Routine Blood Results Explained

A Guide for Nurses and Allied Health Professionals

4th Edition

Andrew Blann

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By

Andrew Blann PhD FRCPath FIBMS

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PREFACE

The past two decades have seen a huge expansion in the responsibilities of a host of health care workers, especially in clinical practice. These expanded roles include:

- examining the patient
- proposing a diagnosis
- venesection (taking blood)
- ordering blood tests
- interpreting the results
- managing the condition.

Previously, these roles were undertaken by medical staff only, but it is now clear that appropriately trained professionals can be equipped to carry out these tasks.

This slim volume has been written to provide help in understanding and interpreting the majority of the normal blood results found in most NHS Hospitals and in General Practice. The text, based on the routine blood report forms sent out from Pathology Departments, has evolved from lecture notes given to various Health Care Professionals (Nurses, Phlebotomists, Pharmacists, Radiographers, and Physiotherapists etc.) attending day-long courses on exactly these topics.

An additional objective is to keep the material simple and focused. Thus, the reader seeking a comprehensive in-depth explanation of a wide number of tests and their exact relationship to various clinical diseases will be disappointed. However, it is impossible to fully understand pathology without a sure grounding in physiology. Hence there will be an adequate and clear explanation of those aspects of the body that are necessary to understand a particular test and its associated problems. Examples are provided that will illustrate particular points; it must be stressed that these are not exact and perfect case reports, merely aids in understanding the concepts developed in the text.

Focusing on ‘routine’ blood tests therefore, by definition, excludes tests less frequently reported. In this volume, tests that will be absent from the general discussion are, for example, platelet volume, red cell mass, magnesium, and reproductive hormones. These omissions are not indicative of lack of importance, merely lack of regular requesting. The emphasis is also on the adult, so that paediatric tests (by and large) will not be covered, but reference ranges are provided.

The objective of this book is to support and enable these professionals to be successful in their new roles. In recognising these roles, wherever possible, each chapter will conclude with a brief case study. However, more complete case reports reflecting the different aspects of primary and secondary care are presented in the concluding chapters.

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ABBREVIATIONS

ACPA	Anti-citrullinated protein antibodies
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ANA	Antinuclear antibodies
APTT	Activated partial thromboplastin time
ARF	Acute renal failure
CKD	Chronic kidney disease
CK	Creatine kinase
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CRP	C-reactive protein
DKA	Diabetic ketoacidosis
DVT	Deep vein thrombosis
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra-acetic acid
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
GFR	Glomerular filtration rate
Hct	Haematocrit
Hb	Haemoglobin
HbA_1c	Glycated haemoglobin
HDL-cholesterol	High density lipoprotein cholesterol
INR	International normalised ratio
LDL-cholesterol	Low density lipoprotein cholesterol
LFT	Liver function tests
LMWH	Low molecular weight heparin
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
NICE	National Institute for Health and Care Excellence
OGTT	Oral glucose tolerance test
PTH	Parathyroid hormone
PSA	Prostate specific antigen
PT	Prothrombin time
PTT	Partial thromboplastin time
PE	Pulmonary embolus
RBCC/RCC	Red blood cell count/red cell count
RhF	Rheumatoid factor
TSH	Thyroid stimulating hormone
U&E	Urea and electrolytes
VTE	Venous thromboembolism
WBCC/WCC	White blood cell count/white cell count

INTRODUCTION: ROUTINE BLOOD RESULTS EXPLAINED

> "…it is estimated that the data received by clinicians from Medical Laboratories constitutes 70 – 80% of the information they rely on to make major medical decisions"
>
> The Biomedical Scientist 2005:49; page 38

Blood tests are important as they provide three times as much information as do all other sources (history, examination, symptoms, imaging etc.) combined. Fortunately, the vast majority of routine blood tests, certainly in routine, emergency, and critical care medicine, fall easily into defined groups – haematology (with blood transfusion), immunology, and biochemistry, together often described as Blood Science. The layout of the volume will therefore follow this pattern. Each of the major sections breaks down into individual chapters and concludes with a dedicated example.

Knowledge is nothing without practice. Therefore, the book will conclude with case studies designed to help the practitioner. These cases will look at both primary and secondary care.

What is done where?

In some Pathology Departments, certain tests are done in the Haematology Laboratory, whilst in other Hospitals the same test may be performed in the Biochemistry or Immunology Laboratory, or perhaps a Department of Blood Science. Examples of this include iron studies, immunoglobulins, C-reactive protein (CRP), and testing for vitamin B_{12}. These latter tests are done on serum obtained from whole blood that has not been anticoagulated, but some tests are performed on blood where clotting has

been prevented by anticoagulants. The reader is referred to their own Pathology Service for the correct tube for the test and the destination of these requests.

Overall, our colleagues in the Pathology Department, regardless of discipline, would far rather set the position clear in a phone call than go through the bother of phoning back that a fresh sample in the correct tube must be obtained.

If in doubt – PHONE!

A note on units. In the real world, of course, results are almost always described as the numbers themselves (e.g., a haemoglobin of 125 or a cholesterol of 5) instead of the more correct way its unit (i.e., 125 g/L and 8 mmol/L). This shorthand is generally accepted, and generally makes life considerable easier. It matters not so much that the correct unit of the average size of a red blood cell is described fully, for example, as 112 fL, or in shorthand simply as 112, but it does matter that the particular cell is much larger than can be expected in health, implying come pathological condition.

The reference ranges

In defining ill-health, we generally use good health as a comparator. Thus, a healthy person can be expected to have a certain blood result profile. However, sometimes these values are not well established and are subject to variation. Furthermore, there are many normal (healthy) people whose blood result may not be in the expected range of values – this does not necessarily mean they are ill. Therefore, the concept of being 'normal' may as well be 'desirable'. So, you could use a 'reference' range, or perhaps a 'target' range. However, for the purposes of this volume, the reference range will be cited.

Haematology, biochemistry, and immunology are very quantitative sciences. Consequently, the reference range is important. The precise definition of the reference range in use at a particular hospital is crucial and may not be transferable to another hospital. This may be because of small differences in the technical manner in which tests are derived. Furthermore, reference ranges may well (or actually should) reflect the local population that the hospital serves, and this is important as different catchment populations may well vary considerably, especially in race and ethnicity.

Reference ranges should also be relevant for the demography of the particular patient, i.e., a male reference range for blood from a man, a paediatric reference range for an infant etc. Therefore, care must be taken when comparing samples and reference ranges. In the future it can be predicted that an age-specific and race-specific reference range may also be produced.

Interpretation

In haematology, biochemistry and immunology, the numbers mean something. Many will seek guidance from, and will base treatment on, these results. One of the first questions is therefore 'is the result acceptable?' This in turn begs the question of acceptability, which in many cases boils down to the reference range, presumably derived from normal, i.e., healthy, individuals. However, merely having a result a fraction outside the reference range does not necessarily imply a serious pathology. Conversely, a result very far from the reference range carries with it an implication of a problem. Several tests, as well as clinical signs, history, etc. are needed to be sure of a particular diagnosis.

Near Patient Testing

Not all routine blood tests are performed in the laboratory. With advances in technology, small analysers have been developed suitable for use literally near the patient (hence NPT). This is also known as point-of-care testing (POTC). Found in locations such as Accident and Emergency, Coronary Care Units and Intensive Care Units, these analysers offer rapid results on almost all routine tests. However, the laboratory is the gold standard, and also offers advice on interpretation.

Key point: The purpose of the laboratory is to provide assistance in the diagnosis and management of disease

PART 1:

HAEMATOLOGY AND BLOOD TRANSFUSION

Objectives and Scope

These are listed in Table 1. The purpose of the Haematology Laboratory is to provide information on blood cells and the ability of the blood to form a clot. The Blood Transfusion Laboratory provides blood components (mostly red cells, but also coagulation factors and the blood protein albumin) for patients at risk of potentially life-threatening situations.

In order to do this, the components of the blood are analysed, almost always in custom-designed equipment. It is taken as understood that all blood tubes and forms must be fully labelled by those taking the blood in order to minimise the risk of (possible fatal) error. Indeed, the laboratory will be well within its rights to decline to test a sample that is incorrectly or inadequately labelled.

There are three basic blood tubes that are used in these disciplines. A full blood count (FBC) is performed on blood that is anticoagulated with ethylene diamine tetra-acetic acid (EDTA). Coagulation tests are invariably done on plasma that is obtained from whole blood anticoagulated with sodium citrate. The erythrocyte sedimentation rate (ESR) may be assessed on blood that is held within its own dedicated glass tube: blood clotting in this tube is also prevented by sodium citrate. However, in some cases, the ESR can be measured on the same sample as is the FBC. For blood transfusion, an EDTA or a tube free of an anticoagulant (providing serum) are often used. Immunologists can work with serum or plasma, but for cell work, the blood must be anticoagulated. Once more, if even in the slightest doubt about which vacutainer to take for whichever test – PHONE!

This is because testing can only be performed on blood that is collected in the correct tube. Failure to do so will, at best, result in a polite phone call from the lab explaining the problem and its remedy. At worse, a report will be returned a day or so later with a comment such as ‘inappropriate

blood sample received, please repeat'. Fortunately, many blood tubes have different coloured tops to help this process and minimise errors.

Fortunately, Haematology can be divided very easily into three different areas. These are the red blood cell (often just described as red cell), the white blood cell (or white cell), coagulation. The most important aspects of each of these are, in turn, anaemia, infection and neoplasia, and thrombosis and haemorrhage. Blood transfusion is easily addressed in its own Chapter, where the avoidance of a transfusion reaction is crucial.

Table 1: Learning Objectives – Haematology and Blood Transfusion

Having completed these notes in a satisfactory manner, the reader will….

1. Appreciate the importance of different anticoagulants and tubes for the different blood tests requested:
    - EDTA (ethylene diamine tetra-acetic acid) for a full blood count
    - Sodium citrate for coagulation
    - The ESR may require its own tube, or the same as the full blood count
    - The blood bank generally needs a sample of clotted blood, taken into a tube with no anticoagulant, or blood taken into EDTA
2. Recognise major areas of interest, i.e.
    - The red blood cell
    - The white blood cell
    - Coagulation
    - Blood transfusion

3. Describe major problems associated with each of these areas, e.g., respectively

    - Anaemia
    - Leukaemia, lymphoma, and myeloma
    - Thrombosis/haemorrhage
    - An incompatible blood transfusion

4. Interpret simple haematological results, e.g.

    - A haemoglobin of 84 g/L
    - A white cell count of 15 x 10^9/L
    - A prothrombin time of 25 seconds

CHAPTER 1

THE RED BLOOD CELL

Key words:

Haemoglobin (Hb)
Red blood cell count (RBCC)
Haematocrit (Hct)

Mean cell volume (MCV)
Mean cell haemoglobin (MCH)
Mean cell haemoglobin concentration (MCHC)

Red cell distribution width (RDW)
Erythrocyte sedimentation rate (ESR)

Key pathological expressions:

Anaemia	Polycythaemia
Thalassaemia	Sickle cell disease

1.1: An explanation of terms

Haemoglobin (Hb): reference range 133-167 in men, 118-148 in women

Haemoglobin is undoubtedly the index most frequently referred to in clinical haematology. It is a protein designed to carry oxygen from the lungs to the tissues, where the oxygen is given up to participate in respiration, the process by which energy is obtained.

The reference range varies between the sexes. Lower levels in menstruating women seem obvious, but in post-menopausal women levels are still lower than age-matched men as the latter produce testosterone to stimulate red

cell production. We know this because men who have lost their testes to accident or disease see their haemoglobin levels fall.

The red blood cell count (RBCC): reference range 4.3-5.7 in men, 3.9-5.0 in women

Haemoglobin is carried in the blood in red cells. These are unusual as they lack a nucleus, thus providing additional flexibility to penetrate the smallest capillaries and so deliver oxygen to distant cells and tissues. They are the most abundant cell in the blood, are often called erythrocytes, and numbers can also vary between the sexes for the same reason as does haemoglobin.

Haematocrit (Hct): reference range 0.35-0.53 in men, 0.33-0.47 in women

This index expresses that proportion, as a decimal (say 0.43) of whole blood that is taken up by all the blood cells. Since there are approximately a thousand more red cells per unit volume than white cells, and twenty times as many (tiny) platelets, the red cells make up the major proportion of the haematocrit. Consequently, at the practical level, it provides an idea of the proportion of red cells that makes up the whole blood pool. The haematocrit also varies with sex.

Mean cell volume (MCV): reference range 77-98

The size of the average red blood cell.

Mean cell haemoglobin (MCH): reference range 26-33

The average amount (mass) of haemoglobin in the cell. It does not take into account the size of the cell.

Mean cell haemoglobin concentration (MCHC): reference range 330-370

The average concentration of haemoglobin inside the average size cell, therefore merging the MCV and MCH.

These three are often described as the red cell indices. Because several of the red cell values are mathematically derived from some of the others, it is entirely possible for any one of them to be outside the reference range whilst the other five are apparently normal. Thus, one must consider all six (and possibly some others) together to obtain a full picture. In most labs, the haematology analyser provides the Hb, RBCC and MCV directly from the blood provided to it, then (simply speaking) calculates the Hct from the RBCC times the MCV, the MCH by the Hb divided by the RBCC, and the MCHC by the Hb divided by the Hct, with adjustments (e.g., multiply or divide by 10 or 100).

Red cell distribution width (RDW): reference range 10-15%

One of the problems with the MCV is that it can mask a number of abnormalities. Being the mean of millions of cells, some are smaller than the mean size, some larger than the mean size. In health, is variation is quite small, so that an MCV of 90 fL may represent a true range in the size of cells from 85 to 95 fL. This variation is quantified as the red cell distribution width (the RDW). An increased RDW, perhaps 20%, shows a wider range of the size of the red cells, with more smaller and large cells than normal, that in turn indicates a pathological state such anaemia.

Erythrocyte sedimentation rate (ESR): reference range <10 mm/hour

The ESR is also a global score of physical aspects of the blood and is simple to understand. The result is obtained by allowing a thin column of blood to settle down under the influence of gravity. As it does so, the red blood cells will separate from the plasma, so that after an hour, a band of clear plasma will sit atop the red cells. The fall in the level of the red cells is then recorded as mm/hour. The effects of platelets and white cells are minimal and are ignored. It is therefore unique in requiring no sophisticated machinery and few technical skills.

An increased ESR can be caused by many factors, including cancer, infections, anaemia, inflammation, renal failure, rheumatoid arthritis, multiple myeloma, and tuberculosis. It is increased soon after myocardial infarction and is also heavily influenced by plasma proteins. Some laboratories have a higher reference range, e.g., <20 mm/hour, especially in the elderly.

Other haematology tests

These include plasma and blood viscosity, iron, ferritin, transferrin, and vitamin B_{12}, although some of these may be performed by biochemists. Reticulocytes (immature red cells) may be increased in anaemia, reflecting the bone marrow's increased red cell production, but not when the bone marrow is seriously suppressed.

1.2: What are red cells for?

The answer to this question is easy: to carry oxygen. This gas is required in the process of respiration, where energy is obtained from molecules such as glucose and certain fatty acids. So, muscles require oxygen for the energy needed to contract, and if this oxygen is insufficient, there may be pain. In the case of muscles of the intestines this may be manifest as abdominal discomfort and cramps: if muscle of the rib cage and diaphragm fails to get enough oxygen this may lead to problems with breathing and shortness of breath. Insufficient oxygen to the brain may lead to forgetfulness, personality changes and what may seem to be early Alzheimer's disease. General symptoms include tiredness and lethargy.

These symptoms of insufficient oxygen (hypoxia) may be due any combination of pathologies. Oxygen in the inhaled air must first cross the alveoli and enter the pulmonary circulation, a process that will be impaired by lung disease such as COPD, emphysema, and pneumonia. But even well oxygenated blood may not be delivered to the body is the heart is functioning poorly, perhaps because of valve disease, cardiomyopathy, heart failure (with an inadequate ejection fraction), left ventricular aneurysm, and the consequences of myocardial infarction. Blood passage around the body may be impaired by atherosclerosis, and in the tissues the movement of oxygen to the cells may be impaired by oedema and cellulitis.

However, if lung function is good, the heart is working well and there is no barrier to blood or oxygen moving into the cells, and the patient still complains of tiredness, lethargy, and shortness of breath etc., then only one major pathology remains.

1.3: Anaemia – an introduction

The oxygen-carrying capacity of the blood is a function not only of the amount of haemoglobin in each cell, but also the number of red cells and,

to a lesser extent, the Hct. In this way someone with a low MCH but a high RBCC may well have the same oxygen-carrying capacity as someone else with a higher MCH but a lower RBCC. Red cells can also contribute to clotting.

Haemoglobin, RBCC, Hct and the three red cell indices MCV, MCH and MCHC are requested to obtain a view of the individual's oxygen carrying capacity. When an individual is having difficulty performing their most basic physiological and lifestyle demands, they could be anaemic. Some authorities define anaemia as a level of haemoglobin below a certain level. However, a haemoglobin value of, say, 115 g/L may well be perfectly adequate for an elderly person with few physiological requirements and a relatively quiet life. Conversely, the same haemoglobin level in a younger person with a very active lifestyle, perhaps including sports, will be inadequate. Thus, the medical state of the individual as a whole person should be considered, not merely an arbitrary number at which one acts. An alternative view of anaemia may be the level at which concern arises, and at which further investigations are considered. Certainly, anaemia should not be seen merely as that level that automatically requires a blood transfusion, a therapy that many consider should be reserved only for life-saving situations (see Chapter 4).

This brings us to an important equation:

Abnormal results x symptoms = disease

What this means if someone has a low haemoglobin (perhaps 115 as above) but is asymptomatic, then they don't have a disease (in this case, anaemia). Conversely, if the patient is tired, lethargic, pale, and short of breath (Table 2) with a haemoglobin of 140, they can't possibly be anaemic. Therefore, their symptoms must be due to some other pathology, maybe several pathologies. However, if the patient does indeed exhibit all those symptoms, and has a low haemoglobin, then they are anaemic, and so warrant further investigation and possibly restorative treatment.

More serious signs of anaemia include jaundice, splenomegaly (a large spleen), hepatomegaly (a large liver), angina, heart failure, and fever, although these may of course arise from other conditions. But first we need more information about the type of anaemia, and how it came about.

Table 2: Signs and symptoms of anaemia

Signs

Pallor (especially of the conjunctiva)
Tachycardia (pulse rate over 100 beats per minute)
Glossitis (swollen and painful tongue)
Koilonychia (spoon nails
Dark urine (a sign of red cell destruction)

Symptoms

Decreased work and/or exercise capacity
Fatigue, lethargy, 'Tired all the time'
Weakness, Dizziness, Palpitations
Shortness of breath (especially on exertion)
Rarely: headaches, tinnitus, taste disturbance

1.4: The aetiology and classification of anaemia

Anaemia may be classified in many ways.

Since red cells are produced in the bone marrow, infiltration by cancer or other cells will inevitably lead to a reduction in the production of the red cells, thus anaemia. Anaemia is also a fundamental aspect of aplastic anaemia, where the other functions of the bone marrow (i.e., the production of platelets and normal white blood cells) are also depressed.

As we shall see, this is also the case in leukaemia, where many abnormal white blood cells make up the tumour. Our present view of medicine offers various drugs in an attempt to solve many problems. However, few, if any, drugs are free of undesirable side effects, and one can be bone marrow suppression. Thus, use of many drugs will call for frequent monitoring to check for the development of, for example, low levels of red blood cells. Azathioprine, for example, is linked to an increase in the size of the red cell.

Poor nutrition will also result in anaemia, and a diet lacking essential factors such as iron, vitamin B_{12} or folate are good examples. However, the diet itself may be adequate, but other factors may cause anaemia, such

as failure to produce special proteins to aid the passage of the minerals and vitamins across the gut wall and into the blood (i.e., malabsorption).

Problems with other organs may also contribute to anaemia. Chronic liver disease may be a factor in anaemia as it produces molecules that store and others that transport essential iron and vitamins around the body (such as the specialised protein transferrin for carrying iron). Intestinal disease or malabsorption may also lead to anaemia, as the ability to absorb essential minerals and vitamins will be impaired. The kidney produces a hormone, erythropoietin, to stimulate the bone marrow to produce red blood cells. Thus, chronic renal failure (chapter 10) may contribute to an anaemia.

Haemolytic anaemia is the bursting, destruction, or inappropriate break-up of red blood cells. Possible mechanisms for this include physical destruction by, for example, a poorly functioning mechanical heart valve, prolonged heavy exercise, or long marches undertaken by the armed forces. Certain individuals are sensitive to drugs (such as antibiotics) that stick onto the surface of red blood cells and render them more susceptible to attack and degradation. High fever may also destroy fragile red cells, as will infections such as malaria.

A sub-type of haemolytic anaemia occurs when antibodies are produced which (erroneously) bind to red blood cells. This is therefore called autoimmune haemolytic anaemia (chapter 6). These autoantibodies will make the cell a target of the immune system and will lead to their elimination, often in the spleen. Indeed, a treatment for certain types of haemolytic anaemia includes splenectomy.

Red cells may be lost by an acute or chronic bleed. The former may include bleeding after surgery, heavy menstrual bleeding, or bleeding by a ruptured blood vessel that may leak into the intestines. If this process is occult, or prolonged, it may lead to a chronic state of blood loss. In these types of anaemia, there is nothing intrinsically wrong with the red cells themselves.

The most common congenital haemoglobinopathies (=disease of haemoglobin) are sickle cell disease and thalassaemia, genetic conditions characterised by qualitative changes in the haemoglobin molecule that severely reduce its ability to transport oxygen. These cells also have a shorter lifespan than cells carrying normal haemoglobin, and both conditions are associated with a variety of clinical conditions such as jaundice and skin ulcers.

There are, of course, many more possible types of anaemia. The above, summarised in Table 3, simply lists major causes.

Table 3: A Simple Classification of Anaemia

1. Depressed red blood cell production from the bone marrow
    - Due to infiltrating cancer (e.g., leukaemia, or secondary's from other primary cancers elsewhere, such as the breast or prostate)
    - Due to total marrow shut down (e.g., aplastic anaemia, or due to drugs, such as cancer chemotherapy)
2. Diet deficiency
    - Iron
    - Vitamins B_{12} and folate
    - Plasma proteins (for building essential carriage and storage molecules)
3. Loss of mature red cells
    - Drugs
    - Fevers, infections
    - Auto-immunity
    - Acute or chronic bleeding
4. Haemoglobinopathy
    - Sickle cell disease
    - Thalassaemia
5. Disease in other organs (such as the liver)

A Cautionary Tale – 1

In the past there was great debate as to the definition of anaemia, such as the haemoglobin count being less than a pre-defined level of 80, 90, 100 g/L or even (marginally) below the reference range. Consider this case report.

The case is a woman in her 30s with homozygous sickle cell disease. She was a fully-trained pharmacist, had worked full-time, had one uneventful and successful pregnancy, and had never received a blood transfusion. When was she last seen in her Clinic in Jamaica her haemoglobin was 39 g/L. Was she anaemic?

In 1997 she emigrated to the USA, and two years later was seen routinely by her family doctor who found a haemoglobin result of 38 g/L. Again – was she anaemic? Her family doctor and colleagues apparently believed so, and she was transfused with six units of blood within 24 hours. This increased her haematocrit from 0.11 to 0.31, but also increased her systolic and diastolic blood pressures by 30 mm Hg each. Nine hours after the last transfusion she reported a headache, subsequently developed cerebral haemorrhage, and later died.

I will not discuss this case further as, I believe, it is self-explanatory. However, the report concludes with '... The award of US $11.5 million recommended by the jury in this case could have been avoided'. Other comments on blood transfusion follow in Chapter 4.

Source: Serjeant, G. Blood transfusion in sickle cell disease: a cautionary tale. Lancet 2003; 361:1659–60.

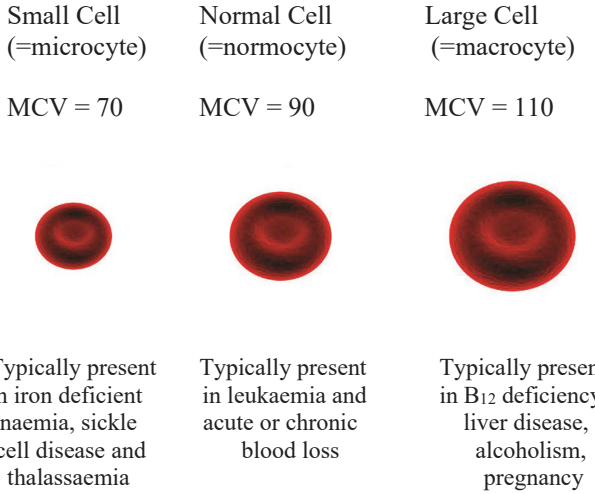
1.5: Size matters!

The red cell index MCV can also be used to further classify anaemia. For example, in the anaemia that follows problems with vitamin B_{12}, the red blood cells are larger than normal (e.g., greater than 98 fL), and are said to be macrocytes, and if the Hb is low and the patient is symptomatic, they

have macrocytic anaemia. Conversely, some haemoglobinopathies and iron deficient states often lead to microcytic anaemia because the red cells are small (e.g., less than 80 fL) and are called microcytes. Finally, a normocytic anaemia may be associated with a normal sized red cell (a normocyte, between 80 and 98 fL) but a lower overall Hb level (figure 1).

A prime reason for a normocytic anaemia will be the sudden loss of a large number of (healthy) red cells, perhaps by an accident, through a perforated duodenal ulcer, or bleeding gastrointestinal cancer. Here, there is nothing intrinsically wrong with the red cells themselves, the anaemia follows from another problem. Treatment therefore follows aetiology: more iron in the diet will not help an anaemia based on malabsorption, but intravenous iron may increase the haemoglobin level.

Figure 1: Red cell size



This illustrates how the three red cell indices can vary. Taking the Hb to be 130 g/L and the red cell count 4.5 x 10^{12} cells/mL in each of these cases with different MCVs, then the MCHC is 412 pg/L in the microcyte, 320 pg/L in the normocyte and 262 pg/L in the macrocyte. This may well be important in certain conditions.

Key point: The most common blood disease is anaemia; its diagnosis and management require regular full blood counts.

1.6: Increased levels of red cell indices

Ideally, the healthy body tightly regulates the numbers and quality of the various aspects of red cells. However, in rare instances, high levels are reported.

Polycythaemia is an excess overall red cell mass and is generally associated with a high haemoglobin, RBCC and haematocrit. This condition may arise from a rare kind of malignancy or over-activity of the bone marrow and is more correctly called polycythaemia rubra vera.

A second cause of increased red cell indices is the response of the bone marrow to reduced levels of oxygen, and is called erythrocytosis. This is understandable in those people living at a very high altitude (up mountains) where the air is very thin and of low oxygen content, but in the UK, this cannot be the case.

The most common form of erythrocytosis is low level of oxygen (hypoxia), and (until recently) the dominant form of this is an unhealthy lifestyle with heavy smoking. A contributing factor is that tobacco smoke contains carbon monoxide, which binds irreversibly to haemoglobin and prevents it carrying oxygen, leading to poor oxygen carrying capacity and thus a pseudo-anaemia. It is also possibly that the low grade pulmonary damage caused by tobacco smoke causes poor oxygen movement across the alveoli. The bone marrow responds by producing high numbers of excess red blood cells in an attempt to improve (restore) oxygen-carrying capacity. Consequently, this places an extra strain on the heart and circulation. Fortunately, the reduction in the rate of smoking means that we are seeing less and less of this problem.

Possibly for the same reason (response to hypoxia), an increased red cell count can be seen in chronic anaemia such as thalassaemia, as the bone marrow attempts to improve the ability of the blood to carry oxygen.

Recommended websites

www.pernicious-anaemia-society.org/
www.nhs.uk/conditions/Anaemia-iron-deficiency-/
www.hbregistry.org.uk/information/haemoglobinopathies.html

Summary of red blood cells

- Haemoglobin (Hb) is the key index used to investigate the major disease of red cells: anaemia.
- To aid precise diagnosis, the causes of the anaemia, and directions for treatment, RBCC, Hct and MCV are frequently referred to.
- Often less useful, but occasionally very enlightening, are MCH and MCHC.
- ESR is a non-specific marker: an abnormal result could reflect a variety of different pathologies.
- The MCV tells us whether the anaemia is microcytic, normocytic, or macrocytic, and so is likely to point to the aetiology and guide therapy.

Case Report 1

A 20-year old female and her family recently moved to this country from the Far East. Following a few weeks' acclimatisation and recovery from jet lag, it became clear to her family that she was consistently tired and lethargic, more so than her siblings, but had no symptoms of infection (e.g., a fever). Blood results were as follows.

