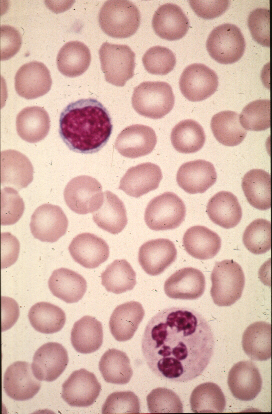
School of Medicine

Haematology

## Graduate Entry year 1 course guide (12/13)

Rlce this box with your Course Image

*Normal Blood -Prof Barbara Bain’s slide collection*

Theme Leader: **Dr Amin Rahemtulla**

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

Haematology

Graduate Entry – Spring course guide

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**SOLE FEEDBACK – *Haematology***

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 |
| **Dr Amit Patel**  Haemapoietic and blood cells- basic laboratory tests- Mechanisms of anaemia and polycythaemia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Nina Salooja**  Anaemia (Iron, vitamin B12, folic acid and anaemia of chronic disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Nina Salooja**  Anaemia (Variant haemoglobins, thalassaemia and haemolytic anaemia) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Prof David Lane** Haemostasis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Prof Mike Laffan** Abnormalities of Haemostasis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Fiona Regan** Blood transfusion |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Donald MacDonald** White cells and Leukaemia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Mark Layton** Sickle Cell anaemia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Carolyn Millar** Haem tutorial |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Dr Nichola Cooper** Haem tutorial |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

| **Lecturer and Lecture Title** | **Please use this box for additional constructive feedback.** |
| --- | --- |
| **Dr Amit Patel**  Haemapoietic and blood cells- basic laboratory tests- Mechanisms of anaemia and polycythaemia |  |
| **Dr Nina Salooja**  Anaemia (Iron, vitamin B12, folic acid and anaemia of chronic disease |  |
| **Dr Nina Salooja**  Anaemia (Variant haemoglobins, thalassaemia and haemolytic anaemia) |  |
| **Prof David Lane** Haemostasis |  |
| **Prof Mike Laffan** Abnormalities of Haemostasis |  |
| **Dr Fiona Regan** Blood transfusion |  |
| **Dr Donald MacDonald** White cells and Leukaemia |  |
| **Dr Mark Layton** Sickle Cell anaemia |  |
| **Dr Carolyn Millar** Haem tutorial |  |
| **Dr Nichola Cooper** Haem tutorial |  |

Haematology

**INTRODUCTION**

The Haematology course is taught in the Spring Term of year 1.

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 1 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 1 teaching of haematology is based mainly on lectures and some videos. This term there will be 7 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 Theme Leader:** Dr Amin Rahemtulla [a.rahemtulla@imperial.ac.uk](mailto:a.rahemtulla@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

* Hoffbrand AV & Mehta A, Haematology at a glance, 3rd ed, Blackwell Science, Oxford, 2009

**Supplementary Haematology reading and reference** (available in Library)

* Hughes-Jones N and Wickramasinghe SN, *Lecture notes on Haematology*, 8th ed, Blackwell Science, Oxford (2009)
* Bain BJ, *A Beginner's Guide to Blood Cells*, 2nd ed, Blackwell Science, Oxford, (2004) ISBN: 1405121750
* Contreras M (ed), *ABC of Transfusion***,** 4th ed, BMJ Publishing group, 2009
* McLelland B (ed), *Handbook of Transfusion Medicine*, 4th edHMSO Publications, 2007

**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 – You can go straight to the [CAL page.](https://education.med.imperial.ac.uk/CAL/index.htm)

**ASSESSMENT**

The course is examined in Paper 1 (Cellular & Molecular Science) – the questions will be in, EMQ and SBA formats. Further details about examinations are provided on the intranet

**TIMETABLE 2012/13 – Spring term 2013**

Details are correct at the time of going to press. Any amendments will be shown on the intranet.

