28

Haematological Investigations in Children

C Halsey, EA Chalmers

The haematology laboratory is able to perform a number of tests to help establish the cause of illness in children. The full blood count (FBC, also known as a complete blood count, CBC) is one of the most basic blood tests performed on children attending hospital or a primary care clinic. All doctors should therefore have an understanding of how the test is performed, possible pitfalls, be able to interpret results and know when more specialised testing or advice is required. Other haematological investigations in routine use include coagulation screens, blood film examination, reticulocyte counts and methods for estimation of iron stores and detection of abnormal haemoglobins. This section will focus on these basic tests and simple algorithms for the subsequent investigation and differential diagnosis of the commonest haemato-logical abnormalities encountered in general paediatric practice. The reader is referred to Chapter 15 for an account of the clinical presentation and management of primary haematological disorders in children.

Full Blood Count

The FBC is a numerical estimate of the number of red cells, platelets and white cells in a given sample of blood along with measurement of the haemoglobin concentration and various red cell indices some of which are directly measured and others derived. Blood is collected into an anticoagulant solution (usually EDTA) and transported to the laboratory. Although counting of each component can be done manually it is now routine to use automated counters in almost all haematology laboratories. These counters recognise cells on the basis of size and physical characteristics. There are two main methods (often used in conjunction). Electrical impedance measurement is based on the fact that blood cells are very poor conductors of electricity. Therefore when cells in a conducting medium are made to flow in single file through an aperture across which an electric current flows, there is a measurable increase in electrical impedance which is proportional to the volume of the cell. In this way cells can be both counted and sized. The second method relies on characteristic patterns of light scatter and absorbance as cells pass through a laser beam, this is particularly useful for the recognition and counting of the different types of white blood cell (to produce a white cell differential count). In addition counters estimate haemoglobin by lysing the red blood cells and measuring the optical density of the resulting solution at an appropriate wavelength. A typical readout from an automated counter is shown in Fig. 28.1.

- Automated blood counters identify cells on the basis of size and laser light scatter patterns. Haemoglobin concentration is measured by lysis of red blood cells and measuring the optical density of the resulting coloured solution
- Because automated machines rely on size as one way to classify cells it is possible to get artefactual results in some situations. For example nucleated red blood cells are often counted as white cells and fragmented red cells are counted as platelets. Any unusual count should be checked manually with a blood film.

Red Cell Indices

In addition to the red cell count and haemoglobin concentration it is clinically useful to know the size of red cells (mean cell volume, MCV) the amount of haemoglobin per cell (mean cell haemoglobin, MCH) and a measure of the variation in size of individual red cells (red cell distribution width, RDW). Collectively these values are known as red cell indices. They are particularly useful in the assessment of likely causes of anaemia (see below).

Blood Film

Examination of a stained blood film is an essential part of the assessment of most haematological disorders. Many haematological diseases have characteristic changes. In some cases it is possible to diagnose a disorder purely from the blood film (e.g. hereditary elliptocytosis) in most cases other confirmatory tests are needed. The blood film may be very useful in identifying artefactual results such as thrombocytopenia caused by platelet clumping. Systemic disease may also produce blood film changes for example sepsis may be accompanied by an increase in immature neutrophils (left shift or band forms), toxic granulation and formation of Döhle bodies (pale blue cytoplasmic inclusions) within neutrophils. These latter changes are particularly useful in assessment of neonates. Figure 28.2 shows some of the characteristic red cell changes seen on the blood film along with common causes for these appearances.

Reticulocyte Count

Reticulocytes are young red cells that have lost their nucleus but still contain substantial amounts of ribosomal RNA, leading to their characteristic bluish purple colour on standard haematoxylin and eosin (H&E) staining of blood films. They can be more easily identified using special stains such as new methylene blue and can be counted manually or on some automated counters. They can be expressed as a percentage of the total red cells or an absolute count. Reticulocyte numbers are very useful in the evaluation of anaemia as they allow a distinction to be made between inadequate marrow production of red cells (associated with a low reticulocyte count) and excessive destruction or loss of red cells in the periphery (usually associated with increased reticulocyte release from the marrow).

Normal Ranges

The synthesis of blood cells and coagulation proteins go through various changes during development (discussed further in Chapter 15). This is particularly marked in the neonatal period and early infancy because of adaptive changes needed for the transition between the uterine microenvironment and the outside world. Therefore when interpreting any haematological value it is important to be aware of age appropriate normal ranges. Table 28.1 gives approximate values for the FBC from birth to adulthood. Normal ranges should ideally be determined using the local population and the actual instruments in everyday use in the laboratory. In paediatrics it is difficult to obtain sufficient numbers of samples from healthy controls and therefore estimates are usually made using published normal ranges.