	Result (unit)	**Reference range**
Haemoglobin	108 g/L	118 - 148
RCC	5.2 x 10^{12}/L	3.9 – 5.0
MCH	20.8 pg	27 – 33
MCV	68 fL	77 - 98
MCHC	330 pg/L	316 - 349
Hct	0.33	0.33 – 0.47
ESR	4 mm/hour	<10
RDW	10-15%	16%

Interpretation

The abnormal results are reduced haemoglobin, MCV, MCH, Hct and RDW, with raised a red cell count. The ESR is within the reference range. With a haemoglobin level below the bottom of the reference range, and the symptoms, we would have little difficulty in describing her result as concerning and would therefore probably label her as anaemic. Very heavy menstrual periods may possibly produce this picture, but as the red blood cells are not simply small (i.e., MCV <77), but are very small (<70), we would have no hesitation in describing a microcytic anaemia, not an anaemia due to simple blood loss by itself. High numbers of red cells may well be a response to the oxygen-carrying problem.

The most common reasons for microcytic anaemia are iron deficiency, sickle cell disease and thalassaemia. Iron status can be easily tested for, and a test for sickle cell disease is also very simple to perform. However, both types of haemoglobinopathies are ultimately diagnosed by another test (chromatography, HPLC). Thus, with a normal iron profile and negative sickle test, thalassaemia would seem to be an appropriate diagnosis.

CHAPTER 2

THE WHITE BLOOD CELL

Key words:

White blood cell count (WBCC)
The differential:
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Blasts/atypical cells.

Key pathological expressions:

Leukocytosis and leukopenia
Leukaemia
Lymphoma and myeloma
Phagocytosis

2.1: An explanation of terms

White blood cell count: reference range 4-10

White blood cells, or leukocytes, defend us from attack by micro-organisms such as viruses, bacteria, and parasites, when raised levels of these cells can be expected. However, increased numbers may also be present in a number of conditions such as rheumatoid arthritis and cancer, and also after surgery and, as we shall see in detail, in leukaemia. Low levels are likely to have several potential causes.

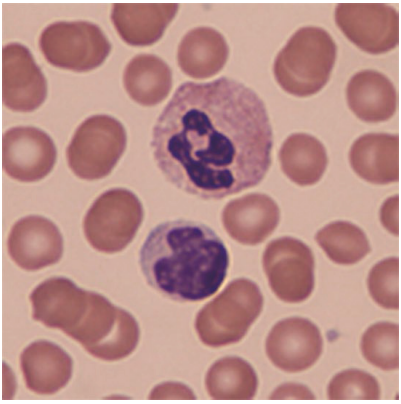
The differential

Haematologists recognise five different types of white blood cells that can be found in the (normal) blood. The differential (or 'diff') reports the numbers of these cells, which are neutrophils, lymphocytes, monocytes, eosinophils, and basophils. A sixth type, blasts, or atypical cells may be

present in certain diseases. The differential is the numbers of the different types of leukocytes, which allows an immediate comparison, often giving clues to pathology, such as leukaemia or inflammation (table 4). The former is described in detail below, the latter in Chapters 5 and 6.

A blood film can be made by smearing a drop of blood on a glass slide, allowing it to dry, and staining it with different dyes. Figure 2 is an example of what you would see down a light microscopy. The two larger bodies in the middle are white cells, characterised by a purple nucleus, surrounded by dozens of smaller red cells.

Figure 2: A blood film



A blood film showing two white cells and numerous red cells

Neutrophils: reference range 2.0 – 7.0

These are the most common white cells, and are known as polymorphonuclear leukocytes (i.e., many different [irregular] shapes, usually three to five), granulocytes (i.e. with granules in the cytoplasm), or simply as polymorphs. The upper cell in figure 2 is a neutrophil. However, basophils and eosinophils also have an irregular nucleus and have granules, so that others may consider these cells also to be polymorphs and granulocytes.

Table 4: White cell differentials

Cell population	Reference range	Result in health	Leukaemia	Acute Inflammation
Total white cell count	4.0 – 10.0	5.9	34.9	14.3
Neutrophils	2.0 – 7.0	3.8	9.3	8.9
Lymphocytes	1.0 – 3.0	1.2	11.5	3.1
Monocytes	0.2 – 1.0	0.5	2.4	1.5
Eosinophils	0.02 – 0.5	0.3	0.8	0.4
Basophils	0.02 – 0.1	0.1	0.4	0.1
Blasts	<0.01	0	10.5	0.3

Units are 10^9 cells/L. Note preponderance of lymphocytes in the leukaemia, and of neutrophils in the acute inflammation

Lymphocytes: reference range 1.0-3.0

The second most common group of white cells, lymphocytes differ principally from neutrophils by the structure of their nucleus, which is more rounded and regular in contrast to the neutrophil's numerous irregular shapes. These cells are also normally smaller than neutrophils, and don't have granules. The lower cell in figure 2 is a lymphocyte. As discussed in Chapter 5, lymphocytes may be classified as B cells or T cells.

Monocytes: reference range 0.2-1.0

These cells often resemble lymphocytes as they also have a round, regular nucleus. However, the nucleus of most monocytes does not take up as much of the cell, maybe up to 80%, whereas the lymphocyte nucleus frequently occupies over 95% of the cell. Monocytes are also larger than lymphocytes. It is often suggested that many monocytes are only temporarily in the blood on their way to the tissues where they become macrophages.

Eosinophils: reference range 0.02-0.5

These cells are so-called because of the reddish colour, due to the chemical makeup of their granules. The nucleus is of composed of just two parts, linked together by a small thread.

Basophils: reference range 0.02-0.1

The least frequent of the normal leukocytes, basophils contain numerous granules that take up different dyes, and so appear as black, violet, or dark blue. They resemble eosinophils in size and nuclear morphology.

Blasts/atypical cells: reference range <0.1

Occasionally the odd cell appears in on a blood film that defies the simple classification discussed above. Such cells, often with unusual characteristics, may be described as blasts or atypical cells. In certain diseases, as will be outlined below, increased numbers of carry pathological implications, some of which are profound. An example of this is in acute leukaemia, where the abnormal blast cells have a strange morphology, and can be of the lymphoid or neutrophil family.

The functions of white blood cells and their role in immunological disease are described in chapters 5 and 6

2.2: Low numbers of cells: leukopenia

This state is often described when the white cell count falls to less than, for example, 3.7 x 10^9/L. However, recall that the definition of the reference range may well include some individuals with leukopenia who are entirely healthy. Nevertheless, persistently low levels are very rare in the absence of a clear explanation.

Almost all cases of leukopenia are associated with the use of certain cytotoxic and immunosuppressive drugs such as are used in the treatment of cancers and to aid the success of a transplant. These drugs, many of which are effectively sophisticated poison, can cause suppression of white cell production in the bone marrow. However, low numbers of neutrophils (i.e., neutropenia) can also be associated with certain drugs such as carbimazole, used to treat hyperthyroidism. If so, cause is therefore reasonably apparent and is reversible. It follows that prophylactic antibiotic therapy is often needed in such patients with neutropenia. However, other drugs can cause the selective loss of lymphocytes (i.e., lymphopenia), and as such these subjects may be at risk of viral infections.

As discussed in Chapter 1, low levels of white cells are also to be found in aplastic anaemia, a condition where the entire bone marrow shuts down, and so is also associated with anaemia and low numbers of platelets.

2.3: High numbers of cells: leukocytosis

As mentioned, high levels of neutrophils and lymphocytes can be found as normal responses to infections and after surgery. The reason for the former is obvious, but the latter is an example of an acute phase response – the body believes it has been attacked, so mobilises white cells in case it needs to defend itself from a bacterial infection. Pathological states also associated with leukocytosis include inflammatory and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE). These will be discussed in Chapters 5 and 6. However, the highest and most serious cases of leukocytosis occur in leukaemia.

2.4 Leukaemia

The high white cell count in leukaemia is not due to an abnormal or persistent response to infections, but to changes in the ways in which white cells develop in the bone marrow. This is due to genetic mutation in the DNA of the cell, so that leukaemic cells stop their development at a certain crucial but early stage and enter the blood not only in an immature state, but also in increased numbers. If this process develops slowly, perhaps over years, then it is said to be chronic, with survival measurable in years. These leukaemic cells are often of a more mature phenotype.

Conversely, if the increase in the white cell count is rapid, maybe over a few weeks, then the leukaemia is said to be acute. Acute leukaemias, frequently characterised by enormous numbers of immature cells (i.e., blasts), are also often much more aggressive than the chronic counterpart, where the increases are more modest, and survival (unless treated appropriately) can be as short as months.

If the major cell in the leukaemia is of the neutrophils lineage, the leukaemia is described as myeloid. When the predominant cell is a lymphocyte, then lymphocytic leukaemia is present. However, if the leukaemia is dominated by blast cells, then it may be described as lymphoblastic. Thus, for the most common we have a combination of these in abbreviations:

- AML = acute myeloid leukaemia
- CML = chronic myeloid leukaemia
- ALL = acute lymphoblastic leukaemia
- CLL = chronic lymphocytic leukaemia

Monocytic leukaemias are rare, whilst eosinophil and basophil leukaemias are very rare indeed. The high lymphocyte count in table 4 points to a lymphocytic leukaemia, with could be CLL or ALL, although the high blast count suggests an ALL.

Because the leukaemia arises in the bone marrow and is characterised by large numbers of these abnormal cells, then the production of other cells within this tissue is also influenced. Thus, anaemia and low levels of platelets (thrombocytopenia) are invariably a consequence of leukaemia, as the tumour grows and thus squeezes out other normal tissues. However, in advanced disease, the leukaemia may escape from the bone marrow and often invade lymphoid tissues of the lymph nodes, liver, and spleen, making them swollen, and described as lymphadenopathy, hepatomegaly, and splenomegaly respectively.

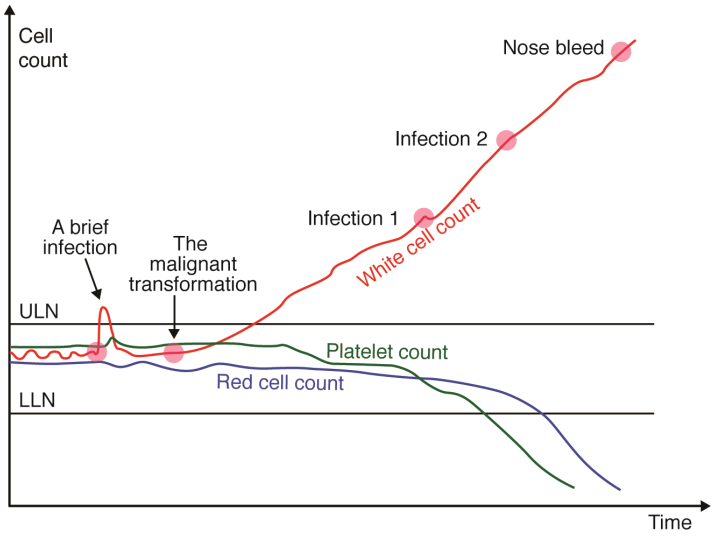
Treatments are aimed at reducing the tumour burden and are generally regimes of cytotoxic drugs. More severe leukaemias demand transplantation of fresh, 'clean' bone marrow stem cells that can be obtained from a donor (i.e., allogeneic, best from an HLA-matched donor) or from the patient themselves (i.e. an autologous transplant).

The natural history of leukaemia

Figure 3 is a representation of changes in blood cells as leukaemia progresses. The initiating event is of a genetic mutation that causes the malignant transformation of a normal cell into a cancer cell. The major laboratory characteristic is the slow and steady rise in the white cell count (red line), and a similar fall in the red cell and platelet counts (blue and green lines). The solid horizontal lines are the upper and lower limits of the normal range. On the left, a brief infection (pink circle) leads to a raised white cell count that soon returns to normal. Sometime after, the malignant transformation of a normal stem cell into a leukaemic stem cell occurs (pink circle), and the white cell count slowly rises, and exceeds the upper limit of normal.

We all experience minor fluctuations in blood results from day to day and week to week – hence the slight variation in the white cell line. Indeed, levels can rise with 'normal' responses to infection, up to levels of the 'teens, say 14 or 15. However as the white cell count continues to rise to about 20 or 25 without an obvious infection (with fevers and sweating), then a malignancy becomes increasingly likely.

Figure 3: The progression of leukaemia



ULN: upper limit of normal. LLN: lower limit of normal. See text for details

As the white cell count rises, the patient will be suffering infections because these cells are immunologically incompetent (pink circles). In parallel, later in the disease, there will be falls in the red cell count and platelets, possibly leading to nosebleeds, leading eventually to the symptoms of anaemia and bruising/bleeding respectively. Eventually, these symptoms will drive the patient to seek our help, and a blood test will make the diagnosis.

This brings us to Table 5, which summarises the key points of leukaemia, from both the perspective of the patient and the laboratory. It is not necessary to see all six conditions fulfilled, but if they are, the diagnosis is unquestionable.

Table 5: Diagnosis of leukaemia

	Patient factor	Laboratory factor
Red cells	Tiredness, lethargy etc. (i.e., the symptoms of anaemia)	Low haemoglobin, low red cell count
White cells	Infections	High white cell count
Platelets	Bruising, bleeding	Low platelet count (thrombocytopenia)

Differential diagnoses of leukaemia

The single most defining criterion of leukaemia is a raised white cell count, but this may be present in severe infections. The most dangerous and life-threatening infection is septicaemia (blood poisoning), where the blood itself is infected with bacteria, which will be confirmed by blood culture. If present, the patient with septicaemia is likely to be in an intensive care unit, with a high temperature and tachycardia, and is probably on industrial doses of intravenous antibiotics.

The white cell count of such a patient is likely to be in the region of 20, perhaps 25 x 10^9 cells/mL, and over 90% of these are likely to be neutrophils. If follows that a patient, maybe feeling poorly and a persistent cough and with a white cell count greater than 25 x 10^9 cells/mL is most unlikely to have septicaemia (especially if most are lymphocytes), and is likely instead to be in the early stages of leukaemia. However, this rule is far from perfect, and the occasional patient presents with a very high white cell in the absence of either of these conditions. An example of this is given in case study 2.

The precise gene mutation causing the leukaemia can be determined in the laboratory and helps in guiding treatment and in estimating survival. Perhaps the most common is called ‘BCR-ABL, and causes the ‘Philadelphia chromosome’ of CML.

2.5 Other lymphoid neoplasia

Whilst leukaemia arises in the bone marrow, other types of lymphoid malignancy can be present in other lymphoid tissues around the body, such as in the liver, spleen, and lymph nodes.

Lymphoma

This disease is most often a cancer of those lymphocytes that make antibodies (B lymphocytes, explained in Chapter 5). Essentially, malignant but inactive lymphocytes take over the normal structure and function of the lymph node. As these small organs are the major sites of antibody production, they are no longer able to help fight infection. A principle example of this type of cancer is a Hodgkin or non-Hodgkin lymphoma, another is a Burkitt's lymphoma. Causes of lymphoma include gene mutations, sometimes driven by a virus.

Lymphomas are often progressive: more and more lymph nodes, often in a chain, become affected and eventually the spleen, liver, and bone marrow (thus possibly leading to anaemia) can become involved. Lymphoma cells are not usually seen in the blood, so a leukocytosis is infrequent. However, a lymphoma may well deteriorate in its later stages into a disease with a leukaemic picture.

Important differential diagnoses of lymphoma are self-limiting cases of lymphadenopathy as may occur in tonsillitis, downstream of an infected wound, and in a clear viral infection such as mumps.

Myeloma

This is a tumour of the B lymphocyte that (normally) makes good antibodies to attack bacteria and viruses. However, unlike a lymphoma, this tumour is found within the bone marrow. Accounting for maybe 10% of all haematological malignancies, and with a median survival of 4 years, it can cause bone pain, and often so much bone is mobilised that serum calcium can rise. As the myeloma cells are making large amounts of an incorrect type of antibody, then the viscosity of the blood rises. A further consequence of this is a very high ESR.

Although centred on the bone marrow, the actual tumour cells of the myeloma (plasma cells) can be found in the blood, especially in advanced disease. So, a myeloma may start out (and possibly remain) centred on the

bone marrow, but these myeloma cells may 'escape' (or are pushed out) into the blood and/or lymph nodes. However, numbers hardly ever rise to those of a 'typical' leukaemia.

Myeloma is ultimately diagnosed by very high levels of serum immunoglobulins using a test called protein electrophoresis, often performed in the Biochemistry laboratory. In some cases, only a small part of the quite large antibody molecule is produced in excess amounts, and this can be detected in the blood or in the urine. This fraction of an entire antibody molecule is called 'Bence-Jones Protein' after its discoverer.

Recommended Websites

www.cancerresearchuk.org/leukaemia
www.lymphoma.org.uk
www.myeloma.org.uk

Summary of white blood cells

- White blood cells defend us from infections, when raised levels of these cells can be found in the blood.
- Increased numbers of neutrophils are found in bacterial infection
- Viral infections are characterised by high numbers of lymphocytes
- The most serious disease associated with inappropriately high levels is leukaemia, a condition where anaemia and low platelets (thrombocytopenia) are also often present.
- Other cancers of white blood cells are lymphoma and myeloma.

Key point: Perhaps the most important aspect of the full blood count is in the interpretation of a raised white cell count – inflammation or leukaemia?.

Case Study 2

A 20 year old student presents with a weeks' onset of influenza-like symptoms, with general malaise, sweating, pyrexia, an intermittent skin rash and a sore throat. As the present symptoms seemed to be more severe and persistent than general 'flu, a blood test was performed:

	Result (unit)	**Reference range**
White cell count	12.1 x 10^9/L	4.0 – 10.0
Neutrophils	5.4 x 10^9/L	2.0 – 7.0
Lymphocytes	5.6 x 10^9/L	1.0 – 3.0
Monocytes	0.35 x 10^9/L	0.2 – 1.0
Eosinophils	0.25 x 10^9/L	0.02 – 0.5
Basophils	0.05 x 10^9/L	0.02 – 0.1.0
Blasts	0.45 x 10^9/L	<0.1

Interpretation

The initial abnormality is a slightly raised white cell count. This justifies the differential, which reports a lymphocytosis but also increased blasts. Although possibly a very early lymphocytic leukaemia, a leading cause of this type of lymphocytosis is a viral infection – the Epstein-Barr virus, a member of the herpes family of viruses.

Notably, the number of blasts exceeds that of the monocytes, which cannot be physiological. However, these are not true malignant blasts, but are simply atypical lymphocytes, perhaps activated by the acute phase response and so seeking other cells that are infected with viruses. A likely primary diagnosis is infectious mononucleosis, which can be confirmed by the Paul Bunel / Monospot test. Treatment is symptomatic, that is, bed rest, fluids, and analgesia. Incidentally, the common name for this condition, glandular fever, is a misnomer, as the bodies occasionally found in the throat are swollen lymph nodes, not glands.

CHAPTER 3

COAGULATION

Key words:

Platelets	Fibrinogen
Prothrombin time (PT)	Partial thromboplastin time (PTT)
International normalised ratio (INR)	D-dimers

Key pathological expressions:

Thrombocytopenia	Thrombocytosis
Haemorrhage	Thrombosis
Anti-thrombotic drugs	Anti-coagulant drugs

3.1: An explanation of terms

Platelets: reference range 143-400

These tiny bodies are fragments of a much larger cell found only in the bone marrow (the megakaryocyte), and form a clot, or thrombus, when aggregated together with the help of the blood protein fibrin. A low platelet count (possibly caused by drugs [such as quinine, sulphonamides, and other antibiotics], poor production, or excessive consumption) is thrombocytopenia – a raised count is thrombocytosis and is often present in infections and some autoimmune diseases.

Fibrinogen: reference range 1.5-4.0

This is one of the more important blood proteins involved in clotting and is made in the liver. It is converted into fibrin by an enzyme, thrombin (itself derived from prothrombin), and is crucial in clot formation. Increased levels are found in inflammation and in smokers, decreased levels due to consumption as part of forming a clot.

Prothrombin time (PT, reference range 11-14) and partial thromboplastin time (PTT, reference range 24-34)

These are laboratory measures of the ability of blood plasma to form a clot, measured in seconds, although this is not necessarily the time taken for clot formation in the body. The PT and PTT measure different parts of the clotting pathway can investigate bleeding disorders and can also be used to monitor the effects of drugs that interfere with different parts of the coagulation system (i.e., the anticoagulants warfarin [by PT] and heparin [by PTT]). Some labs place an 'A' for activated in front of the PTT, thus APTT.

International normalised ratio (INR): reference range 2-3 or 3-4

Warfarin works by interfering with the ability of the liver to synthesise proteins involved in clotting, so that clotting takes longer to happen. The INR is simply the ratio between the time that blood takes to clot normally, compared to the (supposedly increased) time it takes to clot due to warfarin. So, if a normal PT is 12 seconds, and on warfarin the time is 24 seconds, then the INR is 2.0. We use the INR to strike a balance between slowing down the clot-forming process, and the use of too much warfarin, which will interfere with clotting so much that a clot may never happen, and haemorrhage occurs.

Someone at a relatively low risk of thrombosis (for example, with atrial fibrillation), or with a current deep vein thrombosis (DVT) should have an INR between 2 and 3. However, those at high risk of thrombosis, for example, with a mechanical heart valve, or someone who seems to be predisposed to clots, would generally be expected to take enough warfarin to maintain an INR between 3 and 4.

D-dimer: reference range <500 Units/mL*

The break-up of a clot (thrombolysis, or fibrinolysis) results in a number of fragments, the most common being D-dimers. Thus, increased levels reflect clot dissolution, which itself is a likely surrogate of the total mass of thrombosis in the body.

*Reference range may vary between labs, depending on the method they use.

3.2: What is coagulation for?

The coagulation system exists to prevent excessive blood loss. A clot is composed of two elements: platelets and fibrin. Consider a small river or stream that you need to dam. One approach would be to throw a tennis net over the steam, and then float down footballs, tennis balls, basketballs etc. Eventually, the flow of water would be impeded. In this simple metaphor, the tennis net is the fibrin, and the various balls are platelets, red cells, and white cells. Clearly, the tennis net by itself would not stop much water flow, and footballs by themselves would simply be washed away. They have to work together to stop water flow, as do platelets and fibrin to form a clot. The tennis balls and net metaphor is far from perfect; mainly as in real life the balls would not take an active part in stopping water flow. But platelets are very active cells and take a direct part in supporting the fibrin net and making the clot more stable.

The key to understanding thrombosis and haemostasis is the coagulation cascade. This is like a waterfall, where a host of different coagulation molecules come to together in defined order to activate each other, and form thrombin. This converts fibrinogen to fibrin, which forms a clot with platelets (Figure 4). This pathway is important because it shows us the way to inhibit the process of thrombus formation with anticoagulants.

3.3: The causes, consequences, and treatments of increased tendency to form a clot

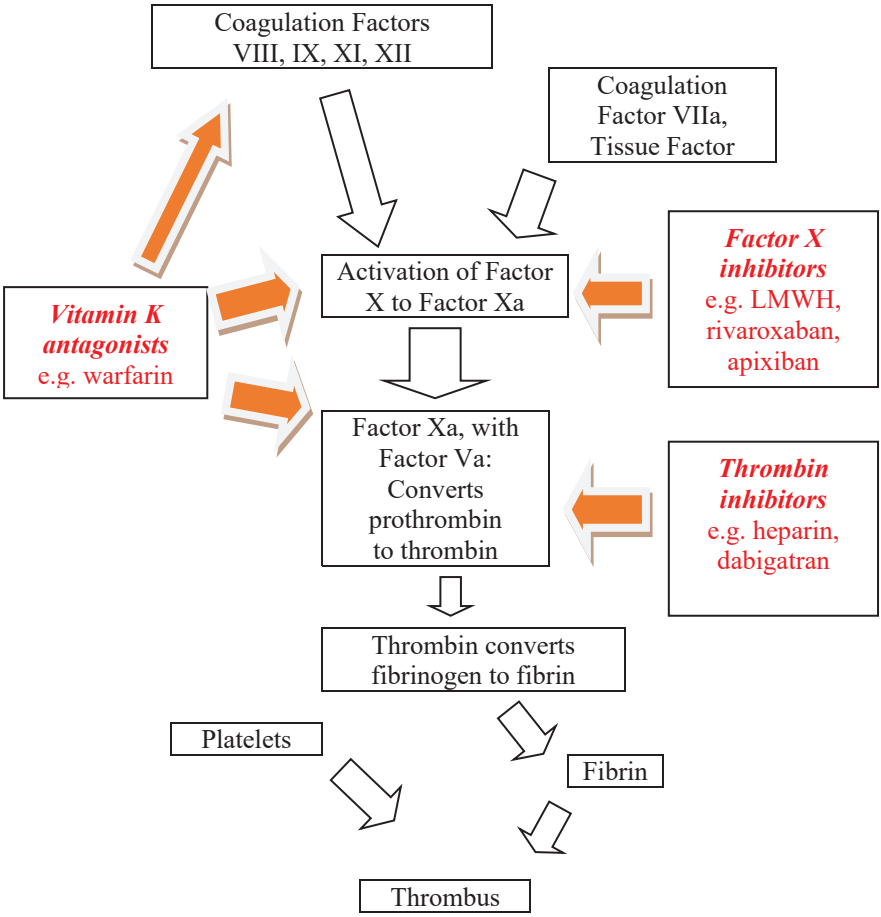
Since a proper word for a clot is a thrombus, then the process that concludes with the formation of a clot is thrombosis. This can occur in the arteries or in the veins.

Clots in arteries

The consequence of an arterial thrombosis that totally blocks that vessel is an infarction. This is where the tissues downstream of the clot die because they are deprived of oxygen and glucose. So, if the clot lies in an artery in the heart, a (quite possibly fatal) myocardial infarction (heart attack) follows. Similarly, a clot in an artery of the brain will cause a stroke. Clots in the legs will cause critical limb ischaemia, as well as considerable pain and, possibly, the development of gangrene. These thromboses are the mainstay of the theory of atherothrombotic disease, the process that will strike perhaps a third of the global population. Doubtless almost all of us are aware of the importance of the risk factors for atherosclerosis

(diabetes, smoking, hypertension and hypercholesterolaemia) that all promote clot formation.

Figure 4: The coagulation cascade



Text in red: anticoagulants. Arrows in orange: target molecules.
LMWH: low molecular weight heparin

Clots in veins

When this happens, it is termed a venous thromboembolism (VTE). The principle vessels burdened are of the legs (where DVT can develop) and of the lungs (where a pulmonary embolus [PE] may arise). Other veins, such as of the arm and shoulder, a rarely burdened.

A DVT leads to a painful, red, and inflamed leg such that walking can be difficult, if not impossible. Blood flow is impeded, and the leg can become swollen by tissue fluid. By itself, DVT is not directly life-threatening, but if the clot breaks up, particles (emboli) can fly around the circulation and cause problems elsewhere. This is the basis of the blockage of a crucial lung vessel by a PE, and will lead to breathlessness and chest pain, and can be fatal. The major risk factors for VTE include surgery (especially orthopaedic), cancer and obesity.

There are several major drugs useful for those at risk of, and needing treatment of, thrombosis.

Anti-thrombotic drugs

These drugs work by inhibiting the function of platelets

Aspirin

This is certainly the most commonly used drug for the treatment and prevention of arterial thrombosis. It is effective, cheap, does not require refrigeration, and one dose (75 mg daily) for all can be given orally. It is now a standard treatment for all who have already had a critical cardiovascular event. It works by reducing platelet activity, so these cells don't get as enthusiastic about forming a clot.

Clopidogrel

Despite its success, some people are intolerant or resistant to aspirin, and so need other drugs, such as clopidogrel, to suppress their platelets. The standard dose is again 75 mg daily. This drug works in different way to aspirin, and the two are often used together after a stroke, heart attack or the placement of an intra-cardiac stent.

Other drugs suppressing platelet activity include prasugrel and ticagrelor.

Anticoagulant drugs

These drugs work by acting on the proteins of the coagulation pathway (figure 4).

Warfarin

Vitamin K, normally obtained in our diet, is an essential requirement in our ability to synthesise key molecules involved in coagulation. So, if our diet is deficient in this vitamin, we can't make the coagulation factors as efficiently and so don't form clots that well. Warfarin works by inhibiting this synthesis activity, so the result is the same – low levels of clotting proteins and so a long time to form a clot. This translates, in real terms, to a protection from thrombosis.

As we have seen, the INR is a simple number that compares the time taken for the blood to clot whilst an individual is on warfarin, compared with the time when he or she is not on the drug. Despite its excellent efficacy, its use is plagued by risk of bleeding and by the variability of the dose between different people: some can be taking 1 mg a day whilst others take 15 mg daily to make their blood equally anticoagulated. Consequently, most patients need to attend an out-patient clinic about every 4 weeks to check that they are taking the right amount of warfarin for their particular INR. However, a serious but rare side effect of warfarin is skin necrosis, often demanding surgery.

Heparin

Heparin is a natural anticoagulant – we all have it in our blood. However, giving extra heparin will help those at risk of thrombosis, such as immediately after orthopaedic surgery. A disadvantage with this agent is that it can’t be taken by mouth, so must be given by sub-cutaneous injection, or intravenously. A further problem is that it can cause dangerously low levels of platelets, and so excessive bleeding, a condition called heparin-induced thrombocytopenia. If this happens, heparin is withdrawn and another anti-coagulant, such as one like hirudin (derived from the leech) or a direct acting oral anticoagulant (DOAC, see below) is an alternative. Like warfarin, the efficacy of heparin can vary from person to person, so it must be monitored in the laboratory by the PTT test.

