

1

Introduction to Blood Science

Learning objectives

After studying this chapter, you should be able to:

- explain key aspects of blood science;
- understand the role of blood science in modern pathology;
- describe the role of blood science in the wider provision of healthcare;
- outline the overlap between different areas of blood science.

In this chapter, we will introduce you to blood science – not only the study of blood, but also how the subject relates with other disciplines in pathology. You will also get a feel for blood science in the wider aspect of healthcare.

1.1 What is blood science?

Put simply, it is the study of blood. However, as with many questions, a short answer is often inadequate, and this is no exception. Blood itself is a dynamic and crucial fluid providing transport and many regulatory functions and that interfaces with all organs and tissues. As such, it has a very important role in ensuring adequate whole-body physiology and homeostasis. It follows that adverse changes to the blood will have numerous consequences, many of which are serious and life-threatening.

Blood itself is water which carries certain cells and in which are dissolved many ions and molecules. These cells are required for the transport of oxygen, in defence against microbial attack and in regulating the balance between clotting (thrombosis) and bleeding (haemorrhage). The blood is also an important distributor of body heat. The blood also carries nutrients from the

intestines to the cells and tissues of the body. Once these nutrients (and oxygen) have been consumed, the blood transports the waste products of metabolism to the lungs and kidneys, from where they are removed (i.e. they are excreted). In some particular diseases and conditions (such as diabetes, myeloma and renal disease), the investigation of urine can be valuable. Although clearly not blood, blood scientists will perform and comment on the analysis of this fluid.

An historical perspective

From the early 19th century, little was known about the make-up of blood, and blood cells in particular, until a way could be found of stopping it clotting once outside the body. Thus, the development of anticoagulants was an important breakthrough. Once this was achieved, it became possible to separate intact blood cells from plasma. This led to the discovery of the differences between serum and plasma, the former obtained from clotted blood.

As the Victorian age progressed, chemists were refining old tests and discovering new ones, and so initiated the development of modern biochemistry. However, the most well-developed disciplines were (what we now call) microbiology and histology. The former was built on study of diseases such as cholera and tuberculosis, and the germ and antiseptic theories of Koch, Lister and others. Histology was benefiting from the development of dyes, enabling the identification of different substances within tissues of the body.

The first organization dedicated to non-medical laboratory workers, the Pathological and Bacteriological Laboratory Assistants Association, the forerunner of today's Institute of Biomedical Science (IBMS), was founded in 1912. Members of this group include biomedical scientists and clinical scientists. Other professional bodies for

laboratory workers include the Association for Clinical Biochemistry, founded in 1953.

Further developments in biomedical science during the remainder of the last century saw the emergence of four disciplines within pathology: biochemistry (also known as clinical chemistry), haematology, histology and microbiology (the latter having evolved from bacteriology in recognition of the role of viruses in human disease). Immunology appeared as a discipline in its own right in the 1970s, followed in the last decades by genetics (possibly also known as molecular biology, or more correctly as molecular genetics).

Therefore, biomedical science has been evolving over the last 200 years, driven by advances in science and technology. This evolution has seen the merging of several of these disciplines (bacteriology and virology into microbiology) and the development of new ones. This principle has also been rolled out for other scientists, such as cardiac physiologists, audiologists and medical physicists. Biomedical sciences (which may also be known as the life sciences), encompassing all those working in modern pathology laboratories, may be classified into three groups: infection sciences, cellular sciences and blood science (Table 1.1).

Therefore, blood science (in common with infection science and cellular science) is simply another step in the development of a particular part of pathology. However, blood science is not simply a group of disciplines thrown together. Haematology and blood transfusion are sisters, and have historically grown up together over the decades. Being based around the functions of certain cells in the blood (leukocytes), immunology is effectively a 'daughter' subdivision of haematology.

The merger of biochemistry with haematology, blood transfusion and immunology at first seems strange. However, all take as their source material blood in special blood tubes called vacutainers, some of which have

anticoagulants to stop the blood from clotting. Furthermore, to some extent, many tests in each of the four disciplines are amenable to measurement in batches by autoanalysers. As we shall see, many diseases call on both haematology and biochemistry, and often immunology. The serious consequences of many diseases may call for the transfusion of red blood cells or proteins to help the blood to clot. The inclusion of genetics in blood sciences comes from the fact that many diseases have a genetic component, such as the bleeding condition haemophilia, and the cancers leukaemia and lymphoma.

The reference range

The function of the laboratory is to provide the practitioner investigating or treating the patient with useful information. This information is almost always numerical; and if so, the particular numbers need to be compared with a range of other numbers to provide the practitioner with an idea of the extent to which a particular result is of concern. This is the set of numbers that we refer to, and hence the term 'reference range'. We prefer this name to alternatives such as 'normal range' or 'target range'.

The expression 'normal range' is inadequate simply because a result that is normal (i.e. is present in a lot of individuals) in a population does not necessarily imply it is desirable. A good example of this is low haemoglobin that may be endemic in some parts of the world, possibly because of malnutrition, genetics and parasites – none of which we would consider healthy. In addition, merely because someone appears healthy (i.e. are asymptomatic), it does not automatically follow that their blood result is satisfactory, and vice versa. Similarly, 'target range' is not fully appropriate as it implies a level of a result that we are trying to achieve – this may never be possible in some individuals, resulting in disappointment and a sense of failure. However, there are cases where a target is a useful objective.

It is also worthwhile discussing where 'normal values' come from. Who is normal? Many people have unsuspected asymptomatic disease that may well impact on blood science. In the past, results from blood donors were considered to be representative of being 'normal', but we now recognize the shortcoming in this definition as blood donors are in fact highly motivated and healthy individuals who are, therefore, on the whole, 'healthier' than the general population.

Individuals who are free from disease are often described as being 'normal'. In a medical setting, someone who is not complaining about any particular condition

Table 1.1 The biomedical or life sciences

Infection sciences

Bacteriology, epidemiology and public health, molecular pathology, virology

Cellular sciences

Cytopathology, genetics, histopathology, reproductive sciences

Blood science

Biochemistry, haematology, blood transfusion, immunology, molecular genetics

(such as chest pain) is said to be asymptomatic. This is not to say that person is free of disease, simply that it is not so bad that it impacts on their lifestyle. It is important to recognize that normality does not always indicate health, but is merely an indication of the frequency of a given condition in a defined population. Some diseases occur with such frequency in the population that they might be considered to be 'normal', such as dental caries. A further example of this is a high level of serum cholesterol, which is asymptomatic, but which predicts, and is a contributor to, cardiovascular disease.

The normal distribution If we examine the distribution of an indicator of health, for example the level of serum cholesterol in a population (Figure 1.1), we can see that it follows a symmetrical bell-shaped curve. Most of the data are in the middle; the result that is present at the greatest frequency (the tallest 'tower', which represents the number of people with that result) is about 4.9 mmol/L. Indeed, the average value (known as the *mean* by statisticians) is 4.9066, which we happily round down to 4.9. This shape has several names, one being the normal distribution. It represents a way we can visualize the spread of values of a particular index in a population. The distribution is called 'normal' because most sets of data (height, weight, serum sodium, haemoglobin, numbers of hairs on the head) take this distribution – it does not make any statement about what is normal or abnormal about the data itself. This type of distribution is also often described as 'bell shaped'.

A close inspection of the distribution in Figure 1.1 shows a small number of low values (on the left, about 2 mmol/L) and an equal number of high values (on the right, about 8 mmol/L). So although the data set ranges from 2 to 8 mmol/L, most values are in the middle, between say 4.2 and 5.6 mmol/L. We can use a mathematical expression to be more precise about this spread, which we call the standard deviation (SD), which is 1 mmol/L. The importance of the SD is that the mean plus or minus two SDs should include 95% of the results, which is 2.9–6.9 mmol/L. It follows that 5% of the results are outside this range.

A common error is to assume that just because someone's result is above or below this 'magical' range of mean plus or minus two SDs this automatically implies this result is abnormal. This assumption is incorrect. The definition of normal or abnormal is generally made independently of the data, usually by a panel of experts. Indeed, the concept of abnormality varies from place to place and over time. Decades ago, before we knew that cholesterol is a risk factor for cardiovascular disease, a cholesterol result of, say, 7.0 mmol/L in a 65-year-old man would probably not have been regarded as abnormal, and so would not have been treated. However, we are now fully aware of the danger of raised serum cholesterol, and so need guidance as to what is normal, and what is abnormal, and so treatable. These days the same serum cholesterol would be regarded as abnormal, and so would be treated.

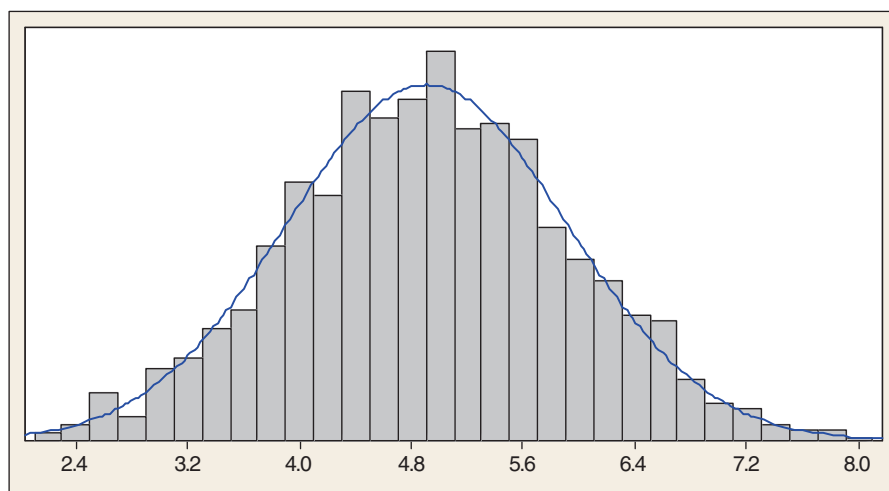


Figure 1.1 The normal distribution.

The non-normal distribution This is the most common alternative to the 'normal' pattern of distribution. A good example of such a distribution is that of another type of fat (lipid) in the blood, the triacylglycerols (also known as triglycerides). In this pattern, the individual data points are not equally spread around the centre of the data set, with the same number of points on either side of the most frequent result (the mean). Instead, the data are skewed, or shifted, to the left or right, and the bell-shaped curve in a normal distribution is not present. This skewed distribution is called non-normal; in itself, it makes no statement of which individual data point is normal or abnormal.