Key Learning Points

- Normal ranges vary with age, especially in infants. Always interpret results in light of age appropriate normal ranges.
- Haemoglobin values are high at birth then fall to a nadir at around 3 months of age before slowly rising again,
- Infants and children up to the age of 4 years have a relative lymphocytosis

Investigation of a Low Haemoglobin in Infancy and Childhood

Although there are a multitude of causes for anaemia in this age range the majority of causes can be ascertained by logical use of relatively few tests. An initial history should focus on the length and speed of onset of symptoms, a dietary history, ethnic origin, any other medical conditions and any family history of blood disorders. When considering the differential diagnosis for any haematological disorder it is useful to divide the causes into those due to underproduction of cells from the bone marrow, those due to peripheral destruction of cells and those due to loss of cells from the circulation (haemorrhage or sequestration). A simple way to narrow down the list of differential diagnoses for anaemia is to look at the red cell indices. There are a limited number of causes of a hypochromic microcytic anaemia or a macrocytic anaemia – the common causes are listed in Table 28.2. When assessing a normocytic anaemia a reticulocyte count is useful to distinguish between marrow production problems and peripheral destruction or haemorrhage. The blood film may also be useful with characteristic changes seen in some red cell haemoglobin or enzyme disorders (see Fig 28.2).

Key Learning Points

When formulating a differential diagnosis for low blood counts (red cells, white cells or platelets) always consider the following mechanisms:

- Reduced production of cells
  - Disorders interfering with normal haemopoiesis such as nutritional deficiency or aplastic anaemia
  - Primary bone marrow failure syndromes
  - Secondary marrow infiltration
- Peripheral destruction of cells
- Loss from the body (eg haemorrhage) or sequestration within the tissues or organs

Hypochromic Microcytic Anaemia

The main differential diagnosis is between iron deficiency anaemia and thalassaemia. Thalassaemia major presents in early infancy with a transfusion dependent anaemia and a characteristic blood film and electrophoretic findings (absent haemoglobin A) and usually presents little diagnostic difficulty. Thalassaemia trait does not produce significant anaemia alone but does give hypochromic microcytic indices often in association with a raised red cell count but relatively normal RDW. In contrast iron deficiency usually gives a low red cell count but a raised RDW (this being a measure of variation in red cell width and therefore raised in the presence of anisocytosis). The blood film is often helpful. Beta thalassaemia trait is characterised by basophilic stippling (see Fig 28.2). Alpha thalassaemia trait has few characteristic features. Iron deficiency gives marked anisopoikilocytosis with hypochromic red cell fragments, pencil and teardrop cells and frequent accompanying thrombocytosis.

Tests to Diagnose Iron Deficiency

Iron deficiency can be sub-divided into initial depletion of iron stores followed by iron deficient erythropoiesis and finally the development of anaemia (see Fig 28.3). Serum ferritin is the first marker of depleted iron stores in the body. It is diagnostic if low, but false negative results can be seen because ferritin is an acute phase reactant and therefore can be elevated with acute inflammation or infection even in the presence of iron deficiency. As iron deficiency progresses the transferrin (measured as total iron binding capacity—TIBC) becomes elevated with reduced serum iron. The ratio of these two results can be expressed as the transferrin saturation. Immediate precursors of haem accumulate (zinc (free erythrocyte) protoporphyrin). Finally a hypochromic microcytic anaemia develops. Other tests include measurement of soluble transferrin receptors (increased in iron deficiency). The gold standard test remains Perls' staining of a particulate bone marrow biopsy specimen for iron but this is rarely necessary.