Low molecular weight heparin (LMWH)

This is a safer form of old-style unfractionated heparin. Although it cannot be taken orally, LMWH is safe enough to be given by subcutaneous injection, even in out-patient clinics and (for the appropriately trained patient) at home and does not need to be monitored by the PTT test. There is also a reduced incidence of heparin-induced thrombocytopenia. There is now a form of LMWH that need be given only once a week.

Direct Acting Oral Anticoagulants (DOACs)

This group consists of two types of drugs: one which directly inhibits thrombin (Dabigatran), others which inhibit Factor Xa (Rivaroxaban, Apixaban and Edoxaban). These drugs are at least as effective and have better safety profiles (some more so) than does warfarin in the prevention of stroke in atrial fibrillation, and of LMWH in the prevention of DVT and PE after orthopaedic surgery. Some have, or are likely to have, licences for the prevention and treatment of DVT and PE in a medical setting, and in cardiovascular disease. Without the need for monitoring with a blood test, DOACs have replaced warfarin and LMWH in many conditions

Anticoagulation Management

We focus on prevention and treatment. All patients coming into hospital must have their risk of VTE assessed, and if high enough, prophylactic treatment given. This is likely to be a LMWH, as it is easy to handle, but in the future one of the DOACs may be used. The practitioner is strongly recommended to refer to their local guidelines, themselves referring to guidelines from the National Institute for Health and Care Excellence (NICE).

At the practical level, patients coming out of surgery (and therefore at risk of thrombosis) need to be anticoagulated immediately. Historically, this has been with a double approach of warfarin and a LMWH, needed because LMWH acts almost immediately, whereas warfarin takes several days to become effective and thus protect against a clot. However, this approach has rapidly fallen out of favour, and many practitioners are giving their patients one of the DOACs instead of this combination of LMWH and warfarin.

Alternatively, other patients may be protected by LMWH only whilst in hospital and be discharged with their own supply so they can dose

themselves at home. This self-dosing is becoming attractive as there is a long history of people with diabetes confidently and safely dosing themselves with insulin. However, this approach is also being superceded by the use of a DOAC. Atrial fibrillation is a major cause of stroke, and prophylaxis must be given. In the past this has been warfarin to target INR 2-3, but this is one area where DOACs may be used with confidence. All these anti-thrombotic and anti-coagulant drugs have side effects, principally haemorrhage, when used in excess or in other situations.

3.4: The causes, consequences, and treatments of reduced ability to form a clot

Haemorrhage is the medical term for bleeding, and we have just noted that excessive anticoagulation can cause minor (e.g., bruising or nosebleeds) or more serious bleeding (with blood loss so severe that it may demand a blood transfusion). Fortunately, bleeding due to anti-platelets, heparins and DOACs can often be managed by withdrawing the drug, but because the effects of warfarin are long-lasting, then the antidote is to give vitamin K orally or by injection.

The best-known coagulation gene defect is haemophilia, caused by lack of a certain crucial component of the coagulation system, Factor VIII (figure 4). Without this factor, bleeding is frequent. Fortunately, the disease is rare and, although always serious, is becoming easier to treat by giving replacements of Factor VII or VIII made by genetic engineering. This revolutionary technology can also provide other clotting factors, which can be used to treat a variety of bleeding disorders.

The most common inherited bleeding disorder is von Willebrand's disease, caused by lack of von Willebrand factor. Unlike haemophilia, this condition is very variable, ranging from virtually asymptomatic to life-threatening. If recombinant clotting factors are unavailable, then excessive bleeding and haemorrhage can be treated with fresh frozen plasma or cryoglobulins, which are purified and prepared from blood transfusion donations. Transfusion of purified platelets is also possible, and all these are generally from the blood bank. This is reviewed in Chapter 4.

3.5: Fibrinolysis and D-dimers

Once a clot has formed, and has done its job in stemming blood loss, it must be removed by a process called fibrinolysis. The enzyme plasmin degrades the clot into small pieces so that blood flow can be restored. Plasmin derives from an inactive precursor, plasminogen, by the activity of tissue plasminogen activator (tPA). A historical treatment of a heart attack or stroke, presumed to follow from a clot in one of the arteries of the heart or brain, was to give tPA (or a closely related product such as streptokinase or alteplase) to help get rid of the clot that is causing the obstruction and the associated symptoms.

A consequence of fibrinolysis is the appearance of fragments clots in the plasma. These can be detected in the laboratory, and of these fragments, D-dimers are the most useful. Thus, increased D-dimers are evidence of fibrinolysis, and so of the presence of a clot being resolved. So, a DVT, being digested by natural fibrinolysis, should be accompanied by raised levels of D-dimers. Unfortunately, many conditions, including cancer, diabetes, smoking and atherosclerosis, are also accompanied by raised D-dimers, so the test is not specific for a DVT. However, around the other way, this test can be used to exclude a DVT as it is just about impossible to have normal D-dimers levels and a DVT.

Increased levels of D-dimers are present in the moderate and severe stages of a COVID-19 infection, and predict hospitalisation, admission to intensive care, and death. Thus COVID-19 is a thrombotic as well as a pulmonary disease.

Recommended websites

www.guidance.nice.org.uk/ KTT16 (on DOACs), NG89 and NG158 (on VTE)
www.rcn.org.uk › ... › Nursing practice issues › CPD online learning
www.haemophilia.org.uk/

Key point: The leading cause of haemorrhage – be it a nose-bleed, excessive bruising, or overt blood loss – is the misuse of an anticoagulant

Summary of coagulation

- The coagulation system exists to minimise blood loss by creating a clot (thrombus) to plug up the hole.
- The clot is composed of platelets in a mesh of fibrin that may also include red blood cells.
- Excessive and inappropriate clotting can lead to possibly fatal disease, and can be retarded by drugs such as aspirin, warfarin, and heparin. The latter two are being superceded by the direct oral anticoagulants (DOACs)
- Fibrinolysis is the dissolution of the clot, and leads to raised D-dimers, molecules that can be used to exclude a DVT or a PE

Case report 3

The wife of a 65 year old man who had recently returned home after a hip replacement calls the GP Surgery to say he has been having a lot of nose bleeds. You advise him to come in for a full blood count and coagulation screen. He does so, and the blood goes off to the Path Lab, who call you back with the results that everything is normal, except a platelet count of 75 x 10^9/L (reference range 143 – 400 x 10^9/L).

Interpretation

The patient has thrombocytopenia (low platelets). This is probably due to the use of a LWMH, used routinely to reduce the risk of a VTE after orthopaedic surgery (hence heparin-induced thrombocytopenia). There are specialist tests to confirm this, but for the time being he needs to stop the LMWH and be given a either a direct thrombin inhibitor (such as argatroban) or one of the DOACs.

CHAPTER 4

BLOOD TRANSFUSION

Key words:

ABO blood groups Rh blood groups Cross-match
Group and Save Blood components and products

Key pathological expressions:

Anaemia Transfusion reaction

4.1: Introduction

Blood transfusion is not a blood test. It is included in the book as it is an important therapy that Practitioners need to be aware of and may even consider in the management of their patients. This is not merely because the objective of blood transfusion has changed markedly over the years, from being a crude instrument to maintain the haemoglobin level, to a therapy to save lives. Additional changes have been the realisation that this is far from a simple and trouble-free treatment to one that can damage, possibly permanently, the health of the recipient. In the UK in 2020 there were over 1.5 million red cell transfusions.

A further development has been the move from transfusing 'whole' blood, which therefore included plasma, white blood cells and platelets, to transfusing only specific components, such as only the red cells. The latter, called 'packed cells', is not only more efficient, but also, without the white cells, platelets, and plasma proteins brings fewer adverse reactions. Furthermore, the 'leftover' plasma from a blood donation can provide other useful blood components (such as clotting proteins) although these can also be produced by genetic engineering.

Each blood group system (of which there are several) consists of two parts.

- Firstly, there are molecules present at the surface of the cells. These define the blood groups and are known as antigens.

- The second aspect is a series of antibodies that recognise these antigen molecules (further details of antibodies and antigens are presented in chapters 5 and 6).

It is the interactions between these two aspects that frustrates the lab scientists who try to match the antigens and antibodies of the patient with those of the blood provided by the donor.

4.2: An explanation of terms

The ABO system

Certain combinations of carbohydrates (sugars) form A and B molecules on the surface of red blood cells. If you have only the blood group A structure on your red blood cells, you are blood group A. Similarly, if you have only group B molecules on your red cells then you are group B. People with both A and B molecules on their red blood cells are group AB, and if you have neither of these structure on your red cells, you are group O.

These A and B molecules act as antigens to generate the production of antibodies in the plasma, but in reverse. Thus, someone of group A has antibodies that recognise group B (i.e. Anti-B). Likewise, group B people have antibodies that recognise group A (i.e., Anti-A). Group AB people have no antibodies, but group O people have both anti-A and anti-B antibodies. This is summarised in table 6.

Table 6: Determinants of ABO Blood Group

Frequency in the UK (%)	Antigen structures on the red cell surface	Antibodies in the plasma
40	A	Anti – B
8	B	Anti - B
3	AB	None
45	None, hence, Group O	Anti-A and anti-B

The Rh system

The Rh system is much more complicated, being composed of dozens of different antigenic molecules, although on a day to day basis, five different structures on the surface of the red cell and commonly dealt with in the blood bank. A full explanation of this is beyond the scope of this book, but in practice, focus is on the molecule known as D (i.e., Rh D), as it is this structure and the antibodies that it can generate that can give rise to haemolytic disease of the foetus and newborn (HDFN) if not correctly treated. About 85% of white Europeans are Rh D positive. Other members of the Rh family of antigens are C, c, E, and e.

The main distinction between the ABO and Rh systems is that in the normal person, there are ALWAYS antibodies to absent antigens. This makes ABO incompatibility potentially fatal as it may results in giant clots and/or destruction of the red cells.

Group and Save

The practitioner requests the laboratory to 'Group and Save' the patient's blood. Our scientist colleagues will then determine the patient's ABO and Rh blood group (Group), but then keep the blood handy (Save, generally in a refrigerator) as it is likely to be needed in a cross-match in the near future. The same blood is also screened for potential antibodies in the patient's blood that may cause problems further down the line. A Group and Save is often requested of patients about to undergo surgery in the next few days or weeks, so that the laboratory has a head start in trying to match some stored blood for the patient.

Cross-Match

This is step two and is requested when the practitioner is absolutely convinced that the patient must be transfused, and a second blood sample may need to be taken. It may be that in an emergency (such as massive haemorrhage after a road traffi accident), the Group and Save, and the Cross-Match happen at the same time.

In practice, your colleagues in the blood bank oversee the mixing of blood from the patient (recipient) with a series of stored bloods (up to 5 or 6) from different donors, to see if there are any matches. This is often done in an analyser. A good match is where the red cells are unaltered by this mixing, and therefore should not react when in the patient. However,

blood that does not match will aggregate, forming small clots, indicating an incompatibility. This is inevitably because the antigens on the cells and the antibodies in the serum recognise each other, and react together, causing blood to clump. It is presumed that the same reaction may happen in the blood vessels: the mixture is incompatible, and the donor pack cannot be given to the patient.

An example of this would be mixing blood from someone of blood group A with that of someone of blood group B. The group A person has group A molecules on their red cells but also antibodies against B in the plasma (i.e., Anti-B). The group B person has the reverse – group B molecules on their red cells and Anti-A antibodies in their plasma. So, when mixed, the group A red cells will be recognised by the Anti-A antibodies, and so will reaction together. Similarly, the group B red blood cells will be recognised by the Anti-B and will also react. Recall from the section on antibodies in the white blood cells chapter that the purpose of antibodies is often to attack and destroy anything they react with. Hence the danger.

The same principle of incompatibility also occurs in other systems, such as antibodies to an Rh antigen or to a Kell antigen being reactive with specific anti-Rh or anti-Kell antibodies.

Blood components and products

The blood pack itself is only red cells (hence 'packed cells') – the donor's plasma has been taken off, and from this blood components and products are prepared. Most of these are to help with haemorrhage, but there are also packs of albumin and of platelets (see section 4.4).

4.3: Why order a blood transfusion?

Firstly, the practitioner will ask - does the patient ***really*** need it? Are there any alternatives - will erythropoietin do the trick? Is autologous transfusion possible? One textbook suggests that a post-operative patient who is asymptomatic with a haemoglobin of 90 g/L probably does not require a transfusion. As mentioned, in the past, ordering a transfusion was often simply because a physician considered it a good thing to do, that it would probably do the patient some good. This would, of course, be fine but for a number of problems. To name but a few:

- In practice, whilst the ABO/Rh systems are the most important, there are a host of other blood group antigens (with names such as

Duffy and Kell) that can generate antibodies. The more transfused a person becomes, then the greater the likelihood that these antibodies can build up to be a real clinical and laboratory issue.

- We are programmed to collect and save iron in stores all over the body. People who are hyper-transfused often have problems in various organs as the build-up of this iron can cause damage to the tissues (haemosiderosis). Since 1 unit of blood contains some 250 mg of iron, then 15 units can more than double the body iron stores.

- Transfused blood can contain pathogenic micro-organisms (viruses, bacteria, parasites), although, gladly, due to screening, this is becoming less of a problem. Infection can also occur via the site of the infusion.

Therefore, not simply because of the above, the present view is that transfusion should be reserved only for those in danger of losing their life or will show a measurable improvement not achievable by other means, and the earlier case report of a woman with sickle cell disease underlines this point. It follows that the requirement for a transfusion can only be made clinically, not in the laboratory. However, much experience is available within the blood bank and advice should be sought if cases fall outside established practice or guidelines.

Indications for blood transfusion

NOT simply because of haemoglobin being less than a certain number. Reasons for ordering a transfusion can be many and varied, but major life-threatening indications include:

- Chronic and serious anaemia refractive to other treatment, e.g., severe cases of the haemoglobinopathies sickle cell disease or thalassaemia

- Life threatening emergencies, e.g., rupture of an aortic abdominal aneurysm, massive blood loss after a road traffic accident or after surgery

- Haemorrhage, e.g., in haemophilia or overdoses of anti-coagulants warfarin or heparin, or thrombocytopenia.

4.4: Blood Components and products

All hospitals need to deal with haemorrhage due to prolonged PT, PTT, decreased fibrinogen, over-anticoagulation with anticoagulants, surgery, disseminated intravascular coagulation, etc.

As discussed in earlier, the Blood Bank can provide not only red cells, but also platelets and coagulation proteins such as fibrinogen, factor VII, and factor VIII that we met in the previous Chapter. Other components are fresh frozen plasma (FFP) and cryoprecipitate. These will be needed by people at risk of haemorrhage, or with actual haemorrhagic blood loss, but who do not need red blood cells, such as those with haemophilia and severe von Willebrand's disease.

As fragments of cells, platelets will also need to be checked for blood group compatibility and are generally given for life-threatening thrombocytopenia. Albumin (generally as a 20% preparation) is also available for people with low levels or who have had heavy burns (although only regional centres will deal with these risky patients) or ascites.

4.5: When things go wrong

Is anything free of possible error? Blood transfusion is not exempt, and an incompatible transfusion can kill. But how can it happen? Errors can and do occur at all places in the 'journey' from of the blood from donor to recipient. However, it is generally recognised that most errors happen in the laboratory and/or once the blood has left the blood bank for its destination. In 2020 there were 39 transfusion-related deaths (most being due to circulatory overload) and 137 cases of major morbidity.

Laboratory error

Clearly the packs of blood arrive from the NHS Blood and Transplant Service (formerly the National Blood Transfusion Service) in good shape, typed for ABO and Rh groups, and screened for major infective agents such as the viruses that cause hepatitis, and HIV. 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. Next, error in the cross-match itself. These are rare because the lab invests heavily in the technology and reagents to ensure that if an adverse reaction is happening, it is detected. However, if the cross-match

goes wrong, which is a false negative, a possible incompatible unit of blood may be issued.

Post-lab error

These are inevitably the wrong blood being given to the wrong patient. The wrong pack of blood may be collected from the Blood Bank issuing refrigerator, or the blood may be given in error to the wrong patient. A common confusion is that two of more patients are to be transfused at the same place at the same time.

Many of these are simply incorrect patient identification, generally by a misunderstood verbal recognition question, or by misreading the patient's ID strip at the wrist.

4.6: What do we do about it?

Early reactions

In an acute setting - how do you know there is a problem? Recall of course that patients may not recognise there is a problem (especially if unconscious!). But what does the practitioner look for? Symptoms and signs of a transfusion reaction vary enormously (Table 7).

Table 7: Signs and symptoms of a potential incompatibility

Symptoms	Signs
Cough, headache Flushing/rash Anxiety, agitation Chills, nausea, and vomiting Trembles/shakes Shortness of breath, chest pain	Fever (temp spike >40^{o}c) Hypotension Oozing from wounds Haemoglobinuria Tachycardia (>100 bpm)

However, there can be acute non-red blood cell reactions. Acute urticarial reactions (e.g., hives) and anaphylaxis may be the result of the recipient responding to the donor's plasma proteins – if so, antihistamines are one possible treatment.

Upon suspicion of a reaction, the infusion should be immediately stopped. All good Hospitals will have a defined protocol that must be followed. Clearly, clinical treatment will depend on the severity of the reaction which, if not too bad, can be rapidly reverse. But severe reactions can be life threatening and will be treated accordingly (e.g., rapid admission to ICU if there is disseminated intravascular coagulation, with ventilation, adrenaline, hydrocortisone). One 'mechanical' way of treating an acute reaction is to try to 'flush' it out – this is attempted by giving fluids, but clearly requires good renal function. If there is massive donor red cell destruction there may well be hyperkalaemia (high potassium).

The donor blood pack will be collected by the Lab Staff, and a blood sample from the patient taken. Both will be thoroughly (re)analysed. Most are subsequently found to be ABO incompatibilities – Rhesus problems are less common.

Late reactions

Problems at 12/24 hours to 3 days may be with major organs such as acute renal failure (with or without haematuria or haemoglobinuria), jaundice, congestive heart failure due to circulatory overload, and pulmonary oedema with or without adult respiratory distress syndrome.

Febrile reactions (generally with a slow rising temperature peaking at over 40^{o}C) are seen in 0.5 – 1% of transfusions may be due to anti-HLA reactions. Later complications (3 - 14 days) after an incompatible transfusion may consist of a 'new' immunological reaction of the recipient to the donor red blood cells, causing the destruction of the latter. This time period may also see transfusion-related infections that have escaped the screening process. There may also be reactions with rare antigens too weak to be detected in the Laboratory.

Post-transfusion purpura is characterised by a severe thrombocytopenia (which can last from 2 weeks to 2 months) and is caused by antibodies to antigens on the surface of platelets. In the short term a thrombocytopenia may develop in the hypertransfused as infused blood is generally platelet-free. If haemorrhaging, then platelet transfusion may be required.

Repercussions

There will of course be an inquest, the findings of which will strengthen the system and so minimise the possibility of a similar error returning. A

report will be forwarded to the relevant authorities e.g., the National Patient Safety Agency, and also to the Serious Hazard of Transfusion (SHOT) group, which in 2020 reported over 4,000 events.

Prevention

Naturally, there are many steps designed to prevent a transfusion: generally, check, check, and check again. Laser bar coding allows the sample to 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. This approach has proven to reduce mistake and serious hazards of transfusion. Indeed, SHOT itself reports that ABO incompatible transfusions show a 54% reduction since 2001/2002. Curiously, SHOT notes the reduction in transfusion related acute lung injury since only male donors have been used for FFP and plasma in platelet concentrates! Blood transfusions outside core hours are less safe – and so this is a SHOT recommendation.

Many wards and Hospitals have their own formal policy, and professional bodies (e.g., Royal College of Nursing, Institute of Biomedical Sciences, British Committee for Standards in Haematology) also offer guidelines.

Recommended websites

http://www.nrls.npsa.nhs.uk/resources/?entryid45=59805
www.learnbloodtransfusion.org.uk .
www.blood.co.uk/
http://www.nhs.uk/conditions/Blood-transfusion/Pages/Introduction.aspx
http://www.shotuk.org/
http://www.bcshguidelines.com/4_haematology_guidelines.html?hilite=transfusion
www.nice.org.uk NG24

Summary

- Blood transfusion is a therapy generally reserved for urgent and life-threatening clinical situations such a blood loss. It is now rarely seen as a temporary ‘cure’ for anaemia where an alternative such as iron may be preferable.
- Major blood groups are A, B, AB, and O, but Rh D is also important.
- Requests are usually to ‘group and save’, and to ‘cross-match’, the latter to ensure compatibility.
- The blood bank also provides platelets, albumin, and coagulation factors.
- Incompatible blood transfusion can be fatal and are proceeded by symptoms such as rash, shortness of breath, headache, and cough.
- Most incompatible reactions are due to clerical and/or identification errors.

Key point: Blood transfusion is an essential but potentially dangerous treatment that should not be undertaken lightly.

PART 2:

IMMUNOLOGY

Objectives and scope

Being concerned with the biology and pathology of white blood cells, immunology is effectively a sub-speciality of haematology. However, there is sufficient immunological disease, and immunological aspects of other disease, for it to warrant a department of its own.

The Immunology Laboratory is generally concerned with antibodies, and normally these help protect us from pathogens. Unfortunately, antibodies can often react with the patient's own tissues, and are called autoantibodies, so causing autoimmunity. The most common such disease being rheumatoid arthritis, another is SLE, but other autoantibodies can cause to disease to specific organs, such as the thyroid. For these tests, often described as serology, serum is required.

However, immunologists may also study certain white blood cells, and this will call for a sample anticoagulated with EDTA. As with other disciplines, the lab cannot process samples delivered in an incorrect tube. Learning objectives for this section are shown in table 8.

Chapter 5 will look at the basics of immunology, chapter 6 looks at clinical aspects of immunological disease, but also on how we can manipulate the immune system to our benefit in transplantation and vaccination.

Key point: A full understanding of COVID-19 is impossible without a knowledge of immunology, which the coming two chapters will provide.

Table 8: Learning Objectives – Immunology

Having completed these notes in a satisfactory manner, the reader will….

1. Be able to describe the major components of the immune system:

- The organs of the immune system
- Cellular immunity
- Humoral immunity

2. Recognise major areas of interest, i.e.

- T and B lymphocytes
- Antibodies and antigens
- Phagocytosis

3. Describe the principal features of inflammation

4. Explain the difference between innate and adaptive immunity

5. Understand key features of immunological disease (autoimmunity, hypersensitivity, and immunodeficiency), transplantation and vaccination.

6. Interpret results such as increased levels of anti-nuclear antibodies and anti-citrullinated protein antibodies

CHAPTER 5

THE BASICS OF IMMUNOLOGY

Key words:

Antigen
Antibody
Inflammation
Immunological organs
Phagocytosis
Immunity

5.1: An explanation of terms

Antigen

A substance (often foreign, such as are on the surface of a virus) that stimulates white blood cells, some of which produce antibodies

Immunological organs

These include the bone marrow (the site of white cell production), the thymus, lymph nodes (where the majority of antibody production takes place), the spleen (a reservoir of neutrophils and site of antibody production), the skin (a barrier) and liver (production of defensive proteins and site of antibody production).

Antibodies

Proteins produced mostly in lymph nodes by combinations of T and B lymphocytes, and other cells, and which recognise antigens. Antibodies belong to the globulin family of proteins, hence immunoglobulin (Ig), of which there are five classes: IgG, IgM, IgA, IgD, IgM.

Phagocytosis

The process by which phagocytes (principally neutrophils, monocytes, and macrophages) ingest and destroy foreign material such as bacteria.

Inflammation

A broadly non-specific process by which parts of the immune system seek to destroy pathogens

Immunity

A complex process by which we develop the ability to defend ourselves from specific pathogens. It consists of two parts – humoral and cellular.

5.2: What are white cells for?

As previously indicated in section 2.1, white blood cells defend us from attack by micro-organisms, and also help in repair and reconstruction following tissue damage. Each white cell has its own functions.

Neutrophils: reference range 2- 7

The main function of neutrophils is to defend us from pathogenic micro-organisms (bacteria and viruses) by phagocytosis. Consequently, high numbers of neutrophils are often associated with bacterial infections. Increased levels in the absence of an infection, such as after surgery or severe exercise, may be explained as part of the body's normal response to physical stress, the so-called ‘acute phase response’, and such raised levels can be expected to fall back to normal as the cause of the stress subsides.

Lymphocytes: reference range 1-3

There are two major types of lymphocytes. B cells, when presented with an antigen, transform into plasma cells, and produce antibodies to that antigen. T cells need to pass through the thymus in order to become fully effective. Almost all B cells need the help of certain T cells, whilst other T cells become cytotoxic, and kill cells expressive antigens.

Mildly increased numbers of lymphocytes in the blood may be expected in conditions that also cause a raised neutrophil count. However, the highest levels often encountered in health are found during attack against viral infections, such as in glandular fever, also known as infectious mononucleosis. In such cases these ‘activated’ lymphocytes may be larger than usual, and sufficiently larger for them to be described as blasts.

However, some viruses actually kill lymphocytes, so that the number in the blood will fall – HIV being such a virus.

Monocytes: reference range 0.2-1.0

Monocytes also defend us from infection by the phagocytosis of bacteria, fungi, and other pathogens, but this also occurs in tissues such as the skin, liver, and lung, where they are described as macrophages. Monocytes also co-operate with lymphocytes in making antibodies, and increased numbers can be present in chronic bacterial and protozoal infections, and in malignancy.

Eosinophils and basophils: reference range <0.5

Eosinophils defend us against infections by large parasites, and are also active in allergy, hay fever, asthma, and skin diseases. High numbers of basophils are rare but may be present in acute hypersensitivity and atopic reactions, and in some leukaemias. Basophils can migrate into the tissues (such as of the lung) where they are described as mast cells.

5.3: How the immune system works

White blood cells defend us from the vast majority of microbial pathogens. This defence can be viewed as having two parts: inflammation and immunity.

Inflammation

An inflammatory response is directed towards an infection and/or acute tissue damage. If the former, neutrophils rapidly mobilise to attack a localised bacterial infection, although such a local response may escape into the blood and lead to septicaemia. Unfortunately, these neutrophils are often a little too enthusiastic and local tissues can also be damaged or destroyed alongside the pathogens. Crush or other injury to tissues can also prompt an inflammatory response in the absence of a clear infection. In both cases, local factors lead to redness, itching and swelling, and injury or infection will prompt an acute phase response, which warns the rest of the body about a possible danger.

White cells at the site of infection/injury release chemical messengers (cytokines) that mobilise the general immune system for a possible attack.

The liver responds by releasing other cytokines and related molecules, and there is a general increase in white cell numbers and activity, and other features such as a rise in temperature and heart rate. These cytokines are part of humoral immunity, which also includes antibodies. A third family of molecules in complement, proteins produced by the liver that help antibodies destroy this target, but which also recruit leukocytes.

In most cases, acute inflammation to a defined pathogenic organism is beneficial, in that it will (ideally) destroy that invader. However, there may be local damage to healthy tissues which hopefully is reversible. But should this fail, then chronic inflammation may develop.

Immunity

This can be separated into two parts. Innate immunity involves non-specific defences such as the barrier effect of the skin, with neutrophils, monocytes, and macrophages. This cellular immunity attacks those micropathogens deemed to be alien, often in an acute inflammatory response. This innate response proceeds without any prior knowledge of the micropathogen, and effectively buys time for the second part, adaptive system to develop antibodies and lymphocytes specific for that micropathogen. A key point about adaptive immunity is that it develops memory, so should the same micropathogen be encountered once more, defence can be rapidly developed.

The immune response

These two different processes come together to form a fully-effective defence, and often co-operate. A good example of this is the symbiosis between the two most common types of white cells. Lymphocytes may make antibodies to bacteria and fungi such as yeast, which when bound are more palatable to the phagocytic neutrophils and monocytes, thus aiding removal of these pathogens.

Thus, for a full and comprehensive defence against micro-organisms, both inflammatory and immunological mechanisms are required. Infections occur when either, or both, of these processes become impaired. Antibodies are not always good as they can take part in autoimmune diseases such as rheumatoid arthritis and thyroiditis (chapter 6), and often frustrate blood transfusion (chapter 4).

CHAPTER 6

CLINICAL IMMUNOLOGY

Key words:

Autoantibodies	Anti-nuclear antibodies
Rheumatoid factor	anti-citrullinated protein antibodies (ACPAs)
IgE/Basophils/Mast cells	

Key pathological expressions:

Autoimmunity	Immunodeficiency
Hypersensitivity	Inflammation

6.1: An explanation of terms

Autoantibodies

These are antibodies that attack our own cells and tissues

Anti-nuclear antibodies (ANAs): reference range <1/40

These are present in many with SLE or systemic sclerosis.

