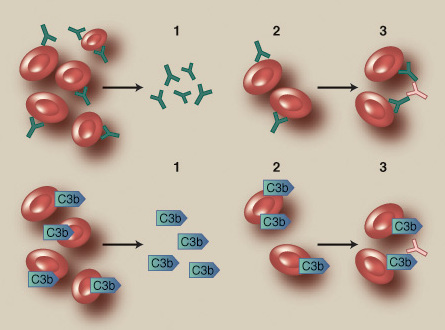
School of Medicine

**Year 2 – 2011/12**

## Molecules Cells and Disease

**Autumn Term Course Guide**

****

**The Coombs' test**  
Tefferi A, Li C. Atlas of Clinical Hematology: Clinical Hematology

Theme Leader: **Dr Keith Gould**tel: 020 7594 3724  
email: k.gould@imperial.ac.uk

Deputy: **Dr James Pease**  
tel: 020 7594 3162  
email: j.pease@imperial.ac.uk

<https://education.med.imperial.ac.uk>

**Theme: MOLECULES CELLS AND DISEASE**

**Year 2 – Autumn Term Course Guide**

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**SOLE FEEDBACK – *Molecules, Cells and Disease***

The following pages provide you with templates on which you can record your thoughts as the course proceeds. At the end of the course you can enter your views onto SOLE.

**Please answer all questions by selecting the response which best reflects your view.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Strongly Agree | Agree | Neutral | Disagree | Strongly Disagree |
| The content of this module is useful. |  |  |  |  |  |
| The support materials available for this module (e.g. handouts, web pages, problem sheets) are helpful. |  |  |  |  |  |
| I receive sufficient feedback and guidance. |  |  |  |  |  |
| Overall, I am satisfied with this module. |  |  |  |  |  |

Please use this box for constructive feedback and suggestions for improvement.

|  |
| --- |
|  |

**SOLE FEEDBACK - INDIVIDUAL LECTURERS**

Please note that for SOLE, a Lecturer’s name will only appear once. This template gives you the opportunity to record your comments about each lecture in the order of delivery.

**On the following section, you have an opportunity to record any comments and constructive feedback you have for each lecturer.**

|  | **The lecture(s) are well structured** | | | | | **The lecturer explains concepts clearly** | | | | | **The lecturer engages well with the students** | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Lecturer and Lecture Title** | Strongly Agree | Agree | Neutral | Disagree | Strongly Disagree | Strongly Agree | Agree | Neutral | Disagree | Strongly Disagree | Strongly Agree | Agree | Neutral | Disagree | Strongly Disagree |
| **Prof. Barbara Bain** Haem 1 – Blood diagnostic parameters |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Prof. Barbara Bain** Haem 2: Anaemia and polycythaemia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Fiona Regan** Haem 3 Blood transfusion |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Donald MacDonald** Haem 4 Abnormal White Cell Count |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Nina Salooja** Haem 5: Iron deficiency |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Nina Salooja** Haem 6 B12 and folate deficiency |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Prof. David Lane** Haem 7 Haemostasis 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Prof. Mike Laffan** Haem 8 Haemostasis 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Nina Salooja** Haem 9 Haemoglobin molecule and thalassaemia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Mark Layton** Haem 10 Sickle cell disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Prof Karim Meeran** Diagnostics 1 Chemical pathology Lab |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Mark Atkins** Diagnostics 2  Virology Lab |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Hugo Donaldson** Diagnostics 3 Bacteriology Lab |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Marjorie Walker** Diagnostics 4  Histopathology and Cytopathology Lab |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Keith Gould** Diagnostics 5 Antibodies as diagnostic tools |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Assad Luqmani** Haem tutorials |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Fiona Regan** Haem tutorials |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Mark Layton**  Haem tutorials |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Diane Hagger** Haem tutorials |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

| **Lecturer and Lecture Title** | **Please use this box for additional constructive feedback** |
| --- | --- |
| **Prof. Barbara Bain** Haem 1  Blood diagnostic parameters |  |
| **Prof. Barbara Bain** Haem 2 Anaemia and polycythaemia |  |
| **Dr Fiona Regan** Haem 3 Blood transfusion |  |
| **Dr Donald MacDonald** Haem 4  Abnormal White Cell Count |  |
| **Dr Nina Salooja** Haem 5 Iron deficiency |  |
| **Dr Nina Salooja** Haem 6 B12 and folate deficiency |  |
| **Prof. David Lane** Haem 7 Haemostasis 1 |  |
| **Prof. Mike Laffan** Haem 8 Haemostasis 2 |  |
| **Dr Nina Salooja** Haem 9 Haemoglobin molecule and thalassaemia |  |
| **Dr Mark Layton** Haem 10 Sickle cell disease |  |

| **Lecturer and Lecture Title** | **Please use this box for additional constructive feedback** |
| --- | --- |
| **Prof. Karim Meeran** Diagnostics 1 Chemical pathology |  |
| **Dr Mark Atkins** Diagnostics 2 Virology Laboratory |  |
| **Dr Hugo Donaldson** Diagnostics 3 bacteriology Laboratory |  |
| **Dr Marjorie Walker** Diagnostics 4 Histopathology and Cytopathology laboratory |  |
| **Dr Keith Gould** Diagnostics 5 Antibodies as diagnostic tools |  |
| **Dr Assad Luqmani** Haem tutorials |  |
| **Dr Fiona Regan** Haem tutorials |  |
| **Dr Mark Layton**  Haem tutorials |  |
| **Dr Diane Hagger** Haem tutorials |  |

**Molecules Cells and Disease**

**INTRODUCTION**

***The Molecules Cells and Disease*** theme is spread across two terms in Year 2.

The **courses** that comprise MCD this year and the **course leaders** are:

Haematology Dr Nina Salooja

Diagnostics Dr Mike Barrett

Microbiology Dr Angelika Grundling

Immunology Dr Keith Gould

Cancer Dr Laki Buluwela & Dr Marjorie Walker

In the autumn term the emphasis is on material that will form a valuable background to your clinical attachment later this term, and consists mostly of lectures. The main course this term is **Haematology** (see below for more detailed comments) together with **Laboratory Diagnostic Methods**.

In the spring term the MCD Theme will concentrate on Cancer (Cellular and Molecular Biology, the Cell cycle, Cancer as a disease, Carcinogenesis), but will also include Immunology and Microbiology.

Teaching will consist of tutorials and practicals as well as lectures, along the lines of MCD in Year 1.

Come prepared. In advance of each session look at this handbook to check what you will be doing, and read through the objectives relating to lectures.

Student’s responsibilities:

We shall endeavour to make sure that the Theme material is delivered to you in the most interesting and fruitful way. In return, we expect that you will make an effort to make the most of what is made available to you, and not to spoil your colleagues’ experience.

Practically, this means that:

You are expected to arrive on time, at the right place. The timetable in this Study Guide gives you the information you need. Make sure you know which is your tutorial group and which practical class (quarter class) you are supposed to go to. Some sessions involve the Brian Drewe Lecture theatre, Reynolds Building, at the Charing Cross (CX) campus, others involve the SAFB lecture theatre on the South Kensington (SK) Campus. All teaching is compulsory. If you cannot attend, a medical note will be expected.

Come prepared. You are expected to have read the relevant material in the Study Guide in advance of tutorials. This makes sure that the learning experience is more beneficial and enjoyable. Much material, including lecture material, is available on the intranet. You are encouraged to make yourself familiar with this material.

Come equipped. This Study Guide and the Lecture Notes are needed daily. Of course you need writing material, a ruler and possibly pens/pencils of different colours, a highlighter and a calculator. The Student Union shop has a good supply of these, on site, were you to forget or lose the essentials.

Please note there are no laboratory practicals in year 2 MCD in the Autumn term.

Be safe. Safety should be foremost in your mind as you embark on a medical career. Follow safety advice scrupulously. Breaches of safety can result in accidents and/or disciplinary procedures.

The Intranet

You will find all of the Study Guide on the intranet for the School of Medicine: <https://education.med.imperial.ac.uk>. You will have to provide your Imperial College username and password to gain access to it. In addition to the Study Guide material with timetables, *etc*., the Intranet provides the lecture handouts, the lectures’ learning objectives and the lecture material itself. Various formats are used: PDF files, Word documents and PowerPoint presentations.

You are also to be encouraged to use the Discussion Boards inside Blackboard which you can access at <http://learn.imperial.ac.uk>

Look at the intranet daily: the Intranet is regularly updated, this Course Guide is not!

**Dr Keith Gould**Theme Leader

**Dr James Pease**

Deputy Theme leader

October 2011

**ABOUT THE HAEMATOLOGY COURSE**

Haematology is the study of the blood and its diseases. The blood is also affected by diseases of many other organs and systems so understanding haematology tests is crucial in all branches of medicine. The haematology course reviews the physiology of blood and bone marrow cells and introduces you to diseases of the blood and bone marrow. It also teaches you about how blood clots, or fails to clot, about an abnormal propensity to form blood clots, about the practice of blood transfusion and about haematological changes as a response to infection, inflammation, injury and surgery.

Medical practitioners in their day-to-day practice are constantly interpreting laboratory results and acting on them. In this course you will start to learn how this is done. Year 2 will provide you with the basic knowledge you need but you will have the chance to deepen your knowledge in other parts of the course. On your clinical attachments in Year 3 you will have another opportunity to learn more about blood diseases and about the haematological abnormalities seen in medical and surgical patients. More haematology teaching will follow in Year 5.

Year 2 teaching of haematology is based mainly on lectures and some videos. This term there will be 10 lectures and a tutorial. All the factual knowledge that you need will be provided in this course book, your lectures and the recommended textbook (you do not need to know everything in this book – the learning objectives will help you to select the most important things and the book will be useful again during your medical and surgical firms). You can supplement this information by using two Computer Assisted Learning (CAL) packages that you will find on the intranet. You have guaranteed computer access to do this next term, but you are welcome to do it much earlier if you wish. In addition, next term you will have a practical class and in both terms you will have small group teaching in haematology. The latter will provide revision of key parts of the course and will give you an opportunity to discuss any problem areas. Otherwise, if you have any difficulty understanding any specific points then you are welcome to email the lecturer concerned – email addresses are provided with the lecture notes. Please note that PowerPoint presentations will normally be placed on the Intranet before the relevant lectures. You therefore have the option to print them off if you want to use them to take notes.

**Haematology Course leader:** Dr Nina Salooja [nina.salooja@imperial.ac.uk](mailto:nina.salooja@imperial.ac.uk)

**Head of Department of Haematology:** Professor Jane Apperley [j.apperley@imperial.ac.uk](mailto:j.apperley@imperial.ac.uk)

### Recommended Haematology text book

Bain BJ, *Haematology: a core curriculum,* (2010) Imperial College Press, ISBN: 978-1-84816-499-4. 336 pages.

Other haematology books you may find useful are:

* Mehta A and Hoffbrand AV, *Haematology at a glance*, 3rd Edition (2009) Wiley-Blackwell, ISBN: 978-1-4051-7970-6. 128 pages.
* Hoffbrand AV and Moss PAH, *Essential haematology*, 6th Edition (2011) Wiley-Blackwell, ISBN: 978-1-4051-9890-5. 468 pages.
* Howard MR and Hamilton PJ*, Haematology: an illustrated colour* *text*, 3rd Edition (2007) Churchill Livingstone/Elsevier, ISBN: 978-0-4431-0362-9. 124 pages.

**Supplementary Haematology reading and reference**

* Hughes-Jones N, Wickramasinghe SN, and Hatton C, *Lecture notes on Haematology*, 8th ed, Wiley-Blackwell, Oxford (2008) ISBN: 978-1405180504. 216 pages.
* Bain BJ, *A Beginner's Guide to Blood Cells*, 2nd Edition (2004), Blackwell Science, ISBN: 978-1405121750. 136 pages.
* Contreras M, *ABC of Transfusion*, 4th Edition (2009) Wiley-Blackwell, ISBN: 978-1405156462. 128 pages.
* McClelland DBL (ed), *Handbook of Transfusion Medicine*, 4th Edition (2007) HMSO Publications, ISBN: 978-0113226771. 92 pages. Can be downloaded from:

<http://www.transfusionguidelines.org.uk/docs/pdfs/htm_edition-4_all-pages.pdf>

**Computer-assisted learning** (CAL - Intranet)

* Dr Bain’s Image Library
* Bain BJ, Interactive Haematology Imagebank, Blackwell Science, Oxford, 1999.

Both these web-based collections are on the Intranet – navigate via links on the Year 2 MCD page or go straight to the Intranet CAL at <https://education.med.imperial.ac.uk/CAL/index.htm>

**ABOUT THE LABORATORY DIAGNOSTICS METHODS COURSE**

This series of 5 lectures is based around a clinical case study and provides an introduction to the use of laboratory-based diagnostic disciplines other than haematology, and which will assist you when attending your clinical attachment later this term.

**Diagnostics Course leader:** Dr Michael Barrett [mike.barrett@imperial.ac.uk](mailto:mike.barrett@imperial.ac.uk)

**ASSESSMENT**

The MCD courses delivered in the Autumn term and those delivered in the Spring term will be examined in a single examination immediately after Easter 2012 covering all courses: - **Molecules, Cells and Disease II** — **Paper 2**

The questions will be in SAQ, EMQ and SBA formats. Further details about examinations will be provided on the Intranet.

**MCD Timetable –Year2 2011/12 – Autumn term**

|  |  |  |  |
| --- | --- | --- | --- |
| **Date and campus** | **Time** | **Lecture topic** | **Lecturer** |
| Monday  **10 October**  CX  Drewe LT | 2 pm | **Haematology 1** Blood diagnostic parameters | Prof Barbara Bain |
| 3 pm | Video **Haematology 2**  Anaemia and polycythaemia | Prof Barbara Bain |
| Friday  **21 October**  CX  Drewe LT | 2 pm | **Haematology 3** Blood transfusion | Dr Fiona Regan |
| 3 pm | Transfusion video |
| 3.30 pm | **Haematology 4** Abnormal White Cell Count | Dr Donald MacDonald |
| Thursday  **10 November** SK  LT1 | 2 pm | **Haematology 5**  Iron deficiency | Dr Nina Salooja |
| 3 pm | **Haematology 6**  B12 and folate deficiency | Dr Nina Salooja |
| Monday  **14 November**  CX  Drewe LT | 9 am | **Haematology 7**  Haemostasis 1 | Prof David Lane |
| 10 am | **Haematology 8**  Haemostasis 2 | Prof Mike Laffan |
| Monday  **21November**  SAFB  LT1 | 9 am | **Haematology 9**  Haemoglobin molecule and thalassaemia | Dr Nina Salooja |
| 10.30 am | **Haematology 10**  Sickle cell disease | Dr Mark Layton |
| 2 pm | **Diagnostics - a case study** |  |
| -Chemical pathology laboratory  -Virology laboratory  -Bacteriology laboratory  -Histopathology and cytopathology laboratory  -Antibodies as diagnostic tools | Prof Karim Meeran  Dr Mark Atkins  Dr Hugo Donaldson  Dr Marjorie Walker  Dr Keith Gould |
| Friday  **25 November**  CX | See separate  timetable | **Haematology 11 & 12**  Tutorials | **Morning**  Dr Assad Luqmani  Dr Fiona Regan  **Afternoon**  Dr Mark Layton  Dr Diane Hagger |

Year 2 MCD course: Haematology Tutorials

Friday 25 November 2011

**Venues:**9th floor Lecture Theatre, Lab Block and Drewe Lecture Theatre, Charing Cross campus

**Tutors:**Dr Assad Luqmani, Dr Fiona Regan, Dr Mark Layton and Dr Diane Hagger

**Tutorials 9th floor** lecture theatre **Drewe** lecture theatre

**Tutors: Dr A Luqmani Dr F Regan**

9.30 -10.30 E1 + E2 + E3a E3b + E4 + E5

10.30 - 11.30 E6 + E7 + F1a F1b + F2 + F3

11.30 -12.30 F4 + F5 F6 + F7

Tutors: Dr M Layton Dr D Hagger

2.00 - 3.00 G1 + G2 + G3a G3b + G4 + G5

3.00 - 4.00 G6 + G7 + H1a H1b + H2 + H3

4.00 - 5.00 H4 + H5 H6 + H7

**Learning Objectives**

These are designed as tasks you should be able to carry out after you have completed the relevant activity. They provide you with a way to assess how well you are keeping up with the material. Note that they are also provided to the external examiners as a guide to what you should know at the end of the course.

