# ✳ CHAPTER 4

Pathophysiology and differential diagnosis of anaemia

Jean-François Lambert, Photis Beris

1. Definition and classification of anaemias

Anaemia is a common condition, particularly in young women and in the geriatric population, and is a significant public health problem in developing countries. Anaemia is defined by the World Health Organisation as haemoglobin (Hb) < 120 g/L in women and Hb < 130 g/L in men. This definition also includes the so-called pseudo anaemic states (pregnancy, cardiac heart failure and hyperproteinaemia) where Hb concentration falls as the result of an increase of the plasma volume. In contrast, a decreased red blood cell mass can be masked by haemoconcentration resulting from a decrease in plasma volume.

Iron deficiency is the most frequent cause of anaemia, closely followed by anaemia of chronic disease (Figure 1).

Figure 1: **Main causes of anaemia**
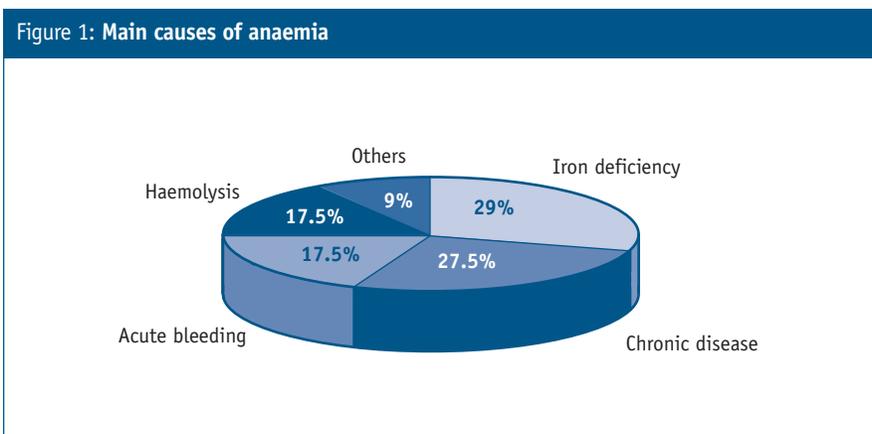
Ref. (1)

There are a number of different ways of classifying anaemia. Until recently classification was based on the red blood size (MCV), but since the introduction of the automated reticulocyte count, which provides an easy way to assess red blood cell (RBC) regeneration, one can now differentiate between hyporegenerative and regenerative anaemia. Basically, the measurement of reticulocytes will inform the clinician whether anaemia is due to a central defect of RBC production or to accelerated destruction (Table 1). For the hyporegenerative group of anaemias, the MCV value remains important in differential diagnosis.

1.1 Hyporegenerative anaemias
As defined by reticulocyte count, hyporegenerative or aregenerative anaemia is present

Table 1: **Classification of anaemias**	
Hyporegenerative	**Regenerative**
Aplastic anaemia	Haemolysis
Pure red cell aplasia	Immune
Myelodysplastic syndrome	Non-immune

Deficiency states
  • Iron
  • Vitamins
	• Congenital: membrane, SS, thalassaemia, enzymopathies, unstable Hb
	• Acquired: PNH, drugs (Pb, Zn and Cu poisoning), microangiopathy, hypersplenism

Marrow infiltration/fibrosis	Haemorrhage (bleeding)
Inflammatory anaemia (anaemia of chronic disease)	
Erythropoietin underproduction	

Hyporegenerative anaemia is defined as a reticulocyte count of < 50x10⁹/L; regenerative anaemia is defined as a reticulocyte count of > 100x10⁹/L. PNH: Paroxysmal nocturnal haemoglobinuria; SS: homozygous sickle cell disease. For reticulocyte count between 50 and 100x10⁹/L, see Section 3: Practical approach to the anaemic patient.

when reticulocytes are less than $50x10^9$/L in absolute value (% reticulocytes x RBC number). This is always due to a deficient bone marrow production of RBC.

1.1.1 Aplastic anaemia
Aplastic anaemia is a rare disease resulting from marrow failure, involving not only red cell precursors, but more primitive progenitors or multipotent stem cells, and usually presents with anaemia associated with a variable decrease of WBC and platelets (pancytopenia). It is defined by a severely hyporegenerative anaemia with pancytopenia. Aplastic anaemia is described in details by P Scheinberg and N Young in chapter 6 of this book.

1.1.2 Pure red cell aplasia (PRCA)
PRCA can be classified as a subgroup of aplastic anaemia as it also involves marrow failure, but only RBC production is affected. The reason to classify it as a separate entity is that PRCA is frequently associated with parvovirus B19 infection, usually not involving the other blood lineages. PRCA can also be associated with other infections, such as cytomegalovirus (CMV), HIV and hepatitis, or may occur as a consequence of drug toxicity or the development of neutralising antibodies to erythropoietin (see below).

1.1.3 Anaemia related to the myelodysplastic syndrome
The myelodysplastic syndrome (MDS) results from a clonal defect affecting the haematopoietic stem cells. The presentation of MDS can be similar to that of

aplastic anaemia (pancytopenia), with refractory anaemia, neutropenia and thrombocytopenia, but a particular characteristic is the associated abnormal maturation of all three cell lines defined as dysplasia, usually with a hypercellular marrow and an increased level of intramedullary apoptosis (2). In the peripheral blood, one usually sees macrocytic anisocytosis with poikilocytosis (abnormal RBC shapes), hyposegmented and hypogranular neutrophils sometimes showing a pseudo Pelger-Huët nuclear appearance (dumbbell-shaped bilobed nuclei), and variably sized hypogranular platelets. This chronic condition often evolves to acute leukaemia.

According to the WHO classification 2008, the MDS syndromes comprise 7 nosological entities (3):

1. Refractory anaemia (RA)
2. Refractory anaemia with ringed sideroblasts (RARS)
3. Refractory cytopenia with multilineage dysplasia (RCMD) with or without ringed sideroblasts
4. Refractory anaemia with excess blasts – 1 (RAEB1, 5-9% marrow blasts)
5. Refractory anaemia with excess blasts – 2 (RAEB2, 10-19% marrow blasts or presence of Auer's rods)
6. Myelodysplastic syndrome – unclassified
7. MDS associated with isolated del(5q).

Of these forms, sideroblastic anaemia will be treated separately in chapter 22. Description of the 5q- syndrome follows in part 2 of the present chapter.

Until recently, the percentage of blasts in the bone marrow was the only predictor of leukaemic transformation. A new prognostic tool, the International Prognostic Score System (IPSS) has been devised based on the retrospective analysis of more than 800 patients (4), and allowed the definition of three efficient prognostic criteria for both overall survival and risk of leukaemic transformation. These criteria are marrow blast percentage, bone marrow cytogenetics and the number of peripheral blood cytopenias (Tables 2 and 3).

1.1.4 Deficiency anaemias

These are the most frequently encountered anaemias. A useful marker is the RBC volume (MCV), which indicates that there is either a nuclear maturation defect or a haemoglobin synthesis defect. In the former case, the impaired cell division process allows a normal intracellular haemoglobin concentration to be achieved after a low number of cell divisions thus resulting in macrocytosis. When haemoglobin synthesis is defective, the immature RBC will divide more times during the slower haemoglobin production process, thus resulting in microcytosis. Vitamin B12 (cyanocobalamin) and folic acid deficiency will present with macrocytic features and iron deficiency with microcytic features.

Table 2: **International Prognostic Score System for MDS**				
	Score			
Prognostic variable	**0**	**0.5**	**1**	**1.5**
Bone marrow blasts (%)	< 5	5-10	-	11-20
Karyotype	Good	Intermediate	-	Poor
Cytopenias	0-1	2-3		

Karyotype: Good: normal, -Y, 5q-, 20q-; Poor: complex (>3 anomalies or chromosome 7); Intermediate: others. Cytopenias: absolute neutrophil count < 1.8x10⁹/L, Hb < 100 g/L, platelets < 100x10⁹/L. Adapted from (4).

Table 3: **IPSS prognostic groups**				
Combined Score	**Risk Category**	**Frequency (%)**	**Median survival (years)**	**Time to 25% probability of evolution to AML (years)**
0	Low	33	5.7	9.4
0.5-1.0	Int-1	28	3.5	3.3
1.5-2.0	Int-2	22	1.2	1.1
≥ 2.5	High	7	0.4	0.2

Four prognostic categories are defined based on the IPPS. A clear separation is observed between groups in terms of overall survival and risk of evolution to acute leukaemia. Adapted from (4).

Iron deficiency remains the most common cause of microcytosis, followed by alpha/beta thalassaemia, Hb E and Hb C (5). Iron deficiency states will present with hyporegenerative and microcytic anaemia. Iron metabolism is finely tuned to regulate intestinal absorption and serum iron level. Iron deficiency is defined as a low serum iron, elevated transferrin iron binding capacity (as a response to deficiency) and low ferritin (reflecting low iron stores). Iron deficiency is very frequent in menstruating women and probably underestimated. Other aetiologies include chronic blood loss (gastrointestinal, phlebotomy), malabsorption (gastrectomy, achlorhydria) or increased needs (pregnancy, breast feeding). The source of blood loss should always be identified when investigating iron deficiency anaemia. In younger patients (<60 years old), the upper digestive tract should be investigated first, while in older patients a colonoscopy may identify bleeding polyps or angiodysplastic lesions (affecting up to 3-5% of people above 60 years).