Rheumatoid factor (RhF): reference range <20

This is an autoantibody to an immunoglobulin and is present about 80% of those with rheumatoid arthritis (RA). It follows that about 20% of people with RA have ‘sero-negative’ disease. It may also be present in SLE, Sjogren’s syndrome and systemic sclerosis

Anti-citrullinated protein antibodies (ACPAs): reference range <7

These autoantibodies recognise the amino acid citrulline, and are important in the diagnosis of RA.

IgE/Basophils/Mast cells

Most, if not all, hypersensitivity response is due to the over-stimulation of basophils and mast cell cells by their binding to the antibody IgE.

6.2: Autoimmunity

A key concept in immunology is the ability of the immune system to recognise its own cells and tissues, being 'self'. It follows that anything that is 'non-self' is expected to be foreign (viruses and bacteria), and potentially pathogenic, so must be destroyed, which is all well and good. The trouble begins when the immune system foolishly considers its own tissues to be 'non-self', and so starts an attack. This process is autoimmunity, and in almost all cases the damage to tissues is caused by a chronic inflammation, hence the suffix -itis on many conditions.

The autoimmune diseases account for a considerable degree of human morbidity and mortality. Table 9 lists some of the cells and organs that are the subject of this attack, and the clinical condition that follows.

Table 9: Cells and organs subject to autoimmune attack

Cells/organ attacked	Clinical consequence
Thyroid tissue	Thyroiditis
Red blood cells	Autoimmune haemolytic anaemia
Joints	Rheumatoid arthritis
The pancreas	Diabetes
The kidney	Glomerulonephritis
Parts of the stomach	Pernicious anaemia
Intestines	Crohn's and coeliac disease, colitis
The liver	Hepatitis
Central nervous system	Multiple sclerosis

A major pathological finding in each autoimmune disease is the presence of a defined auto-antibody (directed towards the organ or cell in question) that actually does the damage. Consequently, the presence of the disease is often ultimately defined by the laboratory finding this auto-antibody.

There is often a family link in autoimmunity, so other members may be at risk of an autoimmune disease if one is diagnosed in a close relative. In addition, many people with an autoantibody to one tissue may also have an autoantibody to a different tissue. Irritatingly, there are few laboratory tests with sufficiently good sensitivity and specificity profiles with which one can make a firm and unequivocal diagnosis.

Connective tissue disease

The most common autoimmune diseases are undoubtedly of musculo-skeletal tissue, mostly RA and SLE, which share several clinical characteristics. The common degenerative bone and joint disease osteoarthritis is not autoimmune. Allied conditions include psoriasis and systemic sclerosis. There are no specific routine haematology or biochemistry blood tests for these conditions although there may well be an abnormal white cell count, ESR and CRP due to the inevitable inflammation. The other major tests are RhF, ACPAs and ANAs, and tests of renal function are often called for, especially in SLE, to detect lupus nephritis.

As elsewhere, the lab provides guidance on diagnosis and the effects of treatment. As many of these diseases are inflammatory, particular treatments (such as immunosuppression) should improve markers of inflammation such as CRP. In this respect the ESR may be used as simple marker of the effects of steroids in certain diseases such as temporal arteritis, so that the daily dose of steroid can be up- or down-titrated as lab tests (which we assume reflect the disease process) are influenced.

Polymyositis and dermatomyositis are inflammatory diseases of muscle and/or skin, so that where there is damage to muscle, there is also likely to be raised creatine kinase. However, this enzyme is also raised in damage to the muscles of the heart and is likely to occur after a myocardial infarction (explained in Chapter 9).

Organ specific autoimmune disease

As listed in table 9, other auto-immune diseases, such as of the thyroid, attack precise organs, so in this case measurement of thyroid stimulating

hormone and thyroxine may be appropriate. If hepatitis is present, liver function tests will be ordered. However, all are characterised by an autoantibody to that particular tissue that can be demonstrated.

6.3: Immunodeficiency

Deprived of a functioning immune system, the subject is open to attack by micropathogens. This is inevitably due to the inability to make antibodies, and of white cells to digest bacteria. Aetiology is generally genetic/congenital, or secondary. The former will be evident in the neonate, whilst the latter will be caused by an external agent, and generally in the adult. As the bone marrow is where white cells (and so the ability to make antibodies) are produced, then stress or damage to this organ, perhaps by cytotoxic chemotherapy or cancer, is a leading cause of immunodeficiency. An established alterative cause of secondary deficiency is infection with HIV, where the virus destroys certain T lymphocytes.

The laboratory demonstration of immunodeficiency is with reduced levels, or the absence, of antibodies. The lab can also show when white cells themselves are dysfunctional – one of the most common such diseases being chronic granulomatous disease.

6.4: Hypersensitivity

There are four types of hypersensitivity, although type 1, allergic hypersensitivity, dominates. Most of us produce a measured and appropriate response to a micropathogen. However, in some, the response far exceeds that which is actually needed, hence hypersensitivity, a condition said to be present in 10% of the population. The factor causing the over-reaction is called an allergen, which causes an allergy.

Common allergens include foodstuffs (peanuts, pecans, pine nuts, walnuts, shellfish, egg albumin, wheat, maize, kiwifruit, milk, soy, chestnut), invertebrate material (house dust mites), plant material (ragweed pollen, poison ivy, certain oaks), drugs (antibiotics, muscle relaxants, painkillers, aspirin, NSAIDs) and venums (insect bites and stings).

Clinical allergy

Interaction of the allergens with IgE on the surface of basophils and mast cells causes the cells to degranulate and release a host of small molecules. These cause a wide variety of symptoms that include:

- Asthma: problems with breathing, with tightness of the chest, cough and wheezing.

- Allergic conjunctivitis, similar to rhinitis, but being inflammation of the conjunctiva, with the same pain, irritation and itching, and with tears.

- Eczema: an umbrella term for several skin conditions that include rash, swelling, dryness and itching.

- Intestinal problems, typically pain and cramps, probably due to the action of histamine by white cells of the intestines.

- Rhinitis, meaning inflammation of the nose, but also of the soft tissues of the nasal passage, with itching, blockage and a mucinous discharge.

- Urticaria, also known as hives, is a raised rash (wheal) most often found on the arms, legs and back, whilst facial urticaria is common with food allergies.

Several of these symptoms can be present concurrently. For example, the most common manifestation of hypersensitivity is hay fever, likely to be a mixture of rhinitis, conjunctivitis and often some pulmonary symptoms such as asthma.

The immunology lab will test for overall levels of IgE, but also allergen-specific IgE. This can identify IgE reactive towards certain grasses, fruit, egg and nuts. Antibodies may also be present in farmer's lung, allergic alveolitis, and pigeon fancier's lung.

Treatment

This depends on the stimulus. Generally, an anti-histamine such as piriton is used to block the histamine receptor. Other treatments such as sodium cromoglicate stabilise the basophil membrane and so suppress degranulation. A variety of agents can be delivered by aerosol directly to the lung in cases of asthma. The agents in these inhalers include salbutamol (a $beta_2$ adrenoreceptor agonist) and other adrenaline agonists (such as epinephrine). For severe disease, glucocorticosteroids are preferred for long-term control.

6.5: Manipulation of the Immune system

A major triumph of immunological science is transplanation, where a foreign body (non-self) from a donor, is placed within a recipient (self). Of course, the recipient should reject the non-self organ or tissue, but is prevented from doing so by matching (as much as possible) the HLA types of the donor and recipient, and by suppressing the immune system of the recipient with drugs such a steroids, azathioprine and tacrolimus. Some of these drugs are also use to suppress an over-active and pathological immune systemic in severe RA and SLE, described above.

The second manipulation is to deliberately introduce a foreign body into someone (a vaccine), which will then induce the formation of an immune response – the process of vaccination. This relies on immunological memory, wherein the immune system is pre-armed to the invander, and so react promptly wih antibody and white blood cell responses. Responsible for the eradication of smallpox, this procedure now extends to protection from viruses such as measles, mumps, rubella, influenza and SARS-CoV-2, which causes COVID-19.

Summary

- The leading class of immunological disease in autoimmunity, which in turn is principally RA and SLE. These diseases are characterised by autoantibodies such as RhF, ACPAs and ANAs.
- Immunodeficiency is present when the body is unable to defend itself from micropathogens. It may be due to damage to the bone marrow or the effects of the human immunodeficiency virus
- Hypersensitivity is present when the body over-responds to a stimulus (an allergen), causing conditions allergic reactions such as asthma, conjunctivitis, and skin rash.
- The immune system can be manipulated to allow transplantation, and in vaccinations.

Recommended websites

www.allergyuk.org
www.nras.org.uk (National rheumatoid arthritis society)
www.immunology.org
http://www.rheumatology.org/practice/clinical/classification/index.asp
www.nice.org.uk (NG100 on RA, NG191 on COVID-19)

Case report 4

An 80 year old man, with a body mass index of 34.8 kg/m^2, complains to his General Practioner of six months of worsening pains in both knees, the pain in the right being markedly greater than that in the left. The pain is minimal upon rising in the morning, but gets worse during the day. The patient is sent for X-rays, a full blood count, ESR, C-reactive protein, ACPAs and rheumatoid factor

The full blood count is entirely normal, but the ESR is very slightly increased at 14 mm/hour (reference range <10 mm/hour). The rheumatoid factor is negative (result 1/20, reference titre <1/40), as is the ACPA (8 EU/mL, reference range <20), CRP at <5 g/L (reference range <5 g/L). The X-ray points to moderate erosions in the right knee, mild erosions in the left, consistent with the history of pain.

Interpretation

The only abnormality is the very marginally increased ESR. The rheumatoid factor titre and ACPA results are well within their reference range. The history points to a chronic connective tissues disease such as osteoarthritis or rheumatoid arthritis. The rheumatoid factor result within its reference range does not exclude the latter, but the lack of any gross abnormalities points to the former. Overweight is the major risk factor for osteoarthritis, and if it were rheumatoid arthritis, the presentation would be different with several sets of swollen and painful joints, and perhaps other factors such as early morning stiffness and more abnormalities in the blood analysis. Normal CRP counts against an overt inflammatory condition. Initial treatment is analgesia with strong advice to lose weight. Surgery may be required if the patient is sufficently fit.

PART 3:

BIOCHEMISTRY

Objectives and Scope

Biochemistry shares are many similarities with haematology and immunology, and there are also numerous instances where the pathophysiology of one spills over to influence the other – renal disease being an excellent example as we shall see.

As for haematology, it is taken as understood that all blood tubes and forms must be fully labelled by those taking the blood in order to minimise the risk of (possible fatal) error. Indeed, the laboratory will be within its right to decline to test a particular sample that is incorrectly or inadequately labelled.

The major difference between these two sciences is that almost all biochemistry is done on serum obtained from clotted blood. Thus, testing can only be performed on blood that is collected in the correct tube. Failure to do so will, at best, result in a polite phone call from the lab explaining the problem and its remedy. At worse, a report will be returned a day or so later with a comment such as 'inappropriate blood sample received, please repeat'. Fortunately, many blood tubes have different coloured tops to help this process and minimise errors. Therefore, again, if in doubt, PHONE.

Again, as for haematology, a note on units. Few with an interest in lipids will disagree that a total cholesterol result of 8.5 is undesirable, even though the correct terms are 8.5 mmol/L. This, however, will certainly be a problem to those (such as Americans) reporting cholesterol in different units of mg/dL. For example, their result would be 325! It matters not so much that the correct unit of, shall we say, serum calcium is given, for example, as 4.5 mmol/L, or in shorthand simply as 4.5, but it does matter that this particular result is very serious and demands immediate attention.

The objectives of the Biochemistry section follow in Table 10.

Table 10: Learning Objectives – Biochemistry

Having completed these notes in a satisfactory manner, the reader will:

1. Appreciate that almost all biochemistry tests need to be done on serum. The principle exception is glucose.

2. Recognise the major areas of interest, i.e.

    - Fluid and electrolytes balance
    - Sodium and potassium
    - The kidney
    - Liver function tests and plasma proteins
    - Atherosclerosis and its risk factors (e.g., diabetes)
    - Calcium and bone
    - Thyroid function

3. Describe major clinical problems associated with each of these areas, such as

    - Dehydration
    - Renal and liver failure
    - Diabetic ketoacidosis
    - Thyroiditis
    - Hypercalcaemia

4. Interpret simple biochemistry results, e.g.

    - A urea of 21.4 with a creatinine of 145
    - A bilirubin of 7 with an alkaline phosphatase of 890
    - A calcium of 1.9 with an albumin of 32
    - An HbA1c result of 50 mmol/mol

5. Understand the basic purpose of the Biochemistry Laboratory.

CHAPTER 7

WATER, UREA, AND ELECTROLYTES

Key words:

Urea and electrolytes
Potassium
Sodium
Osmolality

Key pathological expressions:

Hypernatraemia
Hyperkalaemia
Dehydration
Hyponatraemia
Hypokalaemia
Oedema

7.1: An explanation of terms

Urea and electrolytes (U&Es)

U&Es are undoubtedly the most requested biochemistry tests, reported in units of mmol/L or µmol/L. Urea is a major excretory product of our biochemical metabolism, whilst creatinine is a more specialised product of the protein breakdown. An explanation of the role of urea and creatinine follows in chapter 8.

Electrolytes are charged atoms (ions) or molecules that are involved in the passage of electricity and are often written with a small plus or minus, like Na+ and Cl-, indicating their electrical charge, although, in practice, charge is often ignored. Sodium (Na), hydrogen (H) and potassium (K) are most commonly requested, others are Cl (chloride) and HCO_3 (bicarbonate). The latter is relevant for acidosis. Calcium (Ca) is also an electrolyte, but it belongs in a section of its own (chapter 11).

Sodium: reference range 135-145

This is the primary electrolyte in the blood, as there is more of it than any other. Faced with increases or decreases, we need to consider the equation

concentration = mass/volume, considering changes in sodium in the diet and/or the amount of water in the blood. Since the kidney regulates both these aspects, changes in sodium may indicate problems with this organ.

Hypernatraemia (>145)

Increased serum sodium may be due to a salt rich diet and/or a degree of dehydration – the classical clinical sign of which is loss of elasticity of the skin (the pinch test – although unreliable in the elderly). However, the most common reason is water loss, possibly due to insufficient intake in drinking, excess loss in urine, or losses in sweat and diarrhoea. Clinically, the simplest treatment is to replace fluid orally, if possible. If this is not possible (maybe because the patient is in a coma) then water can be infused as part of a dextrose drip.

Hyponatraemia (<135)

Low levels of sodium may be due to retention of water (low V_{out}) and/or excessive loss of sodium). For this most common in-hospital electrolyte disturbance (15% of patients), very rare causes include insufficient sodium in the diet, and very high levels of proteins or lipids in the blood. A recognised sign of this fluid retention is oedema, although this may not always be present. Oedema is also associated with heart failure and hypoalbuminaemia. In some cases, water retention can be treated with thiazide drugs, but this can be dangerous if the kidneys are not adequately functioning.

Potassium: reference range 3.8-5.0

Many aspects of potassium regulation are similar to sodium (such as intake and outlet), but with a number of crucial differences. The first is that the majority of our potassium is within cells, not in the plasma – the reverse of sodium. A second is the relationship between potassium and hydrogen ions: as hydrogen ions increase (as in acidosis) then potassium is displaced from the cell and enters the plasma. The reverse is true in alkalosis. Overall, (unlike sodium) potassium does not vary a great deal in response to large changes in water balance.

Hyperkalaemia (>5.0)

Increased serum potassium may be due to renal problems (e.g., failure to excrete sufficient potassium), acidosis, or release from damaged cells (e.g. destruction of red bloods cells or tumour cells [perhaps by chemotherapy],

or crush injuries). However, whatever the reason, it may be serious as high levels (for example, >7 mmol/L) can contribute to cardiac arrest because of interference with nerves. Indeed…

High serum potassium kills people!

Therefore, high potassium is the most common and most serious electrolyte emergency, and treatment includes insulin and glucose to get potassium into cells. Other treatments include calcium gluconate, resonium A and dialysis.

Hypokalaemia (<3.8)

Causes of low serum potassium include the reverse of the above, e.g., alkalosis, in addition to loss in diarrhoea and vomiting, from the kidney, or inappropriate use of corticosteroids or thiazide drugs. In the case of the latter, a reactive alkalosis may result. Treatment focuses on replacement, such as oral salts, or a potassium-rich drip supplement.

Osmolality: reference range 275-295

This index measures the sum of all common ions in the blood, and can be determined mathematically (i.e., the sum of individual ions) and technically. A difference between the two is the osmolar gap, and if present implies the presence of another substance, which could be alcohol or a toxin such as glycol.

7.2: Water and homeostasis

Water is fundamental to all blood tests, as the concentration [C] of any substance is given by the mass (amount) [M] of that substance divided by the volume [V] of the fluid it is dissolved in (i.e., water in blood). The process of regulating the concentration of electrolytes (and much else) is homeostasis. In the clinical setting, however, the total mass and total volume of fluids are the difference between in (eating and drinking – M_{in} and V_{in}) and out (excreting – M_{out} as faeces and V_{out} as urine).

This leads to the equation….

$$\text{Concentration} = \frac{\text{Mass}_{in} - \text{Mass}_{out}}{\text{Volume}_{in} - \text{Volume}_{out}}$$

This means that the concentration of something is a balance and will be higher if there is more of it in a fixed volume, or if there is the same amount of it in a smaller volume. So, we must consider the state of hydration or dehydration of our patients. As we shall see, the amount of water in the blood is regulated by the kidney. Therefore, problems with this organ can lead to water retention (and thus, possibly, oedema), or excess water loss (V_{out}) as too much urine is made (diuresis: leading to dehydration). Water loss as excess urine is clear, but not so easy to account for is water loss via sweat, through the lungs (especially on hot days), and in faeces (especially in diarrhoea). These small and separate (by themselves) losses of water can add up to be considerable.

7.3: Intravenous (IV) fluids

It follows from much of the above that loss of fluid balance is important, and can be restored by IV fluids, usually in 500 ml or 1 litre bags. Fluids may also be given to replace losses that can be predicted. Water cannot be given by itself as it will destroy red cells, so must be given as 5% dextrose. Saline (0.9% NaCl) rehydrates plasma and lymph, but not cells, whilst plasma expanders replace fluid deficits in the blood alone. Most clinical situations can be managed with these three fluids, plus 1.26% bicarbonate (to treat acidosis) and concentrated supplements (such as potassium). However, misuse of IV fluids can, of course, cause the reverse of what is being treated, so that monitoring is crucial

Laboratory monitoring is by frequent measurement of serum electrolytes, but clinical assessment (such as pulse, blood pressure, body weight) can also be important. Measurement of fluids taken orally (by drinking) and the volume of urine produced is important, but these will not always add

up to 100% due to 'insensible' losses that are not easy to calculate (from sweat, lungs, and in faeces). Also, if someone is vomiting, this will also compound a possible state of dehydration.

Summary

- Overall, U&Es are often used to monitor renal function
- Consider the equation concentration = mass/volume of water
- Hypernatraemia may be due to decreased water intake, increased sodium intake, or decreased sodium intake with greater decrease in water intake. Rarely, it may be due to renal disease with impaired ability to excrete sodium.
- Hyponatraemia may be due to fluid retention or insufficient sodium, possibly resulting from a poor diet or excess excretion
- Hyperkalaemia can follow from renal failure, acidosis, or damaged cells, and can be life-threatening.
- Hypokalaemia may also follow renal disease, but also from gastrointestinal losses, alkalosis, and misuse of drugs such as thiazides
- Fluids can be rapidly replaced by IV infusion but must be adequately monitored and attention paid to renal functioning.

Because the physiology of much of this opening section relies on good kidney function, then this is often presumed, if not demanded. However, this is clearly not always the case and problems with U&Es are often the result of renal disturbances. Therefore, we now move on to look at this organ in more detail.

Key point: U&Es are the most-requested biochemical test, giving information on renal function and the state of the body's hydration

CHAPTER 8

INVESTIGATION OF RENAL FUNCTION

Key words:

Urea
Glomerular filtration rate (GFR)
Creatinine
Cystatin C

Key pathological expressions:

Acute renal injury
Nephrotic syndrome
Chronic kidney disease
Glomerulonephritis

8.1: An explanation of terms

Urea: reference range 3.3 – 6.7

Produced by the liver, this small molecule is an effective vehicle to remove excess nitrogen from the body

Creatinine: reference range 71-133

This amino acid, a breakdown product of muscle protein, is also rich in nitrogen

Glomerular filtration rate (GFR): reference range >90

This is the leading method for determining renal function. Previously measured by creatinine clearance, it is now reported as an estimate (i.e. eGFR) produced by an equation (the MDRD formula) requiring age, serum creatinine, sex, and whether of black race background.

Cystatin C: reference range 0.6-1.0

Being uninfluenced by muscle mass, this molecule is a more accurate marker of renal function than creatinine, and so the eGFR.

8.2: The kidney

Whilst the heart, brain and lungs are the primary concern in emergency care, the kidney is certainly the next organ in importance. This organ consists of millions of single functional units, nephrons (hence nephrology and nephropathy). The functions of the kidney can be summarised as follows:

- homeostasis: regulating the volume of blood by making urine (diuresis), in maintaining the acid/base balance (i.e., the pH, as may be assessed with hydrogen ions and bicarbonate ions), and in correcting the levels of electrolytes (sodium and potassium).

- endocrine responsiveness and activity: regulating blood pressure via local hormones, influencing the bone marrow in the production of red blood cells, and contributing to the level of serum calcium.

- excreting our metabolic waste products, principally the nitrogen-rich molecules urea, uric acid, and creatinine.

The top part of the mini-organ of the nephron is the glomerulus – the part that interfaces directly with the blood. It is an important filter: most of the good things in blood pass directly through it, and these are then later re-absorbed in the tubes and loop of Henle, so that the leftovers make up the urine. Hence, we are interested in the quality of the function of this small area of the nephron.

8.3: Tests of glomerular function

As mentioned, kidney disease is frequently accompanied by changes in U&Es, but especially in serum creatinine and urea. If the former slowly rises up to 150 µmol/L and beyond to, shall we say, 180, then renal function is deteriorating and the patient is increasingly in need of a referral to a renal physician. Creatinine itself has few physiological effects – it is most useful as a marker of renal function. However, rising urea is dangerous

as it can adversely influence the function of red cells and other cells. Levels may also rise because of blood dehydration.

An alternative test of the integrity of this organ is protein in the urine (proteinuria), which can be determined by dipsticks. We all lose a small amount of protein in this way (generally <30 mg in a 24 hour period), and this is physiologically acceptable. Nonetheless, if this loss becomes considerable then renal function must be deteriorating. An additional marker is cystatin C, although the ultimate test of kidney function is the rate at which blood is filtered by passing over the glomerulus to produce an early form of urine, i.e., the glomerular filtration rate (GFR). There are several free on-line calculators for these methods, some using levels of cystatin C. The practitioner must check with the local path lab to determine which to use.

Generally, as the creatinine rises, then the GFR falls. At the clinical level, the GFR can fall considerably (such as to 15–20% of normal), and the serum creatinine rises appreciably, before the patient becomes symptomatic.

8.4: The aetiology of renal disease

The most common causes of kidney problems can be grouped into three areas…

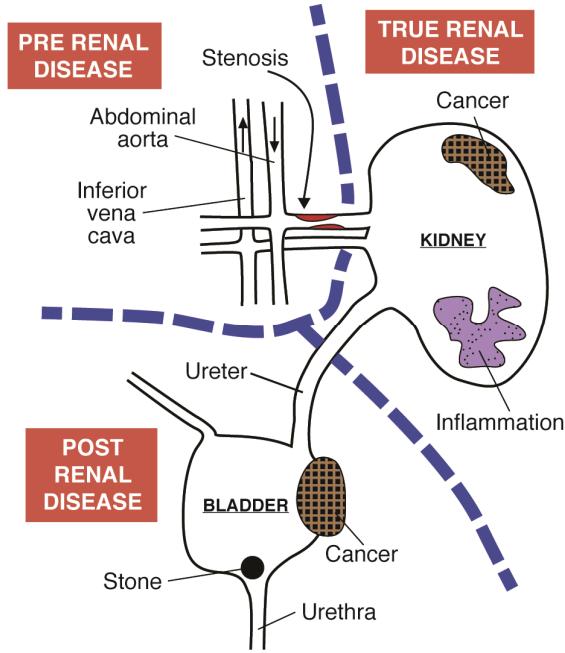
- Pre-renal disease: characterised by factors such as insufficient blood entering the kidney, which may be due to occlusive renal artery stenosis, abdominal aortic aneurysm, or poor cardiac output as may be present in heart failure.

- True renal disease is often seen in septic shock, glomerulo-nephritis (i.e. inflammation of the kidney), in the presence of toxins or amyloid, renal carcinoma (or secondary metastases), and in physical damage with blood loss. This state of the disease may be called acute tubular necrosis.

- Post-renal disease: present if there are problems downstream of the kidney, such as with the ureter, the bladder, or the urethra. Most common causes are kidney stones, carcinoma of the bladder or prostate, benign prostatic hyperplasia, or infections. All these limits or prevent urine from flowing out, so that it will eventually back up to the kidneys themselves.

Thus, in both pre- and post-renal disease, there is nothing intrinsically wrong with the tissues of the kidney itself, or its functioning: the problem is with the tubes at either end (figure 5). However, failure to correct pre- or post-renal disease will inevitably lead to acute tubular necrosis.

Figure 5: The aetiology of renal disease



See text for details

Management of renal disease depends on the particular aetiology and thus the correction of pre/post-renal factors, if appropriate. This may be, for example, antibiotics and immunosuppression for glomerulonephritis, angioplasty for renal artery stenosis, or surgery for bladder carcinoma. Biochemical monitoring will be by U&Es, and clinically by urine production. If the potassium rises dangerously, then dialysis may well be called for.

8.5: Acute renal injury (ARI)

This may be defined by the ratio of the relative rise in urea being greater than the relative rise in creatinine, not simply the levels themselves. Other biochemical abnormalities include acidosis (because the kidney can no longer excrete hydrogen ions) and hyperkalaemia. Of course, the patient is likely to be able to help in describing any changes in making urine. A likely story is of a sudden fall off, or even cessation, in diuresis. Recovery from ARI may be accompanied by a marked diuresis, with a massive increase in urine production (maybe as much as 4 litres a day!), so that fluid balance may need to be checked. Soon after, a return to normal diuresis can be expected.

However, if the damage to the kidney in ARI is excessive, and leads to acute tubular necrosis, it may well become permanently and irreversibly dysfunctional, in which case there will be deterioration to chronic kidney disease.

8.6: Chronic kidney disease (CKD)

CKD is the progressive and (invariably) irreversible destruction of kidney tissues and is typically noted when the eGFR falls below 60. Using U&Es, CKD can be plotted by the relative rise in urea compared to the rise in creatinine. However, unlike ARI, in CKD there is a greater increase in creatinine and a more modest rise in urea.

The consequences of CKD are not unlike those of ARI, principally with disturbances in sodium, hydrogen, and water metabolism – there may be fluid overload or fluid depletion. If present, a metabolic acidosis will be evident with a low level of bicarbonate, and this may also contribute to hyperkalaemia. However, this may independently arise from the increasingly impaired ability to excrete potassium and may be life-threatening. Low levels of calcium, and hence raised parathyroid hormone, may be present as the kidney fails to promote calcium absorption in the intestines. This is due to the kidney's essential part in the synthesis of vitamin D. Failure to help generate this essential metabolite will result in weak bone structure due to hypocalcaemia. In the child this leads to rickets, in the adult to osteomalacia and then renal osteodystrophy.

Urate (the ionised form of uric acid, dependent on pH) is mostly breakdown products of the synthesis of DNA and is excreted by the kidney and in the gut. High levels can arise from increased production (maybe 10% of cases) or decreased excretion (perhaps present in 90%). Thus, with poor excretion, levels in the blood will rise. Various drugs can also cause an increase in levels of this metabolite. Urate is particularly insoluble, so that when levels rise, they will form crystals (e.g. in the synovial joint) and stones (e.g. in the kidney), leading to further problems such as swelling, pain and inflammation, and ultimately to an arthritis (often gout, where there can also be deposits in the skin). Risk factors for hyperuricaemia are alcoholism, use of diuretics, obesity, renal disease, and heart failure.

Clinical features of CKD also include nocturia (resulting from uneven diuresis) and hypertension. Good management will address sodium and water intake, and diuretics may be necessary (depending on the degree of renal (dys)function). Hyperkalaemia may be managed with Resonium A and a low protein diet may help in minimising nitrogen, and thus the need to excrete it as urea and creatinine.

8.7: Management of renal disease

Wherever possible, the cause of the disease must be determined and addressed (Figure 5). ARI must be addressed urgently, and is reversible, treatment depending on aetiology, such as immunosuppression for inflammation. Although CKD is essentially irreversible, its advance can be retarded by treatment of risk factors such as high blood pressure (Table 11). Those with proteinuria, diabetes and microalbuminuria need to safely maintain their systolic blood pressure within a target range 120–129 mmHg and their diastolic blood pressure below 80 mmHg.

