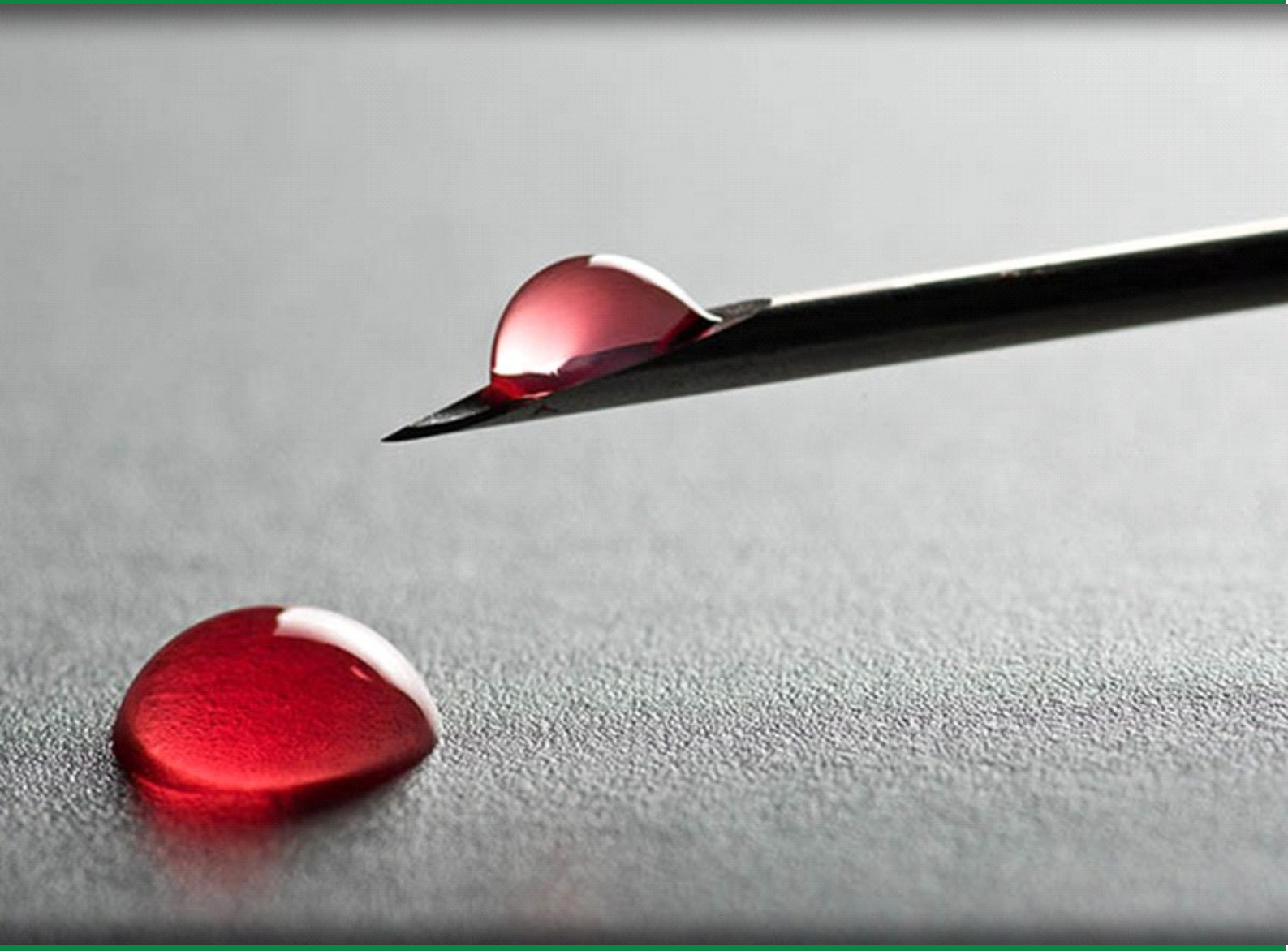
DR. GRAHAM BASTEN

INTRODUCTION TO CLINICAL BIOCHEMISTRY

INTERPRETING BLOOD RESULTS



Dr. Graham Basten

Introduction to Clinical Biochemistry: Interpreting Blood Results

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About the Author

Dr. Graham Basten

De Montfort University

Associate Head of School

School of Allied Health Sciences

Faculty of Health & Life Sciences

Room H1M-2 Hawthorn Building

Leicester LE1 9BH

E-mail: gbasten@dmu.ac.uk

Phone: 0116 207 8639

Fax: 0116 250 6411

Website: http://www.dmu.ac.uk/research/hls/staff/basten.jsp

Twitter: http://twitter.com/grahambasten

Academic Blog: http://isothiocyanates.blogspot.com/

Research Blog: http://grahambastenresearch.blogspot.com/

Short Biography

Dr Graham Basten is Associate Head of the School of Allied Health Sciences at De Montfort University (UK). He holds a PhD from the UK government's Institute of Food Research and has researched and lectured extensively over the past 10 years on clinical biochemistry, nutrition and folate at the Universities of Sheffield and Nottingham (UK). He is a De Montfort University Teacher Fellow and has been nominated for the Vice Chancellor's Distinguished Teaching Award. As a senior lecturer in Clinical Chemistry, and as leader of the undergraduate Projects module, this expertise and experience is transferred to the concise introductory textbooks written for Book Boon.

Select research publications

1. Blood folate status and expression of proteins involved in immune function, inflammation, and coagulation: biochemical and proteomic changes in the plasma of humans in response to long-term synthetic folic acid supplementation. Duthie SJ, Horgan G, de Roos B, Rucklidge G, Reid M, Duncan G, Pirie L, Basten GP, Powers HJ. J Proteome Res. 2010 Apr 5;9(4):1941-50
2. Sensitivity of markers of DNA stability and DNA repair activity to folate supplementation in healthy volunteers. Basten GP, Duthie SJ, Pirie L, Vaughan N, Hill MH, Powers HJ. Br J Cancer. 2006 Jun 19;94(12):1942-7. Epub 2006 May 30
3. Associations between two common variants C677T and A1298C in the methylenetetrahydrofolate reductase gene and measures of folate metabolism and DNA stability (strand breaks, misincorporated uracil, and DNA methylation status) in human lymphocytes in vivo. Narayanan S, McConnell J, Little J, Sharp L, Piyathilake CJ, Powers H, Basten G, Duthie SJ. Cancer Epidemiol Biomarkers Prev. 2004 Sep;13(9):1436-43
4. Effect of folic Acid supplementation on the folate status of buccal mucosa and lymphocytes. Basten GP, Hill MH, Duthie SJ, Powers HJ. Cancer Epidemiol Biomarkers Prev. 2004 Jul;13(7):1244-9

Professional Qualifications and Memberships

- Fellow of the Higher Education Academy (HEA) and National Teacher Fellow Reviewer
- De Montfort University Teacher Fellow
- Member of the Institute of Biomedical Science
- Member of the Phytochemical Society of Europe
- Science Technology STEM Ambassador
- Member and De Montfort University (DMU) Representative for the Society of Biology
- Member of the Sherwood Forest Hospitals NHS Trust

Introduction to Clinical Biochemistry: Interpreting Blood Results

Preface

This book is primarily aimed at undergraduate students reading medicine, nursing and midwifery and subjects allied to health. It will also be useful to professionals undergoing continuing professional development (CPD) or changing to an extended role who require a background covering physiology and pathology for haematology and biochemistry. Since the book uses “example boxes” to explain complex terms in lay language, it should also be accessible to patients and people with a non-clinical background but an interest in the subject. To facilitate this, each chapter has an introductory paragraph guiding the reader to the example boxes if needed and a summary section.

Chapter 1 examines how to interpret results, with the remaining broadly representing a section of the body or a disease type with chapter 9 as a summary. This should enable a read from cover to cover or equally as a reference with each chapter independent. As this book is an introduction to the area, you may be inspired for further training and reading. There are many excellent resources online, too many to list here, although I would recommend starting with your countries’ primary care provider organisation, respected charities, reputable training companies and higher education institutes for further information.

Study with the textbook using key concepts (these are the headings and sub headings). List the key concepts and attempt to write a few words about each section, and then refer back to the text book.

Expert boxes are provided as cues for further reading, as this text is an introductory overview it is not conducive to all readers to cover all aspects in considerable detail.

Example boxes will provide worked examples or case studies

Disclaimer

Reference ranges, normal, abnormal and cut-off values are provided as examples to explain a term or disease setting, these are not transferable to the reader’s own setting. Similarly, definitive diagnosis, prescribing, treatment, monitoring times are excluded; the reader should seek this information from their primary care provider or trusted sources. Case studies are used throughout but should not be used as the basis for a diagnosis. Neither the author nor the publisher accepts any liability for the reader using the information in the book inappropriately.

1. Laboratory tests: Interpreting Results

This chapter introduces the key steps in ensuring that the blood result is as accurate as possible. Whilst true that in most modern hospitals much of the calculations in this chapter will be automated and produced by computer programmes, an understanding of the basic principles is to be encouraged. The non-clinical reader may find the section about reference ranges most useful.

1.1 A typical blood sciences service

Clinical chemistry, chemical pathology and clinical biochemistry are names given to the study of biochemical events or parameters in the body. Increasingly and intuitively, this study has merged with certain aspects of haematology (blood cells and liquid). This has created a new field called “blood sciences” which encompasses sections of both disciplines to create a more logical service to users. Typically a blood science service will have a laboratory manager who will oversee the production of reliable results, and will usually be state registered. Logistically, there will be areas for sample reception, analysis, reporting data and storage of material.

1.2 Variables that may affect a result: Analytical

There are two factors can affect the result of a blood test, analytical or physiological. Analytical error is caused by the service, typically the machine or process which produces the result. Physiological error is caused by people, typically how the blood is collected, whether the patient was fasted or taking medication.

1.2.1 Analytical sensitivity and specificity

Each blood test will have an experimental technique to produce the result. Confusingly, two sets of sensitivity and specificity exist in blood sciences; they have total different meanings and refer to either analytical and physiological (or diagnostic) measures.

Analytical sensitivity refers to the detection limit of the experiment. This is the smallest amount of material of interest that the experiment can detect. As technology has developed it is increasingly easier to measure smaller quantities of material, although at small levels the accuracy and precision may be lower and this can be measured using quality control.

Example: Prostatic Cancer (Cancer of the prostate): A test for this is prostate specific antigen (PSA). PSA increases with the severity of the disease but is also elevated in the early stages, if the method could not detect low levels of PSA then early diagnosis would not be possible.

Analytical specificity refers to whether any other similar chemicals interfere with the test.

Example: Insulin levels may be measured in diabetic management, and it is beneficial to have a test that can differentiate between insulin and proinsulin to avoid an artificially elevated result.