In a typical set of triacylglycerol results from a large population of people, the most frequent result may be, for example, 1.7 mmol/L. This result is called the *median*, and is the middle point of all the individual data points. The smallest value may be, say, 0.5 mmol/L, but the highest may be perhaps 11.0 mmol/L (Figure 1.2). Clearly, therefore, the median of 1.7 is not in the middle of 0.5 and 11.0, unlike the mean cholesterol result from Figure 1.1, where 4.9 is not far from the middle of the full range of 2.2–8.0, that being 5.1 mmol/L.

The point is that that the criteria of what is normal, and therefore acceptable, cannot be used when the particular index (the level of serum triacylglycerol) has a non-normal or skewed distribution. We therefore have to consider a different set of rules, but these are still based on the middle 95% of people. In this case the results from 95% of the people lie between 0.8 and 4.7, so that 2.5% of people have a result less than 0.8 and 2.5% of people have

a result greater than 4.7. This does not mean that someone with a result of 4.8 is abnormal. However, the further away from the median we get, then the more likely that a result is indeed abnormal. Accordingly, we may suspect pathology in the patient with the highest result; that is, 11 mmol/L. They may have a metabolic disease.

Variation in reference ranges It should be noted that reference ranges vary both from hospital to hospital and over time. The former is often because different auto-analysers give a slightly different result on the same sample of blood. Furthermore, the reference range should serve the local population that the hospital serves, and local populations can vary a great deal.

As we improve our knowledge of biomedical science, it becomes clear that some reference ranges need to be changed. In the 1975 edition of a major haematology textbook, the middle of the 'normal' range for the average volume of a red cell in the adult is given as 85 fL. However, in the 2001 edition, in the 'reference range and normal values' table, the mean volume is given as 92 fL. In 1975 the reference range for a type of white blood cell called a neutrophil was $(2.0\text{--}7.5) \times 10^9/\text{L}$, but in 2001 the range is $(2.0\text{--}7.0) \times 10^9/\text{L}$. It follows that in 1975 a result of $7.25 \times 10^9/\text{L}$ was considered to be within the 'normal' range, whereas 26 years later the same result is outside this range, and so may be described as a mild neutrophil leukocytosis (full explanation given in Chapter 5). Whether or not this is actionable is another question.

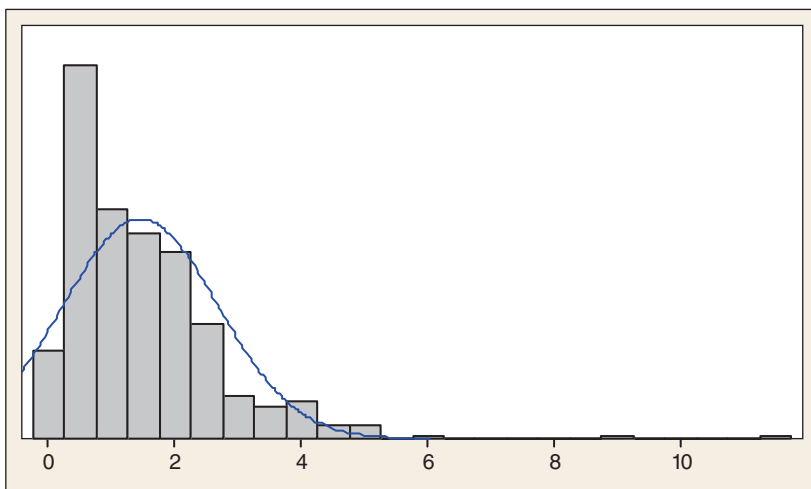


Figure 1.2 The non-normal distribution.

A note on units. Not only do the units of blood tests vary around the world (such as total cholesterol being reported in mmol/L in the UK and as mg/L in the USA), but they also vary in time. Until recently, haemoglobin was reported as g/dL. However, the unit (dL, decilitre) is not fully part of the international system, which reports in terms of the litre (L). Hence, the unit for haemoglobin has transformed into g/L, so that the result of 13.9 g/dL simply becomes 139 g/L.

Interpretation

All routine blood science results sent out (from whichever laboratory) are accompanied by a reference range,

which is often a set of numbers enclosed by brackets (see Figure 1.3). In addition, the laboratory will often draw the attention of the reader to those results that are considered to be out of range and, therefore, worthy of attention. There may be an asterisk or other flag alongside these results. Indeed, for this reason the reference range may also be considered a 'concern range'. This is because a result fractionally outside the reference range does not always carry a serious health hazard. However, the further a particular result is outside the reference range then the more seriously we must address the result, as it may well be the consequence of actual disease and so should be acted upon.

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Surname	BLANN	Address	
Forename	ANDREW	Unit No:	ZC01282337 ASCOT CLINIC (CHEM)
DOB	11.08.54	Dr.D.G.Beevers	
		NHS No:	
Results:			Lab No C.01.0025121.G
Urea	4.1	mmol/l	(3.0-8.3)
Sodium	142	mmol/l	(133-144)
Potassium	3.8	mmol/l	(3.4-5.1)
Creatinine	89	umol/l	(44-133)
Total Protein	65	g/l	(63-84)
Albumin	36	g/l	(35-50)
Bilirubin	7	umol/l	(5-17)
Alk Phosphatase	56	IU/l	(20-130)
Alanine A.T.	38	IU/l	(<50)
Cholesterol	3.7	mmol/l	(3.1-6.5)
Triglycerides	1.25	mmol/l	(0.85-1.98)
HDL-Cholesterol	1.2	mmol/l	(1.2-1.7)
Tests		Check Clinician:	Date Received 09.02.01
CRE,HDL,LFT,LIP,UE			Date Reported 12.02.01

Figure 1.3 Common biochemistry tests. Selected biochemistry results on a presumed healthy middle-aged male. The blood tests themselves are printed out in the first column on the left (headed by urea). The next column is the actual result (in this case, 4.1), and then the units (mmol/L), and finally the reference range on the right (3.0–8.3). Results which are outside the reference range are generally highlighted by an asterisk. The fact that there are no asterisks present means that all results are acceptable and no further testing is required.

Position statement

For these reasons we present a set of reference ranges we consider appropriate. It does not follow that your particular laboratory is wrong merely because it has a different set of ranges. Each practitioner must work to their own local reference range, not to those presented in this volume. They are provided here for perspective and to allow for comparison in the various case studies and examples we will be presenting.

The values we feel are most useful are presented in Appendix 1.

The concept of reference range/normal range/target range is common over all pathology tests, regardless of the particular laboratory producing the data. We will now briefly summarize the different components of blood science.

1.2 Biochemistry

Biochemistry is the study of the chemistry of the molecules, ions and atoms of various elements in the serum or plasma. To do this, the serum or plasma must be separated from the blood cells by the process of centrifugation. Different tests need to be performed on serum or on plasma; and if plasma, then the type of anticoagulant may be important. If in doubt, always consult the laboratory.

The results themselves are generally printed out on to a standard form that finds its way back to the patient's medical notes (Figure 1.3). The information is also held on a computer which can be accessed remotely from the ward or the clinic. It can also be e-mailed to a general practitioner.

Many biochemistry tests can be grouped together. The major biochemistry tests are grouped together according to certain types of physiology or pathology. For example, 'urea and electrolytes' (U&Es) together tell of the function of the kidney, whilst the liver function tests (LFTs) are self-evident. A lipid profile will generally include cholesterol and triacylglycerols; and in confirming and treating a suspected heart attack, several blood tests may be needed.

Urea and electrolytes

These tests include urea, creatinine, sodium and potassium and are described in detail in Chapter 12. The first

two are the body's way of getting rid of the waste products of excess nitrogen, urea being synthesized in the liver. The final point of removal of these molecules is the kidney, so that high concentrations of urea and creatinine are a sign of renal damage. Sodium and potassium are part of our diet, and concentrations in the blood need to be regulated within a fairly tight range. Another function of the kidney is to regulate concentrations of these ions, and also the pH of the blood (the focus of Chapter 13), so a great deal of complex biochemistry is required.

An additional aspect of the concentration of these substances is the amount of water in the blood, which the kidney regulates and, of course, one's drinking of water can vary a great deal during the day. Consequently, changes in sodium and potassium also provide information about renal function. These tests are used to help diagnosis and then monitor the effect of treatment in diseases such as renal cell carcinoma, renal artery stenosis, acute renal failure, chronic renal failure and nephrotic syndrome. If the cause of high concentrations of U&Es is suspected glomerulonephritis, this may be due to auto-immune attack on the kidney, which can be determined by the presence of an autoantibody, calling for the help of the immunology section. The latter is discussed in Chapter 9.

Liver function tests

These tests (detailed in Chapter 17) include bilirubin (a breakdown product of red blood cells), and four enzymes: alkaline phosphatase, gamma glutamyltransferase, alanine aminotransferase and aspartate aminotransferase. Increased concentrations of all five of the LFTs imply malfunction of this organ. However, a problem with these enzymes is that they are also found in other cells and tissues (such as of the heart and bones) and that concentrations are influenced by other factors, such as drugs. Consequently, only one LFT by itself is difficult to interpret.

The liver also makes many proteins, including albumin, so that low concentrations of certain proteins are also indicative of liver disease. If the autoimmune disease primary biliary cirrhosis is suspected, testing for anti-mitochondrial antibodies will be useful. This may be undertaken by the immunology laboratory (Chapter 9).

Lipids, glucose, diabetes and heart disease

The major risk factors for atherosclerosis, the disease process causing most heart disease, are smoking,

hypertension, dyslipidaemia and diabetes. There is a blood test for smoking – cotinine – but the laboratory is most unlikely to offer it or even to agree to send it to a reference laboratory. There are no blood tests for predicting or monitoring hypertension – this is by the clinical measurement of blood pressure with an automatic sphygmomanometer. This therefore leaves lipids and glucose, which are detailed in Chapter 14.