Treatment and care of CKD is therefore conservative, and as renal function slowly deteriorates the patient should be prepared physically and psychologically for dialysis, which is generally needed when the GFR falls to about <25 mls/min. The remaining treatment is transplantation.

Table 11: Stages of Chronic Kidney Disease

Stage	eGFR	Description and management
I	>90	Normal renal function: control whatever cardiovascular risk factors are present
II	60 - 89	Mildly reduced renal function. The stage should not be diagnosed on eGFR alone, but urinalysis, structural abnormalities or genetic factors may be considered. Observe and control cardiovascular risk factors
IIIa	45 - 59	Moderate decrease in renal function, with or without other evidence of kidney damage. More stringent control of cardiovascular risk factors. Consider low dose statin regardless of serum cholesterol, and an ACEI/ARB regardless of blood pressure, target <140/90
IIIb	30 - 44	Marked decrease in renal function, with or without other evidence of kidney damage. Statin and ACEI/ARB likely to be advisable. Check haemoglobin to identify anaemia. Blood pressure target <135/85
IV	15 - 29	Severely reduced renal function. Prophylactic pharmacotherapy mandatory. Measure calcium, phosphate and PTH. Plan for end stage renal disease
V	<15	Very severe (end-stage) renal failure. Preparation for dialysis or transplant.
ACEI = angiotensin converting enzyme inhibitor: ARB = angiotensin receptor blocker: PTH = parathyroid hormone. Blood pressure targets are lower in cardiovascular disease, SLE and diabetes.		

NICE and renal disease

As with other conditions, there are numerous NICE publications, such as NG148, that focusses on AKI, and NICE CG182 on the management of CKD, which also places importance on protein in the urine (proteinuria, detectable with dipsticks), although a better marker of renal damage in the ratio of albumin to creatinine in the urine (hence uACR). In non-diabetics the uACR can be used to direct the use of angiotensin converting enzyme

inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) if the uACR is >30 mg/mmol. However, in diabetics, microalbuminuria (uACR >2.5 mg/mmol in men and >3.5 mg/mmol in women is clinically significant. NG28 on diabetes and NG136 on hypertension are also applicable.

Anaemia may develop as the impaired kidney will fail to produce sufficient erythropoietin; a growth factor required by those stem cells responsible for red blood cell production: NG8 advises on this problem. NG176 offers advice on management of CKD in view of the COVID-19 pandemic.

Websites

eGRF calculators: http://nephron.com/cgi-bin/CGSI.cgi , and
https://patient.info/doctor/estimated-glomerular-filtration-rate-gfr-calculator ,
https://www.nhs.uk/conditions/kidney-disease/
https://www.kidney.org/atoz/content/about-chronic-kidney-disease

Summary

- Although urea and creatinine are the cornerstones of the assessment of renal function, we also consider sodium and potassium.
- Increasing levels of potassium can be life-threatening,
- The gold standard test of renal function is the estimated glomerular filtration rate
- Acute renal injury is often associated with a greater rise in urea than the rise in creatinine.
- Chronic kidney disease is frequently characterised by a greater rise in creatinine than the rise in urea. There may also be anaemia and hypocalcaemia.
- Other features of chronic kidney disease include raised urate, so that a gouty arthritis may develop, and renal osteodystrophy, resulting from failure to participate in the vitamin D pathway.

Case study 5

A man, aged 75, describes making less and less urine over a period of a few days, and complains to his General Practitioner of something not right 'down there', with occasional pain. The doctor, feeling a hard mass in the lower abdomen, sends a venous sample of blood to the local District General Hospital, with the following results.

	Result (Unit)	**Reference range**
Na	140 mmol/L	135–145
K	5.0 mmol/L	(3.8–5.0)
Urea	56.5 mmol/L	(3.3-6.7)
Creatinine	354 µmol/L	(71-133)
eGFR	16 ml/min/1.73m^2	(<90)
Osmolality	285 mOsmol/Kg	275-295

Clue: Compare the rise in urea to the rise in creatinine.

Interpretation

Abnormal results are raised urea and creatinine, pointing to a problem with the kidney. An osmolality result within its reference range denies high concentration of any unknown substances in the blood. The increase in urea is about 10 fold over the middle of the normal range, whilst the increase in creatinine is only about 4 times. Thus, the relative rise in urea is over twice the relative rise in creatinine, pointing to an initial diagnosis of an acute renal problem. This is supported by the clinical history and anuria. The eGFR is profoundly low which, if chronic, would lead to the very dangerous CKD Stage IV.

Diagnosis may be confirmed with imaging (ultrasound) and we can expect a full bladder. A most likely cause of this is blockage of the urethra, maybe by a kidney stone or stones. There seems to be no infection, and a tumour alone would be unlikely to produce such an acute picture.

Key point: Whilst U&Es are important, many practitioners refer simply to the eGFR as the major index of renal function

CHAPTER 9

INVESTIGATION OF LIVER FUNCTION AND PLASMA PROTEINS

Key words:

Liver function tests:	Bilirubin
	Alkaline phosphatase (Alk Phos, or ALP)
	Gamma glutamyl transpeptidase (Gamma GT)
	Alanine aminotransferase (ALT)
	Aspartate aminotransferase (AST)

Key pathological expressions:

Acute hepatic failure	Cholestasis
Chronic hepatic failure	Hepatitis
Jaundice	

9.1: An explanation of terms: the liver function tests (LFTs)

Bilirubin: reference rage <17

When red blood cells come to the end of their life, they are broken down, and the proteins and iron in haemoglobin are recycled. However, the haem molecule is too complex to be recycled, and passes to the liver to be broken down to bilirubin. It is then excreted via the gall bladder and bile duct and so into the intestines, from where some is reabsorbed. Some bilirubin is bound to glucuronide to form conjugated bilirubin, some is unconjugated, and there are several other related metabolites (e.g., bile acids).

If the liver is unable to perform these metabolic functions, perhaps because it is overwhelmed, or that it is damaged, levels of bilirubin in the blood rises, and may lead to jaundice. This classic clinical sign is characterised

by a yellow colouring, most evident in the sclera of the eye and the palms of the hand, and is caused by high serum bilirubin, generally greater than 40 μmol/L. Therefore, there is a large gap between the top end of the reference range (21 μmol/L) and the most common presenting (visual) sign. However, people with a developing jaundice often have abdominal discomfort, lack of appetite, lethargy, tiredness, and general non-specific illness for several days before the jaundice becomes evident. The aetiology of jaundice is most variable.

Alanine aminotransferase (ALT): reference range 5-42

Alkaline phosphatase (Alk Phos, or ALP): reference range 20-130

Aspartate aminotransferase (AST): reference range 10-50

Gamma glutamyl transpeptidase (Gamma GT): reference range 5-55

These four enzymes catalyse a number of reactions involved in intermediate metabolism. They are found within or at the surface of cells of a number of different tissues, but in the highest levels are present in liver cells. Nevertheless, their presence in these non-liver tissues brings the need for caution in interpretation. For example, Alk phos is also present in bone and the placenta, whilst AST and ALT are released by muscle cells following damage/injury, so may be raised after a myocardial infarction.

9.2 Liver function

Just as a single unit of the kidney is the nephron, a single unit of the liver is a hepatocyte (hence hepatitis [inflammation of the liver] and hepatoma [cancer of the liver]). Like the kidney, the liver has a number of functions, but hepatic function is more diverse.

Metabolic activity

The liver synthesises a large number of different proteins, many destined for ‘export’ to the blood. Examples of these include albumin, ferritin, transferrin, complement components, fibrinogen, C-reactive protein (CRP) and cholesterol. So, if the levels of these proteins fall, we consider failure

of production. Alternatively, low levels may arise from excess consumption by metabolic processes, and rarely in malnutrition.

Storage

The liver converts glucose and other carbohydrates into the storage compound glycogen, and it also stores iron in ferritin and haemosiderin, and certain vitamins.

Detoxification

This important metabolic function rids the body of dangerous substances such as plant and animal fungal toxins, paracetamol, and alcohol. It is also where pharmaceutical drugs and anaesthetics are broken up and rendered ineffective.

Although strictly not a liver function test, alpha-feto protein (AFP) is raised in 80–90% of primary liver cancers (hepatoma). Levels are generally not raised when the liver carries metastatic cells from a distant primary tumour. Liver cancers, of whatever aetiology, generally display raised gamma GT, Alk phos, and bilirubin.

9.3: Types of Liver Disease

As with renal disease, we can consider pre-, true-, and post-liver disease (Figure 6).

Pre-liver disease

As mentioned, when red blood cells come to the end of their lifespan they are removed from the circulation and broken up. Un-recycled bilirubin remains in the blood and is eventually cleared, mostly by the liver, and some by the kidney. However, if there is excessive haemolysis, then the liver is unable to keep up purifying the blood of the high levels of bilirubin, then levels rise and can cause jaundice. Thus, there is nothing intrinsically wrong with the liver itself, it just can't keep up with the high levels of bilirubin resulting from excessive red cell destruction.

True liver disease

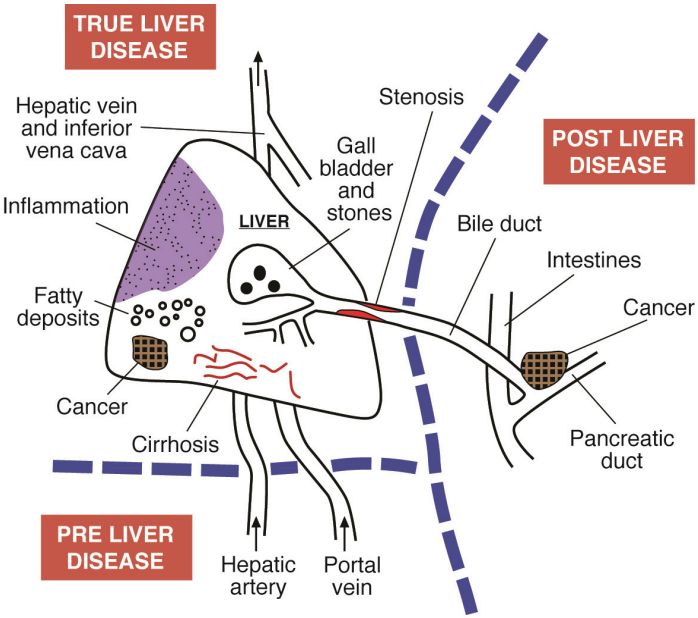
True damage to the hepatocyte (i.e., hepatocellular damage) is often seen cases of poisoning by inhaled solvents in paint spray, some overdoses, or

in viral hepatitis. This would initially be characterised by increases in the aminotransferases ALT and AST, and later by rising bilirubin as the excretory function of the liver deteriorates.

Post-liver disease

A good example of this form is obstruction or stenosis of the bile duct by factors such as gall stones, or inflammation or tumour of the head of the bile duct or pancreatic duct (cholestasis). Thus, again, the initial problem is not the tissue of the liver itself, but with the associated plumbing. However, as with pre- and post-renal disease, failure to act on pre- and post-liver disease will eventually lead to true liver disease.

Figure 6: Types of liver disease



See the text for details

9.4: Aetiology of liver disease

Once again, as with renal disease, we consider the speed of onset. An important differential diagnosis to 'liver' signs and symptoms is pancreatic disease, notably pancreatitis. In this respect, the digestive enzyme amylase is useful, serum levels being increased in this condition.

Acute liver disease

By definition, onset is rapid and can generally be ascribed to a single factor or event. Good examples are poisoning (e.g., organic solvents, carbon tetrachloride, herbicides), drug overdose (e.g. barbiturates, paracetamol), septic shock, severe hypoxia, and acute cardiac failure. In such cases, rising levels of AST and ALT are frequently seen, followed maybe days later by bilirubin. An alternative scenario is sudden blockage of the bile duct by a gallstone (i.e., cholestasis). This would be more likely to be characterised by rising Alk phos and Gamma GT. In some cases, viral hepatitis can be traced to a precise (infective) event.

Chronic liver disease

Again, by definition, we suspect a slow and insidious disease process, although it may also have a precise defining event, and can follow from acute disease. For example, a hepatitis virus infection may be partially treated in the short term, with a modest return of normal liver function, but chronic active hepatitis may result. A further example is cholestasis resulting from a slowly developing blockage, such as a tumour, which the surgeons may be able to treat, although, like chronic renal failure, the chronic liver disease is generally irreversible.

Other common causes of chronic liver disease are alcoholism, fatty liver, and the auto-immune disease primary biliary cholangitis. However, whatever the aetiology, there is rising plasma markers of liver function, although in the terminal stages levels of some will fall as the liver is unable to synthesise them: hence a risk of bleeding due to insufficient clotting proteins. Bilirubin will continue to rise and can be treated by dialysis, although this is clearly only palliative. The only effective treatment is transplantation, although this is, of course, by no means 100% successful.

9.5: Plasma proteins

This short section is relevant here as the liver produces many plasma proteins, others being produced by diverse cells and tissues. The laboratory offers total plasma proteins, and albumin, the latter generally making up about half of all the proteins. The remaining half is made up of dozens of other proteins (such as CRP and the immunoglobulins).

Total proteins: reference range 60-80

As mentioned above, this global score assesses all plasma proteins. Raised total proteins can therefore (in theory) be due to any individual protein, although in practice the only protein to do this is increased levels of immunoglobulins as may be present in an infection, or in a myeloma. Decreased total protein is almost always due to hypoalbuminaemia.

Albumin: reference range 35-50

This molecule is important for a number of reasons, e.g., being a carrier of calcium, bilirubin, and various other substances. Low levels of albumin will therefore have other consequences. Another indication of the importance of albumin is in the maintenance of tissue fluid: low albumin will be a contributing factor in oedema. Hypoalbuminaemia may be due to poor liver function, as it arises in the liver, or in cases of inadequate nutrition or malabsorption or severe nephrotic syndrome, where kidney damage is so great that albumin is lost in the urine (proteinuria).

Globulins: reference range 20-25

The principle member of this group are the immunoglobulins (IgG, IgM, IgA and IgE). Increased levels are expected in infections, reduced levels in immunodeficiency, but high levels may also be expected in myeloma.

C-reactive protein (CRP): reference range <5

This marker of inflammation, like ESR, is typically raised in autoimmune diseases, and in bacterial infections. It will therefore often go together with a raised WBCC. Produced by the liver by the same process as causes pyrexia, it is frequently described as an acute phase protein.

Other plasma proteins

The remaining contributors to total plasma protein pool are numerous individual proteins, present at relatively low levels in health, and many of which we have already come across (table 12). Several are cancer markers, although some are neither fully sensitive and specific for any one cancer or disease. For example, prostate specific antigen may be increased in benign prostatic hyperplasia as well as in prostate cancer, whilst CA-125 may be raised in endometriosis, liver cirrhosis and uterine fibroids.

Table 12: Functions of plasma proteins

Protein	**Function**
Amylase	pancreatic digestive enzyme
Ferritin	iron storage
Fibrinogen	coagulation
Insulin	glucoregulatory hormone
Lactate dehydrogenase	marker of cell damage
Prothrombin	coagulation
Transferrin	iron transport
Cancer marker	**Cancer marked**
Alpha-feto protein (AFP)	liver
CA-125	ovary
Carcinoembryonic antigen (CEA)	Several cancers, but most often colorectal
Prostate specific antigen (PSA)	prostate

Websites

https://www.nhs.uk/conditions/liver-disease/
https://www.mayoclinic.org/diseases-conditions/liver-problems/symptoms-causes/syc-20374502
https://www.cdc.gov/hepatitis/abc/index.htm

Summary

- The aetiology of liver disease, like that of the kidney, considers pre-, true- and post-hepatic causes.
- LFTs include alkaline phosphate, bilirubin, gamma GT, ALT and AST, used to diagnose and investigate conditions such as jaundice.
- Common causes of acute liver disease include cholestasis and viral hepatitis. Chronic liver disease often arises from alcoholism, primary biliary cirrhosis, or from acute hepatic damage.
- Total proteins and albumin are also important in assessing liver function. Low levels may be due to impaired synthesis and/or malnutrition.
- Many plasma proteins have identifiable functions as hormones, enzymes, or as cancer markers.

A Cautionary Tale – 2

Several months after marriage, a young woman developed an irreversibly rapid and fulminant jaundice due to a hepatitis virus infection, that progressed to acute liver failure and so death. The husband subsequently remarried, only to find his second wife suffering almost exactly the same fate. The unfortunate widower identified a third possible spouse, but during their courtship she also became jaundiced, although this time the hepatitis was not fatal.

Investigations into the man found him to be an asymptomatic hepatitis B virus carrier with all liver function tests mildly abnormal. Although clearly (with hindsight) inoculating his three sexual partners with semen loaded with hepatitis B virus, the compassionate nature of the case was clear and the authorities decided against charging him with manslaughter.

From a letter to a learned Journal during the 1980s

Case study 6

A male, aged 68, reports being increasingly tired and lethargic over a six months period, with weight loss, despite his wife's attempts to 'feed him up'. More recently, he complains of abdominal discomfort. Blood results are as follows (normal values in brackets):

	Result (Unit)	**Reference range**
Na	138 mmol/L	(135–145)
K	4.0 mmol/L	(3.8–5.0)
Urea	6.1 mmol/L	(3.3-6.7)
Creatinine	89 µmol/L	(77-133)
eGFR	78 mls/min/1.73m^2	(<90)
Total Protein	60 g/L	(60–80)
Albumin	28 g/L	(35–50)
Alk phos	190 IU/L	(20–120)
AST	89 IU/L	(19-50)
Bilirubin	22 µmol/L	(<17)
ALT	72 IU/L	(5-42)
Gamma GT	215 IU/L	(5-55)
CRP	2.0 mg/L	(<5)

Interpretation

There are no abnormalities in the tests of renal function, so we can discount problems in this organ. However, all five liver function tests are abnormal, so this organ is clearly under suspicion. The low total protein and albumin also indicate liver dysfunction. No increase in CRP points to a lack of an on-going inflammatory response.

Additional tests would include hepatitis virus screening (A, B and C), with ultrasound imaging to check the integrity of the gall bladder. A full blood count would also be in order. The immunology laboratory will help with considering liver disease such as primary biliary cirrhosis. Additional questions to the patient may reveal use of alcohol (although, of course, this may not always be reliable). Levels of lipids may also help, as with non-alcoholic steatohepatitis (fatty liver).

CHAPTER 10

CARDIOVASCULAR DISEASE AND ITS RISK FACTORS

Lead leading cause of this chronic condition is atherosclerosis, is characterised by damage to the blood vessel wall followed by thrombosis and hypertension. The late stages of stroke, myocardial infarction, and critical limb ischaemia (of the leg) are almost always preceded by warning signs of transient ischaemic attack, angina, and intermittent claudication, respectively. However, clinical disease is generally preceded by well-known risk factors: diabetes, smoking, hypertension, and dyslipidaemia. The latter has replaced hypercholesterolaemia, as this incorrectly implies all forms of cholesterol are bad (as we shall see).

There are no direct routine laboratory tests in hypertension, but some tests can assess smoking status, although the laboratory is likely to resist such a request. This leaves diagnosis of diabetes and hyperlipidaemia, and in the monitoring of treatment.

10.1: Diabetes mellitus

Key words:

Insulin
Glycated haemoglobin (HbA1c)
HOMA
Fasting plasma glucose
Oral glucose tolerance test (OGTT)

Key pathological expressions:

Diabetes mellitus
Hyperglycaemia
Impaired glucose tolerance
Diabetic ketoacidosis
Impaired fasting glycaemia
Hypoglycaemia

An explanation of terms

Insulin: reference range 2-10

This hormone, a product of the β-cells of the pancreas, regulates the passage of glucose into the cell. Its absence leads to type 1 diabetes, the body's resistance to it leads to type 2 diabetes.

Blood glucose: reference range fasting 3.3 – 5.5

N.b. blood glucose is perhaps the only routine biochemistry blood test that is not performed on clotted blood and needs to be taken into the anti-coagulant FLOX (check with your laboratory).

Because glucose levels in the blood vary considerably during the day, the timing of the blood test is crucial, with lowest levels in the morning before eating breakfast. Hence the importance of a FASTING sample. The alternative is a random sample. Therefore, random and fasting samples are very different and so have different normal (target) ranges. Plasma levels also give different results from whole blood levels, so that blood from the vein is different from capillary blood from a fingerprick. As always, check with the laboratory.

Glycated haemoglobin (HbA1c): reference range <48

Hyperglycaemia is not benign: it is not simply a situation of too much sugar in the blood. High sugar sticks to other proteins and cells and influences their function. One such interaction is with the haemoglobin within red blood cells, some proportion of which binds the glucose, hence is described as glycated haemoglobin, called in the laboratory HbA1c. As the glucose stays on the haemoglobin, then it remains there for the entire life of that red cell. Consequently, increased HbA1c indicates poor long-term control of the hyperglycaemia, and as such is useful in checking the effect of treatment over a 3 month period. It follows that a high HbA1c may also be taken by many as strongly indicative of diabetes.

n.b. the unit of mmol/mol has the advantage for the presentation of HbA1c as a % (reference range <6.1%) as it adjusts for the total haemoglobin result, and so any potential anaemia.

The oral glucose tolerance test (OGTT)

Many suggest a more dynamic view of an individuals' response to glucose is a better indicator, the leading method being the OGTT. Here, the fasted subject arrives at the laboratory at perhaps 9.00 a.m., gives a blood sample, and then drinks 75g of glucose. Additional blood sample may be taken up to 3 hours, and levels of glucose in the blood are plotted against time. However, many laboratories often simply take a baseline and 2-hour sample. A healthy response sees levels rise to a peak of less than 7.8 mmol/L at 2 hours, after which time levels fall. The response of a diabetic will be increasing glucose from a high fasting level (i.e., greater than 6.1) that rises to peak at over 11 mmol/L (figure 7). Such a response is required on two separate occasions for a firm diagnosis of diabetes.

However, some results do not fall into either of these scenarios. In some, levels may rise rapidly, and exceed the magic 11 mmol/L, but then fall rapidly back into the normal range. Alternatively, levels may not exceed 11 mmol/L, but scarcely fall at all. These and other data demand expert interpretation, and tentative diagnoses of impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) may be offered. These are certainly not diabetes but could be a risk factor for classical diabetes at some time in the future. Therefore, regular monitoring is required.

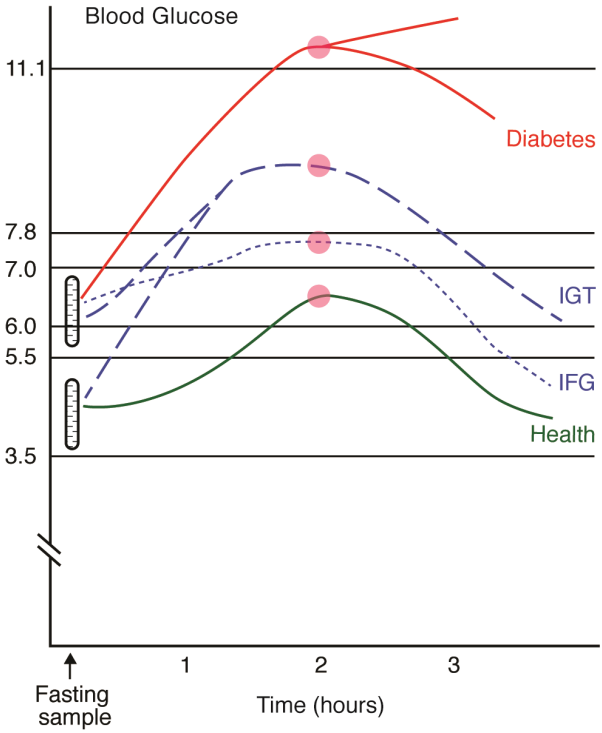
- IGT may be defined as a fasting glucose less than 7.0 mmol/L and a 2 hours glucose between 7.7 and 11.1 mmol/L.

- IFG may be defined by a fasting glucose between 6.0 and 7.0 mmol/L and a 2 hour result less than 7.8 mmol/L.

The homeostasis model assessment (HOMA)

In some circumstances additional knowledge of the state of the patient's glucose metabolism is needed. The HOMA model (now HOMA2) uses paired fasting insulin and glucose concentrations to give data on the declining function of the β-cells and the increase in insulin resistance. Data can be provided by a free on-line service (https://www.dtu.ox.ac.uk/homacalculator/)

Figure 7: The oral glucose tolerance test



The four lines represent changes in blood glucose over the course of the challenge. IGT = impaired glucose tolerance, IFG = impaired fasting glucose

The pathology of diabetes

This condition can arise without an obvious cause (i.e., primary) or may be secondary to factors such as obesity, drugs, pancreatic disease, or Cushing's syndrome. It is the most common endocrine disorder, characterised by hyperglycaemia due to lack or dysfunction of insulin. Complete lack of insulin leads to insulin dependent diabetes mellitus (IDDM, about 10% of diabetics, often occurring in the young), whilst insulin resistance is the basis of non-insulin dependent diabetes mellitus (NIDDM, making up 90%, generally (but not always) arising in later life

and associating with obesity). As more than 50% of adults are overweight (e.g., body mass index over 25) then there is a considerable amount of occult or pre-diabetes.

The pathology of diabetes is established: early hyperglycaemia with symptoms such as polyuria, polydipsia, and weight loss lead to microvascular disease (retinopathy, neuropathy, and nephropathy) and then to atherosclerosis, characterised symptomatically by conditions such as coronary artery disease. Naturally, there is great variety in presenting symptoms and rate of progression from person to person.

Diabetic ketoacidosis (DKA)

This clinical situation is found in cases of severe hyperglycaemia and represents probably the most important crisis a diabetic can face. It is characterised by a cycle of insulin resistance and/or deficiency, with hyperglycaemia, acidosis, and dehydration. Pathophysiologically, two overlapping cycles exist.

The first centres on hyperglycaemia, which invariably leads to glycosuria. It is not enough simply to excrete glucose in urine – it must be excreted with a great deal of water (thus the symptoms of polyuria) leading eventually to dehydration. The consequences of loss of blood volume include hypotension, shock and pre-renal uraemia resulting from a fall in the glomerular filtration rate. This, in turn, leads, via several complex hormones and factors, to insulin deficiency and/or resistance, thus back to hyperglycaemia.

Changes in the 'several complex hormones and factors' also lead to the second part of the cycle. These, alongside insulin problems, can lead to changes in lipid metabolism and the mobilisation of fatty acids from adipose tissues into the blood. Ketone bodies rise, and these can lead to acidosis with an associated hyperkalaemia. This promotes nausea and then vomiting, and so loss of fluids and eventual dehydration.

The laboratory can help in diagnosis, investigation, and treatment of DKA. There are various opportunities to intervene in this cycle, with intravenous fluids to treat dehydration, and insulin to get glucose out of the blood and into cells. This hormone will also help the hyperkalaemia. If the acidosis is severe, then a bicarbonate drip can be used, which may also help address the high potassium.

Hypoglycaemia

Defined by a glucose level less than 2.8 mmol/L, 99% of hypoglycaemic attacks are in patients with IDDM. Cognitive disturbances are common when levels fall below 2.0 mmol/L, followed by ECG changes, coma, and death. Causes include reduced intake of carbohydrates, over-use of insulin, excess exercise, or excess consumption of alcohol. Treatments include oral glucose, chocolate-rich confectionary or jam, or intra-muscular injections of the hormone glucagon. However, if the patient is unconscious intravenous glucose or dextrose will be required.

10.2: Dyslipidaemia

Key words

Total cholesterol
LDL-cholesterol
Triglycerides
HDL-cholesterol
Total/HDL cholesterol ratio

Key pathological expressions

Dyslipidaemia
Hypertriglyceridaemia
Hypercholesterolaemia

An explanation of terms

Total cholesterol: reference range <5.0*

This molecule is of interest as it a major constituent of atheroma, a growth that narrows arteries leading to cardiovascular disease (CVD). It is composed of two forms, dependent on density, i.e., high and low. Broadly speaking, high levels of cholesterol (i.e., hypercholesterolaemia) are linked to morbidity and mortality.

Triglycerides: reference range <1.7*

The second form of lipid is built from and fatty acids. However, the degree which it is linked to CVD has been disputed for decades. Nevertheless, guidelines recommend high levels are treated.

HDL-cholesterol: reference range >1.2

This form is often described as the 'good' cholesterol, as high levels are protective of CVD.

LDL-cholesterol: reference range <3.0*

Conversely, high levels of this form are more closely linked to risk of CVD than are levels of total cholesterol. However, blood LDL cannot be measured directly, but has to be calculated from an equation that needs a fasting triglyceride result.

Total/HDL cholesterol ratio: reference range <4.2*

This ratio is popular as it does not need to be fasting and is required by a leading calculator of the risk of CVD.

*As with high blood pressure, the targets for the treatments of lipids are lower in the presence of other disease, especially diabetes and CVD.