|  |  |  |  |
| --- | --- | --- | --- |
| **Date and campus** | **Time** | **Lecture topic** | **Lecturer** |
| Thursday **31 January** HM-WEC  LTIII | 09.00 – 11.00 | **Haem 1**  Haemopoietic and blood cells- Basic laboratory tests and terminology- Mechanisms of anaemia and polycythaemia | Dr Amit Patel |
| Thursday  **31 January** HM-WEC  LTIII | 11.00 – 13.00 | **Haem 2**  Anaemia (iron - vitamin B12 - folic acid - anaemia of chronic disease) | Dr Nina Salooja |
| Monday  **4 February** HM-WEC  LTIII | 09.00 – 11.00 | **Haem 3**  Anaemia (variant haemoglobins - thalassaemia - haemolytic anaemia) | Dr Nina Salooja |
| Monday  **4 February** HM-WEC  LTIII | 11.00 – 12.00 | **Haem 4**  Haemostasis | Prof David Lane |
| 12.00 – 13.00 | Abnormalities of haemostasis | Prof Mike Laffan |
| Thursday  **28 February** HM-WEC  LTIII | 09.00 – 10.30 | **Haem 5**  Blood transfusion | Dr Fiona Regan |
| Tuesday  **12 February** HM-WEC  LTIII | 9.00 – 11.00 | **Haem 6**  White cells and leukaemia | Dr Donald MacDonald |
|  |  |  |
| Tuesday  **12 February**  HM-WEC SR I &  HM-WEC SR II | 11.00 – 13.00 | **Tutorial** | Dr Carolyn Millar |
| 11.00 – 13.00 | **Tutorial** | Dr Nichola Cooper |
| Thursday  **14 February** HM-WEC  LTI | 09.00 – 10.00 | **Haem 7**  Sickle cell anaemia | Dr Mark Layton |
|  |  |  |

Haematology tutorials

Tuesday 12 February 2013

**Venues**: HM-WEC SR I & HM-WEC SR II

**Tutors**: Dr Carolyn Millar and Dr Nichola Cooper

**Haematology tutorials** HM-WEC SR I HM-WEC SR II

**Tutors: Dr Carolyn Millar Dr Nichola Cooper**

11.00-12.00 Group 1 Group 2

12.00-13.00 Group 3 Group 4

Learning objectives – Graduate Entry 2012/13

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 Haemopoietic and blood cells- basic laboratory tests and terminology- mechanisms of anaemia and polycythaemia** (Dr Amit Patel)

* Explain the origin and function of red cells, neutrophils and platelets
* State the 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
* 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

**2 Anaemia (Iron , vitamin B12 and folic acid deficiency and anaemia of chronic disease)** (Dr. Nina Salooja)

* Describe the role of iron in erythropoiesis.
* List the dietary sources of iron, the factors influencing the absorption of iron, and the causes of iron deficiency.
* Describe the clinical and haematological features of iron deficiency anaemia, 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.

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

**3** **Anaemia (variant haemoglobins, thalassemias and haemolytic anaemia** (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.

**4** **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

**5 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

**6 White cell and leukaemia** (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)

**7 Sickle cell anaemia** (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

**Recommended reading**

Hoffbrand and Mehta: *Haematology at a glance*, Blackwell Science, Oxford, 2005, 2nd Ed. ISBN: 1405126663

Hoffbrand, Moss and Petit, *Essential Haematology*, Blackwell Science, Oxford, 5th ed. 2007

**Supplementary reading**

Hughes-Jones N and Wickramasinghe SN, *Lecture notes on Haematology*, 7th ed, Blackwell Science, Oxford (2003)

Bain BJ, *A Beginner's Guide to Blood Cells*, 2nd ed, Blackwell Science, Oxford, (2004) ISBN: 1405121750

Contreras M (ed), *ABC of Transfusion***,** BMJ Publishing group, 1999

McLelland B (ed), *Handbook of Transfusion Medicine*, HMSO Publications, 2001

**Online resources**

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

**CONTACT DETAILS**

**Haematology Theme Leader:**

Dr Amin Rahemtulla, Department of Haematology 4th Floor Commonwealth Building, Hammersmith Campus.

