should be discontinued to avoid HIT complications and an alternative anticoagulant should be chosen [40]. Recently, two new methods have been proposed for assessing the clinical probability of HIT in the early management of patients suspected of having HIT [41, 42] but they need further validation. Whichever method is used, a careful evaluation is necessary in HIT exclusion or confirmation in order to prevent bleeding risks in thrombocytopenic patients.

6.3 Thrombocytosis in Critically Ill Patients

Elevated platelet counts (>400*10^9/L) are not a common finding among critically ill patients and contrary to thrombocytopenia, thrombocytosis in hospitalized patients has not been investigated at great length. From the etiological point of view, thrombocytosis may be classified as primary or secondary. Whereas the former group includes myeloproliferative or myelodysplastic syndromes, the latter may be either secondary or paraneoplastic. In the ICU patient, the main underlying clinical conditions responsible for thrombocytosis are infection, trauma, splenectomy, hemolysis, bleeding, and drugs such as antifungals, amoxicillin/clavunate, enoxaparin [43]. Two

Table 6.3 The 4 T's score (adapted from [38])

	Points = 2	Points = 1	Points = 0
Thrombocytopenia	>50 % platelet fall *and* Platelet nadir ≥20*10^9/L	50 % fall or Platelet nadir 10–19*10^9/L	<30 % fall or Platelet nadir <10*10^9/L
Timing of platelet count fall	Clear onset between day 5 and 10 or Platelet fall ≤1 day (if heparin exposure within past 30 days)	Consistent with immunization but not clear (e.g., missing platelet counts) or Onset of thrombocytopenia after day 10 or Fall ≤1day if heparin exposure 30–100 days ago	Platelet count fall ≤4 days (without recent heparin exposure)
Thrombosis (or other sequelaes, e.g., skin lesions)	New thrombosis (confirmed); Skin necrosis; Acute systemic reaction postintravenous unfractionated heparin bolus	Progressive or recurrent thrombosis; Erythematous skin lesions; Suspected thrombosis not yet proven	None
Other causes of thrombocytopenia	No other cause for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

other main conditions leading to thrombocytosis are familial (hereditary), due to a mutation responsible for an increase in the production of thrombopoietin [44], and essential thrombocythemia, a condition which may eventually lead to myelofibrosis or leukemia.

Differential diagnosis between primary and secondary thrombocytosis is not always straightforward in the ICU setting. Generally speaking, if thrombocytosis occurs during the stay in an ICU, it is most probably of secondary nature. However, if the patient is admitted urgently and no previous whole blood count is available, hematological consultation and further testing (e.g., *JAK2*) might be useful for the characterization of thrombocytosis [43]. For patients affected by secondary thrombocytosis, risk of thrombotic or hemorrhagic complications is <2 %, irrespective of platelet count.

As far as therapy is concerned, there is no threshold above which platelet removal by apheresis or antiaggregation therapy should be initiated. Risk of thrombosis in these patients must consider associated clinical conditions such as sepsis, trauma, and rheumatic disease, which themselves predispose to venous clot formation. Platelet apheresis is able to reduce platelet counts significantly and is used primarily in patients with myeloproliferative diseases in which either a thrombotic or bleeding event has occurred. Aspirin is administered in thrombocytosis (both primary and secondary) patients who have had a thrombotic event.

References

1. Parker RI (2012) Etiology and significance of thrombocytopenia in critically ill patients. Crit Care Clin 28:399–411
2. Rice TW, Wheeler AP (2009) Coagulopathy in critically ill patients. Part 1: Platelet disorders. Chest 136:1622–1630
3. Stasi R (2012) How to approach thrombocytopenia. Hematology 2012: 191–197

4. Parik S, Bessler M (2012) Recent insights into inherited bone marrow failure syndromes. Curr Opin Pediatr 24:23–32
5. Ballmaier M, Germeshausen M (2009) Advances in the understanding of congenital amegakaryocytic thrombocytopenia. Br J Hematol 146:3–16
6. Kantarjian H, Giles F, List A et al (2007) The incidence and impact of thrombocytopenia in myelodysplastic syndromes. Cancer 109(9): 1705–1714
7. Cazzola M, Malcovati L, Invernizzi R (2011) Myelodysplastic/myeloproliferative neoplasms. Hematology 2011:264–272
8. Finfer SR, Vincent JL (2013) Severe sepsis and septic shock. NEJM 369(9):840–851
9. Kwaan HC (2007) Thrombosis and bleeding complications in malignant hematologic disorders. Hematology 2007:151–157
10. Grabowski GA (2012) Gaucher disease and other storage disorders. Hematology 2012:13–18
11. Arnold DM, Nazi I, Warkentin TE et al (2013) Approach to the diagnosis and management of drug-induced immune thrombocytopenia. Transfus Med Rev 27(3):137–145
12. Mac Manus M, Lamborn K, Khan W (1997) Radiotherapy-associated neutropenia and thrombocytopenia: analysis of risk factors and development of a predictive model. Blood 89(7):2303–2310
13. Shenoy C, Orshaw P, Devarakonda S et al (2009) Occurrence, predictors, and outcomes of post-percutaneous coronary intervention thrombocytopenia in an unselected population. J Interven Cardiol 22:156–162
14. Fontana S, Kremer Hovinga JA, Lammle B et al (2006) Treatment of thrombotic thrombocytopenic purpura. Vox Sanguinis 90:245–254
15. Kitchens CS (2009) Thrombocytopenia and thrombosis in disseminated intravascular coagulation (DIC). Hematology 2009:240–246
16. Provan D, Stasi R, Newland AC et al (2010) International consensus report on the investigation and management of primary immune thrombocytopenia. Blood 115(2):168–186
17. McKenzie CGH, Guo L, Freedman J et al (2013) Cellular immune dysfunction in immune thrombocytopenia (ITP). Br J Haemat 163:10–23
18. Usmani NG, Woda A, Newburger PE (2013) Advances in understanding the pathogenesis of HLH. Br J Haemat 161:609–622
19. Rozman P (2002) Platelet antigens. The role of human platelet alloantigens (HPA) in blood transfusion and transplantation. Transplant Immunol 10:165–181
20. Warkentin TE, Sheppard JA, Heels-Ansdell D et al (2013) Heparin-induced thrombocytopenia in medical surgical critical illness. Chest 144(3): 848–858
21. Peck-Radosavljevic M (2001) Hypersplenism. Eur J Gastroenterol Hepatol 13:317–323

22. Hardy JF, de Moorloose P, Samama M (2004) Massive transfusion and coagulopathy: pathophysiology and implications for clinical management. Can J Anesth 51(4):293–310
23. Thiele T, Selleng K, Selleng S et al (2013) Thrombocytopenia in the Intensive Care Unit – diagnostic approach and management. Semin Hematol 50(3):239–250
24. Dunn AL (2009) Disseminated intravascular coagulopathy. In: Hillyer CD, Shaz BH, Zimring JC, Abshire TC (eds) Transfusion Medicine and hemostasis: clinical and laboratory aspects. Elsevier, Burlington/London
25. Hui P, Cook DJ, Lim W et al (2011) The frequency and clinical significance of thrombocytopenia complicating critical illness. Chest 139(2): 271–278
26. Stansbury LG, Hess AS, Thompson K et al (2013) The clinical significance of platelet counts in the first 24 hours after severe injury. Transfusion 53(4):783–789
27. Trehel-Tursis V, Louvain-Quintard V, Zarrouki Y et al (2012) Clinical and biologic features of patients suspected or confirmed to have heparin-induced thrombocytopenia in a cardiothoracic surgical ICU. Chest 142(4):837–844
28. Warkentin TE, Levine MN, Hirsch J et al (1995) Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. N Engl J Med 332:1330–1335
29. Martel N, Lee J, Wells PS (2005) Risk for heparin-induced thrombocytopenia with unfractionated and low molecular-weight heparin thromboprophylaxis: a meta-analysis. Blood 106:2710–2715
30. Warkentin TE, Sheppard JAI, Sigoin CS et al (2006) Gender imbalance and risk factor interactions in heparin-induced thrombocytopenia. Blood 108:2937–2941
31. Chong BH (2003) Heparin induced thrombocytopenia. J Thromb Haemost 1:1471–1478
32. Amiral J, Bridey F, Dreyfus M et al (1992) Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. Thromb Haemost 68:95–96
33. Newman PM, Chong BH (2000) Heparin-induced thrombocytopenia: new evidence for the dynamic binding of purified anti-PF4–heparin antibodies to platelets and the resultant platelet activation. Blood 96: 182–187
34. Tardy-Poncet B, Piot M, Chapelle C et al (2009) Thrombin generation and heparin-induced thrombocytopenia. J Thromb Haemost 7:1474–1481
35. Greinacher A, Warkentin TE (2006) Recognition, treatment, and prevention of heparin-induced thrombocytopenia: review and update. Thromb Res 118:165–176

36. Warkentin TE (2002) Platelet count monitoring and laboratory testing for heparin-induced thrombocytopenia. Recommendations of the College of American Pathologists. Arch Pathol Lab Med 126:1415–1423
37. Backhoul T, Greinacher A (2012) Recent advances in the diagnosis and treatment of heparin-induced thrombocytopenia. Ther Adv Hematol 314:237–251
38. Linkins LA, Dans AL, Moores LK et al (2012) Treatment and prevention of heparin-induced thrombocytopenia: antithrombotic therapy and prevention of thrombosis, 9th ed: America College of Chest Physicians Evidence-based Clinical Practice Guidelines. Chest 141:e495S–e530S
39. Watson H, Davidson S, Keeling D (2012) Guidelines on the diagnosis and management of heparin induced thrombocytopenia: second edition. Br J Haematol. doi:10.1111/bjh.12059
40. Lo GK, Juhl D, Warkentin TE et al (2006) Evaluation of pretest clinical score (4 T's) for the diagnosis of heparin-induced thrombocytopenia in two clinical settings. JTH 4:759–765
41. Cuker A, Arepally G, Crowther MA et al (2010) The HIT Expert Probability (HEP) Score: a novel pre-test probability model for heparin-induced thrombocytopenia based on broad expert opinion. J Thromb Haemost 8:2642–2650
42. Messmore HL, Fabbrini N, Bird NL et al (2011) Simple scoring system for early management of heparin-induced thrombocytopenia. Clin Appl Thromb Hemost 17:197–201
43. Powner DJ, Hoots WK (2008) Thrombocytosis in the NICU. Neurocrit Care 8:471–475
44. Vannucchi A, Barbui T (2007) Thrombocytosis and thrombosis. Hematology 2007:363–370

Chapter 7
Adverse Transfusion Reactions in Critically Ill Patients

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As transfusion entered routine clinical practice in the mid-twentieth century, it was apparent that the benefits were counterbalanced by unwanted reactions both of infectious and noninfectious nature [1, 2]. Whereas the former received wide attention also by the general population [3], the latter mainly remained of restricted interest to transfusion scientists (and naturally to the patients). It is a well-known fact that in the past 25 years, blood testing and donor selection have had a notable impact on reducing infectious complications [4, 5] and today, noninfectious adverse reactions to transfusion (NIART) are prevalent. If we look at the UK's Serious Hazards of Blood Transfusion hemovigilance data for 2012 [6], of the 538 cases analyzed only 3 were transfusion-transmitted infections; 372 acute transfusion reactions, 42 hemolytic transfusion reactions, 11 transfusion-related acute lung injuries, and 82 transfusion-associated circulatory overload. Hemovigilance data for our region, Friuli Venezia Giulia (North East Italy), are presented in Table 7.1 [7].

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Table 7.1 Adverse transfusion reactions in Friuli Venezia Giulia 2007–2012

Adverse transfusion reactions	2007–2009	2010	2011	2012	Total 2007–2012
Febrile nonhemolytic transfusion reactions (FNHTR)	195	52	70	47	**364**
Allergic transfusion reactions (ATR)	140	39	42	49	**270**
Circulatory overload	20	8	1	3	**32**
Hypotension	14	3	3	3	**23**
Severe dyspnea	4	4	3	1	**12**
Delayed hemolytic transfusion reactions (DHTR)	6	0	1	0	**7**
Anaphylaxis	6	2	1	4	**13**
Transfusion-associated graft versus host disease (TA-GVHD)	1	0	0	0	**1**
Transfusion errors	7	0	5	1	**13**
Transfusion-related acute lung injury (TRALI)	2	0	0	0	**2**
Septic complications	0	1	0	0	**1**
Others	77	15	20	12	**124**
Total adverse reactions	**472**	**124**	**146**	**120**	**862**
Total transfused units	**219,129**	**71,147**	**72,728**	**70,488**	**433,492**
Frequency of adverse reaction per unit (%)	**0.22**	**0.17**	**0.20**	**0.17**	**0.20**

The critically ill patient can be affected by both infectious and noninfectious adverse reactions after a transfusion therapy and the importance of diagnosis is remarkable for the severity of clinical conditions usually treated in an intensive care unit. Transfusion reactions, in fact, can be masked by the severity of the main illness and the lack of active collaboration of the patient [8].

The aim of this chapter is to give an overview of the most common adverse transfusion reactions.

7.1 Infectious Adverse Reactions to Transfusion (IARTs)

IARTs can be caused by viruses, bacteria, and protozoa. Potentially, an undefined number of infective agents are liable to transmit a disease after a transfusion, but we shall consider the most frequent and pathogenic. In this field, it is important to know that not all infectious reactions have the same incidence in different countries, and for this reason the policy of detecting tests varies from USA [9] and Europe, and at the same time among European countries (EU). In this paper, we will focus on Italian policy, which is harmonized with EU regulations.

7.1.1 Viruses

The transmission of viruses after a transfusion therapy is usually due to the presence of the infective agent in the circulation of the donor.

In the past 30 years, the risk of transmitting a virus infection with transfusion has greatly decreased because of the development of microbiological research and new detection techniques (serological and nucleic acid testing (NAT)). At the same time,

more restrictive donor selection criteria and pathogen reduction or inactivation technologies are usually employed to further reduce the risk of infection [10]. Residual risk is due to asymptomatic donors who donate in the "window period."