The pathology of lipids

As with diabetes, hypercholesterolaemia can be primary and secondary: the latter may be due to a poor (fat-rich) diet (with or without obesity), hypothyroidism, alcoholism, chronic renal failure or, indeed, to diabetes. The most common primary cause of raised lipids is genetic. Familial hypercholesterolaemia is the most common, but other include familial hypertriglyceridaemia (raised triglycerides) and familial combined hyperlipidaemia (raised cholesterol and triglycerides).

High levels of total and LDL-cholesterol, and low HDL-cholesterol, are important as predictors of CVD, but they are not, of course, the only such predictors – others include high blood pressure, age, family history of CVD aged <60, male sex, smoking and diabetes. These factors can be entered into a complex formula that hopes to predict and thus identify those at high risk of an adverse event (heart attack or stroke) in the next ten years. Lipid-lowering therapy, such as a statin or ezetimibe, can then be more accurately targeted. One of the most popular equations for determining the risk of CVD is that of QRISK: https://www.qrisk.org/three/index.php

10.3: Cardiovascular disease (CVD)

The three components of CVD are heart disease, cerebrovascular disease, and peripheral artery disease (PAD), the leading pathology being atherosclerosis. There are no specific laboratory tests for the presence of cerebrovascular disease (mostly stroke), or PAD (as may be suggested by intermittent claudication and critical limb ischaemia). However, there are blood tests for the consequences of leading cause of heart disease, that being atherosclerosis of the coronary arteries as is very likely to be present following a myocardial infarction.

Key words

Creatine kinase (CK) CK-MB
Troponin

Key pathological expressions

Angina Acute coronary syndrome(s)
Myocardial infarction

An explanation of terms

Creatine kinase (CK): reference range 55-170

The heart is a heavily modified muscle, so that enzymes present in damaged muscle can be expected to be raised after a heart attack. Such an enzyme is CK. However, CK is also present in skeletal muscle, so that raised levels may also arise from damage to these tissues, such as may be present following heavy exercise (like running a marathon) or in the muscle disease polymyositis.

CK-MB: reference range <25

This is a variant of total CK that is much more specific for heart muscle, it being absent from leg muscle, and so is more useful.

Troponin I: reference range <4

An even more specific marker of myocardial damage is troponin I, another is troponin T. These are not enzymes but actual heart muscle protein, and

so are of even more precise specificity. However, Table 13 provides examples of conditions where there are raised levels in the absence of a heart attack.

Table 13: Indications of raised troponins

Cardiac disease and interventions: Cardiac amyloidosis, cardiac contusion, cardiac surgery, cardioversion and implantable cardioverter defibrillator shocks, closure of atrial septal defects, coronary vasospasm, dilated cardiomyopathy, heart failure, hypertrophic cardiomyopathy, myocarditis, percutaneous coronary intervention, post-cardiac transplantation, radiofrequency ablation, supraventricular tachycardia,

Non-cardiac diseases: Critically ill patients, high dose chemotherapy, primary pulmonary hypertension, pulmonary embolism, renal failure, subarachnoid haemorrhage, scorpion envenoming, sepsis and septic shock, stroke, ultra-endurance exercise (marathon).

Source: British Medical Journal 2004, 328: 1028–9.

Investigation of coronary atherosclerosis

Coronary artery disease may be described in terms of a spectrum that progresses from minimal atheroma/stenosis to a full blown heart attack. Intermediate stages include stable and unstable angina, both being parts of the acute coronary syndromes. Key investigations are an ECG and the blood markers, increases in the latter developing over hours after a presumed infarction, so that serial measurements will be required. The patient's history is also vital, as it may point to the exact time of the chest pain. CK-MB peaks soon after muscle damage, troponins several hours later. 'Liver' enzymes such as ALT and AST may also rise after an acute myocardial infarction.

A potential late consequence of coronary atherosclerosis is heart failure, although this may be a consequence of hypertension. If present, the blood marker brain natriuretic peptide (BNP) will be raised.

NICE and cardiovascular disease

NICE regularly publishes guideline on the diagnosis and management of diabetes (NG3, NG17, NG28, PH35 and PH38), dyslipidaemia (CG71 and KTT3) and cardiovascular disease (CG181, PH15 and PH25).

Websites

http://www.diabetes.org.uk
http://www.evidence.nhs.uk/topic/hypercholesterolaemia
http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001246/

Summary

- There are no laboratory tests specific for stroke, atherosclerosis of the arteries of the legs or hypertension. The laboratory is most unlikely to agree to test for smoking.

- Diabetes can be diagnosed and monitored with fasting glucose and HbA1c; the OGTT is also useful, especially in clarifying the diagnosis and considering impaired glucose tolerance and impaired fasting glycaemia.

- Total cholesterol and HDL-cholesterol can be measured on a random sample; LDL-cholesterol is calculated from a formula that demands a fasting triglyceride.

- Creatine kinase (CK), CK-MB and troponins are used to help diagnose myocardial infarction, BNP for heart failure.

Key point: Circulatory disease was the second most-frequent cause of death in England and Wales in 2020, with over 132,000 fatalities.

CHAPTER 11

CALCIUM, BONE, AND MUSCULO-SKELETAL DISEASE

Key words:

Calcium	Parathyroid hormone (PTH)
Phosphate	Albumin
Alkaline phosphatase	Creatine kinase (CK)

Key pathological expressions:

Hypercalcaemia	Hypocalcaemia
Osteoporosis	Paget's disease
Rickets/Osteomalacia	

11.1: An explanation of terms

Calcium: reference range 2.2 – 2.6

This is the most abundant mineral in the body, and 99% of it is in bone, where it forms calcium hydroxyapatite, a tough and dense quasi-crystalline structure. However, ionic calcium is also required as a co-factor in many enzymes, and for more complex processes such as coagulation, muscle contraction and nerve conduction. Blood levels are regulated by the kidney, and by parathyroid hormone.

Parathyroid hormone (PTH): reference range 1 – 6

A product of the parathyroid gland in the neck, this hormone increases plasma calcium by mobilising bone, by instructing its reabsorption by the kidney, and by prompting the intestines to increase the absorption of calcium from the digesting food. A feedback system operates in those high levels of plasma calcium feed back to the parathyroid gland to suppress the release of PTH.

Phosphate: reference range 0.7 – 1.4

About 85% of body phosphate is in the bone, and there is a marked diurnal effect. It has a ubiquitous and essential presence, being part of numerous metabolic pathways, processes, and molecules, such as ATP. Levels are often reciprocal with calcium.

Albumin: reference range 35-50

Creatine kinase (CK): reference range 55 - 170

Alkaline phosphatase (Alk phos): reference range 20 – 120

These molecules have been described in section 8.5, sections 6.2 and 9.3, and section 8.1 respectively. Albumin is a carrier of calcium, CK may arise from damaged muscle cells, and Alk phos is present in bone.

11.2 Calcium metabolism

About half of our plasma calcium circulates bound to albumin, and it is the unbound balance, the 'free' or ionised calcium, that the parathyroids respond to. This process is pH dependent, so that in acidosis the ionised calcium increases and conversely at an alkalotic pH, it falls. Consequently, in acute respiratory alkalosis, tetany (involuntary muscle contraction) may occur due to the sudden decrease in ionised calcium.

The laboratory measures total calcium, i.e., free and bound, so that interpretation is required. If albumin is low, total calcium may be low as a result, but the free calcium will frequently be satisfactory. Thus, such a situation is not true hypocalcaemia. If there is indeed low (total) calcium and low albumin, then a calculation is required, and the lab will do this for you, reporting adjusted calcium.

Like almost all analytes, we consider the implications of levels above and below the reference range.

Hypercalcaemia

Present in perhaps 5% of in-patients, most cases of high plasma calcium can be explained by a small number of causes:

- Primary hyperparathyroidism results in high levels of PTH that in turn cause the high blood calcium levels. This, in turn, may result from a tumour of the parathyroid gland that secretes PTH.

- Malignancy: most often a tumour secreting a PTH-like protein, or else a tumour (such as a myeloma or secondaries from a breast carcinoma) that has invaded and mobilises bone to make more space for itself, resulting in calcium release from bone and its movement into the blood. In such a case there may well be low or normal PTH levels, and perhaps raised alkaline phosphatase.

- Rare reasons for high calcium include excess calcium intake, immobilisation, thyroid disease, renal disease, and inappropriate use of vitamin D, diuretics, and lithium.

Since calcium is important in nerve conduction, treatment is urgent if levels exceed 3.5 mmol/L, as cardiac arrhythmias, and then arrest may follow. Treatments include vigorous rehydration, a drug from the bis-phosphonate family (especially in myeloma), calcitonin and prednisolone. However, the original cause of the problem must eventually be addressed (e.g., surgical excision of a PTH-secreting adenoma).

Hypocalcaemia

Recall that low calcium in itself is not necessarily true hypocalcaemia: it may be related to a low albumin and adjusted calcium may rise to be within the normal range. Investigation centres on abnormal intake and regulation:

- Hypoparathyroidism resulting from failure of the parathyroid gland, possibly due to autoimmune disease or the excessive treatment for hyperthyroidism, such as over-use of radio-active iodine or poor surgical technique.

- Renal disease: (a) this organ secretes a molecule essential for malabsorption of calcium from the intestines (i.e., secondary malabsorption), (b) damage to the nephron so that calcium is not re-absorbed from the developing urine and is thus excreted.

- Inadequate intake of calcium and/or vitamin D in the diet.

Like hypercalcaemia, clinical features include an abnormal ECG, and treatment must address the cause(s). Oral supplements are one avenue, synthetic vitamin D analogues another. If urgent, a calcium supplement may be added to an intravenous drip.

11.3 Phosphate metabolism

Plasma phosphate is part-controlled by PTH and the vitamin D isoform calcitriol. PTH decreases phosphate reabsorption in the kidneys causing loss of phosphate in the urine and a fall in plasma phosphate concentrations. Calcitriol increases phosphate absorption in the gut and therefore the concentration of plasma phosphate

Hyperphosphataemia

The most common cause of high blood phosphates is renal failure (reduced excretion), others are hypoparathyroidism and cell lysis.

Hypophosphataemia

Low blood phosphate (possibly due to raised PTH) is rare, causes muscle weakness, may lead to respiratory failure, and demands urgent treatment with intravenous supplements. Other causes include treatment of diabetic ketoacidosis with insulin (as the shift of glucose into the cell may cause a similar movement of phosphates) and alkalosis.

Key point: Hypercalcaemia is important as it may be linked to a cancer sending metastases to bone, and can interfere with the heartbeat.

11.4: Bone and musculo-skeletal disease

Metabolic disease

Contrary to popular belief, measurement of calcium usually has little to offer the bone disease that has the highest profile – osteoporosis – although oral calcium supplements as a treatment are advocated. Similarly, Paget's disease is rarely characterised by abnormal calcium or phosphates but a raised Alk phos, indicating increased bone turnover, is common. However, this enzyme may also be raised in hyperparathyroidism and metastatic bone cancer.

There are no helpful blood tests to directly help the diagnosis of osteoarthritis, but lack of vitamin D causes weak, misshapen bones as found in Rickets (in children) and osteomalacia (in adults).

Inflammatory disease

As we have seen in Chapter 6 on Clinical Immunology, this group includes the auto-immune conditions RA, SLE, systemic sclerosis, ankylosing spondylitis, and others. Blood tests for these conditions include the autoantibodies rheumatoid factor, anti-citrullinated antibodies and anti-nuclear antibodies. However, as these diseases often have an inflammatory component, white blood cell count, ESR and CRP may be ordered.

Recall that creatine kinase may also be produced by non-cardiac muscle, so may be increased in conditions such as polymyositis, and can also be increased by the use of certain drugs (statins) designed to lower cholesterol.

Websites

www.arthritisresearchuk.org
http://www.patient.co.uk/doctor/Hypercalcaemia.htm

Summary

- Almost all body calcium and phosphate is in the bone: in the plasma they have a reciprocal relationship.
- Plasma calcium levels are regulated by feedback inhibition by PTH.
- Hypercalcaemia may be driven by inappropriately high levels of PTH or mobilisation of bone stores by a malignancy.
- Hypocalcaemia must first be checked for a low albumin, and if necessary, adjusted. Otherwise, it may be due to failure of PTH, poor diet, or renal disease.
- Marked hypercalcaemia demands urgent action.
- The laboratory is unable to help in most bone conditions, except Paget's disease, where raised alkaline phosphatase alone is a frequent finding, and low levels of vitamin D in Rickets/osteomalacia.
- There are no specific or routine tests for musculo-skeletal disorders, but several tests can help provide a direction.

Case study 7

A 64 year old white male presents with a developing and constant pain in his right leg. There is no erythema, swelling or cellulitis but there is muscle atrophy. The initial prescription is analgesia, a FBC, ESR and D-dimers are taken, and he is listed for rapid access ultrasound.

All blood results are normal as is the ultrasound. A second round of tests are U&E's, LFTs and an X-ray of the legs. The only abnormal blood tests are raised alkaline phosphatase, and the X-ray shows slight bowing of the femur with irregular thinning of the tibia and fibula, which together make the diagnosis of Paget's disease. His treatment is the bisphosphonate drug Risedronate.

CHAPTER 12

INVESTIGATION OF THYROID FUNCTION

Key words:

Thyroid stimulating hormone (TSH)
Free thyroxine (T4)
Free Tri-iodothyronine (T3)

Key pathological expressions:

Thyroiditis
Thyrotoxicosis
Graves' disease
Hashimoto's disease
Goitre

12.1: An explanation of terms

Thyroid stimulating hormone (TSH): reference range 0.2 – 3.5

This hormone, a product of the anterior lobe of the pituitary, directs the production and release of the two thyroid hormones, T3 and T4.

Free thyroxine (T4): reference range 10 – 25

Free Tri-iodothyronine (T3): reference range 4.0- 6.5

These molecules are formed from four and three atoms of iodine, an essential trace metal, bound to thyronine, in the body of the thyroid. Some of each is carried by thyroxine-binding globulin, the remainder is free. In the tissues, T4 is stripped of an iodine atom to leave the more bioactive T3 form.

The role of the laboratory

Not all laboratories will offer all three tests - some only measure T3 or T4. Others will first measure TSH, and if this is within the reference range,

may not measure either of the thyroid hormones, arguing the patients is euthyroid (i.e., in health).

12.2: The thyroid

The thyroid gland is located at the front of the neck, just below the larynx, weighting perhaps 30g. It is a butterfly shaped organ whose two lobes ('wings') lie to the left and right of the trachea and are linked by an isthmus. T3 and T4 have numerous influences on the metabolism of a large number of cells and tissues. These include increasing the basic metabolic rate, production of blood cells (especially red blood cells), regulation of fat, carbohydrate and protein synthesis and storage. They also promote cellular differentiation and growth, influence heart rate and the cardiac cycle, and even the rate of hair growth and density of sub-dermal fat.

Regulation of thyroid hormones

Levels of TSH, T3 and T4 form an enclosed regulatory pathway known as negative feedback, common in endocrinology. Low levels of T3 and T4 are detected by the hypothalamus, which directs the anterior pituitary to release TSH. This acts on the thyroid, resulting in an increase in T3 and T4. Conversely, if there is too much T3 and T4, this will also be detected, leading to a fall in production of TSH. This leads the thyroid to scale back the release of its hormones, which should then fall back to normal.

Goitre

The most common global cause of this swelling of the thyroid is lack of iodine but is diminishingly rare in the UK. Alternatively, the swelling may be a tumour, and this can be considered with a form of biopsy – the fine needle aspirate.

Hyperthyroidism

Also known as thyrotoxicosis, and present in perhaps 1% of the general population, diagnosis is by high T4 and low TSH. However, there are instances of normal T4 but raised T3 in some cases of hyperthyroidism.

- Graves' disease is the consequence of autoimmune attack on the thyroid, with the inevitable inflammation, leading to thyroiditis. It is the most common form of hyperthyroidism, accounting for perhaps 75% of cases.

- Hashimoto's thyroiditis, another type of inflammation, is caused by a different autoantibody. However, many cases of hypothyroidism are also linked to Hashimoto's disease.

- A tumour of thyroid that secretes T3 and/or T4 (that is, an adenoma).

- Sub-acute thyroiditis is characterised by a rapid presentation and often follows a viral infection, such as by the Coxsackie virus. Other forms include a postpartum thyroiditis and a thyroiditis following mechanical damage.

- Use of the certain drugs, such as amiodarone, as is used in atrial fibrillation. Hyperthyroidism may also be due to the excessive use of thyroxine in the treatment of hypothyroidism.

Treatments include anti-thyroid dugs (e.g., carbimazole, which may also cause neutropenia, thus illustrating the close relationship between biochemistry and haematology), radioactive iodine, and surgery. Thyroid function tests are essential in monitoring these treatments.

Hypothyroidism

This is a common insidious condition with significant morbidity, and the subtle and non-specific signs that are often incorrectly associated with other conditions. It is present in 35 of 10,000 people, making hypothyroidism one of the most prevalent endocrine diseases with a frequency second only to diabetes. Indeed, it has been estimated that 3-5% of women have thyroid disease, with up to 10% of those aged over 75 years having sub-clinical hypothyroidism.

This disease is characterised by insufficient or even absent levels of TSH, T3 and/or T4, and be due to failure of the thyroid gland itself (80-90% of cases) or of the pituitary/hypothalamus (5-10%). There are several features:

- The most common cause of primary hypothyroidism in the developed world is the Hashimoto's thyroiditis (as in hyperthyroidism) where the autoantibodies cause progressive destruction of thyroid tissue.

- As indicated above, hypothyroidism may be the result of the over-treatment of hyperthyroidism

- Drug that causes hypothyroidism include lithium, amiodarone (which both reduce T3 and T4 secretion), dopamine and glucocorticoids (decreases TSH secretion) and radioactive iodine (destroys thyroid tissue).

- The most severe form is myxoedema, often noticeable in the face.

Other causes include congenital hypothyroidism in infants due to failure of the thyroid to develop. Lack of treatment leads to cretinism. Diseases or injuries affecting the hypothalamus or pituitary can result in reduced production of TSH and so reduced T3 and T4.

Diagnosis also relies on T4 and TSH, but in the reverse, i.e., low T4 with high TSH. Most cases of hypothyroidism result from destruction or failure of the thyroid gland by autoimmune mechanisms or over-treatment of hyperthyroidism. A rare cause is failure of the pituitary/hypothalamus to produce TSH. Whatever the aetiology, treatment is with thyroxine, and its effect (or lack of) on the thyroid is monitored with blood tests. Key features of thyroid disease are shown in table 14.

Websites

www.british-thyroid-association.org
https://www.nice.org.uk/search?q=thyroid NG145 regards thyroid disease

Summary

- The laboratory generally offers free T4 and TSH

- Hyperthyroidism is most often characterised by high T4 and low TSH

- Hypothyroidism is generally associated with low T4 but high TSH

Table 14: General features of thyroid disease

	Hypothyroidism	**Hyperthyroidism**
Thyroid hormones	Low T3 and/or T4 (underactive thyroid)	High T3 and/or T4 (overactive thyroid)
Levels of TSH	Pituitary failure: low TSH Pituitary overactivity: high TSH	Pituitary failure: low TSH Pituitary excess: high TSH
Common signs and symptoms	Lethargy, dry coarse and pale skin, slow speech and mental function, pallor, hoarse voice, lethargy, constipation	Increased irritability and sweating, tremor, lethargy, breathlessness, muscle weakness
The heart, temperature control, and weight	Bradycardia, Cold intolerance and cool extremities. Weight gain	Tachycardia, palpitations, arrhythmia, heat intolerance and warm extremities. Weight loss
Treatment	Replacement therapy (oral thyroxine)	Suppression of the thyroid (drugs, surgery, radioactive iodine)
Allied clinical conditions	Myxoedema, goitre Hashimoto's thyroiditis anaemia	Exophthalmia, goitre Hashimoto's thyroiditis Graves' disease

Case study 8

A 34-year old man complains of weight loss, palpitations at night, sweats, anxiety, and a dry mouth. His girlfriend adds he talks much more rapidly than six months ago. On examination he is indeed sweaty but the body temperature is not raised. However, blood pressures were 143/86 and the pulse rate was 93 beats per minute. You order thyroid function tests.

	Result (unit)	**Reference range**
TSH	<0.1 mU/L	(0.2 – 3.5)
T3	9.5 pmol/L	(4.0 – 6.5)
T4	32 pmol/L	(10 - 25)

The case is clearly hyperthyroid, with high levels of T4, which also explains the low TSH due to negative feedback. You order an antibody screen which reveals antibodies to the surface of thyroid cells, consistent with a diagnosis of Grave's disease. You start him on 30 mg carbimazole daily, which produces no ill effects after 4 weeks treatment.

The patient feels better after only three weeks, and after six weeks his blood profile, blood pressure and tachycardia have all improved. Fortunately, the neutrophil count is not altered. After six months a trial of a reduced dose is recommended, as long term high dose carbimazole use may burn out the thyroid and render the patient hypothyroid. Most patients are maintained on a dose of 5 - 15 mg daily.

CHAPTER 13

BLOOD GASES AND pH

Key words:

pH
Blood carbon dioxide (pCO_2)
Bicarbonate (HCO_3^-)
Blood oxygen (pO_2)

Key pathological expressions:

Metabolic acidosis
Respiratory acidosis
Metabolic alkalosis
Respiratory alkalosis

13.1: An explanation of terms

pH: reference range 7.35 – 7.45

This is leading index of the concentration of hydrogen ions (H^+) in the blood, the key index of acidity or alkalinity.

Bicarbonate (HCO_3^-): reference range 24 – 29

The acidity of the blood is neutralised by the alkaline nature of this molecule

Blood carbon dioxide (pCO_2): reference range 4.7 – 6.0

The amount of carbon dioxide in the blood

Blood oxygen (pO_2): reference range 12 – 14.6

The amount of oxygen in the blood

13.2: Biochemistry

The p in pO_2 and pCO_2 indicates partial pressure. Rarely, total CO_2 may be requested, being the sum of dissolved CO_2, carbonic acid, and bicarbonate. However, 95% of this total value is contributed by the bicarbonate. pO_2 is often used to monitor the efficiency of oxygen therapy. In blood gas analysis, the bicarbonate is not measured directly but is calculated from the H^+ and the pCO_2 alone, in contrast to 'normal' serum biochemistry, where bicarbonate is measured directly. However, arterial blood is more informative, but obtaining such a sample may be difficult.

The waste products of our normal metabolism include urea and creatinine, to which we can now add hydrogen ions (H^+) and carbon dioxide (CO_2). The former is excreted in urine, the latter at the lungs in our exhaled breath. It follows that disease in either organ will lead to changes in these blood components. Levels of H^+ being too high, and thus the pH being low (tending to, or lower than, pH 7), is acidosis. Conversely, when the pH is high (tending to, or exceeding pH 7.6), with low levels of H+, there is likely to be alkalosis. However, the picture is complex and therefore requires some explanation.

Hydrogen ions can exist by themselves or as part of water, and increased H^+ leads to a low pH and acidosis. However, CO_2 is part of the system as it can combine with water to give carbonic acid, in the following equation:

$$H_2O + CO_2 = H_2CO_3$$

This indeed is how a great deal of carbon dioxide is carried from the tissues to the lungs in red blood cells. The carbonic acid, acting as a weak acid, can itself break down to a hydrogen ion and the bicarbonate ion:

$$H_2CO_3 = H^+ + HCO_3^-$$

The regulation (homeostasis) of this system is quite robust and various checks and balances are in place (mostly in the kidney) to ensure the status quo is maintained. For example, there are various buffer systems (such as bicarbonate, phosphate, haemoglobin, and proteins) designed to keep the level of H^+ acceptable. So, if the level of H^+ falls, more can be generated from carbonic acid. When carbonic acid itself runs low, more can be generated from water and carbon dioxide.

When this system breaks down, the result is acidosis or alkalosis. The primary pathology can depend on respiratory disorders where the primary

pulmonary defect in ventilation affects the pCO_2. An alternative is a metabolic disorder, of which there may be several, such as production of, or ingestion of, certain acids, in such doses that the kidney and/or buffering systems cannot cope, the loss of H^+, and the loss or retention of bicarbonate. Thus, two types of both acidosis and alkalosis are recognised.

13.3: Acidosis (low pH, raised H^+)

There are two forms of acidosis.

Metabolic acidosis

An increased production and/or decreased excretion of H^+, and so low pH, defines this condition. Due to complex buffering with bicarbonate, levels H^+ may fall, and could cause a compensative hyperventilation that will lead to a fall in CO_2. Examples of clinical syndromes increasing H^+ include diabetic and alcoholic ketoacidosis, lactic acidosis and poisoning by methanol, salicylate, or ingestion of acids. Renal failure will lead to failure to excrete H^+ whilst bicarbonate can be lost in severe diarrhoea.

A further molecule of interest is lactate, derived from lactic acid, that may also be helpful in determining the acid/alkaline status of the blood. High levels of related intracellular enzyme lactate dehydrogenase (LDH) are found in the blood when there is cellular damage, as in haemolysis.

Respiratory acidosis

This condition is characterised by a raised pCO_2, often due to retention of CO_2. This in turn may be due to hypoventilation, itself possibly caused by drugs (e.g., narcotics, anaesthetics), nerve (motor neurone disease, poliomyelitis) or muscle problems, stroke, and trauma. Respiratory diseases causing acidosis include bronchitis, asthma, fibrosis, and chronic obstructive airways disease.

13.4: Alkalosis (high pH, decreased H^+)

There are also two forms of alkalosis.

Metabolic alkalosis

Often due to loss of H^+ from the gastro-intestinal tract by vomiting and diarrhoea, there may also be raised bicarbonate. Certain drugs (e.g., thiazide diuretics) may cause H^+ to be lost in urine. Because ventilation is generally normal, pCO_2 levels can be unchanged (or modestly raised) and so bicarbonate rises. Another cause is excess ingestion of alkali solutions (e.g., sodium bicarbonate solution by athletes).

Respiratory alkalosis

The primary causes here is hyperventilation, so that pCO_2 falls. The bicarbonate is generally normal. This may be voluntary, mechanical, or by stimulation of the brain's respiratory centre (by pain, drugs, fever, hypoxia due to high altitude, anaemia and / or pulmonary disease and oedema giving poor perfusion).

13.5: Blood Sampling

Generally speaking, the greatest call for blood gases is in Accident and Emergency, Intensive Care, Neonatal Units, and in Surgery, and is often required urgently. Because of the large gradient in oxygen and carbon dioxide between venous and arterial blood, the latter is used. However, the gases in blood can exchange with those in the air so that the test must be done rapidly, and therefore the blood gas analysers are generally sited close to where they are needed although the Pathology Laboratory may also have an analyser. If blood is to be sent to the Laboratory, it should be carried rapidly in a capped, heparinised syringe, and ideally in crushed ice.

13.6: Clinical interpretations

First, consider the pH, which defines acidosis or alkalosis. Next, look at the pCO_2, and then, if necessary, the level of bicarbonate and pO_2. Interpretation can then follow.

1. Reduced pH: therefore acidosis.

1.1 Decreased pCO_2, therefore metabolic acidosis. Bicarbonate is also generally reduced.

1.2 Normal pCO_2, therefore uncompensated metabolic acidosis. This is likely to be because this is a simultaneous respiratory acidosis with retention of CO_2. Plasma bicarbonate will be reduced.

1.3 Increased pCO_2, therefore respiratory acidosis. If this is simple, bicarbonate will be high. However, if acute, there can be low pH, pCO_2 and bicarbonate (slightly) as the renal responses have yet to develop.

2. Normal pH

This does not always mean there are no metabolic problems: there may be two concurrent competing problems. For example:

2.1 Decreased pCO_2, possibly because of a mixed respiratory alkalosis and metabolic acidosis. Bicarbonate is generally reduced.

2.2 Normal pCO_2, therefore no acid/base disturbance.

2.3 Increased pCO_2, therefore either a fully compensated respiratory acidosis, or a mixed respiratory acidosis and a concurrent metabolic alkalosis. Both lead to a raised level of bicarbonate.

3. Increased pH: therefore alkalosis

3.1 Decreased pCO_2, therefore respiratory alkalosis. If this is uncomplicated, bicarbonate will also be decreased.

3.2 Normal pCO_2, therefore uncompensated metabolic alkalosis. Plasma bicarbonate will be increased.

3.3 Increased pCO_2, therefore another complex disturbance such as metabolic alkalosis with some respiration changes (i.e., hypoventilation) in compensation. Plasma bicarbonate will also be increased.

Management

First, understand the aetiology and define the underlying cause. Where this is not immediately possible, then neutralisation of the pH problem should be undertaken. A short-term treatment of acute and profound acidosis of

either form is intravenous bicarbonate to mop up the excess H^+ ions and restore the pH. However, the sooner or later the cause must be addressed. In respiratory acidosis the objective is to improve alveolar ventilation and so lower the pCO_2, possibly by mechanical means, or by giving oxygen. A saline drip (possibly with potassium supplementation) may be useful to stimulate renal perfusion. A common treatment for the hyperventilation of respiratory alkalosis is to get the subject to breathe into a paper bag. This will generally force more CO_2 in inspired air, and thus into the blood.

