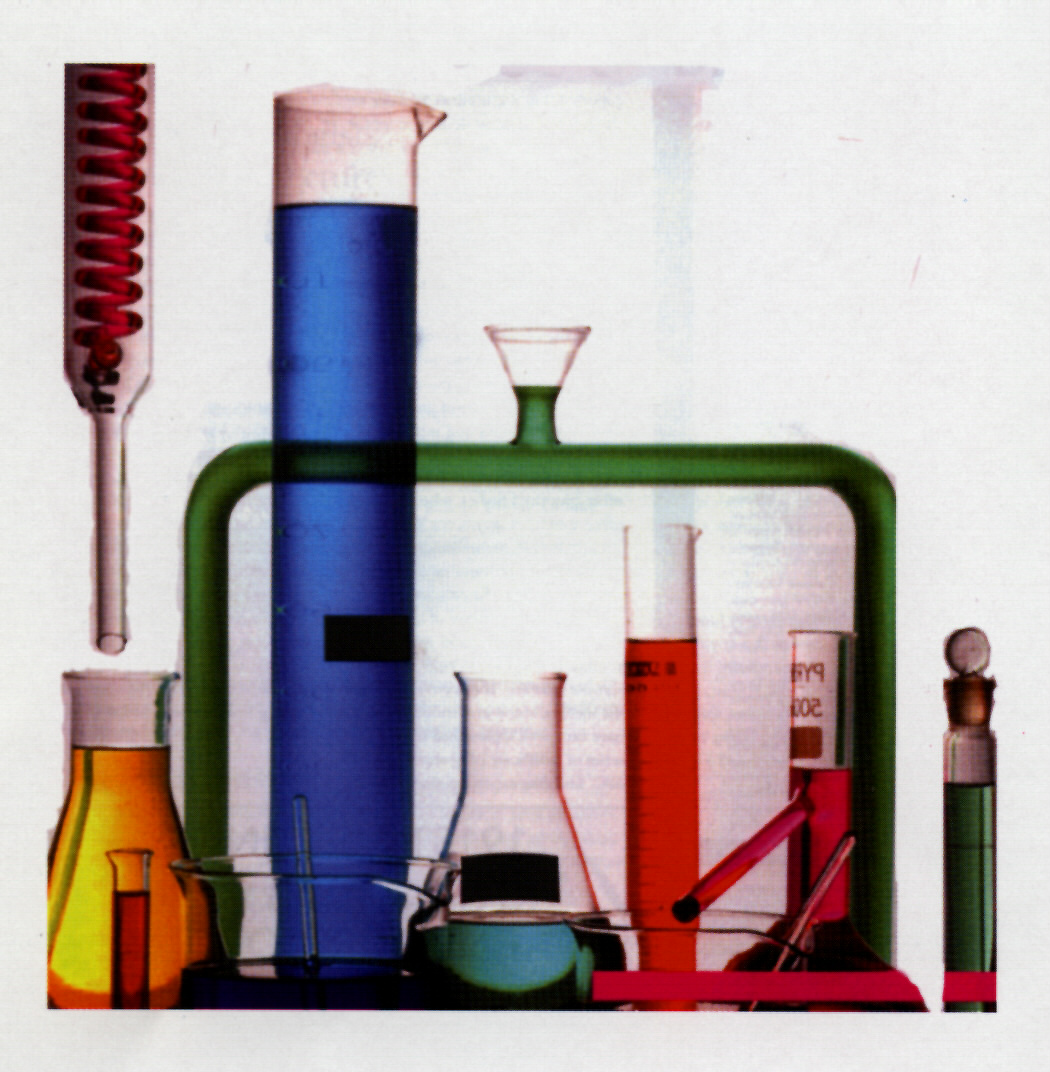
School of Medicine

Year 5 – 2012/13

PATHOLOGY THEME GUIDE

Volume 1 – Week 1

Theme leaders

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Year 5 PATHOLOGY THEME STUDY GUIDE

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SOLE FEEDBACK – PATHOLOGY Week 1

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 – Week 1

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** | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| L**ecturer 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 Mary Sheppard  Vascular & Cardiac |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Prof Margaret Callan Immune Response to Infection / Primary Immune deficiencies 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Prof Karim Meeran Diabetes cases |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Sue Brook  Plasma Proteins |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Margaret Hancock  Metabolic disorders & screening |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Shivani Misra  Hypoglycaemia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Abdul Shlebak Coagulation |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Prof Mike Laffan  Venous thrombosis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Megan Rowley  Blood Transfusion |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Mary Thompson  Connective tissue disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Angela Bailey  Sexually transmitted disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Federico Roncaroli Neurodegenerative disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Alex Rice Respiratory tract infections |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Jaimini Cegla  Therapeutic Drug Monitoring |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Jonathan Hoare  Liver functions tests and cases |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Prof Margaret Callan  Autoimmune disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Margaret Hancock  Metabolic disorders and screening |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Prof Karim Meeran Adrenal disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Nina Salooja Interactive Cases |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Peter Kelleher Allergy |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Candice Roufosse  Transplantation |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Paul Booton |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Alex Everitt Prion Disease |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Peter Choi Renal Function |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Dr Peter Choi Renal Failure |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

| **Lecturer and Lecture Title** | **Please use this box for additional constructive feedback.** |
| --- | --- |
| Dr Mary Sheppard  Vascular & Cardiac |  |
| Prof Margaret Callan Immune Response to Infection / Primary Immune deficiencies 1 |  |
| Prof Karim Meeran Diabetes cases |  |
| Dr Sue Brook Plasma Proteins |  |
| Dr Margaret Hancock Metabolic disorders and screening |  |
| Dr Shivani Misra Hypoglycaemia |  |
| Dr Abdul Shlebak Coagulation |  |
| Prof Mike Laffan Venous thrombosis |  |
| Dr Megan Rowley Blood Transfusion |  |
| Dr Megan Rowley Blood Transfusion1 |  |
| Dr Megan Rowley Blood Transfusion2 |  |
| Dr Federico Roncaroli Neurodegenerative disease |  |
| Dr Alex Rice Respiratory tract infections |  |
| Prof Margaret Callan Autoimmune disease Part 1 |  |
| Prof Margaret Callan Autoimmune disease Part 2 |  |
| Dr Jaimini Cegla Therapeutic drug monitoring |  |
| Dr Margaret Hancock Metabolic disorders and screening |  |
| Dr Nina Salooja Interactive Cases |  |
| Dr Peter Kelleher Allergy |  |
| **Lecturer and Lecture Title** | **Please use this box for additional constructive feedback.** |
| Dr Candice Roufosse Transplantation |  |
| Dr Alex Everitt Prion Disease |  |
| Dr Peter Choi Assessment of Renal Function |  |
| Dr Peter Choi Acute and Chronic Renal Failure |  |

Introduction

The Year 5 Pathology Course covers clinical pathology and aims to give an understanding of the pathology of disease processes and use of the pathology laboratory.

In Years 1 and 2, the Molecules, Cells and Disease Theme provided an introduction to the cellular processes of disease, which was continued in other Themes where pathology specific to an understanding of specific Systems was introduced. Various PBL topics in Years 1, 2 & 3 also included pathology, to varying extents. Also, in Year 3, the pathological basis of several clinical conditions was included. The material covered in Years 1, 2 & 3 therefore underpins the course in Year 5.

At the end of this Theme, you will be expected to:

* Demonstrate a basic knowledge of the principles of haematology, chemical pathology, immunology, microbiology and histopathology.
* Apply this pathological knowledge to making a clinical diagnosis, formulating a treatment plan, and monitoring patient progress and outcome.
* Understand the scientific basis of medicine that underpins your knowledge of clinical practice.
* Be able to request appropriate laboratory tests.
* Understand the consequence of inappropriate sample taking.
* Interpret laboratory data in a clinical context and apply this to your daily practice.
* Recognise pathological patterns and be able to separate complex data into component parts.
* Understand the role of the pathology laboratory as an integrated component of patient care, including ethical and legal aspects.

The Year 5 attachments all comment that having the Pathology Course at the start of the year means that students are better prepared for the attachments that follow, have greater understanding of the concepts and therefore cope better with the clinical attachment.

Study guide and course format

The course is predominately lecture-based but, in response to student feedback, we have increased the number of interactive sessions including small group tutorials, quizzes and CPCs (Clinico-Pathological Conferences).

A comprehensive Study Guide will be issued for the Pathology course. It is in four volumes (one per week) and includes complete learning objectives for each session, lecture notes, reading lists and examples of EMQ’s. Session materials in the printed Guide will be shown in the order of presentation. All new materials will be posted on the Intranet as soon as they become available.

Locations for lectures

All lectures will be held in the Brian Drewe Lecture Theatre, Charing Cross campus.

ASSESSMENT

Formative Assessments

There will be a series of 3 formative examinations during the year. These are conducted in summative exam style using printed answer sheets. The first is held at the end of the course, the second immediately after the Christmas/New Year break, and the final one in the second week of the final attachment. For dates for the second and third “mock”, please check the Intranet. For each of these “mocks”, you will receive your scores after the sheets have been marked and assessed, and you will be able to see your standing as compared to the rest of the year.

Summative Assessment

The summative (Part 5) examination is held at the end of the year (in June) and questions are in Extended Matching Question format. Distinctions are awarded for best performances in the Part 5 Pathology Examination.

Prizes are awarded for the top performances in each of the five courses, and for the best overall performances in the Part 5 paper.

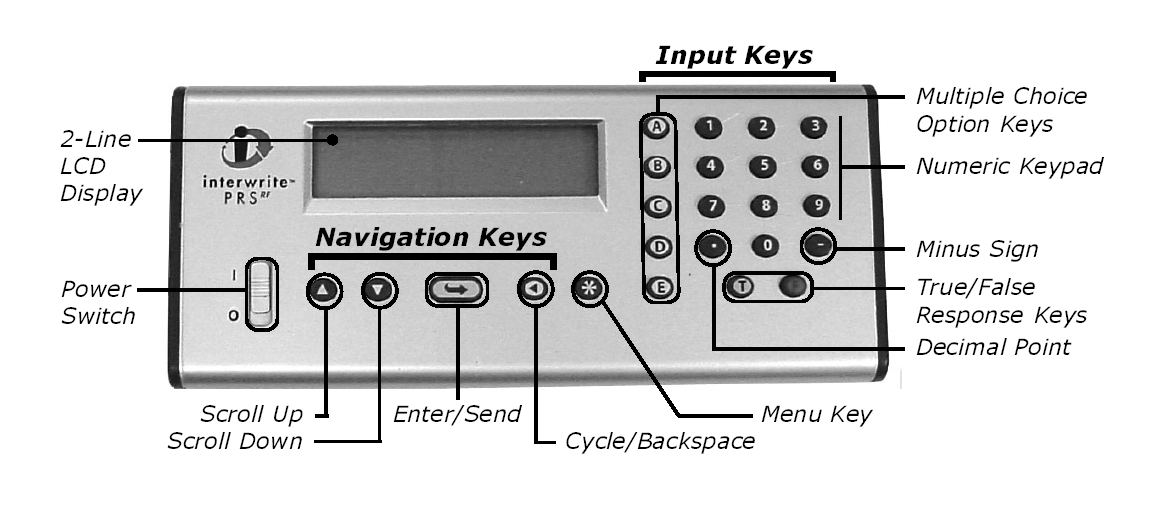
See <https://education.med.imperial.ac.uk/Prizes/index.htm>

Further aids to learning and revision

In addition, over the last several years, students have been encouraged to write EMQs from the session objectives as an aid to their learning and subsequent revision (please see instructions and EMQ template below). These questions will be available on-line but you will also be expected to write your own.

Interactive clickers

On Monday 2nd July, PRS clickers will be distributed. You will be expected to bring a £50 cheque made payable to “Imperial College” – this will be retained as a deposit for your clicker.   
The cheque will be returned to you uncashed in exchange for the clicker, after the mock exam on Friday 29th July. Instructions on how to use the PRS handset are shown below.



1. Only turn on the handset when instructed. **WAIT** till you can all do this together.

**When instructed…**

1. Turn on your handset by sliding the Power Switch up (I = ON).
2. Wait for 4 seconds till “GTCO CalComp” disappears.

**Joining the class**

1. “Scanning classes Please wait” will appear. The clicker will check each possible channel, until it finds the pathology one. Your teacher might know what channel you should connect to, and if you are told what channel to join, you can immediately jump to the correct channel by pressing that number or letter.
2. Press the green “return” key
3. You are now connected and should see: ANS:  
    YEAR 5PATH

**Now wait for a question**

1. ****When the lecturer asks you to respond to a question, press the key that represents your response and press to send it. Then watch for the ‘**Received**’ message to

appear in your handset display.

1. If the ‘**Wait for Q**’ message appears, please wait for your lecturer to start the countdown process before sending your response.
2. If you change your mind, or submit something you didn’t mean to, change your answer and submit again (the green key). Only your FINAL answer will be counted.

**WARNING:** Your handset will go to SLEEP when it is not being used.   
Press the any key to WAKE UP your handset.

Core Curriculum and clarification of policy over non-delivered teaching

The full core curriculum is available on the intranet. It is important to emphasise that learning is the responsibility of students. Academic and clinical staff members provide teaching in order to help students to learn, but it has never been the policy that all areas of intended student learning must be embodied in formal teaching. Students are frequently told that a particular aspect of their learning should be covered by private study.

The same principle applies to teaching that appears in the timetable but is not delivered. While teachers make all possible efforts to ensure that any cancelled teaching is rescheduled, it is not always possible to do so. It is the responsibility of students to cover any such missed material by independent study. Non-delivery of teaching on a particular topic does not mean that the topic will not be tested in examinations.

**Normal ranges**.

You are expected to know the normal ranges for basic haematological and biochemical tests, in particular, Hb, Wc, platelet counts for haematology, and Na, K, U and creatinine for biochemistry. Where needed, other normal ranges will be given in exam questions. It is important to note that rote learning lots of normal ranges will not be helpful, but having examples of real patients abnormalities will compliment your understanding of exam questions, so being exposed to other biochemical tests that you commonly use on the wards will be helpful.

Some comments from previous students about the Pathology theme:

* “Overall, high standard of teaching & very useful in a clinical setting….”
* “An essential course and very important exam. Very useful for the understanding of clinical problems and their causes.”
* “Very well organised theme, and for its clear relevance to clinical medicine, and to the rest of Year 5.”
* “Definitely one of the most useful courses in the MBBS….. After the exam, I wished I'd had had more time to learn rather than cramming it. I felt that had I have had the extra time to put things into context I would be a much better doctor for it.”
* “A well-constructed course dealing with a massive topic. It was a very busy time, but I think the co-ordinators did well in the time available.”
* “The student-written EMQ bank was an interesting idea that was quite useful in the absence of past papers. Its value will surely grow over the years as more questions are added to it.”
* “Very well organised course… The mock test and the student written EMQ's were particularly useful.”
* “The use of the interactive keypads made the lecture interesting and allowed us to test our knowledge, and learn from the mistakes.”
* “Clickers added value to taught sessions wherever they had been used. “
* “CPCs were very popular”
* “Interactive cases were very useful and …. it felt like more active learning was taking place”

**From senior house officer who trained at Imperial**

* “I advised my brother to come to Imperial mainly because of the path course. When doing the course, it seems extremely intense, and many of us wished the exam was immediately after the course, so that we could get on and learn other things. Looking back now I realise that the way we were forced to revise it over the year with the repeat mock quizzes and the links with clinical firms such as paediatrics, obs and gynae rheum, derm and infectious diseases really helped to integrate our knowledge. So much so, that even now on ward rounds and in casualty I remember things we were taught on the path course, and can still remember it. The path course really also helps your understanding of general medicine and starts the preparation for finals. I have to reluctantly agree with the pathologists that having the exam at the end of the year is much better for ones long term remembering and understanding.”