Table 7.2 summarizes information related to the principal virus infections potentially transmitted by transfusion.

7.1.1.1 Management

It is useful for ICU specialists to know the main transfusion-related viral infections. In fact, differently from the main immunological adverse reactions, the symptoms of IARTs can appear some days after transfusion and can be confused with the main disease. Particularly, it is necessary to pay attention to patients with a compromised immunological system who need immediate therapy to stop virus replication.

7.1.2 Bacteria

Bacteria infections following transfusion (Table 7.3) are often derived from microbial flora present on donor skin which contaminate blood products. They can also be due to systemic bacterial infections, though this is a rare event. From 2008, the Italian National Blood Center recommends using the first 40 ml of collected blood for testing, diverting it in tubes during withdrawal.

Regarding the kind of blood components, platelet concentrates are more frequently involved in IARTs, because their storage is at room temperature (22 ± 2 °C). However, medical and nursing staff must inspect the blood component before administration to check for integrity of bags, hemolysis, change in color, gas formation, and clots. Any of these findings must be communicated to the transfusion center to which the product must be returned.

Table 7.2 Main viruses involved in IARTs

Virus	Symptoms	Risk of IARTs	Policy of donor testing and deferral
Hepatitis A virus (HAV) [11]	Only acute phase with jaundice, hepatomegaly, dark urine, anorexia, malaise, fever, nausea, abdominal pain and vomiting	Rare. The transmission is fecal–oral and usually the donor is symptomatic in viral phase. Vaccine is available	No test in routine
Hepatitis B virus (HBV) [11]	The incubation phase is 30–180 days. After this acute phase, sometimes fulminant effect, and in some cases chronic progression (10 %)	Transmission is parenteral, sexual, and perinatal. Vaccine is available	HBsAg serological assay and HBV-DNA NAT
Hepatitis C virus (HCV) [11]	The incubation phase is 15–160 days. The acute phase can be often asymptomatic and chronic progression (50–70 %) is more frequent	Actual residual risk in Italy 0.2×10^6 [12]. Transmission is parenteral, sexual, and perinatal. Vaccine is not available	HCV antibody serological assay and HCV-RNA NAT
Hepatitis D virus (HDV) [11]	The infection is possible only in the presence of HBV. The acute phase can be more severe because of coinfection	Data not available, and in any case lower than hepatitis B	Tests performed for HBV are suitable to prevent the infection

(continued)

Table 7.2 (continued)

Virus	Symptoms	Risk of IARTs	Policy of donor testing and deferral
Human Immunodeficiency Virus (HIV) 1–2 [13]	The incubation phase is 7–28 days. Acute phase with fever, malaise, skin rashes, lymphoadenopathy. After that asymptomatic period for years with persisting viremic phase until the loss of CD4+ lymphocytes	Actual residual risk in Italy 0.4×10^6 [12]. Transmission is parenteral, sexual, and perinatal. Vaccine is not available	HIV 1–2 antibody serological assay and HIV-RNA NAT
Human T-cell Lymphotropic Virus (HTLV) [13, 14]	Most infections are asymptomatic. In some cases tropical spastic paraparesis, T-cell leukemia–lymphoma	Very rare in Italy. Present in tropical areas and Japan. Transmission is parenteral, sexual, and perinatal. Vaccine is not available	Not tested routinely
Cytomegalovirus (CMV) [15]	Acute phase quite asymptomatic or self-limited with fever, malaise, hepatosplenomegaly, and skin rash in immunocompetent patients. The infection is very frequent and increases with age.	Not clinically significant in immunocompetent patients. Dangerous if perinatal and after transfusion in premature infants and hematopoietic stem cell transplantation patients	Serological detection of antibodies in donors and reserved negative blood components for critical situations

West Nile Virus (WNV) [16]	The incubation period is within 28 days after contact and the only acute phase can be asymptomatic or presents fever, headache, vomiting, lymphocytopenia, muscle weakness, and headache. Sometimes sign of peripheral demyelinization and in elderly severe neurological disease	Transmitted through mosquitoes. Vaccine is not available	NAT testing for blood donors coming from endemic areas, usually limited to the warm season
Dengue	The infection is characterized by a different range of outcomes, from asymptomatic viral spread, a mild fever, or a shock syndrome. The first viral phase can be asymptomatic.	Transmitted through mosquitoes in tropical areas. Vaccine is not available	Not tested routinely. NAT testing is available, but usually deferral of donors coming from endemic areas for 28 days

Table 7.3 Main bacteria involved in IARTs

Bacteria	Symptoms	Contamination source and risk	Policy of donor testing and deferral
Treponema pallidum	Agent of syphilis. Incubation period about 7–21 days. Primary phase with the presence of ulcer in the injection site and regional lymphoadenopathy (not present in IARTs). Secondary phase after months with skin rash and later phase after years with neurological and cardiovascular symptoms	Donor blood. Transmission is parenteral, sexual, and perinatal	Antigen serological assay
Staphylococcus spp., *Pseudomonas* spp., *Escherichia coli*, *Enterobacteriacea*	Usually high fever (more than 2 °C), chills, malaise, and diffuse pain	Skin of the donor or devices	Isolation of the agent with microbiological techniques
Borrelia burdgoferi	The agent of Lyme disease. The transmission is by ticks in a sylvatic cycle involving primates. In an early phase a characteristic skin rash is present, while in the later one, after years, neurological and cardiovascular symptoms	Blood of the donor for IARTs.	After tick contact, a donor is deferred for 40 days to donation. If symptoms appear, antibiotic treatment is mandatory until serological resolution

7.1.2.1 Management

Several bacteria are involved in IARTs, but symptoms of infection are usually the same like high fever (an increase >2 °C), chills, malaise, and diffuse pain. If the symptoms appear during transfusion, therapy must be stopped and the residual blood component sent to the transfusion center. It is mandatory to perform a blood culture for identification of microbial agent and begin immediately an antibiotic and antipyretic therapy. Following the laboratory result, pharmacological therapy can be modified to become more effective.

7.1.3 Protozoa

The transmission of protozoa after transfusion is unequivocally due to the presence of the agent in the circulating blood of the donor. Sometimes it is not easy to identify infective donors, because some of these protozoa give no symptoms for years. In Italy, donor selection criteria specify a period of deferral for individuals who were born or visited endemic areas. The main problem with protozoan infections is the globalization in tourism and immigration from countries in which infection is endemic (Table 7.4).

7.1.3.1 Management

A protozoan infection can be detected with a peripheral blood smear which may be followed by a serological assay. The precision of diagnosis is very important for a timely treatment, because often the acute phase is severe and involves different body systems. Chemotherapy is targeted for each different agent and in all cases the diagnosis must be notified to the transfusion center.

Table 7.4 Main protozoa involved in IARTs [17]

Protozoa	Symptoms	Risk of IARTs	Policy of donor testing and deferral
Plasmodium spp.	Agent of different types of malaria. Acute phase of recurrent high fever, hemolysis, chills, jaundice, and hepatosplenomegaly. Possible chronic phase asymptomatic for years	Rare in Italy (not endemic area)	Serological assay, not used routinely. Deferral for 6 months for travellers, 5 years for immigrants from endemic areas. After the disease, the donor is deferred for 3 years after which only plasma for industrial purposes can be donated
Trypanosoma cruzi	Agent of Chagas' disease. Acute phase is self-limited, but chronic phase can be asymptomatic for years until development of gastrointestinal and cardiac symptoms	Rare in Italy (not an endemic area)	Serological assay, not used routinely. Deferral for 3 months for travellers, 5 years for immigrants from endemic areas
Toxoplasma gondii	Acute phase quite asymptomatic in immunocompetent patients. The infection is very frequent and increases with age	Not clinically in immunocompetent patients. Dangerous in pregnancy, immunocompromised individuals like premature infants and hematopoietic stem cell transplantation patients	Serological assay in some donors. Usually leukoreduction of blood components

7.1.4 Emerging Infections

In the past years, numerous emerging infections have been described in different areas of the world. Because of globalization of travel and immigration, it is a challenge for transfusion centers in the prevention of emerging IARTs [18].

The variant of Creutzfeldt–Jakob Disease (vCJD) is transmissible spongiform encephalopathy. Like the primitive CJD, it results from the changing of a prion protein into a protease-resistant form (PrP Sc). Originally, bovine spongiform encephalopathy affected cattle. The use of animal protein in bovine feed diffused the disease in cows. Successively, meat consumption by humans was responsible for the variant of Creutzfeldt–Jakob Disease, which has an earlier onset with neurological manifestations, dementia, and death in 7–38 months. At present, there are no invasive tests available for donors or patients and diagnosis is mainly confirmed postmortem. The policy for blood collection consists in deferring donors who lived in the UK (the area of first onset of the disease) from 1980 to 1992 and donors who present neurological diseases [19].

Severe Acute Respiratory Syndrome (SARS) is a recent disease emerged explosively in Asia in 2004. The coronavirus agent can cause pneumonia with rapid onset and is often fatal. The transmission by transfusion is not clearly detected, but it can be possible in the asymptomatic viral phase. Quarantine and traveler surveillance is employed in airports during the endemic period.

Middle East Respiratory Syndrome (MERS) is due to another *coronavirus* identified in Saudi Arabia in 2012. The virus can affect many types of animals, but recently dromedaries seem to be the most important source of infection for humans. Actually interhuman transmission is not demonstrated. Most infected patients report a severe respiratory disease with acute renal failure and high fatal rates [20]. As during SARS

pandemia, travelers' surveillance in airports is important in the endemic periods.

Xenotropic murine leukemia virus-related virus (XMRV) is a recent discovery and reported in uncertain association with chronic fatigue syndrome (CFS). This disease can potentially be transmitted by transfusion, but more extensive studies are needed to define the pathology [21].

7.1.5 A General Comment

Medical doctors when faced with an infectious disease in a hospitalized patient should always collect an accurate clinical history that must include transfusion of blood components and take into consideration that the viral/bacterial/protozoan infection could be related to a transfusion event. If a transfusion-transmitted infection is suspected, the clinician must contact the transfusion center that will provide a look-back of the blood products and a follow-up of the involved donors.

7.2 Noninfectious Adverse Reactions to Transfusions (NIARTs)

There are many excellent reviews on noninfectious transfusion complications published in journals or as chapters in textbooks [22–24], and this paper does not intend duplicating them. Our aims are to illustrate different criteria with which NIARTs have been classified, mention the most important pathogenetic mechanisms involved, suggest organizational measures that hospitals may adopt to manage NIARTs, and discuss the laboratory's support for NIART diagnosis. Although NIARTs have different grades of severity, it must be underlined that most adverse reactions can occur in critically ill patients.

7.2.1 Classification of NIARTs

There are different ways in which NIARTS can be classified: according to time of presentation (acute, within 24 h or delayed, after 24 h from the transfusion event) or according to pathogenesis (immunologic vs. nonimmunologic). Whereas the former is a more practical classification oriented to clinicians, the latter is of greater interest for the transfusion scientist.

Classification of NIARTs according to pathogenesis (with the exception of transfusion errors, see following paragraph) is presented in Table 7.5.

It may be incorrect to include transfusion errors in Table 7.5, mainly because part of transfusion errors (those which are AB0 incompatible) would be registered under acute hemolytic reactions. However, since transfusion errors are an important source of adverse reactions we believe that it is useful to keep a focus on this type of unwanted event.

Klein and Anstee [24] use a more specific "pathogenetic" classification criterion in that they divide NIARTs into those due to red cell incompatibility, leukocyte antibodies, platelet antibodies, reactions to transfused proteins, and nonimmunological reactions.

7.2.2 Pathogenesis of NIARTs

The aim of this section is to give an overview of the main NIARTs, irrespective of their severity.

Tables 7.6 and 7.7 summarize the main mechanisms responsible for immunological and nonimmunological NIARTs, respectively, as well as the incidences as reported in literature. As far as the latter are concerned, it should be kept in mind that correct estimates are very difficult to obtain and vary according to clinical setting, accuracy of reporting, type of blood component transfused, and whether a transfusion event or number of

Table 7.5 A classification of NIARTS

Mechanism	NIART
Immunological	Acute hemolytic transfusion reaction
	Delayed hemolytic transfusion reaction
	Allergic transfusion reaction
	Anaphylaxis
	Febrile nonhemolytic transfusion reactions (FNHTRs)
	Platelet refractoriness
	Transfusion-associated graft versus host disease (TA-GVHD)
	Transfusion-related acute lung injury (TRALI)
	Posttransfusion purpura (PTP)
	Immunomodulation
Nonimmunological mechanisms	Septic complications
	Red cell hemolysis
	Circulatory overload
	Iron overload
	Hypotension
	Metabolic complications
	Citrate toxicity and hypocalcemia
	Hypothermia
Errors (misidentification, clerical mistakes, etc.)	Transfusion errors (transfusion of a unit to the wrong patient)

units are considered in the denominator. A specific reference is included for all NIARTS.