1.3 Standards

Most tests will have a type of standard to measure quality and ensure the result is as accurate as possible. There are usually two types either primary or secondary. A primary standard is of a known quantity and is often produced externally with certification, they are used to characterise the upper and lower parameters or sensitivity of the test. Often labelled as high, low, calibrators, controls they can also be used to calibrate on-board software of automated analysers. They have a clear advantage in that they can be stored long term and provide a known amount or concentration; a clear disadvantage is that they are usually in a "pure" matrix such as saline or a buffer. I'm confident in declaring that there is no human alive with blood constituted from 100% saline. Hence the need for secondary standards which are usually samples of plasma, serum or whole blood to ensure that test is suitable and consistent in the chosen matrix. This forms the basis for within and between batch variation (see chapter 1.4)

Example: Red cell folate (RCF) primary standards in saline buffer were used to calibrate the machine and create a calibration curve. Red cells from a single volunteer were obtained and lysed (broken open) and RCF was measured many times (several replicates) in one attempt giving a mean (average) RCF of 300nM (secondary standard). Subsequent measures of RCF in the service would include the primary standard but also the secondary standard alongside various patient samples sent for RCF testing.

1.4 Quality Control: Within batch, between batch, external

Each blood test result produced by an accredited laboratory will have quality control procedures in place to ensure that inevitable variations in machine, staff, and temperature do not affect the result.

1.4.1 Within batch variation

This is used to evaluate how good the technique is at giving the same result for identical samples in *one attempt*. This method often identifies whether a machine has bias towards a certain location on the rotor.

Figure 1.1 shows a typical rotor which has 11 samples ready to analyse. H and L are primary standards (see chapter 1.3) and each yellow circle represents the *same* sample in replicate.

With the results from the experiment we can calculate the mean and standard deviation (SD) of the 9 identical yellow samples. Using the mean and standard deviation we can calculate the %Coefficient of Variance (CV)= ((SD /Mean)x100). This data is used to create the Y-Axis of a control plot (see 1.5). A common misconception is that a low CV is by default better than a higher one. The most important thing is that to consider is if the *CV changes*.

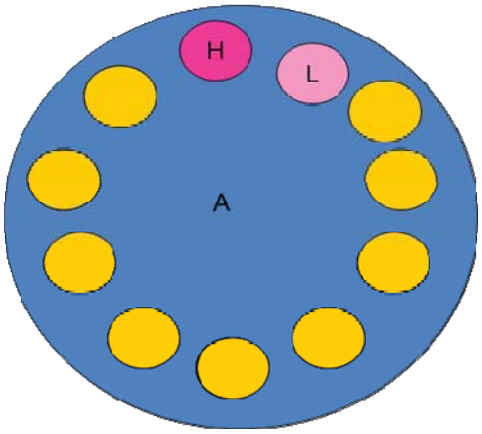

Figure 1.1: Within batch variation

1.4.1 Between batch variation

This is used to evaluate how good the technique is at giving the same result on *separate attempts.* It is used to evaluate for example, if the machine or indeed a different operator will give a different result at a different time. Figure 1.2 shows the next two rotors done after our within batch test. They too have H and L standards (primary) and the *same yellow* sample we used for within batch (secondary) and it is this data (either from the primary, secondary, or both) which is used to create the X-Axis of a control plot (see 1.5) However, we also have the green sample which are *different* patient's samples and the control plot will inform the service if the green results are suitable to be released as accurate..

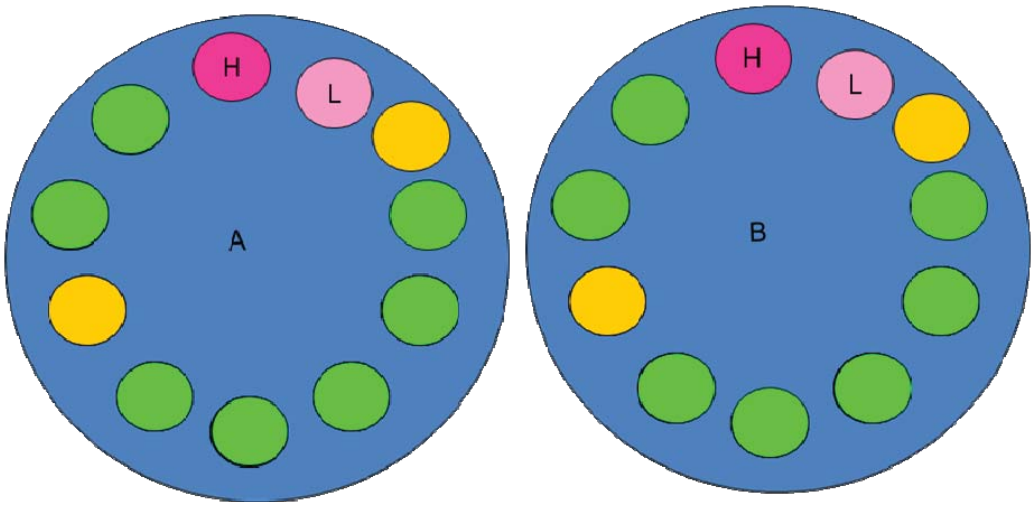

Figure 1.2: Between batch variation

1.4.1 External quality control

This is used to evaluate if you get a different result if the test is done at a different site. Typically, a national organisation will send the *same sample* to different laboratories accredited to produce blood results and measure how similar the returned result is.

1.5 Control Plots

There are two commonly used of control plots, either Levey Jennings or Westgard and both inform the service of the accuracy of the test over time. Excellent online resources for both these plots exist, accessible by any good search engine, and will provide further reading. They usually have a range of the mean plus or minus 3SD (standard deviation) which on normally distributed data is 99.7% confidence. Test results for a patient sample which fall outside of this range are to be considered for rejection. The plots also inform the test is over or under reporting results over time.

Example: 500 samples within batch cholesterol secondary standards were measured. The mean was 200 mg/dL and the SD was 4. Therefore, 2SD = 8, 3SD = 12 and so on. On the Y-axis plot the mean +3SD through to the mean -3SD, so the mean is 200, the mean plus 3SD is 212 and the mean minus 3SD is 188 (Figure1. 3)

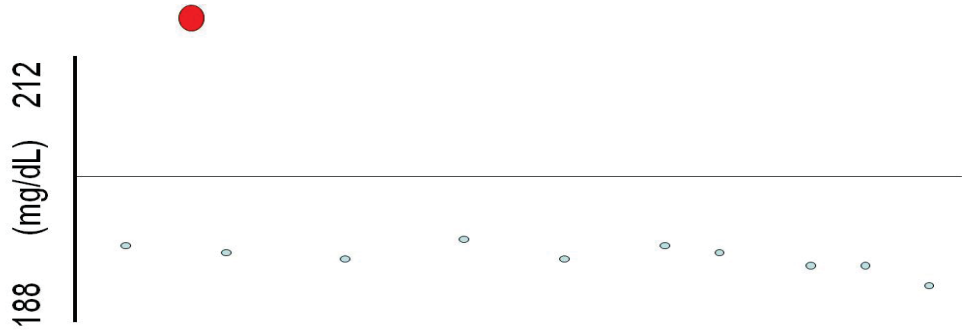

Figure 1.3: Example of a control plot.

On the Y-axis is the mean plus and minus 3 SD determined by the within batch, on the X-axis is the mean value for each between batch test. The red dot is a mean value which is outside the 3SD range and may be considered for rejection. Each blue dot (x10) is the mean value of the between batch over 10 days, a blue dot per day, this shows that this test is under reporting. Think about what a test that over reported may look like?

1.6 Precision, Accuracy, Bias

Precision, Accuracy, Bias are terms used to describe certain parameters of the test. Precision is how close repeated measures of the same sample lie, accuracy is how close the value reported is to the true value and bias describes variables which may affect precision and accuracy and lead to over and under reporting or large random background changes.

A helpful analogy is that of Robin Hood who has 5 arrows and must hit the middle of the red centre bull's eye to win the pageant and release Maid Marian (FIG. 1.4) he needs to be accurate and precise, a reason for not being could be "bias" such as a cross wind, bent arrows, too much wine or mead!

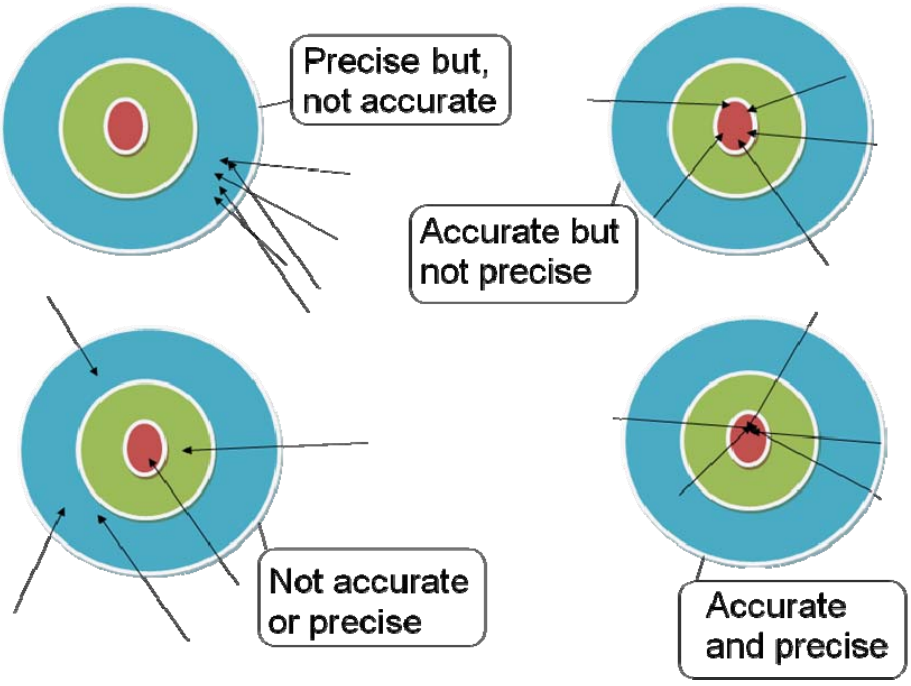

Figure 1.4: Robin Hood at the pageant

1.7 Variables that will affect a result: Physiological

Sections 1.2 to 1.6 looked at how the machinery and experimental method (analytical) can produce errors in the results. In the remainder of the chapter issues caused by people will be explored. Such as, how blood is collected and stored, the difference between plasma and serum, using reference ranges and clinical sensitivity and specificity.

1.7.1 Blood collection and storage techniques

Tourniquets are often used to collect blood samples as they block venous return and cause dilatation enabling identification of entry points. However, due to this phenomenon typically, for each minute of use due to loss of water and electrolytes from plasma, plasma protein increases by up to 1%. The stasis of blood flow can produce different metabolic products such as lactate and if the patient is asked to clench their fist this may cause an artifactual hyperkalemia (elevated potassium levels). Clearly these disadvantages do not justify the non-use of tourniquets but may be worth considering if results appear unlikely based on symptoms or unreliable.

Caution should be given if blood is being taken near an intravenous entry site as to not in effect be taking a sample from the saline or glucose bag itself, a highly unlikely plasma glucose of over 50,000 mg/mL was measured in such a patient!

Other problems include poor patient identification, samples taking more than 72 hours to be transported to the service, incorrect temperature or not protected from light. To reduce this error each test will have a specific blood collection protocol.