**How have we changed the course?**

* Topics for inclusion in CPCs have been re-defined.
* Some areas have been revised to include more clinical orientation.
* Large number of handouts have been revised for July 2012

|  |
| --- |
| Timetable Week 1 |

|  |  |  |
| --- | --- | --- |
|  |  |  |
| **Monday 2nd July** | | |
| 9.00-9.30 |  | Introduction: Prof Karim Meeran, Dr Mike Barrett & Dr Donald Macdonald |
| 9.30-11.00 | Hi | Vascular & Cardiac Pathology (Dr Mary Sheppard) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Im | The Immune Response to Infection / Primary Immune deficiencies – Part 1  (Prof Margaret Callan) |
| 12.15-13.15 |  | LUNCH and CLICKER COLLECTION |
| 13.15-14.15 | Im | The Immune Response to Infection / Primary Immune deficiencies – Part 2  (Prof Margaret Callan) |
| 14.15-14.45 | CP | Clicker training and Diabetes cases (Prof Karim Meeran) |
| 14.45-15.00 |  | BREAK |
| 15.00-15.30 | CP | Plasma Proteins (Dr Sue Brook) |
| 15.30-16.15 | CP | Metabolic disorders and screening 1 (Dr Margaret Hancock) |
| 16.15-17.00 | CP | Hypoglycaemia (Dr Shivani Misra) |
|  |  |  |
| **Tuesday 3rd July** | | |
| 9.00-11.00 | Ha | Coagulation (Dr Abdul Shlebak) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Ha | Thrombosis: aetiology and management (Prof Mike Laffan and Susie Shapiro) |
| 12.15-13.15 |  | LUNCH |
| 13.15-14.45 | Ha | Blood Transfusion 1 (Dr Megan Rowley) |
| 14.45-15.00 |  | BREAK |
| 15.00-17.00 | Ha | Blood Transfusion 2 (Dr Megan Rowley) |
|  |  |  |
| **Wednesday 4th July** | | |
| 9.00-10.30 | Hi | Connective tissue disease, amyloid, sarcoid (Dr Mary Thompson) |
| 10.30-10.45 |  | BREAK |
| 10.45-11.15 | Hi | Neuropathology of neurodegenerative disorders and multiple sclerosis (Dr Federico Roncaroli) |
| 11.15-12.15 | Mi | Sexually transmitted diseases (Dr Angela Bailey) |
|  |  |  |
| **Thursday 5th July** | | |
| 9.00-11.00 | Hi | Respiratory Disease (Dr Alex Rice) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Im | Autoimmune Disease - Part 1 (Prof Margaret Callan) |
| 12.15-13.15 |  | LUNCH |
| 13.15-14.15 | Im | Autoimmune Disease - Part 2 (Prof Margaret Callan) |
| 14.15-15.00 | CP | Therapeutic drug monitoring (Dr Jaimini Cegla). |
| 15.00-15.15 |  | BREAK |
| 15.15-16.00 | CP | Metabolic disorders and screening 2 (Dr Margaret Hancock) |
| 16.00-17.00 | Ha | Interactive cases (Dr Nina Salooja) |
|  |  |  |
| **Friday 6th July** | | |
| 9.00-10.00 | CP | Liver function tests and cases (Dr Jonathan Hoare) |
| 10.00-11.00 | Im | Allergy (Dr Peter Kelleher) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Im | Transplantation (Dr Candice Roufosse) |
| 12.15-13.00 |  | Whoever thought exams were a good idea? (Dr Paul Booton) |
| 13.00-14.00 |  | LUNCH |
| 14.00-15.00 | Mi | Prion Disease (Dr Alex Everitt) |
| 15.00-15.15 |  | BREAK |
| 15.15-16.15 | CP | Assessment of renal function (Dr Peter Choi) |
| 16.15-17.15 | CP | Acute and chronic renal failure (Dr Peter Choi) |
| Week 2 | | |
| **Monday 9th July** | | |
| 9.00-10.00 | Ha | Introduction to leukaemia and CML (Dr. Donald Macdonald) |
| 10:00-11:00 | Ha | Myeloproliferative disorders (Dr Saad Abdalla) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Ha | Myelodysplastic syndromes/Bone marrow failure (Dr Francis Matthey) |
| 12.15-13.15 |  | LUNCH |
| 13.15-14.15 | Ha | Acute leukaemia (Prof Barbara Bain) |
| 14.15-15.15 | Ha | Interactive leukaemia cases (Prof Barbara Bain) |
| 15.15-15.30 |  | BREAK |
| 15.30-16.15 | CP | Adrenal (Prof Karim Meeran) |
| 16.15-17.00 | CP | Forensics (Dr Sue Paterson) |
|  |  |  |
| **Tuesday 10th July** | | |
| 9.00-10.00 | Mi | Respiratory tract infections (Dr Rishi Dhillon) |
| 10.00-11.00 | Mi | Mycobacterial diseases (Dr Graham Cooke) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Mi | Pandemic Flu (Prof Wendy Barclay) |
| 12.15-13.15 |  | LUNCH |
| 13.15-14.15 | Im | Vaccination (Dr Nesrina Imami) |
| 14.15-15.15 | Hi | Lower Gastrointestinal disease (Dr Mike Osborn) |
| 15.15-15.30 |  | BREAK |
| 15.30-16.30 | Ha | Haematology of systemic disease (Dr Donald Macdonald) |
|  |  |  |
| **Wednesday 11th July** | | |
| 9.00-10.00 | CP | Pituitary (Prof Karim Meeran) |
| 10.00-11.00 | Im | HIV Infection (Dr Nesrina Imami) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Im | Immune therapies (Prof Margaret Callan) |
|  |  |  |
| **Thursday 12th July** | | |
| 9.00-10.00 | Mi | Antimicrobial agents I (Dr Annette Jepson) |
| 10.00-11.00 | Mi | Antimicrobial agents II (Dr Annette Jepson) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Ha | Plasma Cell Myeloma & video (Dr Amin Rahemtulla) |
| 12.15-13.15 |  | LUNCH |
| 13.15-15.15 | Im | Case Studies in Immunology (Dr Keith Gould) |
| 15.15-15.30 |  | BREAK |
| 15.30-16.30 | Hi | Liver and biliary disease (Dr Robert Goldin ) |
|  |  |  |
| **Friday 13th July** | | |
| 9.00-10.00 | Mi | Viral Infections in Pregnancy (Dr Lila Paraskevopoulou/Dr H Donaldson) |
| 10.00-11.00 | Hi | Gynaecological pathology (Dr Mary Thompson) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.45 | Im | Malabsorption CPC (Prof Margaret Callan, Dr Marjorie Walker, Dr Tim Orchard) |
| 12.45-13.45 |  | LUNCH |
| 13.45-15.00 | Im | Immunology Revision Quiz (Prof Margaret Callan) |
| 15.00-15.15 |  | BREAK |
| 15.15-16.15 | Mi | GI infections (Dr Eleni Nastouli |
| Week 3 | | |
| **Monday 16th July** | | |
| 9.00-9.45 | Hi | Metabolic bone disease (Dr Ann Sandison) |
| 9.45-10.30 | CP | Calcium handling, bones and renal stones (Dr Yehani Wedatilake) |
| 10.30-11.15 | CP | Neoplastic bone disease (Dr Ann Sandison) |
| 11.15-11.30 |  | BREAK |
| 11.30-12.15 | CP | Uric Acid Metabolism (Dr Ben Jones) |
| 12.15-13.15 |  | LUNCH |
| 13.15-14.00 | CP | Potassium (Dr Amir Sam) |
| 14.00-14.45 | CP | Sodium and fluid balance (Dr Amir Sam) |
| 14.45-15.00 |  | BREAK |
| 15.00-15.45 | Hi | Cytopathology (Dr Corrina Wright) |
| 15.45-16.45 | CP | EMQ examples – bugs and others (Prof Karim Meeran) |
|  |  |  |
| **Tuesday 17th July** | | |
| 9.00-10.00 | CP | Clinical chemistry CPC (Dr Lewis/Prof Meeran/Dr Sandison/Yehani Wedatillake) |
| 10.00-11.00 | CP | ACID-BASE Handling (Dr Jaimini Cegla) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Mi | Opportunistic viral infections (Dr Emilie Sanchez) |
| 12.15-13.15 |  | LUNCH |
| 13.15-14.30 | Ha | Haemolytic anaemias (Dr Mark Layton) |
| 14.30-14.45 |  | BREAK |
| 14.45-15.30 | Hi | Diseases of the pancreas (Dr James Carton) |
| 15.45-16.45 | Hi | Upper Gastrointestinal Disease (Dr Marjorie Walker) |
|  |  |  |
| **Wednesday 18th July** | | |
| 9.00-10.00 | Hi | Skin pathology (Dr Marjorie Walker) |
| 10.00-10.45 | Hi | Non-neoplastic bone and joint disease (Dr Ann Sandison) |
| 10.45-11.00 |  | BREAK |
| 11.00-12.15 | Ha | Obstetric Haematology (Dr Carolyn Millar) |
| 12.15-13.00 |  | Introduction to Year 5 and the Clinical Specialties (Mr Martin Lupton) |
|  |  |  |
| **Thursday 19th July** | | |
| 9.00-10.00 | Hi | Endocrine disease (Prof Terry Cook) |
| 10.00-10.15 |  | BREAK |
| 10.15-11.15 | CP | Update on lipoprotein metabolism, cardiovascular disease & obesity  (Prof Gilbert Thompson) |
| 11.15-12.15 | Mi | CNS Infection & Meningitis (Dr Luke Moore) |
| 12.15-13.15 |  | LUNCH |
| 13.15-15.15 | Ha | Lymphoma: multidisciplinary afternoon (Drs Macdonald, Rice & Marks) |
| 15.15-15.30 |  | BREAK |
| 15.30-16.30 | Ha | Haemopoetic stem cell transplantation (Prof Jane Apperley) |
| 16.30 - 17.00 | Ha | The porphyrias (Dr. Monica Nigher) |
|  |  |  |
| **Friday 20th July** | | |
| 9.00-9.45 | Mi | PUO & Endocarditis (Dr Claire Thomas) |
| 9.45-10.30 | Mi | Zoonoses (Dr Claire Thomas) |
| 10.30-10.45 |  | BREAK |
| 10.45-12.00 | Hi | Urological pathology (Dr Rathi Ramakrishnan) |
| 12.00-12.45 | Mi | Fungal Infections and their Diagnosis (Dr Michael Petrou) |
| 12.45-13.45 |  | LUNCH |
| 13.45-14.45 | Mi | Wound, bone and joint infections (Dr Dunisha Samarasinghe) |
| 14.45-15.45 | CP | EMQ on enzymes, chemistry and zoonoses (Prof Karim Meeran) |
| 15.45-16.00 |  | BREAK |
| 16.00-17.00 |  | Ethics and law in pathology (Dr Ali Mears) |
| Week 4 | | |
| **Monday 23rd July** | | |
| 9.00-11.00 | Mi | Bacterial and Viral vaccines, Antivirals (Dr Mark Atkins) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.30 | CP | Liver Disease CPC (Prof Karim Meeran/Dr Gemma Petts) |
| 12.30-13.15 |  | LUNCH |
| 13.15-14.00 | CP | Enzymes and Cardiac Markers (Dr Radha Ramachandran) |
| 14.00-14.45 | CP | Potassium Handling (Dr Amir Sam) |
| 14.45-15.00 |  | BREAK |
| 15.00-16.00 | CP | Thyroid (Dr Stephen Robinson) |
| 16.00-16.50 | CP | Nutrition (Dr Stephen Robinson) |
|  |  |  |
| **Tuesday 24th July** | | |
| 9.00-10.00 | CP | Electrolyte cases (Dr Amir Sam) |
| 10.00-11.00 | Mi | Neonatal and childhood infections (Dr Marianne Nolan) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Ha | Chronic Lymphocytic Leukaemia and Lymphoproliferative disorder quiz (Donald Macdonald) |
| 12.15-13.00 |  | LUNCH |
| 13.00-14.30 | Ha | Paediatric Haematology (Prof Barbara Bain) |
| 14.30-14.45 |  | BREAK |
| 14.45-16.00 | Ha | Haematology Quiz (Prof Barbara Bain) |
| 16.00-17.00 | Mi | Urinary Tract Infection (Dr Hugo Donaldson) |
|  |  |  |
| **Wednesday 25th July** | | |
| 9.00-10.00 | Mi | Antifungals (Dr Michael Petrou) |
| 10.00-11.00 | Mi | Viral Hepatitis (Dr Janice Main) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.15 | Hi | Breast Pathology (Dr James Carton) |
| 14.00-15.00 |  | ELECTIVES (Dr Mike Barrett) |
|  |  |  |
| **Thursday 26th July** | | |
| 9.00-10.00 | Mi | Hospital acquired infections (Dr Eimear Brannigan) |
| 10.00-11.00 | Hi | Renal disease (Prof Terry Cook) |
| 11.00-11.15 |  | BREAK |
| 11.15-12.30 | CP | Diabetes CPC (Prof Karim Meeran, **Dr Amir Sam**, Dr Paul Lewis) |
| 12.30-13.15 |  | LUNCH |
| 13.15-14.45 | Hi | Infection CPC (Dr Hugo Donaldson, Dr Alex Rice) |
| 14.45-15.00 |  | BREAK |
| 15.00-16.30 | Hi | Cerebrovascular disease and trauma (Dr Rathi Ramakrishnan) |
|  |  |  |
| **Friday 27th July – Tropical Day – Dress Code “Tropical"** | | |
| 9.00-9.15 | Mi | Introduction to Tropical Day (Dr Gareth Tudor-Williams) |
| 9.15-10.00 | Mi | Why toilets are more important than doctors (Dr Oliver Cumming, LSHTM) |
| 10.00-10.45 | Mi | I've got you under my skin (Dr Anthony Solomon) |
| 10.45-11.15 |  | BREAK |
| 11.15-12.00 | Mi | HIV in African children (Dr Gareth Tudor-Williams) |
| 12.00-12.45 | Mi | Fever in the returning traveller (Dr Jim Buckley) |
| 12.45-13.00 |  | Floor show and prizes for best Tropical Gear |
| 13.00-14.00 |  | LUNCH |
| 14.00-15.30 |  | End-of-Course EMQ (Prof Karim Meeran and Dr Mike Barrett) |
| 15.30-16.30 |  | Quiz Answers (Prof Karim Meeran) and Clicker return |

Pathology Museum

The Pathology Museum is on the 11th Floor of the Laboratory block at Charing Cross.