#### HAEMATOLOGY

**1 Blood diagnostic parameters** (Professor Barbara Bain)

* Explain the origin, function and approximate intravascular life span of red cells, neutrophils and platelets
* Explain the function of monocytes, eosinophils and lymphocytes
* List the main physiological factors that influence the rate of red cell production.
* State the approximate intravascular life span of red cells, neutrophils and platelets.

**2 Anaemia and polycythaemia** (Professor Barbara Bain)

* Explain the term anaemia
* Describe the mechanisms underlying the development of anaemia
* Describe the classification of anaemia on the basis of red cell size
* List the common causes of microcytic, normocytic and macrocytic anaemia
* List causes of haemolytic anaemia and describe how you would recognise a haemolytic anaemia
* Explain the possible mechanisms underlying polycythaemia

**3 Blood transfusion** (Dr Fiona Regan)

1. To be able to describe the major significant blood groups and their importance clinically
2. To be able to describe the screening of blood donors undertaken and reasons why
3. To be able to describe the various blood components used and the potential side effects of blood transfusion

**4 Abnormal white cell count** (Dr Donald Macdonald)

* In a leucocytosis (increased white cell count) explain the importance of the differential count and peripheral blood morphology in planning further investigation.
* List the most common causes of an increased neutrophil, eosinophil and lymphocyte count.
* In a lymphocytosis explain how to distinguish between a reactive polyclonal response to infection and a primary lymphoproliferative disorder (a monoclonal or malignant proliferation of lymphocytes such as chronic lymphocytic leukaemia)

**5 Iron deficiency** (Dr Nina Salooja)

* Describe the role of iron in erythropoiesis. dietary sources of iron, absorption of iron, causes of iron deficiency, clinical and haematological features of iron deficiency and the diagnosis and management of iron deficiency.
* Describe the clinical and haematological features of anaemia of chronic disease and explain how this is distinguished from iron deficiency.

**6 Vitamin B12 and folic acid deficiency** (Dr Nina Salooja)

1. Describe the role of vitamin B12 and folic acid in haemopoiesis, dietary sources and absorption of these vitamins, causes of deficiency, clinical and haematological features of vitamin B12 and folic acid deficiency and the diagnosis, further investigation and management of these deficiencies
2. Be able to explain that
3. Synthesis of DNA requires both vitamin B12 and folate
4. Integrity of the nervous system requires vitamin B12
5. Deficiency of either causes anaemia, which is both macrocytic and megaloblastic

**7 & 8 Haemostasis** **and abnormalities of haemostasis** (Professor David Lane & Professor Mike Laffan)

* Describe the normal haemostatic mechanisms including the interactions of vessel wall, platelets and clotting factors
* Describe and distinguish the clinical features of bleeding due to thrombocytopenia and coagulation disorders, respectively
* Describe the use of laboratory tests to assess haemostasis
* Describe the principles of management of disorders of haemostasis

**9** **Haemoglobin molecule and thalassemia** (Dr Nina Salooja)

1. Describe the structure and function of the haemoglobin molecule and list the normal haemoglobins in the fetal, neonatal and adult periods.
2. Describe the genes controlling haemoglobin synthesis and explain how genetic defects lead to α- and β-thalassaemias.
3. Describe briefly the clinical and haematological features of β-thalassaemia major and the principles of management.
4. Describe the haematological features of β thalassaemia trait, how it is diagnosed and why this is important.
5. Describe how β-thalassaemia trait can be differentiated from iron deficiency anaemia and the anaemia of chronic disease.

**10 Sickle cell disease** (Dr Mark Layton)

* Describe the inheritance of clinical and haematological features of sickle cell anaemia (SS)
* Outline principles of management
* Explain the inheritance, clinical significance and diagnosis of sickle cell trait

#### LABORATORY DIAGNOSTIC METHODS

**1 The chemical pathology laboratory** (Professor Karim Meeran)

* List five common diagnostic tests carried out by the chemical pathology laboratory.
* Know how to collect specimens for common tests including electrolytes, urea, glucose and glycosylated haemoglobin.
* Describe a typical chemical pathology request form.

**2 The virology laboratory** (Dr Mark Atkins)

* Appreciate the role of the virology laboratory in the diagnosis of infectious disease.
* List the types of specimen that are commonly sent for virological diagnosis.
* List the laboratory procedures that may be used as part of diagnosis of common viral infections.

**3 The bacteriological laboratory** (Dr Hugo Donaldson)

* Explain the concept of best-guess microbiological diagnosis and the contribution of the laboratory to it.
* Describe how the microbiology laboratory works, what investigations it does and how best to use the laboratory.
* Understand how & when to collect specimens, and which investigations to request.
* Describe the limitations of microbiology laboratory investigations.
* To be familiar with the turn-around times of different investigations, particularly the delays inherent in making cultural diagnoses.
* To know how to interpret laboratory results of the commonly used tests.

**4 The histopathology & cytopathology laboratory** (Dr Marjorie Walker)

* List three situations where histopathology and cytopathology might commonly be used as a diagnostic method.
* Describe the nature of specimens sent for histopathology and cytopathology laboratory diagnosis.
* List two situations where frozen section diagnosis is required.
* Summarise the main steps involved in processing a specimen for routine histopathology diagnosis and indicate the likely time needed to carry out these steps.
* Explain the additional information available from immunohistochemistry, and give an example of when this technique may be used.
* Describe the benefits of the autopsy.
* List 3 benefits of cytology screening.

**5 Antibodies as diagnostic tools** (Dr Keith Gould)

* Explain how antibodies can be generated (experimentally or commercially) for diagnostic purposes.
* Understand the therapeutic and diagnostics use of manufactured antibodies.
* Give examples of types of substance that are typically identified diagnostically by means of antibodies.
* Explain the basic principles of detection methods.
* Describe the advantages in different situations of the types of detection system: for example, enzyme-linked antibodies, radioactively labelled antibodies, fluorescently-labelled antibodies.
* Understand how antibodies may be used in clinical practice.

**CONTACTS**

**MCD Autumn term**

If you have any general queries about the Theme, please contact:

**MCD Theme Leader:**

Dr. Keith Gould  
Immunology, St Mary’s campus  
🖀 020 7594 3724 email: k.gould@imperial.ac.uk

**MCD Deputy Leader:**  
Dr James Pease  
Leukocyte Biology, NHLI, Sir Alexander Fleming Building  
🖀 020 7594 3162 email: j.pease@imperial.ac.uk

If you have queries about the content of individual components of the course, please speak with the teacher concerned or the relevant Course leader.

**Haematology Course leader:**

Dr Nina Salooja  
Department of Haematology, Charing Cross campus  
 email: [nina.salooja@imperial.ac.uk](mailto:nina.salooja@imperial.ac.uk)

**Diagnostics Course leader:**

Dr Michael Barrett  
Sir Alexander Fleming Building, South Kensington campus  
🖀 020 7594 9823 email: [mike.barrett@imperial.ac.uk](mailto:mike.barrett@imperial.ac.uk)

Also, look on the Intranet for more information. <https://education.med.imperial.ac.uk>

October 2011

**HAEMATOLOGY 1  
DIAGNOSTIC PARAMETERS  
TERMINOLOGY AND REFERENCE RANGES**

Prof Barbara Bain ([b.bain@imperial.ac.uk](mailto:b.bain@imperial.ac.uk))

**PHYSIOLOGY**

**OBJECTIVES**

The student should be able to explain the origin, function and approximate intravascular life span of red cells, neutrophils and platelets and the function of monocytes, eosinophils and lymphocytes

The red cells, granulocytes, monocytes and platelets that circulate in the blood are produced in the bone marrow, being ultimately derived from multipotent haemopoietic stem cells. Red cells are produced under the influence of erythropoietin, which is mainly synthesized in the kidney; reduced oxygen supply to the kidney is a stimulus to erythropoietin synthesis. Bone marrow production of granulocytes and monocytes is under the influence of multiple cytokines such as the interleukins and granulocyte- and granulocyte-macrophage colony stimulating factors (G-CSF and GM-CSF). The production of platelets is under the influence of thrombopoietin. The main function of red cells is oxygen transport by haemoglobin. Oxygen delivery is facilitated by the sigmoid oxygen dissociation curve and by the fact that a lower pH, as in metabolically active tissues, lowers the oxygen affinity of haemoglobin and facilitates downloading of oxygen to tissues. Other functions of haemoglobin include transport of carbon dioxide and of nitric oxide. Haemoglobin also acts as a buffer.

The functions and life span of important blood cells are shown in the following table.

|  |  |  |
| --- | --- | --- |
| **Cell** | **Approximate intravascular life span** | **Major function** |
| Erythrocyte (red cell) | 120 days | Oxygen transport |
| Neutrophil | 7-10 hours | Defence against infection by phagocytosis and killing of micro-organisms |
| Monocyte | Several days | Defence against infection by phagocytosis and killing of micro-organisms |
| Eosinophil | A little shorter than neutrophil | Defence against parasitic infection |
| Lymphocyte | Very variable | Humoral and cellular immunity |
| Platelet | 10 days | Haemostasis |

###### TERMINOLOGY and THE CONCEPT OF NORMAL RANGES

**OBJECTIVES** Like many specialized subjects, haematology has its own specialised language. You need to be able to interpret this. You also need to understand how normal ranges are devised and how they are used to interpret blood counts.

The student should therefore be able to

* Recognize the terms commonly used in describing abnormalities in blood counts and films and explain what they mean
* Explain how to assess whether the result of a laboratory test is normal or abnormal

HAEMATOLOGICAL TERMS — during this course you should learn what they mean and be able to explain this when you see them used in context, e.g. in a blood count report.

Write your answers in the boxes below

|  |  |
| --- | --- |
| **anisocytosis** |  |
| **poikilocytosis** |  |
| **microcyte** |  |
| **microcytic anaemia** |  |
| **microcytosis** |  |
| **macrocyte** |  |
| **macrocytic anaemia** |  |
| **macrocytosis** |  |
| **normochromic** |  |
| **normocytic** |  |
| **hypochromic** |  |
| **hypochromia** |  |
| **polychromasia** |  |
| **elliptocyte** |  |
| **spherocyte** |  |
| **target cell** |  |
| **sickle cell** |  |
| **fragment** |  |
| **rouleaux** |  |
| **agglutination** |  |
| **Howell-Jolly body** |  |
| **leucocytosis** |  |
| **leucopenia** |  |
| **neutrophilia** |  |
| **neutropenia** |  |
| **lymphocytosis** |  |
| **atypical lymphocyte** |  |
| **eosinophilia** |  |
| **monocytosis** |  |
| **thrombocytosis** |  |
| **thrombocytopenia** |  |
| **toxic granulation** |  |
| **left shift** |  |
| **hypersegmented neutrophil** |  |
| **reticulocytosis** |  |

**THE CONCEPT OF ‘NORMAL’ RANGES AND INTERPRETATION OF LABORATORY TESTS**

All you **HAVE** to know is

* Normal ranges conventionally describe the results observed in 95% of a healthy population
* Results falling outside the reference range are **PROBABLY** abnormal.

The second of these two statements is something of an oversimplification. The following will give you a better understanding of how normal ranges are derived and how they are used to interpret test results on patients.

**REFERENCE RANGES**

Reference ranges are descriptions of data derived from a sample of a reference population. A reference population has characteristics that have been carefully defined with regard to age and gender and, when relevant, other variables such as state of health, ethnic origin and physiological status (pregnant or not). In addition to these factors, haematological variables are also affected by altitude, cigarette smoking, alcohol intake and whether a tourniquet has been applied for a long time before taking the blood sample.

Reference ranges are commonly given as 95% ranges, i.e. figures that encompass 95% of the data from the reference sample. This is usually the central 95% of the data, i.e. 2.5% of data are excluded at each end of the range. If data have a Gaussian distribution the mean plus and minus 2 standard deviations gives a 95% range. Figure 1 (below) is a histogram of haemoglobin concentrations in 100 healthy women showing a Gaussian distribution.

If data has a non-Gaussian distribution then mathematical transformation of the data is required before analysis. For example, white cell counts have a logarithmic distribution and the mean and standard deviation of the logarithms of the data must be calculated in order to determine the geometric or log mean and the 95% range (Figure 2).

A ’normal range’ is a less strictly defined term than a ‘reference range’. ‘Normal range’ is generally used to mean a range derived from a healthy reference population. Another useful concept is a ‘health-related range’. For some laboratory measurements a 95% range derived from an **apparently** healthy population will include data from patients with a high risk of subsequently developing significant disease. This is so, for example, for measurements of cholesterol concentration. If subjects representing the upper 20% of data have a high risk of developing clinically evident coronary artery disease then it is more relevant to interpret data in the light of whether a laboratory result is predictive of future good health rather than whether it falls within the 95% limits for apparently healthy people.

The following **IMPORTANT POINTS** should be remembered when interpreting laboratory data:

* **A value within the normal range may be abnormal for that individual.** For example, a man whose Hb is usually 16.5 g/dl may suffer a gastrointestinal haemorrhage with a fall of Hb to 14 g/dl which is still within the normal range but is abnormal for him. If previous test results are available from a given individual it is always relevant to consider these when deciding if a result is likely to be abnormal for that particular person.
* **A value outside the normal range may be normal for that individual.** By definition, test results of 5% of healthy subjects are likely to fall outside the ‘normal range’ and if healthy subjects have multiple tests performed there are bound to be one or two which are ‘abnormal’.
* **Reference ranges for healthy and sick individuals usually overlap.** Calculating reference ranges representing 99% of the population reduces the chance of misclassifying a test result on a healthy subject as abnormal but there will be more abnormal results that are not recognised as such.
* **Some haematological variables are dependent on the precise instrument or methodology used.** It is therefore best to use a reference range derived for a particular instrument/method. Those given on the next page relates to a particular instrument. Results from various hospitals may differ slightly.

#### Figure 1

#### 

**Table 1**

**95% RANGES FOR CAUCASIAN ADULTS**

#### Males Females

WBC 3.6-9.2 x 109/l 3.5-10.8 x 109/l

RBC 4.25-5.77 x 1012/l 3.82-4.98 x 1012/l

Hb 13.5-16.9 g/dl 11.5-14.8 g/dl

PCV (Hct) 0.41-0.51 0.36-0.46

MCV 84-99 fl

MCH 27.5-32.7 pg

MCHC 30.9-34.8 g/dl

Platelet count 143-332 x 109/l 169-358 x 109/l

Neutrophils 1.7-6.1 x 109/l 1.7-7.5 x 109/l

Lymphocytes 1.0-3.5 x 109/l

Monocytes 0.2-0.6 x 109/l

Eosinophils 0.03-0.46 x 109/l

Basophils 0.02-0.09 x 109/l

Reticulocytes 20-130 x 109/l

**Figure 2**

**Table 2**

**95% RANGES FOR AFRICAN OR AFRO-CARIBBEAN**

**ADULTS** (when different from above)

**Males Females**

**Afro-Caribbean**

WBC 2.8-9.5 x 109/l 3.3-9.85 x 109/l

Neutrophils 1.0-5.8 x 109/l 1.4-6.5 x 109/l

Platelets 122-313 x 109/l 149-374 x 109/l

**African**

WBC 2.8-7.2 x 109/l 3.2-7.8 x 109/l

Neutrophils 0.9-4.2 x 109/l 1.3-4.2 x 109/l

Platelets 115-290 x 109/l 125-342 x 109/l

**Note:**

To revise terminology and see illustrations of all these abnormalities you can use the two CAL packages or Beginner’s Guide to Blood Cells.

**HAEMATOLOGY 2**

**MECHANISMS OF ANAEMIA AND POLYCYTHAEMIA**

Prof. Barbara Bain ([b.bain@imperial.ac.uk](mailto:b.bain@imperial.ac.uk))

### Learning objectives – you should be able to:

1. Explain the term anaemia
2. Describe the mechanisms underlying the development of anaemia
3. Describe the classification of anaemia on the basis of red cell size
4. List the common causes of microcytic, normocytic and macrocytic anaemia
5. List causes of haemolytic anaemia and describe how you would recognise a haemolytic anaemia
6. Explain the possible mechanisms underlying polycythaemia

**Blood count interpretation**

**To understand blood counts you need to know the meaning of various terms and abbreviations:**

**WBC**  white cell count, the number of white cells in a given volume of blood

**RBC**  red cell count, the number of white cells in a given volume of blood

**Hb**  haemoglobin concentration

**PCV**  packed cell volume, the proportion of a column of centrifuged blood  occupied by red cells

**Hct**  haematocrit, equivalent to the PCV

**MCV**  mean cell volume, i.e. the average size of the red cells

**MCH**  mean cell haemoglobin, i.e. the average amount of haemoglobin in a  red cell

**MCHC**  mean cell haemoglobin concentration, i.e. the average  concentration  of haemoglobin red cell

**Platelet count** the number of platelets in a given volume of blood

**Anaemia** is a reduction in the concentration of haemoglobin (Hb) in the circulating blood below what is normal for a healthy individual of the same age and gender as the individual. Anaemia is usually associated with a reduction in the red blood cell count (RBC) and the haemocrit (Hct) or packed cell volume (PCV).