1.7.2 The difference between plasma and serum

Serum is thought to be a derivative word for “whey” as in “curds and whey” which are the products formed when milk is allowed to clot. The whey is the liquid component whilst the curds are the solid parts. If blood is allowed to clot the liquid component is therefore called serum, if blood is prevented from clotting then the liquid component is called plasma (FIG. 1.6).

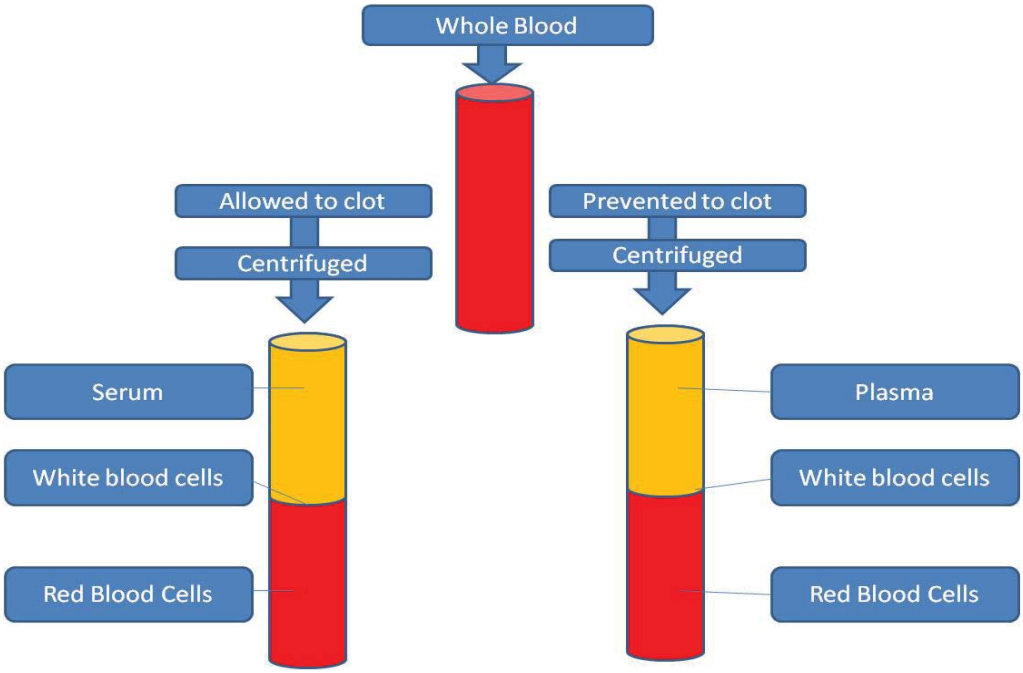

Figure 1.6: The difference between plasma and serum

The main disadvantage of using serum is that the blood has already participated in clotting. In other words, a series of metabolic processes have occurred after the sample was collected but before the sample was measured and this can lead to error in measures like potassium, phosphate, magnesium, aspartate aminotransferase and lactate dehydrogenase. As we will see in alter chapters these are key measures of acute and chronic disease. The advantages of serum is that it can be used to measure constituents which would be destroyed or compromised by the anticoagulant chemicals used in the preparation of plasma samples.

The main disadvantages of using plasma (blood which has not clotted due to the addition of anticoagulants) is the anticoagulants can interfere with certain analytical methods or change the concentration of the constituents to be measured.

The advantages of using plasma samples include “cleaner” samples which have not undergone the clotting process, time saving and a higher yield (up to 20 %)

1.7.3 A haemolysed sample.

If following centrifugation the plasma or serum looks reddish rather than straw yellow, it is likely the sample has haemolysed (FIG. 1.7). In a haemolysed sample some of the red blood cells have lysed (broken open) and their contents have now contaminated the plasma or serum sample. This will cause error in reporting amongst others elevated potassium, magnesium and phosphate. Some analytical methods may be able to negate the effect of the haemolysed sample. Common causes of a haemolysed sample are collection needle gauge too narrow, over vigorous shaking of the sample, underlying haematological disorder, red cells isolated for storage and then stored in water or a non isotonic solution and over physical dispensing of blood from hypodermic syringe to collection tubes.

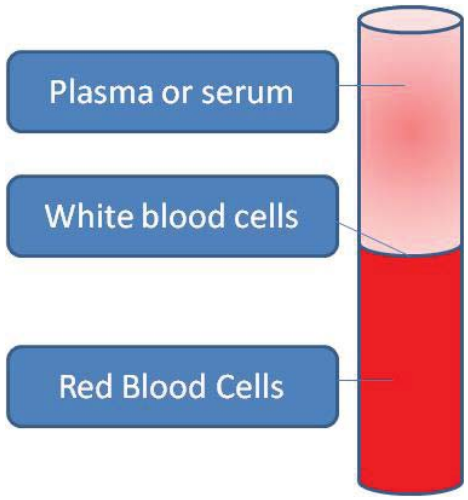

Figure 1.7: A haemolysed sample

1.7.4 Reference ranges

Most people are comfortable with the idea of reference ranges, but what do actually tell us, or rather don't tell us? To create a reference range a number (usually over 120) of volunteers are matched for factors (table 1) and the analyte is measured. Firstly, most ranges have a 95% confidence which means that the top 2.5% of values and the bottom 2% of values are omitted, so it is possible to be healthy but outside the reference range, you are just at the very top or the very bottom which aren't shown (see 1.7.6). Secondly, you should use ranges from unvalidated sources with great care as ranges can vary with age and sex for example.

Age	**Posture**
Blood group	**Race**
Circadian variation	**Sex**
Diet	**Menstrual cycle**
Ethnic background	**Pregnancy**
Exercise	**Time of day**
Fasted	**Tobacco 2ndary**
Geographical location	

Table 1 shows common attributes which are matched to create, or can affect a reference range.

1.7.5 Clinical cut off values

Reference ranges are generally used to identify a range of "normality" a value outside of this may justify further investigation. Values that are outside reference ranges may be matched to case control studies and attributed a disease progression status using clinical cut off values.

Example: Prostate-specific antigen (PSA) is secreted by the prostate and elevation may require further investigation. In a case control trial in men with benign prostate (BPH), normal and cancer (PC) PSA was measured and followed a trend:
0-4 ng/mL (PSA) = normal reference range
4 to 10 ng/mL = BPH but not PC
10-20 ng/mL = Often PC
>20 ng/ML = Almost always PC

1.7.6 Clinical sensitivity and specificity

In order to describe clinical sensitivity and clinical specificity, remember these are different to analytical sensitivity and specificity, we need to think about how references ranges for healthy and diseased patients interact. Figure 1.8 shows a healthy reference range in green and a diseased reference range in red with TN, FP, TP and FN annotated (table 2). Therefore, clinical specificity relates to whether the test can report someone without the disease correctly as being "healthy", conversely clinical sensitivity is whether the test can report someone with the disease correctly as being "diseased". To calculate use the following equations Sensitivity = TP/TP+FN and Specificity = TN/TN+FP

Example: To help to remember clinical sensitivity and specificity there are three strategies:

1) *Human nature is to be sensitive towards people with a disease, remember sensitivity measures the diseased cohort.*
2) *Using figure 1.8 if you draw a green curve (healthy people- specificity) and draw a cut off line, this will give you the healthy people (TN) and those incorrectly assigned diseased as they are on the right of the cut off line (FP). Using these values the equation is therefore TN/TN+FP.*
3) *Using figure 1.8 if you draw a red curve (diseased people- sensitivity) and draw a cut off line, this will give you the diseased people (TP) and those incorrectly assigned healthy as they are on the left of the cut off line (FN). Using these values the equation is therefore TP/TP+FN.*

Patient is...	Test reports...	Description
Healthy	Healthy	True Negative (TN)
Healthy	Diseased	False Positive (FP)
Diseased	Diseased	True Positive (TP)
Diseased	Healthy	False Negative (FN)

Table 2: Summary of TN, FP, TP and FN.

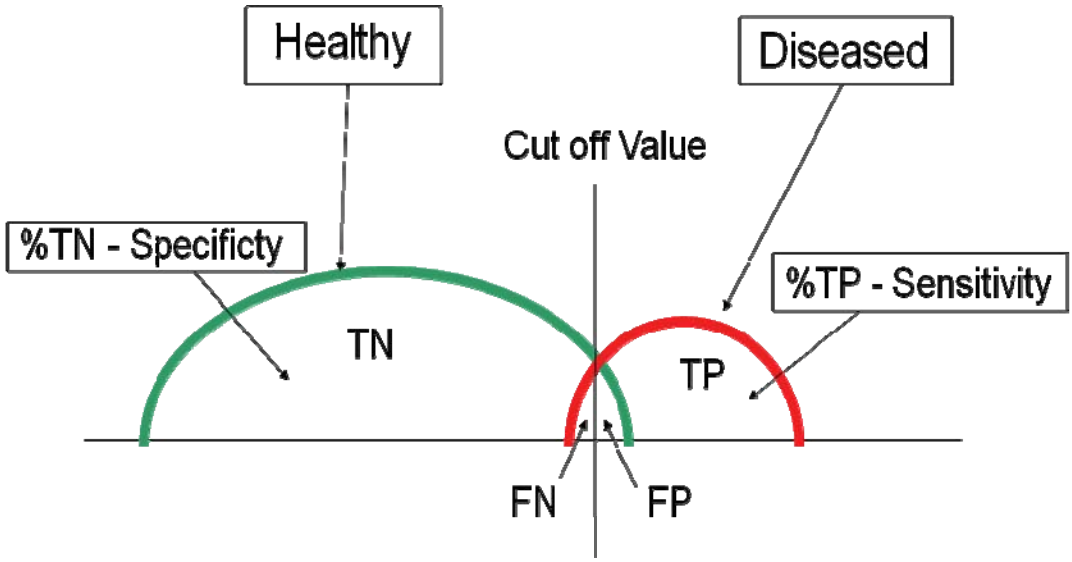

Figure 1.8: Clinical specificity and sensitivity.

The cut off value is the point at which people change from being assigned healthy to disease or the reverse. If we move the cut off value to the far right then everyone who is healthy will be reported as healthy but more diseased people will be missed (wrongly assigned healthy). If we move the cut off value to the far left then everyone who is diseased will be reported as diseased but more healthy people will be wrongly assigned as diseased. It is at this point that factors like cost, screening, reliability of data and most importantly medical ethics are involved, is telling someone they have cancer when they don't as serious as missing someone who does have cancer? These issues and the likely implications of the disease specific intervention (surgical, drugs) will be considered.

Expert further reading: As this text is an introduction, further reading may be needed on predictive value and receiver operator curves to add depth to these issues.

1.8 Summary

This chapter considered factors which can affect the result. These broadly fall into two areas analytical (machine) and physiological (human). Starting with the machine or technique that measures the test, to the blood collection method to variations in the person being tested the chapter highlighted common quality control measures to reduce these errors.

An understanding of how to interpret the results in an essential foundation to understand each of the subsequent tests and theories discussed in the remaining chapters.