**Blackboard Quizzes**

Students love mock exams. There are several available on Blackboard. There are also a small number of compulsory quizzes that should take no longer than 20 minutes each.

Go to <http://learn.imperial.ac.uk>

This will take you to Blackboard.

Enter your username and usual Imperial password.

You have been registered for “Year 5 Pathology (2012-13)”

Click on “Year 5 Pathology (2012-13).”

You will then see a list of quizzes, including revision quizzes.

There are approximately 40 revision quizzes (divided up into 10 for each week).

There are also 4 compulsory quizzes, and a mock exam which MUST be taken before they go off line.

If you have not used Blackboard before, you may click on the “example colours” quiz.

Click on the title of the quiz to run it.

Read the instructions which will tell you how long you have for the quiz.

Turn off all popup-blocking software.

A timer will start as soon as you press “begin quiz”.

**The first quiz should be attempted and marked by 23.59 (midnight) on Thursday 5th July, when it will go offline**. Please try and do this quiz on the evening of Monday 2nd or Tuesday 3rd.

The second quiz will need to be completed by Wednesday 11th July, the third by Tuesday 17th July and the fourth by Friday 20nd July. You will also be expected to complete two mock exams on Blackboard in the last week of the course. The first goes offline at midnight on Wednesday 25th July, and the second on Friday morning, in readiness for the real formative exam on Friday 27th July.

As in the real exam, you have 1 minute per option (about 5 minutes for a five-part question).

Your stored score will be the highest score that you have ever achieved on that quiz. Try and revise before you attempt the mock exam, and see what score you get. It is probably best to do this exam on Tuesday 26th or Wednesday 27th evening

Discussion Board

A Discussion Board is also available for you in Blackboard. Any questions should be posted to the Discussion Board: please do not email individual lecturers.

To complete the quizzes or use the Discussion Board, click on the Blackboard icon on the intranet (or use the URL: <http://learn.imperial.ac.uk>) then login by entering your normal College username and password and select “**Year 5 Pathology 12-13**”.

There may be additional material made available on Blackboard during the course.

Writing your own EMQs

Instructions

* A Word template for writing EMQs is available on the Intranet and from <http://meeran.com>
* You will be allocated a lecture title about which you are invited to write a question.   
  If, in addition, you wish to write questions about other lecture topics to those allocated to you, please feel free to do so.
* Firstly, save and rename the downloaded template using the first nine characters of your surname and forename (all lower case) with a space separating forename if required,   
  e.g. smith ada.doc.
* In the document, insert your name, number and then type out the possible answers   
  (A - P maximum) in alphabetical order. Tip: Use the “SORT” function to rapidly arrange paragraphs into alphabetical order.
* Then write in the questions (1-5 maximum) and put in the answers too.   
  e-mail the document back to me ([emqexam@hotmail.com](mailto:emqexam@hotmail.com) ), and I will put it onto the website as soon as possible.
* If everyone contributes, you will have a bank of over 500 questions which will assist you in your reflection on the topics, and your revision. Do your best to make these correct.
* If you want to submit more than one EMQ, please feel free, and use the same file naming convention but add a number to the filename, e.g. smith ada1.doc, smith ada2.doc.

Pathology EMQ template

Name:

Candidate number:

Theme:

OPTION LIST

|  |  |  |  |
| --- | --- | --- | --- |
| A |  | I |  |
| B |  | J |  |
| C |  | K |  |
| D |  | L |  |
| E |  | M |  |
| F |  | N |  |
| G |  | O |  |
| H |  | P |  |

For each scenario below, choose the most appropriate answer from the list above. Each option may be used once, more than once or not at all.

1.

2.

3.

4.

5.

ANSWERS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1. | 2. | 3. | 4. | 5. |

Recommended Reading List

In addition to the following recommended and suggested reading, some lecturers have highlighted texts in their individual session handouts.

**CHEMICAL PATHOLOGY**

*Required reading and revision:*

Clinical Biochemistry: an Illustrated Colour Text (4th edition) by Gaw A et al. Churchill Livingstone (2008)

Clinical Chemistry (6th edition) by Marshall W and Bangert S. Mosby (2008)

*Reference:*

Clinical Chemistry in Diagnosis and Treatment (6th edition) by Mayne PD (formerly Zilva J,   
Pannell PR and Mayne PD). Edward Arnold (1994)

Clinical Biochemistry: Metabolic and Clinical Aspects (2nd edition) by Marshall WJ and Bangert SK (eds.). Churchill Livingstone (2008)

**HAEMATOLOGY**

*Required reading:*

Haematology at a Glance (3rd edition) by Metha AB and Hoffbrand V. Wiley-Blackwell (2009)

*Supplementary reading and reference:*

Essential Haematology (5th edition) by Hoffbrand AV, Moss PAH, Petit JE. Blackwell Publishing (2006)

Haematology (8th edition; Lecture Notes series) by Hughes Jones N, Wickramasinghe SN, and Hatton C (eds.). Wiley-Blackwell (2009)

A Beginner’s Guide to Blood Cells (2nd edition) by Bain BJ. Blackwell Science (2004).   
E-book available: [http://www3.interscience.wiley.com/cgi-bin/bookhome/117353968](javascript:open_win('http://www3.interscience.wiley.com/cgi-bin/bookhome/117353968'))   
(via library website)

ABC of Transfusion (4th edition) by Contreras M (ed.). BMJ Publishing Group (2009)

Handbook of Transfusion Medicine (4th edition) by McLelland B (ed.). TSO (2007)

Haematology: an Illustrated Colour Text (3rd edition) by Howard MR and Hamilton PJ. Churchill Livingstone (2008)

You might also consider waiting for the following which has been written to follow the Imperial Curriculum.  
Haematology: A Core Curriculum by Bain BJ, Imperial College Press, London, (publication date expected to be December 2010)

**HISTOPATHOLOGY**

*Required reading – these all have the same information but may be in different formats – use the one you like best:*

Pathology (2nd edition) by Stevens A and Lowe J. Mosby (2000)

General and Systematic Pathology (5th edition) by Underwood JCE and Cross S (eds.) Churchill Livingstone (2009)

Pathology: Basic and Systemic by Woolf N. Chapters 1-31. Saunders (1998)

Concise Pathology (3rd edition) by Chandrasoma P and Taylor CR. Prentice-Hall (1998)

Robbins’ Basic Pathology (8th edition) by Kumar V et al (eds.). Elsevier (2007)

**IMMUNOLOGY**

*Recommended reading – choose the one that suits you best:*Immunological factors in disease (chapter 4) in Davidson’s Principles and Practice of Medicine (20th or 21st edition only) by Colledge N, Walker B and Ralston S (eds.). Churchill Livingstone Elsevier (2010)

Flesh and Bones of Immunology (1st edition) by Helbert M. Elsevier (2006)

Problem-based Immunology (1st edition) by Gorczynski R and Stanley J. Elsevier (2006)

Immunology for Medical Students (2nd edition) by Nairn R and Helbert M. Mosby (2007)

*Further reading and reference*

Immunology (6th edition) by Coico R.  Wiley-Blackwell (2009)

Janeway’s Immunobiology (7th edition) by Murphy K, Travers P, and Walport M.  Garland Science (2008)

**MICROBIOLOGY**

*Recommended reading*

Microbiology in Clinical Practice (3rdedition) by Shanson DC. Butterworth-Heinemann (1999)

Lecture Notes on Medical Microbiology (4th edition) by Elliott T et al. Blackwell (2006)

Medical Microbiology (6th edition) by Murray PR, Rosenthall KS and Pfaller MA. Mosby (2009)

Clinical Bacteriology by Struthers JK and Westran RP. American Society of Microbiology (2003)

*Further reading*

Medical Microbiology (4th edition) by Goering R et al (eds.) Mosby Elsevier (2008)

Microbiology and Infection: a Clinical Core Text for Integrated Curricula with Self-assessment (3rdedition) by Inglis TJJ. Churchill Livingstone (2007)

Notes on Medical Microbiology by Timbury M. Churchill Livingstone (2002). Successor to Notes on Medical Virology by Timbury M.

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**Please use the Blackboard Discussion Board for any questions wherever possible, rather than emailing staff directly.**

Handouts for Individual Lectures

These are provided in the order of presentation as known at the date this Guide went to press.

Cardiac Failure, Cardiomyopathy, Valve Disease and Ischaemic Heart Disease

Dr Mary Sheppard

**OBJECTIVES:**

You should be able to:

* List major atherosclerotic (cardiovascular) risk factors
* List the main inflammatory cells within atherosclerotic plaques
* List major inflammatory factors in atherosclerotic plaques
* Discuss main interactions involving inflammatory cell and inflammatory factors in atherosclerosis
* Suggest likely localising factors for atherosclerotic plaques
* Discuss relevance of major mediators of chronic inflammation to atherosclerosis
* Understand the coronary plaque inflammation-plaque rupture-myocardial infarction sequence
* Discuss the mechanism and pathology of complications of acute myocardial infarction
* Discuss the pathology of cardiac failure; discuss common causes of cardiac failure
* Define Cardiomyopathy
* Give main classification of Cardiomyopathy
* Describe the clinical features, mechanism and macroscopic and microscopic pathology of rheumatic heart disease
* Connect clinical features to discussion of predisposing factors, microbiology, likely mechanism and pathology of infective endocarditis
* Correlate clinical features and pathology of mitral valve prolapse
* Describe the different types of pericarditis

***Atherosclerosis & Ischaemic heart disease***

**Summary of major atherosclerotic risk factors:** hypertension, hyperlipidaemia, diabetes, smoking. Comment on obesity as an individual risk factor vs population attributable risk

**Atherosclerosis as a chronic inflammatory disease of intima of large vessels, with sites of predilection at branches and bends**

**Summary of recent evidence implicating shear stress as a factor.** Shear modulates endothelial function via multiple signalling cascades; correlation of plaues with sites of low, turbulent, oacsillating or disturbed flow; protective effect of high laminar shear stress.

**Summary of modern understanding mechanism of atherogenesis** – atherosclerosis as a chronic inflammatory disease of large arteries stimulated by risk factors

**Oxidation and free radicals in atherosclerosis**.

LDL.

Brown & Golstein and LDL-R.

Scavenger receptors.

Scavengers receptors are often pathogen pattern recognition receptors

Scavenger receptors bind modified forms of LDL notable oxidised LDL.

Oxidised LDL the major form in plaques. OxLDL is scavenged by macrophages but at the expense of activation as if by pathogens.

Role of adhesion molecules (e.g. VCAM) and chemokines (eg MCP-1) in macrophage entry to plaque;connection to risk factors.

Activities of activated macrophages in plaques:

* Oxidation (oxidative enymes include NADPH Oxidase, myeloperoxidase, 5-lipoxygenase producing reactive oxygen nitrogen species (RONS) eg superoxide, hydrogen peroxide, HOCl (bleach), HONOO peroxynitrite. NO & free radical damage
* Inflammatory mediators (leukotrienes, interleukins eg IL-1, many more)
* Collagenolysis (MMP family)
* apoptosis releasing cholesterol into lipid core;
* activation of T-cells (antigen presentation)
* activation by T-cells (CD40L, IFNgamma, TNFalpha)
* secretion of growth factors (eg PDGF) (may strengthen fibrous cap)
* clearance (may be beneficial)
* tissue factor expression (IP in thrombosis & plaque rupture)

**Pathology of coronary thrombosis and unstable plaque:** coronary plaque rupture, coronary endothelial erosion and intraplaque haemorrhage. Ruptured plaques contain fewer vascular smooth muscle cells, more inflammatory cells, larger lipid core, thinner fibrous cap.

**Vulnerable plaque & vulnerable patient concept – reinforces atherosclerosis as a multisystem disease**

**Basic pathology of infarction**

Appearances of myocardium at <6hrs (EM changes only) 6-24 hrs (coagulative necrosis) 24-48 hrs (neutrophil infiltrate); 48hrs-5 days (neutrophils, macrophages); 5-7 days (macrophages, fibroblasts, angiogenesis) weeks-months (progressively less cellular scar)

**Complications of acute myocardial infarction**

* Arrythmia eg ventricular fibrillation; structural basis of dysrythmias
* Myomalacia cordis
* Ventricular septal defect
* Papillar muscle rupture
* Left ventricular rupture
* Ventricular aneurysm & dyskinetic segment
* Cardiac failure
* Intracardiac thrombosis
* Thromboembolism
* Deep venous thrombosis (very rare)

**Complications of treatment**

* Reperfusion arrythmias
* Stent complications
* Restenosis
* Bleeding

***Cardiac Failure***

Heart failure is a **final common pathway**. It is defined as inability of the heart to pump blood sufficient for body’s demands.

Two Illustrative case histories of cardiac failure secondary to IHD and cardiomyopathy to compare.

**Pathology**

Dilated heart, with scarring and thinning of the wall of the left ventricle; dilated right ventricle; Heart will show extensive fibrosis and replacement of ventricular myocardium pleural effusions, pulmonary oedema and pulmonary haemosiderin; enlarged liver with histological central venous congestion and cardiac fibrosis, ascites and peripheral oedema of ankles.

**Major causes of Heart Failure**

Ischaemic heart disease

Valve disease

Myocarditis

Hypertension

Cardiomyopathy

***Cardiomyopathy***

**Definition: intrinsic disease of the heart muscle:** one cause of cardiac failure; itself a final common pathway due to multiple defects of myocardium more subtle than blocked coronaries or valves.

**Clinical Features of Cardiomyopathies**

Present with sudden death, palpitations

Can mimic ischemic heart disease in symptoms

Cardiac failure with enlargement on chest film.

Usually no identifiable cause of cardiac enlargement (ischemic, valvular, hypertensive, congenital, etc.)

**Classification of cardiomyopathy**

Principally classified by 1) the physiological abnormality in cardiac contraction (essentially whether the myocardium is too thick, too thin, or too stiff) 2) underlying cause

**Hypertrophic (HCM)**

Defect in myofilament formation; reactive increase in myocyte size

* Left ventricular hypertrophy
* Characteristic fiber disarray on micro)
* Familial in 50% (autosomal dominant, variable penetrance)
* Thickening of septum narrows left ventricular outflow tract
* Can have systolic murmur, arrhythmias, sudden death

**Dilated (=heart failure with no clear anatomic cause)**

Due to progressive loss of myocytes

Dilated heart, heart failure, arrhythmias, sudden death

Causes are many but can be broadly classed:

Idiopathic – probably mostly sequelae of viral myocarditis

Toxic: alcohol, chemotherapy (adriamycin), cobalt

Immunological: e.g. myocarditis incl. viral

Endocrine e.g. hypothyroidism

Genetic / metabolic e.g. glycogen storage disorder

**Restrictive**

Amyloidosis--infiltrates myocardium, characteristic Congo Red staining

Sarcoidosis

Endomyocardial fibrosis (EMF) ,Fibroplastic endocarditis (Loeffler's)

***VALVE DISEASE***

***RHEUMATIC VALVE DISEASE***

***Now rare in the developed world.***

***Cases in older generation or recently migrated***

Streptoccal infection initiate antibodies that crossreact with heart.