It has recently become clear that *Helicobacter pylori* infection is frequently associated with iron deficiency which is either refractory to iron treatment or relapses once iron therapy is discontinued. Competition between *Helicobacter pylori* and the microorganisms responsible for iron absorption is thought to be the cause of iron

depletion and of chronic gastritis (6-8).

Progress in our knowledge about iron metabolism has led to the recognition of the hereditary forms of iron deficiency anaemias. Of these, two recently described entities, mutations in the genes coding for DMT-1 and in matriptase -2 proteins will be described in detail in the second part of the present chapter.

Normal vitamin B12 body stores are around 5 mg, which allows an individual to survive 4-5 years without a supply of exogenous B12. B12 is absorbed from animal products only in the terminal ileum and requires adequate amounts of gastric intrinsic factor (9). Several conditions can lead to B12 deficiency: autoimmune gastritis (Biermer's or pernicious anaemia), partial or total gastrectomy, a diet poor in animal or dairy products, e.g. in vegetarians and especially vegans (who eat neither eggs nor milk products), malabsorption states (chronic ileitis, ileal resection, bacterial overgrowth syndrome), drugs (proton pump inhibitors, metformin, cholestyramine). Despite being considered normal by many clinicians and laboratories, levels below 220 pmol/L should be considered low and should be supplemented, especially in elderly subjects (10). Atrophic gastritis is a frequent phenomenon in the elderly and, in this population, is probably the main cause of B12 deficiency. The morphologic and haematologic features of folic acid deficiency are similar to those of B12 deficiency. However, it should be borne in mind that:

a. in the event of complete deprivation, folic acid stores are only sufficient for 3-4 months;

b. there are no neurological signs, and folic acid supplementation in the case of macrocytosis may precipitate neurological damage due to associated B12 deficiency/low stores;

c. folic acid deficiency may be drug induced, particularly by antimetabolites (methotrexate) or inhibitors of tetrahydrofolic reductase (co-trimoxazole);

d. folic acid malabsorption is extremely rare, so oral supplementation with pharmacological doses is sufficient;

e. the main causes of folate deficiency, other than drugs, are pregnancy, ethanol abuse, inadequate dietary intake (especially in the elderly), and chronic haemolytic anaemia.

1.1.5 Bone marrow infiltration and fibrosis

These states produce the same clinical picture of marrow failure with some degree of blood-marrow barrier rupture resulting in anaemia with or without other cytopenia(s), with immature blood cells circulating in the periphery (myelocytes, metamyelocytes, erythroblasts). A frequent anomaly is the presence of dacryocytes (tear drop RBC). Both haematologic and solid tumours can infiltrate bone marrow. In an advanced stage, diseases such as multiple myeloma, lymphomas, prostate

cancer, lung cancer, breast cancer, and melanoma typically infiltrate the marrow. Primary Myelofibrosis, a myeloproliferative disorder, will slowly induce complete marrow fibrosis with displacement of "strangled" haematopoiesis to the liver and spleen. The peripheral blood will show anaemia and in the advanced stages bi- or pancytopenia with circulating marrow cells at all stages of differentiation.

1.1.6 Anaemia of chronic disease (ACD)

ACD is observed in many conditions associated with chronic inflammation. The pattern of hyporegenerative, microcytic, hypochromic anaemia was thought to be consistent with a disorder of iron metabolism and iron incorporation into the erythroid progenitors, but it was only recently that this was confirmed. The identification of hepcidin, the central regulator of iron homeostasis, allowed us to fully understand this condition (see below).

1.1.7 Anaemia of chronic renal failure

Erythropoietin (Epo) is mainly secreted by the kidneys in response to hypoxia (11). Its action on bone marrow stem cell and erythroid progenitors induces a rapid burst of RBC production. In renal failure, Epo production will gradually decrease and a normocytic aregenerative anaemia will occur. Epo levels usually remain adequate until a decrease of creatinine clearance to less than 30 mL/min (serum creatinine 160µM, 1.8mg/dL). Now that recombinant human Epo is commercially available, all pre-dialysis and dialysis patients benefit from Epo treatment. Anaemia with low Epo levels can also occur in the presence of neutralising antibodies to Epo (12). Such cases have been observed after repeated administration of subcutaneous rhEpo to treat progressive or terminal renal failure.

1.2 Regenerative anaemias

Regenerative anaemia is defined as anaemia with an elevated reticulocyte count (more than $100x10^9$/L). This condition can result either from increased RBC destruction (i.e. haemolysis) or from haemorrhage.

Immune haemolytic anaemia is secondary to an immune mechanism leading to antibody-dependent red cell lysis, with or without activation of complement. Non-immune haemolysis is classified as either congenital or acquired. Congenital haemolytic conditions include erythrocytic membrane defects (spherocytosis, elliptocytosis, acanthocytosis), specific haemoglobin anomalies (sickle cell anaemia, Hb C, Hb H, other unstable haemoglobins and some thalassaemias) and erythrocytic enzyme deficiency (glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency). Acquired haemolytic anaemias can occur as a side effect of drugs, may be secondary to a deficit of phosphatidyl inositol glucosyltransferase (paroxysmal

nocturnal haemoglobinuria), or the result of RBC fragmentation in the microcirculation or in large vessels. Finally, increased RBC destruction is observed in patients with enlarged spleen (hypersplenism).

1.2.1 Immune haemolytic anaemias
These result from the presence of antibodies targeting one or more of the numerous RBC membrane antigens. This condition is frequently associated with other autoimmune or lymphoproliferative disorders, and can also be induced by drugs (hapten-related reactions) or infections (bacterial or viral). Depending on whether complement is activated or not, haemolysis may be intravascular (mediated by complement activation) or extravascular (due mainly to phagocytosis of RBC in the spleen) (see below).

1.2.2 Sickle cell anaemia
Sickle cell anaemia is a prototype of an inherited chronic regenerative anaemia. Anaemia is secondary to Hb polymerisation leading to RBC deformation and lysis. Patients homozygous for the SS mutation will develop a chronic and recurrent haemolytic anaemia that may require RBC transfusions from a young age. Sickle cell anaemia is due to a single nucleotide substitution at codon #6 (glu → val) of the beta chain of haemoglobin. As a consequence, Hb S tends to aggregate into long filaments when it is in the deoxy form (i.e. desaturated), thus deforming the RBC membrane and ultimately inducing cell lysis. The pathophysiology of the disease is related to occlusion of small veins by aggregation of deformed RBC (13), which can finally result in severe ischaemia and painful crisis. The principal sites of veno-occlusion are the bone marrow, the spleen (inducing spontaneous splenic atrophy during the first years of life), the brain and the lungs. Major complications such as stroke, aseptic bone necrosis and acute chest syndrome will seriously alter the quality of life of affected individuals. Treatment is mainly supportive during the acute crises with pain control, oxygen, and i.v. hydration (14). Prevention of frequent crises or severe complications can be achieved by treatment with hydroxyurea (15) or regular transfusions (16). Despite the risks associated with the procedure, stem cell transplantation has been successfully performed in young adults (17, 18).
More details about sickling disorders, haemoglobin variants and thalassaemia syndromes can be found in other Chapters of this book.

1.2.3 Anaemia due to enzymopathies and red cell membrane defects
Red blood cells have an active anaerobic metabolism using glucose as the energy supply. The main metabolic functions include membrane protein maintenance, preservation of haemoglobin iron in the Fe^{3+} status, and modulation of haemoglobin affinity for oxygen. These functions need the regulation of four components: ATP,

NADH, NADPH, and 2,3 diphosphoglycerate. The common enzymatic defects include pyruvate kinase and glucose 6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency results in haemolysis in response to oxidative stress such as drugs, dehydration and fever/infection, while PK deficiency is characterised by a continuous haemolysis due to its activity in the main anaerobic glycolytic pathway. Red cell membrane defects are described in Chapter 16 while enzymopathies are described in Chapter 17 of the present book.

1.2.4 Haemorrhagic anaemia

Haemorrhagic anaemia results from acute bleeding, which may be suspected from the results of complete blood count analysis. A relatively severe anaemia, poorly tolerated by the patient and initially with no increase in regeneration (i.e. normal or low reticulocytes), is probably secondary to bleeding. The reticulocyte response will usually be detectable within 24 hours with an increase of early reticulocytes (elevation of high fluorescence rate, HFR reticulocytes). Frank reticulocytosis will start within 48-78 hours.

2. Selected anaemias

2.1 Pure red cell aplasia

PRCA belongs to the family of aplastic anaemias and is a syndrome limited to central failure of the erythroid lineage only. It is a rare disease, presenting clinically with severely aregenerative anaemia and a bone marrow aspiration showing normal myeloid and megakaryocytic lineages, but a greatly diminished erythroid population with only rare erythroid precursors.

The causes of the erythroid defect can be various, from an autoimmune disorder (often associated with thymoma), a viral infection, drugs or toxic agents, to a congenital primary stem cell anomaly (19).