2. Overview of tests

A typical blood test will aid in diagnosis, screening, evaluate prognosis and monitor interventions or disease progress. The tests generally fall into levels (core to specialist). An increase in test level will usually be justified by an abnormal level one test (Table 1). The tests in level one provide a broad overview of the body and will encompass most common diseases, they are used a starting point for investigation as they are often not specific to one pathology. The likelihood of a definitive diagnosis in increased with more specific tests in levels two and three, these tests tend to be more complex, more expensive and may be performed at specific centres so may take longer to receive the results. The tests, particularly level one, can also be used to *exclude* a diagnosis or organ by pairing normal and abnormal results. In each subsequent chapter salient tests and case studies will use the three level model. Some diagnosis are heavily reliant on blood test results others will have almost no use for a blood test, and the test is used in conjunction with patient observations, clinical technology and physiology (imaging, radiography, lung function, echocardiogram), cytology (cervical smears), microbiology (bacteria), immunology (hayfever) and haematology (blood transfusions)

Level	**Typical Test**
One	Full Blood Count (FBC) Urea and Electrolytes (U&E) Liver Function Tests (LFT) Bone Profile Glucose Amylase Total protein and albumin Thyroid Profile (TFT)
Two	Folate Vitamins i.e. B12 Hormones Trace Elements
Three	Auto-antibodies Tumour markers

Table 3: Shows a typical structuring of common tests

Example: A female patient, 66 years old, with increasing tiredness seeks advice from GP (Table 4).

<table>
<tr><th>Level</th><th>Test</th><th>Result</th></tr>
<tr><td>1</td><td>Full Blood Count</td><td>Low Red Blood Cell Number (RBC)
Low Haemoglobin (Hb)
High Mean Cell Volume (MCV) (Macrocytic)</td></tr>
<tr><td colspan="3">? Macrocytic anaemia justification of further tests</td></tr>
<tr><td>2</td><td>Iron Status</td><td>All markers of Iron Status Normal (this is unlikely to be out of range as the patient is Macrocytic and most Iron anaemias are microcytic: small MCV)</td></tr>
<tr><td>2</td><td>Folate / B12 Status</td><td>Folate Low
B12 Very Low</td></tr>
<tr><td colspan="3">? Macrocytic anaemia caused by either folate or B12 deficiency. Causes could be:
Dietary malnutrition or prescribed drugs (some epilepsy drugs and cancer drugs block folate)
Alcohol dependence (because red blood cells last for 12 weeks, a macrocytic state will reflect long term alcohol intake
Auto-immune disease
Justification of further tests</td></tr>
<tr><td>1</td><td>Liver Function Test (LFT)</td><td>All results normal, so discounts heavy and prolonged alcohol intake</td></tr>
<tr><td>3</td><td>Anti-parietal cell Antibody</td><td>Positive</td></tr>
<tr><td>1</td><td>U&E</td><td>Normal, this confirms that the low RBC is not due to the kidney being unable to produce a chemical called Erythropoietin which makes RBC</td></tr>
<tr><td>2</td><td>Glucose HB1AC</td><td>Normal, this confirms that the loss of feelings in the legs and feet are not diabetic</td></tr>
<tr><td colspan="3">? A reasonable working diagnosis would be that this patient has Pernicious Anaemia, an auto immune destruction of the stomach lining which impairs vitamin B12 absorption. This may take months to become symptomatic to the patient as the body can store up to 20 months of B12. B12 is needed to make healthy red blood cells, but also to help with nerve signals and impulses, so these patients may also have neuropathy (nerve tissue death) in their feet causing an unsteady walk and swelling.
This patient will probably not respond to oral B12 supplementation due to the damage to the stomach so will need an intramuscular injection of B12.</td></tr>
</table>

Table 4: A decision flow showing how different tests would be justified and worked through to arrive at a working hypothesis.

2.1 Summary

This chapter discussed how the tests are structured based on justification. Starting with level one tests which reveal a broad range of possible diseases, and indeed rule out numerous diseases, through to much more specific tests. In your own area you may want to recreate Table 4 using some common diseases and tests you commonly use to help detangle the numerous tests into a structure.

In the following chapter case studies and discussion will follow the levels of testing hierarchy.

3. The blood cells and liquid component: Full Blood Count (FBCs)

This chapter introduces the key words and concepts which are affected by the blood, its cells, liquid components and how it clots. It is helpful to state that, as with the biochemistry sections, this book is about quickly navigating blood tests, it is not an depth review of haematology (the study of blood), although key words and concepts will be discussed.

The chapter is split into two sections, red blood cells and white blood cells.

Name	**Abbreviation**	**What does it mean?**
Red Blood Cell Number	RBC	How many red blood cells
Haemoglobin	Hb	Concentration of Hb
Mean Cell Volume	MCV	What size are the red blood cells
Haematocrit	Hct	What % of the whole blood is made up of red blood cells

Table 5: Example of key red blood cell indices

Name	**Abbreviation**	**What does it mean?**
White Blood Cell Number	WBC	How many white blood cells
White Cell Differential	WBC DIF	What % of white blood cells are made up from each type
Neutrophils	NEU	Type of white blood cell
Lymphocytes	LYM	Type of white blood cell
Basophiles	BAS	Type of white blood cell
Eosinophils	EOS	Type of white blood cell
Blast Atypical cells	ALY	Type of white blood cell often seen in leukaemia

Table 6: Example of key white blood cell indices

Name	Abbreviation	What does it mean?
Platelet Number	PLT	Cells which participate in the clotting process
Prothrombin Time	PT	The time it takes your blood to clot
Partial thromboplastin time	PTT	The time it takes your blood to clot with a chemical to slow down the clotting process (an anticoagulant)
International Normalised Ratio	INR	This is an standardised version of the PT test and is a number rather than a time
D-Dimers	D-Dimer	Can be used to show the presence of a Deep Vein Thrombosis (DVT)

Table 7: Example of key red blood cell indices

3.1 Red Blood Cell Indices

The red cell indices give a valuable specific overview of anaemias (low RBC / Hb) and polycythaemias (high RBC / Hb) whilst in parallel being able to part differentiate alcohol use, kidney problems, liver problems, sickle cell and thalassemia

3.1.1 Red Blood Cell Number

The red blood cells or erythrocytes primarily transport oxygen and some nutrients to organs and tissues. Hence the symptoms of lack of breath, dizziness and tiredness when these cells are low in number. The process of creating RBCs is called erythropoiesis and this is induced by reduced oxygen (hypoxia) supply to the kidneys which causes the release of the hormone erythropoietin (EPO). The RBC will last around 12 weeks or 120 days; this has an advantage and a disadvantage. It is a great long term marker but will take longer than most tests to see if the intervention is correct (see expert box)

What would cause a reduced oxygen supply (hypoxia) and thus an increase in RBC production?

- Smoking – Carbon Monoxide replaced oxygen and is bound to the RBC
- Altitude – High altitude contains less oxygen
- *Chronic obstructive pulmonary disease (COPD)*
- Blood loss
- Kidney damage: The kidney is unable to produce EPO – ask for an EPO or U&E test?
    - Think about how different blood tests are linked in this example an FBC and an U&E

Why do men and women have different amounts of red blood cells?

- Men have a larger blood volume
- Women may lose blood due to menstrual loss
- Muscle mass in men is higher and thus oxygen usage is higher
- Testosterone can also induce erythropoiesis

3.1.2 Haemoglobin

Haemoglobin (Hb) is the oxygen carrying protein matrix in the RBC. As with RBCs men will often have higher concentrations of Hb. Many people can be asymptomatic for Hb and whilst a key measure it should be considered in parallel with other red cell indices because of compensation.

Low Hb but high RBC
High Hb but Low RBC
Both of these patients will have similar oxygen carrying capacities.

Further reading: Hb is a complex protein which has roles in diseases such as diabetes and conditions like sickle cell.

3.1.3 Mean Cell Volume

So our patient has a low RBC and probably a low Hb but what could have caused this? The mean cell volume is a measure of the **size** of the red blood cell and is measured in femto litres (fL) with a normal cell (normocytic) being between 80 and 100 fL. Small cells are called microcytic, micro = small and cytic = cell and are usually less than 80 fL, with large cells (macrocytic) being over 100 fL.

Since iron and nutrients like folate and vitamin B12 are needed to make red blood cells we can now start to differentiate between the sizes. Microcytic cells are usually seen in iron deficient cases. Macrocytic in deficiencies with folate and or B12, since these may be dietary or more complex we should also consider higher level tests such as parietal cell antibodies for B12 and alcohol usage for folate as alcohol blocks the uptake of folate in the stomach (figure 3.1)

Further reading:

- Reticulocytes are immature red blood cells and can indicate if the intervention to correct the anaemia is working quicker than waiting 12 weeks for the RBCs
- Some drugs can interact with folate, list them and think what size RBC may be seen in these patients

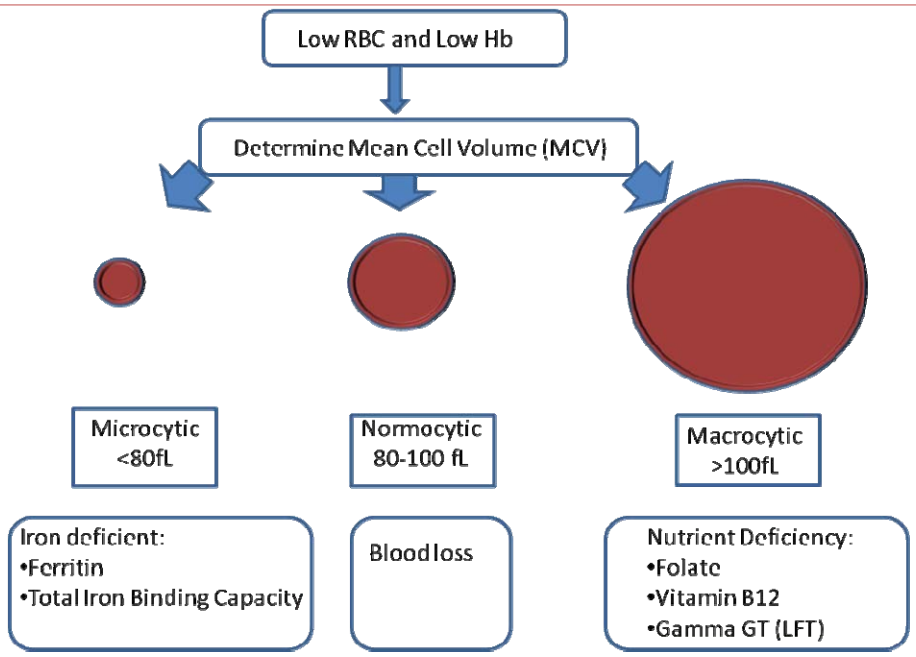

Figure 3.1: Mean cell volume (MCV)

3.1.4 Haematocrit (HCT)

The haematocrit is a % of the whole blood is made up of erythrocytes (RBCs) and is a therefore a crude marker of red cell number (figure 3.2). The HCT, like most red cell indices should be viewed in line with the other markers because traditionally HCT can have a wide CV (see analytic variation in chapter one) and so a patient may have HCT outside of the range but all the other red cell markers are normal.