Inflammation affects all 3 layers of the heart **Pan**carditis

***Acute rheumatic fever***

Acute disease - mainly children. **Pan**carditis in 40% ie endocarditis, myocarditis, pericarditis

* **Endocarditis:** vegetations, predominantly left-sided valves (almost always mitral)   
  Mitral > Aortic > Tricuspid > Pulmonic. Mitral alone 48%, Mitral + aortic 42%
* **Myocarditis:** characterised by Aschoff bodies in myocardium, Anitschkov myocytes;
* **Pericarditis:** fibrinous + friction rub.
* **Extracardiac manifestations:** Rheumatic polyarthritis, Subcutaneous Nodules, erythema marginatum, Sydenham's chorea Prone to recurrence
* ***Chronic rheumatic valve disease***

Mainly adults. Sequela of earlier rheumatic fever. Lower grade, more protracted, valvular scarring.

Same valve predilection as acute (ie almost always mitral, mitral + aortic about 40%) but a more scarring pattern producing: Thickening of valve leaflet; Fusion of commissures; thickening, shortening and fusion of chordae tendineae (mitral)

***AORTIC STENOSIS***

May be classified by site: subvalvular, valvular, supravalvular

***Valvular Aortic Stenosis***

* + Acquired (usually rheumatic)
  + Degenerative (ie calcific aortic stenosis)
  + Congenital (usually bicuspid)
* **Rheumatic** (chronic rheumatic endocarditis)
  + Fused commissures, thickening at lines of closure
  + Almost always accompanied by mitral involvement
  + More common in middle-age, females > males
  + Onset of murmur in mid-adult life, usually
* **Degenerative** (ie calcific aortic stenosis)
  + Pathogenesis uncertain; likely inflammatory component and statin-sensitive
  + Calcium deep in sulci, on cusps (not on lines of closure and commissures)
  + Main cause of isolated aortic stenosis
  + More common in elderly, and in males; Onset of murmur late
* **Congenital** Three types: (bicuspid most common)

***AORTIC regurgitation***

* **Rigidity** - rheumatic, degenerative: valve too stiff to shut
* **Destruction** - microbial endocarditis: valve has a hole
* **Collapse** - prolapse through VSD, or due to myxomatous degeneration
* **Aortic & valve annulus dilatation**: leaflets will not meet in the middle (e.g. connective tissue disease)
  + Marfan's Syndrome
  + Dissecting aneurysm
  + Syphilitic aortitis
  + Ankylosing spondylitis

***Myxomatous Mitral Valve/ Mitral Valve Prolapse***

Mid systolic click, late systolic murmur

Stretched, redundant mitral valve

*. COMPLICATIONS*

* Atrial dilatation & dysrythmia (typically AF)
* Secondary infective endocarditis
* Some patients have sudden cardiac death (uncommon)
* Chest pain, breathlessness, congestive heart failure

***TYPES OF ENDOCARDITIS***

* Non-infective (Non-microbial)
* Verrucous (acute rheumatic fever)
* Atypical verrucous (Libman-Sacks) (systemic lupus)
* Non-bacterial thrombotic - NBTE (marantic)

***Infective (Microbial) endocarditis***

* Mainly bacterial or fungal (viral, rickettsial rare)
* Destroys valve tissue
* Thrombus with microorganisms deep within it
* Acute and subacute merged into single classication as **infective endocarditis, due to name of organism**. Pathogenesis fundamentally the same, but virulent organisms destroy tissue faster
* **Less virulent organisms** often enteric (Strep. viridans, most common)
  + Lengthy course - months if untreated
  + Previously damaged valve (rheumatic, congenital defect, previous surgery)
* **Virulent organisms, focally destructive (Staph. aureus, Strep. faecalis, others).** 
  + Fulminant course - weeks only, if untreated
  + Previously normal valve
  + Bypass of normal immune defences eg intravenous drug use,
  + Sudden onset, high fever, patient prostrated
  + Murmur(s)
* Death due to heart failure, valve perforation, sepsis
* Treatment: IV antibiotics, resect infected valve

***COMPLICATIONS OF INFECTIVE ENDOCARDITIS***

* Cardiac: Valve destruction: Perforation of cusp or leaflet, rupture of chordae tendineae, abscess, fistula, obstruction of valve or outflow tract
* **Extracardiac**
  + **Emboli** to major organs, septic or bland, mycotic aneurysm formation
  + **Immune complex** focal segmentalglomerulonephritis from immune complexes (not actually focal "embolic"); Other immune complex vasculitis (petechiae and/or splinter hemorrhages, in skin, mucosae, conjunctiva, retina)

***PERICARDITIS***

* **Definition:** Inflammation of the pericardium
* **Types and Causes**
  + Fibrinous (MI, uremia)
  + Purulent (pyogenic infection e.g. staphylococcus)
  + Granulomatous (TB)
  + Hemorrhagic (tumor, TB, uremia)
  + Fibrous (a.k.a.Constrictive) (arises from any of above**)**
* ***Pericardial effusion:*** Serous fluid in pericardial sac, Usual cause: Chronic heart failure
* ***Haemopericardium:*** Myocardial rupture from myocardial infarction, Trauma

The immune response to infection

Margaret Callan

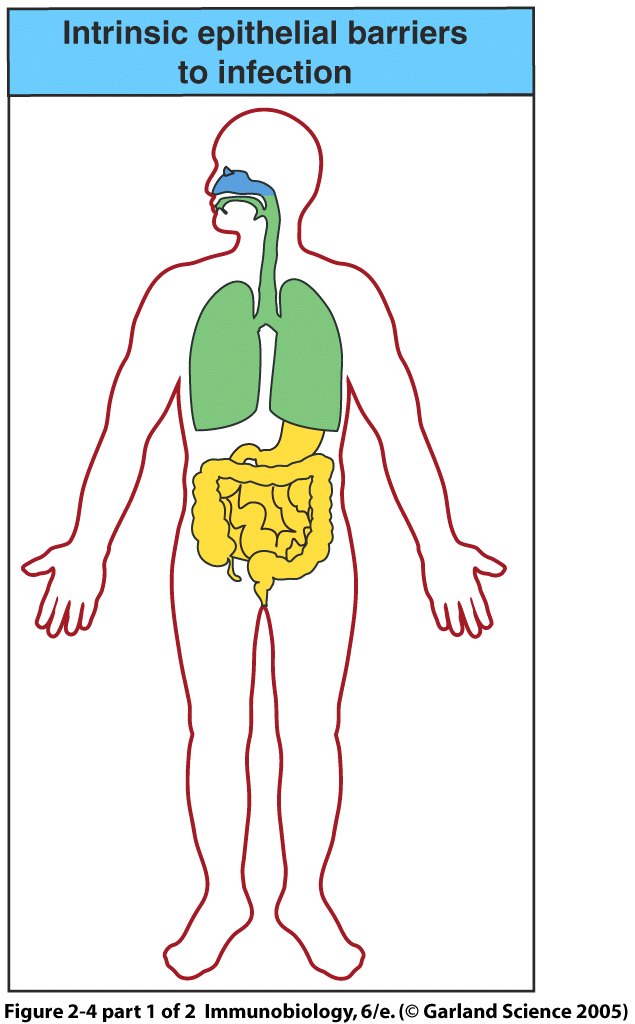
***Objectives***

Review basic principles of immunology

* + 1. Constitutive barriers to infection
    2. Cells of the innate immune system
    3. Cells of the adaptive immune system
    4. Complement

|  |  |
| --- | --- |
| ***Classification of the immune system***  **Innate immune system** |  |
| *Components* | *Features* |
| Cells:  Macrophages, Neutrophils, Eosinophils, Basophils, Mast cells, Natural Killer cells | Essentially identical responses in all individuals |
| Proteins:  Complement, Acute phase proteins, Cytokines | Genetically encoded receptors that have been selected in evolution for ability to recognise structures that are likely to be associated with infectious organisms (‘pathogen recognition receptors’ that recognise ‘pathogen associated molecular patterns’) |
|  | Initial line of defence  Stimulates the acquired immune response |
|  |  |
| **Acquired immune system** |  |
| *Components* | *Features* |
| Cells:  B lymphocytes, T lymphocytes | Person-specific responses |
| Proteins:  Antibody, Cytokines | Antigen receptors generated by semi-random process of gene rearrangement thereby creating a population of cells with a very wide repertoire of receptors  Receptors have very high specificity for antigens  Clonal expansion of relevant cells following antigen exposure  Capacity for memory |
|  |  |

***1. Constitutive barriers to infection***

* 

Commensal bacteria

Mucous membranes:

Physical barriers

Secretory IgA

Lysozyme, Antimicrobial peptides, Lactoferrin

Cilia

Skin: Tightly packed keratinised cells

Physiological factors: low pH low pO2

Sebaceous glands: hydrophobic oils, lysozyme, ammonia, antimicrobial peptides

***2. cells of the innate Immune system***

**phagocytes – MACROPHAGES AND NEUTROPHILS**

*Defence particularly against bacteria and fungi*

Initiation and amplification of the inflammatory response

Neutrophils are rapidly mobilised from bone marrow

Macrophages are derived from monocytes and are resident in tissue

Express ‘pattern recognition receptors’ (eg Toll-like receptors) that recognise ‘pathogen associated molecular patterns’ (PAMP)

Express Fc receptors to allow recognition of antibody/antigen complexes

Ingest and kill microorganisms (oxidative and non-oxidative killing)

Macrophages can subsequently present peptides from ingested microorganisms to T cells

Scavenge cellular and infectious debris

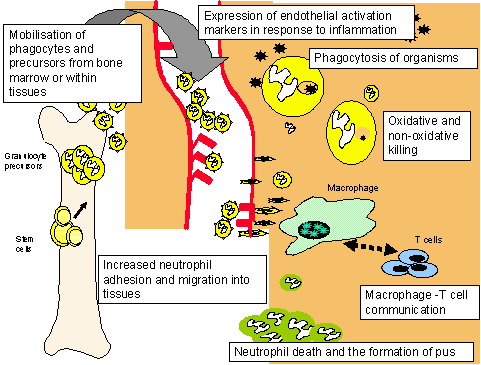
Produce inflammatory molecules (cytokines and chemokines) which regulate other components of the immune system

Resolution and repair

**basic steps involved in phagocyte function**

|  |
| --- |
| *Phagocyte recruitment* |
| Mobilisation from bone marrow |
| Migration to site of infection |
| *Find and catch the organism* |
| Chemotaxis |
| Endocytosis |
| Formation of phagolysosome |
| *Kill the organism* |
| Oxidative killing  Non-oxidative killing |

**What happens to phagocytes in response to infection**

****

**Natural killer cells**

*Defence against intracellular pathogens and malignancy as well as having important role in activating dendritic cells to promote the adaptive immune response*

* + Express inhibitory receptors for self-HLA to prevent damage to healthy self cells
  + Express a wide range of activatory receptors
  + Cytotoxic capacity (ie kill target cells)
  + Express cytokines

**dendritic cells**

*Prime the adaptive immune response*

Reside in peripheral tissues

Express cytokine receptors to ‘detect inflammation’

Express pattern recognition receptors and Fc receptors

Capable of phagocytosis

Mature into cells with enhanced antigen presenting capacity

Migrate to lymph nodes where they prime the adaptive immune response

Express cytokines which help regulate development of immune response

***3. CElls of the Acquired immune system***

***THe anatomy of the acquired immune response***

**PRIMARY LYMPHOID TISSUE**

Where B and T cells mature (Bone marrow and thymus)

**SEcondary lymphoid tissue**

Where B and T cells are primed by antigen (Lymph nodes, spleen, mucosal associated lymphoid tissue)

***T Lymphocytes***

**Physiology**

Arise from haematopoetic stem cells in bone marrow

Each T cell undergoes ‘T cell receptor gene rearrangement’ in which T cell receptor alpha and beta chain segments are selected and rearranged in a semi-random manner to create a population of cells bearing a very wide repertoire of distinct receptors. Potentially >>109 receptors can be created.

Exported as immature cells to the thymus where they undergo:

i) Selection for CD4 or CD8

ii) Selection for receptors of intermediate affinity for self HLA. Negative selection of T cells expressing receptors of high affinity for self HLA is termed ‘central tolerance’ and is an important mechanism protecting against autoimmunity.

Mature T lymphocytes enter the circulation and reside in lymph nodes and secondary lymphoid follicles

Following priming by antigen presented by ‘antigen presenting cells’, specific T cells undergo massive clonal expansion to allow them to mediate an effective response. Following control of expansion many of these T cells die by apoptosis, but a pool of ‘memory cells’ remains.

**Function**

Defence against wide range of pathogens, including viruses, bacteria and fungi

Immunoregulation

**Classification of T lymphocytes**

*CD4+ (“helper”) T lymphocytes*

Recognise peptides that are usually derived from extracellular proteins and presented on HLA Class II molecules (HLA-DR, HLA-DP and HLA-DQ)

Produce cytokines. Types of cytokines produced further divides CD4+ T cells into Th1 and Th2 cells

Th1 cells produce IL-2, IFNγ and lymphotoxin

Th2 cells secrete IL-4, IL-5 and IL-10

Th17 cells secrete IL-17 and IL-22

Tregs express CD25 and Foxp3 and secrete IL-10

Tr1 cells secrete IL-10 and TGF beta

Important role in ‘helping’ development of B cell response and CD8 T cell response. Note that CD4 T cells are required for process of ‘isotype switching’ and ‘somatic hypermutation’ of B cells; in absence of CD4 T cell function the B cell response will therefore be restricted to an IgM response (without development of IgG and IgA responses)

*CD8+ (“cytotoxic”) T lymphocytes*

Specialised cytotoxic (killer) cells

Recognise peptides that are usually derived from intracellular proteins in association with HLA class I (HLA-A, HLA-B, HLA-C)

Kill cells directly

Important role in immunity against intracellular pathogens such as viruses and mycobacteria

***B Lymphocytes***

**Physiology**

Arise from haematopoetic stem cells in bone marrow. Like T cell receptors, B cell receptors are created by semi-random gene rearrangement to create a diverse repertoire of receptors. B cells bearing receptors with high affinity for self are deleted in the bone marrow (‘central tolerance’) as a mechanism for preventing autoimmunity.