e-mail: [a.rahemtulla@imperial.ac.uk](mailto:a.rahemtulla@imperial.ac.uk)

**HAEMATOLOGY 1a**

**Haemopoietic and blood cells- basic laboratory tests and terminology**

Dr Amit Patel ([amit.patel@imperial.ac.uk](mailto:n.panoskaltsis@imperial.ac.uk))

**PHYSIOLOGY**

**OBJECTIVES**

The student should be able to:

* Explain the origin and function of red cells, neutrophils and platelets
* State the 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

The red cells, granulocytes, monocytes and platelets that circulate in the blood are produced in the bone marrow, being ultimately derived from haemopoietic multipotent progenitor 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 | 6-10 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 | 6 hours | Defence against parasitic infection |
| Lymphocyte | Very variable | Humoral and cellular immunity |
| Platelet | 7-10 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
* State approximate normal ranges for white cell count (WBC), haemoglobin concentration (Hb), mean cell volume (MCV) and platelet count in adult men and women of northern European origin and know that normal ranges in children and in people with African ancestry differ from those of adult northern Europeans

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. The data given on the next page relates to a particular instrument. Results from various hospitals may differ slightly.

The figures illustrating the distributions of haemoglobin and white cell counts are shown on the next two pages together with tables of 95% ranges for some blood parameters.

#### 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 values for Caucasians, see table 1 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. You will have the opportunity to see many of these abnormalities in the practical class in the Spring Term.

**HAEMATOLOGY 1b**

**Mechanisms of anaemia and polycythaemia**

Dr Amit Patel (amit.patel@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:**

|  |  |  |
| --- | --- | --- |
| **Abbreviation (units)** | **Meaning** | **Definition** |
| **WBC (x 109/l)** | white cell count | the number of white cells in a given volume of blood |
| **RBC (x 1012/l)** | red cell count | the number of red cells in a given volume of blood |
| **Hb (g/dl or g/l)** | haemoglobin | haemoglobin concentration |
| **PCV (l/l)** | packed cell volume | the proportion of a column of centrifuged blood occupied by red cells |
| **Hct (l/l)** | haematocrit | equivalent to the PCV |
| **MCV (fl)** | mean cell volume | the average size of the red cells |
| **MCH (pg)** | mean cell haemoglobin | the average amount of haemoglobin in a red cell |
| **MCHC (g/l or g/dl)** | mean cell haemoglobin concentration | the average concentration of haemoglobin red cell |
| **Platelets (x 109/l)** | 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).

# Mechanisms of anaemia include:

1. Reduced production of red cells by the bone marrow
2. Loss of blood from the body
3. Reduced survival of red cells in the circulation (called haemolysis)
4. 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

1. Microcytic
2. Normocytic
3. Macrocytic

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

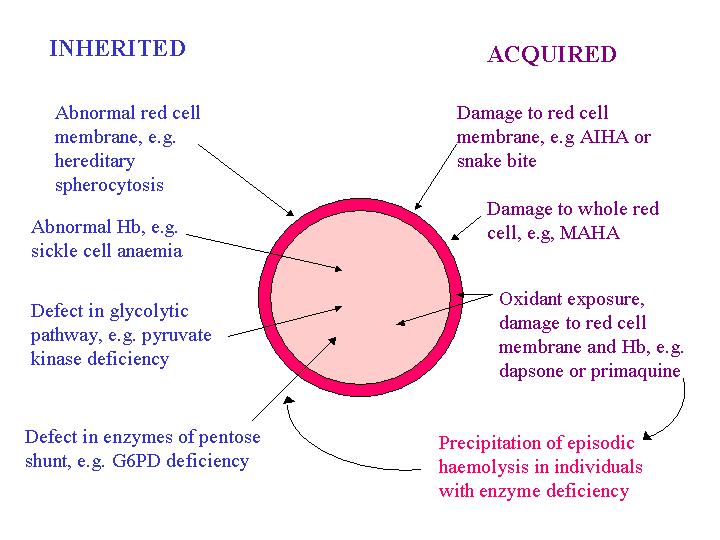
* Megaloblastic anaemia resulting from a deficiency of vitamin B12 or folic acid
* Liver disease
* Excess alcohol intake
* An increased proportion of young red cells newly released from the bone marrow