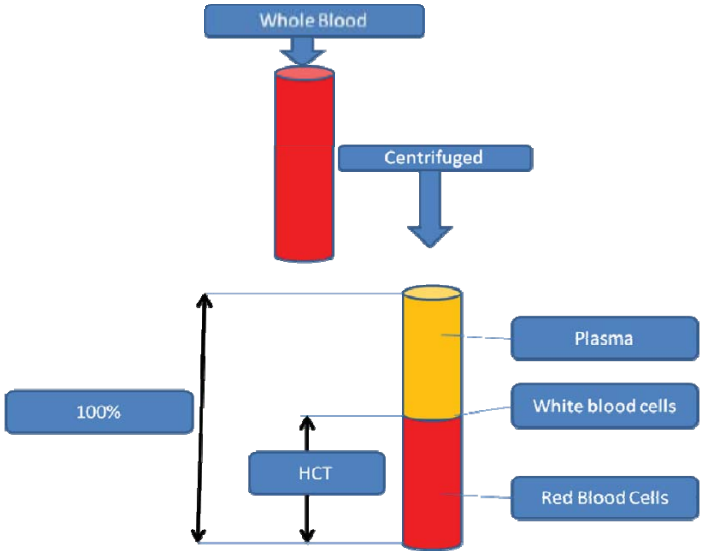

Figure 3.1: Haematocrit (HCT) is the measure of how many red blood cells contribute to the whole blood amount.

3.2 White Blood Cell Indices

The primary role of white blood cells (leukocytes or WBCs) is defence, although they are also key in diagnosing cancers and autoimmune disease.

3.2.1 White Blood Cell Number

The overall WBC count is an overview of infection and inflammation and rise if the patient becomes infected, has a trauma injury or an autoimmune event. Almost all of these events are driven by C - reactive protein (CRP) a chemical which causes WBC development and recruitment (see chapter 4).

3.2.2 White Cell Differential

The white cell differential is similar in theory to the haematocrit and represents the percentage of each type of white blood cell in the white blood count.

Example: Patient A has a Neutrophil % of 50, so half the white blood cells are neutrophils. The patient has a bacterial infection and the % is now 70 demonstrating that more of the white blood cells are now neutrophils, expected given the case?

3.2.3 Neutrophils

Neutrophil numbers will increase in response to CRP and this is usually seen in:

- Bacterial infection
- Acute Phase Response
- Auto-immune disease
- Inflammation (a broken bone for example)

3.2.4 Lymphocytes

Lymphocytes respond to viral infections and will increase in number, apart from one type of virus which is HIV/AIDS in which case the lymphocyte count may fall. There are two types of lymphocytes B cells and T cells; together they produce an antibody or direct killing response.

3.2.5 Basophils and Eosinophils

Basophils and eosinphils are specialised cells thought to play a role in asthma, hay fever allergies and hypersensitivities like irritable bowel syndrome.

Further reading, the different types of white blood cells have been overviewed in this book to enable the reader to quickly understand what each type primarily responds to.

3.2.7 Blast Atypical cells

Blast atypical cells are commonly seen in leukaemia cancers are immature white blood cells, probably being overproduced at the tumour site. These can be viewed in line with an increase white cell count and decreased red cell and platelet count as the leukaemia causes a switch to over produce immature white cells.

There are several graphs produced which chart white cell number rising and platelet and red cell numbers falling, try to draw one these graphs.

Example: Lymphoma states often see very little antibody production as the immature over produced B cells are in the lymph system compromising the ability of the lymphatic system to make antibodies

Example: Myeloma states often see large production of nonsense antibodies from the B cells in the bone marrow. A fragment of these antibodies is called a bence jones protein. If the tumour is over producing nonsense antibodies what will happen to the plasma viscosity and erythrocyte sedimentation rate? (See chapter 4)

3.3 Clotting Indices

Clotting indices are used primarily in the management of bleeding, for example before or after an operation. They are also used for patients on anticoagulation therapy such as heparin or warfarin (chemicals which slow the blood's ability to clot).

3.3.1 Platelet Number

Platelets are cells which assist in the formation of the clotting. The platelet number will provide information about the patient's ability to clot, with a low platelet number usually meaning a longer time for the blood to clot.

3.3.2 Prothrombin Time

Prothrombin time or PT is the time in seconds it takes the patient blood to clot. This test result can vary depending on laboratory system used. A longer PT means that the patient's blood is taking longer to clot.

3.3.3 Partial thromboplastin time

Partial thromboplastin time or PTT or aPTT, with the "a" meaning "activated", is a measure of how well the patient's blood ***would*** clot. A sample of blood is "clot activated" and the time taken to clot is measured in seconds. Like the PT a longer aPTT means that clotting will generally take longer.

3.3.4 International Normalised Ratio

International Normalised Ratio or INR is a standardised PT test. The patient's blood PT results are compared to an internationally standardised PT sample, and the ratio is reported rather than seconds this means that INR is usually 1. If INR is 2 this means that the blood will take twice as long to clot and so on.

How much longer would a blood of an INR of 3 take to clot?

3.3.5 D-Dimers

A d-dimer is a fragment of a clot which has been broken down by the body in a process called fibrinolysis. It is therefore used in the management of pulmonary embolism and deep vein thrombus (DVT) patients.

A high positive result means that there must have been (or still is) a thrombus (clot), although whilst a negative result probably means no thrombus, care should be made to ensure that the patient does not have an impaired fibrinolysis pathway. This would lead to:

- Higher risk of thrombus formation initially
- Inability to break down the thrombus
- Lack of D-Dimers – however the thrombus is still present.

False positives are possible with liver disease and post surgery so this is an example of a test whish also requires confirmation from additional studies such as leg Computer Tomography CT scan.

Further reading on this area should cover the extrinsic and intrinsic (contact activation) coagulation pathway and clotting factors. You may also want to learn about how the different anticoagulants work, which determines whether to use heparin, warfarin or both, why is this?

3.4 Summary

This chapter discussed the key "blood" components of a typical blood results, often called the FBC or full blood count. Disease pathologies of anaemias, leukaemia, infections, inflammation and clotting ability would be at least partly addressed by these tests.

Attempt to put these tests into a hierarchy or tests and start to list additional more specialised tests specific to your area.

4. Autoimmune and inflammation

Inflammation is a response to injury, infection, autoimmune, indeed any tissue site damage. It has two pathways exudative (liquid) and cellular. Autoimmune diseases are when the white cells destroy self tissue rather than bacteria or exogenous material, often autoimmune diseases will present as inflammation and so both are discussed in this chapter. In this chapter we will start by building up a picture of inflammation through a sequence of figures (Figure 4.1 to 4.4).

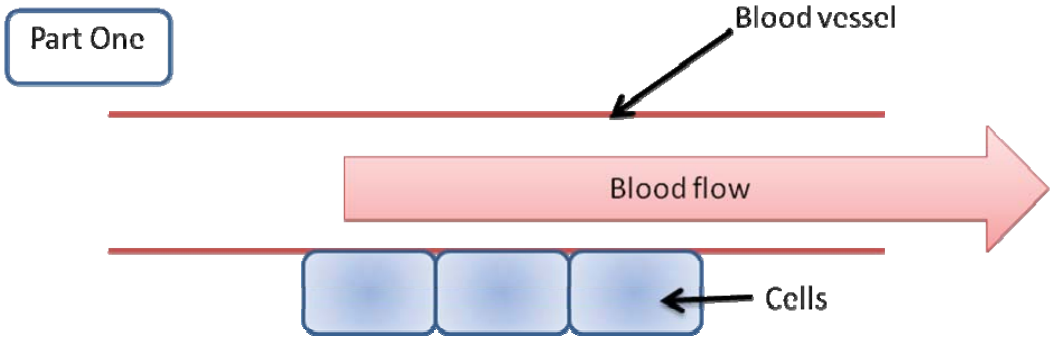

Figure 4.1: Inflammation is this example is not present, but the figure shows a normal state with cells, a blood vessel and blood flow.

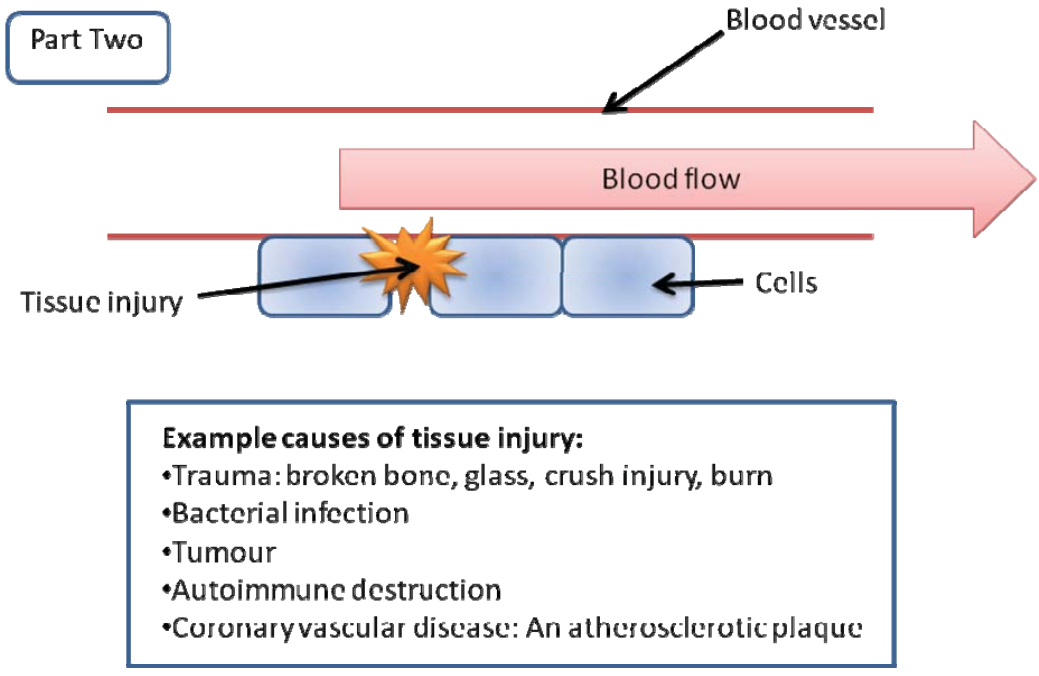

Figure 4.2: A tissue injury (examples are given in the figure, can you think of anymore?) has occurred.