Tests to Diagnose Thalassaemia

In order to understand tests for thalassaemia properly it is necessary to be aware of the composition of haemoglobin and the developmental changes that occur in the use of various globin chains, these are discussed on page xx chapter 15. Diagnosis of thalassaemia is usually made by tests that separate the haemoglobin molecules on the basis of electrical charge; this allows quantitation of the normal haemoglobins HbA ($\alpha_2\beta_2$), HbA_2 ($\alpha_2\delta_2$) and HbF ($\alpha_2\gamma_2$) and also detection of abnormal haemoglobins that contain amino acid changes that alter charge (such as sickle cell HbS). The two main methods in use are haemoglobin electrophoresis and high performance liquid chromatography (HPLC). Beta thalassaemia major can be diagnosed by the complete absence of Haemoglobin A on haemoglobin electrophoresis, provided the test is performed before transfusion of the patient. Beta thalassaemia trait usually shows an elevated HbA_2 level above 3.5% (normal ranges will vary from lab to lab), care should be taken in the presence of iron deficiency as this may reduce the HbA_2 level back into the normal range- results should be repeated after iron replacement in any iron deficient individual. Alpha thalassaemia major is usually diagnosed antenatally or at the time of birth of a severely hydropic infant since all the normal haemoglobins present at birth contain α-chains. Three α gene deletions, so called HbH disease can be diagnosed by electrophoretic detection of Haemoglobin H (β_4 tetramers) or by staining a blood film with brilliant cresyl blue- the β_4 tetramers in the red cells are stained dark blue and produce a golf-ball like appearance. The diagnosis of alpha thalassaemia trait (one or two gene deletions) is suspected by the presence of hypochromia and microcytosis in the absence of iron deficiency and with a normal HbA_2 measurement. As it does not produce clinically significant disease, definitive diagnostic investigations (genetic testing for individual mutations) are usually reserved for antenatal patients at significant risk of alpha thalassaemia major in their offspring. Diagnostic investigations for thalassaemia are summarised in Table 28.3.

Macrocytic Anaemia

The cause of a macrocytic anaemia in children is often obvious from the history. History of concurrent or past illnesses, symptoms and signs of malabsorption, and a detailed drug history are important. B_{12} and folate, liver function tests, a reticulocyte count and thyroid function should be measured in unexplained cases. Causes are listed in Table 28.2.

Normocytic Anaemia

As mentioned above a reticulocyte count is particularly useful in distinguishing reduced marrow production from increased destruction of red cells. The blood film can also give clues as to the most likely cause and best initial tests. A simple algorithm is given in Fig. 28.4.

Haemolytic anaemias are a large subgroup of normocytic anaemias. The combination of jaundice (unconjugated hyperbilrubinaemia), reticulocytosis and anaemia suggests a haemolytic process. Further tests for haemolysis include serum haptoglobin measurement (proteins present in normal plasma which can bind free haemoglobin and are then removed from the circulation by the reticuloendothelial system), urinary haemosiderin (an iron storage protein derived from the breakdown of free haemoglobin in the renal tubular system) and urobilinogen (a natural breakdown product of bilirubin excreted in the urine). These are summarised in Table 28.4. A key test in establishing the cause of haemolysis is the direct Coombs test (DCT), also called the direct antiglobulin test (DAT). This tests for the presence of antibody bound to the red cell surface by the use of reagents containing anti-IgG and anti-complement that cause agglutination of the cells as shown in Fig. 28.6. A positive DCT indicates a likely immune cause for the anaemia. If the DCT is negative then tests for red cell enzyme defects (G6PD and pyruvate kinase assays), haemoglobinopathies (haemoglobin electrophoresis and sickle solubility test) and membrane disorders (demonstration of increased osmotic fragility of cells, protein analysis by SDS-Polyacrylamide gel electrophoresis or more recent dye binding tests) may need to be performed.

If the reticulocyte count is normal or low it is likely that the anaemia is due to a problem with red cell production in the marrow. A bone marrow aspirate and trephine (see section on white cell disorders below) may be needed to help establish the cause. Lack of red cell precursors in the marrow can be seen as an isolated phenomenon in transient erythroblastopenia of childhood (TEC) or inherited red cell aplasia (Diamond-Blackfan anaemia). If part of a pancytopenia, then aplastic anaemia or

hypoplastic myelodysplastic syndrome may be the cause. Occasionally acute leukaemias can present with an aplastic phase followed several weeks to months later by the development of ALL.