A congenital form of PRCA, called the Blackfan-Diamond syndrome is characterised by an isolated erythroid hypoplasia, and mostly occurs as sporadic cases with occasional familial transmission following an autosomal recessive or dominant pattern. No clear gene defect has been identified, but a primary stem cell anomaly is proposed. In some cases, an increased rate of erythroid progenitor apoptosis has been suggested. The clinical picture is a low birth-weight child associated with abnormal facial proportions. Thumb malformations are frequent. Anaemia is profound with macrocytosis and a low reticulocyte count associated with severe erythroid hypoplasia in the bone marrow.

Acquired PRCA can be seen in an acute or chronic setting. Acute PRCA is associated

with parvovirus B19 infection. The virus specifically targets the erythroid precursors through the P membrane antigen and temporarily suppresses the production of red blood cells. Parvovirus infection is also known as "fifth disease" or *erythema infectiosum* and frequently affects children. A transient decrease of red cell production will marginally affect most otherwise healthy persons, but individuals with an increased red cell turnover, as seen in chronic haemolytic states, are susceptible to severe anaemia requiring transfusion support until spontaneous resolution of the infection. Another group of patients at high risk are those under immunosuppressive therapy or with decreased immunity (HIV, stem cell transplantation recipients); in such cases the parvovirus infection will be persistent and lead to severe anaemia (20).

Patients infected with parvovirus B19 will present with aregenerative anaemia associated with very low reticulocyte counts. The absence of high fluorescence reticulocytes (i.e. young reticulocytes with high RNA content) is typical for this condition. Bone marrow aspirate will show a markedly increased M:E ratio with rare persistent proerythroblasts, which occasionally show a vacuolated cytoplasm resulting from the viral insult (Figure 2). If immunity is intact, a patient with chronic haemolytic anaemia and parvovirus B19 infection will temporarily require blood transfusions, but will recover a normal red blood cell production within a few weeks. However, immunosuppressed patients are at high risk of chronic parvovirus infection and will need passive immunotherapy with i.v. immunoglobulins which can help to eradicate the infection in most of the cases (Figure 3).

Figure 2: **Parvovirus infected proerythroblast**
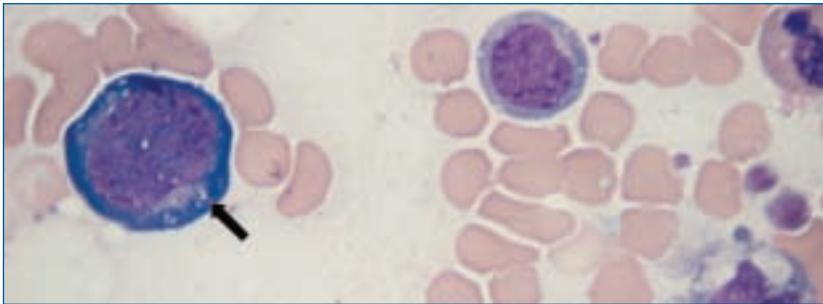
This picture shows the bone marrow aspirate of a kidney transplant recipient who developed a pure red cell aplasia three months after transplantation. He was receiving sirolimus as immunosuppressive therapy. The arrow shows small vesicles in the cytoplasm of a proerythroblast. These vesicles contain viral particles as shown by electronic microscopy.

Figure 3: **Parvovirus B19 infection**
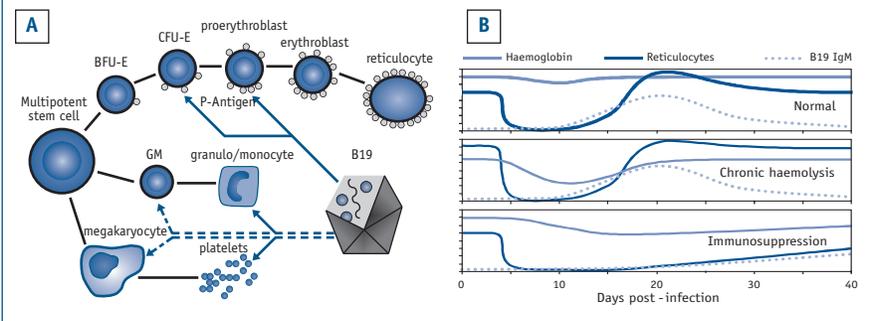
A. Specific targeting of P antigen allows parvovirus to infect late erythroid progenitors (from CFU-E onwards). B. Changes in haemoglobin, reticulocyte count and anti-parvovirus IgM during three classical situations (normal, chronic haemolysis and immunosuppressed patients). Chronic haemolysis induces a high RBC turnover and demands an elevated production; parvovirus infection will induce a severe, although relatively short, aregenerative anaemia. In contrast, immunosuppressed patients will show a progressive anaemia of long duration if untreated.

Chronic PRCA can occur in patients with underlying lymphoproliferative disorders, thymoma, autoimmune disorders, or systemic lupus erythematosus (SLE). Etiologic evaluation must always be undertaken.

2.2 B12 deficiency anaemia

Between 1847 and 1880, Adisson, Biermer and Ehrlich described a severe form of anaemia associated with megaloblastic features and neurological disturbance. Slowly progressive and invariably causing death, it was called pernicious anaemia. In 1927 the first effective treatment was administered to patients thanks to the work of Whipple, Minot and Murphy consecrated by a Nobel prize in 1934. A diet containing large quantities of liver allowed correction of anaemia and of neurological signs. In the same period, Castle identified a factor produced in the stomach (intrinsic factor) that was found to improve Hb values of patients with pernicious anaemia (21). Intrinsic factor is a 45 kD protein produced by gastric parietal cells with a low affinity for cobalamin. The synthesis of vitamin B12 (cyanocobalamin, 1948) allowed a simple treatment of this otherwise lethal condition.

Vitamin B12 absorption is dependent on different digestive enzymes and proteins. Figure 4 depicts the absorption process.

In order to be adequately absorbed, all the following conditions must be achieved: sufficient dietary intake, release of B12 by acid and pepsin, sufficient pancreatic enzyme to free B12 from haptocorrin, secretion of normal amounts of intrinsic factor

Figure 4: **Vitamin B12 absorption process**
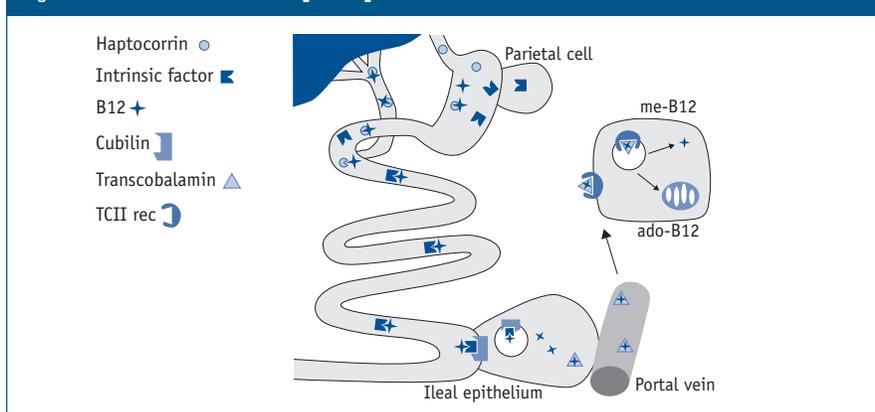
Vitamin B12 is released from food, by gastric acid and pepsin and initially is fixed by haptocorrin present in saliva. In the duodenum the alkaline environment and proteases release haptocorrin, allowing fixation to intrinsic factor. A specific ileal receptor, cubilin, allows specific absorption and transfer of B12 to transcobalamin in the blood circulation. Final utilisation by cells is driven by the transcobalamin receptor. TCII: transcobalamin II; me-B12: methylcobalamin; ado-B12: adenosylcobalamin.

and a normal ileal mucosa to bind B12-IF complex.

Once absorbed into body cells, vitamin B12 is used in two important biochemical pathways: methylcobalamin (in conjunction with folic acid) is required for the synthesis of methionine, which in turn allows methylation of DNA and proteins, while adenosylcobalamin is required for the synthesis of succinyl CoA and synthesis of fatty acids.

Vitamin B12 deficiency can originate from multiple aetiologies, summarised in Table 4.

The diagnosis of B12 deficiency is based on a serum level below ~135 pmol/L. This limit should be raised in elderly persons, where a level below 220 pmol/L should be considered pathologic. The same rule applies to younger individuals displaying macrocytic anaemia and hypersegmented neutrophils.

Haematologic features include macrocytic anaemia with MCV generally over 110 fl, leucopenia and thrombocytopenia. The peripheral smear will show megalocytes (macrocytes with an oval shape), macrocytosis and the presence of hypersegmented neutrophils (as a rule, more than 2% with 5 segments or at least 1% with 6 segments). In severe deficiency, ineffective marrow erythropoiesis leads to intramedullary haemolysis with a high plasma LDH and decreased haptoglobin (22). Pernicious anaemia is the most frequent cause of B12 deficiency with an estimated 4% of women and 2% of men affected in the general population. A direct autoimmune

Table 4: **Aetiology of B12 deficiency**	
Cause	**Condition**
Insufficient intake	• Strict vegans (no milk, cheese or eggs)
Absorption defect	• Blind loop syndrome
	• Post infectious (tropical sprue)
	• Crohn's disease
	• Diphyllobothrium latum (clearwater fish)
Intrinsic factor defect	• Pernicious anaemia
	• Atrophic gastritis (elderly)
	• Post gastrectomy
	• Proton pump inhibitors

attack on the gastric parietal cell leads to complete extinction of intrinsic factor. The autoantibodies are directed against both parietal cells and intrinsic factor. The identification of anti-parietal cell autoantibodies is more sensitive, while the anti-intrinsic factor antibodies are more specific. About 70% of patients with pernicious anaemia will produce detectable levels of such autoantibodies.