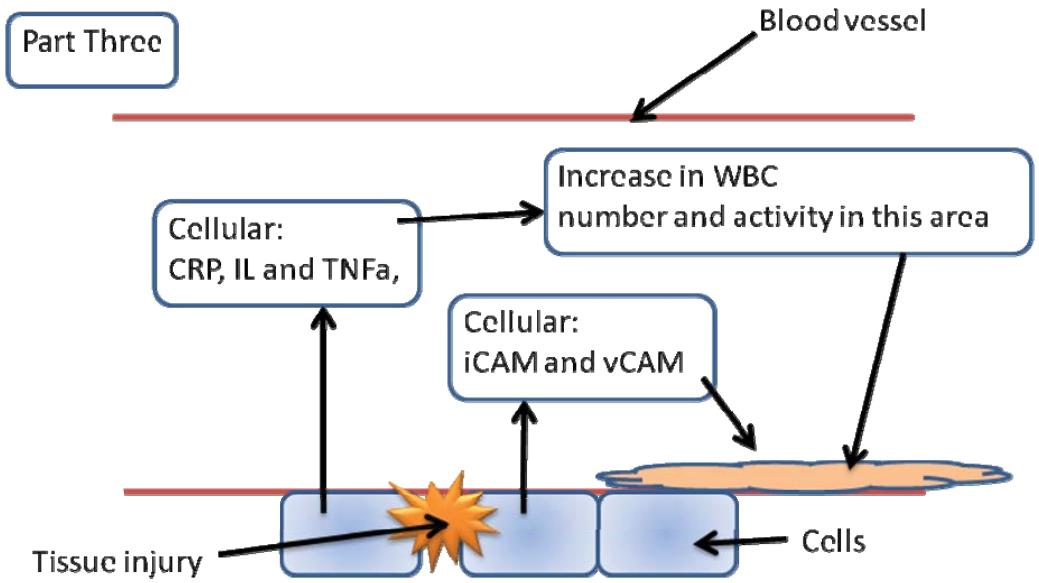

Figure 4.3: The tissue injury has caused a cellular response through the release of C Reactive Protein (CRP), Interleukins (IL), and Tumour Necrosis Factor alpha (TNFa) (there are others) and these, particularly CRP is generally relative to the amount of tissue damage. These chemicals attract white blood cells, particularly neutrophils and monocytes. There is also a cellular response which releases cellular adhesions molecules (iCAM and vCAM), these also help to attract white cells to the area.

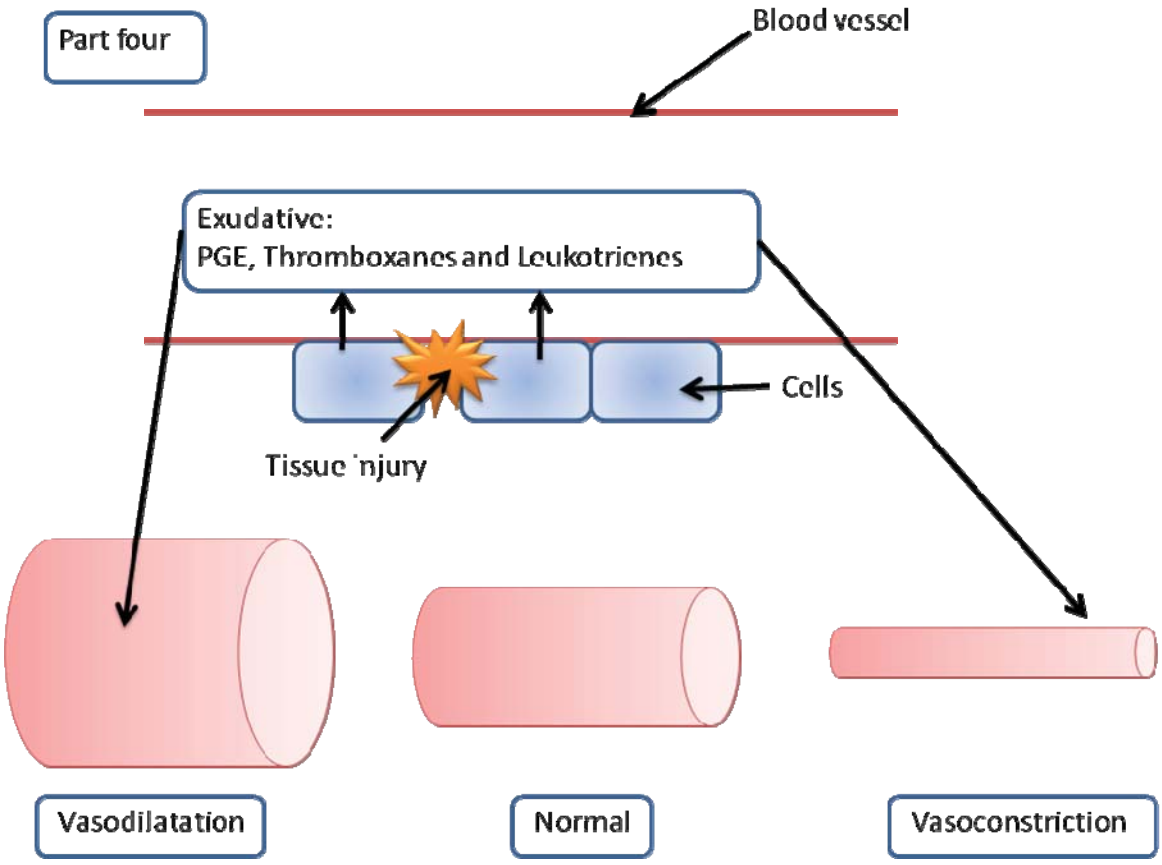

Figure 4.4: The tissue injury has also caused an exudative response, prostaglandins, thromboxanes and leukotrienes. These, via the enzyme cycloxygenase (COX), can cause vasodilatation (widening of lumen, more blood flow) before the site of injury and vasoconstriction (narrowing of lumen, less blood flow) after the site of injury. This blood stasis (slowing or stop) can then cause oedema (swelling and redness). It also facilitates more white cells to the area and helps to prevent the bacteria or tissue leaving the area.

4.1 Inflammation and CRP

Figures 4.1 to 4.4 show the progression of inflammation following a tissue injury. C Reactive Protein (CRP) is a key chemical in this process and is therefore a suitable marker for tissue injury. Generally, the more tissue injury that has occurred, the more CRP is released. Since CRP can be linked to vascular injury and is often raised by coronary vascular disease (CVD) it is increasingly being used as a preventative marker for heart attack risk.

4.2 Plasma Viscosity

Plasma viscosity or PV is a measure of the pressure (Pascals) that the plasma exerts on the vasculature. The lumen (diameter) of the blood vessels are a specific size to allow the correct amount of blood to pumped around, like a domestic central heating system. It is a therefore a general indicator of what ***extra*** is in the plasma that would not normally be present; these will take up more space in a set diameter and increase the pressure. These ***extras*** could be increased concentrations of antibodies, white blood cells, red cells (size and number) and plasma liquid volume. This makes PV a good global marker for various diseases. A general rule of is that the higher the PV the more ***extras*** are in the plasma and more disease pathology is present.

Why would PV be elevated in inflammation?

4.3 Erythrocyte Sedimentation Rate

Erythrocyte Sedimentation Rate or ESR can be re-written as “how fast do the red cells fall?” ESR is a measure of how far the red cells fall in a column of blood in 1 hour. ESR is therefore a surrogate marker of cancer, inflammation, anaemia and soon after MI (plasma enzymes) because fibrinogen causes the red blood cells to stick together making them heavier, causing them to sink faster through the plasma. Generally the higher the ESR the worse the disease pathology (usually inflammation is).

4.4 The inflammation trilogy

Why do CRP, PV and ESR all appear to be raised in inflammation and what other test is usually raised?

The tissue injury causes an inflammation cellular response, which produces elevated CRP. The CRP will increase white cell count (particularly neutrophils and monocytes) which will raise PV, if the injury is caused by bacteria and the monocytes infiltrate the cells then the CRP will be significantly raised. These ***extras*** will raise PV. The tissue injury will drive a rise in fibrinogen (needed to cause a clot) the fibrinogen will cause the red blood cells to stick together and thus ESR will raise, the final measure in the inflammation trilogy. A final word is that the original CRP response may drop but the PV and ESR will remain high until the extras and the fibrinogen stuck RBCs are removed.

Neutrophils can be activated in different ways; this can be seen in the ANCA test which occurs in inflammation and damage of the kidneys and lungs.

4.5 Autoimmune Diseases

An autoimmune disease occurs when the white blood cells either produce antibodies which are directed to the host or they directly release toxic chemicals to destroy the host tissue. Either of these events will result in the inflammation trilogy driven by tissue injury caused by the patient’s own white blood cells.

So, to begin with most autoimmune disease, at level one, will have a raised WBC, increased neutrophil differential, CRP, PV and ESR.

The next step is to differentiate between the different types of autoimmune disease. At this stage the blood tests will be used along with patient history and examination, for example ankylosing spondylitis (AS) will be primarily seen in the patient's back, whilst rheumatoid arthritis (RA) is more likely in digits.

Two common tests are HLAB27 (usually seen in AS) and RF (usually seen in RA), these are proteins expressed on the cell surface which make them more susceptible to destruction by the patient's own white blood cells. Most patients are either positive or negative for either HLAB27 or RF, although some patients can have AS or RF and be negative for HLAB27 or RF, it is not known why this is case.

There are other antigens and antibodies used to diagnose autoimmune diseases such as ANA, Anti-RHO, Anti-SSA.

4.6 Summary

This chapter discussed the key components of inflammation and autoimmune diseases. Disease pathologies of anaemias, autoimmune, leukaemia, cancers, heart disease risk, infections and inflammation and clotting ability would be at least partly addressed by these tests.

Attempt to put these tests into a hierarchy or tests and start to list additional more specialised tests specific to your area.

5. Liver function test (LFTs) and Enzymes

The liver function tests or LFTs are often used to diagnose liver diseases, but they are also invaluable in measuring the toxicity of drugs used to treat other diseases.

The liver has three main roles:

1. Detoxification: The liver takes compounds which are harmful and not very soluble and makes them less harmful and more soluble (so easier to store or excrete); this is mainly done with enzymes.
2. Storage: The liver, for example, stores glycogen which is the stored form of glucose.
3. Production: The liver also produces some of the chemicals we have already discussed like CRP, fibrinogen and transferrin.

Start to link the tests and key concepts learnt so far. Example, why would a patient with RA being treated with a drug, also have her LFTs measured?