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**

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

Anaemia

Red cells phagocytosed by macrophages and destroyed

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

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 | Altitude, e.g., Himalayas or Andes  Hypoxia, e.g., cyanotic heart disease, 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 |

**TEST YOURSELF** (Answers to all tests will be found on [page 75](#hanswers) onwards)

## QUESTION 4

A blood film from an anaemic patient is described as showing hypochromia, microcytosis and poikolocytosis.

This means:

A. There are large red cells T/F

1. There is increased variation in shape T/F
2. The red cells are paler than normal T/F
3. The reticulocyte count is high T/F
4. The white cell count is increased T/F

#### QUESTION 5

A 58-year-old man living in London is found to have a haemoglobin concentration of 19.2/g/dl.   
This could be due to:

1. Chronic bronchitis and emphysema T/F
2. Haemolysis T/F
3. Vitamin B12 deficiency T/F
4. Carcinoma of the kidney T/F
5. Living above sea level. T/F

#### QUESTION 6

Here is an example of a blood count on a 35-year-old Portugese woman. Compare her test results with the normal ranges (in brackets) and decide which of her values are abnormal. Then answer the questions below.

WBC 4.0 x 109/l (NR 3.5 – 10.8 x 109/l)

RBC 1.38 X 1012/l (NR 3.82 – 4.98 x 1012/l)

Hb 5.8 g/dl (NR 11.5 – 1.48 g/dl)

Hct 0.172 l/l (NR 0.36 – 0.46 l/l)

MCV 125fl (NR 84 – 99 fl)

MCH 42.2 pg (NR 27.5 32.7 pg)

MCHC 33.8 g/dl (NR 30.9 – 34.8 g/dl)

Platelet count 92 x 109/l (NR 169 – 358 x 109/l)

Reticulocyte count 20 x 109/l (NR 20 – 130 x 109/l)

A. The patient has quite a severe anaemia T/F

B. The red cells are macrocytic T/F

C. Iron deficiency anaemia is likely T/F

D. The cells are likely to appear hypochromic T/F

E. There is a reticulocytosis T/F

**HAEMATOLOGY 2ª**

**Anaemia (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. Three 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
3. soluble transferrin receptors (Tfr)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Comment** | **Iron deficiency** | **ACD** | **Thalassaemia Trait** |
| Hb | Non-specific screening | Low | Low | Normal or low |
| MCV | Differential diagnosis of type of anaemia | Low (normal in early stages) | Low or normal | Low |
| Serum Iron |  | Low | Low | Normal |
| TIBC |  | Raised | Normal or Low | Normal |
| Ferritin | Specific to IDA when low | Low | Normal or raised | Normal |
| Soluble TfR | Dependant on erythroid activity | High | Normal | Normal or slightly raised |
| BM iron stores | Gold standard for iron status | Absent | Increased | Normal or increased |

**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)

**TEST YOURSELF**

### 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) |
|  |
|  |

**HAEMATOLOGY 2b**

**ANAEMIA (VITAMIN B12 and FOLIC ACID)**

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# 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)

TEST YOURSELF

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

HAEMATOLOGY 3

ANAEMIA (VARIANT HAEMOGLOBINS- THALASSAEMIA- HAEMOLYTIC ANAEMIA)