As B12 stores are sufficient for about 5 years before deficiency leading to clinical symptoms, pernicious anaemia will develop slowly. Nowadays, the full clinical picture - with severe intramedullary haemolysis and severe neurological symptoms with demyelinisation leading to weakness and paraplegia - occurs only rarely.

Treatment with parenteral vitamin B12 will lead to a rapid increase of reticulocytes (within 48-72 hours) and subsequent correction of anaemia. Neurological symptoms tend to respond slowly and may be irreversible depending on severity and duration of B12 deficiency or if folic acid was given without B12 in combined deficiencies. In very severe deficiencies, one should follow the plasma level of potassium, as the rapid restoration of erythropoiesis in the bone marrow may lead to hypokalaemia. Usually, cyanocobalamin is given i.m. or s.c. as 1 mg dose repeated weekly for one month, then monthly to bimonthly according to blood levels. In the absence of intrinsic factor, about 1% of ingested B12 is absorbed through the ileal mucosa. Thus, a daily oral dose of 1mg can be sufficient to maintain steady levels in patients not willing to receive regular injections (23).

2.3 5q- syndrome
The characteristics of the 5q– syndrome (according to Van den Berghe, subsequently restricted to cases with marrow blasts < 5% by the WHO classification) are (24):
• Female preponderance
• Severe anaemia

- Pronounced macrocytosis
- Normal or moderately decreased leucocytes
- Normal or moderately increased platelets
- Rare AML transformation (10%) (compared with other forms of MDS)
- Prolonged survival
- Characteristic dysmegakaryopoiesis (large monolobulated megakaryocytes with eccentric nucleus, see also Figure 5)
- No or moderate blast excess (restricted to marrow blasts < 5% in the WHO classification)
- Isolated 5q deletion.

Figure 5: **Dysplastic megakaryocyte**

Typical dysplastic megakaryocyte (large monolo-bulated megakaryocyte with eccentric nucleus) in the bone marrow of a female patient suffering from the 5q- syndrome. Wright staining, 1000X magnification.

In the bone marrow we find the characteristic dysmegakaryopoiesis (large monolobulated megakaryocytes with eccentric nucleus) with hypoplasia of the erythroid precursors. There is no or moderate blast excess (restricted to marrow blasts < 5% in the WHO classification). Finally cytogenetic studies confirm the diagnosis by demonstrating the isolated 5q deletion.
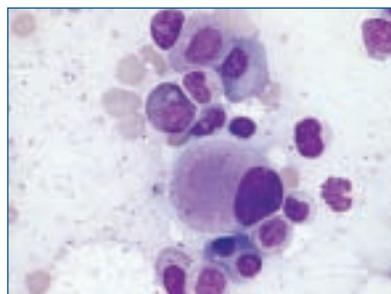
5q- syndrome has a good prognosis and according to prospective randomised studies between 70 and 80% respond to treatment with the newly developed immunomodulator drug lenalidomide (25). This treatment is cost-effective as compared to iterative transfusion and chelation.

2.4 Anaemia of chronic disease

2.4.1 Pathophysiology

Anaemia of chronic disease is the second most prevalent form of anaemia after iron deficiency anaemia. It is typically found in patients with any kind of infection (viral, bacterial, parasitic, and fungal), in patients with cancer or in patients with autoimmune disorders such as rheumatic arthritis, SLE, and other vasculitides. It is also frequently seen in solid organ recipients who develop chronic rejection.

Recent advances in our knowledge of iron metabolism and regulation as well as of Epo function and secretion have improved our understanding of the pathophysiology of this kind of anaemia. In inflammatory diseases, cytokines like IL-1, IL-6 and TNF-α are secreted in conjunction with bacterial lipopolysaccharides. These mediators

induce the production of hepcidin by the liver. It is now known that hepcidin inhibits duodenal absorption of iron as well as iron release from macrophages (26). This subsequently leads to decreased availability of iron for erythroid progenitor cells, thus turning RBC production towards iron-restricted erythropoiesis mimicking true iron deficiency. Ferroportin is also downregulated by the proinflammatory stimuli, further blocking the release of iron from macrophages. In patients with anaemia of chronic disease, the proliferation and differentiation of erythroid precursors are impaired by IFN-α1, -β1, -γ1, TNF-α, and IL-1. IFN-γ appears to be the most potent inhibitor of these inflammatory cytokines.

It was also shown, at least *in vitro*, that IL-1 and TNF-α directly inhibit the expression of the Epo gene and Epo receptor, thus further decreasing erythroid proliferation and increasing erythroid apoptosis (27).

In summary, chronic inflammation leads to anaemia in three different ways: first, at the iron level, second at the Epo-Epo receptor level and finally at the erythroid precursor level.

2.4.2 Laboratory evaluation

Anaemia is usually mild to moderate (Hb levels rarely below 80g/L) with a low reticulocyte count. Serum iron is low, as is transferrin saturation. Ferritin, in contrast, is normal or increased and soluble transferrin receptor (sTFR) is normal. Anaemia of chronic disease can be distinguished from iron deficiency anaemia by a low ratio of sTFR over the decimal logarithm of the ferritin (less than 1). In the case of iron deficiency, the ratio is often over 2 (28). MCV is normal or slightly decreased. A severe microcytosis indicates co-existent iron deficiency or a thalassaemic condition. When dealing with the diagnosis of anaemia of chronic disease, it is mandatory to examine the biochemical and clinical evidence of inflammation as well as to look for an underlying cause of iron deficiency. In fact, the precise diagnosis and subsequent treatment of the underlying disease is essential for the improvement/correction of this type of anaemia.

Finally, patients originating from the thalassaemia belt region should be evaluated for a possible β-thalassaemia, which is also the most common haemoglobinopathy in Africa and Southeast Asia.

2.5 Genetic forms of iron deficiency anaemia

2.5.1 DMT-1 mutations

DMT1 is a transmembrane protein encoded by the SLC11A2 gene located on chromosome 12 (29). It is involved in iron absorption by the enterocytes in the duodenum and in iron transport from the microsomes to the cytoplasm in the

erythroblasts. In case of a mutation affecting the function of DMT-1 iron absorption in the duodenum continues because the absorption of haem iron is not disturbed. (In fact, in meat-eating humans it is estimated that about 2/3 of absorbed iron comes from haem.) Thus in humans, a mutation in DMT1 protein will primarily affect iron utilisation and not absorption, leading to a severe microcytic iron deficiency anaemia with increased iron stores. To date mutations in the gene encoding DMT1 has been described in three families (30-32). All the affected patients presented a severe hypochromic, microcytic anaemia with hepatic iron overload: Hb between 74 and 85 g/L; MCV between 51 and 54 fl; MCHC between 287 and 296 g/L. Serum iron and serum transferrin saturation was high while serum ferritin was low or normal at the time of diagnosis: 5, 6 and 20 years old respectively. It is expected that ferritin levels will be high in older affected patients. The disease is autosomal recessive and the anaemia is present from birth.

2.5.2 Mutations in matriptase-2 gene

Matriptase-2 is an essential regulator of iron homeostasis. In mice as well as in humans, mutations in the $Tmprss6^{-/-}$ gene lead to severe iron deficiency anaemia. This state is characterised by reduced ferroportin expression (shown in the mouse model) and both animals and humans have high hepcidin levels (33, 34). Recent studies have demonstrated that TMPRSS6 (Matriptase-2) is a transmembrane protease suppressor of hepcidin gene expression. *In vitro* studies showed that it acts via hepatic haemojuvelin (35). Mutation in matriptase leads to the IRIDA disease (Iron-Refractory, Iron-Deficiency Anaemia). Nine patients have so far been described (36-38). All of them presented from birth with a moderate to severe anaemia with severe microcytosis (MCV from 49 to 65 fl), with typical iron deficiency state (low serum iron and serum transferrin saturation, high serum transferrin receptor). Typically when hepcidin levels were measured high levels were always found, reflecting the absence of matriptase function. Oral iron administration is ineffective and response to parenteral iron administration is partial.

2.6 Immune haemolytic anaemia

2.6.1 Introduction – classification

Immune haemolytic anaemia is caused by the presence of RBC autoantibodies or RBC alloantibodies. The former situation is usually called autoimmune haemolytic anaemia (AIHA), and can occur as a primary (idiopathic) condition or secondary to a defined disease process or drug therapy. Alloantibodies are formed during pregnancy, after transfusion or post haematopoietic stem cell or solid organ transplantation (Table 5). RBC autoantibodies causing AIHA are further classified as warm or cold autoantibodies

depending on the temperature at which they react optimally to cause AIHA: 37°C or less than 15°C respectively. Thus on the basis of serologic and clinical investigations, AIHA can be further classified into warm type, cold agglutinin syndrome and paroxysmal cold haemoglobinuria (Figure 6).