# Anaemia can be caused by:

* Reduced production of red cells by the bone marrow
* Loss of blood from the body
* Reduced survival of red cells in the circulation (called haemolysis)
* Increased pooling of red cells in an enlarged spleen

Anaemia can be classified not only by mechanism but also by the size of the red cells. This has the advantage that cell size gives important clues to the likely cause of the anaemia. Anaemia can be classified on the basis of cell size as

* Microcytic
* Macrocytic
* Normocytic

In a **microcytic anaemia** red cells are small. They are referred to as microcytes. The size of red cells can be judged by looking at a blood film with a microscope or by measuring the mean cell volume (MCV) on an automated blood cell counter. Microcytic cells are usually also hypochromic, i.e. they appear pale when looked at with a microscope. The anaemia is therefore described as hypochromic microcytic. The common causes of microcytosis are

* Iron deficiency anaemia
* Anaemia of chronic disease
* Thalassaemia

Microcytosis is a result of reduced synthesis of haemoglobin. This can be caused by reduced synthesis of haem (iron deficiency or anaemia of chronic disease) or reduced synthesis of globin (thalassaemia).

In **a macrocytic anaemia** red cells are larger than normal. They are referred to as macrocytes. The size of red cells can be assessed by examining a blood film or by noting an elevated MCV.   
Important causes of macrocytosis include

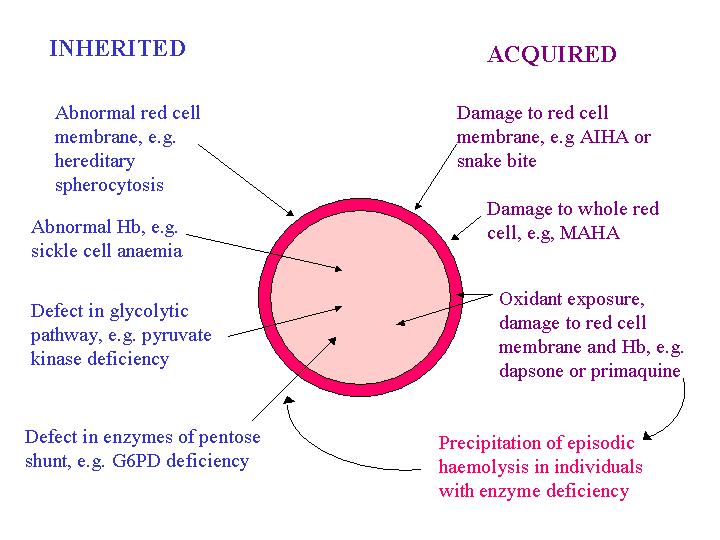
* Megaloblastic anaemia resulting from a deficiency of vitamin B12 or folic acid
* Liver disease
* Excess alcohol intake
* An increased proportion of young red cells newly released from the bone marrow

In **normocytic anaemia** the red cells are usually normally staining as well as normal in size so the anaemia is referred to as normochromic normocytic. Important causes include:

* The early stages of iron deficiency and the anaemia of chronic disease
* Renal failure
* Recent blood loss
* Bone marrow failure or infiltration

A **haemolytic anaemia** may be inherited or acquired. It may be caused by defective red cells (e.g. hereditary spherocytosis) or by a defect outside the red cells (e.g. fragmentation by fibrin strands deposited in capillaries, referred to as a micro-angiopathic haemolytic anaemia). Haemolysis may be mainly extravascular (e.g. increased removal of defective red cells by splenic macrophages in hereditary spherocytosis) or mainly intravascular (e.g. incompatible blood transfusion in which antibody destroys cells in the circulation). The following diagram summarises the causes of haemolytic anaemia.

**CAUSES OF HAEMOLYTIC ANAEMIA**

****

In macrocytic and normochromic normocytic anaemias it is often necessary to think about the mechanism of anaemia as it may not be immediately obvious from the patient’s history and blood film. The reticulocyte count is very important in this. It involves exposing living red cells to a dye that is taken up by young red cells so that they can be counted. An approximately equivalent observation on a routine blood film would be polychromasia. Polychromasia means that cells have a blue tinge, caused by ribosomal RNA in young red cells, in addition to the pink colour of the haemoglobin – hence ‘polychromasia’ - ‘many colours’. An increased reticulocyte count is seen as a response to haemolytic anaemia and recent blood loss and also as a response to treatment with iron, vitamin B12 or folic acid. A reduced reticulocyte count is seen when there is reduced output of red cells from the bone marrow.

If haemolytic anaemia is suspected, diagnosis is aided by

* The detection of morphologically abnormal red cells, e.g. spherocytes, elliptocytes, fragments.
* Evidence of increased red cell breakdown, e.g. increased serum bilirubin (unconjugated) and lactate dehydrogenase (LDH)
* Evidence of an increased bone marrow response, e.g. polychromasia and an increased reticulocyte count

The following diagram summarises what happens in haemolysis and shows you the tests (in **bold**) that can be used if you suspect haemolysis.

Stimulation of bone marrow**,** polychromasia and reticulocytosis

**INTRAVASCULAR HAEMOLYSIS**

Anaemia

Red cell destroyed in **circulation**

Haemoglobin in plasma

Haemoglobin released from red cells

When all haptoglobin is saturated, free haemoglobin is filtered by the kidney

Haemoglobin binds to haptoglobin and the complex iscleared by liver

Haemoglobin and, later, haemosiderin in the urine

Low serum haptoglobin

**EXTRAVASCULAR HAEMOLYSIS**

Anaemia

Stimulation of bone marrow**, polychromasia** and **reticulocytosis**

Red cells phagocytosed by macrophages and destroyed

**Red cell destruction leads to increased serum bilirubin and LDH and faecal and urinary bile pigments**

Don’t worry if, at this stage, this seems all very complex. Further half days of Blood course will deal with microcytic and macrocytic anaemias.

POLYCYTHAEMIA

Polycythaemia is the opposite of anaemia. There are too many red cells produced and the Hb is too high. You can classify polycythaemia as follows:

|  |  |
| --- | --- |
| Type of polycythaemia | Example |
| Physiological | Newborn baby |
| Appropriate erythropoietin secretion | Residence in Himalayas or Andes  Hypoxia due to cyanotic heart disease or severe chronic lung disease |
| Inappropriate erythropoietin secretion | Erythropoietin abuse by athletes  Erythropoietin secreted by renal cysts or tumours or other tumours |
| Not mediated by erythropoietin but due to intrinsic bone marrow disease | Polycythaemia vera |

HAEMATOLOGY 3  
BLOOD TRANSFUSION, DONOR SELECTION, TESTING OF DONATIONS AND PRE-TRANSFUSION TESTING

Dr Fiona Regan ([fiona.regan@imperial.nhs.uk](mailto:fiona.regan@imperial.nhs.uk))

**Objectives**

* To be able to describe the major significant blood groups and their importance clinically
* To be able to describe the screening of blood donors undertaken and reasons why
* To be able to describe the various blood components used and the potential side effects of blood transfusion

# TESTING PATIENT SAMPLES

**Blood Group Systems**

1. **The** **ABO system:** is important because people have naturally occurring antibodies that are IgM, reactive at 37ºC and capable of activating complement. They are, therefore, able to cause potentially fatal haemolysis if incompatible blood is transfused.

|  |  |  |  |
| --- | --- | --- | --- |
| **Frequency in population (UK)** | **Blood group** | **Antigens** | **Antibodies** |
| 46% | O | nil | anti-A and anti-B |
| 43% | A | A | anti-B |
| 8% | B | B | anti-A |
| 3% | AB | A and B | nil |

**Genes:** O, A, B - O is *'recessive'* to others

**e.g.** Group O = OO Group A = AO or AA  
Group AB = AB Group B = BO or BB

1. **The** **Rh system:** the most important antigen is D

D positive = RhD positive = Rh positive

D negative = RhD negative = Rh negative

|  |  |  |  |
| --- | --- | --- | --- |
| **Frequency in population (UK)** | **Blood group** | **Antigens** | **Antibodies** |
| 85% | RhD positive | D | - |
| 15% | RhD negative | nil | after exposure to RhD pos blood from transfusion or fetus, patient can make anti-D |

**Genes:** D is dominant

*d* is recessive (no actual antigen)

**e.g.** Group RhD neg = dd  
Group RhD pos = DD or Dd

1. **Other red cell antigens:**

It is impossible to provide *'identical'* or fully matched red cells for transfusion. In practice, only two blood group systems are taken into account - ABO and RhD.

In order to provide ABO and Rh compatible blood, it is necessary to test the blood groups (or *'group'*) the recipient. An antibody screen should be performed at the same time as grouping, on the plasma of all patients due to be transfused (*'group & screen'*).

It is relatively common for a D-negative woman to become sensitised through exposure to fetal D pos red cells during pregnancy, as her partner is likely to be D-positive (fetomaternal leakage of red cells across the placenta occurs commonly at the time of delivery, but also silent bleeds are not uncommon and may occur during late pregnancy). If an RhD-negative woman develops anti-D antibodies, then in the next pregnancy, the IgG antibodies can cross the placenta and destroy fetal red cells - causing hydrops fetalis or haemolytic disease of the new-born (hdn). It is therefore important **not** to sensitise RhD negative girls or women of child bearing age, by transfusing RhD positive blood.

**Blood Grouping**

Red cells are grouped for ABO and RhD by taking the patient's red cells, incubating with antibodies of known specificity, and observing for agglutination – indicating the patient has the corresponding antigen.

**Selection of Blood:** blood selected for transfusion should be ABO and RhD compatible; plus the purpose of compatibility testing is to ensure that the recipient does not have antibodies against blood group antigens present in donor blood selected for transfusion, which could cause haemolysis.

***'Antibody screen'*** of recipient plasma to exclude **any** clinically significant immune antibodies. Recipient plasma is incubated with 2 or 3 different fully typed *'screening'* red cells, which are known to possess all the blood group antigens which matter clinically. If the screen is negative, any donor blood which is ABO (and D) compatible can be given. If positive, the antibody must be identified with the use of a large panel of red cells; donor units that lack the corresponding blood group antigen are then chosen for cross matching with the recipient's plasma prior to transfusion.

**Compatibility test** done between donor red cells and recipient plasma = ***'cross-match'***.

**BLOOD DONORS**

**Careful Donor Selection**

Blood is collected in the UK only from volunteer, unpaid donors, who are between 17-70 years of age. Donors are excluded if they have any disease that might make blood donation hazardous, e.g. cardiovascular/ neurological disease, or if their blood would be hazardous for the recipient (risk of viral, bacterial or parasitic infections, certain diseases or drugs). Donor education and self-exclusion of individuals who are at high risk of having contracted blood-borne infectious diseases are essential to ensure that subjects who are in an early infectious stage, but who have not sero-converted (they have not yet developed antibodies, i.e. in the *'window period'*) are not accepted as blood donors.

**Common causes of donor exclusion:**

1. High risk groups:
2. Men or women who are infected with HIV, hepatitis B or C
3. Men or women who have injected illegal or non-prescribed drugs, including body-building drugs, at any time
4. Men or women who have ever been given money or drugs for sex
5. Men or women who have had sex in the last 12 months with:
6. anyone in the above groups
7. a man or woman who may have ever had sex in parts of the world where HIV/AIDS is very common (this includes most African countries except those bordering the Mediterranean)
8. Men who have had oral or anal sex with another man with or without a condom or other form of protection
9. Women who have had sex in the last 12 months with a man who has had oral or anal sex with another man with or without a condom or other form of protection

#### Tests Undertaken on Blood Donations

*1. Group & screening:*

Every blood donation has the ABO and RhD blood group determined. Also, the other Rh blood groups, namely, C, c, E, e and the K blood group are determined on most donations in the UK.

Every donation is tested to ensure that no strong clinically significant red cell antibodies are present in the donor's plasma, so that any transfusions containing plasma will only contain ABO antibodies.

*2. Infection testing:*

**NB:** The most important step in maintaining a safe blood supply is rigorous donor selection and self-exclusion of subjects at high risk of transmitting blood-borne agents. Testing donations for the relevant agents adds to safety, but no test can pick up all infections, especially early ones. The tests done on every blood donation are shown in table below. In addition, giving blood only to patients who really need it reduces the risk to patients.

Tests performed on donated blood in UK

\*Ab=antibody: Ag=antigen

|  |  |
| --- | --- |
| **Infections** | **Tests done** |
| HIV  hepatitis B  hepatitis C  HTLV  Syphilis | anti-HIV 1+2 Ab; PCR  HBsAg  anti-HCV Ab; PCR  anti-HTLV Ab  TPHA (Ab test) |
| Plus some donations:  CMV (cytomegalovirus) T.Cruzii Malaria | anti-CMV Ab  anti-T. cruzii Ab  anti-Malarial Ab |

**Prion Disease: -** Prion proteins have been found in membranes of lymphocytes and platelets and the prions of variant Creutzfeldt-Jacob disease (CJD) are found in lymphoreticular tissues. There have been 4 cases in the UK of variant CJD transmitted by transfusion of blood or blood products in humans, where donors who were entirely well, donated then years later developed vCJD. A blood test to exclude any donor with vCJD is not yet available

**BLOOD COMPONENTS AND PRODUCTS**

450ml blood is collected from a donor into a sterile plastic bag containing anti-coagulant. Over the last 25 years, the emphasis in blood transfusion has changed. It is no longer the aim to provide unseparated whole blood, because very few patients require all the components in blood. With improved diagnosis of coagulation factor deficiencies, modern aggressive chemotherapy regimes, bone marrow transplantation, and improved technology, it has become routine to treat patients only with those components which are required - for example platelets, red cells, factor VIII, etc. Component therapy enables more efficient use of blood donations, and less waste of valuable resources. Also mainly due to the publicity given to transfusion-transmitted infections, clinicians are starting to become more conscious than ever that blood should be prescribed only when there is no safer alternative therapy, e.g. iron therapy, intraoperative salvage etc.

To reduce the risk of vCJD through transfusion in the UK:

(i) Plasma from UK donors is no longer used for fractionation

(ii) All blood products are **LEUCODEPLETED** to remove white blood cells

**1 UNIT = WHOLE BLOOD OR BLOOD PRODUCTS DERIVED FROM ONE SINGLE BLOOD DONATION**



|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Storage (°C)** | **Shelf life** | **Dose** | **Outcome** |
| **Red cells** | 2-6° | 35 days | 1 unit | Hb rise 1g/dl in adult |
| **Platelets** | keep room temperature agitated | 5 days | 1 pool | >10x109/l increase  - check platelet count after |
| **FFP** | frozen | 2 years | 12-15ml/kg | Response to FFP transfusions should be measured clinically and by post-transfusion coagulation tests |
| **Cryoprecipitate** | frozen | 2 years | 10 donors | Test coagulation function |

**A) Red cells** - less than 1% of blood is used as *'whole'* blood in the UK; it is deficient in labile clotting factors and functional granulocytes and platelets. Most blood is given as *'SAG-M’,* where red cells are more concentrated after plasma removal, so that plasma can be used for other purposes, plus this avoids fluid-overloading patients during transfusion.

**B) Platelet Concentrates** - available in two forms.

1. Pooled platelets - platelets from 4 donations pooled to constitute a single adult dose. (Commonest)

2. Or from a single donor by cell separator machine, equivalent to 4 single donations of platelets.

**Indications:**

***i) Preventative:***

1. Prophylaxis due to thrombocytopenia (with decreased platelet production e.g. chemotherapy, bone marrow transplant, aplastic anaemia) or defective platelet function
2. Bleeding becomes likely when platelet count is less than 10x109/L, but can occur at higher levels when there is fever, infection, platelet dysfunction (e.g. post cardiac bypass).