7.2.3 Transfusion Errors

Transfusion of a RBC unit to the "wrong patient" is a very significant problem in transfusion medicine although it is only part of a wider problem of hospital adverse events due to misidentification [35]. It is estimated that a transfusion error

Table 7.6 Pathogenesis of NIARTs with an immunological mechanism

NIART	Pathogenesis in brief	Incidence
Acute hemolytic transfusion reaction [25]	Binding of recipient antibodies (usually anti-A, anti-B, or anti-A,B) to incompatible RBCs. Activation of complement cascade, intravascular RBCs lysis, release of Hb in plasma. DIC. TRANSFUSION ERRORS MAIN UNDERLYING CAUSE	1:38,000–1:70,000
Delayed hemolytic transfusion reaction [25]	Usually secondary immune response with increase in antibody titres following "re-challenge" with incompatible RBCs. No complement activation or only up to C3. Destruction of RBCs occurs in extravascular space by MPS (mainly spleen and liver). Drop in Hb, increase in bilirubinemia 3–15 days posttransfusion	1:5,000–1:11,000
Allergic transfusion reaction [24]	Mild IgE-mediated reactions against soluble substances present in plasma; release of histamine leads to urticaria and pruritis	1:100–1:33
Anaphylaxis [26]	Severe IgE-mediated reactions against plasma proteins. Histamine and other biological mediators are responsible for severe systemic reactions, which lead to laryngeal edema, lower airway obstruction, hypotension. Recipients with congenital IgA deficiency are particularly at risk; in this case, the reaction is mediated by high-titre anti-IgA	1:20,000–1:50,000
Febrile nonhemolytic transfusion reactions [27]	Anti-HLA or other anti-leukocyte antibodies react with WBCs present in RBC or platelet units. Complement binding leads to WBC lysis and release of pyrogens (TNF-α, Il-1, Il-6). Transfusion of "old units" (mainly platelets) containing cytokines may also be responsible	PLTs:1:100

(continued)

Table 7.6 (continued)

NIART	Pathogenesis in brief	Incidence
Platelet refractoriness [28]	HLA antibodies (due to previous pregnancies, transplants, or transfusions) are more commonly implicated, followed by anti-ABO or anti-HPA. Platelets become coated with HLA antibodies and are then removed by MPS	Not available
Transfusion -associated graft versus host disease [29]	Viable lymphocytes present in transfused unit are not recognized as foreign and exert an alloimmune response toward the recipient's cells leading to rash, abdominal pain, diarrhoea, liver function abnormality, and bone-marrow suppression 2–30 days following transfusion	Not available (very rare)
Transfusion-related acute lung injury [30]	Donor anti-HLA or anti-granulocyte antibodies bind to the host's granulocytes causing pulmonary leucostasis and complement-mediated leucocyte activation. This leads to endothelial damage in pulmonary capillaries through release of proteolytic enzymes and toxic oxygen metabolites from neutrophils. Alternative hypothesis: cumulative effect of 2 conditions: First, adherence of patient's neutrophils to pulmonary vascular endothelium and second, presence of lipids or cytokines or leucocyte antibodies in transfused plasma cause further neutrophil activation and endothelial damage	1:5,000–1:190,000
Posttransfusion purpura [31]	Anti-platelet antibodies (usually anti-HPA-1a) in recipient react with transfused platelets leading to their elimination. The reaction, however, for some as yet unexplained mechanism may also regard the patient's own platelets	Not available (very rare)
Immunomodulation [32]	Is matter of controversy. Donor factors (WBCs or other) would be responsible for immunosuppression in host	Not available

Table 7.7 Pathogenesis of NIARTs with nonimmunological mechanism

NIART	Pathognesis in brief	Incidence
Septic complications [33]	Transfusion of unrecognized septic blood components, mainly platelets	Not available
Red cell hemolysis (nonimmunological) [25]	Transfusion of RBCs damaged by excessive heating or freezing/thawing. Inappropriate administration of medication simultaneously to RBC units. Transfusion of contaminated RBC units or RBCs from donors with congenital RBC defects (G6PD deficiency)	Not available
Circulatory overload [34]	Occurs in patients with compromised cardiac status who cannot cope with increased intravascular volume; if cardiac output cannot be maintained, pulmonary edema results	<1 %
Iron overload [24]	Is seen in multitransfused patients and is due to the fact that iron intake with transfusions is very high compared with the capability of excretion (1 unit contains ~200 mg of iron whereas daily excretion amounts to 1 mg). Chelation therapy is essential to limit damage related to deposit of iron in vital organs (heart, pancreas, liver, gonads, etc.)	In all cases of multitransfused recipients. Variable degree
Hypotension due to ACE inhibitors [23]	Patients receiving ACE drugs may experience hypotension if transfused with components (mainly platelets) filtered with bedside leukoreduction filters. Bradykinin is a vasodilatory peptide which is released when blood comes into contact with negatively charged surfaces	Not available

(continued)

Table 7.7 (continued)

NIART	Pathogenesis in brief	Incidence
Metabolic complications [24]	Neonates and small children are mainly at risk. Increase in potassium, ammonium. Acidosis. Changes in RBCs occur as units age (storage lesion)	Not available
Citrate toxicity and hypocalcemia [24]	Transfusion of large volumes of citrated blood may lead to a decrease of ionized calcium levels which can have a negative effect on cardiac contractility	Not available
Hypothermia [24]	May occur when large volumes of cold blood are transfused at high rates mostly to neonates and children	Not available

RBC red blood cells, *Hb* hemoglobin, *HLA* Human Leukocyte Antigens, *WBCs* white blood cells, *HPA* Human Platelet Antigens, *MPS* Mononuclear Phagocyte System, *ACE* Angiotensin Converting Enzyme, *DIC* disseminated intravascular coagulation

occurs about 1:16,000 transfused units, and in the majority of cases it is due to misidentification of the patient. An AB0-incompatible transfusion error will occur 1:33,000 units; 50 % of these will give rise to hemolysis but the mortality incidence due to an incompatible transfusion is calculated as being 1:800,000 [4]. These figures are surely underestimated due to the legal implications of reporting a transfusion-associated mistake. It is interesting to note that the Joint Commission International accreditation system [36] quite rightly consider transfusion errors as "sentinel events," which implies that a thorough root-cause analysis must be performed in the health facility where the event occurs. Today, technology for the prevention of transfusion errors is available [37] and hospitals should consider its implementation.

7.2.4 Organizational Measures for the Management of NIARTs

Every health facility practicing transfusion therapy should have a system in place to ensure the highest possible level of safety for the patients. Figure 7.1 depicts the main critical points for a safe transfusion. Transfusion requests originate in the ward and clinicians first of all should ask themselves whether their patient does in fact need the transfusion. This means that clinicians should rely on guidelines for which there is a wide consensus on the appropriate use of blood. An important role in this regard is played by the Hospital Transfusion Committee, which is the ideal forum in which these documents are prepared and shared by the hospital medical staff. Clinicians should always bear in mind that the safest transfusion is the one which is not performed. A written request form must be made specifying correct patient data (name, surname, place, and date of birth), condition requiring transfusion, ward, type and number of blood components required, urgency of transfusion, blood group and

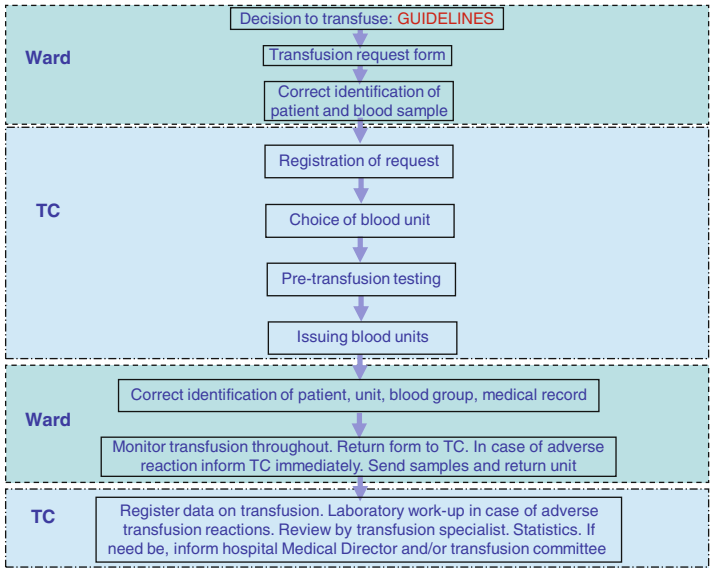

Fig. 7.1 Duties and responsibilities of ward and transfusion center (*TC*) in order to guarantee a safe transfusion

red blood cell antibody status if previously determined, history of sensitization episodes, previous NIARTs, signature of medical doctor. Fulfilling the request form correctly is the first step toward a safe transfusion. The second step relates to withdrawing blood samples; patient must be identified at the bedside; when possible the patient should be asked his/her name and surname and date of birth, otherwise the patient record must be used. Here again it must be pointed out that many transfusion errors are made at this truly critical point. Traceability (an important ingredient of safety) is enhanced by bar codes on the request form and sample labels. Moreover, as far as AB0 and Rh testing is concerned, it should be performed previous to the request form on a separate blood sample. This is to reduce the

risk of mistakes due to swapping of patients. The nurse or medical doctor who withdraws blood should sign the label on blood sample tube to testify that patient identity has been checked.

The request form and sample is then sent to the transfusion center; the date and time of arrival must be registered and the coherence of data reported on the request form and on the sample label checked. Each center should have a policy that clearly states when samples should be rejected. Transfusion centers should have computer software (with adequate backups) on which to store patient data (as already mentioned, barcodes are very useful and should be used whenever possible). The best softwares are those that allow a complete traceability "from vein to vein" (donor to recipient) thus permitting to perform look-back studies should it be necessary.

The transfusion technician should check that the patient for whom blood components are being requested has been tested for AB0 and Rh and has undergone red cell antibody screening (if red blood cells are needed). The transfusion center should have a Standard Operating Procedure (SOP) in place specifying what type of compatibility testing is performed for which type of patient (e.g., type and screen for nonimmunized recipients, cross-matching for immunized patients). The SOP should also specify what tests should be performed in case of very urgent cases. Once the request form is registered, pretransfusion testing can be performed. If we consider safety at 360° as it ought to be, we must also mention that quality of testing plays a major role in transfusion safety. This means that a quality management system should be in place in the immunohematology laboratory foreseeing SOPs for all activities (testing, blood component preparation, and storage), internal quality control on reagents and blood components (including sterility), external proficiency testing (for blood groups, antibody screening, and identification), maintenance program for instruments, a training program for the staff, and a quality improvement scheme.

Once testing is completed (an AB0 direct test should be performed on all samples accompanying the request form), compatible units are chosen according to an SOP; this document should also state in which conditions nonideally compatible units can be issued (e.g., group 0 Rh positive red blood cell units for 0 Rh negative recipients). The issuing of blood components should be registered on the software, which also prints a form that accompanies the units on which patient data number and blood group of unit are reported. This "transfusion form" ideally should be divided in two with one section that must be returned to the transfusion center testifying that the patient has been transfused; should adverse reactions be observed, these should be registered on this form. Units are then sent to the ward (in appropriate containers at controlled temperature). Before transfusion, personnel (in Italy a nurse and medical doctor) should check, with the aid of a checklist, the patient's identity (possibly asking name, surname, and date of birth), blood group on patient record, blood unit label, and transfusion form. This check is pivotal for the safety of transfusion since it is the last chance for a transfusion error to be stopped. In fact, it has been published many times that a superficial pretransfusion check at the patient's bedside is the single most frequent cause of transfusion mistakes.

Transfusion then starts and must be monitored for the appearance of adverse reactions. It is of fundamental importance for clinicians and nurses who are directly involved in transfusing patients to be aware of the different NIARTs in order to recognize them promptly and give appropriate treatment. It is a good measure to register in the patient's record the blood pressure, heart rate, and temperature before and after the end of transfusion. One of the difficulties concerns differential diagnosis of NIARTs in that many signs and symptoms are common to more than one reaction; Table 7.8 reports some signs and symptoms of NIARTs (for a more thorough description see specific references).

Table 7.8 Some signs and symptoms of more common NIARTs

NIART	Onset	Fever	Urticaria/ rash	Shock	Respirat. distress/ dyspnea	(Shaking) chills	Increase bilirubinemia	Drop Hb	Drop PLTs	Hemoglobi-nemia	Hemoglo-binuria	DIC
Acute hemolytic transfusion reaction	Immediate	X		X	X	X				X	X	X
Delayed hemolytic transfusion reaction	3–10 days	X					X	X				
Allergic transfusion reaction	Immediate		X									
Anaphylaxis	Immediate			X	X							
Febrile nonhemolytic transfusion reaction	Immediate	X				X						
Transfusion-associated graft versus host disease	2–30 days		X				X					

(continued)

Table 7.8 (continued)

NIART	Onset	Fever	Urticaria/ rash	Shock	Respirat. distress/ dyspnea	(Shaking) chills	Increase bilirubinemia	Drop Hb	Drop PLTs	Hemoglobi-nemia	Hemoglo-binuria	DIC
Transfusion-related acute lung injury	<6 h	X			X							
Posttransfusion purpura	5–10 days								X			
Circulatory overload	Immediate				X							

A second difficulty concerns the fact that fortunately, the majority of NIARTs are rare events and therefore personnel may have little experience in dealing with them. Third, in some cases (patients in intensive care units for example) symptoms may be heavily modified by the patient's clinical condition (hemoglobinuria may be the only sign of an acute hemolytic transfusion reaction in patients under anesthesia). Finally, for reactions occurring at considerable time period from transfusion (post-transfusion purpura, delayed hemolytic transfusion reaction, transfusion-associated graft vs. host disease), establishing a link between symptoms and the transfusion event may not be so obvious.

Once an acute NIART is suspected or diagnosed (during transfusion), TRANSFUSION MUST BE STOPPED IMMEDIATELY, but a line must be kept for infusion if necessary. The transfusion center must be promptly informed and a blood sample together with the remains of the transfusion unit must be sent to the transfusion center together with a description of signs and symptoms (on transfusion form). Treatment must be started immediately and specialized opinion may be sought from an intensive care unit specialist or from a nephrologists or other specialist. A synthesis of the most important therapeutic and preventive strategies for immunologic NIARTs is shown in Table 7.9. Data regarding any NIART should be registered on the patient's medical record for future preventive measures.