Investigation of Anaemia in Neonates

Anaemia is the commonest haematological abnormality seen in neonates. The spectrum and causes of disease are somewhat different than in older children. There are key differences in red cell physiology in neonates that contribute to the different modes of presentation in this age group. Although the haemoglobin tends to be high initially (due at least in part to haemoconcentration and placental transfusion prior to cord clamping), erythro-poiesis is then switched off at the time of birth and haemoglobin falls to a nadir of around 10 g/dl by the age of 8 weeks. This fall is exaggerated in premature infants – so called anaemia of prematurity. In addition premature babies are particularly vulnerable to iatrogenic anaemia secondary to blood loss associated with frequent blood testing. The MCV is high in neonates and differences in red cell membrane composition can make some haemolytic red cell disorders such as hereditary pyropoikilocytosis and hereditary spherocytosis worse in the neonatal period. In contrast, the enzymopathy glucose-6-phosphate dehydrogenase deficiency (G6PD) is not usually associated with significant haemolysis in the newborn period (unless the baby is exposed to oxidant stress) but may present with severe jaundice which is thought to be haepatic in origin. Haemolysis may also be antibody mediated due to Rhesus or ABO incompatibility between mother and infant. Increased red cell destruction puts the baby at risk of kernicterus caused by high bilirubin levels. Hence it is important to be aware of the possibility of haemolysis in all newborn babies.

Anaemia presenting soon after birth may also be due to haemorrhage pre, during or post delivery. Feto-maternal haemorrhage can be diagnosed by performing a Kliehauer test on the mother – this test looks for the presence of fetal haemoglobin containing cells in the maternal circulation by virtue of their ability to resist acid elution of haemoglobin. In multiple pregnancies that share a placental circulation twin-to-twin transfusion may also occur, producing one polycythaemic twin and one anaemic one. An algorithm for the diagnosis of neonatal anaemia is given in Fig. 28.5.

Key Learning Point

The causes and presentation of anaemia are different in neonates due to differences in red cell physiology. It is important to diagnose haemolytic disorders early to reduce the risk of kernicterus

Polycythaemia

High haemoglobins and haematocrits can be due to increased numbers of red cells (true polycythaemia) or dehydration leading to a decreased plasma volume (relative polycythaemia). In children true polycythaemia is usually due to a secondary cause such as hypoxia from cyanotic congenital heart disease leading to increased erythropoietin production. Occasionally kidney tumours can secrete erythropoietin. Primary erythrocytosis (a myeloproliferative disease relatively common in adults) is extremely rare in children. Neonates have higher incidences of polycythaemia usually secondary to placental insufficiency or delayed clamping of the cord. The blood viscosity increases exponentially with haematocrits above 0.65 therefore these infants are often treated with exchange transfusion – the evidence for benefit from this is lacking.

White Cell Disorders

The white cells in the blood can be subdivided into different subpopulations with distinct functions. These are listed in Table 28.5 along with the main causes of high or low counts for these cells.

Low White Cell Counts (Leucopenia)

The commonest and most important white cell deficiency is that involving neutrophils (neutropenia) since this can be associated with an increased risk of serious infection. It can be caused by a defect in bone marrow production either affecting this cell type alone (isolated neutropenia), or as part of a

general failure of the bone marrow to produce mature blood cells (pancytopenia). Alternatively neutropenia can result from peripheral destruction of neutrophils by antibodies or their redistribution to the tissues or sites of injury. Normal ranges for neutrophils vary between ethnic groups and are particularly low in black Africans. This is thought to represent a different distribution of neutrophils between the tissues and the circulation, rather than an overall lower total body count. Neutrophil numbers circulating in the bloodstream rise after exercise and as a stress response.

Lymphopenia can follow acute infections or periods of immunosuppression. Lymphocyte counts are generally higher in neonates. In neonates with lymphopenia and serious infections the possibility of an inherited immunodeficiency should be borne in mind.

High White Cell Counts (Leucocytosis)

A high neutrophil count often accompanies infection (neutrophilia) and can be a useful marker of sepsis. Very high neutrophil counts with evidence of immature precursors in the bloodstream are sometimes called a leukaemoid reaction. This can be seen with overwhelming sepsis, marrow infiltration by a solid malignancy, a severe stress response such as status epilepticus or burns. Leukaemia itself can present with high or low white cell numbers and is usually suspected by the combination of an abnormal blood count (white cells high or low, usually with accompanying anaemia and thrombocytopenia) with a blood film that shows a population of immature precursors (blast cells). Blast cells vary in appearance with the different subtypes of leukaemia but are generally larger than normal cells with a large nuclei and open chromatin (see chapter 15, Figs 15.15 and 15.16). Myeloid blast cells may have rod-like inclusions in their cytoplasm called Auer rods.

The definitive diagnosis of leukaemia usually requires bone marrow examination, this allows detailed study of the appearance of the red cells (morphology) as well as analysis of various specific proteins expressed by the cells (immunophenotyping) and genetic abnormalities (molecular genetics and cytogenetics) which help classify the leukaemia further and guide treatment.