Mature B lymphocytes found mainly in bone marrow, lymphoid tissue, spleen

B cells may be stimulated by antigen to secrete IgM

B cells usually require CD4 T cell help to undergo affinity maturation (ie refining receptors to improve affinity for antigen) and isotype switching (ie switch from IgM to expressing other heavy chains) within germinal centres of secondary lymphoid organs to emerge as memory B cells or plasma cells capable of secreting IgG, IgA,

**Function**

Antibody production

Antigen presentation

**Immunoglobulins**

Soluble proteins made up of two heavy and two light chains with same specificity as the B cell receptor expressed by the B cell/plasma cell

Heavy chain determines the antibody class (IgM, IgG, IgA, IgE, IgD)

Antigen is recognised by the antigen binding regions (Fab) of both heavy and light chains

Effector function is determined by the constant region of the heavy chain (Fc)

*Antibody function*

Identification of pathogens

Recruitment of other components of immune response to remove pathogens, including complement, phagocytes and natural killer cells

Neutralisation of toxins

Particularly important in defence against bacteria of all kinds

*Memory is a key feature of B cell activation*

The lag time between antigen exposure and the production of antibody is decreased (to 2-3 days) following second exposure to antigen

The titre of antibodies produced is greatly increased

The response is dominated by IgG antibodies of high affinity

***4. Complement***

> 20 tightly regulated, linked proteins

Produced by liver

Present in circulation as inactive molecules

May be activated by one of three pathways that, when triggered, activate other proteins in a biological cascade. All pathways converge on C3

Classical Mannose binding lectin Alternative

C1, C4, C2 BIP

C3 cleavage

Membrane attack complex

C5-9

Activated complement components:

Increase vascular permeability and stimulate cell trafficking

Opsonise pathogens

Activate phagocytes and mast cells

Opsonise and so help to solubilise immune complexes

Form ‘holes’ in pathogen membranes (membrane attack complex).

Important role in innate & antibody-mediated immunity. Critical role in defence against encapsulated bacteria

Immunodeficiency

**Margaret Callan**

***Objectives***

Be familiar with the clinical features of immunodeficiency

Know the common forms of immunodeficiency

Understand how to investigate possible immunodeficiency

Know how to approach management of immunodeficiency

***Overview of immune deficiencies***

**Classification of immunodeficiencies**

*Primary*

Rare: 1:10,000 live births

>100 primary immune deficiencies now described

*Secondary*

|  |  |
| --- | --- |
| **EXAMPLES OF CONDITIONS ASSOCIATED WITH SECONDARY IMMUNE DEFICIENCY** | |
| **Infection** | Human immunodeficiency virus |
|  | Measles |
|  | Mycobacterial infection |
| **Treatment interventions** | Immunosuppressive therapy |
|  | Anti-neoplastic agents |
|  | Corticosteroids |
|  | Bone marrow transplantation |
|  | Radiation injury |
| **Malignancy** | Haematological malignancies including |
|  | Solid tumours |
| **Biochemical and** | Malnutrition |
| **nutritional disorders** | Renal insufficiency |
|  | Type 1 and type 2 diabetes |
|  | Specific mineral deficiencies, e.g. iron, zinc |
| **Other conditions** | Burns |
|  | Asplenia/hyposplenism |

*Physiological*

Neonates

Pregnancy

Old age

***Clinical features suggestive of primary immunodeficiency***

Recurrent infections

Two major or one major and recurrent minor infections in one year

Unusual organisms

Unusual sites

Unresponsive to oral antibiotics

Chronic infections

Early structural damage

*Other features*

Failure to thrive

Oral thrush

Skin rash (eczema)

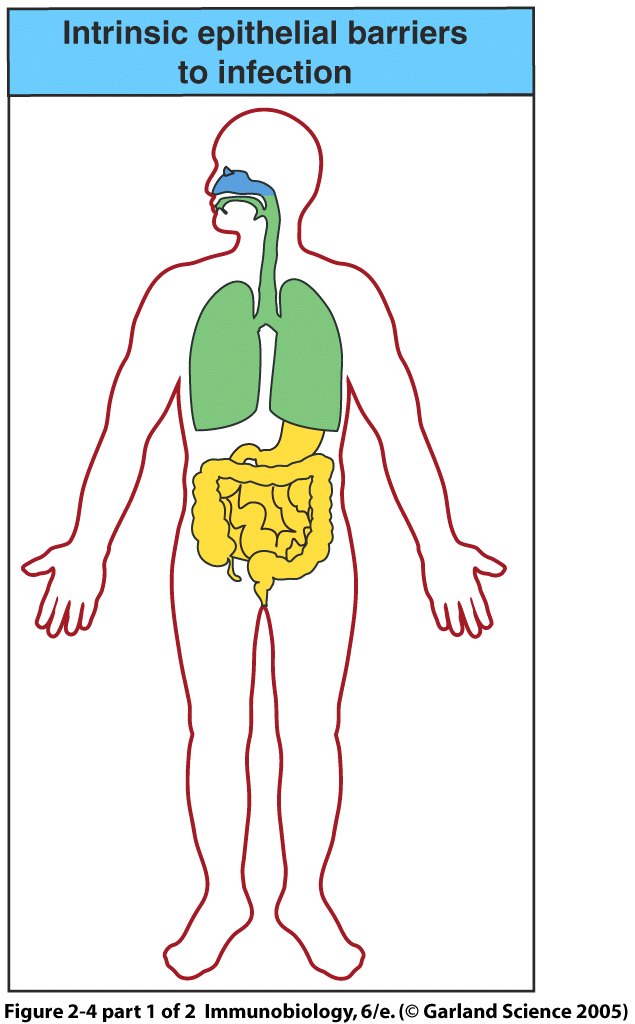
Chronic diarrhoea

Mouth ulceration

Other autoimmune disease

Family history

***1. failure of Constitutive barriers to infection***

* 

Failure of commensal bacteria: C difficile infection following antibiotic use

Failure of immunity associated with mucous membranes: IgA deficiency

Failure of skin: Burns patients

***2. immunodeficiency involving phagocytes***

**Clinical features of phagocyte deficiencies**

Phagocytes are important in initial defence particularly against bacteria and fungus. Deficiencies therefore result in bacterial and fungal infections, often with deep abscess formation and particularly involving skin and mouth.

Recurrent deep bacterial infections, especially

Staphylococcus Aureus

Enteric bacteria

Mycobacteria: MTB and atypical

Recurrent fungal infections

Candida

Aspergillus

**types of Phagocyte deficiencies**



**Investigation of phagocyte function**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *Neutrophil count* | *Pus formation* | *Leukocyte adhesion markers* | *Nitroblue test (NBT) or Dihydrorhodamine test (DHR) of oxidative killing* |
| *Kostmann syndrome (congenital neutropaenia)* | *Absent* | *No* | *Normal* | *Negative – no neutrophils* |
| *Leukocyte adhesion defect* | *High during infection* | *No* | *CD18 absent* | *Normal* |
| *Chronic granulomatous disease* | *Normal* | *Yes* | *Normal* | *Negative* |

**management of phagocyte deficiencies**

*Aggressive management of infection*

Infection prophylaxis

Septrin

Itraconazole – anti-fungal

Oral/intravenous antibiotics

Surgical draining of abscesses

*Definitive therapy*

Bone marrow transplantation to ‘replace’ defective population

Interferon gamma therapy for Chronic granulomatous disease

***3. immunodeficiency involving b and t lymphotyes***

**Clinical features of B and T lymphocyte deficiencies**

CD8 T cells are particularly important in protection against intracellular infection including viruses and mycobacteria. B cells are particularly important in protection against extracellular pathogens (eg many bacteria) and toxins. CD4 T cells provide ‘help’ to both these arms of the adaptive immune response. Deficiencies in T and B cells therefore increase susceptibility to infection, broadly as follows.

* T cell deficiency
  + Viral infections
    - Eg Cytomegalovirus
  + Fungal infection
    - Eg Pneumocystis, Cryptosporidium
  + Some bacterial infections – esp intracellular organisms
    - Eg Mycobacteria tuberculosis, Salmonella
  + Early malignancy
* Antibody deficiency (NB CD4 T cell deficiency will lead to IgG and IgA deficiency)
  + Bacterial infections
    - Eg Staphylococcus, Streptococcus
  + Some viral infections
    - Enterovirus
  + Toxins
    - Eg Tetanus, Diptheria

**Types of B and T lymphocyte immune deficiencies**

**Combined B and T lymphocyte immune deficiencies - SCID**

*Severe combined immune deficiency* is caused by defects in lymphoid precursors

Many molecular defects cause a similar clinical phenotype

X-linked SCID due to mutation involving IL2 gamma chain accounts for ~45% cases

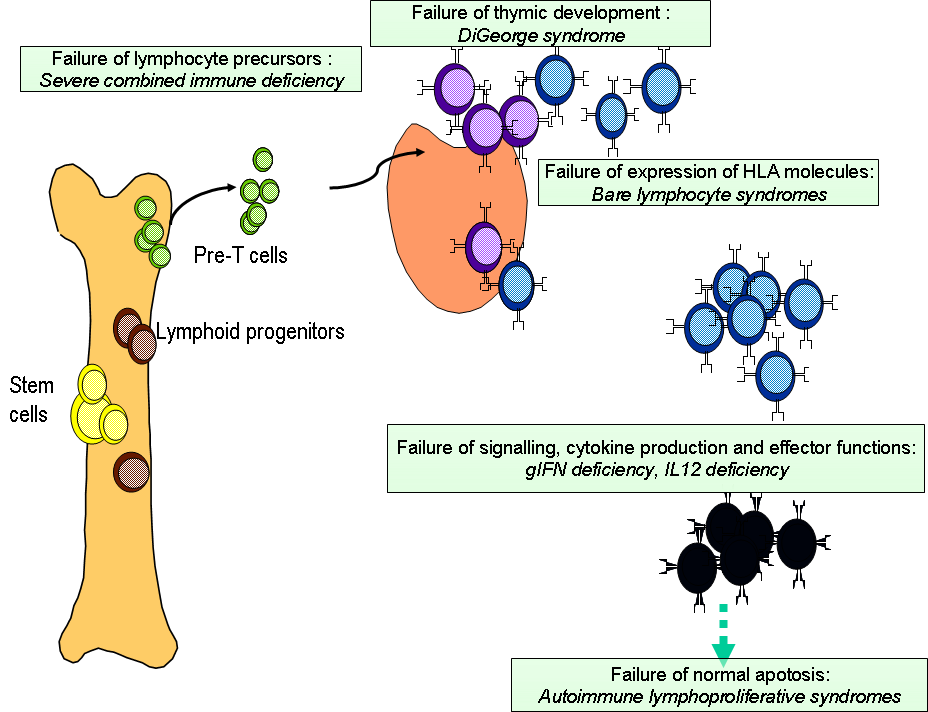
Results in the combined failure of B and T cell maturation. Note that in X-linked SCID B cell numbers may be normal although function is compromised.

Associated with recurrent bacterial, fungal and viral infections soon after birth.

Bone marrow transplantation is the only established treatment option

**primary T lymphocyte deficiencies**

Remember that if CD4 T cells are deficient then the germinal centre reaction with B cell affinity maturation and isotype switching will be compromised so that IgM but not IgG or IgA can be produced.

**

*DiGeorge syndrome*

Failure of development of the 3rd/4th pharyngeal pouch, associated with deletion of 22q11

Immunological abnormality reflects absent thymic development

Other abnormalities include malformation of the aortic arch, hypocalcaemia, tracheo-oesophageal fistulae, cleft lip and palate

Characterised by very low numbers of mature T cells despite normal development in the bone marrow.

*Bare lymphocyte syndromes*

Caused by absent expression of HLA molecules within the thymus: if HLA class I molecules are affected, CD8+ lymphocytes fail to develop (BLS type 1); if HLA class II molecules CD4+ lymphocyte fail to develop (BLS type 2).

Failure to express HLA class I also associated with a systemic vasculitis syndrome caused by uncontrolled activation of natural killer cells.

*Other primary T cell defects*

Defects of T cell activation

Defects of cytokine production or receptors (Note that defects in IFN gamma or IL12 and their receptors increase susceptibility to atypical mycobacterial infection)

Abnormalities of T cell death

**Investigations for suspected T cell deficiencies**

Total lymphocyte count and differential

Remember that lymphocyte counts are normally much higher in children than in adults

Quantitation of lymphocyte subpopulations by flow cytometry

Serum immunoglobulins and protein electrophoresis

IgG and IgA are surrogate marker of functional CD4 T cells

Functional tests of T cell activation and proliferation may be useful if signaling or activation defects are suspected

HIV test

**Management of suspected T cell deficiencies**

Refer early to specialist teams

Infection prophylaxis: eg anti-pneumocystis and anti-fungal prophylaxis (but no live vaccines)

Aggressive management of specific infections

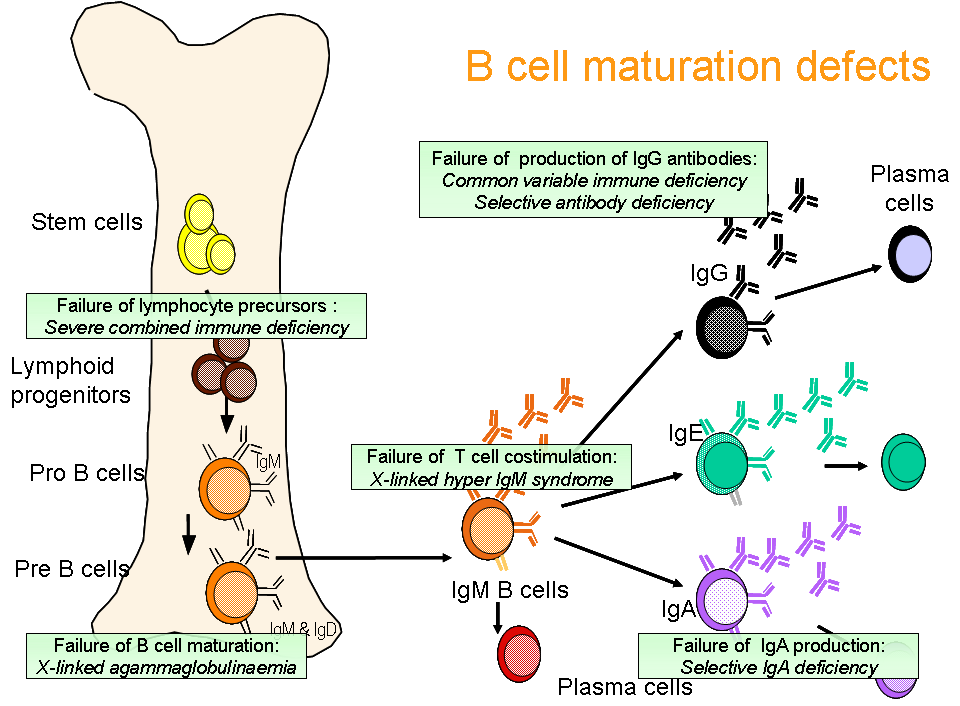
Immunoglobulin replacement indicated if disease is associated with defective antibody production

Bone marrow transplantation in BLS

(Gene therapy)

(Thymic transplantation in DiGeorge’s syndrome)

**primary B cell and antibody deficiencies**

**

*Bruton’s X-linked hypogammaglobulinaemia*

Defective B cell tyrosine kinase gene causes failure to produce mature B cells

Results in absence of circulating antibody after first 3-6 months

*Selective IgA deficiency*

Prevalence = 1:600

2/3 individuals asymptomatic; 1/3 have recurrent respiratory tract infections

Genetic component, but cause as yet unknown

*Common variable immune deficiency*

Heterogenous group of disorders, often adult onset

Disease mechanism unknown

May be associated with antibody mediated autoimmune disease

*Hyper IgM syndrome*

Boys present in first few years of life with recurrent bacterial infections and failure to thrive. Also pneumocystis carinii, malignancy, autoimmune diseases

Failure to express CD40 ligand on activated T cells means that activated T cells cannot provide “help” for B cell differentiation to IgG / IgA secreting plasma cells or memory B cells. Ig M is produced and is usually present at high levels.