Dyslipidaemia Total cholesterol is actually made of two components: low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol. We know that high concentrations of LDL are a risk factor for cardiovascular disease. We also know that low concentrations of HDL are a risk factor for cardiovascular disease. Therefore, to use the word ‘hypercholesterolaemia’ (i.e. high total cholesterol) as a risk factor for atherosclerosis is to fail to understand the value of measuring LDL and HDL. This is why we now prefer the word ‘dyslipidaemia’, as it does not focus on LDL or HDL.

It is still a matter of debate as to whether or not isolated high concentrations of triacylglycerols are an independent risk factor of atherosclerosis in the general population. As we shall see, there are abnormalities in lipid metabolism when disease is associated with high triacylglycerols, but these are uncommon. Triacylglycerols show a strong diurnal variation (i.e. up or down at different times of the day), so that the best sample is first thing in the morning, before breakfast, and is called a fasting sample.

Diabetes This risk factor is probably the most prevalent treatable and preventable risk factor for atherosclerosis. It is characterized by high blood glucose (hyperglycaemia). The trouble is that glucose, like triacylglycerols, also has a diurnal variation, and so calls for a fasting sample. Diabetes mellitus is not simply about too much glucose in the blood, but how this glucose is handled by insulin, the hormone required (amongst its other roles) for moving glucose out of the blood and into cells. A good way of looking at this is to take a sample of blood before and 2 h after having had a drink that contains 75 g of glucose. This test is called the oral glucose tolerance test.

Glucose can easily enter the red blood cell, and stick to the haemoglobin, leading to glycated haemoglobin (HbA_{1c}). This stickiness is irreversible, so that the haemoglobin stays sugary for its entire lifetime; that is, approximately 120 days. This means that a one-off

measurement of HbA_{1c} effectively provides a long-term view of the degree of hyperglycaemia.

Cardiovascular disease The serious, acute, clinical aspects of cardiovascular disease are myocardial infarction (heart attack) and stroke, where the arteries of the heart and brain (respectively) are attacked by the combined effects of the risk factors. There are no major acute consequences of atherosclerosis of arteries of the groin and the leg – but these are long-term and chronic diseases.

There are no blood tests to help diagnose the consequences of disease of the arteries of the groin, legs and brain. However, the consequence of disease of coronary arteries (a heart attack) is damage to the muscle cells of the heart. This causes the release of various enzymes and other molecules, including creatine kinase and troponin. Measurement of these molecules is important in differentiation of chest pain caused by a myocardial infarction from chest pain caused by other factors, such as muscle strain or gastrointestinal problems.

Calcium, phosphate, magnesium and bone disease

Bone is made almost exclusively of calcium and phosphates, so that abnormal plasma concentrations of these ions can be indicative of bone disease. A key enzyme in bone metabolism is alkaline phosphatase, but this enzyme is also part of the LFT panel. Vitamin D and concentrations of parathyroid hormone are also important in bone health, where the major diseases are osteoporosis, osteomalacia (called rickets in children), Paget’s disease and osteomyelitis. In some cases the blood protein albumin may be part of a bone panel as this molecule can carry calcium. The importance of these indices, and their related diseases, are explained in Chapter 15.

Hormones and endocrine disorders

Although diabetes is the most common endocrine disease, there are several others that can be tested for in the laboratory, and these are detailed in Chapter 18. These include disease of the thyroid, where thyroxine, triiodothyronine and thyroid-stimulating hormone can be measured. Several metabolic conditions (Addison’s disease, Cushing’s disease, diabetes insipidus) focus on the adrenal glands, and the measurement of cortisol, adrenocorticotrophic hormone and vasopressin may be informative.

Other blood tests in this area include those for growth hormone, testosterone, estradiol (oestrogen), progesterone, follicle-stimulating hormone and luteinizing hormone. Some of these are released from the pituitary, others from the gonads (ovary and testes). Measurement of these hormones is undertaken when assessing reproductive disorders and subfertility.

Many endocrine conditions have an autoimmune basis, so that the immunology laboratory will be needed in order to find presumed autoantibodies, as are listed in Chapter 9. Indeed, the red blood cell disease pernicious anaemia is also part-diagnosed with an autoantibody.

Other tests

Naturally, there are dozens of other tests of undoubted value in the diagnosis and management of human disease. Unfortunately, the pathology laboratory does not have an infinite budget, and it can only offer those tests most commonly requested. However, regional or specialist laboratories may offer rare tests if there is a sufficiently large demand and if critical mass is achieved. The most common biochemistry tests are listed in Table 1.2.

The laboratory can also test for drugs. This is clearly important in cases of accidental or deliberate overdose, where concentrations of the drug (such as paracetamol) can be crucial in treatment. We often need to assess concentrations of prescribed drugs in the plasma. These

include levels of lithium (a treatment of bipolar disease), digoxin (to treat certain types of heart conditions) and methotrexate (in cancer and rheumatoid arthritis). Collectively, this is described as therapeutic drug monitoring, and is the subject of Chapter 21.

1.3 Blood transfusion

Blood transfusion is not a blood disease, or a test, but it is blood science and a form of therapy. Its objective has changed over the years, from being a crude instrument to maintain haemoglobin at a certain level, to a therapy that saves lives. Additional changes have been the realization that a blood transfusion is far from a simple and trouble-free treatment, and that it can promote damage, possibly permanently, to the health of the recipient. A further development has been the provision of blood products such as platelets, albumin and coagulation factors, provided by the National Health Service (NHS) Blood and Transplant (NHSBT) service (formerly the National Blood Transfusion Service).

Blood groups

All blood group systems consist of two parts. First, certain molecules present at the surface of the red blood cell, also known as ‘antigens’ (an antigen is a structure that invokes an antibody response). The second aspect is a series of corresponding antibodies that recognize these antigenic molecules. The ABO system is based on the presence of two molecules, A and B, that may be present on the surface of red blood cells. Some people have one or the other (group A or B), some have both (group AB) and some have none (group O). There are also plasma antibodies that recognize blood group structures, but these are the reverse of your blood group. So if you are group A, you will have antibodies that will recognize group B (i.e. anti-B). Likewise, group B people have antibodies that recognize group A (i.e. anti-A). Group AB people have no anti-A or anti-B antibodies in their plasma, but group O people have both anti-A and anti-B antibodies. This is summarized in Table 1.3.

The second most important blood group system is the rhesus (Rh) system. It is very much more complicated, being composed of over 40 recognized glycoproteins, although on a day-to-day basis five different structures on the surface of the blood cell are commonly dealt with in the blood bank. In practice, we tend to focus on the molecule known as D (i.e. RhD). The main distinction

Table 1.2 Common biochemistry tests

Urea and electrolytes

Urea, creatinine, sodium, potassium

Liver function tests

Bilirubin, alkaline phosphatase, gamma glutamyltransferase, alanine aminotransferase, aspartate aminotransferase

Bone panel

Calcium, phosphate, vitamin D, parathyroid hormone

Atherosclerosis and its risk factors

Total cholesterol, HDL, LDL, triacylglycerols, glucose, HbA_{1c}, creatine kinase, troponin

Endocrine and metabolic disease

Thyroxine, tri-iodothyronine, thyroid-stimulating hormone, adrenocorticotrophic hormone, cortisol, vasopressin

The pituitary and reproduction

Testosterone, estradiol, progesterone, follicle-stimulating hormone, luteinizing hormone, growth hormone

Table 1.3 ABO blood group factors.

Antigenic structures on the red cell surface	Antibodies in the plasma
A	Anti-B
B	Anti-A
AB	No antibodies
O	Anti-A and Anti-B

between the ABO and Rh systems is that, in the normal person, there are always anti-A and anti-B antibodies in the absence of reciprocal molecules on the red blood cell surface (which makes ABO incompatibility potentially fatal), but people with the D molecule on the red cell surface do not have a corresponding antibody in the plasma. There are also hundreds of other blood groups of diminishing frequency and importance, such as those of Lewis, Kidd and Duffy.

Blood groups present a serious challenge to the transfusion scientist. An incorrect transfusion may well precipitate a major clinical crisis in the recipient, and is termed an incompatible transfusion, which could prove fatal.

The practice of blood transfusion

Basic training in blood transfusion demands competency in several techniques: the determination of blood group, antibody screening and the cross-match.

Blood group determination The request to ‘Group and Save’ (G&S) is made by a practitioner with the implication that a blood transfusion may be needed in the near future. The request is to find out the patient’s blood group (*Group*), but then keep the blood handy (*Save*, generally in a refrigerator). Most blood banks determine the ABO and Rh groups.

Antibody screening Many of us have natural antibodies to blood groups A and B, but exposure to other people’s tissues, perhaps by a previous blood transfusion or pregnancy, may induce the formation of antibodies to other blood groups. If present in the recipient, such antibodies may cause serious problems, and so it is prudent to test the recipient’s blood for antibodies to other groups.

Cross-match Not every G&S is translated into a request to cross-match. But when the call comes, scientists will mix red cells and plasma (that potentially contain antibodies) from the patient with a sample

from different packs of donor blood. A good match is where the red cells are unaltered by this mixing, and therefore should not react together when in the patient.

However, blood that does not match will aggregate, forming small clots, indicating an incompatibility. This is inevitably because the molecules on the red cells and the antibodies in the serum or plasma recognize each other, and react together, causing blood to clump. It is presumed that the same reaction may happen in the blood vessels; hence the danger. The same principle of incompatibility also occurs in other systems, such as antibodies to Rh molecules (such as D).

Blood components (previously blood products) The blood bank can provide not only red cells, but also platelets and coagulation proteins such as fibrinogen, factor VIII, factor VII, fresh frozen plasma and cryoprecipitate. The latter will be needed by people at risk of, or with actual haemorrhage (uncontrolled bleeding). Albumin is available for people with critically low concentrations, have had heavy burns, or have ascites (fluid in the abdomen).

Clinical aspects of blood transfusion

No treatment is 100% safe or effective. Therefore, does the patient *really* need a transfusion? Alternatives may be possible, such as transfusion of the patient’s own blood, which is possible using cell salvage systems. Therefore, transfusion should be reserved only for those in danger of losing their life, or when it will show a measurable improvement not achievable by other means.

Sources of error Errors can and do occur in all laboratories, regardless of discipline, and so also in the ‘journey’ of blood from the donor to the recipient. However, it is generally recognized that most errors happen in the laboratory and/or once the blood has left the blood bank for its destination. However, with increasingly rigorous safety checks, errors are becoming increasingly rare.