7.3 Concluding Remarks

Blood transfusion is a complex procedure and guaranteeing a safe transfusion requires a joint effort from the clinician and transfusion specialist. As we all know, a "zero risk" transfusion does not exist and thus risk management systems must be implemented since knowing the extent of risk is the first step for

Table 7.9 Therapy and prevention for some immunological NIARTs

NIART	Therapeutic strategy	Preventive strategy
Acute hemolytic transfusion reaction	STOP TRANSFUSION, support blood pressure (low dose dopamine), support urine output (diuretics). Plasma if needed to correct DIC. Analgesics if necessary	Correct patient identification at all stages. Infuse slowly at beginning of transfusion
Delayed hemolytic transfusion reaction	Usually no treatment is necessary. Antipyretics	Patient in successive transfusions must receive cross-match negative, antigen negative RBCs
Allergic transfusion reaction	Interrupt transfusion; administer antihistamines (oral or IV). If urticaria and pruritis disappear, try continuing slowly	Premedication with anti-histamines. Steroids if severe. For refractory cases, use washed RBCs or PLTs.
Anaphylaxis	STOP TRANSFUSION. Epinephrine, antihistamines, steroids, fluids, oxygen	For IgA-deficient patients, use IgA-deficient blood components
Febrile nonhemolytic transfusion reaction	STOP TRANSFUSION, antipyretics	Premedication with antipyretics, leukocyte-reduced components
Platelet refractoriness	Monitor platelet counts at 1 and 24 h posttransfusion. Rule out nonimmunologic factors	HLA compatible platelets or cross-match negative platelets
Transfusion-associated graft vs. host disease	Rarely therapy is effective (steroids, immunosuppressive agents)	Gamma-irradiation of cellular blood components for selected cases
Transfusion-related acute lung injury	STOP TRANSFUSION. Oxygen, support respiration, Intensive Care Unit for intubation	Defer donors implicated in TRALI cases
Posttransfusion purpura	Intravenous immunoglobulin	For successive transfusions, use HPA compatible platelets

controlling it. This means that statistics must be prepared on all types of NIARTs and discussed at the Transfusion Committee. One of the obstacles that prevent NIARTs from being successfully managed is underreporting; this is due to underrecognition in the ward but also due to a reticence from clinicians who may be worried that, for example, a transfusion error may lead to legal problems. An anonymous form for adverse event reporting must be available in the wards. Clinicians must feel adequately supported by the transfusion laboratory which must perform all necessary tests in a timely manner. The final word goes to training; continuous updating and "refresher" courses should be given to both nurses and doctors working at the patient's bedside as well as to personnel working in transfusion centers to allow prompt recognition, laboratory diagnosis, effective treatment, and implementation of prevention strategies in order to guarantee an as-safe-as-possible transfusion for our patients.

References

1. Rossi E, Simon T, Moss GS, Gould SA (1996) Transfusion into the next millennium. In: Rossi E, Simon T, Moss GS, Gould SA (eds) Principles of transfusion medicine, 2nd edn. Williams and Wilkins, Baltimore, pp 1–11
2. Franklin IM (2001) Introduction. In: Murphy MF, Pamphilon DH (eds) Practical transfusion medicine. Blackwell Science, London, pp 3–12
3. Gedye R (1993) German AIDS scandal infects Europe. BMJ 307:1229
4. Goodnough LT, Shander A, Brecher ME (2003) Transfusion medicine: looking to the future. Lancet 361:161–169
5. Perkins HA, Bush MP (2010) Transfusion associated infections: 50 years of relentless challenges and remarkable progress. Transfusion 50:2080–2099
6. Bolton-Maggs P, Thomas D. Annual SHOT Report 2012. www.shotuk.org
7. De Angelis V, Tillati S, Totis V, Dolfini P, Franchi D, Mazzi G, Zandomeni L (2012) Il sistema trasfusionale del Friuli Venezia Giulia nel 2012. (available from authors on request)
8. Kuriyan M, Carson JL (2004) Blood transfusion risk in the intensive care unit. Crit Care Clin 20:237–253

9. Goodnough LT (2013) Blood management: transfusion medicine comes of age. Lancet 381:1791–1792
10. Custer B (2013) Update on pathogen reduction technology. ISBT Sci Ser 8:80–84
11. Dienstag JL, Isselbacher KJ (1998) Acute viral hepatitis. In: Harrison's principles of internal medicine, 14th edn. McGraw Hill, New York, pp 1677–1692
12. Velati C, Romanò L, Fomiatti L, Baruffi L, Zanetti AR, the SIMTI Research Group (2008) Impact of nucleic acid testing for hepatitis B virus, hepatitis C virus and human immunodeficiency virus on the safety of blood supply in Italy: a 6-years survey. Transfusion 48(10): 2205–2213
13. Fauci AS, Longo DL (1998) The human retroviruses. In: Harrison's principles of internal medicine, 14th edn. McGraw Hill, New York, pp 1105–1111
14. Stramer SL, Foster GA, Dodd RY (2006) Effectiveness of human T-lymphotropic virus (HTLV) recipient tracing (lookback) and the current HTLV-I and –II confirmatory algorithm, 1999 to 2004. Transfusion 46:703–707
15. Hirsh MS (1998) Cytomegalovirus and human herpesvirus types 6,7 and 8. In: Harrison's principles of internal medicine, 14th edn. McGraw Hill, New York, pp 1092–1095
16. Francis RO, Strauss D, Willliams JD, Whaley S, Shaz BH (2012) West Nile virus infection in blood donors in the New York City area during the 2010 seasonal epidemic. Transfusion 52:2664–2670
17. Shaz BH (2009) Transfusion transmitted diseases. In Hillyer CD, Shaz BH, Zimring JC, Abshire TC (eds) Transfusion Medicine and Hemostasis. Clinical and Laboratory aspects. Elsevier, Burlington pp 361–371
18. Dodd RY (2012) Emerging pathogens and their implications for the blood supply and transfusion transmitted infections. BJH 159:135–142
19. Zhou S, Fang CT, Schonber LB (2008) Transfusion transmission of human prion diseases. Transfus Med Rev 22:58–69
20. Guery B and the MERS CoV Study Group (2013) Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. Lancet 381:2265–2272
21. Simmons G, Glynn SA, Komaroff AL et al, for the Blood XMRV Scientific Research Working Group (2011) Failure to confirm XMRV/MLVs in the blood of patients with chronic fatigue syndrome: a multi laboratory study. Science 334:814–817.

22. Petrides M (2001) Adverse effects of transfusion. In: Petrides M, Stack G (eds) Practical guide to transfusion medicine. AABB Press, Bethesda, pp 117–150
23. Perrotta PL, Snyder EL (2001) Non-infectious complications of transfusion therapy. Blood Rev 15:69–83
24. Klein HG, Anstee DJ (2005) Some unfavourable effects of transfusion. In: Klein HG, Anstee DJ (eds) Mollison's blood transfusion in clinical medicine, 11th edn. Blackwell Science, Oxford, pp 666–700
25. Klein HG, Anstee DJ (2005) Haemolytic transfusion reactions. In: Klein HG, Anstee DJ (eds) Mollison's blood transfusion in clinical medicine, 11th edn. Blackwell Science, Oxford, pp 455–495
26. Gilstad CW (2003) Anaphylactic transfusion reactions. Curr Opin Haematol 10:419–423
27. Brand A (2002) Immunological aspects of blood transfusion. Transpl Immunol 10:183–190
28. Rebulla P (2005) A mini-review on platelet refractoriness. Haematologica 90:247–253
29. Sage D, Stanworth S, Turner D, Navarrete C (2005) Diagnosis of transfusion-associated graft-vs. host disease: the importance of short tandem repeat analysis. Transfus Med 15:481–485
30. Bux J, Sachs UJH (2007) The pathogenesis of transfusion-related acute lung injury (TRALI). BJH 136:788–799
31. Rozman P (2002) Platelet antigens. The role of human platelet alloantigens (HPA) in blood transfusion and transplantation. Transpl Immunol 10:165–181
32. Blajchman MA (2005) Transfusion immunomodulation or TRIM: what does it mean clinically? Hematology 10:208–214
33. Blajchman MA, Goldman M (2001) Bacterial contamination of platelet concentrates: incidence, significance, and prevention. Semin Haematol 38:20–26
34. Popovsky MA (2006) Pulmonary consequences of transfusion: TRALI and TACO. Transfus Apher Sci 34:243–244
35. Murphy MF (2006) Errors in transfusion medicine. Blood Ther Med 5:51–57
36. Joint Commission International Accreditation Standards for Hospitals, 5th edn (2014) http://www.jointcommissioninternational.org
37. Murphy MF, Kay DS (2004) Patient identification: problems and potential solutions. Vox Sang 87(Suppl 2):197–202

Chapter 8
Drugs and Blood Cells

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8.1 Introduction

Adverse drug reactions (*ADRs*) are negative side effects of drug therapy. From a pathogenetic point of view, ADRs are classified in two major types: *type A* ADRs, which are defined as dose-dependent, since they are attributable directly to the mechanism of action of the drug; and *type B* ADRs, which are defined as dose-independent, since they are mainly immune-mediated. Whereas type A ADRs are often predictable, and sometimes avoidable, conversely those of type B are almost always unpredictable in the first appearance. Many therapeutic drugs have been associated with ADRs that may affect circulating blood cells and blood forming tissues. Among drug-induced blood cytopenias, thrombocytopenia is the most represented.

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There are few recent epidemiological studies investigating the incidence of drug-induced blood dyscrasias. Indeed, the incidence appears to be low, on a population basis, as rough estimates account for less than 10 episodes per million inhabitants annually, even if underreporting has often been advocated. Nevertheless, drugs were recognized as the second leading cause of all cytopenias, accounting for 20–40 % of cases [1].

In terms of mortality, a study carried out in 1984 in the USA estimated 1,350 deaths attributable to drug-induced blood dyscrasias in that year. Approximately 30 % of fatal blood dyscrasias were caused by therapeutics drugs. Aplastic anemia was the leading cause of death, followed by thrombocytopenia, agranulocytosis, and hemolytic anemia [2].

ICU patients are particularly vulnerable to ADRs for several reasons, including the high number of coadministered medications and the peculiar pathophysiological status that may alter the pharmacokinetics of many drugs. The frequency of ADRs in the ICU is reported to be around 12 %, ranging between 2.3 and 34.1 % [3]. More than two-thirds of these ADRs are of type A. As far as the target of ADRs in ICU patients is concerned, a recent study highlighted that bone marrow and circulating blood cells rank first, accounting for 30 % of total cases, with thrombocytopenia being responsible for more than half of these events. Nervous system, endocrine apparatus, and liver follow. The most common causative drugs were amphotericin B, diltiazem, vancomycin, midazolam, and piperacillin/tazobactam [4].

The pathophysiology of drug-induced cytopenias is far from being definitive, mainly because a unified explanatory theory is still lacking. Various mechanisms, each specific for single drug reactions, have been gradually described.

As a general rule, drug-induced cytopenias could be referred to two main categories: those due to bone-marrow suppression, which are frequently of *type A*, and those due to increased lysis of circulating cells, which are *type B* ADRs [5]. Classification of ADRs due to drug-induced hematological disorders is depicted in Table 8.1, together with some examples. As far as the mechanism

Table 8.1 Adverse Drug Reaction Classification applied to drug-induced hematological disorders

Type	Characteristic	Mechanism		Examples
Type A	Dose-dependent Predictable			Chemotherapy-induced myelosuppression
Type B	Dose-independent Unpredictable	Nonimmune-mediated		Primaquine-induced hemolytic anemia, metformin-induced megaloblastic anemia, methotrexate-induced megaloblastic anemia, clozapine-induced neutropenia
		Immune-mediated	IgG-mediated cytotoxicity (type II Gell and Coombs)	Penicillin-induced hemolytic anemia, cephalosporin-induced hemolytic anemia, methyldopa-induced hemolytic anemia, aminopyrine-induced neutropenia
			Immune-complex deposition (type III Gell and Coombs)	Quinine-induced hemolytic anemia, heparin-induced thrombocytopenia, bevacizumab-related thrombocytopenia

of drug-associated cytopenias is concerned, most immune-mediated ADRs are antibody-mediated cytotoxic reactions (*type II*) and immune complex-mediated reactions (*type III*), according to the *Gell and Coombs classification*. Nonimmune-mediated cytopenias are typically due to *idiosyncrasy*, that is, an extreme susceptibility toward some drugs which is in relation to genetic and/or environmental factors. Enzymatic deficiencies (e.g., glucose-6-phosphate dehydrogenase deficiency responsible for primaquine-induced hemolytic anemia) or reactive intermediate metabolites (as in the case of clozapine-induced neutropenia) are most frequently responsible for these aberrant reactions.

From a clinical point of view, drug-induced hematological disorders are quite difficult to diagnose for at least three reasons: they occur rarely, clinical presentation may be indistinguishable from that induced by other causes of dyscrasias, and often the available literature is not exhaustive [6]. Moreover, they may have multifaceted clinical presentations, either in terms of the blood cell lines which are involved or of the time of onset after drug exposure, this spanning from few hours to several weeks.

From this analysis it follows that hematological ADRs represent challenging threats for clinicians, as phenotypic unpredictability is common place.

The aim of this chapter is to focus on the epidemiological, pathogenetic, clinical, and therapeutical aspects of drug-induced blood dyscrasias. Each blood cell line is considered separately for ease of consultation.

8.2 Drug-Induced Thrombocytopenia

8.2.1 Epidemiology

Drug-induced thrombocytopenia (*DIT*) was first noted in 1865, when Vipan reported the occurrence of purpura in patients treated with quinine. Nowadays, several epidemiological studies, performed both in the USA and in Europe, estimated the

minimum incidence of DIT at about 10 cases per million population per year in the general population, but higher estimates were shown in some settings, such as among hospitalized patients and among the elderly [7].

Among ICU patients, thrombocytopenia is a particularly frequent occurrence, as it was reported in 15–58 % of admitted patients, and DIT is reported in up to 25 % of acutely ill patients [8].

8.2.2 Causative Drugs

More than 200 drugs have been reported as possible causes of DIT and the list is expected to become longer in the ensuing years in the light of the increasing number of new biotherapeutic drugs which are becoming clinically available [9]. Among the various attempts at classifying levels of evidence of DIT, George et al. proposed in 1998 some specific criteria for the assessment of probability of causal relationship for DIT, on the basis of which four different levels of evidence were defined [10]. Essentially, four different criteria were identified: (1) Drug administration came before thrombocytopenia, and complete and sustained recovery was obtained after drug withdrawal; (2) the suspected drug was the only one administered before the onset of thrombocytopenia, or the other drugs were continued or reintroduced without any persistence of thrombocytopenia; (3) other etiologies of thrombocytopenia were excluded; (4) reexposure to the suspected drug led to recurrence of thrombocytopenia. On the basis of these criteria, four different levels of evidence were defined: Definitive: 4 criteria met; probable: the first 3 criteria are met; possible: criteria 1 is met; unlikely: criteria 1 is not met [10].