**Investigation of B cell deficiencies**

Total lymphocyte count and differential

Remember that lymphocyte counts are normally much higher in children than in adults

Quantitation of lymphocyte subpopulations by flow cytometry

Serum immunoglobulins and protein electrophoresis

Specific antibody responses to known pathogens

Measure IgG antibodies against tetanus, Haemophilus influenzae B and S. pneumoniae

If specific antibody levels are low, immunise with the appropriate killed vaccine and repeat antibody measurement 6–8 weeks later

Failure to mount a response indicates a significant defect in antibody production.

Functional tests have generally superceded IgG subclass quantitation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Serum immunoglobulins** | | | | **Circulating lymphocyte numbers** | |
|  | **IgM** | **IgG** | **IgA** |  | **B cells** | **T cells** |
| **Selective IgA deficiency** | + | + | - |  | + | + |
| **Brutons X linked agammaglobulinaemia** | - | - | - |  | - | + |
| **Hyper IgM syndrome** | +++ | - | - |  | + | + |
| **Common variable immune deficiency** | + | +/- | +/- |  | + | + |

**Management of B cell deficiencies**

* Aggressive treatment of infection and infection prophylaxis
* Immunoglobulin replacement is mainstay of treatment
  + derived from pooled plasma from thousands of donors
  + contains IgG antibodies to a wide variety of common organisms
  + Usually administered intravenously every 3–4 weeks
  + Aim of maintaining trough IgG levels within the normal range
  + Treatment is life-long
* Bone marrow transplantation in some situations
* With the exception of selective IgA deficiency, immunisation is generally not effective because of the defect in IgG antibody production

***4. Immunodeficiency involving complement***

**Clinical features of complement deficiencies**

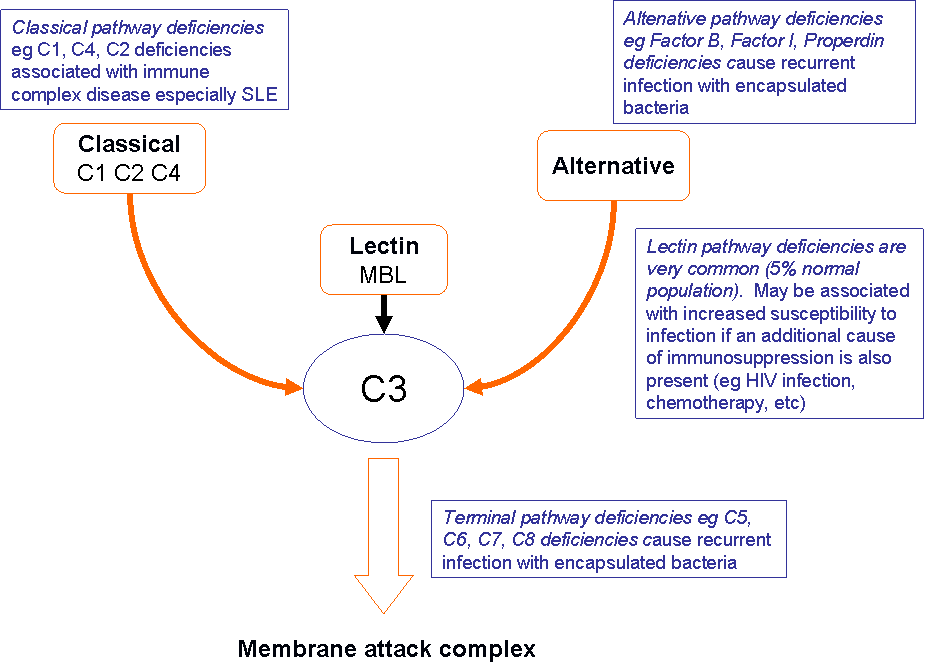
Functions of complement include promoting inflammation, facilitating phagocytosis of pathogens, direct damage to pathogens (via membrane attack complex) and solubilising immune complexes.



Complement deficiency will therefore result in increased susceptibility to infection, particularly by encapsulated bacteria.

Deficiency of the early components of the classical pathway result in increased risk of SLE (failure to solubilise immune complexes)

**types of complement deficiencies**



**Investigation of the complement pathway**

*Quantitation of complement components*

C3, C4 routinely measured

(Also C1 inhibitor – decreased in hereditary angiodema)

Other components not routinely quantified, but can be performed if deficiency is suspected

*Functional complement tests*

CH50 (“haemolytic complement 50”)

AP50 (“alternative pathway 50”)

**Management of patients with complement deficiencies**

* Vaccination
  + Boost protection mediated by other arms of the immune system
  + Meningovax, Pneumovax and HIB vaccines
* Prophylactic antibiotics
* High level of suspicion
* Screening of family members

Diabetes – Clinical Cases

Prof Karim Meeran

# Learning Objectives

1. To understand the acid/base abnormalities that occur in patients with type 1 diabetes.

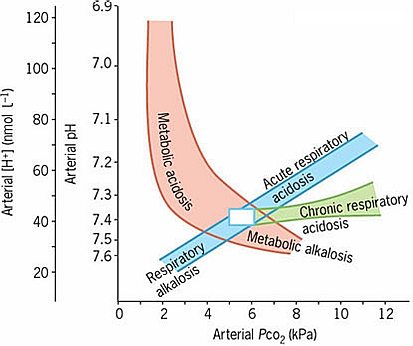
2. To understand the use of chemical pathology to determine the anion gap in patients with diabetes, and to be able to interpret this.

3. Know how to calculate the osmolality and interpret the results

4. Interpret data in from electrolyte results to give a differential diagnosis.

-----------------------------------------------------------------------------------------------------------

Draw graph of CO2 versus pH in the space below.



**pH= - log 10 ([H+])**

**Case 1**

A 16-year-old arrives unconscious in casualty. His mother said that he had been very ill for the last few days, with vomiting and complaining of breathlessness. Investigation reveal:

pH 6.85. pCO2 = 2.3 kPa (NR 4-5) pO2 = 15 kPa (normal)

What is the acid-base abnormality?

Na: 145. K 5.5 Urea 10.0mM. Glucose 25 mM Chloride = 96mM Bicarb=4mM

Why is he unconscious?

**How to calculate the plasma osmolality:**

Formula: Osmolality = charged molecules + uncharged molecules

= cations + anions + urea + glucose

Cations (Na, K) = anions (Cl, HCO3, others), otherwise the blood would be charged!

Write down the final formula for calculating osmolality here:

What is the plasma osmolality?

**How to calculate the Anion Gap:**

Cations (Na + K) = anions (Cl, HCO3, **others**), otherwise the blood would be charged!

“Others” known as “anion gap”. They represent the usually unquantified anions, including phosphate, sulphate, lactate, ketones and any added anions.

Write down the final formula for calculating the anion gap here:

AG=

What is the normal anion gap?

What is the anion gap in this patient?

**Case 2**

A 19-year-old who is known to have had type 1 diabetes for several years, presents unconscious to casualty.

pH 7.65. pCO2: 2.8 kPa. Bicarb 25mM (normal). pO2 15 kPA (normal)

What is the acid-base abnormality?

Further results:

Na=140, K=4.0, Bic = 24mM, Cl = 100, Glucose = 1.3 mM

What is the anion gap?

What is the diagnosis?

**Case 3**

A 60-year-old man presents unconscious to casualty with a long history of polyuria and polydipsia. Investigations reveal:

Na 160 mM, K 6.0 mM, U=50mM, Glucose = 60mM

What is the osmolality?

Why is he unconscious?

**Case 4**

A 60-year-old man, know to have had type 2 diabetes for several years who is on a good diet and metformin, presents to casualty unconscious. His urine is negative for ketones.   
Investigations reveal:

Na: 140 mM, K=4.0mM, U=4.0 mM, pH=7.10, Glucose = 4.0 mM

pCO2 = 1.3 kPa, Cl = 90 mM, Bicarb = 4.0 mM

What is the osmolality?

What is the anion gap?

What is the acid-base disturbance?

Why is he unconscious?

**Definition of Diabetes**

This is only really relevant to type 2 diabetes, where patients only slowly become unwell. Patients with type 1 diabetes present with ketoacidosis, so there is no argument about whether or not they have diabetes. However type 2 diabetes can be more difficult because patients might have fasting plasma glucose values close to 7.0 mmol/l. To diagnose diabetes, it is important that the patient has symptoms of diabetes. If they have a random (i.e. not necessarily fasting) plasma glucose of >11.1 mmol/l then it is highly likely that they have diabetes.

If they have a fasting plasma glucose > 7.0 mmol/l, they have diabetes. If the fasting glucose is greater than 7.0 then the patient has diabetes, and no further testing is needed.

Normal fasting plasma glucose < 5.5 mmol/l according to the American Diabetes Association and <6.1 mmol/l according to the World Health Organisation. It is irritating for students that both values are widely used, but we will ensure that for exam purposes that this difference is not a problem. For the rest of this guide, we are going to use the ADA guidelines.

Thus, a glucose level between 5.5 mmol/l and 7.0 mmol is known as **“impaired fasting glucose (IFG)”.**

Alternatively, an oral glucose tolerance test (OGTT) can be performed. This is performed by giving 75 grams of glucose in approximately 300 ml of water and can be useful if patients are particularly obese or have impaired fasting glucose. Patients who have a glucose 2h after an OGTT that is greater than 11.1 also have diabetes. Thus, to have diabetes one needs to have **either** a fasting plasma glucose greater than 7.0mmol/l **or** a 2 hour value greater than 11.1 mmol/l.

Some patients who have a fasting glucose of less than 7.0 mmol/l will have a 2 hour value of between 7.8 and 11.1. These patients have “**impaired glucose tolerance (IGT)**”.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **NORMAL** | **IFG** | **IGT** | **Diabetes** |
| 0 mins | <5.5 | 5.5 – 7.0 |  | >7.0 mmol/l |
| 120 mins | <7.8 mmol/l |  | 7.8 – 11.1 mmol/l | >11.1 mmol/l |

All the values above apply to PLASMA glucose. To measure this, the blood sample needs to be collected in fluoride oxalate and centrifuged to remove the red cells. If this is not done, then you are measuring WHOLE BLOOD glucose. In this case, the values above need to be changed:

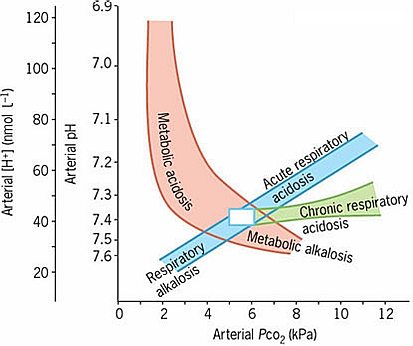
7.0 plasma is 6.1 whole blood.

7.8 plasma is 6.7 whole blood.

11.1 plasma is 10.0 whole blood.

This occurs because red cells occupy approximately 40% of blood volume, and the glucose within those cells is lower than outside the cells.

Occasionally, patients with type 2 diabetes who have a fasting glucose only just above 7.0 mM, might be able to lose weight (by strict dieting and exercise) so that they become “non diabetic”. This is definitely worth striving for, but unfortunately most patients aren’t motivated enough to keep the weight off, and so the diabetes returns shortly afterwards. However people forced to keep on diet and exercise can stay non diabetic. For evidence that type 2 diabetes is preventable with diet and exercise, see the landmark trial, the Diabetes Prevention Programme (New Engl J Med Feb 7th 2002 Vol 346 (6) pages 393 – 403.



Mild hypokalaemia is generally asymptomatic. Symptoms are more likely to occur below 3.0 mmol/L Hypokalaemia may present with muscle weakness, reduced gut motility and cardiac arrhythmias.

***CAUSES OF HYPOKALAEMIA include:***

##### Renal loss

Diuretics

Mineralocorticoid excess (e.g. aldosterone/corticosteroids)

Renal tubular disease (e.g. Renal tubular acidosis)

##### Intestinal loss

Diarrhoea, vomiting or fistula

##### Redistribution

Glucose and Insulin, alkalosis

Plasma Proteins

Dr Sue Brook

(Handout by Dr. Jaimini Cegla)

**Learning Objectives**

* To know the functions of the commonly measured proteins found in plasma
* To understand the factors which contribute to the concentrations of these proteins in plasma
* To know the changes to the concentrations of these proteins in disease
* To understand the usefulness of measurements of these proteins in plasma and other body fluids in which they occur.

**General**

Plasma total protein concentration in health: 60-80 g/L.

Sum of many individual proteins, ranging in concentration from ng/L or less for hormones and enzymes, through ug/L (e.g. ferritin), mg/L (eg CRP) up to 40 g /L for plasma albumin.

|  |  |  |
| --- | --- | --- |
| **Class** | **Protein** | **Serum Conc (g/L)** |
|  | Albumin | 40 |
| α1-globulin | α1-antitrypsin | 2.9 |
| α2-globulin | Haptoglobulins  Caeruloplasmin | 2.0  0.35 |
| β-globulin | Transferrin  LDL  Complements | 3.0  1.0  1.0 |
| γ-globulins | IgG  IgA  IgM  IgD  IgE | 14.0  2.5  1.5  0.03  trace |

**Functions of plasma proteins:**

1. Transport
2. Humoral Immunity
3. Maintenance of oncotic pressure
4. Enzymes
5. Protease Inhibitors
6. Buffering

**Factors influencing protein concentrations:**

1. Synthesis rate

2. Catabolic rate

3. Distribution in body fluids

**Specific Plasma proteins**

**1. Albumin:**

Structure: Single polypeptide chain with MW of 67,000 daltons.

Normal plasma concentration: 33-47 g/L

Functions: Oncotic pressure, source of amino acids, buffer,

ligand binding eg bilirubin.

In Disease Almost always a reduction. Increase only seen in severe dehydration.

Behaves as a negative acute phase protein, reduced levels due to decreased synthesis, increased capillary permeability and renal and gut losses.

**2. C-Reactive Protein:**

Archetypal acute phase reacting protein. Plasma concentration <10 mg/L (probably <1 in most individuals). Increases markedly in the acute phase response (may rise above 500).