***ii) Therapeutic****: for treatment of bleeding due to thrombocytopenia or dysfunction*

1. Massive blood transfusion (dilutional thrombocytopenia)
2. Platelet dysfunction of cardiac bypass, aspirin

***NB: Autoimmune Thrombocytopenia (AITP):*** platelet transfusions are rarely indicated because there is rapid destruction of all platelets by the autoantibody. In this disease, platelets are required only for life-threatening bleeds.

Important to monitor clinical response (not just rise in platelet count).

**White Cells -** very rarely used except when severe infections occur in neutropaenic patients not responding to antibiotics/ antifungal drugs

1. **Fresh Frozen Plasma – FFP**

**Plasma -** contains clotting factors/ albumin/ immunoglobulins, water, electrolytes

1. Once thawed (at 30-37ºC) - deterioration of clotting factors - use ASAP
2. Use ABO compatible, as plasma contains anti-ABO group antibodies

**Indications** - very few definite indications. Should be given only in patients who are bleeding actively and have abnormal clotting tests or are receiving anticoagulant therapy and need urgent surgery.

**D) Cryoprecipitate**: separated from other plasma constituents by freezing fresh plasma and then allowing it to thaw at 4º-8ºC overnight. Approximately 3% of the FFP forms a residue - fails to redissolve = cryoprecipitate. Contains factor VIII and fibrinogen. Stored frozen in a small vol of plasma (approximately 15ml). When thawed quickly for use, it redissolves in plasma.

**Indications:** (i) treatment of DIC, together with other blood components  
 (ii) fibrinogen deficiency

## E) Blood Products - by fractionation of plasma

1. **Albumin** - human albumin solution (HAS) 4.5%. A safe product that is pasteurised and has never been implicated in the transmission of infections.

Clinical uses: - very few; a highly overused product  
 - hypoproteinaemia, burns, extensive surgery and plasma exchange

2. **Factor VIII Concentrate** - Large pools of plasma (2,000->5,000 donations) subjected to fractionation and heat treated to eliminate viral transmission.

Clinical uses: - treatment of haemophilia A (prophylaxis and acute bleeding)  
 - von Willebrands' disease

NB: Recombinant factor VIII is now given to all new haemophiliacs in the UK.

3. **Factor IX Concentrate**

Clinical uses: - treatment of Christmas disease or Haemophilia B (again, recombinant IX available)

4. **Normal Human Immunoglobulin:** prepared from pooled normal human plasma and contains a mixture of immunoglobulins present in the healthy adult population.   
Available as IM or IV preparations.

Indications for use: (a) mostly by IV route - as replacement in immunodeficiency states, ITP or autoimmune haemolytic anaemia. (b) by IM route - prevention of certain infections (by providing broad antibody cover from normal population) e.g. hepatitis A, measles, rubella.

5. **Specific Immunoglobulins:** fractionated from plasma from selected donors who have a high titre of a specific antibody (from hyperimmune donors) e.g. anti-D Ig, hepatitis B Ig, varicella zoster Ig, rabies Ig, tetanus Ig, CMV Ig.

HAEMATOLOGY 4  
ABNORMAL WHITE CELL COUNT

##### Dr. Donald Macdonald ([d.h.macdonald@imperial.ac.uk](mailto:d.h.macdonald@imperial.ac.uk))

## Learning Objectives

* In a leucocytosis (increased white cell count) explain the importance of the differential count and peripheral blood morphology in planning further investigation.
* List the most common causes of an increased neutrophil, eosinophil and lymphocyte count.
* In a lymphocytosis explain how to distinguish between a reactive polyclonal response to infection and a primary lymphoproliferative disorder (a monoclonal or malignant proliferation of lymphocytes such as chronic lymphocytic leukaemia).

The Full Blood Count (FBC) is a frequently requested investigation, which yields much useful information. This includes a total White Cell Count (WBC) along with an analyser generated white cell differential count. It is vital to consider both the total WBC and the differential count. There are limitations in the machine differential count particularly when abnormal white cells are present. It is therefore often necessary to examine the peripheral blood film microscopically to identify morphological features.

White cells consist of two main groups:

1) Phagocytes; including monocytes and granulocytes, the subtypes of the latter including neutrophils basophils and eosinophils

2) Immunocytes; which consist of T and B lymphocytes. These cell types will react in response to different stimuli.

Both cell groups are present throughout body tissues and play a central role in the response to infection mediated via phagocytosis and soluble proteins of the immunoglobulin and complement system.

**Investigating a leucocytosis (raised WBC)**

When an elevated WBC is identified it is necessary to first look at the automated differential. Is the leucoytosis due to an elevation of a particular cell type i.e. an increase in one cell type only e.g. a lymphocytosis, a neutrophilia or an eosinophilia, or alternatively an increase in all cell types. The next stage is to examine the blood film. This will provide further information such as whether only mature cells are present in the peripheral blood (PB) or immature forms such as myeloblasts and lymphoblasts (precursor/immature blood cells, normally confined to the bone marrow and not seen in peripheral blood except in diseases of the marrow such as leukaemia) are present. Morphology will also identify other features, including reactive changes such as toxic granulation in neutrophils. This approach allows correctly planned further investigation. For example an elevated total white cell count due only to the presence of mature eosinophils might suggest an underlying parasitic infection, whereas an elevated total white cell count due to the presence of immature blast cells, identified by microscopy suggests an underlying leukaemia.

# Important causes of elevated white cell counts

**Neutrophilia**

Neutrophilia is defined as an absolute neutrophil count > 7.5x109/l (adults.)

Common explanations for a neutrophilia seen in clinical practice are

* Bacterial infection. Probably the commonest cause is an acute bacterial infection e.g. chest, or urinary tract. The neutrophil count is raised and morphology may show toxic granulation. The presence of increased numbers of cytoplasmic granules and vacuoles.
* Inflammation and tissue necrosis e.g. appendicitis, myocardial infarct auto-immune tissue damage.
* Underlying neoplastic disease such as carcinoma or lymphoma may produce a reactive neutrophilia due to the aberrant production of stimulatory cytokines.
* Myeloproliferative disorders such as chronic myeloid leukaemia (CML) (also known as chronic granulocytic leukaemia – CGL) With CML, less mature forms such as myelocytes and rarely myeloblasts are present and basophilia is usually present
* Demargination: neutrophils within the blood stream are divided between the circulating and the marginated granulocyte pool. Physical exercise and acute, severe physical stress can increase the circulating neutrophil count by moving neutrophils from the endothelial surface of small blood vessels into the flowing blood. Corticosteroids raise the neutrophil count by other mechanisms.

# Eosinophilia

An eosinophil count of > 0.4 x109/l is designated an eosinophilia. The most common causes in different parts of the world are

* Parasite infestation e.g. schistosomiasis, filariasis.
* Atopic allergic conditions such as eczema and asthma
* Pulmonary eosinophilia.
* Hodgkin’s disease a cancer of the lymphatic system which may produce a reactive eosinophilia.

# Monocytosis

Monocytosis is uncommon but may be seen in certain chronic bacterial infections, which do not produce a neutrophil response, such as tuberculosis, brucellosis and typhoid. It may also occur in chronic myelomonocytic leukaemia.

Response to pyogenic bacterial infection

**Increase in cell numbers**: Infection by pyogenic bacteria will result in tissue damage and the production and release of a range of inflammatory cytokines. Amongst these may be factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) that will stimulate granulocyte and monocyte production by the bone marrow. More importantly in the acute response there will be the early release from the BM of less mature cells. This will result in an increase in the circulating granulocyte count and a left shifted appearance in the peripheral blood.

**Chemotaxis**: The phagocytes will circulate in the peripheral blood, at the site of infection they will move out of the circulation and into the tissues moving to the site of inflammation in response to chemotactic factors.

**Phagocytosis**: The neutrophils and monocytes will encounter foreign material that has been opsonised by immunoglobulin or complement. Using their Fc C3b receptors they are able to recognise and phagocytose the foreign material.

**Killing and digestion**: Ingested material will be killed within the phagocytic vacuoles by both oxidative and non-oxidative mechanisms.

# Lymphocytosis

A lymphocyte count > 4.0 x 109/l (adults) There are many causes of a lymphocytosis however they can be divided into two categories. Primary lymphocytosis, a malignant clonal proliferation of lymphocytes e.g. lymphocytic leukaemia, or lymphoma. Secondary reactive lymphocytosis a polyclonal reactive proliferation as a result of infection or inflammation.

# Reactive lymphocytosis

* Infections: Epstein Barr virus (EBV), Cytomegalovirus (CMV), Toxoplasma, Rubella, Adenovirus, Varicella-Zoster, Infectious Hepatitis, Pertussis, Tuberculosis, Brucellosis.
* Autoimmune disorders.

When a lymphocytosis is identified in a FBC the blood film must be examined for the presence of

* Atypical/reactive lymphocytes seen in mononucleosis syndromes.
* Immediate response to acute stress (e.g. heart attack or other severe pain).
* Small lymphocytes and smudge cells seen in chronic lymphocytic leukaemia.
* Primitive blasts seen in acute lymphoblastic leukaemia.

# Distinguishing between primary and reactive/secondary lymphocytosis

A full blood count may reveal the presence of an increased lymphocyte count. This can broadly be considered to be due to either a neoplastic proliferation of lymphocytes (a form of lymphoma or lymphoid leukaemia) or, alternatively, it may be a reaction to an underlying disorder such as a viral infection, for example ‘glandular fever’ (infectious mononucleosis). The approach to diagnosing the cause of a lymphocytosis would consider the age, clinical features and laboratory investigation. In the laboratory, morphology may simply reveal mature lymphocytes. The presence of abnormal forms such as smear cell (lymphocytes damaged by blood film preparation) or blast cells are suggestive of a lymphoproliferative disorder such as leukaemia or lymphoma. If required, further laboratory tests can distinguish between monoclonal (primary) and polyclonal (reactive) lymphocytes. Individual B lymphocytes express either κ or λ light chains on the cell surface. In a population of monoclonal B cells only one immunoglobulin light chain type, either κ or λ will be present whereas in a reactive increase in B cells there will be a mixed population of κ and λ expressing cells. A more demanding assay using the T cell receptor genes can be used to study the rarer finding of a T cell lymphocytosis.

**HAEMATOLOGY 5  
IRON DEFICIENCY**

Dr Nina Salooja  
([nina.salooja@imperial.ac.uk](mailto:nina.salooja@imperial.ac.uk))

Learning Objectives

* Describe the role of iron in erythropoiesis, dietary sources of iron, absorption of iron, causes of iron deficiency, clinical and haematological features of iron deficiency and the diagnosis and management of iron deficiency.
* Describe the clinical and haematological features of anaemia of chronic disease and explain how this is distinguished from iron deficiency.

**IRON METABOLISM**

Iron is an essential component of many haem-containing molecules including **enzymes** and the oxygen carrying compounds **haemoglobin** and **myoglobin.**

Dietary iron is found in large amounts in red meat, offal and to a lesser extent other animal products. It is also found in relatively large amounts in green vegetables. Because free iron is toxic and because there is no mechanism for excretion of iron the absorption of iron from food is strictly regulated by the intestinal mucosa and this is governed by the amount of iron in the body.

Other factors that affect iron absorption include the form of iron. Haem consists of a protoporphyrin ring with an iron atom at its centre. Haem is better absorbed than free iron (up to 10% absorption) and its absorption is not adversely affected by other food components. In contrast, non-haem iron (i.e. Fe2+ and Fe3+) from vegetable sources are less well absorbed (1-2% absorption) and may be affected by other dietary factors. Factors which improve non-haem iron absorption include acid pH, ascorbic acid (e.g. glass of orange juice) and digestive enzymes, whilst those that inhibit iron absorption include alkaline pH, phytates and phosphates (e.g. cups of tea).

The total amount of iron in an adult is between 3-5 grams. This is divided into three pools:   
(i) a metabolic pool in haemoglobin and myoglobin (2-3 grams), (ii) a storage pool in ferritin and haemosiderin of up to 1 gram and (iii) a proportionately small but extremely important transit pool which consists mainly of plasma protein-bound iron of which the most important component is transferrin-bound iron (about 3 milligrams).

# Iron compounds in the body

## Metabolic pool

Haemoglobin 2500 mg

Myoglobin 500 mg

## Storage pool

Ferritin and haemosiderin 0-1000 mg

## Transit pool

Plasma protein-bound iron 3 mg   
e.g. transferring -bound

**Transferrin** is a glycoprotein made in the liver with two binding sites for iron. It interacts with a **transferrin receptor** on the surface of erythroblasts. The complex is internalised; the iron is removed from the transferrin, which is then recirculated. Iron itself will act as a positive regulator of erythropoiesis and expression of the gene that codes for ferritin. Iron is a negative regulator for expression of the gene that codes for transferrin receptor.

**Hypochromic microcytic anaemias**

This is a term used to describe an anaemia where the red cells contain less haemoglobin than normal [low mean cell haemoglobin (MCH)], have a lower concentration of haemoglobin [hypochromia, low mean cell haemoglobin concentration (MCHC)] and are small (microcytic low mean cell volume MCV). The three commonest causes for this type of anaemia are **iron deficiency, anaemia of chronic disease** and **the thalassaemias.**In milder forms of thalassaemia there is often microcytosis without anaemia.

**Iron deficiency anaemia (IDA):**

Iron deficiency is the most important cause of anaemia on a worldwide basis. The major cause of iron deficiency is **BLOOD LOSS**. Additional causes are **dietary deficiency, increased needs** and **malabsorption.** . Often there is more than one cause of deficiency such as a poor diet in menstruating women or in growing children.

**Blood loss.** The main sources of **blood loss** are uterine in women of childbearing age group, followed by gastrointestinal blood loss, which may be overt or occult.

**Dietary deficiency** occurs in vegans and vegetarians with unbalanced diets poor in iron but can also occur in non-vegetarians.

**Increased needs** occur during childhood and especially during the pubertal growth spurt and during child bearing.

**Malabsorption** is a less common cause of iron deficiency

**Treatment:** iron replacement is simply, effectively and cheaply effected with oral iron compounds and the most commonly used is ferrous sulphate. Side effects include constipation and indigestion and may reduce compliance. Compounds containing less iron (ferrous fumarate or ferrous gluconate) may be better tolerated. In case of difficulties, iron can be given parenterally (IM or IV).

**Assessment of iron status:**

Several parameters can be useful including serum iron, total iron binding capacity (TIBC), transferrin saturation, serum ferritin and a visualisation of haemosiderin in bone marrow aspirates using the Prussian blue reaction.

***The serum ferritin*** is PARTICULARLY USEFUL in clinical practice as it is low in uncomplicated iron deficiency and normal in thalassaemia trait and normal or raised in anemia of chronic disease. As ferritin is an acute phase reactant, however, it may be normal or increased in patients where iron deficiency co-exists with chronic inflammatory conditions.

Anaemia of chronic disease. Typically the serum iron is low but the ferritin is normal or raised. Total iron binding capacity is normal or reduced.

Iron deficiency. Typically the serum iron is low and the ferritin is also low. The total iron binding capacity is increased.

Thalassaemia trait. Iron levels, total iron binding capacity and ferritin levels are all normal.

Anemia of chronic disease plus iron deficiency. This is not uncommon. Additional tests which may help to establish whether a patient is iron deficient are:-

1. blood film….you may see changes of iron deficiency, such as elliptocytes
2. bone marrow aspirate….slides can be stained to see whether or not iron stores are present

**FILL IN THE MISSING SECTIONS**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Iron deficiency** | **ACD** | **Thalassaemia Trait** |
| Hb |  |  |  |
| MCV |  | Low or normal |  |
| Serum Iron |  |  |  |
| Ferritin | Low |  |  |
| Transferrin |  |  |  |
| Transferrin saturation |  |  | Normal |

**Anaemia of chronic disease (ACD)**

This is an anemia associated with chronic inflammatory, infectious or neoplastic conditions. ACD can cause a mild to moderate normocytic or microcytic hypochromic anaemia. In ACD the inflammatory markers such as CRP (C-reactive protein) and ESR (erythrocyte sedimentation rate) are raised. The serum ferritin may also be raised and there is accumulation of excess iron in the bone marrow storage pool but with a block in iron incorporation into erythroblasts, which may lead to reduced haemoglobin synthesis and hypochromia. Difficulties arise in differentiating between ACD and IDA and in certain cases the two coexist; in these patients the serum ferritin may be within the low normal range.   
A bone marrow aspirate may be required to distinguish between the two conditions.   
The pathogenesis of ACD is complex but it usually responds to treatment of the underlying disorder.