Websites

http://www.patient.co.uk/doctor/Metabolic-Acidosis.htm
http://www.rightdiagnosis.com/a/alkalosis/symptoms.htm
http://www.labtestsonline.org.uk/understanding/conditions/acidosis/

Summary

- Arterial blood must be measured as soon as possible after it has been obtained and must be transported in ice.
- Acidosis can be respiratory (e.g., following lung disease as in COAD [chronic obstructive airways disease]) or metabolic (due to overproduction of, and/or failure to excrete H^+).
- Alkalosis can be respiratory (e.g., due to hyperventilation) or metabolic (e.g. loss of gastric secretions).
- A simple treatment of acidosis is with an infusion of bicarbonate. Treatment of respiratory alkalosis is by breathing into a paper bag, of metabolic alkalosis by the addressing the root cause.

Key point: Whilst blood gas analysis is the gold-standard scientific test of oxygenation of the blood, it can also be determined with a pulse oximeter, attached to a finger, ideally giving a result of >95%. It is often used on the ward to check oxygen transfer at the lung, and if low (as in pneumonia) may result in oxygen delivered to the patient via a nasal tube or mask.

PART 4:

CASE REPORTS

CHAPTER 14

CASE REPORTS IN PRIMARY CARE

There is a growing trend for patients to be investigated and managed within their own General Practice. The appropriately trained practitioner can take and order blood tests, which then may be sent to the local Path Lab for analysis, which then returns the results, often by E mail. It is then for the practitioner to interpret and act on these results. The following scenarios reflect this process.

For clarity, units for the results are not shown. If needed, they are presented in page 155. However, recall that reference ranges differ between laboratories, so are not necessarily appropriate in your own practice.

Case report 1

A 69-year-old man pops into a 'Well Man' clinic whilst out shopping with his wife. He generally considers himself to be in good health, being a non-smoker, physically active (playing golf twice a week) and is not overweight. Blood results are passed to the Practice for your interpretation.

Interpretation

Abnormal results are calcium, alkaline phosphatase, cholesterol, triglycerides, and glucose. Normal LFTs and normal renal function (eGFR 75 ml/min/1.73m^2 = Stage II CKD) exclude these organs as the source of raised alkaline phosphatase. Triglycerides and glucose could be raised as this is almost certainly a non-fasting sample. A repeat (fasting) test, to include PTH, is needed to confirm the abnormalities and help explain the raised calcium. HDL-cholesterol also needs to be determined. A 'bone' problem may be present. The high cholesterol probably needs to be treated, initially by a 3 month trial of a low fat diet. Without blood pressure or smoking history, we cannot say if this case is at a high risk of a cardiovascular event, but if the high cholesterol is genuine a statin is likely to be offered.

Results for Case report 1

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	138	K	3.8-5.0	3.8
Urea	3.3- 6.7	5.3	Creatinine	71-133	92
T protein	60 – 80	69	Albumin	35 – 50	39
Calcium	2.2-2.6	2.75	Gamma GT	5-55	50
Bilirubin	<17	9	Alk phos	20-120	145
ALT	<5-42	30	PSA	<4.0	2.1
Cholesterol	2.5-5.0	6.7	Triglycerides	<1.7	2.85
Glucose	3.5-5.5	7.0	CRP	<5	1.5
White cell count	4 – 10	6.5	Red cell count	4.3-5.7	5.1
MCV	77 – 98	86.5	Haemoglobin	133-167	146
Neutrophils	1.7 – 6.1	4.5	Lymphocytes	1.0-3.2	1.5
ESR	<10	12	Platelets	140–400	265

Case report 2

A female, aged 53 years, is married with three children. A frequent blood donor, she reported several months of general tiredness and malaise when attending for a donor session. Found to be slightly anaemic, her donation was politely rejected and she was advised to visit her General Practitioner. The following results are returned for your interpretation.

Interpretation

Consider first that her symptoms may reflect the menopause, but this will not influence the laboratory results. Sodium, potassium, urea, and creatinine are all acceptable, suggesting good renal function, although the eGFR is 51 ml/min/1.73m^2 (Stage III CKD). Both calcium and PTH are raised, and the low T4 with high TSH suggests hypothyroidism. Liver function tests and proteins are normal. The high calcium is certainly a concern and demands investigation. Given the physical location of the parathyroids, the thyroid problem (possible autoimmune in origin) may be causing the calcium irregularities.

Results for Case report 2

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	136	K	3.8-5.0	4.8
Urea	3.3 – 6.7	6.3	Creatinine	71-133	104
T protein	63 – 84	65	Albumin	35 – 50	42
Calcium	2.2 – 2.6	2.82	PTH	1 - 6	7.5
Bilirubin	<17	12	Alk phos	20-130	105
ALT	5 - 42	25	Gamma GT	5 - 55	30
Free T4	10-25	7	TSH	0.2-3.5	7.5
White cell count	4 – 10	5.4	Red cell count	3.9-5.0	4.0
MCV	77 – 98	91.0	Haemoglobin	118-148	106
Neutrophils	2.0 – 7.0	3.5	Lymphocytes	1.0 – 3.0	1.6
ESR	<10	16	Platelets	140-400	225

Blood donors are screened for a low haemoglobin, confirmed with the formal test, and the abnormal ESR confirms a pathology. This may be linked to the hypothyroidism, as T4 can act as a growth factor to stimulate red cell production. Referral to an endocrinologist is required, who will establish the extent of the thyroid and parathyroid pathology.

Case report 3

A male aged 37 years, a frequent traveller abroad, visits his family doctor with a long history of feeling generally tired and unwell, but without any precise symptoms. On examination there is tenderness on the right side of the abdomen. His diet is good, he reports no sweating or fevers, and there are no changes in his bowel habits or frequency of urination.

Interpretation

The renal profile is good but there is evidence of liver problems with all tests being abnormal. The history is suggestive of a long-term problem and questioning would have considered anything that would have provoked an acute insult.

Results for Case report 3

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	136	K	3.8-5.0	4.9
Urea	3.0 – 6.7	6.2	Creatinine	71 – 133	88
T protein	60 – 80	75	Albumin	35 – 50	40
Bilirubin	<17	25	Alk phos	20-120	155
ALT	5-42	45	Gamma GT	5-55	154
CRP	<5	6.5	AST	10-50	53
White cell count	4 – 10	8.9	Red cell count	4.3 – 5.7	5.5
MCV	77 – 98	88.0	Haemoglobin	133-167	152
Neutrophils	2 - 7	5.8	Lymphocytes	1 - 3	1.9
Monocytes	0.2 – 1.0	0.6	Eosinophils	0.02 – 0.5	0.7
ESR	<10	15	Platelets	140-400	312

Other, more intrusive questioning may be required and supportive tests such as ultrasound would help.

Differential diagnoses include poor outflow of bile from the gall bladder, perhaps due to an obstruction or maybe a tumour at the head of the bile duct, or maybe alcoholism. Certainly, a hepatitis virus screen should be done as this may indicate a low-grade infection, perhaps even chronic active hepatitis. Is the frequent foreign travel relevant?

The haematology profile is normal except a monocytosis, an eosinophilia and a raised ESR. The white cell abnormalities could coincidental, but alongside the ESR are likely to be pathological. Is there a parasite present? Quite possibly, as the total white cell count is within range, but the neutrophil count is close to the top of the reference values, and there are raised eosinophils. All tests should be repeated, and in addition, testing for hepatitis virus infection.

Case report 4

Part 1

A female, aged 59 years, reported progressive weight loss and tiredness over six to nine months. Over the last three months she also noted a growing mass in the right iliac fossa, but no pain, and even more recently was becoming 'unwell'. On examination there was also a mild splenomegaly. Initial blood results were as follows.

Test	Ref. range	Result	Test	Ref. range	Result
Na	135–145	147	K	3.8–5.0	4.8
Urea	3.0–6.7	7.9	Creatinine	71–133	129
T protein	60-80	66	Albumin	35 – 50	33
Calcium	2.2–2.6	2.65			
Bilirubin	<17	10	Alk phos	20-120	706
ALT	5- 42	30	CA-125	0 - 30	580
Cholesterol	2.5–5.0	4.5	Triglycerides	<1.7	2.7
TSH	0.2 -3.5	7.3	Free T4	10 – 25	4
White cell count	4 – 10	7.8	Red cell count	3.9–5.0	3.7
MCV	77 – 98	88.8	Haemoglobin	118-148	110
Neutrophils	2 - 7	5.0	Lymphocytes	1 – 3	2.0
ESR	<10	15	Platelets	143– 400	213

Interpretation

All renal indices are within the reference range, although the high end of the scale, giving eGFR 39 ml/min/1.73m^2 = Stage III CKD. Albumin is a little low, with marginally raised calcium. However, the most abnormal routine result is the markedly raised alkaline phosphatase, but this pales into insignificance compared to the very high CA-125, a marker (principally) of ovarian cancer, but also present in cervical, breast, bowel, and other cancers. Thyroid tests are consistent with hypothyroidism, and the haematology results support the diagnosis of normocytic anaemia, possibly secondary to the hypothyroidism and/or the presumed cancer.

There is a clear urgent referral to the local oncology service to determine the cause of the raised CA-125. The other abnormalities (such as the thyroid question) will be addressed in turn. The isolated raised alkaline phosphatase does not reflect liver disease and may be related to the cancer.

Part 2

The same women returns 18 months later, with vague and non-specific symptoms, complaining of feeling unwell.

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	139	K	3.8-5.0	4.1
Urea	3.0 – 6.7	3.5	Creatinine	71-133	93
T protein	60 - 80	63	Albumin	35-50	33
Calcium	2.2 – 2.6	2.45			
Bilirubin	<17	15	Alk phos	20-120	90
ALT	5 – 42	35	Gamma GT	5 - 55	164
AST	10 - 50	23	Cholesterol	2.5-5.0	4.0
Triglycerides	<1.7	2.9			
White cell count	4 – 10	5.2	Red cell count	3.9-5.0	4.3
MCV	77 – 98	97.1	Haemoglobin	118-148	120
Neutrophils	2 - 7	3.2	Lymphocytes	1 - 3	1.4
ESR	<10	12	Platelets	140-400	274

Interpretation

We presume the woman has had treatment for unspecified abdominal cancer (bowel/ovary). The abnormalities are (as initially) a low albumin and raised trigylcerides, but there is a new raised gamma GT. In comparison with the initial result, renal function has improved (eGFR 57 ml/min/1.73m^2) and the low red cell indices, hypercalcaemia and raised alkaline phosphatase have all resolved. It is tempting to speculate this is the result of the (surgical?) treatment of the cancer.

However, they raised gamma GT is annoying, and may perhaps be a late effect of chemotherapy or radiotherapy to the liver, but this seems unlikely

as this is only the abnormal LFT. The same argument applies to the hypothesis of liver metastases. We need another CA-125 result. However, an alternative is simple excess alcohol intake: this itself causes gamma GT and triglycerides to rise. Alcohol also increases the MCV, as seems likely in this case. Looking again, we see the cholesterol has fallen by 0.5 mmol/L, and the bilirubin increased by 50%, which may support the alcohol hypothesis, although this may reflect a degree of malnutrition. Use of alcohol is a common psychological prop after cancer surgery, although a referral to counselling may be a good move.

Case report 5

Interpret these oral glucose tolerance tests. Data are the serial levels of glucose (units: mmol/L) after the 75g of glucose taken orally.

Case and notes	Pre-load glucose	1 hour Later	2 hours later	3 hours later
1. Male, 65, obese, often tired, and thirsty with some diarrhoea	8.1	11.5	16.2	12.2
2. Male, 41, dipstick glycosuria found at a well-man clinic	6.5	7.8	8.9	7.4
3. Female, 62, complains of a dry mouth and skin, hair loss	7.4	12.8	14.7	9.9
4. Male, 25, high finger-prick glucose at a church fete	4.2	6.2	6.9	5.2
5. Female, 75, high random glucose on GPs clinic machine	6.3	7.0	7.6	6.9

Interpretation

Cases 1 and 3 are both barn-door diabetes, with high fasting and high 2-hour levels of glucose. The second case is impaired glucose tolerance, with the 2-hour result between 7.7 and 11.1 mmol/L. Case 4 shows no signs of a glucose problem with a normal response, suggesting the church fete result was incorrect. Case 5 has a good 2-hour response (<7.8 mmol/L) but a high fasting result, hence impaired fasting glycaemia. n.b. An alternative definition of diabetes is a high HbA1c, perhaps >48 mmol/mol / 6.5%, but refer to local guidelines.

Case report 6

An 82-year old man presents with a four month history of excessive and progressive tiredness and lethargy, and occasional pain in some bones, but not his joints. During the recent winter he twice needed antibiotics to treat a chest infection. On examination he was thin, pale and with a small lump in his left axilla. The blood test results were as follows:

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	139	K	3.8-5.0	4.8
Urea	3.0 – 6.7	7.5	Creatinine	71-133	121
T protein	60 - 80	85	Albumin	35-50	38
Calcium	2.2 – 2.6	2.75			
Bilirubin	<17	12	Alk phos	20-120	145
ALT	5 – 42	32	Gamma GT	5 - 55	54
AST	10 - 50	20	CRP	<5	7.5
White cell count	4 – 10	8.0	Red cell count	4.3-5.7	4.1
MCV	77 – 98	82	Haemoglobin	133-167	98
Neutrophils	2 - 7	3.2	Lymphocytes	1 - 3	3.8
ESR	<10	85	Platelets	140-400	157
Monocytes	0.2-1.0	0.3	Atypical cells	<0.1	0.5

Interpretation

There are many abnormalities. These are low haemoglobin and red cells, with high ESR, lymphocytes, atypical leukocytes (which exceed the number of monocytes), total protein, calcium, alkaline phosphatase, and CRP. eGFR is 53 ml/min/1.73m^2, giving Stage III CKD.

With low red cell indices and a history of tiredness and lethargy, the patient is anaemic, and an MCV in the reference range makes this a normocytic anaemia. The greatest abnormality is the ESR, one of the primary reasons for which is rheumatoid arthritis. However, with no history of overt joint pains, we can probably exclude this. Although the lymphocyte count is raised, this could conceivably be due to an infection, but few infections can, by themselves, cause such a grossly abnormal ESR. The increased number of atypical leukocytes demands attention.

The key abnormalities are the raised alkaline phosphatase, calcium, and total protein. These results would prompt a second round of investigations: the raised calcium may be due to mobilisation from bone or may be due to primary hyperparathyroidism, which would be helped by knowledge of the PTH concentration. An alternative is that the hypercalcaemia is due to bone metastases, a possible source being the prostate, hence the request for the measurement of PSA. The hyperproteinaemia would prompt a request for serum protein electrophoresis.

A low PTH rules out primary hyperparathyroidism as a cause of the hypercalcaemia, and a low PSA would also effectively rule out prostate cancer. But key test is electrophoresis, which if reporting a paraprotein, effectively makes the diagnosis of myeloma. This would account for the abnormal ESR with high calcium and alkaline phosphatase. The normocytic anaemia is an established aspect of this, whilst the raised lymphocyte count and atypical cells may be some plasma cells which have escaped the bone marrow. Other investigations would be X-rays and bone scans to assess the extent of the infiltration of the tumour into the skeleton. However, treatment is likely to be palliative.

Case report 7

A 63 year old man with type 2 diabetes, a body mass index of 28.5 kg/m^2, mildly reduced renal function, and a below knee amputee, is seen at his home by the Community Matron. She is concerned that his remaining foot feels cool and is pale and takes some blood. Whilst doing so, the patient

recalls a blood test was done “months and months ago”. On examination today his systolic/diastolic blood pressure is 142/82 mm Hg. His medications are amlodipine 5 mg once a day, frusemide 40 mg twice a day, ramipril 5 mg once a day, simvastatin 20 mg once a day and metformin 1000 mg once a day. A week later the District Nurse telephone and asks for an opinion. You review his blood results and compare them with bloods taken nine months ago.

Test	**Ref. range**	**9 months ago**	**Now**
Haemoglobin	133 – 167	132	129
MCV	77 – 98	85.5	86.4
Red cell count	4.3 – 5.7	4.9	4.7
Haematocrit	0.35 – 0.55	0.42	0.41
Platelets	143 – 400	296	301
ESR	<10	8	12
HbA1c	<48	71	78
White cell count	4 - 10	7.6	7.9
Neutrophils	2 - 7	5.0	5.6
Lymphocytes	1 - 3	2.0	1.8
Sodium	135 - 145	138	141
Potassium	3.8 – 5.0	4.4	5.0
Urea	3.3 – 6.7	9.1	9.3
Creatinine	71 – 133	112	168
Bilirubin	<17	9	12
Gamma GT	5 - 55	35	45
AST	10 - 50	43	38
Alk phosphatase	20 - 120	120	112
Total protein	60 - 80	68	65
Albumin	35 – 50	33	29
uACR	<2.5	3.6	8.3
Total cholesterol	2.5 – 5.0	4.9	5.1
HDL-cholesterol	>1.2	1.4	1.3

Interpretation

The low haemoglobin and normal MCV point to a normocytic anaemia at both time points. The HbA_{1c} is high in the initial sample and has increased further in the second sample, indicative of deteriorating glycaemic control. Nine months ago, the marginally acceptable total cholesterol has gone out of range. Regarding renal function, the urea is consistently high, but the creatinine has increased markedly: the eGFR (ideally >90 ml/min/1.73m^2) has fallen from 61 to 38. The albumin has fallen and the uACR has become considerably worse.

The health of this diabetic seems have deteriorated markedly in the nine months between the samples. Haemoglobin is definitely low and the patient is likely to be anaemic, and the HbA_{1c} has become more adverse. Anaemia is not uncommon in diabetes, but a clear link is often uncertain. However, the most concerning aspect is the rise in creatinine and parallel fall in eGFR. This gives a clear diagnosis of chronic renal failure, falling from Stage II to Stage IIIb, and is mirrored by the adverse uACR. The latter may be linked to the reduction in serum albumin, which may be because of loss into the urine through a damaged glomerulus.

Risk factor management

There are several aspects needing attention. This patient needs a lower total cholesterol, certainly <4.5 mmol/L, maybe even <4 mmol/L, as should be achieved by increasing the simvastatin to 40 mg a day. Similarly, the blood pressure target in general health of <140/90 is lower in high risk groups, and should be <135/85, although as an amputee and therefore a history of atherosclerosis perhaps a target of <130/80. There may also be a need to juggle those drugs (such as ACE inhibitor ramipril) whose plasma levels are influenced by renal function. This patient is at risk of suffering a major cardiovascular event (myocardial infarction, stroke) and would certainly benefit from 75 mg aspirin daily. Finally, the hyperglycaemia needs to be addressed, and there are a number of options, including insulin, normally reserved for type 1 diabetics. There may also be a case for a visit to the Day Hospital, where Consultants can meet in a multi-disciplinary team to discuss options.

These data give the patient a QRISK (https://www.qrisk.org/three/index.php) 10-year risk of a major CVD event of 32%, and a ‘healthy heart’ age of 81 years. NICE guidelines CG127 (on hypertension), CG87

(newer agents in diabetes), CG119 (diabetic foot problems) and CG67 (lipid modification) apply.

Case report 8

A 48 year old woman with a body mass index of 26.5 kg/m^2 complains of generalised aches and pains (which are getting worse), with some early morning stiffness, especially in the legs and the back. On examination, there is some eczema. She is advised to take simple analgesics, take regular exercise, lose weight, and a blood test is taken for rheumatoid factor, which comes back as negative.

Six months later she returns, saying the pains have got worse, and are how in both left and right wrists, and some finger joints in both hands. She is also on the maximum doses of paracetamol. On examination there is also tenderness in various muscles. The rheumatoid factor is repeated, which comes back weakly positive. All this prompts a full haematology, biochemistry and immunology screen aimed at a connective tissue disease.

Test	**Reference range**	**Result**	**Test**	**Reference range**	**Result**
White cell count	4 – 10	7.9	Red cell count	3.9 – 5.0	4.2
MCV	77 - 98	85	ESR	<10	21
Lymphocytes	1 – 3	1.9	Neutrophils	2 – 7	5.5
Haemoglobin	118-148	121	Platelets	143 - 400	367
Na	135 - 145	140	K	3.8 – 5.0	4.4
Urea	3.3 – 6.7	6.7	Creatinine	71 - 133	98
Bilirubin	<17	10	Alk phos	20 – 120	45
AST	10 – 50	21	ALT	5 – 42	27
Gamma GT	5 - 55	45	HsCRP	<3.5	6.7
ACPA	≤7	31	ANA	≤1/40	1/20
RhF	≤20	45			

HsCRP = high sensitivity CRP. ACPA = Anti-citrullinated protein antibodies. ANA = Anti-nuclear antibodies. RhF = Rheumatoid factor

Interpretation

The initial present symptoms are vague, but on hindsight were clear harbingers of something more sinister, and it is likely that this woman is suffering from an inflammatory connective tissue disease. The only very slightly raised body mass index is likely to exclude osteoarthritis (overweight/obesity being the greatest risk factor). This exclusion is supported by the raised ESR, as osteoarthritis is a disease whose inflammation is limited to the joint and with minimal systemic effects.

The most common disease in this group is rheumatoid arthritis, commonly attacking middle-aged women, and the positive RhF and ACPA, and history point to this diagnosis. However, the ultimate diagnosis demands both clinical and laboratory features, according to formal guidelines such as those of the European League against Arthritis. The raised CRP indicates on-going inflammation, as part of an acute phase response. The red cell indices are within reference values, but are low, and may fall, leading to the anaemia of chronic diseases.

Management will focus on reducing symptoms, and the laboratory markers, which are assumed to be surrogates of disease severity. NICE clinical guideline NG100 (rheumatoid arthritis) applies.

Case report 9

A family gathers for the funeral of a 62-year old man who died unexpectedly of a heart attack. He had no obvious signs or symptoms of cardiovascular disease. His widow notes the man's older brother and a cousin also died quite young, and the man's sister recall their own father died of a stroke whilst still working. This prompts a general 'call to health' for the entire family. Blood results show raised cholesterol (>8 mmol/L) in two of the man's children and in a younger sister

Interpretation

With a strong family history of premature cardiovascular disease, a genetic link is implied. The family is tested for genetic mutations known to cause familial hypercholesterolaemia, and all those with high levels of cholesterol do indeed test positive. Depending on the particular case, statins or other lipid-lowering drugs are in order.

CHAPTER 15

CASE REPORTS IN SECONDARY CARE

Almost by definition, these cases are likely to be more serious, and so the diagnosis must be determined rapidly and accurately. The on-site pathology laboratory and other resources provide crucial help. Once more, units for these results are (generally) not shown. If needed, they are presented in page 155. And again, recall that reference ranges differ between laboratories, and so cannot be used in your own practice.

Case report 10

A man of unknown age, but looking perhaps 50, is brought to Accident and Emergency (A&E) by the police, having been found collapsed in the street. He is poorly dressed, unshaven and unkempt. On examination there was no evidence of violence, concussion, or alcohol abuse, but he was thin and poorly communicative with dry lips and tongue. His pulse was 104, and his blood pressure was 95/65. The staff in A&E take blood for routine tests, but also for drugs of abuse, induce the man to drink some water and put up a saline drip.

Interpretation

This is a reasonably clear case of dehydration where the clinical signs and laboratory tests concur. There is pre-renal uraemia (and so acute renal failure), low total protein and low albumin. LFTs are acceptable and the normal glucose excludes coma or other neurological state linked to diabetic ketoacidosis. Renal function is poor with eGFR 44 ml/min/1.73m^2 = Stage III CKD. The red cell count is a little raised (erythrocytosis) and this may also reflect the dehydration.

The case appears to be self-neglect, possibly with malnutrition. The patient's body mass index is 18.5 kg/m^2. After bed rest with rehydration, a referral to Social Services seems appropriate. The drugs of abuse tests are all negative.

Blood results for Case report 10

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	150	K	3.8 - 5.0	4.6
Urea	3.3 – 6.7	22.9	Creatinine	71 - 133	155
T protein	60 – 80	58	Albumin	35 – 50	31
Bilirubin	<17	9	Alk phos	20 - 120	140
ALT	5 – 42	26	Gamma GT	5 - 55	45
Glucose	3.5 – 5.5	2.8	CRP	<5	1.5
White cell count	4 – 10	5.8	Red cell count	4.3-5.7	5.9
MCV	77 – 98	93	Haemoglobin	133-167	142
Neutrophils	2 – 7	3.9	Platelets	140-400	225
ESR	<10	5	Lymphocytes	1 - 3	1.4

Case report 11

A 55-year-old male cave explorer was trapped for 7 hours after the roof of a cave collapsed on him. Upon rescue, he was in marked pain and was immediately given analgesia, being suspected of having sustained fractures and crush injuries to the back and legs. In A&E, he was disorientated and confused with rapid breathing, a pulse of 110 and systolic/diastolic blood pressures of 100/70 mm Hg. Blood gases were normal and he was adequately oxygenated (pulse oximetry 98%). Bloods were taken, a saline drip put up, and he was sent to X-ray. As he returned, the bloods were available on the hospital pathology webpage.

Interpretation

With such a history, we can expect multiple abnormalities resulting from the trauma. High sodium, with high-ish urea and creatinine suggest dehydration due to water deprivation, which seems obvious with the clinical picture. The raised creatine kinase suggests damage to muscle cells, but the CK-MB within the normal range suggests that this is not of cardiac origin. Low glucose may be explained by temporary starvation of at least 7 hours, possibly longer.

Blood results for Case report 11

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	149	K	3.8 – 5.0	5.3
Urea	3.3 – 6.7	6.5	Creatinine	77 - 133	123
T protein	60 – 80	72	Albumin	35 – 50	42
Bilirubin	<17	19	Alk phos	20 -120	115
ALT	5 - 42	35	Gamma GT	5 - 55	44
Cholesterol	2.5 – 5.0	6.7	Triglycerides	<1.7	2.85
Glucose	3.5 – 5.5	2.9	Creatine kinase	55 - 170	225
CK-MB	<25	13			
White cell count	4 – 10	9.5	Red cell count	4.3 – 5.7	5.5
MCV	77 – 98	92	Haemoglobin	133-167	150
Neutrophils	2 - 7	6.5	Lymphocytes	1 – 3	2.0
ESR	<10	8	Platelets	140-400	325

Bilirubin is high-ish, and this may be due to release from damaged red blood cells, which, alongside damaged muscle cells, may part-explain the high potassium. However, the red cell profile is normal, but this does not preclude internal haemorrhaging. The high neutrophil count is likely to reflect the general trauma and does not (yet, perhaps) imply a bacterial infection. Treatments include rehydration (which has already begun with the saline drip) with added dextrose, but also insulin (the latter to restore the hyperkalaemia) and glucose (to prevent hypoglycaemia that would probably result from the insulin), and then admission to a medical ward. Anticoagulation would need to be considered at it is likely he is at risk of a venous thrombosis. However, the possibility of internal haemorrhage must also be considered. More complex imaging (MRI/CT) would also be considered.

Case report 12

A woman in her mid-twenties is brought to the Accident and Emergency Unit at midnight unconscious having collapsed at a night club. Her friends do not consider her to be drunk but say she may well have hit her head as she fell. The subject is poorly responsive, pale, and clammy, and with a high breath rate of about 25 –30/minute and a Glasgow coma score of 11 (poor result 3, good result 15). Blood pressures are 110/70, pulse rate 90/minute. Serum biochemistry and blood gases are ordered.

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	147	K	3.8 – 5.0	3.7
Urea	3.3-6.7	7.5	Creatinine	71 – 133	85
Glucose	3.5-5.5	4.0	Bicarbonate	24 - 29	24
Bilirubin	<17	15	Alk phos	20 - 120	90
ALT	5 – 42	35	Gamma GT	5 - 55	14
pCO_2	4.5 – 6.1	3.8	pH	7.35-7.45	7.47
White cell count	4 – 10	5.0	Red cell count	3.9 – 5.0	4.7
MCV	77 – 98	93.4	Haemoglobin	118-148	131
Neutrophils	2 - 7	3.3	Lymphocytes	1 – 3	1.2
ESR	<10	4	Platelets	140-400	301

Interpretation

The abnormal serum results are slight hypernatraemia and uraemia, with serum bicarbonate at the bottom end of the normal range. The blood pressure is not too bad but there is somewhat of a tachycardia. The blood gas result is consistent with respiratory alkalosis (raised pH and low pCO_2, low-ish bicarbonate), which fits in with the hyperventilation (about twice the normal rate). Note the report of possible head injury that may have involved the brain stem.

A check of the woman's handbag found an inhaler, implying a mild lung problem, and antibiotics, implying an unknown infection. Normal glucose rules out diabetic ketoacidosis as a cause of the collapse. Electrolyte changes may be secondary to a pre-renal problem, possibly dehydration. Also consider the possibility of drug abuse causing a respiratory stimulation. The situation does not seem to be life threatening – the respiratory rate is not extremely high; the Glasgow coma score (based on eye opening, verbal responses and motor responses) is moderate (11 on a scale of 3 [poor] to 15 [good]) – so that rest and observation may be all that is required. A saline drip may help the pre-renal uraemia.