Packs of blood arrive from the NHSBT service already typed for ABO and Rh, and screened also for major infective agents, mostly viruses. However, the blood sample from the recipient may be labelled incorrectly. The next source of error may be the incorrect labelling of the same portion of each potential donor pack. Further errors are possible during the cross-match itself. Fortunately, these are rare because the laboratory invests heavily in the technology and reagents to ensure that

Table 1.4 Some signs and symptoms of a transfusion reaction.

Symptoms	Signs
Cough	Fever (temp. spike >40 °C)
Flushing/rash	Hypotension
Anxiety	Oozing from wounds
Chills	Haemoglobinaemia
Nausea and vomiting	Haemoglobinuria
Tremble/shakes	Tachycardia

if an adverse reaction is happening it is detected as rapidly as possible. However, if the cross-match goes wrong, which is a false negative, a possible incompatible unit or units of blood may be issued.

Responses to an incompatible transfusion Symptoms and signs of a transfusion reaction vary enormously (Table 1.4), but if suspected, it should be immediately stopped. All good hospitals will have a defined protocol that must be followed. Treatment will depend on the severity of the reaction, which, if not too bad, can be rapidly reversed. Problems may occur perhaps days after the transfusion, and include renal failure and jaundice, the latter being an indication of destruction of red cells.

Post-transfusion purpura (bruising) is characterized by a severe thrombocytopenia (low platelets, which can last from 2 weeks to 2 months) and is caused by antibodies to molecules on the surface of platelets. If heavily haemorrhaging, then the transfusion of platelets may be required.

Repercussions Naturally, there are many steps designed to prevent a transfusion error, such as the use of laser bar-coding so the sample can be traced from the requesting blood sample all the way back to the patient. Many hospitals have a policy of at least two members of staff checking that the blood they are about to transfuse into one of their patients is the right type. This approach has proven to reduce mistakes and serious hazards of transfusion. Chapter 11 has more complete details about blood transfusion.

1.4 Genetics

Genetic diseases are surprisingly common. Perhaps 0.5% of all newborn infants have a chromosomal abnormality, a figure that rises to 7% if the infant is stillborn; and

20–30% of all infant deaths are related to abnormal genetics. Many ‘adult’ diseases have a genetic component, and this proportion is highest in the cancers, where inherited susceptibility accounts for 15% of cases.

The broad title ‘genetics’ includes several other names, such as molecular biology or molecular genetics. The newest recruit to the pathology laboratory, genetics has yet to find its place alongside the other disciplines, but doubtless will eventually do so. One of the reasons that molecular genetics has not been rapidly and extensively adopted is that in many cases the techniques, such as Southern blotting and the polymerase chain reaction, are very sophisticated and demand highly skilled scientists and complex equipment, which together generate a high unit cost. Accordingly, this service is often offered at regional level, and is frequently attached to a university teaching hospital. However, with advances in technology, many methods are becoming simplified, so that they can be offered by a district general hospital.

A further aspect of a genetics service is its function. Our present healthcare system starts with the patient complaining about a particular problem, or in response to a coincidental finding. The next step will be investigations, ideally a diagnosis, and then management (cycles of treatment and testing to ensure the treatment is effective). This model works well for well over 90% of problems, and so does not call for a genetics input. It follows that genetics is often called upon to help in confirming or refuting a diagnosis. There are dozens of instances where a particular disease has a genetic component, but this need not always be confirmed.

Genetic disease in families

However, a valuable service offered by the genetics service is to confirm or refute a potential diagnosis in someone who is asymptomatic, but in whom occult (hidden) disease may be suspected, such as in a strong family history of a particular disease. Of those cancers caused by gene mutations, multiple endocrine neoplasia (lesion present on chromosome 10), von Hippel–Lindau syndrome (chromosome 3) and Wilm’s tumour (chromosome 11) are the most common. However, one of the strongest family ‘cancer’ genes *BRCA-1* (found on chromosome 17) is closely linked to the development of breast cancer. A second mutation also linked to breast cancer is *BRCA-2*, present on chromosome 13, and both *BRCA-1* and *BRCA-2* are linked to ovarian cancer. Some gene effects are so strong that one can say with a firm level of confidence that the individual has a likelihood of

actually developing a particular disease at some time in the future. This is called penetrance.

Penetrance

Some particular gene mutations (the genotype) always produce a physical problem such as a disease (the phenotype). If so, we say there is complete (100%) penetrance. A good example of this is haemophilia, caused by one of several possible mutations in the gene for coagulation factor VIII so that low levels or even none of the molecule is produced. The mutation is always present and active: you cannot have ‘partial’ haemophilia. A second, allied, haemorrhagic condition is von Willebrand’s disease, caused by one of several mutations in the gene for von Willebrand factor. These mutated genes have varied penetration into the disease. In some, penetrance is high and the disease is severe (complete absence of von Willebrand factor). However, in others, the mutation causes a partial reduction in concentrations of the protein, and so minor bleeding, or the disease may even be largely asymptomatic, in which case penetrance is low.

BRCA-1 has a penetrance of over 50% for breast cancer, and over 30% for ovarian cancer, by the age of 70. The predictive power of this marker was demonstrated over a decade ago by the case of a middle-aged woman whose female relatives all suffered breast cancer and who were positive for *BRCA-1*. Although her breasts were entirely normal by established tests, she opted for bilateral mastectomy. Early neoplastic changes were subsequently found in both breasts. More recently, chemotherapy with tamoxifen and surgical removal of the ovaries are options.

Genes, chromosomes and DNA

All the information regarding the working of the body is carried by an individual’s deoxyribonucleic acid (DNA). An individual piece of information, such as an instruction to synthesize haemoglobin, is carried by a particular section of DNA: a gene. The sum total of an individual’s gene and DNA make-up is their genome. The DNA itself is a long chain of nucleic acids, wrapped about certain proteins (histones), and which form into chromosomes. Normally, the information that is carried in genes is tightly controlled, but if it goes wrong, and a section of a particular gene is altered, the resulting product may be abnormal, and we say the gene is mutated.

A good example of this is the case of sickle cell disease. Haemoglobin has evolved as a specialized protein to

carry oxygen, but a certain mutation in a gene for haemoglobin means that there is a different amino acid sequence of the abnormal haemoglobin, which means it is less able to carry oxygen. This can lead to the symptoms of anaemia, and so is called sickle cell anaemia. However, the sickle gene has variable penetrance: in some it causes considerable discomfort, but in others the consequences of the same mutation can be mild. The mutation can develop ‘naturally’, but in most cases it is inherited from one or both parents.

Chromosomal disorders

Some abnormalities are present at the level of the whole chromosome. A normal individual has 46 chromosomes in their nucleus, two pairs of 22 (autosomes) and two sex chromosomes (an X and a Y in males and two X chromosomes in females). This collection of chromosomes within the nucleus is called a karyotype.

Perhaps the best-known chromosomal disease is Down syndrome, which is caused by an extra copy of chromosome 21, so that the karyotype is 47 chromosomes. An extra copy of chromosome 13 defines Patau syndrome. Klinefelter syndrome is characterized by an extra X chromosome in males (i.e. XXY), whilst Turner’s syndrome is characterized by the presence of only one X chromosome in females (i.e. XO), so that the karyotype is 45 chromosomes. Disorders of entire chromosomes almost always have complete penetrance into the genotype (it is not possible to have ‘partial’ Down’s syndrome).

There can also be sections of a chromosome missing (a deletion), an extra part fused on to a chromosome (a duplication), sections of DNA swapped between chromosomes (a translocation), and a section of DNA the wrong way round (an inversion). Examples are provided in Table 1.5.

Table 1.5 Chromosome abnormalities.

Abnormality	Example of condition (location of lesion)
Deletion	Prader–Willi syndrome (chromosome 15) Wilm’s tumour (chromosome 11) DiGeorge syndrome (chromosome 22)
Duplication	Charcot–Marie–Tooth disease (chromosome 17)
Translocation	Chronic myeloid leukaemia (chromosomes 9 and 22) (the Philadelphia chromosome)
Inversion	Acute myelomonocytic leukaemia (chromosome 16)

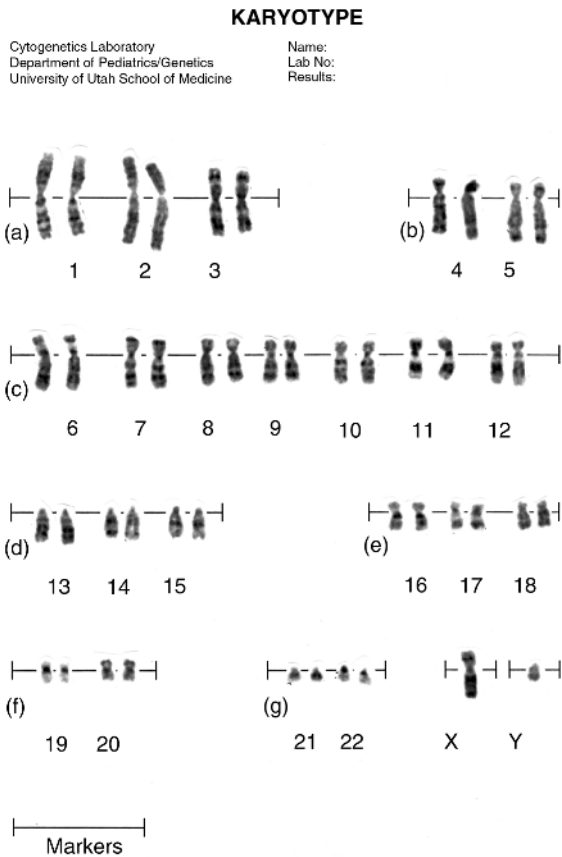


Figure 1.4 A normal karyotype: 46 chromosomes arranged in 23 pairs, dependent on the length and the banding patterns, of 22 pairs of autosomes and two sex chromosomes. (Iles & Doherty (eds) 2012, Fig. 3.14, p. 104. Reproduced with permission of John Wiley & Sons, Ltd.).

Determination of a subject's karyotype requires growing some of their cells (often lymphocytes) in tissue culture, and then arresting their growth cycle at a key point in mitosis. At this point the chromosomes are not bunched together in the nucleus but are spread out and so can be stained and identified (Figure 1.4).