Increases 6-8 hrs after tissue damage (trauma, infection, inflammation), & peaks after 24-48 hrs. Stays elevated if there is continuing stimulus (by cytokines such as interleukins [IL-1 & IL-6]). In the well individual, slight increases in CRP are an independent risk factor for cardiovascular disease.

**3. Immunoglobulins:**

Plasma concentrations (adult): IgG 5.4-16.1 g/L

IgA 0.8-2.8 g/L

IgM 0.5-1.9 g/L

IgD no clinical significance

IgE approx 1 ug/L

Polyclonal increases occur in a wide range of infectious and autoimmune diseases.   
The **pattern** of increase may give most information.

IgE is associated with type 1 hypersensitivity. Specific IgE antibodies to a range of environmental antigens can be measured.

Monoclonal increase (paraprotein) usually indicates a B cell neoplasm.

Myeloma – IgG 60%, IgA 20%, BJP only 20%, IgM <1%, IgD<1%, IgE rare.

IgM paraproteins occur in lymphoma, Waldenstroms macroglobulinaemia, leukaemia.

Indicators of malignancy in myeloma – immune paresis, high and rising pp level, BJP present. Monoclonal gammopathy of unknown significance (MGUS) must be monitored.

Immunoglobulin deficiency may be primary (genetic) or secondary.

**4. α-1-antitrypsin**

Physiology: Major antagonist of serine proteases released at site of tissue injury.   
Positive acute phase reactant.

Clinical: Genetics important, over 59 alleles of the protease inhibitor gene (Pi) are known.

The incidence of some phenotypes in UK is:

Pi Frequency serum level disease

M 80% normal none

S 0.25% 60% normal none

SZ 0.17% 37% normal Lung or liver as adult

ZZ 0.03% 15% normal Liver as neonate

**5. Transferrin**

Physiology: Main plasma iron transport protein.

Negative acute phase protein.

Clinical: % saturation of transferrin (serum iron/TIBC x 100, normal = 20-45%) is used clinically in assessment of iron deficiency and iron overload.

**6. Caeruloplasmin**

Physiology: Copper containing protein with oxidase activity. Acts as scavenger for superoxide radicals. Positive acute phase reactant.

Clinical: Wilson’s disease (autosomal recessive deficiency) leading to liver disease (40% cases), or neurological, psychiatric or haematological disorders.

**7. Tumour markers**

PSA (prostate specific antigen) prostate ca

AFP (alpha fetoprotein) hepatic ca

CA19-9 pancreatic masses

CA125 ovarian ca and pelvic masses

CEA (carcinoembryonic antigen) colorectal ca

βhCG (human chorionic gonadotrophin) gestational trophoblastic disease

**Proteins in other body fluids**

**Urine:** Normal urine protein <150 mg per day. Proteinuria due to renal disease causes increased excretion of a range of plasma proteins, the pattern reflecting the type of kidney damage.

Microalbuminuria is a marker for the early onset of renal disease in diabetes.

**CSF:** Normal CSF protein is mainly an ultrafiltrate of plasma, 0.15-0.45 g/L.

Non specific increases seen in trauma, infection, spinal block, etc.

“Oligoclonal” IgG may be seen in demyelinating diseases and some infections.

**Pleural effusions:** Transudate: protein <25 g/L. Exudate: protein >35 g/L.

**Transudative pleural effusions**: congestive heart failure, liver cirrhosis, hypoalbuminaemia, peritoneal dialysis.

**Exudative pleural effusions**: parapneumonic effusions, malignancy, pulmonary embolism, miscellaneous (>40, eg pancreatitis, collagen vascular disease e.g. rheumatoid arthritis and SLE, tuberculosis, post- myocardial infarction syndrome, asbestosis, drug reactions, chylothorax, haemothorax).

|  |
| --- |
| **EMQ - Plasma Proteins** |

**OPTION LIST**

|  |  |  |  |
| --- | --- | --- | --- |
| **A** | Albumin | **3** | Haptoglobin |
| **B** | Alpha-1-acid glycoprotein | **4** | Immunoglobulin |
| **C** | Alpha-1 antitrypsin | **5** | Pre-albumin |
| **D** | Alpha-2 macroglobulin | **6** | Thyroxine-binding globulin |
| **E** | Apolipoprotein | **7** |  |
| **F** | Caeruloplasmin | **8** |  |
| **1** | C-reactive protein | **9** |  |
| **2** | Fibrinogen | **0** |  |

**For each description below, choose the most appropriate protein from the list above. Each option may be used once, more than once or not at all.**

1. Deficiency is associated with movement disorders and liver disease

2. is a protein is low in nephrotic syndrome

3. is a major antagonist of serine proteases.

4. Levels fall during intravascular haemolysis.

5. Can be used as a marker for cardiovascular disease.

**ANSWERS**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **1.** | **2.** | **3.** | **4.I** | **5.** |

Metabolic Disorders and Screening 1

Dr Maggie Hancock

**Topics to address:**

* scope of inherited disorders and inherited metabolic disorders (IMD)
* concept of predictive values
* current UK neonatal screening for IMD and future prospects

Inherited disorders may be chromosomal, polygenic or monogenic (Mendelian).

Online Mendelian Inheritance in Man, OMIM ™ is a free, continuously updated catalogue of human genes and genetic disorders of Mendelian inheritance (<http://www.ncbi.nlm.nih.gov/omim/>).

Inherited metabolic disorders (IMD) are often referred to as inborn errors of metabolism. These are permanent, inherited biochemical disorders. IMD are caused by the lack of a functional gene product in the form of an enzyme, transmembrane transporter or similar protein, which then results in a blockage of the corresponding metabolic pathway. There are currently approx. 600 recognised IMD; they are individually rare but cause substantial morbidity and mortality. Recent epidemiological data from an ethnically diverse UK population calculated a birth prevalence of **1 in 784 live births** (excluding FH and diseases detected by neonatal screening). The majority of diagnoses (72%) were made by the age of 15 years and one third by the age of 1 year.

The symptoms of defective enzyme activity may relate to the lack of an end product, the build-up of precursors or to the synthesis of abnormal metabolites.

Diagnosis of IMD is largely made by identifying these biochemical abnormalities; mutation analysis is currently used mainly for disease confirmation and kinship screening (UK Genetic Testing Network, <http://www.ukgtn.nhs.uk>).

Approximately half of all IMD can be treated biochemically, although the success of such treatment is variable. Even in treatable disorder, damage incurred during metabolic decompensation is largely irreversible. It is for this reason that we have a national neonatal screening programme.

**Phenylketonuria (PKU) is the paradigm for Neonatal Screening Criteria:**

Well defined disorder

Associated with significant morbidity and mortality

Treatable, and early treatment makes a difference

Period before onset during which intervention improves outcome

Known incidence in relevant population

Ethical, safe, simple and robust screening test with acceptable sensitivity and specificity

**Sensitivity/Specificity/Predictive value**

|  |  |  |
| --- | --- | --- |
| Test | Positive | Negative  Sensitivity:  (TP / (TP + FN)) \* 100 |
| Condition |
| Present | True positive (TP) | False negative (FN)  Specificity:  (TN / (TN + FP)) \* 100 |
| Absent | False positive (FP) | True negative (TN) |

Positive predictive value (PV +ve) = (TP/ (TP+FP)) \* 100

Negative PV (PV –ve) = (TN/ (FN+TN)) \* 100

Predictive value depends upon disease incidence; as a rule of thumb, a lower incidence of disease is associated with a lower positive predictive value.

**UK Newborn Screening at days 5-8 of life**

(<http://www.newbornbloodspot.screening.nhs.uk> )

c. 600,000 live births/year

PKU Phenylalanine hydroxylase def. (OMIM 261600)

Incidence 1:10000, Blood spot phenylalanine

*CH Congenital hypothyroidism, Incidence 1:400, Blood spot TSH*

CF Cystic fibrosis (OMIM 219700), Incidence 1:2500

Blood spot immune reactive trypsin (IRT) + mutation analysis

HbSS Sickle cell disease (OMIM 603903), Incidence: 1:2000

Blood spot Hb electrophoresis

MCADD Medium chain acyl-CoA dehydrogenase def. (OMIM 201450), Incidence 1:10000, Blood spot octanoyl carnitine

Tandem-Mass Spectrometry (MSMS) permits screening for up to 42 additional disorders, some of which are so-called secondary targets.

Candidate disorders for extended UK screening are:

Homocystinuria (non B6 dependent) *Amino acid disorder*

Isovaleric acidaemia *Organic aciduria*

Glutaric aciduria type I *“*

Maple syrup urine disease *“*

Long chain acyl CoA dehydrogenase deficiency *Fatty acid oxidation disorder*

Hypoglycaemia

Dr Shivani Misra

**Objectives:**

To understand

* The clinical presentation of hypoglycaemia
* Its diagnosis and investigation.
* Maintenance of blood glucose levels
* Reactive and non-fed hypoglycaemia

**What is hypoglycaemia?**

1. **Symptoms of hypoglycaemia (Whipple’s triad)**

Rapid fall in glucose: symptoms are adrenergic (hunger, tremors, palpitation, sweating, weakness, anxiety).

Gradual fall: may include somnolence, poor coordination, poor concentration, focal neurological signs to seizures and coma.

Chronic hypoglycaemia may present as personality changes, defective memory, psychosis and/or dementia.

1. **In the presence of low plasma glucose**. Commonly used cut-off is < 2.5 mmol/l, individual thresholds vary between 2.3 – 3.0 mmol/l.
2. **Symptoms are relieved by glucose administration.**

**Neuroglycopaenia – the importance of glucose**

Basic metabolic rate = 2,000 kCal/day of which the brain needs 400 kCal/day in the form of glucose.

Carbohydrate stores:

* ECF glucose 15 g = 60 kCal
* Muscle glycogen 400 g = 1,600 kCal (cannot be exported as glucose).
* Liver glycogen 100 g = 400 kCal

In the non-fed state glucose is spared by other tissues using free fatty acids for energy and brain adapts to use ketone bodies to supply 50% energy during fasting.

**Mechanisms of maintaining blood glucose**

* Glucose transporters:
  + Brain – high affinity, low capacity, Liver/pancreas - low affinity, high capacity, Muscle/adipocytes - insulin dependent
* At blood glucose >6.0 mmol/l
  + High hepatocyte glucose stimulates glycogen**esis** and fat synthesis (VLDL)
  + Glucose entering pancreas stimulates release of preformed **insulin**.
  + Glucose passes into muscle and adipocytes
* At blood glucose c. 4.6 mmol/l
  + Low intracellular hepatocyte glucose stimulates glycogenolysis.
  + Low insulin limits glucose passage into muscle and adipocytes.
  + Insulin-dependent lipase inhibition is lifted permitting lipolysis
* At blood glucose c. 3.8 mmol/l
  + Pancreatic **glucagon** stimulates hepatic gluconeogenesis from glycerol/amino acids.
  + The liver starts to produce ketone bodies (beta-hydroxybutyrate/acetoacetate)
  + Rate of glucose passage into brain begins to fall
* At blood glucose c. 3.0 mmol/l
  + **Cortisol** further stimulates hepatic gluconeogenesis
  + **Adrenalin** and **GH** further stimulate lipolysis (oppose insulin)

Ketone body synthesis is controlled by a positive feedback on pancreatic insulin release.

**Drug-induced hypoglycaemia**

Effects more profound in liver/renal disease

* Alcohol, quinine, suphonylureas and pentamidine all stimulate insulin

release with the latter associated with eventual destruction.

* Salicylates (mechanism unknown)
* Non-selective beta blockers prevent glucagon-mediated   
  glycogenolysis/gluconeogenesis.
* In the diabetic patient, insulin-induced hypoglycaemia may be the result of excess administration, insufficient carbohydrate intake or excess exercise. Patient may not be aware of symptoms; it has been suggested that HbA1c levels below 5.2% (DCCT aligned) indicate a danger of hypoglycaemia.

**Autoimmune Causes:**

Insulin receptor-binding antibodies may occur in autoimmune disease such as SLE, primary biliary cirrhosis or Hashimoto’s.

Autoimmune insulin syndrome in which antibodies are directed to insulin has been reported, rarely, most subjects have been Japanese].

**Reactive, or fed, hypoglycaemia**

Accelerated gastric emptying causing amplified insulin release or early diabetes,

Rarely needs investigation, best diagnostic tool is the mixed meal test.

Exception is in paediatric population because of IMD e.g. galactosaemia and fructose-1-phosphate deficiency

**Hypoglycaemia with inappropriate insulin response (insulin high, C-peptide high)**

* Islet cell hyperplasia Diabetic mother

PHII

Beckwith-Weidemann

* Insulinoma presents with symptoms of chronic hypoglycaemia

80% benign solitary adenoma

4-8% MEN type 1 (parathyroid, pancreatic, pituitary).

**Hypoglycaemia with appropriate insulin response (insulin low, C-peptide low)**

* Non-islet cell tumour (NICT) due to big-IGF II secreted by mesenchymal/haematopoietic tumours.
* Adrenal insufficiency (Addison’s crisis or CAH)
* Pituitary failure (neonates/children and elderly)
* Isolated ACTH or GH deficiency.
* Alcohol induced (due to low gluconeogensesis, ketones high)

Inherited metabolic diseases:

Carbohydrate disorders (including GSD Type 1)

Fatty acid oxidation defects

**Pancreatic pro-insulin is broken down to insulin and C-peptide. High insulin with low C peptide suggests exogenous administration but note that insulin and C-peptide are unstable in blood.**

**NB:Hypoglycaemia is a metabolic emergency / get the samples / then TREAT**

Coagulation and Haemostasis

Dr Abdul Shlebak

**Learning objectives**

After studying this section you should confidently be able to:

* Name the main systems involved in preventing and arresting blood loss.
* Outline the contribution of the vascular system to haemostasis
* Outline the contribution of platelets to haemostasis
* Describe the classical and modern theories of blood coagulation
* Outline blood coagulation
* Outline the fibrinolytic system and its potential clinical application
* Understand the mutual interdependence of the various systems involved in haemostasis

**NORMAL HAEMOSTASIS**

**The understanding of normal haemostatic mechanisms can not be overemphasised.   
It provides an insight to the contribution of different components of the system, helps the diagnosis and the appropriate treatment of patients with haemostatic failure.**

Blood coagulation (haemostasis) is a host defence mechanism that protects the integrity of the vascular system after tissue injury. It is responsible for minimising blood loss. It is critical that blood clot formation in response to a breach in the vascular endothelium occurs rapidly but without systemic activation of the coagulation cascade or extensive local extension of thrombosis resulting in vascular occlusion.

**Q1. The normal haemostatic response to vascular damage depends on co-ordinated interactions between (what are the three major components?):**

a…………………………………………………………………………………………

b…………………………………………………………………………………………

c…………………………………………………………………………………………

An immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the injured area. The reduced blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines and thrombaxane A2 from platelets, and the fibrinopeptides produced during fibrin formation, may also have vaso-constrictive activity.