Warm type AIHA can occur at any age and can be acute, transient or chronic. Drug induced AIHA is almost always associated with a warm antibody.

Cold agglutinin syndrome usually occurs in older patients, and is due to the presence of an IgM antibody optimally reacting at cold temperatures. When the specificity is polyclonal, the aetiology is mycoplasma or viral infections. If the IgM is

Table 5: **Classification of immune haemolytic anaemias**		
Autoantibody		**Alloantibody**
Primary	**Secondary**	
• Idiopathic	• Lymphoproliferative disorders • Infections • Drug-induced • Systemic autoimmune disorders • Other	• Pregnancy • Transfusion • Post-transplantation

Ref. (39)

Figure 6: **Relationships between the different types of autoantibodies and aetiologic factors**
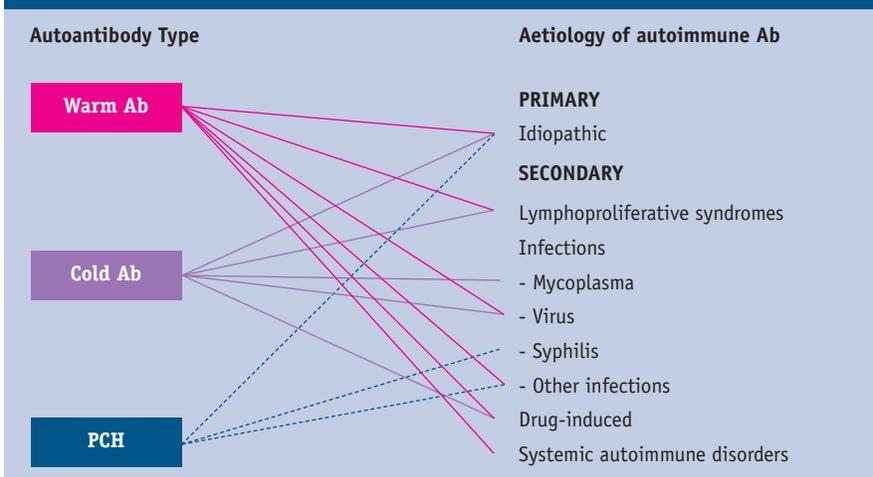
Ab: antibody; PCH: Paroxysmal cold haemoglobinuria (Donath–Landsteiner antibodies)

monoclonal, it is secondary to a lymphoproliferative disease or a monoclonal gammopathy of unknown significance.

Paroxysmal cold haemoglobinuria is usually seen in children and manifests as an acute transient haemolytic episode in association with an infectious state. The chronic form is actually the rarest form of AIHA and is seen in adults suffering from syphilis (Donath–Landsteiner antibodies).

Drug-induced immune haemolytic anaemia (DIIHA) is rare. To date, about 100 different substances have been implicated, for which there is reasonable published evidence to support an immune aetiology for a DIIHA and/or positive direct antiglobulin test. Currently, the most common drugs associated with DIIHA are cefotetan (Cefotan®) and cefriaxone (Rocephin®) (40).

Haemolytic transfusion reactions (HTR) (apart from ABO mismatched transfusions) are due to recipient production of antibodies to donor RBC antigens. HTR may be acute and severe occurring shortly after a transfusion and may present with fever and/or chills, hypotension, dyspnoea, tachycardia and signs of intravascular haemolysis and acute renal failure. HTR may also be mild and manifest days to weeks after the transfusion. The manifestation will then be anaemia without severe intravascular haemolysis. These delayed reactions are usually well tolerated. As discussed below, factors influencing the outcome of HTR include the characteristics of the RBC antigen, the type of antibody, production of mediators and predisposing conditions in the patient.

More than 50 different RBC antigens can cause haemolytic disease of the foetus and newborn (HDFN) by alloimmunisation of the mother. However, only anti-Rh (D), -C and –Kell commonly appear in large series of severe HDFN necessitating intrauterine transfusion. To avoid such haemolytic complications, complete maternal and paternal RBC antigen phenotype and genotype testing is essential for screening pregnancies at risk. Assessment of the patient with RBC alloimmunisation in pregnancy requires a DNA reference laboratory and well-trained obstetricians/gynaecologists. Diagnosis and clinical management of a mother with RBC sensitisation during the first pregnancy or with a previously affected infant is beyond the scope of this book.

Immune haemolysis is one of the adverse effects that can occur following haematopoietic stem cell or solid organ transplantation. Table 6 lists the most frequent causes of haemolysis following HSCT that have an immune aetiology. A distinct syndrome of immune haemolysis following transplantation has become known as the "passenger lymphocyte syndrome". It has been attributed to the proliferation of and antibody production by "passenger" lymphocytes present in the blood vessels of the transplanted organ or in the stem cell transfusion. This phenomenon has been described for most solid organ transplantations (kidney, liver, lung, heart and pancreas). It is remarkable that the few lymphocytes administered

Table 6: **Differential diagnosis of immune haemolysis following HSCT**
Minor ABO blood group incompatibility
Major ABO blood group incompatibility a) immediate b) early post transplantation c) late post-transplant haemolysis of newly produced RBC by residual isohaemagglutinins
RBC alloantibodies targeting blood groups other than ABO
Autoimmune haemolytic anemia
Passive transfer of antibodies by: a) plasma transfusion b) platelet transfusion c) i.v. immunoglobulins

Ref. (41)

with the transplant are capable of proliferating sufficiently to produce enough antibody to cause haemolysis of the recipient RBC within a few weeks of transplantation. The clinical course of a typical case is illustrated in Figure 7.

2.6.2 Pathophysiology of immune haemolytic anaemia

Drug induced antibodies may be divided into two main types:
- Drug-independent antibodies, i.e. the presence of the drug is not required for their detection in the test system
- Drug-dependent antibodies.

The first type of antibody is similar to typical warm autoantibodies and associated with cladribine, fludarabine, levodopa, methyldopa and procainamide. The aetiology of these "true" autoantibodies is still unknown. It is believed that the above-mentioned drugs directly affect the immune system creating an autoimmune disease.

There are two types of drug-dependent antibodies. The penicillin (or hapten) type reaction is due to a covalent binding to the RBC membrane causing an allergic reaction; the antibody reacting to the membrane-bound drug induces an extravascular (splenic) haemolysis. The second type of drug-dependent antibody is called the immune complex type. These antibody-drug immune complexes trigger complement activation and result in acute severe intravascular haemolysis. In this situation, the RBC is an innocent bystander. A third situation, which is not fully understood, leads to the formation of autoantibodies against RBC components. Figure 8 shows a proposed unifying hypothesis of drug- induced antibody reactions.

Recently a new mechanism for drug induced haemolytic anaemia has been described. It seems that DIIHA is the result of non-immune protein adsorption onto drug-treated

Figure 7: **Clinical course of a "passenger lymphocyte syndrome" within a few days after minor ABO mismatched BMT (donor 0+, recipient B+)**
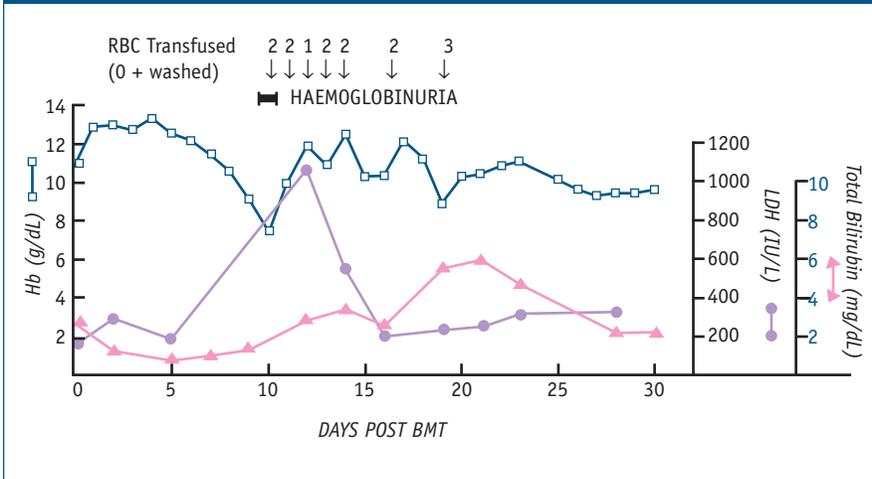
The chart shows haematologic and biochemical evidence of haemolysis. Fourteen units of packed 0+ cells were required to maintain an adequate haemoglobin level between day 10 and day 19 post-BMT. Note that the haemoglobin, LDH and total bilirubin were normal for the first 5 days post-BMT. During the haemolytic episode, no incompatible isohaemagglutinins were infused; RBC transfused were washed group 0; platelets were group B or 0 washed and resuspended in fresh AB plasma. (Reproduced from How J et al., Blood 1986; 67: 177-81 with permission).

RBCs. Drugs that have been described as causing haemolysis by such a mechanism are cefotetan, cisplatin, oxaliplatin, and the beta-lactamase inhibitors (42).
In systemic autoimmune disorders and cases of lymphoproliferative disorders with a warm autoantibody (85% of chronic lymphocytic leukaemias), we are dealing with a polyclonal IgG anti-RBC. Cold agglutinin associated with a lymphoproliferative syndrome is almost always monoclonal and of the IgM type.