Gene disorders

The sequence of nucleotides in a particular section of a chromosome that have a defined function (such as ultimately generating a protein) make up a gene. Changes in this sequence can give rise to genes that generate a different protein; an example of this is normal haemoglobin and sickle haemoglobin. The ability to detect differences in genes has been a crucial step forward in

biomedical science, and those pioneers developing the techniques were justly rewarded with Nobel Prizes. With these tools, geneticists have identified hundreds of thousands of alternative forms of different genes, and these give rise to the desired variation in the human condition. However, other variations are unwelcome as they cause disease, and most of these can be classified as deletions or as translocations. Some of these mutations cause cancer, and so are called oncogenes.

Deletions These mutations are simply sections of DNA that are missing, so that genes fail to generate a molecule that functions correctly. For example, a normal gene may produce a protein whose amino acid sequence 'spells' a sensible word such as

***** understandable *****

A deletion mutation in the gene may produce a protein that is missing some amino acids, so that the word now 'spells' something unrecognizable, such as

***** undeandable *****

Although some mutations are beneficial, many are associated with disease. A good example of the former is a deletion of a section of a gene for a lymphocyte molecule called CCR5, which confers resistance to the human immunodeficiency virus (Chapter 11). However, the list of gene deletions leading to harmful mutations is considerable. One of the best known is a deletion in the dystrophin gene on the X chromosome that causes high levels of calcium in muscle cells, and that is the basis of Duchenne muscular dystrophy.

Cystic fibrosis is caused by deletion of three DNA bases in a gene on chromosome 7 that codes for a structure on the cell membrane with a role in regulating chloride and sodium ion transport in and out of the cell. The consequences of the abnormal gene are increased mucus secretion that ultimately causes the disease, mostly of the lungs and digestive tract.

Translocations Diseases caused by this process develop as a result of two different sections of DNA being brought together, and so a new section of DNA is created that codes for a new gene. For example, on one chromosome a 'nonsense' DNA sequence may 'spell'

*****unfortu

whilst another section of DNA on a different chromosome may have a sequence that ‘spells’ an equally obscure word such as

nate * * * * *

If a translocation happens, the two sections of gene could merge and produce a new protein, which may be unfortunate. Translocations commonly cause cancer, an example of which is a gene called *PAX8* on chromosome 2 becoming adjacent to a gene called *PPAR-gamma* on chromosome 3. This often results in follicular thyroid cancer. Translocations of a part of chromosome 1 to chromosome 6, chromosome 14 or chromosome 19 are found in many cases of malignant melanoma.

Oncogenes An important series of deletions involves oncogenes – genes that cause cancer – and in many cases this is to do with the rate of growth of the cell and its differentiation. One such gene is *MYC* (pronounced ‘mick’) located on chromosome 8, which codes for a protein involved in the regulation of many other genes. Inappropriate activation of *MYC*, therefore, leads to abnormal gene expression, which in turn can lead to cancers. A good example of this is the translocation of *MYC*, so that it becomes adjacent to a gene on chromosome 14 that codes for part of an immunoglobulin molecule. This translocation is often found in Burkitt’s lymphoma. Another oncogene is *SRC* (pronounced ‘sarc’), acting on internal cell enzymes also involved in signalling. Fascinatingly, the gene sequence of *SRC* resembles a virus that in chickens causes a tumour of muscle tissues.

An important group of genes are the tumour suppressors, whose function is clear. Many cases of retinoblastoma are caused by a mutation in the *RB1* gene on chromosome 13. A function of the protein product of the normal *RB* gene is to regulate the cell cycle and cell growth. The mutated *RB1* gene produces an abnormal protein that fails to regulate cell growth and that can lead to cancer. However, the pathology of this type of retinoblastoma is an example of a two-hit disease, as a second factor in addition to *RB1* is needed to cause the disease. Both *BRCA* genes are tumour suppressors: the normal genes code for proteins that help repair broken and damaged DNA. The abnormal protein produced by the mutated gene fails to repair damaged DNA, which may then precipitate a cancer.

Genetics as an independent blood science

Genetics has the power to unequivocally define a disease, and also, by the absence of a mutation, to deny a disease.

However, current research can provide a clue as to which treatments, such as a particular combination of cytotoxic drugs, are likely to be more successful, as different chemotherapy can target the products of different mutations. Indeed, knowledge gained from the molecular genetics of a certain type of chronic leukaemia has led to the development of a completely new and very successful class of drugs that treat only this type of cancer.

The preceding section has introduced the value of genetics in many different types of diseases, including cancer. Indeed, it could be argued that clinical genetics is in fact a collection of methods called on by pathologists to help with diagnosis. Accordingly, this textbook will not have a separate chapter on genetics. Instead, each individual chapter will emphasize genetics where and when it is appropriate. Examples of the impact of genetics in the other blood sciences include:

- haemoglobinopathy, leukaemia, haemophilia, von Willebrand’s disease, Factor V Leiden, and hereditary haemochromatosis in haematology;
- alpha-1-trypsin deficiency, LDL cholesterol receptor, cystic fibrosis and 21-hydroxylase deficiency in biochemistry;
- ABO and rhesus blood groups in transfusion science;
- the heritability of autoimmune disease, X-linked agammaglobulinaemia, variation in human leukocyte antigens (HLAs), and DiGeorge syndrome in immunology.

All these conditions, and others where genetics impacts into a particular blood science, will be explained in their own chapter. Other examples of molecular genetics used by non-blood science disciplines are shown in Table 1.6.

Table 1.6 Molecular genetics across pathology.

Test	Discipline	Disease
Hepatitis C virus	Virology	Chronic hepatitis
CK19, with or without mammaglobin	Histology	Breast cancer intra-operation lymph node investigation
HER2 (human epidermal growth factor receptor 2)	Histology	Breast cancer
Chlamydia	Microbiology	Population screening
<i>KRAS</i> (Kirsten rat sarcoma viral oncogene homolog)	Histology	Colorectal cancer

Blood science angle: Haematology and molecular genetics

Certain haematology malignancies, including polycythaemia rubra vera, essential thrombocytopenia and myelofibrosis, are often characterized by increased numbers of red blood cells and platelets. In the past, these diseases have been diagnosed by a combination of blood tests and examination of the bone marrow, and difficulties in specificity and sensitivity of these tests often brought false positives and false negatives.

Janus kinase 2 (JAK2) is a protein involved in a cell's response to growth factors. In 2005, several groups linked a mutation in the gene for this molecule (*JAK2 V617F*) that effectively conferred a hyperreactivity of cell to growth factors, and which went a long way in explaining the high cell counts in these diseases.

This link is now so powerful that the presence of *JAK2 V617F* now makes the diagnosis of polycythaemia. This is a classic example of molecular genetics providing a new diagnostic tool. Moving to populations, a study of almost 50 000 Danes demonstrated that this mutation is also a risk factor for ischaemic heart disease and deep vein thrombosis. Further details of JAK2 are presented in Chapter 4.

1.5 Haematology

Haematology can be summarized in four areas: the blood film, the full blood count, coagulation and haematinics. These are used in the diagnosis and management of a broad range of diseases of the blood.

The blood film

The blood film provides the opportunity to view the components of blood under a microscope. A drop of blood, generally from the same tube that provides the full blood count, is smeared on to a glass slide and is allowed to dry in air at room temperature. It is then fixed, stained, allowed to air dry and examined under a light microscope. We can also use the shape of the nucleus to help classify the different types of white blood cells; this is called morphology.

Figure 1.5 shows a typical blood film. The principal features in the centre are two white blood cells, characterized by a nucleus that has taken up stains so that it appears purple. Note that in these two cells the structure of the nucleus is different. We will return to the importance of this in Chapter 5. A second feature is the large number of roughly round cells that are all a single colour; these are red blood cells. The third feature is the single

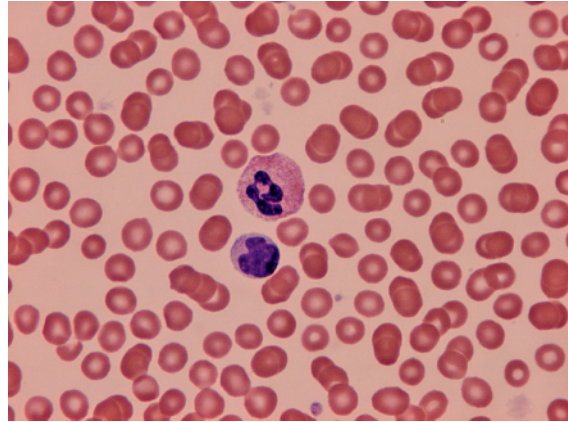


Figure 1.5 A blood film. (Courtesy of D. Bloxham, Cambridge University Hospitals NHS Foundation Trust).

small purple body to the lower right of the white blood cells; this is a platelet.

Autoanalysers now provide an excellent breakdown of the different types of blood cells, so the blood film has become less important and is now not routinely examined. However, there is still a place for examination of a blood film with a microscope that may be necessary to confirm the autoanalyser's profile or to perform other assessments of the blood. The same principle is also used to look at bone marrow, which is where blood cells are generated.

The full blood count

This is the most requested and valued blood test in haematology. It provides a package of information on red blood cells, white blood cells and platelets (Figure 1.6).

Red blood cells These are unusual among cells of the body as they lack a nucleus. Consequently, they are relatively easy to identify in a blood film (Figure 1.5). Red cells carry haemoglobin (Hb), an iron-containing protein that absorbs oxygen from areas of high oxygen content (i.e. at the lungs) and then releases it in areas where oxygen levels are low (e.g. in the tissues). The haematocrit (Hct) expresses that proportion of whole blood that is taken up by all the blood cells as a decimal (e.g. 0.42) or as a percentage (e.g. 42%). The reference range for haemoglobin, red cell count and haematocrit varies between the sexes, with lower levels in women.

The red blood cell indices comprise three measures of different aspects of the red cell, its size and the amount of

Sandwell and West Birmingham Hospitals NHS Trust.