For comprehensive lists of drugs causing DIT, on the basis of these and of other criteria, the readers are referred to some recent reviews [11–13] and to the following URL: http://www.ouhsc.edu/platelets.

Some of the most relevant drugs with definite or probable evidence for DIT are listed in Table 8.2. Among drugs that are

Table 8.2 Drugs with definite or probable evidence for causality of drug-induced thrombocytopenia

Drug category	Level of evidence: definitive	Level of evidence: probable
Analgesics	Acetaminophen, diclofenac, meclofenamate, tolmetin,	Ibuprofen, naproxen, oxyphenbutazone, sulindac
Anti-infective drugs	Amphotericin B, cephalotin, ethambutol, interferon-alfa, isoniazid, methicillin, nalidixic acid, novobiocin, quinine, piperacillin, rifampicin, sulphisoxazole, TMT-SMX, vancomycin	Ampicillin, fluconazole, oxytetracycline
Antiepileptics		Carbamazepine, oxcarbazepin, phenytoin
Antineoplastics	Aminoglutethimide, rituximab, tamoxifen, trastuzumab	
Antirheumatics	Levamisole, sulfasalazine	Gold salts, infliximab
Cardiovascular drugs	Abciximab, alprenolol, amiodarone, amrinone, atorvastatin, heparin, diazoxide, digoxin, eptifibatide, methyldopa, minoxidil, nitroglycerine, oxprenolol, quinidine, tirofiban	Captopril, clopidogrel, hydrochlorothiazide, procainamide, rosuvastatin, simvastatin, ticlopidine
Gastrointestinal drugs	Aminosalicylic acid, cimetidine	Ranitidine
Psychotropic drugs	Chlorpromazine, diazepam, haloperidol, lithium, thiothixene	
Other drugs	Danazol, deferoxamine, diatrizoate, diethylstilbestrol, difluoromethylornithine, iopanoic acid, naphazoline, meglumine	Glibenclamide

Based on data from George et al. [10]

frequently used in the ICU setting, those deserving major attention are heparin, sodium valproate, some antibiotics, and the GPIIb/IIIa inhibitors (tirofiban, eptifibatide, and abciximab).

It has been estimated that approximately 1–5 % of patients treated with heparin may experience *heparin-induced thrombocytopenia* (*HIT*). It occurs ten times more frequently with unfractioned heparin than with low-molecular-weight heparins, and with the highest frequency among orthopedic patients [14].

Thrombocytopenia is the most common hematologic abnormality associated with *sodium valproate*. Its incidence has been reported between 5 and 40 %, regardless of absolute values of plasma drug concentrations [15].

As far as antimicrobials are concerned, a Dutch retrospective case-controlled study in hospitalized patients showed that beta-lactams were associated with an increased risk of DIT, with an odds ratio of 7.4 [16]. Among the various beta-lactams, *piperacillin* is considered to be one of the most frequently associated with DIT [17]. Also vancomycin is shown to be an important cause of DIT [18]. Trimethoprim–sulfamethoxazole-induced thrombocytopenia was estimated at 1 in 25,000 patients [8]. Recently, linezolid was shown to be frequently associated with dose-dependent DIT in patients on long-term treatment [19].

The prevalence of DIT caused by GPIIb/IIIa inhibitors is estimated between 0.1 and 2 % of cases [20–22].

Among other potential causes of thrombocytopenia to take into account for differential diagnosis versus DIT, it should not be overlooked that iodinated contrast media, some common beverages (tonic water and bitter lemon), some herbal remedies (Jui herbal tea), and foods (tahini sesame seeds and *Lupinus termis* beans) may be involved [23].

8.2.3 Pathogenesis

DIT may occur through two major pathogenetic mechanisms [23].

The first is a nonimmune-mediated mechanism, which in most cases leads, in a dose-dependent fashion, to bone-marrow suppression with pancytopenia. This is typically related to several antineoplastic agents that may block cell proliferation. Additionally, other drugs that may cause nonimmune-mediated DIT may be linezolid, thiazide diuretics, colchicine, and amrinone.

The second mechanism, which is more common and challenging, is immune-mediated and yields to a specific lysis of circulating platelets. Indeed, six different mechanisms are currently advocated for immune-mediated DITs (Table 8.3) [21, 24]. Among these, the *hapten-induced antibody theory* was originally advocated to explain hemolytic anemia caused by high-dose *penicillin*, and it is less certain whether this mechanism may be applied also to DIT. This process involves the covalent binding of the penicilloyl group of an opened beta-lactam ring to the amino groups of glycoproteins in the platelet membrane with consequent perturbation of the antigen processing of protein that ends up with a new antigenic structure, or neo-*epitope*, toward which a specific immune response is elicited.

Drug-dependent antibody theory has been recently revisited and an integrated view of previous theories is now well-accepted. According to this mechanism, antibodies causing DIT may react weakly with epitopes or glycoproteins on the surface of platelet membranes, increasing their avidity to their targets when a specific drug, presenting structural polar affinities, interacts noncovalently both with platelets and with antibodies. Consequently, drug is trapped between antibodies and platelets, and this strengthens the interaction. Whether the production of antibodies is promoted by the drug itself or whether it derives from a pool of naturally produced immunoglobulins is not well-understood. Drugs recognized to belong to this category are *quinine*, *quinidine*, *nonsteroidal anti-inflammatory drugs*, and some sulfonamide antibiotics.

Table 8.3 Mechanisms of immune-mediated drug-induced thrombocytopenia

Type	Mechanism	Clinical consequence	Examples
Hapten-dependent antibody	Drugs covalently bind to platelet glycoproteins producing a neoantigen that elicits an immune response	Hemorrhage	Penicillin, cephalosporin
Drug-dependent antibody	Antibodies bind a complex formed by drugs noncovalently linked to platelet glycoprotein	Hemorrhage	Quinine, quinidine, sulfonamides, NSAIDs
Ligand-induced binding site	Drugs bind to platelet GPIIb/IIIa producing a neoantigen that elicits an immune response	Hemorrhage	Eptifibatide, Tirofiban
Drug-specific antibody	Antibodies react to murine component of the drug	Hemorrhage	Abciximab
Autoantibody induction	Drugs induce antibodies that reacts with platelet glycoprotein in absence of drug	Hemorrhage	Gold salts, procainamide, rituximab, infliximab
Immune-complex	Antibodies bind drug-PF4 immune-complex and cross-link between platelets, leading to aggregation	Thrombosis	Heparin

Reprinted from Kenney and Stack [25] with permission from Archives of Pathology & Laboratory Medicine. Copyright 2009 College of American Pathologists

Tirofiban and eptifibatide are synthetic small molecules that react with the arginine-glycine-aspartic acid (RGD) recognition site on platelet *glycoprotein IIb/IIIa*, preventing this receptor from binding fibrinogen, thus blocking platelet aggregation and thrombus formation. These drugs, by binding to the GPIIb/IIIa complex on the platelet surface, may induce conformational changes leading to the emergence of cryptic domains with resultant antibody formation and platelet destruction (*ligand-induced binding site*).

Another antiplatelet agent causing thrombocytopenia is *abciximab*, a chimeric monoclonal antibody specific for GPIIIa, that may induce the production of specific antibodies which recognize the murine component of the molecule itself, and platelets are consequently destroyed as bystanders when coated with this drug (*drug-specific antibody*).

Some drugs may promote the production of autoantibodies (*autoantibody induction*), which may last for a long period of time even after drug discontinuation, leading to chronic autoimmune thrombocytopenic purpura. Typical drugs in this category are *gold salts*, once used in the treatment of rheumatoid arthritis, and *procainamide*. In recent years, numerous reports have suggested that DIT arising after treatment with the monoclonal antibodies *rituximab*, *infliximab*, etanercept and efalizumab should be referred to this mechanism.

The last type of immune-mediated mechanism is due to *immune-complex formation*, and is typical of *heparin* and heparin-like drugs. Essentially, heparin links to platelet factor 4 (PF-4) on platelet surface. This aggregate, in turns, binds to heparin-induced immunoglobulin G (HIT-IgG), whose Fc portion cross-links with FcγRII on the same platelet or on adjacent platelets, thus promoting further activation of platelets, release of PF-4, and platelet aggregation.

8.2.4 Clinical Presentation

Whereas the *time of onset of pancytopenia* due to cytotoxic myelosuppression may take several weeks to become of clinical

concern, conversely the onset of symptoms due to immune-mediated mechanisms is generally more rapid. Indeed, the onset depends on the time to mount the immunologic response. In case of primary immunization, frequently it takes 2–3 weeks before clinical appearance. However, the onset of thrombocytopenia may occur in less than 24 h if significant drug-induced antibody titer due to recent exposure exists, or in 3–10 days if the antibody titer has fallen after previous exposure [25].

A notable exception to this rule is represented by platelet inhibitors tirofiban, eptifibatide, and abciximab, whose naturally occurring antibodies may lead to acute thrombocytopenia within hours of the first exposure [22].

Most patients experiencing DIT usually have moderate-to-severe thrombocytopenia (platelet count less than 55,000 platelets/μL), and platelet counts lower than 20,000/μL are relatively frequent. Clinical presentations of DIT may be petechial hemorrhages, bruising, and epixtasis. Systemic symptoms such as chills, fever, nausea, and vomiting may often anticipate bleeding signs. Major bleeding was reported in 9 % of patients with DIT, whereas minor bleeding occurred in 28 % [8]. Life-threatening conditions due to platelet drop to 1,000 platelets/μL could also be expected. In these cases, patients could experience gastrointestinal, genitourinary, intracranial, or pulmonary hemorrhage [25].

Differently from other DITs, heparin may be associated with two different types of thrombocytopenia. The first, known as *HIT-1*, is a spontaneously resolving benign form characterized by a mild decrease in platelet count, rarely below 100,000/μL. The second type, described as *HIT-2* and considered the typical HIT-form, occurs 5–10 days after the initiation of therapy and is associated with a more than 30–50 % drop in platelet count [26]. About one-third of HIT occurs within few days since initiation of therapy, and is usually associated with reexposure to heparin within 100 days since the first episode [27].

Interestingly, in contrast to the usual course of DIT, platelet nadir in HIT is less severe (~55,000 platelets/μL), since counts

below 20,000/μL occur in less than 10 % of cases. Of note, HIT has a different clinical evolution with respect to other DITs. The major clinical complication of HIT is *thrombosis* as a consequence of the immune-complex mechanism with platelet activation and consumption. The most frequent clinical scenario is represented by venous thrombosis, with pulmonary embolism being the most common fatal complication. Conversely, spontaneous hemorrhage is uncommon. Interestingly, some authors have postulated the "iceberg" model of HIT [28, 29]. This model postulates that whereas a significant proportion of patients may produce HIT–IgG while on treatment with heparin, indeed only a smaller proportion become thrombocytopenic and an even smaller proportion may develop thrombotic complications.

8.2.5 Management

The unexpected occurrence of thrombocytopenia in a patient with a recent history of drug exposure should always give rise to the suspicion of DIT. In most cases the identification of the putative drug is quite challenging, as therapeutic schedules often include polytherapy, especially in the ICU. For this reason, a stepwise approach in the diagnosis of DIT is recommended [25, 30].

After excluding other causes of DIT, such as pseudothrombocytopenia (i.e., spurious in vitro causes of low platelet count), disseminated intravascular coagulation, or thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, a causal relationship should be evaluated according to the criteria proposed by George et al. [10]. Although the diagnosis of DIT is usually based mainly on clinical criteria, definitive confirmation of the suspicion of DIT may come through the identification of *drug-induced antiplatelet antibodies*. Unfortunately, testing is technically challenging and, with the notable exception of heparin, few laboratories may provide with these antibody assays. Two

major categories of test are available, namely immunoassays and functional tests [23, 26]. Immunoassays measure immunoglobulins associated with or bound to platelets. Among the different available methods, *flow cytometry* is the most rapid and sensitive for the detection of antibodies induced by the most common causative drugs.

Functional assays, as for example the *^{14}C-serotonin release assay* and the *heparin-induce platelet aggregation test*, measure antibody-induced changes in platelet activation. These tests are widely applied for the diagnosis of HIT, but rarely for other DIT.

Overall, it should also be taken into account that in patients with a highly suggestive clinical history of DIT, laboratory tests may sometimes result falsely negative. This could be due to some technical problems (i.e., insolubility of many drugs in in vitro testing) [31].

As far as the treatment of DIT is concerned, discontinuation of the causal medication is of paramount importance. After drug discontinuation, the prognosis of DIT is generally excellent. Platelet count recovers to more than 100,000/μL generally in 1–10 days, with a median of about 7 days [25].

Patients presenting with severe thrombocytopenia should be aggressively treated with platelet transfusions in order to avoid fatal intracranial or intrapulmonary haemorrhage. The role of high-dose corticosteroids and of intravenous immunoglobulins in DIT remains controversial [20].

Platelet transfusions should not be applied to HIT, as they could increase the rate of thrombotic complications [32]. In patients with HIT, clinicians may face the need to continue the anticoagulative treatment. The choice of low-molecular-weight heparins and of warfarin is not recommended, the former because of the high cross-reactivity with HIT-IgG and the latter for reports of necrotic complications in acute DIT [33]. Conversely, *direct thrombin inhibitors* (lepirudin, argatroban, bivalirudin) or heparinoids (danaparoid) should be used. Oral anticoagulation shift toward warfarin should be applied only

after platelet count recover to more than 150,000/μL and after an overlap with direct thrombin inhibitors for at least 5 days [33].

8.3 Drug-Induced Anemia

8.3.1 Epidemiology

There are several types of *drug-induced anemias*, namely *immune-mediated hemolytic anemia* (*IHA*), nonimmune-mediated hemolytic anemia, sideroblastic anemia, megaloblastic anemia, and methemoglobinemia.