# 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.

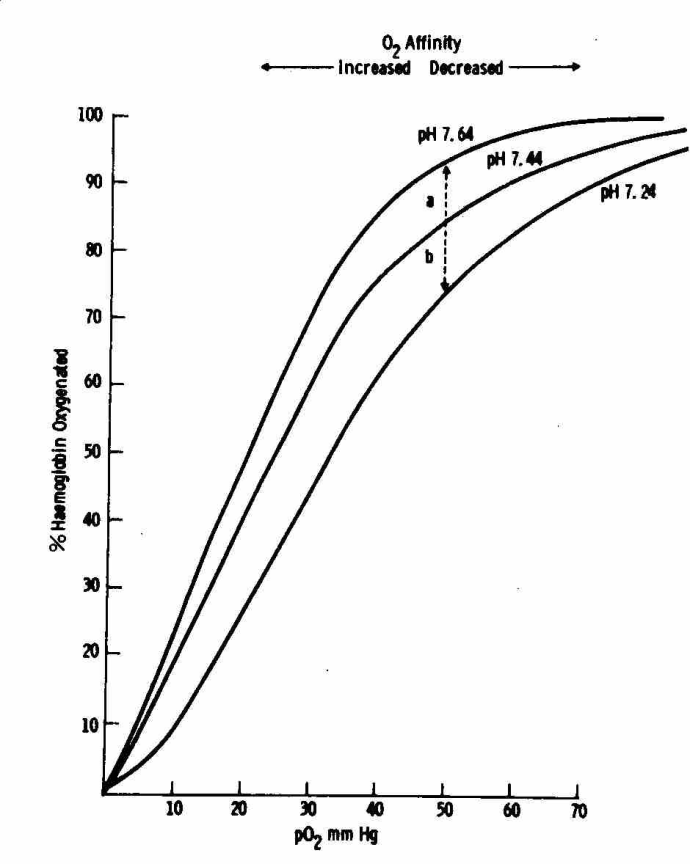
Several different types of globin proteins exist each encoded by their own gene (s). The globin genes are located in two clusters. The alpha cluster is found on chromosome 16 and contains the genes for α globin (adult variety) and   
ζ globin (zeta globin - an embryonic variant). The alpha genes are 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.

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## BThalMajor3

Subcutaneous infusion of desferrioxamine in beta thalassaemia major

**HAEMATOLOGY 4**

**HAEMOSTASIS AND ABNORMALITIES OF HAEMOSTASIS**

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**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, Ehlors-Danlos syndrome (inherited).

The platelet has its origin in the bone marrow. Haemopoeitic stem cells give rise to megakaryocyte precursers which undergo nuclear replication, then maturation before migrating to the marrow sinusoids and fragmenting into platelets.





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. ADP, released from platelet granules, and thrombin are other activators. Aspirin acts as an antiplatelet agent by irreversibly inhibiting one of the enzymes of this pathway, cyclo-oxygenase. 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. The bleeding time is selectively used to monitor the platelet-vessel wall interaction and platelet aggregation is performed to monitor platelet dysfunction or von Willebrand factor deficiency.

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, 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 Willebrands disease is another inherited disorder. Its inheritance is usually autosomal dominant. Bleeding in this disorder may be of characteristically vascular 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 5

Blood transfusion

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**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**

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 haemolysis if incompatible blood is transfused, i.e.

|  |  |  |  |
| --- | --- | --- | --- |
| **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

In 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 fetus 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

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'* donor and recipient. An antibody screen should be performed at the same time as grouping, on the serum of all patients due to be transfused (*'group and screen'* or *'type and screen'*).

It is relatively common for a D-negative woman to become sensitised through 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 a subsequent 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 and woman 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.

**Selection of Blood**

Blood selected for transfusion should be ABO and RhD compatible. 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.

***'Antibody screen'*** of recipient serum to exclude **any** clinically significant immune antibodies. Recipient serum 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 serum prior to transfusion.