2.6.3 Diagnosis of haemolytic anaemia
The first step is to demonstrate red cell destruction, which may be intravascular or extravascular. In each case, a regenerative anaemia is found with a reticulocyte count >100x10^9/L. In extravascular haemolysis, indirect bilirubin and LDH are increased, but to a lesser degree than in intravascular haemolysis. Haptoglobin is generally low and free Hb is elevated. RBC morphology and platelet count can help to diagnose a fragmentation syndrome (micro or microangiopathy). The presence of spherocytes (also called microspherocytes) indicates immune haemolytic anaemia

Figure 8: **Different types of drug-induced antibody reactions**
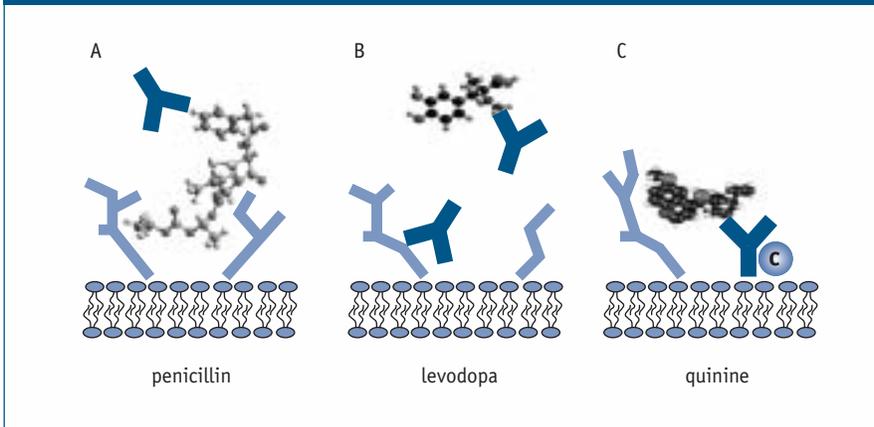
*Drugs (haptens) bind loosely or firmly to the RBC membrane and antibodies can be raised to: **A.** the drug directly (penicillin-like reaction); **B.** membrane compounds showing some similarities to the drug conformation (levodopa); **C.** drug in circulation; the drug-antibody complex attaches to the RBC membrane, secondarily causing complement (C) activation and intravascular lysis through an immune complex mechanism (quinine). Ref (40).*

or familial spherocytosis. The Coombs test (direct antiglobulin test) is a key test for distinguishing immune haemolytic anaemia from other haemolytic processes and especially from familial spherocytosis (Figure 9). Furthermore, it provides some information on the nature of the antibody: IgG in case of warm, IgM in case of cold antibody fixing complement. Serologic studies looking for viruses and autoantibodies (HIV, HBC, HCV, ANA, rheumatoid factor), serum protein electrophoresis and full body CT-scan help to determine the possible cause of immune haemolytic anaemia should also be done. In general, there is a 50% chance that immune haemolytic anaemia will be secondary to a lymphoproliferative syndrome or an autoimmune disease, and a 50% chance that it will be primary. Thus the diagnosis of idiopathic autoimmune haemolytic anaemia is one of exclusion.

When drug-induced haemolysis is suspected, it is advisable to test the patient's serum as well an eluate from the patient's RBC against RBC in the presence and absence of the suspected responsible drug. For details of technical methods that can be used for this type of investigation, the reader is referred to a recent specialised text (43).

When a haemolytic crisis is triggered by infection, it would be reasonable to rule out an enzymopathy or PNH. Finally, a patient originating from a thalassaemic or

Figure 9: **Direct Coombs test**
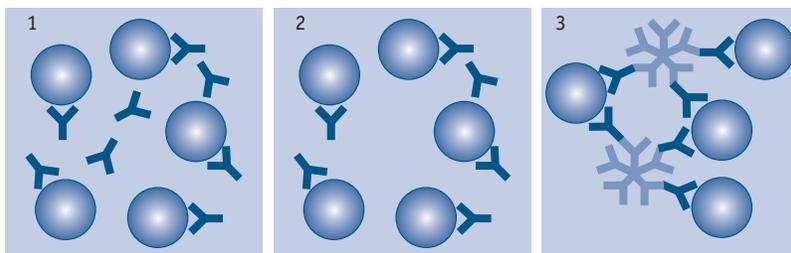
1. Whole blood is drawn; 2. RBC are washed so only membrane attached antibodies remain; 3. Anti-human IgM is then added, thus provoking the agglutination of antibody-bearing RBC.

tropical region, with a positive familial history and anaemic since a young age, strongly suggests a haemoglobinopathy. Laboratory testing for such pathology is described elsewhere in this book. A working algorithm for diagnosis of regenerative anaemia is shown on page 135.

2.7 Non-immune haemolytic anaemia

2.7.1 Paroxysmal nocturnal haemoglobinuria (PNH)
PNH is an acquired haemolytic syndrome caused by a deficit in phosphatidyl inositol glucosyltransferase (coded by the PIG-A gene) (44). This enzyme assures the correct attachment of type III protein to the cell membrane through a GPI-anchor. Many surface proteins from the haematopoietic lineages are dependent on this mechanism. At least two RBC proteins, CD59 and CD55, are responsible for protecting the cells from complement-induced cell lysis. Figure 10 shows a schematic view of the interaction between CD59 (protectase) and the complement system. The final complement complex C5b-C6-C7-C8 normally interacts with C9 to form the membrane attack complex, creating a lytic pore in the RBC membrane. RBC are highly dependent on this mechanism as they physiologically transport complement on their membranes through the blood circulation.

PNH will present with acute episodes of intravascular haemolysis producing haemoglobinuria, mostly at night (due to an increased nocturnal activity of complement). When testing for the absence of type III proteins at the cell surface of haematopoietic cells, one sees that not only are RBC lacking CD59 and CD55, but

Figure 10: **Protective mechanism against red cell lysis by the membrane attack complex (MAC) through a GPI anchored protein (protectin, CD59)**
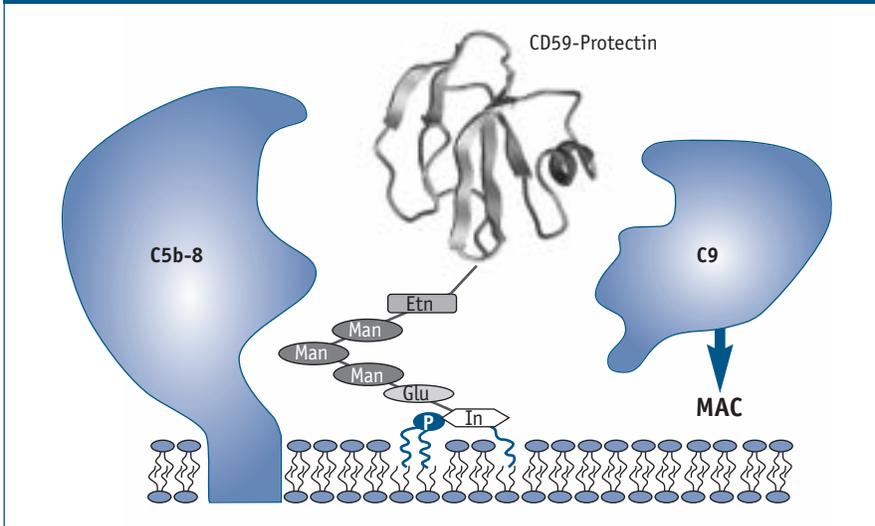
The normal interaction between the C5b-C8 complex and C9 is blocked, thus avoiding the formation of the membrane attack complex. In PNH, the inability to synthesise the GPI-anchor confers a lack of resistance to complement and results in chronic haemolysis. CD59 is the major actor, but CD55, another type III protein, also plays a role. Glu: glucosamine; Man: mannose; In: inositol; Etn: ethanolamine.

also that lymphocytes, monocytes and neutrophils are lacking CD56 and CD57, both of which are attached through a GPI anchor. This supports the hypothesis that PNH is a primary haematopoietic stem cell defect secondary to clonal alteration of a single stem cell. The clonal nature of the phenomenon can also be observed in aplastic anaemia. In this autoimmune disorder, one of the hypotheses is that a GPI-anchored protein may be the target of the immune attack. Thus lacking type III protein might confer a survival advantage to the PNH clone (45).

2.7.2 Microangiopathic disorders

Microangiopathy can be defined as a non-immune haemolysis associated with peripheral platelet destruction. The classical syndromes, called thrombotic thrombocytopenic purpura (TTP) and the haemolytic uraemic syndrome (HUS), despite being similar in their presentation, clinical course and severity, have been now clearly identified as separate pathophysiological entities. A common differential diagnosis is paraneoplastic microangiopathy, a sort of localised intratumoural

disseminated intravascular coagulopathy rapidly leading to death if the primary malignancy is not brought under control. Because the disease course and treatment can differ substantially, the general approach to the patient presenting with TTP-HUS should be systematic in order to define a clear aetiology.