Unfortunately, exhaustive epidemiological data on drug-induced anemia are available only for some of these forms. The estimated incidence of *drug-induced immune hemolytic anemia* (*DIIHA*) is about 1 case per million individuals. However, the real incidence of DIIHA is probably underestimated for some reasons: firstly, because of misdiagnosis with classical *autoimmune hemolytic anemia* (*AIHA*), which has been reported to occur in 1 in 80,000 of the population; and secondly, because serological confirmation of DIIHA is usually performed only in the presence of severe hemolysis, this leaving less relevant causative drugs potentially undiscovered [34].

Epidemiological data on the other types of drug-induced anemias are still sparse or totally absent. *Megaloblastic anemia* is reported to occur in 9 % of patients on continuous treatment with biguanides (phenformin or metformin), and between 3 and 9 % of patients on treatment with methotrexate [35]. *Non immune hemolytic anemia* is a major side effect of the antiviral ribavirin and it occurs in more than two-thirds of patients with HCV hepatitis treated with the dual combination ribavirin–interferon [36]. *Sideroblastic anemia* is well-known for some antimicrobials, such as isoniazid and chloramphenicol, the former requiring regular pyridoxine prophylaxis in every

antitubercular regimen. Some reports have suggested that linezolid could induce anemia in long-term treatments, even if less frequently than thrombocytopenia [37].

8.3.2 Causative Drugs

Several drugs that are commonly prescribed can induce anemia as ADR [38]. The most notable examples, with the underlying mechanism and with the type of anemia, are reported in Table 8.4.

DIIHA has been widely investigated since the first report of suspected hemolysis due to the antiepileptic drug mephenytoin in 1953 [39]. In the 1980s, the most common drugs reported to cause DIIHA were methyldopa (67 %) and penicillin (23 %). Subsequently, the spectrum of implicated drugs has progressively changed and second- and third-generation cephalosporins, in particular *cefotetan* and *ceftriaxone*, were reported as responsible for more than 80 % of DIIHA [34].

Differently from what occurred with drug-induced thrombocytopenia and with drug-induced neutropenia, for which well-defined criteria of eligibility for causative drugs have been established, conversely well-defined criteria are still lacking for DIIHA. The most comprehensive update on DIIHA listed 125 drugs on the basis of the number of reports available in the literature and of a positive direct antiglobulin test (DAT), which was performed in almost all cases [40]. Drugs with the highest frequency of DIIHA include second- and third-generation cephalosporins, nonsteroidal anti-inflammatory drugs, in particular *ibuprofen* and *diclofenac*, and the chemotherapeutic oxaliplatin [40]. As far as the other type of anemias are concerned, the most important causative drugs for nonimmune-mediated hemolytic anemia are *primaquine*, sulphamethoxazole; for sideroblastic anemia, isoniazid and pyrazinamide; for megaloblastic anemia, methotrexate, trimethoprim/sulfamethoxazole,

Table 8.4 General scheme of drug-induced anemia, with relative mechanism of action and main examples

Phenotype of anemia	Mechanism of anemia	Common medications
Hemolytic anemia	Immune (DIIHA)	
	DDAB	Cephalosporins
		Cefotetan
		Ceftriaxone
		Penicillin
		NSAIDs
		Quinine/Quinidine
	DIAB	Fludarabine
		Methyldopa
		Levodopa
		Beta-lactamase inhibitors
	Nonimmune	
	G6PD deficiency	Primaquine
		Nitrofurantoin
		Sulfamethoxazole
		Nalidixic Acid
	Other	Ribavirin
Sideroblastic anemia	Pyridoxine deficiency	Isoniazid
		Pyrazinamide
	Copper chelation	Penicillamine
	Myelosuppression	Chloramphenicol
		Linezolid
		Busulfan
		Tetracycline
Megaloblastic anemia	Folic acid deficiency	Methotrexate
		Trimethoprim/ Sulfamethoxazole
		Pyrimethamine
		Sulfasalazine
		Triamterene
		Anticonvulsants
		Phenobarbital
		Phenytoin
		Primidone
	Cobalamin deficiency	Metformin
		Omeprazole

Table 8.4 (continued)

Phenotype of anemia	Mechanism of anemia	Common medications
Variable: sideroblastic/ megaloblastic/ aplastic anemia	Myelosuppression	Antiviral Zidovudine Didanosine Stavudine Lamivudine
Methemoglobinemia	Indirect oxidizers	Dapsone Primaquine
	Direct oxidizers	Metoclopramide Benzocaine Prilocaine Phenazopyridine

Based on data from Shander et al. [38]
DIIHA drug-induced immune hemolytic anemia, *DDAB* drug-dependent antibodies, *DIAB* drug-independent antibodies, *NSAIDs* nonsteroidal anti-inflammatory drugs, *G6PD* glucose-6-phosphate dehydrogenase

phenobarbital, and *phenytoin*; and for methemoglobinemia, dapsone, benzocaine, and prilocaine [41].

Finally, it is worth noting that some first-generation antiretroviral drugs have been reported as potential causes of dose- and time-dependent macrocytic anemia (zidovudine and to a lesser extent didanosine and stavudine) [42, 43].

Table 8.5 classifies drugs responsible for drug-induced anemia on the basis of the number of reports available in the literature [40].

8.3.3 Pathogenesis

Hemolytic anemias could be due to both an immunological (DIIHA) and a nonimmunological mechanism. The pathogenesis of DIIHA has been debated for years, and has recently converged to the so-called unifying hypothesis, which is summarized in Table 8.6 [44]. According to this hypothesis, the mechanisms

Table 8.5 Most common drugs causing drug-induced anemia stratified according to the number of references available in the literature

Drug category	≥5 references	<5 references
Analgesics	Acetaminophen, diclofenac, mefenamic acid, phenacetin, tolmetin, sulindac	Aceclofenac, aminopyrine, aspirin, etodolac, fenoprofen, ibuprofen, nabumetone, naproxen, propyphenazone, suprofen
Anti-infective drugs	Ampicillin, cefazolin, cefotetan, cefoxitin, ceftazidim, ceftriaxone, cephalotin, chloramphenicol, ciprofloxacin, interferon, isoniazid, nafcillin, nalidixic acid, PAS, penicillin G, piperacillin, ribavirin, rifampicin, streptomycin, tetracycline, zidovudine	Acyclovir, amoxicillin, amphotericin B, cefamandole, cefixime, cefotaxime, cefpirome, ceftizoxime, cefuroxime, cephalexin, chloramphenicol, chlorpheniramine, clavulanic acid, cloxacillin, didanosine, erythromycin, ethambutol, fluconazole, ketoconazole, levofloxacin, linezolid, mefloquine, minocycline, moxalactam, nitrofurantoin, norfloxacin, pyrazinamide, pyrimetamine, rifabutin, sulbactam, sulfasalazine, sulfisoxazole, suramin, tazobactam, teicoplanin, ticarcillin, trimethoprim/sulfamethoxazole, vancomycin
Antiepileptics		Mephenytoin, phenobarbital, phenytoin
Antineoplastics	Cisplatin, cladribine, fludarabine, oxaliplatin	6-Mercaptopurine, carboplatin, fluorouracil, imatinib mesylate, lenalidomide, melphalan, methotrexate, teniposide

Cardiovascular drugs	Hydrochlorothiazide, methyldopa, procainamide, quinidine, quinine	Captopril, furosemide, hydralazine, streptokinase, triamterene
Gastrointestinal drugs	Cimetidine	Ranitidine
Psychotropic drugs	Chloropromazine, levodopa	Thiopental sodium
Other drugs	Catechin, chlorinated hydrocarbons, chlorpropamide	Antazoline, azapropazone, carbimazole, chaparral, cyclofenil, cyclosporin, diethylstilbestrol, dipyrone, fenfluramine, fluorescein, insulin, methadone, tacrolimus, tartrazine, tolbutamide, zomepirac

Based on data from Garratty et al. [40]

Table 8.6 Mechanisms of drug-induced immune hemolytic anemia (DIIHA)

	Drug-dependent antibody		Drug-independent antibody	
Type of reaction	RBC coating	RBC membrane interaction	True autoantibodies	Nonimmunologic protein adsorption
Mechanism	Drug binds strongly to RBC membranes, leading to IgG antibodies directed to drug epitopes	Drug noncovalently binds to RBC surface, forming a part-drug–part-membrane neoantigen target for antibodies	Antibodies reacting mainly to RBC membrane components	Drug alters RBC surface so that serum proteins are adsorbed onto the RBC membrane
Type of hemolysis	Extravascular hemolysis	Intravascular hemolysis	Extravascular hemolysis	Extravascular hemolysis
Onset of hemolysis	Gradual RBC destruction	Abrupt RBC destruction (few hours)	Gradual RBC destruction	Gradual RBC destruction
Diagnosis	DAT+: IgG	DAT+: IgG, C3	DAT+: IgG	DAT+: IgG
Examples	Penicillin (high-dose), cefotetan	Ceftriaxone, piperacillin, NSAIDs	Fludarabine, cladribine, methyldopa, levodopa, procainamide	Beta-lactamase inhibitors, cisplatinum, carboplatinum, oxaliplatinum

Based on data from Pierce and Nester [46]
RBC red blood cell, *DAT* direct antiglobulin test

of DIIHA are based on the interactions between pathological antibodies, causative drugs, and red blood cells (RBCs). Briefly, human antibodies responsible for DIIHA are classified as drug-dependent or drug-independent according to the way by which they react with the complex formed by the causative drug and the RBC surface. *Drug-dependent antibodies* may react directly with the drug or both with the drug and the RBC membrane components, whereas *drug-independent antibodies* react mainly with the RBC membrane components and only minimally with the drug. A further mechanism based on drug-independent antibodies involves a nonimmunological adsorption of serum protein on RBC membrane.

DIIHA are mainly caused by IgG and, in the case of antibodies interacting with both drug and RBC membrane components, also by the complement. In this latter case, the hemolysis is intravascular, rapid, and life-threatening. Otherwise, the coated RBCs undergo extravascular destruction via Fc-receptor recognition by splenic macrophages [45, 46].

Nonimmune-mediated hemolytic anemia may occur frequently as a consequence of hereditary *glucose-6-phosphate dehydrogenase deficiency* (*G6PD*) or of the use of *ribavirin*.

G6PD deficiency is the most frequent red cell enzymopathy associated with hemolysis. This cross-linked disorder affects predominantly males and leads to the intracellular reduction of glutathione, which is the only source for restoration of reduction potential in RBCs. Consequently, in case of exposure to oxidative agents, whether drugs (such as some antimalarials, sulfonamides, and analgesic) or foods (e.g., fava beans), hemoglobin denaturates, cross-links, and precipitates intracellulary, forming the so-called Heinz bodies inclusions, which are removed in the spleen. Then, the altered RBCs undergo both intravascular and extravascular destruction [47].

Hemolytic anemia associated with ribavirin is caused by drug accumulation in erythrocytes, depletion of ATP via formation of ribavirin triphosphate and hemolysis due to oxidative membrane damage [48].

Sideroblastic anemias are characterized by the presence in the bone marrow of erythroblasts with an excessive accumulation of iron in their mitochondria (sideroblasts), due to an impaired heme biosynthesis. Various steps in heme biosynthesis could be inhibited, ultimately leading to myelosuppression. In particular, isoniazid inhibits the enzyme apotryptophanase leading to pyridoxine deficiency. Penicillamine and triethylene tetramine dihydrochloride, both used for the treatment of Wilson disease, can lead to copper deficiency due to excessive chelation, causing a defective protoporphyrin IX. *Chloramphenicol* may interfere with ferrochelatase, inducing a reversible dose-dependent anemia. Linezolid, progesterone, and busulfan are also potential causes of direct inhibition of myelopoiesis [38].

Megaloblastic anemias may be induced by drugs interfering with the metabolism of folic acid or of vitamin B_{12} (cobalamin) with consequent inhibition of DNA synthesis. *Methotrexate* is an irreversible inhibitor of dihydrofolate reductase, an enzyme that regenerates intracellular tetrahydrofolic acid, which plays an important role in DNA–thymine synthesis. Similarly, *trimethoprim–sulfamethoxazole*, pyrimethamine, and triamterene have been reported to cause megaloblastic anemia, but to a lesser extent and especially in patients with a preexisiting deficiency of cobalamin or of folate. First-generation anticonvulsants, such as phenytoin and phenobarbital, have also been reported to induce megaloblastic anemia, but mainly through interference with folic acid absorption [38].

Decreased cobalamine levels have been observed with long-term use of omeprazole [49] and metformin [50], as a consequence of decreased intestinal absorption. Nevertheless, the clinical impact of these interactions requires further investigation.

Methemoglobinemia is a functional anemia that results from an excessive oxidation of normal hemoglobin, impairing oxygen transport and leading to tissue hypoxia. Direct oxidizers commonly used in the ICU setting include the local anesthetics

benzocaine and *prilocaine*, the former being responsible for the most seriously elevated methemoglobin levels. However, the most common cause of methemoglobinemia is *dapsone*, which in a recent series accounts for 42 % of cases [51].

8.3.4 Clinical Presentation

Drug-induced hemolytic anemias generally develop within few days in the case of most DHIIAs, or after longer periods (weeks or months) when related to myelosuppression. Symptoms such as chills, fever, vomiting, nausea, and abdominal pain are often present [45].

Among DIIHA, rapid complement-mediated intravascular hemolysis leading to renal failure, shock, and disseminated intravascular coagulation might rarely be induced by some cephalosporins (cefotetan and ceftriaxone) or NSAIDs [45].

8.3.5 Management

In the presence of drug-induced anemia, drug discontinuation is fundamental and almost always resolutive, irrespective of the type of anemia. Rarely, blood transfusions could be necessary in the most severely ill patients.