(Handout modified from a previous version from Dr. Saad Abdalla)

**HAEMATOLOGY 6  
B12 and FOLATE DEFICIENCY**

Dr Nina Salooja ([nina.salooja@imperial.ac.uk](mailto:nina.salooja@imperial.ac.uk))

# Learning Objectives

# The student should be able to

1. Describe the role of vitamin B12 and folic acid in haemopoiesis, dietary sources and absorption of these vitamins, causes of deficiency, clinical and haematological features of vitamin B12 and folic acid deficiency and the diagnosis, further investigation and management of these deficiencies

**And to be able to explain that**

1. Synthesis of DNA requires both vitamin B12 and folate
2. Integrity of the nervous system requires vitamin B12
3. Deficiency of either causes anaemia, which is both **macrocytic** and **megaloblastic**

**MACROCYTIC ANAEMIA**

This is defined as anaemia with an increase in the mean cell volume (MCV) of the red cells, e.g. as measured by an automated full blood count machine. A raised MCV means that the red cells produced are larger than normal. Causes of a raised MCV are as follows:

1. Vitamin B12 or folate deficiency
2. Liver disease
3. Hypothyroidism
4. Excessive alcohol consumption
5. Drugs e.g. azathioprine, zidovudine
6. Haematological disorders

a) myelodysplasia

b) aplastic anaemia

c) reticulocytosis e.g. chronic haemolytic anaemias

**Megaloblastic anaemia**

This is defined by an abnormal but distinct morphological appearance of early and developing red cells. As the nucleus and cytoplasm of normal red cells mature, they demonstrate characteristic morphological features, readily discernible by light microscopy. The earliest recognisable erythroid cell in the bone marrow is the proerythroblast. This is a large cell with dark blue cytoplasm, reflecting the high RNA content. The nucleus contains only slightly condensed chromatin, which can have a lacy appearance. The proerythroblast gives rise to a series of progeny called erythroblasts, which contain progressively less RNA and more haemoglobin; late erythroblasts have pink rather than blue cytoplasm and are normally confined to the bone marrow. Meanwhile the nuclear chromatin becomes more condensed as cells mature from proerythroblasts to early, intermediate and late erythroblasts and the nucleus is extruded completely from the latter to form reticulocytes. These may be found in the peripheral blood and are the precursors of mature red blood cells. In megaloblastic anaemias, there is asynchrony between the maturation of the nucleus and cytoplasm and thus a nucleus with a unclumped chromatin and lacy appearance may be seen even in late (pink) erythroblasts. These abnormal cells are called megaloblasts, whereas erythroblasts showing normal maturation are called normoblasts. As a result of the delayed maturation of the nuclei, many red cells die in the bone marrow and the activity of red cell production increases to compensate. This is referred to as ineffective erythropoiesis.

White cells also show characteristic morphological abnormalities. In the bone marrow, myeloblasts successively give rise to promyelocytes, myelocytes, metamyelocytes and neutrophils. In megaloblastic anaemias the metamyelocytes may be 2-3 times the normal size (“giant metamyelocytes”) and neutrophils have hypersegmented nuclei.

**Peripheral blood**

Although the term megaloblastic refers to changes visible in the bone marrow, certain associated abnormalities are visible in the peripheral blood as follows:

a) Red blood cells often show variation in size (anisocytosis)

b) The mean cell volume (MCV) is high.

c) The haemoglobin concentration (Hb) may be low as a result of ineffective erythropoiesis.

d) Hypersegmented neutrophils can be seen.

e) The white count &/ or platelet count may also be low

**Causes of megaloblastic anaemia** include vitamin B12 and/or folate deficiency. Drugs which interfere with DNA synthesis directly, or with the metabolism of vitamin B12 or folate (e.g. methotrexate) will also cause a megaloblastic change.

**Causes of haematinic deficiencies**

Always consider the following:

1. inadequate intake
2. increased demand
3. inadequate absorption
4. excessive losses or utilization

**Vitamin B12**

1. Inadequate intake is rare

\* Vitamin B12 is found in animal products, so vegans are at risk

\* Abnormal bacterial flora in the small bowel (e.g. associated with stagnant loops) can consume vitamin B12

2. Increased demands are usually readily covered by the vitamin B12 stores, which are relatively large in relation to daily needs and usually sufficient to last for many years.

3. Absorption of B12 is complicated and failure of absorption is the commonest cause of B12deficiency

B12 is absorbed in the ***small bowel*** following combination with intrinsic factor.   
Intrinsic factor is made in the ***stomach***.

B12 absorption may be impaired in the following situations:

1. reduction in active intrinsic factor

post gastrectomy

autoimmune gastric atrophy **(“pernicious anaemia”)**

1. small bowel disease

surgical resection

Crohn’s disease

coeliac disease

4. Excessive losses: this is not a common cause of B12 deficiency

**Consequences of vitamin B12 deficiency**

* Megaloblastic anaemia
* Neurological problems:

a) peripheral neuropathy

b) subacute combined degeneration of the spinal cord

1. optic neuropathy
2. dementia

**Laboratory diagnosis of B12 deficiency**

* blood count and film
* serum B12 level

The **Schilling test** may be necessary to determine the CAUSE of the B12 deficiency.

Radiolabelled B12 is given orally and its excretion in the urine is measured, after first having saturated the serum B12-binding proteins by giving an intramuscular injection of non-radio-active B12. Clearly, any radiolabelled B12 detected in the urine must have been successfully absorbed in the small intestine. If the excretion is low then the test is repeated with the addition of intrinsic factor. If this restores the excretion of B12 to normal it is possible to conclude that the defect lies with a lack of intrinsic factor secretion. The detection of anti-parietal cell and anti-intrinsic factor antibodies in the blood, particularly the latter, would be additional evidence that a patient had pernicious anaemia.

**Folate**

1. Inadequate intake is common. Folate is found in animal and plant products but is readily destroyed by cooking, canning and processing. Poor nutrition, for example in the elderly, alcoholics or those living in poverty is a common cause of folate deficiency

2. Increased demand is also a common cause of deficiency

* Physiological: pregnancy, lactation, adolescence, premature babies
* Pathological: an excessive turnover of cells as may occur with haemolytic anaemias, malignancy, erythroderma.

3. Absorption of folate occurs in the duodenum and jejunum. This is rarely a cause of folate deficiency unless there is widespread disease of the small bowel such as coeliac disease.

4. Excessive losses: this is not a common cause of folate deficiency.

**Consequences of folate deficiency**

* megaloblastic anaemia
* neural tube defects in developing fetus
* ? increased risk of coronary artery disease if associated with variant enzymes in folate metabolic pathway.

## Laboratory diagnosis of folate deficiency

* FBC and film
* Serum folate and red cell folate (red cell folate gives a better indication of body stores of folate whereas serum folate reflects recent intake)

**HAEMATOLOGY 7 & 8**

**HAEMOSTASIS AND ABNORMALITIES OF HAEMOSTASIS**

Professor David Lane and Professor Mike Laffan  
([d.lane@imperial.ac.uk](mailto:d.lane@imperial.ac.uk) and [m.laffan@imperial.ac.uk](mailto:m.laffan@imperial.ac.uk))

**Learning Objectives**

The student should be able to:

• Describe the normal haemostatic mechanisms including the interactions of vessel wall, platelets and clotting factors

• Describe and distinguish the clinical features of bleeding due to thrombocytopenia and coagulation disorders, respectively

• Describe the use of laboratory tests to assess haemostasis

• Describe the principles of management of disorders of haemostasis

Haemostatic plug formation is essential to prevent blood loss from intact vessels and to arrest bleeding from injured vessels. A series of linked processes are involved:

1. Vessel constriction occurs as a local contractile response to injury. This is in itself sometimes sufficient to temporarily restrict blood loss from a wound in small blood vessels.
2. Almost immediately following vessel injury, platelets adhere to the subendothelial structures. There are a number of molecules mediating the adhesion reaction, including extravascular collagen, von Willebrand factor and membrane-glycoproteins. Activation of platelets, particularly by adenosine diphosphate (ADP) and thrombin, then results in aggregation and the formation of an unstable platelet plug.
3. Stabilisation of the unstable plug is achieved by the tissue factor activation of the blood coagulation system and formation of fibrin clot.
4. Finally, the clot is lysed by the fibrinolytic enzyme system and blood vessel repair is initiated.

Vessel disorders causing bleeding arise because of impaired interaction of platelets with the compromised vessel. Examples are scurvy, senile purpura, allergic vasculitis (acquired) and haemorrhagic telangiectasia, Ehlers-Danlos syndrome (inherited).

The platelet has its origin in the bone marrow. Haemopoeitic stem cells give rise to megakaryocyte precursers which undergo nuclear replication without cytoplasmic division, then maturation before migrating to the marrow sinusoids, extending proplatelets through the endothelial wall and fragmenting into platelets in the circulation.





The lifespan of the platelet is ~10 days, and ~1/3 of the platelets are sequestered in the spleen. An important metabolic pathway in platelets converts membrane phospholipids to thromboxane A2, which can activate platelets. Aspirin acts as an antiplatelet agent by irreversibly inhibiting one of the enzymes of this pathway, cyclo-oxygenase. ADP, released from platelet granules, and thrombin are other activators. The adhesive and aggregation reactions of platelets are mediated through surface membrane glycoproteins (Glp), Glp Ib, Glp Ia-IIa and Glp IIb/IIIa.

Platelet disorders may be acquired (common) or inherited (rare). Acquired disorders include a low platelet count (thrombocytopenia) and drug-induced effects on function. Thrombocytopenia can arise from three mechanisms:

1) failure of platelet production (e.g. aplastic anaemia, leukaemia, vitamin B12/folate deficiency)

2) shortened platelet half life (e.g. auto-immune thrombocytopenia, thrombotic thrombocytopenia, infections, drugs) and

3) increased pooling of platelets in an enlarged spleen.



There are three laboratory tests to monitor platelets, the platelet count, the bleeding time and platelet aggregation. The most important of these is the platelet count, as progressive reduction of platelets dramatically increases the risk of bleeding. Platelet aggregation is performed to monitor platelet dysfunction and can be used to measure von Willebrand factor activity. The bleeding time is now rarely used in clinical practice although it is sometimes required if it is necessary to assess vessel wall function.

The blood coagulation system is portrayed as an enzymatic cascade by which trace initiators generate large amounts of thrombin to clot fibrinogen and aggregate platelets. Traditionally, the cascade is described in terms of the intrinsic, extrinsic and common pathways.





This description is still useful for understanding the use of laboratory tests. However, it is important to realise that in vivo the intrinsic pathway (initiated by factor XII activation) plays a minimal role in normal haemostasis. The main initiator is thought to be tissue factor, exposed by vessel damage, which forms an activation complex with factor VIIa. Tissue factor/ factor VIIa also activates the intrinsic pathway at the level of factor IX. Certain of the clotting factors (factors VII, IX, X and prothrombin) bind to phospholipid in order to activate their substrate factor. Phospholipid binding requires Vitamin K dependent post translational modification of certain amino acids (“Gla” residues) and is mediated by Ca2+ ions.

Factors VIII and V, once activated, accelerate these membrane-dependent reactions on the platelet surface, enabling clotting to be localised to the sites of vessel injury.

Anticoagulant control of blood coagulation is achieved with the drugs heparin (which accelerates the inhibition of thrombin and other clotting proteinases by antithrombin) and warfarin (which inhibits the post translational Gla modifications of certain of the clotting proteinases, thus reducing their activities).

The main tests of coagulation function are the activated partial thromboplastin time (APTT) and the prothrombin time (PT). The APTT monitors the function of the intrinsic and common pathways and is used to control the level of heparin in patients. The PT monitors the extrinsic and common pathways and is used to control levels of oral anticoagulant (warfarin) in patients. These tests are also used to detect inherited coagulation abnormalities such as haemophilia A and B (factor VIII or factor IX deficiency: APTT used) and acquired disorders such as disseminated intravascular coagulation (both tests).

Fibrinolysis is initiated by fibrin clot formation. This facilitates the activity of the main plasminogen activator, tissue plasminogen activator (tPA). tPA activates plasminogen to plasmin, which lyses the fibrin clot in a specific reaction.

In broad terms, bleeding due to platelet-vWF and coagulation defects can be distinguished clinically.   
The former are characterised by superficial bleeding into the skin and mucosal membranes and by bleeding immediately following injury. Bleeding from coagulation factor deficiencies tends to be into deep tissues, muscles and joints. It is often delayed after injury but is prolonged and can be severe. The haemophilias are well known sex linked recessive disorders of this latter type. Von Willebrand disease is another inherited disorder. Its inheritance is usually autosomal dominant. Bleeding in this disorder may be of characteristically vascular (e.g. nosebleeds, menorrhagia and prolonged bleeding from superficial cuts) due to its importance in the platelet-vessel wall interaction, but because this factor is a carrier for factor VIII, it can also resemble the haemophilias.

Treatment of bleeding arising from acquired platelet and coagulation factor abnormalities requires identification and treatment of the underlying conditions. If it cannot be corrected then replacement therapy may be possible. Inherited deficiencies (e.g. haemophilia, von Willebrand disease) tend to be treated by replacement therapy. Drugs are available to inhibit (tranexamic acid) or enhance (tPA) fibrinolysis.

HAEMATOLOGY 9  
THE HAEMOGLOBIN MOLECULE AND THALASSEMIA

# Dr Nina Salooja

### Learning Objectives

1. Describe the structure and function of the haemoglobin molecule and list the normal haemoglobins in the fetal, neonatal and adult periods.
2. Describe the genes controlling haemoglobin synthesis and explain how genetic defects lead to α and β thalassaemias.
3. Describe briefly the clinical and haematological features of β thalassaemia major and the principles of management.
4. Describe the haematological features of β thalassaemia trait, how it is diagnosed and why this is important.
5. Describe how β thalassaemia trait can be differentiated from iron deficiency anaemia and the anaemia of chronic disease.

Haemoglobin (Hb) is a protein molecule found in red blood cells. Each molecule of haemoglobin consists of 2 pairs of globin protein chains together with 4 haem groups. Each haem group consists of a protoporphyrin ring with an iron atom at its centre and a single haem group sits in a pocket formed by a single globin chain.

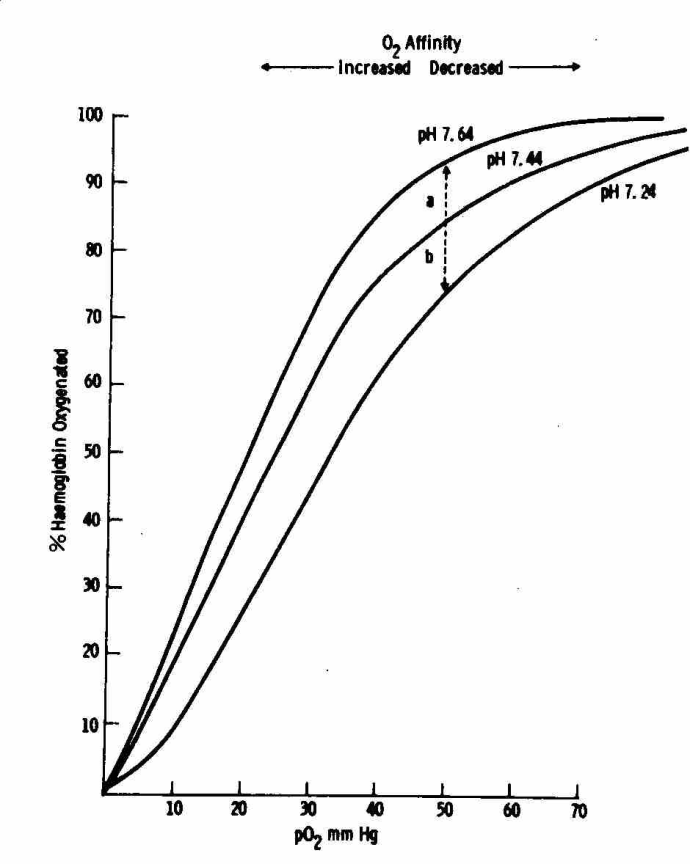
Several different types of globin proteins exist each encoded by their own gene (s). The globin genes are located in two clusters. The alpha cluster is found on chromosome 16 and contains the genes for α globin (adult variety) and   
ζ globin (zeta globin - an embryonic variant). The alpha genes are duplicated so that there are two functional alpha genes within an alpha cluster. The beta cluster is found on chromosome 11 and contains the genes for β globin, and δ globin (adult varieties), γ globin and ε globin (fetal and embryonic variants respectively).