Haematology, City Hospital

Surname	Address	Unit No.	OPD - CITY
Forename		Consultant/CP	
D.O.B.		Prof G.Y.H.Lip	
Sex			
NHS No.		Lab No. C13.4250574.P	

Ethnic group: White - British

Clinical Details:

HB	145	g/L	(125-180)	ESR	2	mm/h	(1-14)
MCV	94.6	fL	(79.0-99.0)				
WBC	5.8	10 ⁹ /L	(4.0-11.0)				
PLT	241	10 ⁹ /L	(150-450)				
RBC	4.62	10 ¹² /L	(3.50-6.50)				
HCT	0.44	L/L	(0.38-0.54)				
MCH	31.4	pg	(27.0-34.5)				
MCHC	332	g/L	(316-365)				
NEUT	3.38	10 ⁹ /L	(1.70-7.50)				
LYMPH	1.85	10 ⁹ /L	(1.00-4.50)				
MONO	0.49	10 ⁹ /L	(0.20-0.80)				
EOS	0.06	10 ⁹ /L	(0.00-0.50)				
BASO	0.01	10 ⁹ /L	(0.00-0.10)				

RANDOM SAMPLE

F: Please note new units for Hb & MCHC (Now reported in g/L).
e.g a Hb previously reported as 12.5g/dL will from 27/03/13
be reported as 125g/L

Tests: ESR, FBC	Check Clinician	Date collected 13.05.13 14:30
Report Run: 73	Specimen: Blood	Date received 13.05.13 16:19
		Date reported 13.05.13

Figure 1.6 The full blood count.

haemoglobin it contains. These indices are: the mean cell volume (MCV), the volume of the average (mean) red blood cell; the mean cell haemoglobin (MCH), which reports the average amount (mass) of haemoglobin in the average cell; and the mean cell haemoglobin concentration (MCHC), the average concentration of haemoglobin in a given volume of red cells. The red cell is discussed in detail in Chapters 3 and 4.

White blood cells White blood cells, or leukocytes, defend us from attack by microorganisms (viruses, bacteria and parasites), when raised levels of these cells can be expected. However, increased numbers (i.e. a leukocytosis) may also be present in a number of diseases. There are five different types of white cells: the neutrophil, lymphocyte, monocyte, eosinophil and basophil. Each can be defined on morphological grounds, but also by their function, as is explained in Chapters 5 and 6.

Neutrophils are the most common leukocytes, and also the most common polymorphonuclear leukocyte, so

named because they have an irregular nucleus (the upper cell in Figure 1.5). They are called neutrophils because they take up dyes at a neutral pH. The second most frequent group of leukocytes are the lymphocytes. Their nucleus is round and regular and takes up almost the entire cell (the lower cell in Figure 1.5). Monocytes, the largest white cell, also have a regular nucleus, but the nucleus takes up perhaps two-thirds or three-quarters of the cell. Eosinophils are so-called because of the reddish colour, owing to the chemical make-up of their granules. Basophils are the least frequent leukocytes; they contain numerous granules that take up different dyes, and so appear as black or dark blue.

Platelets These are not true cells, but are small fragments of the cytoplasm of a larger cell found only in the bone marrow (the megakaryocyte). They form a clot, or thrombus, when aggregated together with the help of the blood protein fibrin and so reduce blood loss. This process is focused upon in Chapters 7 and 8.

A low platelet count (thrombocytopenia) may be caused by drugs, poor production (as may be present in disease of the bone marrow) or by excessive consumption. This condition can lead to an increased risk of bruising and bleeding. The converse, a raised count, is thrombocytosis and is often present in many physiological and pathological situations. These include infections, after surgery, some autoimmune diseases and after short but intense bouts of physical activity. A high platelet count may lead to thrombosis.

Erythrocyte sedimentation rate The erythrocyte sedimentation rate (ESR) is a global score of physical aspects of the whole blood. The result is obtained by measuring a band of plasma on top of a thin column of blood that has settled after standing for an hour. An ESR can be abnormal in a large number of conditions, including inflammation, infection, the acute-phase response, after surgery, anaemia, leukaemia and almost all forms of cancer. Indeed, it follows that an abnormal ESR is present in most patients in hospital.

Haemostasis

Haemostasis is the balanced orchestration of interactions between blood vessels, blood cells and plasma proteins. Together they keep the blood in a fluid state, and also limit and stop bleeding upon damage to the blood vessel. We have already mentioned platelets are part of the full blood count.

The clot (or thrombus) is generated by platelets and fibrin, which together form a net that traps red cells; but clots can occur without red cells. The laboratory offers tests on the ability of the blood to generate these clots. The inappropriate formation of a clot can lead to a disabling deep vein thrombosis or, more seriously, a stroke or even, in the lung, death. Conversely, inability to form a clot can also lead to serious disease due to excessive bleeding (haemorrhage).

Prothrombin time The prothrombin time (PT) assesses the ability of plasma to form a clot based on certain components of the coagulation pathway. It employs a reagent called thromboplastin that activates part of the coagulation system. The time taken from the addition of thromboplastin to the patient's plasma to generation of a fibrin clot is the PT itself, which is recorded in seconds.

Activated partial thromboplastin time This test assesses the ability of a different series of coagulation proteins from those of the prothrombin to form a clot. Patient's plasma is again incubated with a complex collection of reagents, and the time taken to clot from the addition of the calcium ions is also recorded in seconds.

Fibrinogen An adequate level of fibrinogen is crucial if coagulation factors such as prothrombin and thrombin are to have their desired effect. The laboratory measurement of fibrinogen activity is performed using a modified version of the thrombin time where the patient's plasma is induced to clot.

Pathology of thrombosis and haemostasis Thrombosis is the most common ultimate cause of death, such as is caused by a clot in an artery of the heart or the brain. However, a great deal of disease is also caused by clots in veins (venous thromboembolism), mostly of the leg (deep vein thrombosis) and the lung (pulmonary embolism). High numbers of platelets in the blood can also predispose to thrombosis. Consequently, the ability of the laboratory to assess these disease processes is at a high premium.

We can intervene in the process of thrombosis with drugs such as warfarin, heparin and aspirin. However, in many cases, the activity of the former two drugs needs to be checked with an appropriate blood test, such as the prothrombin time and activated partial thromboplastin time. A serious problem with these drugs is that too much warfarin or heparin can grossly reduce the ability to form a clot, which therefore results in bleeding. There are several examples of diseases where coagulation factors are 'naturally' lacking or are ineffective, haemophilia being a good example, where spontaneous or accidental haemorrhage is a constant concern.

Haematinics

A small number of vitamins and minerals are needed for blood cells to be generated effectively, and all must be provided by a healthy diet. These include iron, vitamin B₁₂, vitamin B₆ and folate. Deficiencies in any one of these micronutrients may result in impaired production of red blood cells by the bone marrow and therefore result in anaemia.

Iron This micronutrient is the key atom in the middle of the haemoglobin molecule where the oxygen is carried.

It is placed within a complex molecule, haem, by an enzyme called ferrochelatase, a process that happens within stem cells in the bone marrow. If there is not enough iron being placed in haem, an iron-deficient anaemia will result. However, the root of the problem may be not enough iron in the diet (malnutrition), or perhaps the inability of this iron to cross the gut wall and enter the blood (malabsorption, which may be related to, for example, inflammatory bowel disease).

The polar extreme of too little iron is too much iron. This can cause the disease haemochromatosis, and in many cases the ultimate cause is a gene mutation; another is too many blood transfusions.

Vitamin B₁₂ The second most common type of deficiency is of vitamin B₁₂, which is required by key enzymes in the synthesis of haem, a process that happens in the cytoplasm and mitochondria of bone marrow stem cells. Only a tiny amount of the vitamin is needed, so that malnutrition rarely causes the anaemia. Instead, the major cause of the anaemia is not being able to absorb enough of the vitamin across the gut wall, so is a form of malabsorption, the most frequent type being due to autoimmune disease of the stomach, and if present this is called pernicious anaemia. There are no known examples of problems caused by too much vitamin B₁₂.

The laboratory in micronutrient deficiency The opposing features of microcytes (with a low MCV, often due to iron deficiency) and macrocytes (with a high MCV, often due to vitamin B₁₂ deficiency) are key steps in the diagnosis and understanding of anaemia. Treatment of these deficiencies, in many cases, is simply by replacing the missing micronutrient. However, if there is malabsorption, the oral route is not appropriate, and the iron and vitamin will need to be given by injection or infusion. The laboratory is then needed to ensure the treatment is effective – which is that the size of the red cell becomes normal and that the haemoglobin rises.

Haematological disease

These tests and processes are used to diagnose and manage a large number of diseases, such as cancers (leukaemia, lymphomas and myeloma), different types of anaemia (caused by lack of micronutrients or perhaps by the destruction of red cells (haemolysis)) and by excessive bleeding (haemorrhage) or clotting (thrombosis).

Table 1.7 Common routine haematology blood tests.

Tests performed on anticoagulated whole blood

Full blood count: provides information on red blood cells, white blood cells and platelets

ESR: a physical property of whole blood

Tests performed on plasma

Coagulation tests: prothrombin time, activated partial thromboplastin time, fibrinogen (important in thrombosis and haemorrhage)

Tests performed on serum or plasma

Micronutrients iron, vitamin B₁₂ and folate

Proteins transferrin and ferritin (used to investigate different types of anaemia)

Table 1.7 summarizes key features of laboratory haematology.

1.6 Immunology

The laboratory and clinical aspects of immunology are certainly the most recent developments in mainstream biomedical science. The British Society for Immunology was founded as recently as in the mid 1970s. Indeed, the subspecialty of immunology can be said to have grown out of haematology (since the function of white blood cells is immunological defence), and in many hospitals the immunology laboratory is physically linked to the haematology laboratory. Furthermore, immunology is the basis of almost all reactions in the blood transfusion laboratory.

Laboratory aspects of this subject can be simply classified into serology (literally the study of serum) and cell biology.

Serology

From an immunological perspective, serology focuses on the assessment of levels of antibodies to particular pathogens or tissues, total levels of the immunoglobulins, complement, and other proteins.