Microangiopathy is generally defined as the combination of haemolysis associated with RBC fragments (schizocytes) and thrombocytopenia due to peripheral consumption. Depending on aetiologic factors, fever, renal failure or neurologic dysfunction (confusion, lethargy) may also be present.

During recent years, the mechanisms leading to microangiopathy have been elucidated. In TTP, a von Willebrand factor cleaving protease (Adamts13) has been shown to be absent or decreased. In normal states, endothelial cells produce vWF and multimers tend to aggregate at their surface. The serum metalloprotease, Adamts13, is responsible for cleaving the multimers and thus avoiding platelet aggregation and activation. The absence or decreased activity of Adamts13 leads to platelet activation and formation of a platelet clot leading to sheering and fragmentation of RBC upon contact. The process consumes most of the platelets, leading to moderate to severe thrombocytopenia and clinical purpura. The Adamts13 defect can be congenital or acquired in association with an autoimmune reaction raising antibodies against Adamts13, drugs (ticlopidin, cyclosporin, tacrolimus, mitomycinC), or some infections (viral) (46, 47).

HUS is secondary to direct damage to the endothelial membrane, leading to vWF multimer aggregation and platelet activation. In this situation, the normal Adamts13 is overwhelmed by the abundance of vWF, which also leads to microangiopathy. In this condition, the kidney microvasculature seems to be more sensitive than that of other organs; renal failure is the main result, although other organs may be involved (heart, brain, gastrointestinal tract). The classical situation leading to endothelial damage is the gastrointestinal infection by the verotoxin-producing *E. coli* HO157-H7. The secretion of verotoxin will damage the endothelium leading to microangiopathy (48).

Malignant hypertension is another cause of fragment-associated haemolysis, usually with mild thrombocytopenia. Bringing the blood pressure under control will result in immediate correction of the haemolysis.

Figure 11 summarises the current understanding of the pathophysiology of microangiopathic disorders and classifies these entities according to the presence or absence of the Adamts 13 protease.

2.7.3 Haemolysis secondary to toxic agents and other causes

Heavy metals such as lead and copper can typically cause haemolysis. In lead intoxication, only acute exposure will provoke haemolytic anaemia. The diagnosis

Figure 11: Classification of microangiopathic disorders
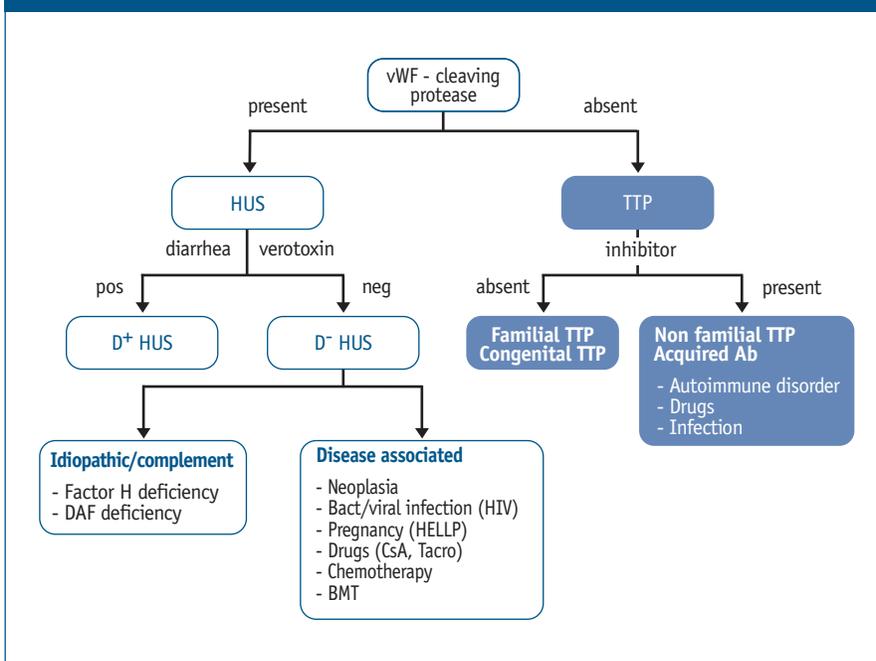
Von Willebrand factor cleaving protease (Adamts13) dosage is central to the diagnostic procedure (adapted from Micha Furlan, personal communication). D+ HUS and D- HUS, diarrhoea verotoxin positive or negative HUS.

is based on serum lead levels (> 40µg/dL or > 1.9µM/L). The blood film examination will reveal RBC basophilic stippling, reflecting lead-induced sideroblastic anaemia. A gum lead line due to the deposition of sulfide may also be seen. As a divalent cation, lead binds to protein sulfhydryl groups. Central nervous system (CNS) and bone marrow are the main organs affected. The toxic effect of lead poisoning is related to a severe inhibition of pyrimidine 5'nucleotidase activity as well as inhibition of glycolysis at the hexokinase step. Haem synthesis is also inhibited through ALA synthase, ALA dehydrase and ferrochelatase. The accumulated pyrimidines impair RNA breakdown resulting in aggregates of partially degraded ribosomes causing the basophilic stippling. Occupational exposure is the main source of intoxication in adults (cable factories, furnaces, glass and pottery industry, welding), but non-professional exposure is also possible through lead-loaded house paints (principally in toddlers living in badly maintained housing) or use of insufficiently fired

ceramics. The clinical presentation of acute poisoning generally includes abdominal pain and regenerative anaemia reflecting haemolysis. Chronic poisoning more frequently affects children, hypochromic anaemia and mental retardation being the only signs of intoxication (49).

Copper is an important catalyst of haem synthesis and iron absorption. Copper toxicity is rare and primarily affects the liver. Gastrointestinal and neurological toxicity occur when blood levels achieve 3 mg/L, but occasionally, Coombs negative haemolytic anaemia may be the sole sign of copper accumulation. Haemolysis results from oxidative damage to the red cell not necessarily associated with a G6PD deficiency (50).

Finally, acquired acanthocytosis is due to a severe alteration of RBC lipid membrane composition (51). Infections with plasmodium and clostridium can cause a direct mechanical haemolysis.

3. Practical approach to the anaemic patient

We propose to consider systematically the medical history, physical signs and laboratory findings in patients with all types of anaemia. Table 7 summarises the key points to look for in order to identify possible aetiologic factors.

Laboratory investigation will be targeted according to positive key points in the history and physical examination. However, a complete blood count with morphology of RBC, white blood cells and platelets should be part of the investigation of every case of anaemia. Bone marrow examination should be reserved for cases of anaemia of central origin, or where marrow infiltration is suspected, in particular in lymphoproliferative disorders.

Figure 12 gives algorithms for a diagnostic approach to anaemia. As discussed earlier, the most important measurement is the reticulocyte count, which distinguishes between aregenerative ($< 50x10^9$/L) and regenerative ($> 100x10^9$/L) anaemia. However, in practice, many cases have a reticulocyte count between 50 and $100x10^9$/L which makes it difficult to classify them as hyporegenerative or regenerative; they also sometimes represent an anaemia of mixed origin. In such cases, clinicians should consider causes of regenerative anaemia as well as partial inhibition of erythroid production (for example, acute bleeding when iron stores are almost exhausted or when there is an important inflammatory state). Sometimes, this kind of anaemia is a real diagnostic challenge and, for that reason, we suggest a systematic assessment according to Table 7. In elderly patients in particular, iron studies, and measurement of B12 and folate, as well as bone marrow aspirate and biopsy should be performed in the absence of a clear-cut diagnosis.

Anaemia is the most frequent human pathology. It may reflect a primary defect of

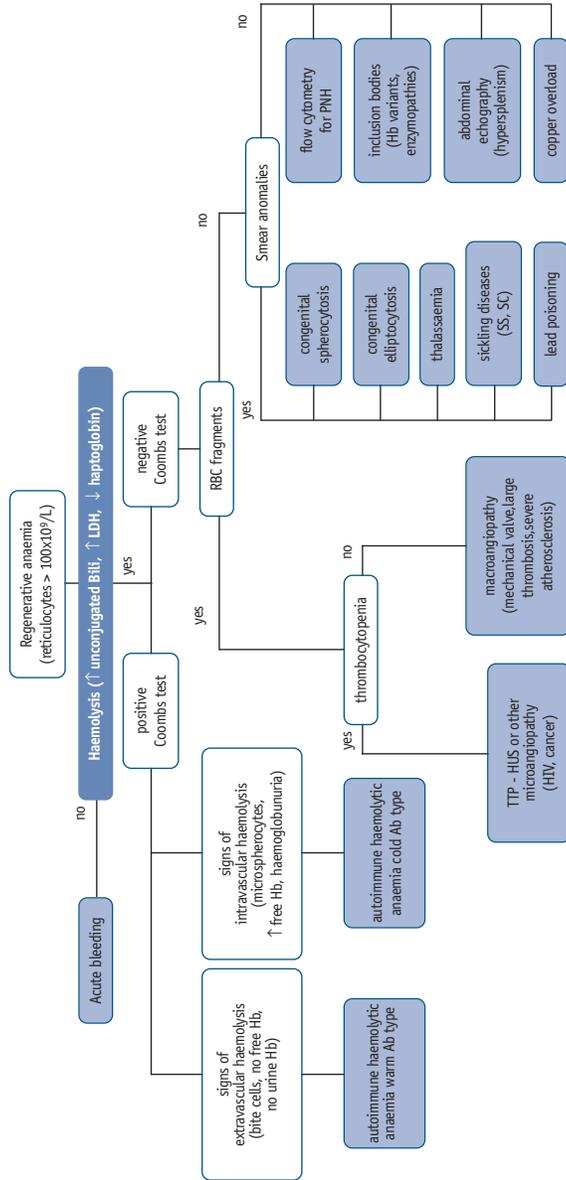| Table 7: **Key points to search for when investigating anaemia**		
History	**Physical examination**	**Laboratory investigations**