There are 6 common variants of Hb. Three are transient embryonic haemoglobins. Hb F (α2γ2) is the predominant haemoglobin of fetal life and is also found in large amounts at birth. Hb A (α2β2) is quantitatively the major Hb (>95%) in adults and is found together with some HbA2 (α2δ2) (2-3.5%) and traces of HbF (α2γ2).

The main function of Hb is the carriage of oxygen from the lungs to the tissues. To facilitate this the Hb molecule can exist in two spatial configurations. Deoxy haemoglobin exists in a tight (T) configuration and has a relatively low affinity for oxygen. Oxygen molecules are taken up sequentially by the 4 haem groups and at some point the partially liganded Hb molecule switches to a relaxed (R) configuration which has a markedly higher affinity for oxygen.

This can be represented diagramatically by the oxygen dissociation curve.

On the Y axis is the oxygen saturation which is defined as the fractional occupancy of the oxygen binding sites, and on the X axis is concentration of oxygen which is expressed as its partial pressure.

The binding of oxygen by haemoglobin is regulated by specific molecules in its environment. H+ ions, CO2 and 2,3-DPG (an organic phosphate compound) all stabilise the T form of the oxygen molecule by forming H bonds and thus decrease the oxygen affinity of the molecule. This is represented on the oxygen dissociation curve as a shift to the right *i.e* a higher concentration of O2 is needed for maximum O2 saturation if the concentration of CO2, H+ ions or 2,3-DPG are high. Thus in metabolically active tissues where the concentration of H ions and CO2 are high, oxyhaemoglobin will assume the T configuration and give up oxygen readily. Conversely in the lungs where CO2 is exhaled, oxygen affinity is higher. This effect of CO2 on the affinity of Hb for oxygen is called the Bohr effect.

**The thalassaemias** are disorders in which there is underproduction of one of the types of globin chains of adult haemoglobin and are called alpha or beta thalassaemia according to the chains affected. Globin genes are transcribed into messenger RNA which is processed before translation into protein. Underproduction of a globin chain may therefore result from deletion of part (or all) of the gene, or else genetic mutations which lead to defects in transcription, mRNA processing, translation or stability of the final protein product. Different sets of mutations develop in different parts of the world. They probably arose independently and were expanded by selection possibly in relation to malaria

### Alpha thalassaemia

Alpha chains are found in HbA and HbF so alpha thalassaemia may present clinically *in utero*. Alpha thalassaemia is usually (>80% cases) due to a deletion of one or more alpha genes and since each alpha cluster (one on each chromosome) has two alpha genes, four syndromes are possible as follows each with an increasing degree of anaemia and associated morbidity: α+ trait where one locus fails to function, α0 trait where two loci on the same chromosome are dysfunctional, Hb H disease with three loci affected and Hb Bart’s hydrops fetalis where all four loci are defective and death *in utero* is the norm. α+ thalassaemia is particularly common in Africa and in those of African descent and α0 thalassaemia is particularly common in SE Asia.

### Beta thalassaemia

Most types of β thalassaemia are due to point mutations and over 100 different mutations have been described. In the absence of beta chains, alpha chains accumulate and precipitate in the bone marrow causing cell death; this is called ineffective erythropoiesis.

Cells which do manage to mature and enter the circulation contain β-chain inclusions and are removed by the spleen which subsequently enlarges. The anaemia stimulates erythropoietin production and this causes expansion of the bone marrow in the skull and long bones.

A simple clinical classification of β thalassaemia takes into account the severity of anaemia and need for regular transfusions and is not dependent on the underlying genetic changes. Thus, a patient with thalassaemia major has profound anaemia and requires regular blood transfusions to survive. A patient with thalassaemia intermedia, has anaemia but does not require regular blood transfusions.

Patients with thalassaemia major usually present within the first year of life with failure to thrive, and general malaise. Splenomegaly and bony deformities of the skull are characteristic and bone changes in the long bones may be associated with recurrent fractures. Without transfusion the children usually die by the age of 7. If blood transfusions are commenced in infancy, however, then early growth and development may be normal. The blood transfusions are themselves associated with considerable morbidity due predominantly to iron overload but also as a result of the transmission of blood borne viruses (*e.g*. hepatitis B and C and HIV). Each unit of blood contains 200mg iron and this accumulates in the liver, heart and endocrine glands. The effects of this start to appear by the end of the first decade. Secondary sexual development may be delayed or absent, the normal adolescent growth spurt fails to occur and diabetes, hypoparathyroidism and adrenal insufficiency may become apparent. In addition, progressive liver and cardiac damage occur and liver damage from the iron overload may be exacerbated further by infectious hepatitis. Death usually occurs before the age of 25. Removal of iron is difficult. Currently the most successful drug is an iron-chelating agent called desferrioxamine. This is not an ideal medication since it is not orally active and must be administered by a subcutaneous infusion over several hours on several occasions a week. Furthermore, it is expensive. Iron chelation does improve the outcome of thalassaemia, however, and in the transfused and chelated patient, survival into the 4th decade is possible. Death is usually (60%) a result of cardiac failure secondary to iron overload.

Bone marrow transplantation has the potential to cure thalassaemia major and should be considered in transfusion-dependent thalassaemics under the age of 16 years who have an HLA-identical sibling greater than 18 months of age

### Beta thalassaemia trait

Heterozygotes for a beta thalassaemia gene are said to have β-thalassaemia trait. These carrier states are usually clinically silent, and can be referred to as thalassaemia minor. They can, however, be identified in the laboratory on the basis of abnormal red cell indices. Typically, patients with β-thalassaemia trait have smaller red cells than usual (microcytosis) and a reduced mean cell haemoglobin (MCH) with a normal mean cell haemoglobin concentration (MCHC). The red cell count is usually raised and the haemoglobin level is normal or slightly reduced. If β thalassaemia trait is suspected then the level of HbA2 should be measured and is typically raised. If levels are equivocal even on repeat testing, and there is no evidence for coexisting iron deficiency then DNA analysis could be considered. There are two situations in which identifying patients as having β thalassaemia trait is of value. Firstly, the microcytosis may be misinterpreted as iron deficiency if the raised red cell count and normal MCHC are not noted. If these patients are then put on long term iron they can become iron overloaded. Secondly, it is important to identify pregnant patients with

thalassaemia trait so that their partners can be tested and the couple can be counselled about their chance of having a baby with clinically significant thalassaemia and can be offered further testing.

[n.salooja@imperial.ac.uk](mailto:n.salooja@imperial.ac.uk)

## BThalMajor3

Subcutaneous infusion of desferrioxamine in beta thalassaemia major

**HAEMATOLOGY 10  
SICKLE CELL DISEASE**

# Dr Mark Layton

*Lecture notes prepared by Dr Nina Salooja and Prof Barbara Bain*

**LEARNING OBJECTIVES**

The student should be able to

1. Describe the inheritance of clinical and haematological features of sickle cell anaemia (SS)
2. Outline principles of management
3. Explain the inheritance, clinical significance and diagnosis of sickle cell trait

Sickle cell anaemia results from a single amino acid change in the haemoglobin molecule. The blood disorder that results has long been a source of major interest to doctors and scientists. In 1949 Pauling and associates deduced that this disease was a result of a change in a protein by an allelic change in a single gene. As such, this was the first demonstration of a molecular disease. Although genetically simple, sickle cell anaemia is clinically complex and relating a single amino acid change in a single protein in a single cell type to the variable clinical manifestations represents a scientific challenge. In recent years considerable efforts have been made towards ameliorating the clinical picture which, as you will see, can be severe.

Sickle haemoglobin (HbS) differs from HbA by a single amino acid. The defect is in the β globin chain and results in replacement of glutamic acid at position 6 of the β chain by valine. 3-D models of the deoxyhaemoglobin indicate that the residue at position 6 sits on the surface of the protein. Although glutamate is a highly polar amino acid, the side chain of valine is distinctly nonpolar and this alteration markedly reduces the solubility of deoxyhaemoglobin. These molecules can then polymerise within the red cell, which distorts and undergoes a characteristic shape change: the sickled cell. These cells have a marked decrease in deformability. In addition, the formation of intracellular polymers is associated with red cell membrane changes, which make the red cells particularly “sticky” to vascular endothelium.

Sickle cell anaemia refers to a condition in which there are two βS genes and no normal β genes so that the individual cannot produce any normal β chain and therefore cannot produce any haemoglobin A (see Table). The term sickle cell disease is a more general one that covers also other conditions that lead to formation of sickled red cells such as co-inheritance of haemoglobin S and either haemoglobin C (another β chain variant) or β thalassaemia trait.

|  |  |  |  |
| --- | --- | --- | --- |
| **Condition** | | **β genes** | **Haemoglobins present  (in addition to A2 and F)** |
| Sickle cell disease | Sickle cell anaemia | βSβS | S |
| Sickle cell/haemoglobin C disease | βSβC | S and C |
| Sickle cell trait | | βSβA | A and S |
| Normal | | ΒAβA | A |

The sickle β globin gene (βS) is spread widely throughout Africa, the Middle East, Mediterranean countries and India. The frequency of sickle cell carriers is up to 1 in 4 in West Africans and I in 10 in Afro-Caribbeans, and has reached high levels in these populations because the carrier state protects against malaria. Currently there are more than 10 000 patients with sickle cell disease in Britain.

Only adult Hb is affected because HbF does not have any beta chains. The problems therefore start at 4-6 months or older, after the HbF level decreases and the adult Hb level increases. As sickled red cells become trapped in the small blood vessels, circulation is impaired and there is damage to multiple organs. In children, infarcts of the small bones of the hands of feet may occur and lead to a painful dactylitis called the “hand-foot“ syndrome and, as a later result, shortening of the digits. In adults, generalised pains are more typical and result from oxygen deprivation of tissues and avascular necrosis of the bone marrow. The effects of sickling in various organs are listed next:

Bones dactylitis / osteomyelitis/ avascular necrosis of the hip

Kidneys haematuria and failure to concentrate urine, papillary necrosis

Brain stroke

Lungs “chest crisis”

Spleen splenic sequestration/ hyposplenism

Skin skin ulcers

**Terminology**

***Infarct*** death of tissue due to loss of blood supply

***Dactylitis*** inflammation of a digit (in this case resulting from infarction of bone)

***Avascular necrosis*** death of tissue as a result of loss of its blood supply

***Osteomyelitis*** infection of bone (dead tissue is susceptible to bacterial infection)

***Splenic sequestration*** pooling of large numbers of red cells in the spleen (see below)

***Hyposplenism*** reduced function of the spleen (in this case, as a result of recurrent interruption of the blood supply leading to death of splenic tissue)

***‘Chest crisis’*** hypoxia resulting from death of lung tissue

Sickled cells are fragile and have a shortened life span (***haemolysis***), which results in anaemia. The affinity of HbS is lower than that of HbA so it gives up oxygen more readily to tissues and anaemia is often well tolerated. In an attempt to compensate for the shortened red cell life span there is an increased turnover of red cells and the body’s supply of folic acid can become low. The shortened life span of the red cells makes patients with sickle cell disease particularly susceptible to the effects of parvovirus B19 infection. This virus infects red blood cell precursors and stops red cell production for up to a week. In the setting of a short red cell life span, if red blood cell production stops for even short period of time the Hb level can fall dramatically; this is called an aplastic crisis. Children are also at risk of another sort of crisis called a splenic sequestration crisis. Abdominal pain, pallor and shock together with a large spleen and low haemoglobin are indicative. The reticulocyte count is raised in a sequestration crisis but in an aplastic crisis it is much lower than normal.

In one large survey the median life expectancy for men and women with homozygous sickle cell anaemia was 42 and 48 years respectively and the causes of death were:

21% associated with a painful crisis

14% associated with a chest syndrome

9% associated with renal failure

7% associated with infection

6% perioperative

**Note**: you do NOT have to memorise these figures – they are just to give you an idea of the long term clinical features of this disease

# Laboratory features and diagnosis

1. The **blood count** shows a low Hb *e.g.* 6-9 g/dl and raised reticulocyte count
2. **Blood film** shows sickled cells. Also it may show signs of hyposplenism, namely the presence of Howell-Jolly bodies (which are nuclear remnants usually removed in the spleen) and target cells.
3. Simple screening tests.

These tests depend on the decreased solubility of haemoglobin S when the oxygen tension is low. One such test is a **sickle solubility test** in which a reducing agent is added to diluted blood and leads to formation of many sickle cells (in sickle cell trait as well as in sickle cell anaemia). This makes the blood turbid. A positive result must be confirmed by Hb electrophoresis.

1. Definitive diagnosis requires **haemoglobin electrophoresis** (or an equivalent test)as well as a sickle solubility test. Electrophoresis separates proteins according to their charge; this varies according to the pH at which electrophoresis is carried out. So, at alkaline pH, HbS separates readily from Hb A and F. However, there are some non-sickling haemoglobins (called HbD and HbG) that run with HbS – this is why a sickle solubility test is also needed. In sickle cell anaemia no HbA is detected and there is a variable (5-15%) amount of HbF and a small amount of HBA2. Patients with sickle trait have Hbs A and S (plus small amounts of HbA2 and HbF).

# Management

1. **Painful crisis**

Factors known to precipitate a crisis should be avoided. Fast, adequate pain relief with strong analgesics should be given and precipitating factors should be reduced by rehydration, warmth and additional oxygen as necessary. Infection should be excluded by chest X-ray and appropriate cultures, *e.g*. of urine and blood. If infection is present, antibiotics are needed.

1. **Folic acid 5mg /day**
2. **Vaccination to protect against pneumococcal infection**
3. **Prophylactic penicillin** to prevent some of the infections caused by hyposplenism
4. **Blood transfusion**

a) top up blood transfusion e.g. if aplastic or sequestration crises

b) exchange blood transfusion if life threatening/severe disease such as a stroke, or chest crisis. An exchange transfusion aims to reduce the HbS to less than 20%

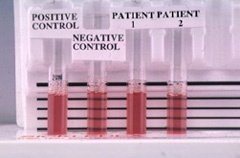
**NOTE:** top-up blood transfusion is NOT a treatment for painful crisis; it will increase blood viscosity and may make the painful crisis worse

1. **Stem cell transplantation**

Consider in children with severe disease. Currently survival is 90-95%.

# Sickle cell trait

This is the carrier state for sickle cell disease. This does not affect life expectancy, and is often clinically silent. However it needs to be identified because certain situations can provoke sickling e.g. anaesthesia, high altitude, air travel in unpressurised planes. All patients from ethnic groups in whom βS occurs should be screened prior to surgery. Sickle trait must also be identified in pregnant women so that their partners can be tested and appropriate counselling given and action taken if necessary.



A sickle solubility test (from Bain BJ, Interactive Haematology Imagebank, Blackwell Publishing, Oxford, 1999 — available on Intranet)

[m.layton@imperial.ac.uk](mailto:m.layton@imperial.ac.uk?subject=MCD-Sickle%20Cell%20Disease)

DIAGNOSTICS 1  
INTRODUCTION TO CHEMICAL PATHOLOGY

Professor Karim Meeran ([k.meeran@imperial.ac.uk](mailto:k.meeran@imperial.ac.uk))

#### Learning objectives

1. List five common diagnostic tests carried out by the department of chemical pathology
2. Know how to collect specimens for common tests including electrolytes, urea, glucose and glycosylated haemoglobin
3. Describe a typical chemical pathology request form

The Department of Chemical Pathology processes samples and measures the concentrations of many important metabolites. Changes in the concentration of metabolites can suggest particular illnesses.

The most commonly requested tests include:

1. **Electrolytes** (including, sodium and potassium).
2. **Urea and creatinine**. High levels suggest failure of renal excretion of these substances and hence renal failure.
3. **Calcium and phosphate**
4. **Markers of liver function** (liver enzymes). Only very small amounts of liver enzymes should enter the bloodstream. Damage to the liver may result in extra amounts of these enzymes leaking into the blood. Particular diseases seem to be associated with particular patterns of liver enzymes. Enzymes commonly measured include
   1. alkaline phosphatase
   2. aspartate amino-transferase (AST)
   3. alanine amino-transferase (ALT)
   4. gamma glutamyl transferase (GGT)
5. **Hormone assays** are done within a subdivision of the chem. Path department (endocrinology). Hormones commonly measured include thyroxine, TSH and cortisol.
6. **Glucose**. This can be rapidly measured using a glucose sensitive stick which can be undertaken in the Ward/clinic/home. A more accurate method is carried out within the laboratory. Red cells will consume glucose, even after it is out of the patient, unless they are poisoned.