Bone Marrow Examination

Bone marrow examination is performed for further assessment of haematological disorders where production of cells from the bone marrow is thought to be abnormal. In children it is often performed under general anaesthesia although local anaesthetic can be used if appropriate. The usual site for aspiration is the posterior iliac crest. A large bore needle is used to penetrate the bony cortex and enter the marrow cavity. Bone marrow is then aspirated and spread on glass slides, preferably immediately. If a good specimen is obtained then a granular appearance should be seen (see Fig 28.7). Further samples can be taken in appropriate anticoagulant or medium for cytogenetics, immunophenotyping and molecular genetic tests. In many cases a bone marrow trephine can also be taken – this involves introducing a longer needle below the cortex and taking a core of tissue that can then be fixed in formalin and sectioned for pathological examination.

Platelet Disorders

Platelets are small cytoplasmic fragments produced from megakaryocytes in the bone marrow and are important for the initiation of haemostasis and may have as yet poorly understood roles in inflammation. As with white and red cells, platelet disorders can be subdivided on the basis of high and low numbers.

High Platelet Counts (Thrombocytosis)

High platelet counts are usually reactive i.e. not primary bone marrow disorders but secondary to iron deficiency, ongoing inflammatory processes or infection. Very high platelet counts (>1500 × 10^9/l) can be associated with an increased risk of thrombosis. Primary thrombocytosis (essential thrombocythaemia) is rare in children.

Low Platelet Counts (thrombocytopenia)

Again these can be classified according to the underlying problem ie inadequate bone marrow production or peripheral destruction/consumption (see Table 15.3 chapter 15). Unexpectedly low platelet counts should always be confirmed by examination of a blood film as artefactually low platelet counts are not uncommon either due to partial clotting of the sample or platelet clumping, the latter is often an *in vitro* phenomenon due to EDTA dependent antibodies. The commonest cause of true thrombocytopenia in children is immune mediated peripheral destruction – idiopathic thrombocytopenic purpura (ITP). Unfortunately there is no diagnostic test for this condition so it remains a diagnosis of exclusion. It is characterised by the sudden onset of bruising and/or bleeding in an otherwise well child, often with a history of an antecedent viral infection or more rarely post immunisation. There should be no other abnormalities in the blood count and no lymphadenopathy or organo-megaly on examination. In these cases careful examination of a peripheral blood film is sufficient but in the presence of any abnormal or suspicious features a bone marrow examination should be performed to exclude leukaemia. The bone marrow in ITP shows increased numbers of normal megakaryocytes (as shown in chapter 15 Fig. 15.11). Although the disease is immune mediated platelet associated antibodies show high false positive and negative results and are therefore not useful in making or excluding the diagnosis.

Platelets may also be consumed in the periphery and a low platelet count almost always accompanies established disseminated intravascular coagulation (see Table 28.6). Giant haemangiomas (Kasabach–Merritt syndrome) or splenomegaly may also sequester and destroy platelets.

Lack of marrow production of platelets often accompanies marrow infiltration by diseases such as leukaemia. Other bone marrow failure syndromes such as Fanconi's anaemia can also present initially with low platelets.

It is also possible to have a platelet function disorder. The commonest of these are Glanzmann's thrombae-sthenia, usually associated with a normal platelet count, and Bernard-Soulier syndrome, associated with a moderate to severe thrombocytopenia. Both are due to different platelet glycoprotein defects and can be diagnosed by platelet function testing and flow cytometry.

In neonates causes of thrombocytopenia vary depending on the gestation and clinical condition of the baby. In well term neonates alloimmune thrombocytopenia due to the transplacental passage of maternal anti-platelet antibodies directed against foreign paternal antigens on the babies platelets (akin to the red cell disorder Rhesus haemolytic disease of the newborn) needs to be excluded. In preterm neonates benign gestational thrombocytopenia may be seen soon after birth but later appearance of thrombocytopenia often heralds sepsis.

Coagulation Testing in Infants and Children

Interpreting the results of coagulation screening requires some basic knowledge of the coagulation cascade. Coagulation tests are performed in the laboratory (*in vitro*) and do not faithfully replicate the circumstances seen in the body (*in vivo*). The interpretation of laboratory tests often places a lot of emphasis on extrinsic and intrinsic pathways but these sequences of activation probably do not play a major role in the initiation of clotting *in vivo*. Despite this the concept of extrinsic and intrinsic pathways is useful to be aware of when faced with an abnormal coagulation screen and is shown in Fig. 15.12.