The key molecule in immunology is the antibody, and all antibodies are immunoglobulins (Igs). They are classified into one of five groups, dependent on their protein make-up – IgG, IgM, IgA, IgD, IgE – and all have a common structure, which is often presented as a ‘Y’ shape (Figure 1.7). All have two ‘heavy chains’, of which there are five types (A, D, E, G and M) which define their

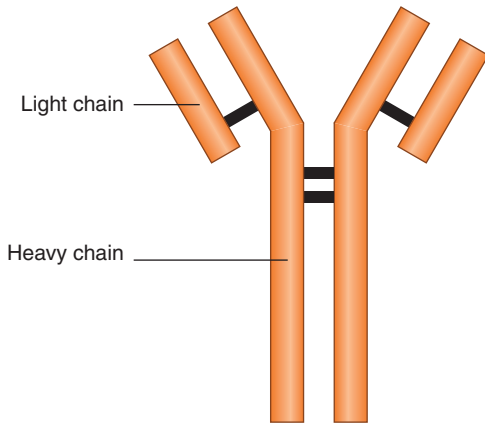


Figure 1.7 An antibody molecule.

class, but also two ‘light’ chains’, which can be one of two types: kappa or lambda. All antibodies are made by B lymphocytes, most in the lymph nodes and (to a lesser degree) in the spleen, and to do this they need the cooperation of T lymphocytes and perhaps some specialized monocytes. Antibodies are found in the plasma and also as part of the membrane of B lymphocytes. These important cells and molecules are revisited several times, in Chapters 5, 6, 9, 10 and 11.

As with different roles of the various white blood cells, the different antibody classes have both specific and common functions, but all are designed to bind to structures believed to be foreign. These foreign structures are called antigens. However, some antigens are not foreign – such as the AB blood group molecules – and this is the basis of the incompatible blood transfusion that scientists in the blood bank seek to avoid.

Complement is a family of nine molecules (C1–C9) that have a number of functions. Some are broken-down fragments that influence the cells of the blood vessel wall (endothelial cells) and attract certain white blood cells, whilst others promote the ingestion of bacteria by those white blood cells. Others come together to form a complex that can punch holes in the walls of certain cells and bacteria, resulting in their destruction. Details of complement are found in Chapter 9.

An alternative aspect of serology is the ability to detect plasma antibodies to defined pathogens, and this effectively proves the presence of an infection with the particular bacteria or virus. This is of interest to microbiologists, who seek evidence of a possible pathogen and so determine the most appropriate form of antibiotic or antiviral chemotherapy. Many inflammatory states, with

or without an infection, are characterized by increased concentrations of a c-reactive protein (CRP). Consequently, in many cases, this molecule defines immunological activity within the body. However, CRP may be measured not in an immunology laboratory but in biochemistry.

Cells

Cellular aspects of immunology focus on two areas: the enumeration and function of neutrophils and of lymphocytes, which can be achieved morphologically on a blood film, or by identifying cell-specific molecules on the surface (the CD family of molecules). Neutrophil function can be assessed by the ability of the cell to attack and digest common pathogens such as yeast (the process of phagocytosis), and by their ability to switch on certain biochemical pathways designed to destroy bacteria. There are no commonly used tests of monocyte function.

Perhaps the most common lymphocyte investigation is to determine the proportions of the two major classes of lymphocytes; that is, T cells and B cells. An extension of this is the T lymphocyte subgroup, mostly the number of the CD4 subset, as this is important in those infected with the human immunodeficiency virus (HIV). Almost all of this work is performed with a machine called a fluorescence-activated cell scanner; this technique is also of interest to haematologists, as there are many other leukocyte groups that can be assessed and that are important in leukaemia. The fluorescence-activated cell scanner can also help diagnose diseases of the membrane of the red cell and of platelets.

Immunopathology

The most successful immune responses are characterized by the serological aspects working together with the white cells to defeat the infection. However, this can be defective and result in disease. Like many different pathological states, immunological disease can be classified according to two extremes: where there is an excessive or inappropriate activity (too much), and where an immune response is weak, or even absent altogether (too little). These topics are examined in Chapter 9.

An inappropriately excessive immune response

Ideally, an infection is countered in physiology by the immunological system with minimal adverse side effects for the body, the most common being a fever and perhaps some aches and pain. However, if the response to an

infective agent is strong, these side effects can also be strong, and may actually cause illness. An example of this is where an acute infection (such as of the lungs) transforms into a chronic inflammation that requires immunosuppressive chemotherapy. An overactive immune response can also cause allergic reactions, hypersensitivity and asthma.

However, there are many inappropriate responses where, instead of attacking a foreign invader, the immune system attacks the body. These conditions are collectively called ‘autoimmune diseases’ perhaps the most well-known being rheumatoid arthritis. All autoimmune diseases are characterized by an abnormal antibody; that is, an autoantibody, generally to the cell or tissue that is presumed to be abnormal and so is being attacked. Thus, an autoantibody to the thyroid causes thyroid disease, and we have already noted the autoimmune attack of certain cells of the stomach that causes pernicious anaemia.

A weak or absent immune response Failure to mount an effective immune response to an infectious agent inevitably results in the success of the pathogen. This immunodeficiency may be due to problems with the cells, with proteins or with both.

A common cause of cellular immunodeficiency is the effect of cytotoxic chemotherapy as in the treatment of many cancers. This happens because these sophisticated poisons damage the bone marrow as well as the tumour, resulting in fewer blood cells, and so a lack of the white blood cells that are needed to attack microbes. HIV preferentially attacks and destroys T lymphocytes, which are required to attack cells infected with viruses and are also needed to help B lymphocytes make antibodies. As mentioned in Section 1.4, DiGeorge syndrome is characterized by lack of a thymus and so lack of T lymphocytes. In the case of HIV, this eventually leads to the complete destruction of the immune system and the acquired immunodeficiency syndrome (AIDS). Other cellular deficiency diseases include chronic granulomatous disease, where neutrophils can ingest but are unable to kill bacteria.

A consequence of HIV/AIDS, and contributor to the heavy burden of infections, is falling levels of antibodies (hypogammaglobulinaemia), but this can also be seen in myeloma and leukaemia. However, a complete lack of antibodies, agammaglobulinaemia, is also known. This lesion is a defective gene on the X chromosome that leads to inactive B lymphocytes. Specific deficiencies in individual antibody classes are known, such as lack of IgA.

Table 1.8 Immunology

<i>Tests performed on serum/plasma</i>	
Total immunoglobulin levels	
Levels of specific antibody classes (IgG, IgM)	
Complement components	
Antibodies to defined pathogens, e.g. bacteria	
Autoantibodies, CRP	
<i>Tests performed on cells</i>	
Neutrophil function	
T and B lymphocytes	
T cell subsets (CD4, CD8)	
<i>Immunological disease</i>	
Overactivity:	
hypersensitivity, allergy autoimmune disease, chronic inflammation	
Underactivity (immunodeficiency):	
hypogammaglobulinaemia, lack of, and/or failure of function of lymphocytes and neutrophils	

Total deficiencies of complement components that lead to major disease are exceedingly rare, but some diseases, such as systemic lupus erythematosus, are often associated with low levels of some complement components. Table 1.8 presents a summary of immunological matters.

1.7 The role of blood science in modern healthcare

It has been estimated that, in hospitals, 75% of the information needed to make a clinical judgment, be it a diagnosis or a decision to initiate, change or stop treatment, comes from the laboratory. Put around the other way, the laboratory provides three times as much information as do all other sources (history, signs, symptoms, imaging, etc.) combined. Whether or not this staggering statistic is different in primary care is not known. Therefore, healthcare professionals provide a huge resource for the practitioner facing the potential or actual patient.

Blood science in human disease

There are many ways to classify human disease. One is to consider three broad areas of pathology: cancer, connective tissue disease (such as rheumatoid arthritis, osteoarthritis and their allied conditions) and cardiovascular disease (principally heart attack and stroke, to include their risk factors of diabetes and hyperlipidaemia).

Together, these constitute 70–80% of the healthcare burden of the developed world. The remaining conditions include, for example, infections, endocrine diseases and psychiatric illness.

One reason for bringing together the major disciplines into blood sciences is the fact that ‘pure’ diseases of haematology, biochemistry or immunology are exceptionally rare. The pathological basis of many diseases is multifactorial, and so demands a more comprehensive understanding of pathology than any one discipline in itself can provide.

Almost all congenital disease (present at birth or noted in the immediate neonatal period) is genetic, and in some cases will need to be formally confirmed with molecular genetics. Several have been mentioned in Section 1.4. Non-genetic congenital diseases will generally have been acquired from the mother, and these are generally metabolic and infectious diseases, such as some types of diabetes, HIV infection and syphilis. Others may be caused by drugs taken during pregnancy, such as thalidomide and warfarin, or perhaps by lack of adequate nutrition, such as the relationship between folic acid and neural tube defects.

Cancer We can consider cancer in two aspects: genetics and environment. In some cancers, these aspects are separate, and in some they are combined. Some are apparent at birth or shortly after, whereas others develop later in life. The disease may manifest as a lump or growth (breast, lymph node) or as unexplained weight loss, pain, an unexplained blood clot in the leg and excessive tiredness.

Investigation of cancer in an adult will inevitably call for many blood tests and genetic analyses, as well as imaging such as X-ray. In many cases these may also indicate cause, although for some the likely cause may be clear, such as tobacco smoking and lung cancer. Many cancer markers can be measured in the blood, such as CA-125 and breast cancer, and carcinoembryonic antigen (CEA) in gastrointestinal cancer (Chapter 19). Many of these will be measured in the biochemistry laboratory. However, numerous cancers are associated with non-specific changes, such as an increased ESR and a normocytic anaemia.

Connective tissue disease These include diseases of bone, joints, muscle, tendon, ligaments, collagen and skin. Epidemiologists tell us that this group has a high prevalence, with osteoarthritis and rheumatoid arthritis at the top of the list. The former is more common, and is

likely to trouble over half of the population at some stage of their life. The biggest risk factor for osteoarthritis is obesity. Rheumatoid arthritis affects approximately 3% of the population, affecting three times as many women as men, and rises with age. Rheumatoid arthritis brings an increased risk of cardiovascular disease and death.