What poison is used for this purpose? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Case Study**

Billy is a 26-year-old who has blood taken by his GP.

The chemical pathology department undertake the following tests using the GP request form.

* U + E
* LFT
* Glucose

1. What can you infer from the electrolytes? (Na and K).

2. If the potassium is raised, what important question does the lab need to check?

3. What can you infer from the discrepancy between the urea and the creatinine?

4. Liver “function” tests include albumin and bilirubin as well as the liver enzymes.

5. What can you infer from Billy’s Albumin result?

6. What can you infer from the Bilirubin result, and what would you see on careful examination of Billy’s eyes?

7. Looking at the rest of the form, what are “cardiac enzymes”?

**DIAGNOSTICS 2  
THE DIAGNOSIS OF INFECTION AND THE USE OF   
THE VIROLOGY LABORATORY**

Dr Mark Atkins ([m.atkins@imperial.ac.uk](mailto:m.atkins@imperial.ac.uk?subject=MCD-Virology%20Lab%20lecture))

**Learning objectives**

* Appreciate the range of viruses that can cause human disease.
* Understand what tests are available for diagnosing viral infections.
* Know what clinical samples to take to enable you to make the correct diagnosis.

When diagnosing a viral illness, taking a good history and performing a clinical examination can often give you the diagnosis. Some infections are easy to diagnose such as shingles and chicken pox. However many have more subtle signs. When talking a history it is important to include, vaccination history, travel (especially in the previous 3 weeks), contact with animals/pets, contact with infected persons and occupation. This information may give you some important clues.

Diagnosis depends on the clinical findings, the detection of specific antibodies and/or the detection of a virus in the appropriate clinical sample.

# Virological tests- ideal tests should have the following qualities

## High specificity i.e. have a low level of cross reactivity.

## Sensitive- detect the virus or the antibody at very low levels

## Rapid- results should be available in a timely fashion.

## Non-invasive. This reduces the risks of the procedure and makes then easier to repeat if necessary.

## Cost effective. Most virology tests only cost a few pounds each but some of the molecular tests are significantly more expensive, so use them wisely.

# Diagnostic methods

## Cell culture

## Electron microscopy (EM)

## Antibody detection e.g. HIV antibody

## Antigen detection e.g. HBsAg in hepatitis B infection or RSV antigen in respiratory sample

## Genome detection – e.g. using PCR to detect viral DNA or RNA

## Quantification of antigens and genomes (now essential for diagnosis and monitoring of HIV, HBV and HCV)

# Samples to take will depend on the disease being investigated

## Throat swab - for virus isolation (in virus transport medium, VTM) - useful in the diagnosis of enteroviruses and respiratory viruses.

## Stools - for EM and Rotavirus EIA (in sterile pot) - for the diagnosis of enteroviruses and viruses that cause diarrhoea such as rotavirus, astrovirus, adenovirus, noroviruses, etc.

## CSF - PCR for herpes and enteroviruses (in sterile container, VTM (viur transport medium) not required) - for the diagnosis of viruses causing meningitis or encephalitis such as HSV, VZV, enteroviruses, mumps, etc.

## Nasopharyngeal aspirate (NPA) - for respiratory viruses using Immunofluoresence (IF) or PCR, such as RSV, influenza A&B, adenovirus, parainfluenza viruses, SARS etc.

## Urine - virus isolation or PCR depending on which viruses you are interested in (in sterile container), e.g. BK virus, CMV, etc.

## Blood (clotted) - for antibody detection

## Blood (EDTA) - for PCR. Used for detection and quantification of HIV, HBV and HCV.

Biopsy samples can be useful in certain circumstances e.g. brain biopsy in encephalitis.

# Electron microscopy

## Virus structures can be visualised using an electron microscope

## Used mostly for stool samples.

# Immunofluorescence (IF)

## Useful for the direct detection of viral antigens in clinical samples (eg respiratory viruses)

## Can be used for typing and culture confirmation

## Relatively quick and inexpensive but subjective and very dependent on the skill of the technician and the quality of the sample

# Enzyme Immuno assays (EIA’s)

# Detection of antibodies and antigens using immunoassays.

## EIA’s (enzyme immunoassays)

## Western blots

## RIBA’s (recmbinant immunoblot assays useful for eg typing anti-HIV 1 &/or 2)

## Specific, sensitive and relatively easy to automate.

## Can be adapted to detect specific antibody classes e.g. IgM IgG or IgA.

## Sensitive and can quantify amounts of antibody (e.g. anti –HBs antibody)

## Adaptable to antibody or antigen detection

## Examples include HIV antibody and antigen, Hepatitis A,B,C serology, rubella, mumps, parvo etc

# EIA’s for HIV antibody detection

## Detection of specific antibody is an indirect method of detecting infection.

## Non-specific reactions can be a problem, therefore it is important to use multiple formats (generally use 3 different assays).

## Interpretation of results must take the clinical circumstances into account.

# Viral gene detection and quantification

## Polymerase chain reaction PCR (a target amplification system)

## bDNA (signal amplification system)

## Both assays used to measure “viral load” in HIV, HCV HBV infection.

# Polymerase chain reaction

## Target amplification to allow detection and quantification over very large dynamic ranges (> 5-8 logs)

## Can be very sensitive (as low as 1 genome copy)

## Can subtype viruses from PCR products

## Problems with contamination. This can be overcome using “Real Time” PCR.

# “Real Time” PCR. Advantages

## Quantification during linear phase gives better reproducibility, precision and dynamic range.

## Readily adapted to multi-plexing i.e. detect multiple viruses in the samples simultaneously.

## Closed tube monitoring eliminates contamination

# Summary

Many virus infections can be diagnosed by the detection of specific antibodies. This is particularly so for HIV, HCV and HAV.

The detection of viruses in the appropriate clinical sample using cell culture, immunofluorescence or PCR can be diagnostic during acute or chronic infections.

Modern detection methods e.g. PCR can be applied to most viruses. These are very powerful diagnostic tools but they must be used with caution.

## Antibody and antigen detection will be supplemented by genome detection and quantification for a wider range of viruses

## Resistance testing, genotyping and other DNA/RNA based tests are become more widely used.

## However, as with all tests, the results need to be interpreted in the clinical context of the patient.

DIAGNOSTICS 3  
THE DIAGNOSIS OF INFECTION AND THE USE OF THE   
BACTERIOLOGY LABORATORY

Dr Hugo Donaldson

**Learning Objectives:**

1. Explain the concept of best-guess microbiological diagnosis and the contribution of the laboratory to it.
2. Describe the investigations the microbiology laboratory does and their limitations
3. Describe the limitations of microbiology laboratory investigations.
4. Be aware of the turn-around times of different investigations, particularly the delays inherent in making cultural diagnoses.
5. To know how to interpret laboratory results of the commonly used tests.

The initial diagnosis of infection is based on the principles of making an informed best-guess clinical diagnosis.

Present illness: date of onset, symptoms, signs (esp. rashes)

Past history: previous infections particularly with resistant organism e.g. MRSA, hospitalizations, travel, antimicrobial use.

**Types of investigation made by the microbiology lab:**

* **Most microbiology samples are cultured on agar plates, which takes time:**

For organisms to multiply sufficiently: usually 24-48 hours

(Some need longer incubation: e.g. TB, brucella, actinomycetes)

To culture again for antibiotic sensitivities: another 24 hours

* **Microscopy**

direct under the microscope – urine

various stains (Gram, Ziehl-Nielsen (ZN), *etc*.) – pus, tissue fluids

fluorescence, with conjugated antibodies to specific antigens

* **Direct antigen detection** (particle agglutination tests, ELISA)
* **Molecular probes and amplification** (PCR, *etc*.)
* **Serology**: looking for antibodies as evidence of infection/immunity

**Optimal time of Collection of Specimen**

* In the acute phase of illness and before staring antimicrobials
* Collection from proper site, avoiding contamination by normal flora
* Prompt transport to lab since micro-organisms multiply in transit
* Adequate quantity and appropriate number of specimens
* Acute sera and Convalescent sera (paired), for rising antibody titres

**EXAMPLES:**

**Microbiological examination of Urine**

* Bedside: Naked eye - clear, cloudy, haemorrhagic.   
  note: although these are NOT microbiological investigations dipstick tests for nitrites, leucocytes, blood, protein, bilirubin, ketones may provide indication of there being an infection. Nitrites strongly suggest bacteriuria as many species of gram-negative bacteria convert nitrates to nitrites.
* Microscopy: WBC (pyuria suggests infection), RBC (may also indicate tumour/microemboli/trauma), epithelial cells (suggest the specimen has been contaminated during collection), crystals, casts.
* Culture on MacConkey agar (urine should be sterile so any microbial growth is potentially significant in an appropriately taken sample)
* Quantitative colony count for “significant” bacteruria (>105 bacteria/mL)
* Antibiotic sensitivity testing of bacteria that grow

**Microbiological examination of faeces**

* Naked eye, consistency, blood stained, colour, presence of worms
* Microscopy: ova, cysts, parasites
* Culture on inhibitory media – e.g. deoxycholate-citrate agar (DCA), selenite (Faeces contains 1012-14 bacteria per gram, so selective media are used to suppress background ‘flora’ organisms)
* Certain organism such as *Vibrio cholerae* are not looked for routinely therefore it is important adequate clinical information is provided on the request to allow the appropriate laboratory investigations to be carried out.
* Toxin detection (*Clostridium difficile*)
* Special stains, *e.g.* for cryptosporidia

**Examples - microscopy**

* Gram stain of CSF, joint fluid, purulent exudates.
* ZN/auramine stain of *e.g*. sputum, for TB
* FTA (fluorescent treponemal antibody) for antibodies to *T. pallidum*

**Examples – direct antigen detection**

* Meningococcal antigen in CSF
* *C. difficile* toxin in faeces
* Legionella and Pneumococcal antigen in urine

**Examples – PCR**

* Chlamydia in genital specimens
* Rapid PCR for MRSA

**Investigations on Billy** (case study)

**Diarrhoea**

* **Microscopy of stool for parasites**, especially giardia, amoeba (which cause diarrhoea but not rashes) and for higher parasites (may cause rashes but rarely diarrhoea). Stool culture for the common bacterial pathogens – salmonella, campylobacter, shigella. Stool result often negative.
* **Rash/skin lumps**. Often viral aetiology. Also, infected insect bites, syphilis, gonococcal infection, typhoid, endocarditis, systemic parasites.   
  ? Take skin biopsy – prolonged cultures for TB, fungi.

DIAGNOSTICS 4  
CELLULAR PATHOLOGY

# Dr Marjorie M. Walker ([mm.walker@imperial.ac.uk](mailto:mm.walker@imperial.ac.uk))

**Aims & Objectives:**

The aim of this lecture is to gain a broad understanding of how histopathology and cytopathology aid in diagnosis of disease.

By the end, students should be able to:

* List 3 situations where histopathology and cytopathology might commonly be used as a diagnostic method.
* Describe the nature of specimens sent for histopathology and cytopathology laboratory diagnosis.
* List 2 situations where frozen section diagnosis is required
* Summarise the main steps involved in processing a specimen for routine histopathology diagnosis and indicate the likely time needed to carry out these steps.
* Explain the additional information available from immunohistochemistry, and give an example of when this technique may be used
* Describe the benefits of the autopsy
* List 3 benefits of cytology screening

**Pathology** is the medical science and specialty practice that deals with all aspects of disease, but with special reference to the essential nature, the causes, and development of abnormal conditions, as well as the structural and functional changes that result from disease processes - more simply it is the study of disease. The literal translation of pathology from Greek (pathos, -logos) is "the words of suffering." Pathologists study: the causes of disease; how various internal and external derangements or injuries affect certain cells and tissues; the progression of disease in the human body (its pathogenesis); how disease is manifest (its clinical expression and the lesions produced); and methods for monitoring disease progression. Pathology provides a scientific foundation for clinical medicine and serves as a bridge between the basic sciences and patient care.

**Histopathology** encompasses surgical and autopsy pathology and histopathologists make diagnoses on tissue - biopsy material, surgical specimens removed at operation, make rapid diagnoses when necessary at operations by use of “frozen sections”. The autopsy (post mortem) is performed to determine the cause of death of a patient, explain why treatment was unsuccessful, to show the spread of disease and to educate doctors regarding disease processes.

**Cytopathologists** make diagnoses on cells - cellular specimens – sputum, body fluids which contain cells, cervical smears and tissue obtained by fine needle aspirate (FNA). This is a less invasive technique than obtaining tissue for a biopsy and thus has advantages to the patient.

**Biopsies are taken for numerous reasons.** Common conditions that require biopsies are skin lesions (inflammatory and suspected tumours). If a tumour is suspected clinically this may be removed intact, with a margin of normal skin to ensure excision of the tumour. However a rash may simply require a punch biopsy to determine the nature of the inflammation.

Endoscopy enables the clinician to view the gastrointestinal tract. Biopsies are commonly taken from the stomach to exclude for example cancer, the duodenum to exclude coeliac disease and the large bowel to confirm cancer or diagnose inflammatory bowel disease.

Bronchoscopy allows a view of the trachea and bronchi and biopsies of suspected tumours or inflammatory lung conditions can be taken

Liver biopsies can be performed under imaging guidance for tumours or for diagnosis of liver disease.

Renal biopsies are taken to determine the nature of glomerulonephritis and other renal disease

**Cytopathology** is very helpful in diagnosis of lung tumours and is non invasive.

Cytopathology can also aid diagnosis in endoscopy when brushings of the tumour can be taken. It is used in cervical and breast screening. Fluids such as urine and ascities can be examined for cells,

Surgical specimens removed at operation are all sent to Histopathology for diagnosis and to determine if a tumour is completely excised.

The histopathologists and cytopathogists will generate a report of the examination of the specimen, which is sent to the clinician responsible for the patient. It is important that the report gives a conclusive diagnosis and a statement of tumour excision and prognostic features – for example if surrounding lymph nodes are involved or the tumour spread on to the peritoneum.

# How long does a histopathology result take to reach the clinician?

Specimens for histopathology (biopsies and whole tissue) must be “fixed” in formalin, a preservative that stabilises protein bonds and prevents autolysis. The tissue then must be processed, sections cut and stained and the histopathologist must write the report.

For large specimens, allowing for overnight fixation or longer if the specimen is large or fatty, this process should take 2-3 days. Small biopsies can be processed in a day and if a rapid diagnosis is required then rapid processing takes 4-5 hours.

A very rapid diagnosis may be required during an operation – is this a tumour? Is the margin of excision adequate? Is this lymph node involved? Have I got the parathyroid?

Frozen section tissue is received in the laboratory without any fixative. A small sample is selected and frozen rapidly. A thin section is cut on a microtome contained in a refrigeration cabinet, fixed rapidly and then stained with haematoxylin and eosin. An answer can be given by phone within 20 minutes.

# How are sections obtained?

All specimens are allocated an identity number and logged in to the computer.

During the daily cut up of specimens the pathologist or biomedical scientist selects and describes the tissue samples to be examined. Some specimens (for example biopsies) are processed whole, while larger specimens (e.g. mastectomies, colonic specimens) have a few selected pieces removed. Each selected piece of tissue is placed in a small perforated plastic container with a lid. This plastic cassette receives the laboratory number. These tissue blocks are processed to paraffin wax. This small sample of the tissue must have the water extracted by dehydration through alcohols to be impregnated with paraffin wax that allows the block to be cut to thin sections using a microtome and the section mounted on glass slides. Sections are usually cut at around 5 microns thick - a micron is one thousandth of a millimetre!

The number of blocks cut every day varies from 50-400 and several sections may be taken from certain blocks – levels through the tissue to extract maximum information. The sections are rehydrated and stained with haematoxylin and eosin (H&E). Haematoxylin stains nuclei blue and eosin the cytoplasm pink. The sections are then viewed using the microscope.