It is now thought that the key initiating event *in vivo* is exposure of tissue factor in response to endothelial damage. Tissue factor activates factor VII to form a complex, TF-VIIa, which cleaves factor X to its active form Xa. Xa can convert prothrombin to thrombin with low efficiency but this generation of small amounts of thrombin then activates feedback loops to increase coagulation factor activation. Factor VIII (activated by thrombin) and factor IX (activated by TF-VIIa and factor XI) form a complex VIIIa-IXa known as tenase. Tenase generates activated factor X with great efficiency. Thrombin also activates factor V and a Xa-Va complex is formed which cleaves prothrombin to form thrombin. Thrombin generation leads to conversion of fibrinogen to fibrin with subsequent crosslinking by factor XIII. This pathway is summarized in Table 28.6.

When to perform a coagulation screen

Coagulation screens should not be a routine blood test. They should be performed in any child with unusually severe or unexplained bleeding or in very unwell children with suspected

disseminated intravascular coagulation. They can also be performed prior to high-risk invasive interventions. A good bleeding history needs to be taken to determine the need for investigation and to help guide appropriate tests. This includes a history of abnormal bleeding in the patient or relatives in response to haemostatic challenges such as tooth extraction, cuts and minor operations as well as a history of maenorrhagia in older females. Some clinically significant bleeding disorders can have a normal coagulation screen (in particular some von Willebrand's disease and Factor XIII deficiency see Table 28.7) and some abnormal coagulation screens do not lead to a clinical risk of bleeding (e.g. Factor XII deficiency or lupus anticoagulant). Therefore the results of testing always need to be interpreted in the light of a clinical history.

Key Learning Point

Do not rely on coagulation screening as the sole indicator of bleeding risk. History of bleeding in response to a haemostatic challenge is just as important

Coagulation Tests

When performing a clotting screen care should be taken during venepuncture to avoid activation of clotting as this can produce artefactually low results. Samples should be from a free flowing vein, in particular heel prick samples are unsuitable in neonates. Care should be taken to avoid contamination with heparin – a particular problem when sampling is from an indwelling venous catheter. Like the FBC it is very important to be aware of normal ranges for the clotting screen especially in neonates who tend to have significantly prolonged values compared to older children. In addition values vary considerably between different automated analysers and may therefore vary between hospitals, local normal ranges should always be used.

Initial screening tests should comprise:

1. *Prothrombin time* (PT) –this is a test of the overall activity of the extrinsic pathway. It measures the activity of factors II, V, VII and X and is also dependent on adequate fibrinogen levels.
2. *Activated partial thromboplastin time* (APTT) – this is a test for the overall activity of the intrinsic pathway and measures factors II, V, VIII, IX, X, XI and XII, it also requires adequate fibrinogen levels.
3. *Thrombin time* (TT) – prolonged by quantitative and qualitative disorders of fibrinogen, the presence of inhibitory factors such as fibrin/FDPs and the presence of heparin.
4. Fibrinogen level.
5. Platelet count.

Results of these tests along with clinical history can guide subsequent investigation. Bleeding times are generally unhelpful. A diagnostic algorithm is shown in Table 28.8.

The typical findings in disseminated intravascular coagulation are shown in Fig. 28.6, although coagulation screening is useful in this disorder the primary therapy for DIC is treatment of the underlying cause. Replacement of coagulation factors with fresh frozen plasma or cryoprecipitate should be guided by the patient's clinical condition and presence of other risk factors for bleeding rather than treating the abnormal clotting screen *per se*.

Factor Assays

Clinical aspects of inherited coagulation disorders are discussed in chapter 15. Inherited factor deficiencies may initially be suspected on the coagulation screen and confirmed by direct assay of the clotting factor. In the case of suspected von Willebrand's disease, von Willebrand Factor (vWF) should be measured both quantitatively (vWF antigen) and qualitatively (a functional test such as a ristocetin cofactor assay). This is because low levels of vWF or normal levels of dysfunctional vWF can cause the disease. vWF can also rise with stress and therefore repeated testing may be needed to exclude disease especially in young children who are difficult to venepuncture.

Platelet Function Testing

Besides a platelet count and assessment of platelet morphology by light microscopy it is possible to assess platelet function in a number of ways. Historically a bleeding time has been used as a global test of platelet function but it is difficult to standardize and not very predictive of bleeding risk. Currently the three commonest techniques in use are platelet aggregation studies (looking at aggregation in response to various stimulants such as epinephrine), flow cytometry (to assess expression of glycoproteins on the platelet surface) and use of a platelet function analyzer (PFA-100, an automated machine that measures the ability of platelets to form a plug under shear stress).