**Compatibility test** done between donor red cells and recipient serum = ***'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:**

High risk groups for human immunodeficiency virus (HIV) infection:

1. Men and women who are infected with HIV
2. Men who have had sex with another man at any time since 1977
3. Men and women who have misused drugs by injecting themselves at any time
4. Men and women who have had sex at any time since 1977 with men or women who live, or have lived in African countries (except those bordering the Mediterranean)
5. Men and women who have had sex with anyone in the above groups
6. Men and women who are the sexual partners of a haemophiliac
7. Men and women who are prostitutes
8. Men and women who have had sex with a prostitute

#### Tests Undertaken on Blood Donations

Blood grouping: every blood donation has the ABO and RhD blood group determined. Additionally, 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 (which might destroy the recipient's red cells) are present in the donor's plasma.

**Theoretical risks**

**Prion Disease?: -** Although prion proteins have been found in membranes of lymphocytes and platelets and it has been shown that the prions of variant Creutzfeldt-Jacob disease (CJD) are found in lymphoreticular tissues, there is no evidence so far that CJD or variant CJD can be transmitted by the transfusion of blood or blood products in humans, though it has been transmitted by transfusion in sheep. However there are concerns over vCJD, in light of our lack of knowledge on how this agent is spread.

**NB:** The most important step in maintaining a safe blood supply is rigorous donor selection and self-exclusion of subjects at risk of transmitting blood-borne agents. Testing donations for the relevant agents adds to safety. The agents that may be transmitted by blood transfusion 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  Syphilis | Anti-HIV 1+2 Ab  HBsAg  Anti-HCV Ab  TPHA (Ab test) |
| Plus on some donations:  CMV (cytomegalovirus) T.Cruzi Malaria | Anti-CMV Ab  Anti-*T. cruzi* Ab  Anti-Malarial Ab |

**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, autologous predeposit, intraoperative salvage etc.

To avoid the theoretical risk of CJD 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 | 12 months | 12-15ml/kg | Response to FFP transfusions should be measured clinically and by post-transfusion coagulation tests |
| **Cryo** | frozen | 12 months |  | Test coagulation function |

**A) Whole Blood** - 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'*.

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

1. Pooled platelets - platelets from several donations (4-5) pooled to constitute a single adult dose. (Commonest)

2. Or from a single donor by cell separator machine, equivalent to 4-5 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 neutropenic patients not responding to antibiotics/ antifungal drugs

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

**C) Fresh Frozen Plasma - FFP**

1. Once thawed (at 30-37ºC) - rapid deterioration of clotting factors - use immediately
2. Red blood cell contamination minimal; use ABO and Rh compatible

**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 young 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: mostly by IV route as replacement in immunodeficiency states, ITP or autoimmune haemolytic anaemia. Prevention of certain infections, e.g. hepatitis A, measles, rubella by IM route.

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.

#### ADVERSE EFFECTS OF TRANSFUSION

The transfusion of blood or its components is usually a safe and effective procedure. Nevertheless, adverse effects may follow transfusion. Some effects are preventable, but others are not.

**a) Immediate complications of transfusion (within 1-2h). ABO incompatibility and bacterial infection are the two commonest causes of death shortly after transfusion.**

|  |  |
| --- | --- |
| **Immunological:** | **Non-Immunological:** |
| * Haemolytic transfusion reaction (ABO incompatibility) * Febrile, non-haemolytic reaction * Urticarial rash * Anaphylactic reaction * Transfusion related acute lung injury (TRALI) | * Bacterial contamination ± endotoxic shock * Congestive cardiac failure (overload)   Hypothermia  Hyperkalaemia  Hypocalcaemia  {   * In large volume transfusions * Air embolism (rare) if air in tubing |

**b) Delayed complications of blood transfusion**

|  |  |
| --- | --- |
| **Immunological:** | **Non-Immunological, mainly infectious:** |
| * Delayed haemolytic transfusion reactions (other blood group antibodies) * Post-transfusion purpura * Graft-versus-host disease * Immunomodulatory effects  (eg: increased patient infections) | * Viruses   Hepatitis: B & C most important  HIV 1 and 2  HTLV (human T-lymphotrophic virus)I and II  Parvovirus  CMV   * Other   Malaria  Babesiosis  Brucellosis  Trypanosomiasis (Chagas' disease)  Syphilis   * Iron overload |

**Autologous Blood**

Sometimes, as an alternative to transfusing donor blood, the patient can have their own.