The diagnosis of DIIHA is based on serological findings suggestive of hemolysis (increased indirect serum bilirubin, low serum haptoglobin, increased serum LDH; hemoglobinemia and hemoglobinuria in case of intravascular hemolysis) together with a positivity of the *direct antiglobulin test* (*DAT*), which is devoted to identify whether or not the anemia is immune-mediated [52].

Of note, the presence of a positive DAT is quite challenging, since clinicians are faced with differentiating between DIIHA

and AIHA. This differential diagnosis is fundamental for clinical management, considering that only in case of AIHA the use of steroids is recommended [38, 46].

Ribavirin-induced hemolytic anemia occurs in almost all patients treated for HCV-related hepatitis, even if with different degree. Strategies for management include ribavirin dose reductions, administration of an agent that stimulates erythropoietin production or blood transfusions. Of note, dose adjustments of ribavirin should be of limited entity, since it has been demonstrated that maintenance of dose of at least 80 % of the initial dose is critical for optimal sustained virologic response in HCV patients treated with dual therapy based on ribavirin and interferon [36].

Drug-induced anemia resulting from vitamin deficiencies could be easily managed through exogenous supplementation (e.g., pyridoxine and folinic acid following *isoniazid* and metothrexate administration, respectively).

Emergency treatment is necessary in case of high methemoglobin concentrations. Chocolate-brown arterial blood, cyanosis, reduced oxygen saturation, and the measurement of methemoglobin on arterial blood gas analysis made the diagnosis certain. Cessation of the inducing agent, prompt administration of methylene blue and oxygen should be provided [17].

8.4 Drug-Induced Neutropenia

8.4.1 Epidemiology

The annual incidence of *drug-induced neutropenia* is estimated at about 1.6–15.4 cases per million population in Europe and in the USA [53, 54]. The incidence increases with age, with more than half of cases occurring in patients aged 65 years or over, and is approximately twofold higher in women than in men.

Interestingly, neutropenia has a specific feature among blood dyscrasias, since it is the only one mostly related to drugs, the other etiologies being involved in less than 10 % of cases [6]. Case fatality is around 10 %, largely depending on a rapid and correct use of antibiotic treatment in case of systemic infections.

8.4.2 Causative Drugs

It is well-known that almost all antineoplastic drugs may induce direct cytotoxicity with bone-marrow suppression and neutropenia.

In this section, only *nonchemotherapic drugs* responsible for *drug-induced neutropenia* will be considered. The identification of nonchemotherapic drugs responsible for severe drug-induced neutropenia is extremely challenging among ICU patients due to the frequent concomitant presence of polytherapy and of frailty and/or of critical clinical conditions. Criteria for rational approach in identifying drug-induced neutropenia are based on international consensus agreements [55] (7). According to these standardized causality assessment criteria that are reported on Table 8.7, 125 drugs have been identified as definitive or probable cause of acute neutropenia [53], most of which are reported in Table 8.8. Most of drug-induced acute neutropenias are due to the following drugs: carbimazole, *clozapine*, dapsone, *dipyrone*, methimazole, penicillin G, *trimethoprim–sulfamethoxazole*, procainamide, propylthiouracil, rituximab, sulfasalazine, and *ticlopidine*. Odds ratios for acute drug-induced neutropenia have also been estimated for some high-risk drugs: the highest odds ratios are associated with *methimazole* (230.9), followed by ticlopidine (103.2), calcium dobesilate (77.8), sulfasalazine (74.6), dypirone (25.8), trimethoprim–sulfametoxazole (25.1), and carbimazole (16.7) [53]. *Clozapine* was reported to induce neutropenia in almost 1 % of patients, particularly in the first 3 months of treatment [56].

Table 8.7 Criteria for establishing a causative relationship of drug-induced agranulocytosis

Criterion	
1	Acute agranulocytosis occurred during therapy or within 7 days after drug withdrawal and did not resolve spontaneously during continuous therapy[a]
2	Absence of concurrent disease or other drugs that may have caused acute agranulocytosis (history of congenital or immune neutropenia, recent infectious disease, radiotherapy, chemotherapy, immunotherapy, and existence of an underlying hematological disease)
3	Increase in neutrophil count to more than 1.5×10^9 cell/L within 1 month after drug discontinuation
4	Existence of a satisfactory rechallenge procedure or of a definitive pharmacologic explanation for acute agranulocytosis (e.g., confirmation of causality by detecting drug-dependent antineutrophil antibodies)
Levels of evidence	
I	Definitive: All 4 criteria are met
II	Probable: Criteria 1, 2, and 3 are met
III	Possible: Criteria 1 is met
IV	Unlikely: Criteria 1 is not met

Based on data from [53, 63, 66]

[a]for rituximab-induced, delayed-onset neutropenia, this window has been extended to 6 months

8.4.3 Pathogenesis

Drug-induced neutropenia may be caused by two different mechanisms: one is immune-mediated while the other is related to direct cytotoxicity [21]. The immunological mechanisms include both cell-mediated response, as in the case of activated T-lymphocytes in late-onset neutropenia after rituximab therapy, and antibody-mediated response (leukoagglutinines), as in the case of most beta-lactams and chincona derivatives.

Direct damage to myeloid precursors or even the bone-marrow microenvironment plays a role in most other cases.

Table 8.8 Nonchemotherapeutic drugs with definite or probable evidence for causality of drug-induced neutropenia

Drug category	Level of evidence: definitive	Level of evidence: probable
Analgesics	Aminopyrine, diclofenac, diflunisal, dipyrone, ibuprofen	Acetaminophen, bucillamine, fenoprofen, mefenamic acid, naproxen, pentazocin, phenylbutazone, piroxicam, sulindac
Antiarrhythmics	Dysopyramide, procainamide, quinidine	Ajmaline, amiodarone, aprindine
Anti-infective Agents	Ampicillin, carbenicillin, cefotaxime, cefuroxime, flucytosine, fusidic acid, imipenem, nafcillin, oxacillin, penicillin G, quinine, ticarcillin	Abacavir, amodiaquine, amoxicillin, cefamandole, cefepime, ceftriaxone, cephalexin, cephalotin, cephapirin, cephradine, chloroguanide, clarithromycin, cloxacillin, dapsone, indinavir, isoniazid, mebendazole, nifuroxazide, nitrofurantoin, norfloxacin, penicillin-procaine, piperacillin, terbinafine, TMT-SMX, vancomycin, zidovudine
Antiepileptics	Phenytoin	Carbamazepine, lamotrigine
Antineoplastics	Amygdalin	Aminoglutethimide, flutamide, imatinib, nilutamide, rituximab
Antirheumatics	Infliximab, levamisole	Gold, penicillamine, sulfasalazine
Antithyroid drugs	Propylthiouracil	Carbimazole, methimazole
Cardiovascular drugs	Clopidogrel, methyldopa, ramipril, spironolactone	Bepridil, bezafibrate, captopril, metolazone, ticlopidine, vesnarinone

(continued)

Table 8.8 (continued)

Drug category	Level of evidence: definitive	Level of evidence: probable
Gastrointestinal drugs	Cimetidine, metoclopramide	Famotidine, mesalazine, metiamide, omeprazole, pirenzepine, ranitidine
Psychotropic drugs	Chlorpromazine, clozapine, fluoxetine	Amoxapine, clomipramine, cyanamide, desipramine, dothiepin, doxepin, imipramine, indalpine, maprotiline, meprobamate, methotrimeprazine, mianserin, olanzapin, thioridazine, ziprasidone
Other drugs	Calcium dobesilate, mebhydrolin	Acetosulfone, acitretin, allopurinol, chlorpropamide, deferiprone, prednisone, promethazine, riluzole, ritodrine, tolbutamide, yohimbine

Based on data from Andersohn et al. [53]

A dose-dependent inhibition of granulocytopoiesis has been described with carbamazepine, valproic acid, methimazole, and with phenothiazines, other than with several antineoplastic chemotherapic agents such as doxorubicin, cyclophosphamide, busulfan, and methotrexate [57]. Interestingly, some drug-induced neutropenias could also be due to mixed mechanisms. The antithyroid drug *propylthiouracil* is reported to induce neutropenia on the basis of a complement-mediated mechanism in some individuals and on the basis of nonimmune mechanisms in others [58].

Actually, the pathogenesis of drug-induced neutropenia is quite heterogeneous, often involving the impairment of different cellular pathways in a multistep series of events, and sometimes also the patient genotype [59]. The most notable example of this complexity is represented by *clozapine-induced neutropenia*. This antipsychotic undergoes oxidation to a reactive nitrenium ion, an unstable intermediate metabolite that interacts with sulfhydryl groups in the glutathione cycle, depletes intracellular ATP, ultimately rendering neutrophils highly susceptible to oxidant-induced apoptosis [60]. It has also been supposed that clozapine and aminopyrine could also stimulate NADPH oxidase and myeloperoxidase in the generation of reactive oxygen species (ROS) in neutrophils [61].

Association between certain histocompatibility antigens and the occurrence of neutropenia has also been described. For example, *HLA-B27* and *HLA-B38* are risk factors for *clozapine-induced neutropenia*, while HLA-B35 might be protective [62].

8.4.4 Clinical Presentation

Patients with drug-induced neutropenia usually present with fever (*febrile neutropenia*); general malaise including chills, myalgia, arthralgia; nonspecific sore throat; or severe deep infections. Neutropenic patients are highly susceptible to almost all

type of bacterial or fungal infections whose occurrence depends on the degree and duration of neutropenia. Severe neutropenic patients may frequently develop sepsis. In elderly patients clinical manifestations are generally more severe, with severe sepsis or septic shock reported in two-thirds of them. Moreover, in this setting, anemia and thrombocytopenia are associated with neutropenia in at least 30 and 10 %, respectively [63].

In some cases of drug-induced neutropenia, neutropenia may be associated with suppression of neutrophil precursors in the bone marrow, whereas in other cases, immature myelocytes remain preserved (myeloid maturation arrest) [21].

As far as the time of onset is concerned, the median duration of treatment before *onset of drug-induced neutropenia* may be extremely variable. Interestingly, this issue was addressed in a recent systematic review [53]. The time of onset ranged between 2 days for dipyrone and 60 days for levamisole, and it was of 20 and 40 days for beta-lactams and antithyroid agents, respectively. The time to recovery of neutrophil count after drug discontinuation usually ranges between 4 and 24 days.

8.4.5 Management

The diagnosis of drug-induced neutropenia could be formulated once the criteria reported on Table 8.7 have been fulfilled. Differential diagnosis in adults includes a limited number of clinical conditions, such as neutropenia secondary to sepsis, neutropenia due to hematological diseases (e.g., myelodysplasia or bone-marrow suppression) or hypersplenism, neutropenia secondary to peripheral destruction of polymorphonuclear cells (e.g., Felty's syndrome, systemic lupus erythematosus, Sjögren's syndrome), neutropenia determined by nutritional deficiencies (e.g., cobalamin and folate deficiencies) [63].

Immediate discontinuation of the offending drug is the first and foremost intervention to undertake. For drugs at high risk,

such as clozapine, ticlopidine, and antithyroid drugs, routine monitoring of neutrophil count should be carried out during use in order to assess the potential development of neutropenia.

Source control of potential infections may include both prophylactic and therapeutic approaches. The role of prophylactic antibiotics has not been fully established nor validated [64]. The occurrence of sepsis requires prompt empiric broad spectrum antibiotic therapy that must be subsequently tailored according to the susceptibility of the isolated microorganisms whenever feasible. Addition of empiric antifungal therapy should be considered for patients with persistent fever despite broad-spectrum antibiotics [64].

The use of granulocyte colony stimulating factor, namely *filgrastim* (G-CSF) and *pegfilgrastim*, may foster granulopoiesis and reduce incidence, severity, and duration of neutropenia [65]. A sound evidence on the efficacy of hematopoietic growth factors in drug-induced neutropenia is growing as several studies have highlighted statistically significant lower rates of infectious and fatal complications, and reduced durations of hospitalizations and global costs with the use of such agents [63].

Overall, with an appropriate management that includes a wise antibiotic stewardship and a proper administration of hematopoietic growth factors, the mortality rate from idiosyncratic drug-induced neutropenia was reported around 5 % [66].

8.5 Drug-Induced Aplastic Anemia

Drug-induced aplastic anemia is related to a failure of all myeloid lines in bone marrow. Its incidence has been found at a rate of 2.34 per million inhabitants per year in a European cohort [67], whereas it appears to be two- to threefold more common in Asia than in Europe. The physiopathology is largely unknown; though, it has been hypothesized that both immune

and nonimmune process might be involved. Environmental exposures to *benzene* among industrial workers and past experiences with the use of *chloramphenicol* were historically the most relevant demonstration of *drug-induced aplastic anemia* [68]. Relative risk assessment for other drug classes has been carried out [56, 67] and significant evidence of an association emerged for the following drugs (relative risk is reported in parenthesis): penicillamine (49); gold (19); carbamazepine (13); allopurinol (4.6); furosemide (2.8); chloramphenicol (2.7); sulphonamides (2.1); nonsteroidal anti-inflammatory drugs such as butazones, indomethacin, diclofenac, and naproxen (2.8–3.9). Moreover, the anticonvulsant drug *felbamate* was found to carry an aplastic anemia risk approximately ten times higher than that of carbamazepine [69].

As for all the other drug-induced cytopenias, all suspected medications should be discontinued. Drug-induced aplastic anemia is treated like the idiosyncratic form of the disease, and responds to therapy at about the same rate. Immunosuppressive therapy by means of antithymocyte globulin and cyclosporine produces significant improvements in survival, with 5-year post-immunosuppression rates increasing from 40 % in the 1980s to about 80 % in patients treated after 2003 [68].