• Positive family history of anaemia
• Ethnic origin
• Acuteness of onset of anaemia
• Bleeding: stool, urine, lungs, menses
• Infection: Parvovirus B19, Hepatitis, HIV
• Phlebotomy
• Jaundice, dark urine
• Petechiae
• Symptoms from other organs (CNS, gastritis)
• Masses, nodes
• Alcohol consumption
• Diet: low fruit, no meat
• Pica (abnormal food habits)
• Drugs
• Previous transfusion
	• Pallor, icterus, petechiae
	• Temperature
	• Lymph nodes
	• Enlarged spleen
	• Tachycardia, hypotension
		• WBC and platelet count (aplastic anaemia)
		• Reticulocyte count (red cell aplasia)
		• MCV (iron or vitamin deficiency, thalassaemias)
		• RBC morphology:
		  - double RBC population (transfusion, sideroblastic anaemia, high HbF)
		  - RBC agglutination (cold agglutinins)
		  - schizocytes (microangiopathy)
		  - spherocytes (immune haemolysis)
		• Bilirubin, LDH, haptoglobin, Coombs test (haemolysis)
		• Kidney function (Epo deficiency)
		• Occult blood in the stools (chronic blood loss)
		• C-reactive protein (inflammation)
		• Iron studies + vitamins (serum iron, TIBC, ferritin, B12, folates) depending on MCV

erythropoiesis or accompany a pathological state of other tissues or organs. Precise diagnosis is sometimes difficult and demands both clinical and laboratory examination of the patient. While investigating anaemia, one should try to determine a pathophysiological mechanism corresponding to the patient's condition and which would not only explain laboratory findings, but also the signs and symptoms observed.
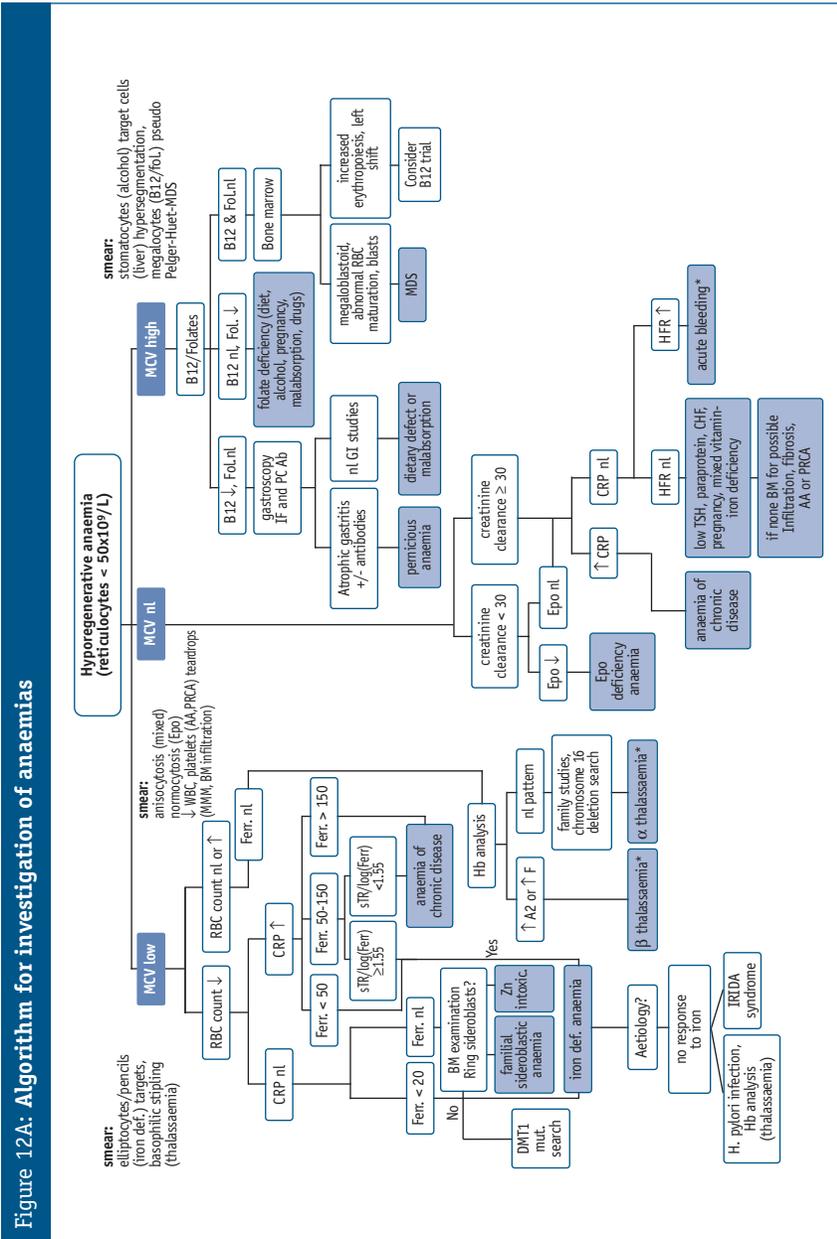
Figure 12A: **Algorithm for investigation of anaemias**

*Hyporegenerative anaemias. Nl: normal; Ferr: ferritin. * These pathologies may also have a reticulocyte count between 50-100x10⁹/L*

Figure 12B: **Algorithm for investigation of anaemias**

Regenerative anaemia
(reticulocytes > 100x10⁹/L)

Haemolysis (↑ unconjugated Bili, ↑ LDH, ↓ haptoglobin)

no

Acute bleeding

yes

positive
Coombs test

negative
Coombs test

yes

signs of
extravascular haemolysis
(bite cells, no free Hb,
no urine Hb)

autoimmune haemolytic
anaemia warm Ab type

signs of
intravascular haemolysis
(microspherocytes,
↑ free Hb, haemoglobunuria)

autoimmune haemolytic
anaemia cold Ab type

RBC fragments

yes

thrombocytopenia

yes

TTP - HUS or other
microangiopathy
(HIV, cancer)

no

macroangiopathy
(mechanical valve,large
thrombosis,severe
atherosclerosis)

no

Smear anomalies

yes

congenital
spherocytosis

congenital
elliptocytosis

thalassaemia

sickling diseases
(SS, SC)

lead poisoning

no

flow cytometry
for PNH

inclusion bodies
(Hb variants,
enzymopathies)

abdominal
echography
(hypersplenism)

copper overload

*Regenerative anaemias. Nl: normal; Ferr: ferritin. * These pathologies may also have a reticulocyte count between 50-100x10⁹/L*

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Multiple Choice Questionnaire

To find the correct answer, go to http://www.esh.org/iron-handbook2009answers.htm

1. Which one of the following statements is not true in case of 5q- syndrome?
a) There is pronounced macrocytosis .... ☐
b) Patients present anaemia with thrombocytopenia .... ☐
c) The bone marrow aspirate shows pronounced dysmegakaryopoiesis ...... ☐
d) Transformation to AML is ≤ 10% .... ☐

2. Which one of the following features is typical of vitamin B12 deficiency?
a) Left shift of neutrophils with numerous band forms .... ☐
b) Pancytopenia with circulating peripheral erythroblasts and elevated LDH .... ☐
c) Hypokalaemia .... ☐
d) Seizures .... ☐

3. In the anaemia of chronic disease, all the following statements are true except one:
a) The ratio of sTFR/log(Ferritin) is elevated .... ☐
b) The severity of anaemia is mild with Hb generally > 8g/dL .... ☐
c) Hepcidin, the central regulator of iron homeostasis, is increased .... ☐
d) Ferritin is normal or elevated .... ☐

4. Which one of the following statements is not true?
a) TMPRSS6 is a transmembrane protein which suppresses hepcidin gene expression .... ☐
b) Patients with mutations in TMPRSS6 gene present with iron deficiency anaemia and low hepcidin levels .... ☐
c) Patients with iron deficiency secondary to TMPRSS6 mutation do not respond to oral iron administration .... ☐
d) To develop clinically the disease (hypochromic-microcytic anaemia) both matriptase-2 genes (maternal and paternal) should be mutated .... ☐

5. All the following are true regarding microangiopathic disorders except one:
a) Regenerative anaemia, increased schizocytes and low platelets counts are typical findings of peripheral blood formula .... ☐
b) Coagulation studies show normal PT, normal aPTT, and normal or elevated fibrinogen .... ☐
c) Microangiopathy related to drugs is antibody mediated and unrelated to Adamts 13 protease .... ☐
d) Verotoxin-producing E. coli HO157-H7Q is the classical agent causing haemolytic uremic syndrome .... ☐

NOTES