There are 2 main options:

**1. Pre-operative autologous deposit**

Patients can donate a few units of their own blood in the 5 weeks leading up to a planned operation – provided that they are fit enough and are given iron supplements and that the operation will not be postponed.

**2. Cell Salvage**

During large '*bloody*' operations the patient's blood can be salvaged, washed and centrifuged and given back to the patient - provided the area operated on is not contaminated with bacteria or malignant cells.

**Supplementary reading**

Contreras M (Ed), ABC of Transfusion, BMJ Publishing group, 1999

McLelland B (Ed), Handbook of Transfusion Medicine, HMSO Publications, 1996

**TEST YOURSELF – QUESTION 7**

7a. Complete the following table

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

7b. Complete the following diagrams to show the possible blood groups their offspring could have:



7c. Could a group A male be the father of a group O child with a group O mother?

7d. What are the 2 commonest causes of death immediately following transfusion.

7e. What blood group can you safely give to any patient in a dire emergency, and why?

7f. Here is an example of a short answer question (SAQ) about blood transfusion

7g. List the important viruses that can be transmitted by blood transfusion

7h. 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

HAEMATOLOGY 6

White cells and leukaemia

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

## Learning Objectives

* Explain, in a leucocytosis (increased white cell count), 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.
* Explain, in a lymphocytosis, 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 leucocytosis 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 7

## 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 | βSβS | S |
| Sickle cell/haemoglobin C disease | βSβC | S and C |
| Sickle cell trait | | βSβA | A and S |
| Normal | | ΒAβA | 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%

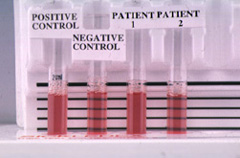
**NOTE:** top-up blood transfusion is NOT a treatment for painful crisis; it will increase blood viscosity and may make the painful crisis worse

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)

Haematology tutorials

Tuesday 12 February 2013

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.

**Details of the tutorial groups will be provided on the Intranet as the course proceeds.**

**Venues**: HM-WEC SR I & HM-WEC SR II

**Tutors**: Dr Carolyn Millar and Dr Nichola Cooper

**Haematology tutorials** HM-WEC SR I HM-WEC SR II

**Tutors: Dr Carolyn Millar Dr Nichola Cooper**

11.00-12.00 Group 1 Group 2

12.00-13.00 Group 3 Group 4

**TEST YOURSELF ON HAEMATOLOGY**

#### Question 8

#### 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 9**

##### 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 10**

#### 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 11

##### 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 12**

#### 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 13

**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 14

#### 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 | Question 6 A T  B. T  C. F  D. F  E. F |
| (ii) A. F  B. T  C. T  D. F  E. T | Question 4A. F B. T  C. T  D. F  E. F |  |
| Question 2 F  T  F  D. T  E. F  F | Question 5 A. T  B. F  C F  D. T  E. F |  |

Question 7a. 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 |

**7b. 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..

**7c. Could a group A male be the father of a group O child with a group O mother?**

YES

**7d**. **What are the 2 commonest causes of death immediately following transfusion.**

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

**7e**. **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.

7f. 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 8  A. F  B. T  C. T  D. F E. T | Question 11 A. F  B. T  C. T  D. F  E. T | Question 14 A. F  B. T  C. F  D. T  E. T |
| Question 9A. F B. F  C. T  D. F  E. F | Question 12 A. F  B. T  C. T  D. F  E. F |  |
| Question 10A. F B. F  C. T  D. F  E. F | Question 13 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

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