Rheumatoid arthritis certainly has a genetic component, linked with certain HLA molecules, and as a typical autoimmune disease will attract the interest of the immunology laboratory and so the measurement of rheumatoid factor. The chronic inflammation will also be noted by haematologists, with a raised ESR and (often) a low haemoglobin leading to a normocytic anaemia. Rheumatoid arthritis is a systemic disease, and spreads out of joints to attack other organs such as the liver and kidney, in which case the biochemistry laboratory can offer LFTs and U&Es respectively. Osteoarthritis is restricted to joints, so is unlikely to be linked with grossly abnormal blood tests. These issues are discussed in Chapter 9.

Cardiovascular disease This complex and multifactorial condition is dominated by atherosclerosis, which has four major risk factors. Two of these (dyslipidaemia and diabetes) and a consequence of atherosclerosis (such as myocardial infarction) can be tested for in the biochemistry laboratory (Section 1.2). In many cases, and certainly in middle age, atherosclerosis is acquired from lifestyle, but in later life it seems an inevitable consequence of ageing. Indeed, it has been argued that, as the pathophysiology of this disease is so complex, genomics will never be useful in arterial thrombosis. However, despite this, there are several clear examples of the influence of gene mutations on the disease process of atherosclerosis, such as polygenic and familial hypercholesterolaemia.

Table 1.9 shows some examples of diseases where the pathology impacts into each of the blood science disciplines.

Blood scientists: who are they?

These are simply scientists working in blood science, and they can be employed in one or more of the major disciplines we have been looking at. There are several different types of scientists, the most numerous being biomedical scientists, with smaller numbers of clinical scientists and biochemists.

The major professional body for scientists in the pathology laboratory (whether NHS or private) is the IBMS, being the natural home of biomedical scientists,

Table 1.9 Blood science and human disease.

	Haematology	Biochemistry	Immunology
Cancer (e.g. malignant myeloma)	Anaemia ^a and thrombocytopenia	Increased calcium resulting from bone resorption, increased U&Es reflecting renal damage	Acute-phase response, increased cytokines, paraproteinaemia
Connective tissue disease (e.g. SLE)	Anaemia ^a	Increased U&Es, reflecting renal damage	Acute-phase responses, autoantibodies
Cardiovascular disease (e.g. atherosclerosis)	Thrombosis	Increase lipids and glucose	Low-grade inflammation

SLE: systemic lupus erythematosus.

^aBlood transfusion: a treatment for profound anaemia and blood loss regardless of aetiology (rarely called upon in cardiovascular disease, except perhaps ruptured abdominal aortic aneurysm).

although clinical scientists are also members. Many clinical scientists with a background in biochemistry are members of the Association for Clinical Biochemistry. However, the diversity in the biomedical and blood sciences and the requirement to specialize at higher staff grades (such as Agenda for Change level 7 and upwards) often demands membership of additional professional bodies. These include, for example, the British Society for Haematology, the British Blood Transfusion Society and the British Society for Immunology, although a host of other professional and quasi-professional bodies exist, such as the Health Professions Council (HPC), to which practicing biomedical and clinical scientists are required to register.

The IBMS provides a formal structure of examinations and diplomas, from which follow levels of membership (Licentiate, Member, Fellow), position statements on professional practice, and standards of proficiency. It also provides the basis of keeping its members up to date, via a series of education modules summarized as ‘continuing professional development’ (CPD). One of the requirements of both the IBMS and HPC is that members complete sufficient CPD to warrant registration.

Clinical scientists may also be members of the Royal College of Pathologists. The award of Fellow may be obtained by examination or by the submission of a thesis of research publications. Whilst an MSc is mandatory for those at higher grades, scientists of all disciplines may well have a PhD.

However, the individual professional groups are being drawn together into one, as healthcare scientists, with a defined career pathway and specific training.

The role of the higher education institutions

Historically, many universities provided, and continue to provide, formal education in biomedical science, and so in blood science. Many do this by merging their existing modules in the subdisciplines. However, in order for these to be of value to the student practitioner they must be validated and approved by external bodies that include the IBMS. In this way, the particular university benefits from ratification from the profession, and the IBMS ensures that its new recruits have an appropriate background and are well on the way to formal competency, and so state registration.

More recently, the government has licensed certain higher education institutions to provide formal courses on blood science at undergraduate and postgraduate levels. These are linked to formal training courses with placements in hospitals. Foremost among these are those administered by the National School for Healthcare Science.

Training in blood science

Whilst the terms ‘biomedical scientist’ and ‘clinical scientist’ were made protected titles in law during 2003, there is as yet no clear definition of a blood scientist. However, in their document ‘Modernising Scientific Careers’, the UK Government has determined the existence of this speciality and that a formal training system is set up to deliver trained blood scientists to NHS hospitals. This training is in conjunction with selected higher education institutions, and operates at various levels:

- Associates and assistants – this includes National Vocational Qualification and foundation degrees, underpinned by an awards and qualifications framework;
- Practitioner Training Programme (PTP) – undergraduate level;
- Scientist Training Programme (STP) – postgraduate entry, pre-registration training;
- Higher Specialist Scientific Training (HSST) – at doctoral level.

Figure 1.8 shows the scientist training programme.

These initiatives lead to the following grades of employment, generally with increasing responsibility, and increasingly focusing on management. This process therefore provides a uniform career structure that can be accessed at different levels, dependent on the qualifications and experience of the individual. The grades of healthcare scientist are as follows:

- Healthcare science assistant
- Healthcare science associate
- Healthcare science practitioner
- Healthcare scientist

- Senior healthcare scientists
- Consultant healthcare scientist.

This pathway is also present in the other NHS scientist groups, such as audiologists and respiratory physiologists.

1.8 What this book will achieve

The objective of this book is to provide a firm foundation in the new discipline of blood science. It will also provide an informed view of each particular discipline that will be attractive for those experienced in another (i.e. haematology for biochemists, and biochemistry for haematologists). Naturally, in order to produce a volume of manageable size, not all aspects of all disciplines can be addressed. For further details of particular subdisciplines, a reading list is provided.

All these tests and procedures felt to be of major value in blood science will be discussed in more detail in the chapters that follow, and can be grouped together as follows:

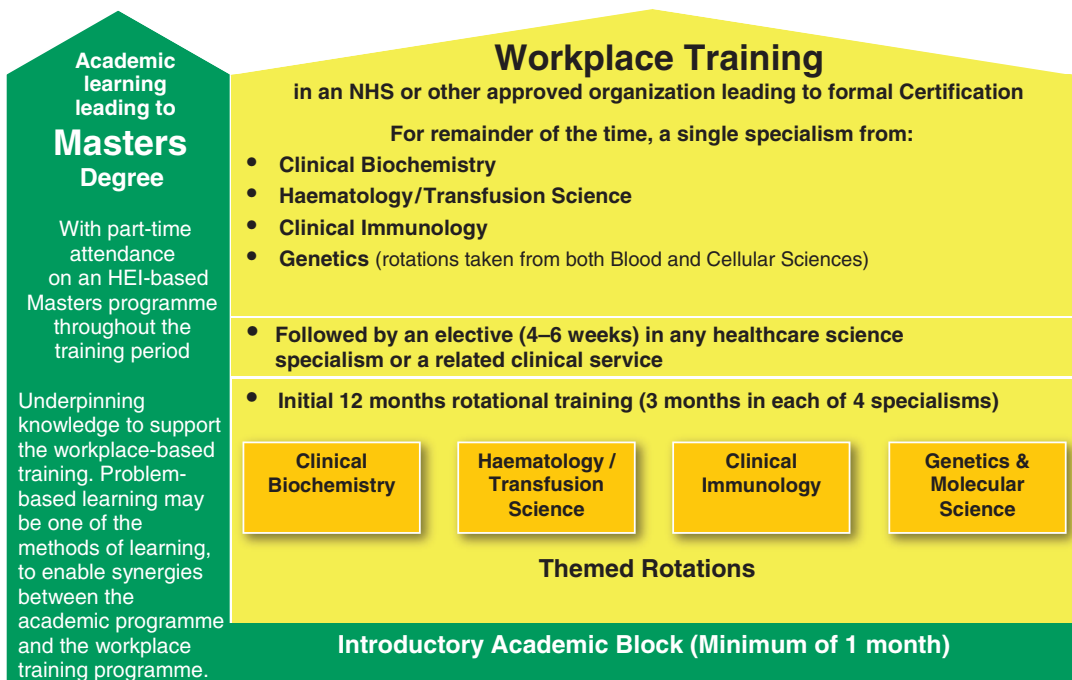


Figure 1.8 Training of blood scientists. (<http://www.nhs.uk/explore-by-career/healthcare-science/modernising-scientific-careers/>)
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- Chapter 2 will examine some basic techniques in blood science
- Chapters 3–8 will consider haematology
- Chapters 9 and 10 will look at immunology
- Chapter 11 will discuss blood transfusion
- Chapters 12–21 will focus on biochemistry
- Chapters 22 will have a number of case reports in blood science.

Naturally, although particular chapters will focus on their particular topic, each will also refer to other aspects of the other disciplines of blood science. This is particularly relevant for molecular genetics, which has a role in each of the other disciplines.

Summary

- Historically, biomedical science has arranged a number of well-established disciplines: biochemistry, haematology and blood transfusion, histopathology and microbiology.
- Recently, immunology has been included as a biomedical science, whilst molecular genetics is the most recent addition to the family.
- The biomedical sciences have been reinvented as blood science, cellular science and infection science.
- Biochemistry, haematology, blood transfusion and immunology make up blood science. However, there is also a place for molecular genetics, as this new discipline has a place in each of the established disciplines.
- Blood science is set to become a major force in health-care-based biomedical science.
- In turn, biomedical science, alongside other diagnostic specialities such as imaging, audiology and cardiology, make up the life sciences.
- The major professional groups in the laboratory are biomedical scientists and clinical scientists, although in time it is likely that these will merge.

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Web sites

Modernizing scientific careers and healthcare scientist training:

- <http://www.nhscareers.nhs.uk/details/Default.aspx?Id=2105>
- http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_113275
- <http://www.nhsemployers.org/PLANNINGYOUR-WORKFORCE/MODERNISING-SCIENTIFIC-CAREERS/MSD/Pages/MSD.aspx>.

The Association for Clinical Biochemistry: <http://www.acb.org.uk/>

The Health and Care Professions Council: www.hpc-uk.org

The Institute of Biomedical Science: www.ibms.org

The National School for Healthcare Science: <http://www.nshcs.org.uk/>