Case report 13

This 55-year-old woman has been in intensive care for 3 weeks with an E Coli septicaemia being treated with ciprofloxacin. Previously acidotic and developing hypotension (SBP/DBP 110/65, despite fluid support), she was placed on warfarin and briefly on LMWH because of the risk of venous thromboembolism. However, these were cut back because of bleeding per vagina, and gross obesity (accurate BMI impossible but estimated >35) prevented many investigations. Other problems include pyrexia (38.1˚C) and bilateral effusive sacral and buttock ulcers/sores. Psychologically she was anxious and depressed, refusing physiotherapy and the Dietician's suggestion of a naso-gastric tube. Basal crackling in both lungs was noted with respiration shallow and forced.

Interpretation

We already know this woman is severely ill and has numerous problems. The major haematology points are the normocytic anaemia (note bleeding per vagina, low Hct and low red cell count) and marked neutrophil leukocytosis. There is also prolonged PT (due to warfarin?) and APTT (due to what?). Fortunately, she has sufficient platelets and fibrinogen to maintain haemostasis.

Blood results for Case report 13

Test	Ref. range	Result	Test	Ref. range	Result
Na	135-145	143	K	3.8 – 5.0	4.7
Urea	3.3 – 6.7	10.2	Creatinine	77 – 133	114
T protein	60 - 80	48	Albumin	35 – 50	23
Calcium	2.2 – 2.6	1.76	CRP	<5	37
Bilirubin	<19	7	Alk phos	20 - 120	140
ALT	5 - 42	30	AST	10 - 50	63
Gamma GT	5 - 55	63			
Haemoglobin	118 - 148	80	MCV	80-98	90
Red cell count	3.9 – 5.0	2.5	White cell count	4 – 10	20.0
Neutrophils	2 - 7	17.9	Lymphocytes	1 – 3	1.5
Haematocrit	0.33-0.45	0.23	Platelets	140-400	210
Prothrombin time	11 – 14	19	APTT	24 – 34	55
Fibrinogen	1.5 – 4.0	2.0	ESR	<10	25

APTT = activated partial thromboplastin time

Major biochemistry points are uraemia (virtually to be expected), a profoundly low albumin, and low total proteins with low calcium. High CRP and ESR are to be expected in view of the septicaemia and add nothing of value. Other abnormalities are of minor clinical significance. Clinical history implies self-neglect and malnutrition, lung involvement, and the sores may be infected and/or a source of blood and fluid loss. She was about to have a 4-pack blood transfusion but died during the night.

Case Report 14

A 66-year-old man of African descent self-presents to Accident and Emergency with a short (2 – 3 week) history of a recently developing tiredness and lethargy. He appears thin but otherwise seemed well. The following blood result was obtained:

Blood results for Case report 14

Test	Ref. range	Result	Test	Ref. range	Result
Na	133-148	143	K	3.3-5.6	4.3
Urea	3.0 – 8.3	5.0	Creatinine	44-133	92
Uric acid	<420	630	Albumin	35 – 50	35
Bilirubin	<21	24	Alk phos	20-130	257
ALT	<41	304	Gamma GT	<64	104
Calcium	2.2 – 2.6	1.97	CRP	<5	51
Haemoglobin	133-167	25	MCV	80 – 98	91
Red cell count	4.3-5.7	1.85	White cell count	4 – 10	656
Neutrophils	2 - 7	72.1	Lymphocytes	1.0-3.2	13.1
Monocytes	0.2 – 1.0	13.1	Atypical cells	<0.1	558
Haematocrit	0.35-0.53	0.17	Platelets	140-400	30
ESR	<10	38	APTT	24 – 34	38
Prothrombin time	11 – 14	31	Fibrinogen	1.5-4.0	1.0

Interpretation

With the triad of an exceptionally high WBCC (especially atypical cells), blasts, low haemoglobin, and low platelets, and the history, this is clearly an acute leukaemia. The high number of neutrophils suggests an AML, but this needs to be confirmed with other tests. Other changes are to be expected: low red cell count and haematocrit.

However, there are other abnormalities – notably raised PT and PTT, as if the patient were on active anticoagulation, which he was not. This may reflect the extent to which the leukaemia has moved from the bone marrow to the liver. Indeed, there is clear evidence of the disease spreading, notably raised liver enzymes, and this may account for the prolonged clotting times, as the production of coagulation factors by a damaged liver would be impaired, as witnessed by low levels of fibrinogen

Remarkably, renal function seems to be unimpaired (eGFR 92 ml/min/1.73m^2). Raised CRP and ESR simply reflect very severe disease,

although an infection cannot be discounted. Shortly after this report was received, the patient was due to be transferred to the leukapheresis suite in an attempt to reduce the load of abnormal (leukaemic) cells in his blood. However, he suffered a fatal cardiac arrest whilst in transfer. The case also illustrates how rapidly this acute myeloid leukaemia developed and influenced other laboratory tests.

Key point: This case, from the author's own hospital, underlines the body's ability to remain functioning despite an extreme pathological condition. With an astonishingly high white cell count, and very low red cell count, the patient's blood resembled strawberry milkshake.

Case report 15

A 73 year old diabetic female presented to Accident and Emergency with progressive shortness of breath and a two week history of diarrhoea. Her medications included aspirin, blood pressure lowering drugs irbesartan and hydrochlorothiazide, and metformin. On examination she was oligouric (making little urine), disorientated and confused, with a respiratory rate of 32 breaths/minute, very low systolic/diastolic blood pressures of 76/44 mm Hg, and a very high pulse rate 125 beats/minute.

Interpretation

The haematology shows a minimally increased ESR, but a markedly abnormal HbA_1c implying months of hyperglycaemia. As for the biochemistry, there is hyperkalaemia, raised urea and creatinine, the latter giving a low eGFR, synonymous with stage 4 chronic kidney disease. LFT's are normal, but there is markedly raised lactate and hyperglycaemia. All blood gas indices are low, except the oxygen partial pressure.

The picture is of clear diabetic lactic acidosis, possibly a diabetic ketoacidosis, but ketones have not been directly measured. Precipitating factors include the diarrhoea, predisposing to dehydration (hence the low blood pressure) leading to the impaired perfusion of organs. The normal white cell count and CRP rule out septicaemia, which commonly causes an acidosis. Indeed, factors precipitating this condition include infections, clearly not present in this case.

Blood results for Case report 15

Test	Ref. range	Result	Test	Ref. range	Result
Red cell count	3.9 – 5.0	4.7	White cell count	4 - 10	7.0
Haemoglobin	118 – 148	129	MCV	77 - 98	85
Platelets	143 – 400	216	ESR	<10	12
Neutrophils	2 – 7	4.5	Lymphocytes	1 – 3	1.8
Na	135 - 145	135	K	3.4-5.1	5.9
Urea	3.3 – 6.7	15.5	Creatinine	71 - 144	279
eGFR	>90	15.3	Albumin	35 – 50	39
Bilirubin	<17	8	Alk phos	20-120	47
AST	10 – 50	39	ALT	5 – 42	29
Gamma GT	10 -50	39	Lactate	0.7-1.9	17.4
CRP	<5	<5	Glucose	3.3-5.5	9.0
HbA_1c	48 – 58*	80			
pH	7.35-7.45	6.72	pCO_2	4.7-6.0	1.87
pO_2	12.0-14.6	14.1	Bicarbonate	24 – 29	12

HbA1c results: *%, *Reference range for known diabetics

Urgent treatment is demanded and is likely to be led by the need to vigorously rehydrate, probably with intravenous fluids, but also supplements of bicarbonate to reverse the acidosis. This is also likely to help the hyperkalaemia, but an additional treatment is likely to be insulin, but if so, there must be sufficient plasma glucose. The borderline low sodium may be due to the osmotic diuresis caused by the hyperglycaemia. Once stable, a complete overhaul of the patient's care is required.

PART 5:

APPENDICES

APPENDIX 1

SPECIAL SITUATIONS: PREGNANCY, PAEDIATRICS, AND THE ELDERLY

Appendix 1 brings together three separate areas that individually would not normally warrant too much attention in the 'routine' sense. There are changes in blood results that arise in pregnancy, in early life, and in late life, so that a combined approach will be taken. However, it must be stressed that clinical decisions in these situations demand a high level of experience and interpretation.

Pregnancy

It is little surprise that there are numerous changes to a woman's blood whilst she is carrying a child. However, these are entirely physiological: indeed, pregnancy is an indication of good overall health – the pregnant woman should not automatically be viewed as a patient. Reference ranges in pregnancy are present in Appendix 3.

Anaemia

This is perhaps one of the best-known haematology features of pregnancy, and is probably due to a number of factors, such as increased plasma volume. This increases by up to 40% (but with wide individual variations) during the first two trimesters whereas the red cell mass increases by perhaps only 25%. The consequences are often a fall in haemoglobin (e.g., to 100 - 110 g/L) in weeks 16 to 40. An additional reason to explain the anaemia is the increased demand for iron by the foetus.

The British Nutrition Foundation recommends the healthy female aged 11-50 needs 14.8 mg of iron daily, with no extra in pregnancy, but an extra 6 mg when lactating in months 0 - 4, then 2.5 mg extra thereafter. Therefore, she will be at risk of an iron-deficient anaemia without a change in diet. In the immediate post-partum period, blood loss during labour needs to be replaced and so extra iron will be needed. There is also an extra need for

micronutrients folate, thiamine, riboflavin, and vitamins A, C and D, and supplements may be needed to reduce the risk of certain anaemias and of neural tube defects in the infant. Red cells can also increase their size, as is assessed by the mean cell volume (MCV), often by 5 to 10 fL

Thrombosis

Anticoagulation must be considered even before a woman becomes pregnant as warfarin can cross the placenta to produce foetal abnormalities and is therefore contraindicated. So, if a woman on warfarin (regardless of the reason, such for antiphospholipid syndrome, DVT and pulmonary embolism (PE), as introduced in Chapter 3) is considering pregnancy, she needs to come off warfarin and use LMWH. Monitoring of the latter, if necessary, would be by the blood test for coagulation factor Xa.

Pregnancy in itself brings a risk of DVT and/or PE, but this risk is generally so small that anticoagulation with a LMWH is not called for. However, if a woman does have a DVT whilst pregnant, she will probably need to go onto a LMWH, the activity of which needs to be monitored by levels of factor Xa. The reasons for this increased risk of VTE are unclear but may be related to increased levels of clotting factors such as prothrombin, factor VIII, and fibrinogen.

There may also be a reduced ability to remove a clot (fibrinolysis) once formed. For those who have had a single VTE, even with a family history of thrombophilia, the initial dose should be five days of a higher weight-adjusted LMWH (preferably in two divided doses) followed by a prophylactic dose for the rest of the pregnancy. This will need to be continued post-partum with at least six weeks of either warfarin or LMWH, although some women may need anticoagulation for up to 6 months.

Once the baby has been delivered, the woman is even more at risk of DVT or PE. Potential reasons why this entirely healthy condition confers such a risk of thrombosis include a reduction in the risk of post-partum haemorrhage, or possibly that the growing uterus slows venous blood return up the inferior vena cava in the lower abdomen. The risk of DVT in the six weeks post-partum is threefold the ante-partum risk, whilst the risk of PE is eight fold higher. Diagnosis of VTE is impaired by the fact that pregnancy alone can confer raised D-dimers so that non-invasive methods such as ultrasound are required. Post-partum, warfarin can be used, and DVT can be managed as non-pregnant women, but not with unfractionated

heparin as LMWH safety data is better. The Royal College of Obstetrics and Gynaecology (www.rcog.org.uk) have their own guidelines for managing risk of VTE.

Perhaps 8 to 10% of women will experience a fall in their platelet count during pregnancy, and this may be to below the normal range (i.e., a thrombocytopenia). However, this is rarely associated with a risk of significant bleeding.

Apart from DVT and PE, the pregnant woman is at risk of a very rare type of haemolytic anaemia (which manifests with elevated liver enzymes (LFT's: Chapter 8) and low platelets (hence the acronym HELLP). Another dangerous condition is a generalised coagulopathy called disseminated intravascular coagulation (DIC) that may call for admission to an intensive care unit. If DIC and HELLP co-exist, then the induction of labour or caesarean section may be indicated.

Biochemistry

The numerous physiological changes during pregnancy mean that many indices applicable in non-pregnant woman are simply not valid (Appendix 3). The weight gain of perhaps 12 kilograms includes about 5 litres of water, perhaps a litre of which contributes (as in haematology) to a haemodilution. A consequence of this is that the glomerular filtration rate rises so that urea and creatinine levels are lower.

Diabetes, if either present before pregnancy, or developing during pregnancy (i.e. gestational diabetes), is associated with fetal mortality and morbidity (such as high birth weight and respiratory distress). Thus adequate control of hyperglycaemia is required, although excess glucose in the urine (i.e. glycosuria) is common because of changes in renal function and so does not necessary imply a glycaemic pathology.

One of the more common and serious complication of pregnancy, pre-eclampsia, is characterised early on by hypertension and later by ankle oedema. The laboratory can generally demonstrate increasing serum urate and later rising serum urea and creatinine (i.e. the reverse of a healthy pregnancy) resulting from a falling GFR. A further consequence is proteinuria – generally detected by a urine dipstick. If untreated, pre-eclampsia leads to life-threatening eclampsia.

Prolactin is known to be higher in women than men (103 - 497 mU/L and 86-324 mU/L respectively), with levels up to 8,000 in pregnancy. Similarly, oestrogen, progesterone, testosterone, luteinising hormone, follicle stimulating hormone all vary between the sexes, and in women dependent on their age and the phase of their menstrual cycle.

Immunology and blood transfusion

Many laboratories offer ante-natal testing for antibodies to a number of pathogens that include hepatitis B (surface antigen and its antibody), HIV, syphilis, and rubella. Antenatal screening for Rh D will identify those D negative women who are likely to need anti-D immunoglobulin prophylaxis to reduce the risk of future haemolytic disease of the newborn (HDN). At a later stage it is possible to determine if this has been successful by testing for anti-D antibodies. Other antibodies that can be tested for include the K antigen and the c antigen.

Paediatrics

That the transition from intra-uterine to independent life demands numerous changes and so differences in a child's blood compared even to adolescents and adults is hardly surprising. Once more, these are entirely physiological. A crucial aspect of paediatric blood science is the need to refer to local reference ranges and guideline. Notably, such values in textbooks often demonstrate considerable variation. A second caveat is the variation in the definition (days, weeks, months, and years of life) of neonate, infant, child and even adolescent.

Haematology

The haemoglobin, red cell count and mean cell volume vary considerably and change rapidly in the first three months of independent life, and the transition to 'adult' levels of many blood indices may take additional months and even years. Indeed, haemoglobin levels at birth may be regarded in adult terms as polycythaemia (e.g., 160 to 190 g/L in the neonate compared to perhaps 125 to 170 g/L in the adult), rise a little more in the 24 hours that follow, and then fall. By six months, levels are around the lower limit of normal for an adult (maybe 125 g/L). Consequently, the definition of anaemia should not be made according to adult values. Causes of anaemia, if present, include haemolytic disease of the newborn, infections and haemoglobinopathy (the latter largely sickle cell disease and

thalassaemia). Notably, perhaps 30% of all new cases of haemoglobinopathy could not be predicted by the genetic status of the parents and would thus be due to unforeseen mutation.

Prematurity brings its own special problems, one of which may be an anaemia due to nutritional deficiencies such as iron and vitamin B_{12}. Insufficient vitamin K may lead to disorders of haemostasis with bruising and bleeding as certain coagulation proteins are reliant on this micronutrient. Healthy infants have 'adult' levels of platelets, but neonatal thrombocytopenia is present in up to a quarter of babies referred to neonate intensive care units.

Biochemistry

The kidney should be fully formed and operational by week 36 of gestation although the glomerular filtration rate in relatively low at birth. Thus, serum creatinine rises in the immediate neonatal period, falls after about 4 weeks, and then remains low for perhaps five years, when levels rise to those of adults.

The liver is a relatively late-developing organ and in the first few weeks of life may not be capable of metabolism all its own bilirubin (previously managed by the mother) resulting in jaundice. However, this should resolve rapidly as the liver undergoes maturation. However, jaundice in the first 24 hours is almost all pathological - more concerning reasons for jaundice include haemolytic disease of the newborn or abnormalities of the liver. It follows that the premature child will be at risk of additional metabolic problems.

Levels of oestrogen, progesterone, testosterone, luteinising hormone, follicle stimulating hormone are all low or absent before puberty. Indeed, increased levels of these hormones, possibly arising from a gonadal malignancy before this stage, nevertheless often define precocious puberty.

Neonatal screening

Several pathological conditions are apparent within the first year of birth and can be screened from in the first few days of life, often on a spot of blood obtained about a week about birth. Perhaps the best known biochemistry examples of this are for congenital hypothyroidism and phenylketonuria (PKU). The former (present in 1/3,500 live births in the

UK), if untreated, can result in irreversible cretinism – the most common cause being failure of the thyroid gland to develop adequately and produce the thyroid hormones T3 and thyroxine (Chapter 11). This deficiency, once detected, results in raised TSH – the basis of the relevant blood test and so the diagnosis. If discovered, treatment is with small doses of thyroxine that can be increased to an adult dose as the child approaches its teenage years.

PKU (present in 1/10,000 live births in the UK) is the result of an error in amino-acid metabolism (lack of a particular enzyme), the clinical consequences of which include neonatal irritability, poor feeding, eczema, and fair hair with blue eyes. The later consequence of mental retardation can be minimised with a diet low in the amino acid phenyl-alanine.

In paediatrics, a positive or equivocal result should always be confirmed by a repeat test.

The Elderly

The process of aging is inevitable and is associated with many changes as physiology merges with pathology. Abnormal laboratory results are more common in the aged because they have more disease, and the increased use of prescription drugs is likely to cloud a diagnosis. There are relatively few instances where the reference range varies with age in the absence of clear disease. These include renal function, manifesting as a deterioration in the GFR with age: indeed, both common calculators input age into their equations. With decreasing fertility in both sexes, there are reduced levels of sex hormones, but most marked in women around the age of 50.

Levels of growth hormones peak in the teenage years and fall markedly and progressive after the age of 20. Some consider that ESR rises ‘normally’ with age, although others contend that this reflect a low level of an as yet asymptomatic pathology. Similarly, others are prepared to accept a lower level of haemoglobin in elderly that would stimulate an investigation in someone a decade younger. Age related increased in the reference range for prostate specific antigen: age 50 – 59: $\leq$3, age 60 - 69: $\leq$4, age >70: <5.

Notably, as witnessed in studies of vaccinations for COVID-19, the ability to generate antibodies to a defined pathogen falls with age. This may account for an increased frequency of infections in this group.

APPENDIX 2

PHYSIOTHERAPY AND PODIATRY

The changes in the NHS have brought about the opportunity for Physiotherapy and Podiatry practitioners to take a leading role in the diagnosis and management of their patients. Blood tests can help in both of these processes.

Physiotherapy

There are several separate areas of the body and its diseases to consider.

Musculoskeletal disease

In seeking to clarify a diagnosis of leg, hip and back pain, tests of inflammation may be useful in differentiating osteo- and rheumatoid arthritis (RA). The former is unlikely to have abnormal inflammatory markers (ESR, CRP, white cell count) whereas these are common in the latter. A raised rheumatoid factor (RhF) and/or anti-citrullinated protein antibody (ACPA) leads to a strong suspicion of RA, but joint disease and clinical history are also important. However, both may be raised in systemic lupus erythematosus, but an abnormal level of anti-nuclear antibodies are common, as are autoantibodies to DNA. Other diseases in this area include polymyalgia rheumatica (PMR, literally rheumatic pain of many muscles) and temporal arteritis, but neither of these have precise tests. However, as will all such diseases increased ESR and CRP can be expected. Muscle wasting diseases such as polymyositis may be associated with increased creatine kinase.

Ankylosing spondylitis (AS) lacks a definitive blood test, as the disease is generally restricted to the lower vertebrae and pelvis. However, many patients are positive for HLA-B27, although this has a particularly poor sensitivity and specificity (that is, many people with this HLA do not have AS, and many AS patients are negative for B27. Do not expect abnormalities in bone markers such as calcium, and increased systemic

markers of inflammation are unlikely. Muscle wasting diseases such as polymyositis may be associated with increased creatine kinase.

Bone and orthopaedics

Lack of vitamin D is the cause of osteomalacia (Ricket's in children), whilst Paget's disease (associated with bone pain and deformity) is often linked with a raised alkaline phosphatase. Gout is caused by crystals of uric acid/urate in the tissues and joints. They are the result of increased levels of uric acid/urate, the most common causes of which are (in order) excess alcohol (> 42 units/week), use of diuretic drugs, renal Impairment, and obesity (BMI > 30). Gout can be differentiated from pseudogout as a common form of the latter are calcium pyrophosphate crystals, and so is also known as chondrocalcinosis and pyrophosphate arthropathy.

Bone pain may be due to myeloma and secondary deposits of cancer (such of the prostate and breast). In such cases, increased bone markers (calcium, alkaline phosphatase) may be helpful. There are no markers of breast cancer, but in prostate cancer levels of prostate specific antigen (PSA) are often increased. Physiotherapy after surgery, especially orthopaedic, may need to take account of anticoagulation.

Stroke

There are no tests for this condition, or its precursor of transient ischaemic attack, although it may have been precipitated by abnormal lipids, high blood pressure and/or atrial fibrillation.

The chest/respiratory

Pains in the chest are symptoms of both coronary artery disease and pulmonary embolism, but both of these should be addressed urgently in Accident and Emergency. There are no specific blood tests in chronic obstructive pulmonary disease, although non-specific markers of inflammation may be raised. In severe and acute asthma there may be raised IgE, but pulmonary function tests are probably more important.

For details of the classification of rheumatic diseases, see the website of the American college of Rheumatology at:
http://www.rheumatology.org/practice/clinical/classification/index.asp

Podiatry

Although these professionals are principally interested in the leg below the knee, disease of this organ may have systemic manifestations. Perhaps the leading blood tests in podiatry are for diabetes, which are fasting plasma glucose, the OGTT and HbA1c, as discussed extensively in Chapter 9. Those podiatrists about to undertake debridement and other surgery need to be aware of the use of anticoagulants and so a risk of excessive bleeding (Chapter 3).

Other diseases of relevance for podiatrists include:

- Osteomalacia/Rickets: caused by lack of vitamin D
- Osteomyelitis: no very valuable blood tests, although general tests of inflammation (ESR/white cell count/CRP) may be helpful. This condition is generally diagnosed clinically and by X-ray of the foot, where there is likely to be bone destruction.
- Paget's disease: alkaline phosphatase is essential, and X-ray may be used to define abnormal bone structure.
- Rheumatoid arthritis/osteoarthritis: as above, general tests of inflammation, but also RhF, ACPA and ESR (Chapters 5 and 9).
- Muscle wasting diseases such as polymyositis may be associated with increased creatine kinase.
- Gout in a painful and swollen toe may be confirmed with levels of uric acid, and also U&Es.

Notably, many of these are of interest to physiotherapists.

APPENDIX 3

ADULT REFERENCE RANGES FOR COMMON BLOOD RESULTS

(n.b. these may be different from those of your own hospital, and so you must practise according to the local laboratory)

Analyte	**Reference range**	**Units**
Alanine aminotransferase	5 – 42	IU/L
Albumin	35 – 50	g/L
Alkaline phosphatase	20 – 120	IU/L
Amylase	30 - 120	IU/L
Anti-citrullinated antibodies	<7	U/mL
Anti-nuclear antibodies	<1/40	Titre
Aspartate aminotransferase	10 – 50	IU/L
Basophils	0.02 – 1.0	10^9/L
Bicarbonate	24 – 29	mmol/L
Bilirubin	<17	µmol/L
Blast/atypical cells	<0.1	10^9/L
Calcium	2.2 – 2.6	mmol/L
Cholesterol (total)	<5.0	mmol/L
C-reactive protein	<5	mg/L
Creatinine	71 – 133	µmol/L
Creatine kinase	55 – 170	U/mL
Creatine kinase MB	<25	U/mL
Cystatin C	0.6 – 1.0	mg/mL
D-dimers*	<500	Units/mL
eGFR	>90	ml/min/1.73m^2
Eosinophils	0.02 – 0.5	10^9/L
Erythrocyte sedimentation rate	<10	mm/hour

Fibrinogen	1.5 – 4.0	g/L
Gamma-glutamyl transferase	5 – 55	IU/L
Globulins	20 – 25	g/L
Glucose (fasting)	3.5 – 5.5	mmol/L
Haematocrit (female)	0.33 – 0.47	L/L
Haematocrit (male)	0.35 – 0.53	L/L
Haemoglobin (female)	118 – 148	g/L
Haemoglobin (male)	133 – 167	g/L
HDL cholesterol	>1.2	mmol/L
Insulin	2 – 10	mU/L
International normalised ratio	2 – 3 or 3 – 4	
Lactate	0.7 – 1.9	mmol/L
Lactate dehydrogenase	240 – 480	IU/L
LDL cholesterol	<3.0	mmol/L
Lymphocytes	1.0 – 3.0	10^9/L
Magnesium	0.8 – 1.2	mmol/L
Mean cell haemoglobin	26 – 33	pg
Mean cell haemoglobin conc.	330 – 370	pg/L
Mean cell volume	77 – 98	fL
Monocytes	0.2 – 1.0	10^9/L
Neutrophils	2.0 – 7.0	10^9/L
Osmolality	275 – 295	mOsm/kg water
Parathyroid hormone	1 – 6	pmol/L
Partial thromboplastin time	24 – 34	seconds
pCO_2	4.7 – 6.0	kPa
pO_2	12 – 14.6	kPa
pH	7.35 – 7.45	$-\log_{10}[H^+]$
Phosphate	0.7 – 1.4	mmol/L
Plasma viscosity (at 25°C)	1.5 – 1.72	mPa/s
Platelets	143 – 400	10^9/L
Potassium	3.8 – 5.0	mmol/L
Protein	60 – 80	g/L
Prothrombin time	11 – 14	seconds

PSA (prostate specific antigen)**	<3 to <5	ng/mL
Red blood cell count (female)	3.9 – 5.0	10^{12}/L
Red blood cell count (male)	4.3 – 5.7	10^{12}/L
Red cell distribution width	10 – 15	%
Rheumatoid factor	<20	IU/mL
Sodium	135 – 145	mmol/L
Thyroid stimulating hormone	0.2 – 3.5	mU/L
Thyroxine (Free T4)	10 – 25	pmol/L
Tri-iodothyronine (Free T3)	4 – 6.5	pmol/L
Triglycerides	<1.7	mmol/L
Troponin I	<4.0	ng/mL
Urate/uric acid	0.14 – 0.43	mmol/L
Urea	3.3 – 6.7	mmol/L
White blood cells	4.0 – 10.0	10^{9}/L

*Dependent on method, **Age dependent.

Key point: The reader is reminded that blood result reference ranges can change over time and from laboratory to laboratory. He/She must work to the range provide by their own laboratory.

Selected biochemistry and haematology normal/reference values in pregnancy

Analyte	Unit	Non-pregnant normal range	Pregnant normal range
ALT	IU/L	5 - 42	2- 33
Albumin	g/L	35 - 45	26 - 42
Alk Phos	IU/L	20 – 120	30 - 230
AST	IU/L	10 - 50	3 - 33
Bicarbonate	mmol/L	24 - 29	18-26
Bilirubin	umol/L	<17	<14
Calcium	mmol/L	2.2-2.6	2.2-2.8
CRP	mg/L	<5	<20
ESR	mm/hour	<10	4 - 85
Fasting glucose	mmol/L	3.5 – 5.5	4.0 – 4.4
Fibrinogen	g/L	1.5 – 4.0	2.0 – 6.0
Gamma GT	IU/L	5 – 55	2 - 26
Haematocrit	Proportion	0.33 - 0.47	0.28 – 0.41
Haemoglobin	g/L	118 - 148	95 – 140
MCV	fL	77 – 98	82 – 103
Platelets	10^9/L	143 – 400	150 - 430
Potassium	mmol/L	3.8 – 5.0	3.3 – 5.1
Prothrombin time	Seconds	11 – 14	8 - 12
Red cell count	10^9/L	3.9 – 5.0	2.8 – 4.5
Sodium	mmol/L	135 - 145	129-147
TSH	mU/L	0.2 – 3.5	0.4 – 4.0
Total protein	g/L	60 - 80	56 - 76
Urea	mmol/L	3.3 – 6.7	1.5 - 3.8
White cell count	10^9/L	4 - 10	5.6 – 16.9

Recall that many indices vary between the sexes even in the absence of pregnancy. Some results vary according to trimester. As with other ‘normal/reference ranges’ this is provided for perspective and should NOT be used in your clinical setting as your local normal/reference ranges will apply.

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