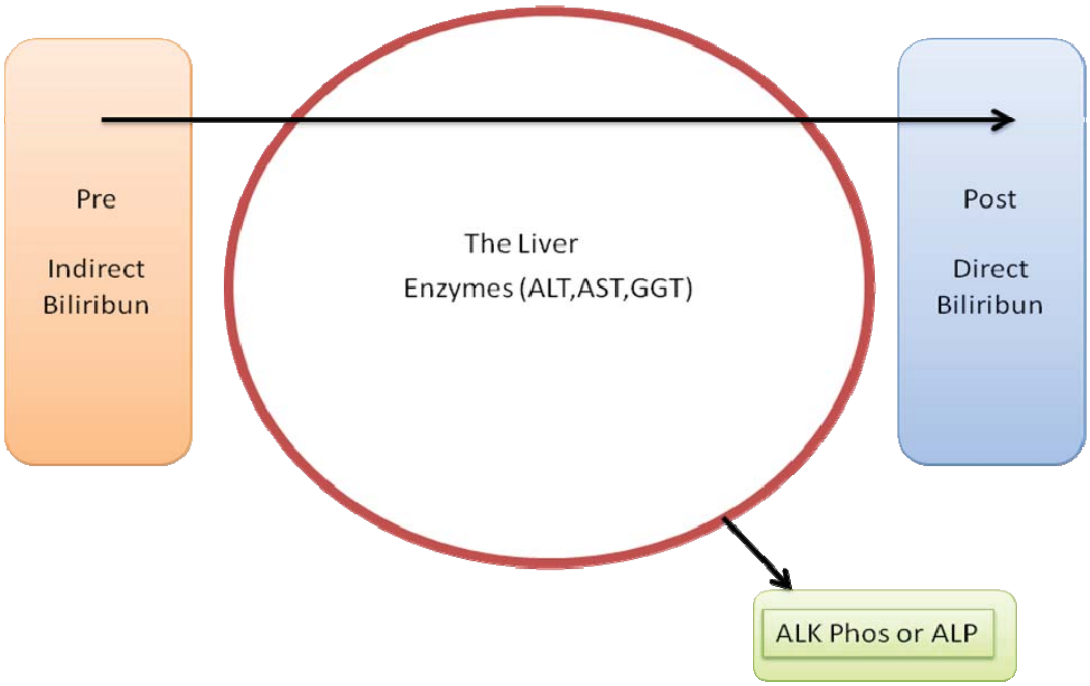

Figure 5.1: Each one of the liver tests provides "geographical" information. Bilirubin is more indicative of the "plumbing" to (pre) and from (post) the liver. The enzymes ALT, AST and GGT are mainly found inside the liver whilst ALP is more indicative of damage to the biliary tree (bile duct etc).

5.1 Enzymes

Enzymes are biological materials with catalytic properties, that means they take a substrate and convert it to a product, much like a toaster (the enzyme) which takes bread (the substrate) and converts it into toast (the product).

Enzymes are present in two forms either constitutive and (or) inducible, the former are present at all times and work all the time and will have a specific metabolic role, the latter tend to be "switched on" more in response to more harmful chemicals being present.

5.1.1 Measuring an enzyme

The enzyme can be measured by looking at how much substrate has been used or by how much product is formed. The latter is usually used and this is usually done visually either the formation of colour of the removal of colour as the product is formed.

Example: Alkaline phosphatase can be measured by the conversion of the colourless substrate 4-nitrophenyl-phosphate to the coloured product 4-nitrophenol.

5.1.2 Activation energy

The activation energy is the amount of energy needed for a reaction to take place. The presence of an enzyme reduces the activation energy and this helps regulate metabolic functions.

Example: The catalase reaction needs about 90 kJ mol-1 to work alone with no enzyme, but in the presence of the catalase enzyme only about 1 kJ mol-1 is needed. This makes enzymes more energy efficient and regulating as less energy is needed and the body can make certain reactions only work in teh presence of an enzyme because without them they would need to much energy.

5.1.3 Factors which affect enzyme rate

Temperature, pH, enzyme concentration, and substrate concentration can all affect the enzyme rate (or how quickly the enzyme works).

The human body is about 37^{o}C, thus most enzymes have an optimum temperature around this point, too hot or too cold and the enzymes will stop working.

The human body has a pH of about 7, thus most enzymes have an optimum pH around this point, apart from a set of enzymes in the stomach. In the stomach the pH is acidic (pH 1-2) and thus these enzymes have an optimum rate at pH 1, which means they won't work well outside of the stomach in pH 7, which is good as these enzymes break down food which is protein, carbohydrate and fats, the same materials that the body is made of!

5.1.4 Factors which inhibit enzymes

Enzymes can be made to stop working (inhibited) by three ways, competitive, non-competitive or allosteric. In competitive inhibition the inhibitor is very similar to the substrate and ***competes*** for the site binding site, like fake plastic toast (inhibitor) will fit in the same slot as the bread (substrate). Thus, the more competitor you have the more likely that inhibition will occur.

In non-competitive inhibition the inhibitor binds at a ***different site*** on the enzyme to that of the substrate, once bound the inhibitor changes the shape of the substrate site thus preventing the substrate from binding.

These two options allow the body to regulate its processes via ***negative feedback*** using a allosteric inhibition. This is usually when the product of an enzyme reaction is the allosteric inhibitor of an enzyme in earlier in the pathway, thus temporarily switching off the chain.

5.1.5 Extracellular vs. Intracellular

We can measure two types of enzymes, either secretory (extracellular) or intracellular.

Secretory enzymes such as amylase or those that assist in blood clotting are naturally present in the plasma, thus higher or lower than normal levels will usually indicate either a tumour, or organ failure or blockage of that which secretes it.

Intracellular enzymes are usually only found inside the cells and have intracellular metabolic roles. They are present in low levels in the plasma due to cell turnover, leaking and cell death. Very high levels of these enzymes indicate damage to the organ which contained them. We can see this in the liver enzymes.

List as many secretory (extracellular) or intracellular enzymes and their associated organs as you can.
How specific are they to each organ?
What does this mean to assigning enzymes to organs?

5.1.5 Isoenzymes

There are several versions of the same enzyme called isoenzymes and these are more organ specific. For example the enzyme lactase dehydrogenase (LDH) is a good global marker of tissue damage but is present in almost all organs so not specific enough to allow for conclusive diagnosis. However, LDH has 5 isoenzymes and these are distributed differently in different organs. After a heart attack (myocardial infarction) then LDH1 is seen in high levels whereas in acute hepatitis it is usually LDH5 that is high.

5.2 Bilirubin

A product of red blood cell recycling, bilirubin is harmful and not very soluble. The liver enzyme UGT1A1 modifies the bilirubin to make it less harmful and more soluble. Pre hepatic (before the liver) bilirubin is called “indirect” then in the liver (via UGT1A1) it is converted to “direct” bilirubin. It is possible therefore to look at the ration between indirect and direct to evaluate liver plumbing and performance.

High levels of indirect would indicate a liver problem or pre-hepatic block, high levels of direct would indicate that the liver is able to convert the bilirubin but there could be a post hepatic blockage.

We can also start to link the tests discussed thus far, since bilirubin is a product of red cells, a very high red cell count could lead to very amounts of bilirubin, more than the liver can remove. This leads to an artificially high bilirubin concentration, the liver is not compromised or diseases it simply has too much bilirubin delivered to it to remove.

Albumin is a key protein which not only provides plasma pressure and is a key source of protein for metabolic functions; it is also a “chaperone” for other chemicals such as calcium and ***bilirubin*** so a check of albumin levels could be helpful if bilirubin levels are abnormal.

What would happen to a patient’s bilirubin levels if:

- The liver cells (hepatocytes) were immature (young, like in a baby) and were unable to break down bilirubin?
- The patient had a faulty UGT1A1?
- The patient had very high red blood cell number?
- The patient had liver failure?
- The patient was on a long term protein restricted diet?

5.3 Liver Enzymes

We have previously discussed what an enzyme is and how it works. In this section the intracellular liver enzymes will be expanded upon, remember that these enzymes take substrates which are harmful and not very soluble and make them less harmful and more soluble. A rise in these enzymes usually means that the liver is working harder to remove harmful chemicals, either that there are more harmful chemicals (liver toxicity as seen in overdose) or that the liver's ability to remove harmful chemicals is reduced (liver damage as seen in hepatitis or alcoholism).

5.3.1 Aspartate aminotransferase (AST)

Aspartate aminotransferase or AST can be used to detect a liver injury or an active or chronic liver proble, shock, low blood pressure or any hypoxia (low oxygen). As we have discussed enzymes are not always specific to one organ and since the heart also contains AST this may also rise if the patient has had a heart attack.

5.3.2 Alanine Aminotransferase (ALT)

Alanine Aminotransferase or ALT may indicate active hepatitis from any cause, including virus, drug or toxin. The patient with RA treated with a drug could have her ALT measured because this enzyme generally breaks down commonly used drugs.

If AST and ALT are high and outside of reference range then the ratio can also be used, if AST concentrations are higher than ALT then this is a poor prognosis and often seen in alcohol hepatitis, if ALT is higher than AST then this is often seen in viral hepatitis..

5.3.3 Gamma-glutamyl transferase (GGT)

Gamma-glutamyl transferase or GGT is often used a marker for alcohol intake. It does respond in self reporting healthy people who "binge" drink (excessive) and can remain high for up to weeks after the alcohol consumption. However, in a similar way to diabetes, in long term alcoholic patients, because the liver tissue is so damaged and sclerotic GGT have the ability to respond.

We can also start to link the tests discussed this far in the example of a suspected alcoholic patient who has an elevated GGT. Their mean cell volume (MCV) is very high and their folate is very low, this would indeed indicate excessive alcohol intake, but for how long? How long does a red blood cell last?

5.3.4 Alkaline phosphatase (ALP)

Alkaline phosphatase or ALP or ALK Phos is commonly found in the biliary tree and bile ducts, a blockage in this system will cause an elevated ALP. However, it is also found in the bone, kidney and in the placenta (in high levels) so caution is required to exclude these other organs and systems. There are tests which are specific to these organs but these are usually requested in order to exclude as above, after initial high levels of ALP.

5.4 Summary

This chapter discussed the key components of the liver function tests or LFTs. The role of the LFT is varied from determining actual liver disease to confirming or indeed excluding the liver in more complex diseases. Bilirubin is a useful marker of the plumbing pre and post the liver whilst the liver enzymes are helpful in determining liver toxicity and viability.

The LFTs discussed thus far are single dimension. Research shows that the LFTs may provide very different results for example between an alcoholic (single dimension) and an alcoholic who also takes marijuana and heroin.

Attempt to put these tests into a hierarchy or tests and start to list additional more specialised tests specific to your area and now also link these to other tests such as FBCs.

6. Kidney function tests and electrolytes (U&Es)

The U&Es means ***u***rea and ***e***lectrolytes, and are a marker of kidney function and the level of electrolytes. In a similar fashion to the LFTs, U&Es are often used to diagnose renal (kidney) diseases, but they are also invaluable determining dehydration and cardiac risk.

The kidney has three main roles:

1. Blood pressure and urine regulation primarily using the water driving electrolyte sodium (Na).
2. Exocrine such as red cell production through EPO and bone metabolism through production of calcitriol (vitamin D metabolite)
3. Excretion of metabolites such as urea, creatinine and uric acid (a cause of gout)

Start to link the tests and key concepts learnt so far. Why could an elderly patient with kidney problems have a low red blood count?

6.1 Electrolytes

The electrolytes are chemicals with a charge, either positive or negative. This means that they can interact with water in the case of sodium or cellular impulses in the case of potassium.

6.1.1 Sodium (Na)

Sodium is the primary electrolyte in the control of water. Low concentrations are called hyponatraemia whilst high concentrations are called hypernatraemia. The majority of sodium is present outside the cell and so if subject to water (saline) intake as part of hydration therapy.

Further reading on how high salt diets, high water intake or low intake etc will affect sodium levels, these are mass and volume hydration therapies.

6.1.2 Potassium (K)

Potassium is a primary intracellular electrolyte that modulates electrical signalling within the cell controlling vesicles and channels. A high level of potassium in the plasma is called hyperkalaemia, and these elevated amounts can cause damage to tissue exposed to the plasma. The most acute of these is cardiac muscle explaining why high potassium levels causes great damage to the heart and induce a myocardial infarction.