Heparin

The presence of contaminating heparin in a sample is often initially suspected by the combination of a prolonged APTT with a significantly prolonged thrombin time (this test is exquisitely sensitive to heparin). A number of methods exist to try and confirm whether the abnormal result is due to heparin or not. These include a reptilase time (which measures the same pathway as the TT but is unaffected by heparin) or methods to neutralize the heparin using protamine sulphate.

Monitoring of Anticoagulant Therapy

Therapeutic anticoagulation in children is used to prevent or treat thrombosis. Heparin and warfarin are the two main agents in use. Heparin comes in two main formulations – standard unfractionated heparin and low-molecular weight heparin. The former is monitored by the APTT with a therapeutic range of 1.5-2.5 times normal control values. Low molecular weight heparin therapy does not prolong the APTT and needs to be monitored by anti-Xa levels. Warfarin therapy prolongs the PT but in order to standardize results between laboratories this level is converted into an international normalized ratio (INR), the target INR varies depending on the indication for anticoagulation.

Table 28.1: Normal ranges for the FBC in infancy and childhood

Haemo-globin (g/dl)	*Hct*	*MCV (fl)*	*WBC ($x10^9$/l)*	*Neutro-phils ($x10^9$/l)*	*Lympho-cytes ($x10^9$/l)*	*Mono-cytes ($x10^9$/l)*	*Eosino-phils ($x10^9$/l)*	*Baso-phils ($x10^9$/l)*	*Platelets ($x10^9$/l)*
14.9-23.7	0.47-075	100-128	10-26	2.7-14.4	2.0-7.3	0-1.9	0-0.85	0-0.1	150-450
13.4-19.8	0.41-0.65	88-110	6-21	1.5-5.4	2.8-9.1	0.1-1.7	0-0.85	0-0.1	170-500
9.4-13.0	0.28-0.42	84-98	5-15	0.7-4.8	3.3-10.3	0.4-1.2	0.05-0.9	0.02-0.13	210-650
10.0-13.0	0.3-0.38	73-84	6-17	1-6	3.3-11.5	0.2-1.3	0.1-1.1	0.02-0.2	210-560
10.1-13.0	0.3-0.38	70-82	6-16	1-8	3.4-10.5	0.2-0.9	0.05-0.9	0.02-0.13	200-550
11.0-13.8	0.32-0.4	72-87	6-17	1.5-8.5	1.8-8.4	0.15-1.3	0.05-1.1	0.02-0.12	210-490
11.1-14.7	0.32-0.43	76-90	4.5-14.5	1.5-8.0	1.5-5.0	0.15-1.3	0.05-1.0	0.02-0.12	170-450
12.1-15.1	0.35-0.44	77-94	4.5-13	1.5-6	1.5-4.5	0.15-1.3	0.05-0.8	0.02-0.12	180-430
12.1-16.6	0.35-0.49	77-92							

Reproduced from Pediatric haematology. 3rd edition. Eds Arceci, Hann & Smith. Blackwell publishing August 2006

Table 28.2: Causes of anaemia classified on red cell indices

Hypochromic	*Normocytic*	*Microcytic*
Iron deficiency	Haemorrhage (acute)	Vitamin B_{12}/Folate deficiency
Thalassaemia	Haemolysis-AIHA	Reticulocytosis

Sideroblastic anaemia	Haemoglobinopathy- sickle cell disease	Myelodysplasia
Chronic disease	Red cell membrane defect- Hereditary spherocytosis	Hypothyroidism
Lead poisoning	Red cell enzyme defect –G6PD, Pyruvate kinase	Drugs
	Marrow infiltration- malignancy	Liver disease
	Aplastic anaemia	Scurvy
	Transient Erythroblast- openia of childhood	
	Bone marrow failure syndromes	
	Anaemia of chronic disease	

Diagnosis electrophoresis	*Genetic defect*	*Blood film*	*Haemoglobin*
Beta thalassaemia major (pretransfusion), high HbF	2 β gene mutations	Severe hypochromic microcytic anaemia, nucleated red cells, target cells	Absent HbA
Beta thalassaemia trait	1 β gene mutation	Basophilic stippling, hypochromia and microcytosis but normal or borderline low haemoglobin	Raised HbA_2 >3.5%
Alpha thalassaemia major HbF (incompatible with survival beyond embryonic period) HbBart's and HbH	4 α gene deletions	Very severe anaemia, nucleated red cells	Absent HbA, A2 and Presence of embryonic HbPortland and
Haemoglobin H disease	3 α gene deletions	Anaemia, target cells, teardrop cells and fragments HbH bodies on special staining of film	HbH
Alpha thalassaemia trait (may be detected programmes)	1 or 2 α gene deletions	Hypochromia, microcytosis	2-8% Hb Bart's at birth on neonatal screening