# Additional stains

Most diagnoses can be made by simple H&E staining, however additional stains can help diagnosis. According to the dyes or chemicals used it is possible to stain certain tissue components selectively. For example, silver nitrate solutions may be used to identify melanin pigment, fungi, calcium deposits and certain types of fibres. Other dyes are used to show glycogen, mucins and types of structure within the tissues. It is also possible to demonstrate tuberculosis bacilli by the Ziehl-Neelsen methodand bacterial infections by Gram’s method. However for difficult tumours immunocytochemistry techniques are needed which use antibody systems to label antigens within tissues. Thus epithelial, mesochyme, nerve, prostate tissues can be specifically identified.

# The autopsy

Autopsies may be performed by authority of the Coroner (suspicious/ unknown cause of death) or by permission of the relatives of the decreased – the questions that can be answered include:

* What happened as a terminal event?
* Was the treatment successful but complications occurred?
* Where did the cancer originate?
* Was my diagnosis correct?

# Cytopathology

Cervical smears are prepared preserved (fixed) with alcohol, stained by a special stain called Papanicolaou and permanently preserved. Most of the preparation methods within the laboratory are automated. Preparatory work is carried out by well-trained and experienced MLSOs. Slides are read by trained screening technical staff and abnormalities found checked by consultants.

Cervical smears are performed to pick up cervical cancer as part of the screening programme - Screening only has value if these (and other) criteria can be met:

1. Test is easy and non invasive
2. High take up in the population
3. A significant number of cases can be detected.
4. Something can be done about the disease.

However there are problems with for example, cervical screening:

1. Failure to obtain a satisfactory sample (smear).
2. Failure to make a proper smear.
3. Poor staining.
4. Poor interpretation

DIAGNOSTICS 5  
ANTIBODIES AS DIAGNOSTIC TOOLS:  
Immunology in Diagnostics

Dr Keith Gould

**Key Concepts**

* Following infections, patients’ sera contain antibodies, some of which may neutralise the infective agent
* There are different classes of antibody proteins – immunoglobulins (Igs) with overlapping and unique functions
* The high degree of specificity of antibodies for their target antigens is the basis of many diagnostic tests.
* The ability to immunise animals to obtain antibodies with defined specificities
* Coupling of reporter molecules to antibodies
* Hybridoma technology and monoclonal antibodies
* Genetically engineered antibodies
* Therapeutic antibodies

**TECHNOLOGY**

* **Antibody Manufacture**
  + Polyclonal antibody production
  + Monoclonal antibody production
  + Engineered antibodies
* **Antigen-Antibody Interaction**
  + Immunoprecipitation
  + Haemagglutination
  + Agglutination
* **Labeling with reporter molecules**
  + Radioisotopes
    - Radioimmunoassays (RIA)
    - Autoradiography
  + Enzymes
    - Enzyme-linked immunoassays (ELISA)
    - Immuno-enzyme staining
  + Fluorescent probes
    - Fluorescent microscopy
    - Flow Cytometry
  + Magnetic Beads
    - Cell separation
  + Coloured Beads
    - Luminex assays

**USE OF MANUFACTURED ANTIBODIES**

**Therapeutic**

* Prophylactic protection against microbial infection
* Anti-cancer therapy
* Removal of T-cells from bone marrow grafts
* Block cytokine activity

**Diagnostic**

* Tissue typing
* Blood group serology
* Immunoassays
  + Hormones
  + Antibodies
  + Antigens
* Immunodiagnosis
  + Infectious diseases

**ANTIBODIES IN CLINICAL PRACTICE**

**Clinical Immunology**

* Immunodeficiency
* Malignancy
* Autoimmunity
* Inflammation
* Tissue typing and transplantation

**Pathology**

* Clinical Chemistry
* Haematology and Blood Transfusion
* Medical Microbiology
* Histopathology

**Antibodies produced by the patient**

**Manufactured antibodies**

**BACK TO THE CASE:-**

|  |  |
| --- | --- |
| **Signs & Symtoms** | **Immunological Concerns** |
| * vague aches and pains * Loss of appetite Weight loss * “Glands” up in his neck * Fever, rash, small red patches, some lumpy * Diarrhoea | * Immune complexes * Effect of poor nutrition on bone marrow cells * Immune activation * Acute Phase, activation, complexes * Protein-losing enteropathy |

|  |  |
| --- | --- |
| **Immunological Work Up** | |
| **First Line Tests**   * FBC and differential * CRP, ESR, etc. * Immunoglobulins * Complement C3 & C4 * Lymphocyte subsets * Autoantibody screening tests | **Second Line Tests**   * Complement Functions * Specific antibodies * Neutrophil functions * Lymphocyte functions * Molecular tests |

Useful link

A website created for the annual Schools Science Conference project and showing how a modern Immunology Lab functions:

[http://www.science4u.info/virtuallab/index.htm](%20http:/www.science4u.info/virtuallab/index.htm)

HAEMATOLOGY 11 & 12

Haematology tutorials

Friday 25 November 2011

It is very important that you revise all the haematology lectures that you have had to date before your tutorial as it is meant to build on and to consolidate the information you have been given in the lectures.

If there is anything you do not understand this is the chance to ask.

**Venues**: 9th floor Lecture Theatre, Lab Block and Drewe Lecture Theatre,   
Charing Cross campus

**Tutors**: Dr Assad Luqmani, Dr Fiona Regan, Dr Mark Layton and   
Dr Diane Hagger

**Haematology tutorials** **9th floor** lecture theatre **Drewe** lecture theatre

**Tutors: Dr A Luqmani Dr F Regan**

9.30 -10.30 E1 + E2 +E3a E3b+E4 +E5

10.30 - 11.30 E6 + E7 + F1a F1b + F2 + F3

11.30 -12.30 F4 + F5 F6 + F7

Tutors: Dr M Layton Dr D Hagger

2.00 - 3.00 G1 + G2 + G3a G3b + G4 + G5

3.00 - 4.00 G6 + G7 + H1a H1b + H2 + H3

4.00 - 5.00 H4 + H5 H6 + H7

**TEST YOURSELF ON HAEMATOLOGY**

### Question 1

### Case history

A 23-year-old vegetarian Indian presents to her GP with fatigue. She has 2 children aged 2 and 3 and since her last pregnancy she has suffered from very heavy menstrual bleeding. Her FBC shows:

WBC 5.2 x 109/l (normal range 3.5-10.8)

RBC 3.42 x 1012/l (normal range 3.82-4.98)

Hb 8.0 g/dl (normal range 11.5-14.8)

MCV 75 fl (normal range 84-99)

MCH 23.3 pg (normal range 27.5-32.7)

MCHC 31.0 g/dl (normal range 30.9-34.8)

Her serum ferritin is 8 μmol/l (normal range 15-300).

### (i) Likely causes of the abnormal blood count are:

1. iron deficiency anaemia T/F
2. vitamin B12 deficiency T/F
3. folic acid deficiency T/F
4. anaemia of chronic disease T/F
5. haemolytic anaemia T/F

(ii) Likely underlying causes include:

1. dietary deficiency of folic acid T/F
2. dietary deficiency of iron T/F
3. increased menstrual blood loss T/F
4. malabsorption of vitamin B12 T/F
5. increased iron utilisation during previous pregnancies T/F

|  |
| --- |
| SAQ |
| List three clinical features of moderately severe iron deficiency anaemia (3 marks) |
|  |
| Fill in the following table indicating how serum parameters may change in the anaemic conditions shown (3 marks) |
| |  |  |  | | --- | --- | --- | |  | Iron deficiency anaemia | Anaemia of chronic disease | | Serum ferritin |  |  | | Serum iron |  |  | | Serum transferrin or iron binding capacity |  |  | |
| Name a disease that can cause anaemia of chronic disease and explain briefly the mechanism of anaemia (4 marks) |
|  |
|  |

Question 2

1. Fruit and vegetables are a rich source of vitamin B12 T/F
2. Chronic blood loss may lead to iron deficiency T/F
3. Vitamin B12 is maximally absorbed in the colon T/F
4. Peripheral neuropathy may be a feature of vitamin B12 deficiency T/F
5. Serum ferritin is typically reduced in the anaemia of chronic disease T/F
6. The MCV is usually low in vitamin B12 and folic acid deficiency T/F

Question 3

Case History

A 46-year-old Indian vegetarian man with a past history of ileal resection for Crohn’s disease presents with an Hb of 11.6g/dl and an MCV of 121 fl.   
The most likely explanation of the haematological abnormality is:

1. Iron deficiency resulting from inadequate diet T/F
2. On-going blood loss from Crohn’s disease T/F
3. Dietary folate deficiency T/F
4. Pernicious anaemia T/F
5. B12 malabsorption as a result of ileal resection T/F

**TEST YOURSELF – QUESTION 4**

4a. Complete the following table

|  |  |  |
| --- | --- | --- |
| ABO Blood Group | ABO antigens on red cells | ABO antibodies in plasma |
| O |  |  |
| A |  |  |
| B |  |  |
| AB |  |  |

4b. Complete the following diagrams to show the possible blood groups their offspring could have:



4c. Could a group A male be the father of a group O child with a group O mother?

4d. A patient is bleeding post surgery, what components does he need if:

* 1. his platelet count is normal and his coagulation tests (PT and APTT) are prolonged?
  2. His PT and APTT are prolonged and his fibrinogen is very low?

4e. What blood group can you safely give to any patient who needs blood in a dire emergency, and why?

4f. Here is an example of a short answer question (SAQ) about blood transfusion:

List the important viruses that can be transmitted by blood transfusion

Outline how transmission of viral infection by blood transfusion can be reduced other than by testing of blood

Note: in a SAQ there are no extra marks for being verbose; do NOT attempt to write a mini-essay

#### Question 5

#### A high neutrophil count would be expected in:

A. Infectious mononucleosis T/F

B. Pneumococcall pneumonia T/F

C. Myocardial infarction T/F

D. Viral meningitis T/F

E. Gangrene of a leg due to vascular obstruction T/F

**Question 6**

##### The platelet normally survives in the bloodstream for:

##### One hour T/F

1. One day T/F

C. 10 days T/F

D. 100 days T/F

E. 120 days T/F

**Question 7**

#### A bleeding time test is usually performed to assess:

1. The intrinsic coagulation pathway T/F
2. The intrinsic coagulation pathway T/F
3. Platelet function T/F
4. Heparin effect T/F
5. Warfarin effect T/F

##### Question 8

##### A high haemoglobin concentration (Hb) could result from:

##### A. Living beside the Dead Sea T/F

##### B. Chronic hypoxic lung disease T/F

##### C. Renal cysts T/F

##### D. Chronic renal failure T/F

##### E. Cyanotic congenital heart disease T/F

**Question 9**

#### Macrocytosis would be expected in:

A. Iron deficiency T/F

B. Vitamin B12 deficiency T/F

C. Folic acid deficiency T/F

D. Vitamin D deficiency T/F

E. Calcium deficiency T/F

#### Question 10

**A male blood donor who is blood group B Rh negative and who has never been transfused would be expected to have, in his plasma**

A. Anti-A T/F

B. Anti-B T/F

C. Anti-O T/F

D. Anti-D T/F

E. Anti-d T/F

#### Question 11

#### The following suggest that anaemia is caused by haemolysis:

A. Low reticulocute count T/F

B. Increased plasma bilirubin T/F

C. Microcytic red cells T/F

D. Blood film showing spherocytes T/F

E. Polychromasia T/F

##### GENERAL QUESTIONS TO USE FOR EXAM REVISION

Although not strictly in Exam Format, these True/False questions may be of some use in your revision.

Haemolytic anaemia causes an increase in serum direct (conjugated) bilirubin T/F

A low serum ferritin concentration is strongly suggestive of iron deficiency T/F

Tissue invasion by parasites can cause an increased eosinophil count T/F

Iron deficiency is more common during periods of rapid growth T/F

Blood for transfusion should be stored at 370C T/F

Vitamin B12 deficiency can have deleterious effects on the nervous system T/F

A low reticulocyte count is useful evidence that anaemia has been caused by haemolysis T/F

Infectious mononucleosis usually results from primary infection by   
the varicella-zoster virus T/F

Vitamin D is essential for synthesis of coagulation factor T/F

Thrombocytopenia can cause petechiae and easy bruising T/F

Heparin is useful for the immediate treatment of pulmonary embolism T/F

In an emergency, group AB RhD-negative red cells can be transfused   
into patients of any blood group T/F

Bone pain and hypercalcaemia are common clinical features of multiple myeloma T/F

Hodgkin’s disease often presents with cervical lymphadenopathy T/F

The high haemoglobin concentration in polycythaemia rubra vera results from increased erythropoietin secretion T/F

Essential thrombocythaemia can result from poor bone marrow production or increased   
destruction of platelets T/F

Oral iron is useful for the anaemia of chronic disease T/F

##### ANSWERS TO “TEST YOURSELF” QUESTIONS

|  |  |  |
| --- | --- | --- |
| Question 1 (i) A. T  B. F  C. F  D. F  E. F | Question 3 F   1. F 2. F 3. F 4. T |  |
| (ii) A. F  B. T  C. T  D. F  E. T |  |  |
| Question 2 F  T  F  D. T  E. F  F |  |  |

Question 4a. COMPLETE THE FOLLOWING TABLE

|  |  |  |
| --- | --- | --- |
| ABO Blood group | ABO antigens on red cells | ABO antibodies in plasma |
| O | none | Anti-A and anti-B |
| A | A | Anti-B |
| B | B | Anti-A |
| AB | A and B | none |

**4b. Complete the following diagrams to show the possible blood groups their offspring could have:**

**Mother group O** **Father group AB**

**(Genes**:..O.. and ..O..**)** **(Genes:**..A.. and .. B..**)**

**Genes:**..OA.. ..OB.. **Genes:**..OA.. ..OB..

**Group** ..A.. **Group** ..B.. **Group**..A .. **Group**..B..

**4c. Could a group A male be the father of a group O child with a group O mother?**

YES

**4d**. **What are the 2 commonest causes of death immediately following transfusion.**

(1) ABO – incompatible transfusion  
(2) Bacterial contamination of blood

**4e**. **What ABO and Rh group blood can you safely give to any patient in a dire emergency, and why?**O negative – because there are no ABO antigens on red cells which recipient antibodies could interact with and Rh negative, so that patient will not make antibodies if they are Rh D positive.

4f. Here is an example of a short answer question (SAQ) about blood transfusion

List the important viruses that can be transmitted by blood transfusion

Hepatitis B… HTLV1

Hepatitis C… Hepatitis A

HIV………… non-A non-B non-C hepatitis

CMV………. Parvovirus B19

Outline how transmission of viral infection by blood transfusion can be reduced other than by testing of blood

‘Lifestyle’ questions to exclude high risk donors such as homosexuals, bisexuals, previous I.V. drug users, prostitutes, individuals from, sub-Saharan Africa, sexual partners of high-risk individuals including haemophiliacs.

Avoid unnecessary transfusions.

Use autologous rather than donor blood.

Note: in a SAQ there are no extra marks for being verbose; do NOT attempt to write a mini-essay.

|  |  |  |
| --- | --- | --- |
| Question 5  A. F  B. T  C. T  D. F E. T | Question 8 A. F  B. T  C. T  D. F  E. T | Question 11 A. F  B. T  C. F  D. T  E. T |
| Question 6A. F B. F  C. T  D. F  E. F | Question 9 A. F  B. T  C. T  D. F  E. F |  |
| Question 7A. F B. F  C. T  D. F  E. F | Question 10 A. T  B. F  C. F  D. F E. F |  |

ANSWERS TO REVISION QUESTIONS

Haemolytic anaemia causes an increase in serum direct (conjugated) bilirubin. F

A low serum ferritin concentration is strongly suggestive of iron deficiency. T

Tissue invasion by parasites can cause an increased eosinophil count. T

Iron deficiency is more common during periods of rapid growth. T

Blood for transfusion should be stored at 37o C. F

Vitamin B12 deficiency can have deleterious effects on the nervous system. T

A low reticulocyte count is useful evidence that anaemia has been caused by haemolysis F

# Infectious mononucleosis usually results from primary infection by the varicella-zoster virus. F

Vitamin D is essential for synthesis of coagulation factors. F

Thrombocytopenia can cause petechiae and easy bruising. T

Heparin is useful for the immediate treatment of pulmonary embolism. T

In an emergency, group AB RhD-negative red cells can be transfused into patients of any blood group. F

Bone pain and hypercalcaemia are common clinical features of multiple myeloma. T

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The high haemoglobin concentration in polycythaemia rubra vera results from increased erythropoietin secretion. F

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