Further reading on how K modulates insulin release in beta cells and how hydrogen ions (H) can replace K inside cells, increasing plasma K levels.

6.2 Urea and Creatinine

Urea and creatinine are general markers of renal function. Urea is a product of general cellular metabolism whilst creatinine is a specific product of muscle breakdown. A high level (outside of the reference range) of urea is indicative of ***acute*** renal dysfunction whilst a high level of creatinine is indicative of ***chronic*** renal dysfunction.

The BUN (used in the USA) or urea (used in Canada and Europe) ratio to creatinine can be used to determine disease aetiology.

6.3 Glomerular filtration rate (GFR)

The GFR can be used to assess renal function. There are two types, actual and estimated. In actual GFR the equation UV/P is used where U and P are the concentrations of creatinine in the urine and plasma respectively, with V being the amount of urine produced in 1 minute. Since creatinine levels vary during the day for V and U to be accurate the urine needs to be collected over 24 hours, making this test time consuming for the patient. To avoid the 24 hour collection of urine, an estimated GFR or eGFR can be used in which only the plasma creatinine is used. eGFR has some limitations but can be successfully used to identify patients which would need an actual GFR.

GFR is subject to a reduction with age, generally from age 35 the GFR falls by approximately 10% in self reported healthy people, emphasising the importance of using the correct reference ranges.

6.4 Uric acid and gout

Uric acid (urate) is a product of DNA breakdown and is normally excreted by the kidney. In renal dysfunction with a low GFR, the uric acid concentration rises. Urate at high concentrations is not soluble and the insoluble crystals deposit in interphalangeal articulations (joints in hand and feet) and can cause gout. Other causes of gout include purines, which are proteins found in certain foods like port and pate, and psuedogout which is caused by calcium pyrophosphate crystals.

To assist in differentiating the types of gouts, allopurinal treatment can redress urate gout, diet restriction will reduce “gouty attacks” from food purines, whilst visualising the crystals can help identify psuedogout.

6.5 Summary

This chapter discussed the key components of the kidney function tests or U&Es and gout. The role of the U&Es is varied from determining actual renal disease to confirming or indeed excluding the kidney in more complex diseases.

Attempt to put these tests into a hierarchy or tests and start to list additional more specialised tests specific to your area and now also link these to other tests such as FBCs.

7. The bone and calcium (bone profile)

99% of the calcium stored in the body is stored as bone, with the remaining 1% “free” in circulation in the plasma. Of this remaining 1% about half is bound to albumin (see chapter 6) and half is actively participating in cellular functions. The bone therefore has three main roles:

- Storage of calcium and other chemicals
- In the bone marrow assisted production of red and white cells
- Physical support for the body.

7.1 Corrected calcium

Since part of the free calcium is chaperoned or bound to albumin we need to take this into account with corrected calcium. The equation for this is total Ca + 0.02 (47-[Albumin]) although this is likely to be calculated for you in the laboratory. However, it is worth thinking about how albumin can affect calcium concentration.

7.2 Calcium control

Since 99% of calcium is stored in the bone it is this site which undergoes bone metabolism to release calcium (osteoclast cells) and then subsequently "remineralised" or put back into the bone by osteoblast cells.

Think about what would happen to the calcium levels and bone thickness if:

- Too much calcium is removed
- Not enough calcium in the diet to replace that removed
- The cells which remove and put back the calcium are dysfunctional and the amounts taken out and put back are unequal.

7.2.1 Parathyroid hormone (PTH)

Parathyroid hormone or PTH is produced in the parathyroid gland (this is not the thyroid!) and assists in bone metabolism and calcium control. PTH and vitamin D have the following effects to ***raise*** calcium levels:

1. Release more calcium from the bone
2. Excrete less calcium in the kidney
3. Absorb more calcium through the gut

Vitamin D is a vital part of this process and originates in the skin under the action of UV energy from sunlight, with the subsequent metabolites calcidiol (stored in the liver) and calcitriol (stored in the kidney). It is calcitriol which, with PTH, helps to modulate calcium levels.

Elevated calcium (Hypercalceamia, plasma corrected Ca >2.8 mM, with >3.5 being life threatening) can lead to lethargy depression, stomach pains, sickness and diarrhoea, with long term renal stones and cardiac problems. PTH (therefore Ca) can be elevated in four ways; the first is a normal response to low calcium. The remaining three are described below:

1) Primary hyperparathyroidism: Primary means "at source" so this is when the parathyroid gland itself overproduces PTH, probably caused by a tumour of the parathyroid gland with no negative feedback.
2) Secondary hyperparathyroidism: Secondary means not at source and could be a renal problem which is causing persistently low Ca levels, due to over excretion. These low Ca levels will induce the parathyroid hormone to over produce Ca to compensate.
3) Tertiary hyperparathyroidism: This is almost a mixture of primary and secondary in that end stage renal failure and caused persistently low Ca, inducing PTH production, but over a period of time the parathyroid gland has lost a negative feedback response and becoming hyperplastic (hyperplasia, cellular turnover is increased)

The hormone responsible for negative feedback is calcitonin.

Think about what would happen to the calcium levels and bone thickness if:

- PTH was overproduced?
- The patient was vitamin D deficient?
- The patient has a kidney problem?
- What are phosphates and bisphosphonates?
- The parathyroid hormone was damaged or surgically removed?

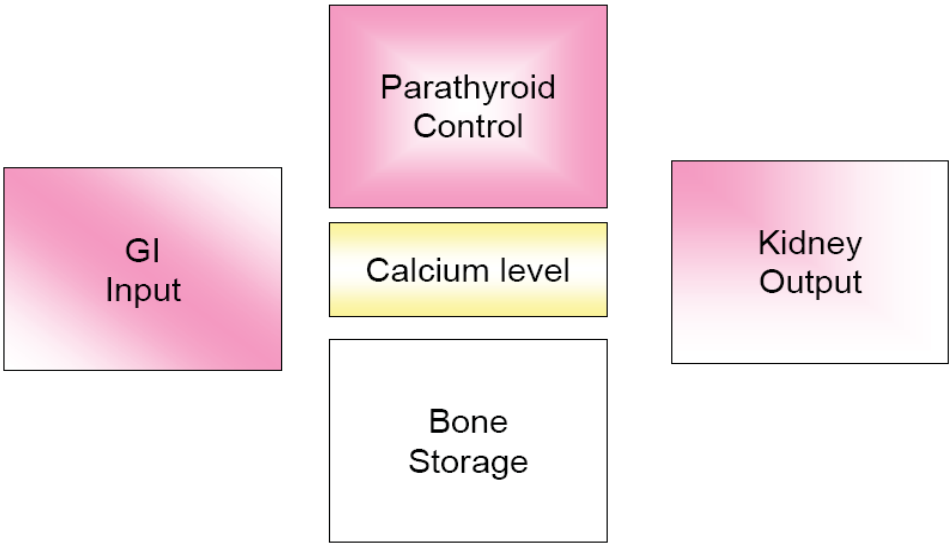

Figure 7.1: The organ systems involved in calcium control. (GI is gastrointestinal)

7.3 Bone diseases

Bone diseases can be caused by variety of factors, but most will involve bone turnover, PTH, vitamin D and or calcium.

7.3.1 Osteoporosis

A common bone disorder, which is often difficult to detect with a blood test, as the bone re-modelling (taking Ca and putting it back in) is working normally, just accelerated. The main reason for this is thought to be oestrogen which may be the “speed limiter” of the process. In post-menopausal women oestrogen levels fall and the regulation of re-modelling is lost, increasing the bone turnover. This why the adolescent bone mineral density is important as it is thought that the more bone you have pre-menopause the more this will withstand an increase turnover and is less likely to fracture.

7.3.2 Paget's disease

In Paget's disease the osteoclast (taking Ca out) are dysfunctional, increased in size, number and activity. There is also an increase in bone collagen and alkaline phosphatase in the plasma due to the over activity of the osteoclast cells. The result is bone which is poorly defined and leads to protrusions often seen in the face and back.

7.3.3 Osteomalacia and rickets

This is generally caused by vitamin D deficiency and has characteristic "bowed" legs. Osteomalacia or rickets in children can also be caused by GI or renal complications, mineral (Ca and P) deficiency or Fanconi's syndrome which is poor re-absorption.

7.3.4 Differentiating between bone diseases

Create a table with bone diseases as rows and levels of Calcium, Phosphate, PTH, Alk Phos, Calcidiol and Calcitriol (1,25 DHCC) as columns, and compare and contrast each analyte relative to the disease.

7.4 Summary

This chapter discussed the key components of the bone profile test. The role of the bone and calcium is varied from determining actual renal disease to confirming or indeed excluding the bone in more complex diseases.

Attempt to put these tests into a hierarchy or tests and start to list additional more specialised tests specific to your area and now also link these to other tests such as FBCs, U&Es, LFTs and Alk Phos.

8. Summary

We have discussed some key tests and concepts in this textbook. Each section provides an introduction to the key areas with example boxes and cues for further reading. The following strategy could aid learners further:

1) Read the book
2) Complete the example boxes and seek advice from your tutor or other more in-depth textbooks for the answers
3) The headings for the further reading should allow specific additional information to be gained. Use reputable sources such as recommended text books and validated internet resources. The idea with further reading is to build your knowledge in layers, only adding a layer once you have tested your knowledge at that level. A key understanding of the basics is fundamental allows for more engaged and complex thought.
4) Test your knowledge: Use past examination paper, questions in other textbooks. A great resource is clinical case studies, you can even make up cases to test your theory, see example box below.
5) Put the tests in hierarchy order and list them in columns, then draw lines between tests if you think they will interact with each other.

A male patient, aged 75 complaining of back ache attends the GP surgery.

- Urea is very high
- Alkaline Phosphatase is high
- All other tests are fine (bone profile, FBC and LFT) so unlikely that the high ALP could have come from the bone or liver.

This could confirm an acute renal problem such as a kidney infection, or a kidney stone which has moved causing acute pain and the back pain is in the right place for a renal problem.

However, if we also look at the patient's prostate specific antigen (PSA, see chapter 1) levels they were very high.

The patient was subsequently diagnosed with prostate cancer, which was causing the acute renal dysfunction due to blocking the area. The cancer has since metastasised to the bone, so FBCs and bone profiles are also measured, why?

As the text is introductory, it is not possible to cover all aspects of blood tests, or indeed clinical chemistry. Instead, some of the most common tests have been discussed, although the next level of tests discussed would have been and should be subject to further reading:

- Heart attack markers: AST, LDH, Troponins, CKmb, myoglobin and B-type natriuretic peptide
- Lipids: HDL, LDL, cholesterol, triglycerides, oxLDL
- Diabetes: Plasma glucose, glucose tolerance test, HbA1C
- Thyroid: TRH, TSH, T3 and T4
- Tumour markers: Ca-125, Ca-119, Ca-153, oncogenes and specificity.

As the integration of clinical chemistry and haematology is embedded by increased automation the number, speed and accuracy of tests will improve significantly, thus improving the lives of each person which is behind the test being requested. However, with this increased automation comes an imperative that understanding of the underlying concepts must remain sound.