Due to increased red cell destruction	Raised serum unconjugated bilirubin Raised urinary urobilinogen Reduced plasma haptoglobins
Due to increased red cell production	Raised reticulocyte count
Due to presence of damaged red cells	Abnormal morphology – spherocytes, bite cells, fragments Increased osmotic fragility

Cell type decreased count	*Function*	*Causes of increased count*	*Causes of*
Neutrophil viral or fulminant	Innate immunity, control of	Bacterial infection	Infection –
	bacterial and fungal infection,	Inflammatory disorders and tissue	bacterial
	Role in phagocytosis of dead and	necrosis	Autoimmune
	damaged cells as part of	Severe marrow stress- haemorrhage or	Marrow
failure			
	inflammation	haemolysis	Drugs
		Steroid therapy	African race
		Myeloproliferative disorder (rare in children)	
Lymphocyte including acute	Adaptive immunity	Acute infection especially viral e.g. Epstein	Acute stress
	Control of viral infection	Barr virus (glandular fever)	infection,
burns, surgery, trauma			
		Chronic infection- TB, toxoplasmosis	Steroids,
Cushing's syndrome			
		Acute leukaemia	
	Immunodeficiency- congenital and		
			acquired
including use of			
	immunosuppressive drugs		
Monocyte failure	Part of reticuloendothelail system-	Chronic bacterial infections (TB)	Marrow
	macrophage precursors in transit	Lymphoma	Drugs
		Juvenile myelomonocytic leukaemia	
		Associated with neutropenia	
Eosinophil failure	Inflammatory responses	Parasitic infection	Marrow
	Response to parasitic infection	Allergy, atopy	Cushing's
syndrome			
		Skin diseases	Acute stress
e.g. burns			
		Hodgkin's disease	Drugs
Basophil	Largely unknown, blood	Chicken pox	As above
	counterpart to tissue mast cell	Myeloproliferative diseases	
		Hypothyroidism	
		Ulcerative colitis	

1. Prolonged APTT
2. Prolonged PT
3. Prolonged thrombin time
4. Low fibrinogen
5. Low platelet count
6. Raised fibrin degradation products or D-Dimers
7. Red cell fragmentation on the blood film

- Factor XIII deficiency
- Glanzmann's Thrombaesthenia / other platelet function disorders
- von Willebrand's disease
- Vascular disorders

APTT	PT	TT	Fibrinogen	Platelets	Possible Diagnosis
Prolonged	Normal	Normal	Normal	Normal	Factor VIII, IX. XI deficiency (Haemophilia A, B or C) von Willebrand's disease Lupus Anticoagulant Factor XII/ Contact factor deficiency
Prolonged	Prolonged	Prolonged	Normal or low	Normal or low	Heparin Liver disease Fibrinogen deficiency Vitamin K deficiency DIC
Prolonged	Prolonged	Normal	Normal	Normal	Vitamin K deficiency Warfarin Factor II, V, VII, X deficiency
Normal	Prolonged	Normal	Normal	Normal	Warfarin therapy Factor VII deficiency

Fig. 28.1: A typical computer generated readout from an automated blood cell analyser (Sysmex XT2000i)

Fig. 28.2: Red cell changes seen on blood films and their common causes (a) Spherocytes (b) Sickle cells (c) Bite cells (d) A pencil cell (e) Basophilic stippling (f) Target cells (g) Red cell fragments. Arrows point to the abnormal cell type

Fig. 28.3: Stages of iron deficiency

Fig. 28.4: Investigation and causes of normocytic anaemias

Fig. 28.5: Investigation and causes of neonatal anaemia

Fig. 28.6: The direct Coombs test

Fig. 28.7: Example of bone marrow aspirate specimen spread on a slide and stained with H&E, note the granular appearance at the top of the smear

Fig. 28.8: **Haematological finding in disseminated intravascular coagulation: A revised coagulation cascade. Thick dashed arrows indicate low efficiency pathways. Thin dashed arrows indicate**
feedback activation loops. Boxes indicate complexes formed on phospholipid surfaces