References

1. Benichou C (1994) Adverse drug reactions: a practical guide to diagnosis and management. Wiley, New York
2. Hine LK, Gerstman BB, Wise RP, Tsong Y (1990) Mortality resulting from blood dyscrasias in the United States, 1984. Am J Med 88(2):151–153
3. Reis AM, Cassiani SH (2011) Adverse drug events in an intensive care unit of a university hospital. Eur J Clin Pharmacol 67(6):625–632. doi:10.1007/s00228-010-0987-y
4. Park S, In Y, Suh GY, Sohn K, Kim E (2013) Evaluation of adverse drug reactions in medical intensive care units. Eur J Clin Pharmacol 69(1):119–131. doi:10.1007/s00228-012-1318-2

5. Vandendries ER, Drews RE (2006) Drug-associated disease: hematologic dysfunction. Crit Care Clin 22(2):347–355, viii. doi:10.1016/j.ccc.2006.02.002
6. Heimpel H (1996) When should the clinician suspect a drug-induced blood dyscrasia, and how should he proceed? Eur J Haematol Suppl 60:11–15
7. van den Bemt PM, Meyboom RH, Egberts AC (2004) Drug-induced immune thrombocytopenia. Drug Saf 27(15):1243–1252
8. Priziola JL, Smythe MA, Dager WE (2010) Drug-induced thrombocytopenia in critically ill patients. Crit Care Med 38(6 Suppl):S145–S154. doi:10.1097/CCM.0b013e3181de0b88
9. Everds NE, Tarrant JM (2013) Unexpected hematologic effects of biotherapeutics in nonclinical species and in humans. Toxicol Pathol 41(2):280–302. doi:10.1177/0192623312467400
10. George JN, Raskob GE, Shah SR, Rizvi MA, Hamilton SA, Osborne S, Vondracek T (1998) Drug-induced thrombocytopenia: a systematic review of published case reports. Ann Intern Med 129(11):886–890
11. Reese JA, Li X, Hauben M, Aster RH, Bougie DW, Curtis BR, George JN, Vesely SK (2010) Identifying drugs that cause acute thrombocytopenia: an analysis using 3 distinct methods. Blood 116(12):2127–2133. doi:10.1182/blood-2010-03-276691
12. Garbe E, Andersohn F, Bronder E, Salama A, Klimpel A, Thomae M, Schrezenmeier H, Hildebrandt M, Spath-Schwalbe E, Gruneisen A, Meyer O, Kurtal H (2012) Drug-induced immune thrombocytopaenia: results from the Berlin Case-control Surveillance Study. Eur J Clin Pharmacol 68(5):821–832. doi:10.1007/s00228-011-1184-3
13. Arnold DM, Kukaswadia S, Nazi I, Esmail A, Dewar L, Smith JW, Warkentin TE, Kelton JG (2013) A systematic evaluation of laboratory testing for drug-induced immune thrombocytopenia. J Thromb Haemost 11(1):169–176. doi:10.1111/jth.12052
14. Martel N, Lee J, Wells PS (2005) Risk for heparin-induced thrombocytopenia with unfractionated and low-molecular-weight heparin thromboprophylaxis: a meta-analysis. Blood 106(8):2710–2715. doi:10.1182/blood-2005-04-1546
15. Acharya S, Bussel JB (2000) Hematologic toxicity of sodium valproate. J Pediatr Hematol Oncol 22(1):62–65
16. ten Berg MJ, Huisman A, Souverein PC, Schobben AF, Egberts AC, van Solinge WW, van den Bemt PM (2006) Drug-induced thrombocytopenia: a population study. Drug Saf 29(8):713–721
17. Wright RO, Lewander WJ, Woolf AD (1999) Methemoglobinemia: etiology, pharmacology, and clinical management. Ann Emerg Med 34(5):646–656

18. Von Drygalski A, Curtis BR, Bougie DW, McFarland JG, Ahl S, Limbu I, Baker KR, Aster RH (2007) Vancomycin-induced immune thrombocytopenia. N Engl J Med 356(9):904–910. doi:10.1056/NEJMoa065066
19. Pea F, Viale P, Cojutti P, Del Pin B, Zamparini E, Furlanut M (2012) Therapeutic drug monitoring may improve safety outcomes of long-term treatment with linezolid in adult patients. J Antimicrob Chemother 67(8):2034–2042. doi:10.1093/jac/dks153
20. Aster RH, Bougie DW (2007) Drug-induced immune thrombocytopenia. N Engl J Med 357(6):580–587. doi:10.1056/NEJMra066469
21. Aster RH (2010) Adverse drug reactions affecting blood cells. Handb Exp Pharmacol 196:57–76. doi:10.1007/978-3-642-00663-0_3
22. Aster RH, Curtis BR, McFarland JG, Bougie DW (2009) Drug-induced immune thrombocytopenia: pathogenesis, diagnosis, and management. J Thromb Haemost 7(6):911–918. doi:10.1111/j.1538-7836.2009.03360.x
23. Chong BH, Choi PY, Khachigian L, Perdomo J (2013) Drug-induced immune thrombocytopenia. Hematol Oncol Clin North Am 27(3):521–540. doi:10.1016/j.hoc.2013.02.003
24. Visentin GP, Liu CY (2007) Drug-induced thrombocytopenia. Hematol Oncol Clin North Am 21(4):685–696, vi. doi:10.1016/j.hoc.2007.06.005
25. Kenney B, Stack G (2009) Drug-induced thrombocytopenia. Arch Pathol Lab Med 133(2):309–314. doi:10.1043/1543-2165-133.2.309
26. Reilly RF (2003) The pathophysiology of immune-mediated heparin-induced thrombocytopenia. Semin Dial 16(1):54–60
27. Warkentin TE, Kelton JG (2001) Temporal aspects of heparin-induced thrombocytopenia. N Engl J Med 344(17):1286–1292. doi:10.1056/nejm200104263441704
28. Kelton JG (2002) Heparin-induced thrombocytopenia: an overview. Blood Rev 16(1):77–80. doi:10.1054/blre.2001.0189
29. Warkentin TE (1999) Heparin-induced thrombocytopenia: a clinico-pathologic syndrome. Thromb Haemost 82(2):439–447
30. George JN, Aster RH (2009) Drug-induced thrombocytopenia: pathogenesis, evaluation, and management. Hematology Am Soc Hematol Educ Program 153–158. doi:10.1182/asheducation-2009.1.153
31. Bougie D, Aster R (2001) Immune thrombocytopenia resulting from sensitivity to metabolites of naproxen and acetaminophen. Blood 97(12):3846–3850
32. Jang I-K, Hursting MJ (2005) When heparins promote thrombosis: review of heparin-induced thrombocytopenia. Circulation 111(20):2671–2683. doi:10.1161/circulationaha.104.518563
33. Arepally GM, Ortel TL (2006) Clinical practice. Heparin-induced thrombocytopenia. N Engl J Med 355(8):809–817. doi:10.1056/NEJMcp052967

34. Garratty G (2009) Drug-induced immune hemolytic anemia. Hematology Am Soc Hematol Educ Program 73–79. doi:10.1182/asheducation-2009.1.73
35. Weinblatt ME (1985) Toxicity of low dose methotrexate in rheumatoid arthritis. J Rheumatol Suppl 12(Suppl 12):35–39
36. Hynicka LM, Heil EL (2013) Anemia management in patients with chronic viral hepatitis C. Ann Pharmacother 47(2):228–236. doi:10.1345/aph.1R513
37. Bernstein WB, Trotta RF, Rector JT, Tjaden JA, Barile AJ (2003) Mechanisms for linezolid-induced anemia and thrombocytopenia. Ann Pharmacother 37(4):517–520
38. Shander A, Javidroozi M, Ashton ME (2011) Drug-induced anemia and other red cell disorders: a guide in the age of polypharmacy. Curr Clin Pharmacol 6(4):295–303
39. Snapper I, Marks D, Schwartz L, Hollander L (1953) Hemolytic anemia secondary to mesantoin. Ann Intern Med 39(3):619–623
40. Garratty G, Arndt PA (2007) An update on drug-induced immune hemolytic anemia. Immunohematology 23(3):105–119
41. Mintzer DM, Billet SN, Chmielewski L (2009) Drug-induced hematologic syndromes. Adv Hematol 2009:495863. doi:10.1155/2009/495863
42. Weinberg RS, Chusid ED, Galperin Y, Thomson JC, Cheung T, Sacks HS (1998) Effect of antiviral drugs and hematopoietic growth factors on in vitro erythropoiesis. Mt Sinai J Med 65(1):5–13
43. Tseng A, Conly J, Fletcher D, Keystone D, Salit I, Walmsley S (1998) Precipitous declines in hemoglobin levels associated with combination zidovudine and lamivudine therapy. Clin Infect Dis 27(4):908–909
44. Arndt PA, Garratty G (2005) The changing spectrum of drug-induced immune hemolytic anemia. Semin Hematol 42(3):137–144
45. Salama A (2009) Drug-induced immune hemolytic anemia. Expert Opin Drug Saf 8(1):73–79. doi:10.1517/14740330802577351
46. Pierce A, Nester T (2011) Pathology consultation on drug-induced hemolytic anemia. Am J Clin Pathol 136(1):7–12. doi:10.1309/ajcpbvljzh6w6rqm
47. Dhaliwal G, Cornett PA, Tierney LM Jr (2004) Hemolytic anemia. Am Fam Physician 69(11):2599–2606
48. Jarvis SM, Thorn JA, Glue P (1998) Ribavirin uptake by human erythrocytes and the involvement of nitrobenzylthioinosine-sensitive (es)-nucleoside transporters. Br J Pharmacol 123(8):1587–1592. doi:10.1038/sj.bjp.0701775
49. Ruscin JM, Page RL 2nd, Valuck RJ (2002) Vitamin B(12) deficiency associated with histamine(2)-receptor antagonists and a proton-pump inhibitor. Ann Pharmacother 36(5):812–816

50. Filioussi K, Bonovas S, Katsaros T (2003) Should we screen diabetic patients using biguanides for megaloblastic anaemia? Aust Fam Physician 32(5):383–384
51. Ash-Bernal R, Wise R, Wright SM (2004) Acquired methemoglobinemia: a retrospective series of 138 cases at 2 teaching hospitals. Medicine (Baltimore) 83(5):265–273
52. Zantek ND, Koepsell SA, Tharp DR Jr, Cohn CS (2012) The direct antiglobulin test: a critical step in the evaluation of hemolysis. Am J Hematol 87(7):707–709. doi:10.1002/ajh.23218
53. Andersohn F, Konzen C, Garbe E (2007) Systematic review: agranulocytosis induced by nonchemotherapy drugs. Ann Intern Med 146(9):657–665
54. Strom BL, Carson JL, Schinnar R, Snyder ES, Shaw M (1992) Descriptive epidemiology of agranulocytosis. Arch Intern Med 152(7): 1475–1480
55. Benichou C, Solal Celigny P (1991) Standardization of definitions and criteria for causality assessment of adverse drug reactions. Drug-induced blood cytopenias: report of an international consensus meeting. Nouv Rev Fr Hematol 33(3):257–262
56. Kaufman DW, Kelly JP, Jurgelon JM, Anderson T, Issaragrisil S, Wiholm BE, Young NS, Leaverton P, Levy M, Shapiro S (1996) Drugs in the aetiology of agranulocytosis and aplastic anaemia. Eur J Haematol Suppl 60:23–30
57. Bhatt V, Saleem A (2004) Review: drug-induced neutropenia–pathophysiology, clinical features, and management. Ann Clin Lab Sci 34(2): 131–137
58. Fibbe WE, Claas FH, Van der Star-Dijkstra W, Schaafsma MR, Meyboom RH, Falkenburg JH (1986) Agranulocytosis induced by propylthiouracil: evidence of a drug dependent antibody reacting with granulocytes, monocytes and haematopoietic progenitor cells. Br J Haematol 64(2):363–373
59. Palmblad J, Papadaki HA, Eliopoulos G (2001) Acute and chronic neutropenias. What is new? J Intern Med 250(6):476–491
60. Williams DP, Pirmohamed M, Naisbitt DJ, Uetrecht JP, Park BK (2000) Induction of metabolism-dependent and -independent neutrophil apoptosis by clozapine. Mol Pharmacol 58(1):207–216
61. Tesfa D, Keisu M, Palmblad J (2009) Idiosyncratic drug-induced agranulocytosis: possible mechanisms and management. Am J Hematol 84(7):428–434. doi:10.1002/ajh.21433
62. Dettling M, Cascorbi I, Roots I, Mueller-Oerlinghausen B (2001) Genetic determinants of clozapine-induced agranulocytosis: recent results of HLA subtyping in a non-jewish caucasian sample. Arch Gen Psychiatry 58(1):93–94

63. Andres E, Federici L, Weitten T, Vogel T, Alt M (2008) Recognition and management of drug-induced blood cytopenias: the example of drug-induced acute neutropenia and agranulocytosis. Expert Opin Drug Saf 7(4):481–489. doi:10.1517/14740338.7.4.481
64. Andres E, Zimmer J, Affenberger S, Federici L, Alt M, Maloisel F (2006) Idiosyncratic drug-induced agranulocytosis: update of an old disorder. Eur J Intern Med 17(8):529–535. doi:10.1016/j.ejim.2006.07.012
65. Crawford J (2002) Clinical uses of pegylated pharmaceuticals in oncology. Cancer Treat Rev 28(Suppl A):7–11
66. Andres E, Maloisel F, Kurtz JE, Kaltenbach G, Alt M, Weber JC, Sibilia J, Schlienger JL, Blickle JF, Brogard JM, Dufour P (2002) Modern management of non-chemotherapy drug-induced agranulocytosis: a monocentric cohort study of 90 cases and review of the literature. Eur J Intern Med 13(5):324–328
67. Montane E, Ibanez L, Vidal X, Ballarin E, Puig R, Garcia N, Laporte JR, Catalan Group for Study of Agranulocytosis and Aplastic Anemia (2008) Epidemiology of aplastic anemia: a prospective multicenter study. Haematologica 93(4):518–523. doi:10.3324/haematol.12020
68. Young NS, Kaufman DW (2008) The epidemiology of acquired aplastic anemia. Haematologica 93(4):489–492. doi:10.3324/haematol.12855
69. Pellock JM, Faught E, Leppik IE, Shinnar S, Zupanc ML (2006) Felbamate: consensus of current clinical experience. Epilepsy Res 71(2–3):89–101. doi:10.1016/j.eplepsyres.2006.06.020