**Q2. Discuss the role of platelets in the initial phase of the haemostatic mechanisms?**

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**platelet plug by fibrin** Thrombin generated at the site of injury converts soluble fibrinogen into fibrin and also potentiates platelet aggregation and secretion. Thrombin also activates factor XI which amplifies the intrinsic pathway activity. Furthermore, it activates factor XIII which covalently cross-links the fibrin meshwork. A meshwork of fibrin anchors and extends the platelet plug. The fibrin component increase. As the fused platelets autolyse and after a few hours the entire haemostatic plug is transformed into a solid mass of cross-linked fibrin. During the same time frame the plug begin to autodigest due to the incorporation of plasminogen and tissue plasminogen-activator (t-PA) in the plug resulting in plasmin generation (see fibrinolysis).

**Q3. The coagulation cascades are of fundamental importance in understanding haemostasis, discuss the coagulation pathways with special reference to the intrinsic and extrinsic components.**

**Intrinsic pathway (flow chart) Extrinsic pathway (flow chart)**

………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………

Recent evidence suggests that the contact factor system does not play any role *in vivo* coagulation. The dominant pathway for blood coagulation is via factor VII which is activated by tissue factor. Activation of factor IX is mainly by factor VII. Factor XI *in vivo* is activated by thrombin and is important only at sites of major trauma or operation. Describe the main differences between in vivo and in vitro coagulation.

**Q4. a) Elaborate on the current understanding of in vivo and in vitro coagulation mechanisms**

**b) why the old model is unsatisfactory in explaining *in vivo* coagulation and c) What is the role of vitamin K?**

***In vitro* coagulation *In vivo* coagulation**

……………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………

***HAEMOSTATIC REGULATION***

**Q 5. a) Discuss the physiological role of the fibrinolytic pathway.**

…………………………………………………………………………………………………………………………………………………………………………………………………………………………

**READ:**

Pasi. J. The Coagulation Cascade-Old and New, *CME Bulletin Haematology* 1997; **1**: 3-4.

**b) How is unchecked thrombus formation prevented? c) Enumerate the known natural anticoagulants.**

**NORMAL HAEMOSTASIS**

1.

1. Vessel wall (endothelium and subendothelium): The active role of endothelial cell in preserving vascular integrity is well established. This cell provides the basement membrane , collagen, elastin and fibronectin of the subendothelial connective tissue. Loss or damage to the endothelial lining results in both haemorrhage and activation of the haemostatic mechanism. The endothelial cell also has an active role in haemostatic response. Synthesis of tissue factor, prostacyclin, vWF, plasminogen activator, antithrombin III and thrombomodulin, the surface protein responsible for activation of protein C, provides agents which are vital to both platelet reaction and blood coagulation.
2. Platelets: Are derived from the cytoplasm of bone marrow megakaryocytes and are the smallest of blood cells. They are disc shaped, anucleate cells with relatively complex internal structure reflecting its specific haemostatic function. The normal platelet count is 150-400 x 109/l.

The platelets contain the following granules with their specific biological functions:

|  |  |  |
| --- | --- | --- |
| **Location** | **Compound** | **Function** |
| **α granule** | Platelet factor 4 | Neutralizes heparin effect |
| β-thromboglogulin | Promotes fibroblast chemotaxis |
| Platelet derived growth factor | Mitogen for fibroblast;  Chemotaxis for neutrophils, fibroblasts and smooth muscles |
| von Willebrand factor | Adhesion molecule; carrier for VIII protecting it from proteolysis |
| Thrombospondin | Promotes platelet-platelet interaction |
| Fibroenctin | Adhesion of platelets and fibroblasts |
| **Dense granule** | ADP | Aggregation of platelet |
|  | ATP | Source of ATP |
| Serotinin | Vasoconstriction |
| Calcium | Coagulation; platelet function |

The contents of both α- and dense granules may be released via a system of surface-connecting tubules, during platelet function.

1. Circulating proteins with procoagulant, anticoagulant and fibrinolytic activities.

**2.** **Platelet reactions and primary haemostatic plug formation:**

Following a break in the endothelial lining, there is an initial adherence of platelets to exposed connective tissue (Fig. 1). Both platelets and vascular endothelial cells contain biochemical pathways for the metabolism of arachidonic acid (Fig. 2). The platelet adhesion is potentiated by von Willebrand's factor (vWF). Collagen and thrombin generated at the site of injury cause the adherent platelets to release their granules which include ADP, serotonin, fibrinogen, lysosomal enzymes and heparin-neutralising factor (PF-4). Collagen and thrombin activate platelet prostaglandin synthesis leading to the formation of thromboxane A2 which potentiate platelet release reactions, platelet aggregation and also has powerful vasoconstrictive ability. Released ADP causes platelets to swell and aggregate. Additional platelets from the circulating blood are drawn to the area of injury which continues the growth of the haemostatic plug which soon covers the exposed connective tissue. Released platelet granule enzymes, ADP and thrombosthenin may all contribute to the consolidation of the accumulated platelet plug. Prostacyclin, produced by endothelial and smooth muscle cells in the vessel wall adjacent to the area of damage , is important in limiting the extent of initial platelet plug. This unstable plug produced usually sufficient to provide temporary control of bleeding. Definitive haemostasis is achieved when fibrin formed by blood coagulation is added to the platelet mass and by platelet-induced clot retraction.

**Fig. 1. Scheme of haemostasis**.  
**Fig. 2 Metabolism of Arachidonic Acid**



**3. Coagulation**

The coagulation cascade involves sequential activation of a number of blood clotting factors resulting in the formation of fibrin. Figures 3 & 4 shows how the coagulation operates both *in vitro* (Fig. 3) with the classical waterfall model whereas (Fig.4) shows what is thought to occur *in vivo*.

Conventionally, the coagulation cascade has been divided into intrinsic, extrinsic and final common pathways. Intrinsic pathway: the negatively charged subendothelium activates factor XI, which in turn leads to activation of factor XI, which activates factor IX. In association with calcium and the cofactor VIII, activated factor IX activates factor X on the membrane surface provided by platelet phospholipid (platelet factor 3). The intrinsic pathway is mediated via the contact factor system; following limited activation, factor XII activates prekallikrein to kallikrein, which in turn activates factor XII reciprocally and fully. High molecular weight kiniogen (HMWK) is a nonenzymatic accelerator of these interactions. Extrinsic pathway: tissue factor activates factor VII which in turn activates factor X. Final common pathway: activated factor X in association with cofactor factor V on phospholipid surface and calcium converts prothrombin to thrombin. Thrombin converts fibrinogen to fibrin.

**Fig 3.**



***4. a) In vivo* vs. *in vitro* coagulation (The role of factor VII-tissue factor complex)**

The classical waterfall hypothesis described above fails to represent accurately what happens *in vivo* haemostasis. This is may be shown by considering the following points. Firstly patients that have an inherited deficiency of factor XII, prekallikrein or HMWK have no clinical bleeding problems, yet have extremely prolonged aPTTs. This clinical observation demonstrates that these proteins are probably not important components of blood coagulation *in vivo*. Similarly factor XI deficiency is not always associated with bleeding and its role therefore unclear, whereas patients with factor VII deficiency bleed abnormally, although the intrinsic pathway is intact. Thirdly factor VII-tissue factor is known to activate not only factor X but also factor IX. In the classical pathway this activation is not required. Tissue factor is a natural constituent of many non-vascular cells. Tissue factor on such cells is able to initiate blood coagulation. These points suggest a more central role for the tissue factor-F VII complex.

**b) The revised hypothesis of coagulation**

The findings of direct activation of factor IX by tissue factor-factor VII led to the revision of the coagulation cascade, with tissue factor-factor VII and factor X central to the model. This model also takes into account the novel feedback inhibition of factor-VIIa-tissue factor produced by tissue factor pathway inhibitor (TFPI). Fig 4. is believed to represent more accurately the processes that occur   
*in vivo*.

**Fig 4.**

**c) The role of vitamin K in blood coagulation:**

Coagulation factors II, VII, IX and X as well as protein C and protein S are dependent on vitamin K for their normal function. These are synthesized in an inactive form that cannot bind calcium ions. This ability is confered by a post-translational modification which involves γ carboxylation of glutamic acid residues. Vitamin K *in vivo* continuously cycles between three forms: vitamin K quinone, vitamin K hydroquinone and vitamin k epoxide. The  carboxylation reaction is coupled to the conversion of vitamin K hydroquinone to the epoxide form. Thus in vitamin K deficiency,  carboxylation fails and a non-carboxylated forms of factors II, VII, IX and X and protein C and protein S are released into the circulation. Although they are immunologically identical to the normal proteins, these proteins induced by vitamin K absence or antagonism (PIVKAs) cannot bind calcium ions, and thus cannot bind to phospholipid surfaces.

**FEEDBACK TO SECTION II:**

**REGULATION OF HAEMOSTASIS**

**5. a) Fibrinolysis:**

Fibrinolysis (like coagulation) is a normal haemostatic response to vascular injury. Deposition of fibrin is accompanied by activation of fibrinolysis (Fig 5). Fibrinogen and fibrin are substrates for the proteolytic action of plasmin. Unlike the highly specific action of thrombin on fibrinogen, which results in the cleavage of only two pairs of tony fibrinopeptides A and B, plasmin cleaves fibrinogen and fibrin at multiple sites producing a variety of split (degradation) products. Plasmin is normally present in its inactive zymogen form, plasminogen, in blood, urine, and tissue fluids. Major activation of the fibrinolytic system follows the release of the tissue plasminogen activator (t-PA) from endothelial cells. t-PA is a serine protease that binds to fibrin. This enhances its capacity to cinvert thrombus-bound plasminogen into plasmin. This fibrin dependence of t-PA action strongly localizes plasmin generation by t-PA to the fibrin clot. Release of t-PA occurs after such stimuli as trauma, exercise or emotional stress. Activated protein C stimulates fibrinlysis by destroying plasmin inhibitors of t-PA. Therapeutic t-PA and urokinase are produced by recombinant DNA technology. The fibrinolytic agent streptokinase is a peptide produced by haemolytic streptococci. It forms a complex with plasminogen, which converts other plasminogen molecules to plasmin.

Plasmin has a wider range of activity than thrombin, hydrolysing both arginine and lysine peptide bonds in a wider range of substrates.

Tissue plasminogen activator is inactivated by PAI-1. Circulating plasmin is inactivated by potent inhibitors a2-antiplasmin and a2macroglobulin. This prevents widespread destruction of fibrinogen and other coagulation factors.

**Fig 5. Overall reactions of fibrinolysis.**

**b) Natural anticoagulants**

The major naturally occurring anticoagulant pathways that prevent unchecked thrombus formation include:

1. Antithrombin III: anti-thrombin III (AT-III) is a single-chain glycoprotein (61000 daltons) which is synthesized in the liver and endothelium. ATIII is the main physiological inhibitor of activated coagulation serine proteases (Fig 6). It inactivates thrombin, activated factor X, IX, XI.   
   ATIII activity is greatly accelerated (2000-fold) by heparin. Because of this ATIII is sometimes known as heparin co-factor I.
2. Heparin co-factor II: is a single chain glycoprotein which is also synthesized in the liver. It complexes with thrombin in a 1:1 stoichiometric ratio, thereby inactiating it. In contast to ATIII, heparin cofactor II is specific for thrombin, having no inhibitory activity against the other serine proteases. Its activity is amplified 1000-fold by heparin.



**Fig 6. Coagulation regulation by antithrombin.**

3) Protein C/S pathway: Protein C is a vitamin K dependent protein that plays a dual role in haemostasis by inhibiting blood coagulation and stimulating fibrinolysis. Upon activation by thrombin in the presence of a cofactor called thrombomodulin, activated protein C inhibits the coagulation cascade by incativating factor VIIIa and factor Va, thereby reducing the rate of thrombin generation (Fig 7). Protein S is required as a cofactor for protein C activity. Thrombomodulin is present in tight association with vascular endothelium. It forms complexes with thrombin in a 1:1 stocihiometeric ratio. Complexed thrombin activates protein C several thousand times faster than free thrombin, but does not clot fibrinogen, activate factors V and VIII or aggregate platelets. Thrombomodulin-bound thrombin can still be inhibited by ATIII.

Protein S is a single chain glycoprotein synthesized in the liver and endothelium. It is a vitamin K dependent protein but is not a serine protease. Activated protein C complexes with protein S and calcium ions on platelets and at the endothelial surface. The inhibitory activity of complexed protein C is greatly amplified.

A schematic representation of the two principle anticoagulant pathways, known to be important in the regulation of coagulation proteinase activity. On the left side of each diagram is a simplified view of the coagulation cascade with its “procoagulant” feedback lops by which thrombin activates factors V and VIII. To the right, are the “anticoagulant” pathways by which excessive activation of coagulation is prevented. These pathways involve antithrombin (which directly inhibits the coagulation proteins such as factor Xa and thrombin), and PC/PS (which inactivates factors Va and VIIIa).



**Fig 7. Coagulation regulation by protein C/protein S.**

4) Tissue factor pathway inhibitor (TFPI) is emerging as the most important regulatory mechanism in *vivo* coagulation. TFPI is synthesized by the endothelial cells and circulate in plasma bound to low density lipoproteins. It is also present in platelets and bound to heparan sulphate at the endothelial surface. TFPI inhibits coagulation by binding to factor Xa and TF:VIIa complex, thereby inhibiting their proteloytic activity.

**Fig 8. Coagulation regulation by TFPI**

Fig 8 demonstrates the downregulation of tissue factor pathway activity. Neither components of the clot initiating complex (TF + VIIa) can be inhibited separately. A natural plasma component, tissue factor pathway inhibitor (TFPI), halts continued direct generation of factor Xa. Thus, continued Xa formation early becomes dependent on ongoing activation of factor X by the Ixa-VIIIa-phospholipid complex.

**SUMMARY**

* In health, a dynamic equilibrium exists between mechanisms that promote clot formation and those that oppose cot formation.
* Substances such as adrenaline, ADP, kinins and thromboaxanes mediate vasoconstriction.
* Vascular endothelium is the major source of vonWillbrand factor (vWF), thrombomodulin and tissue factor pathway inhibitor (TFPI).
* Vascular endothelium produces prostacyclin, a vasodilator and potent inhibitor of platelet aggregation.
* Vascular endothelium expresses TF, the primary activator of blood coagulation.
* There are 4 phases of platelet haemostatic function: adhesion to the surface, shape change, release of granule contents and aggregation.
* Platelet activation rapidly results in the formation of a primary haemostatic plug.
* The coagulation factors are synthesized primarily in the liver, although vWF is produced by the endothelial cells and megakaryocytes.
* Coagulation factors II, VII, IX, X, protein C and protein S are dependent on vitamin K for their normal function. In vitamin K deficiency inactive forms are produced called PIVKAs (proteins in vitamin K absence).
* The classical concept of coagulation has been superseded, although it remains important for the interpretation for laboratory results.
* In the current model, coagulation is initiated by contact between factor VII or VIIa and tissue factor which is expressed by subendothelial cells exposed at sites of vascular damage.
* The major inhibitors of the blood coagulation pathway are ATIII, heparin cofactor II, PC/PS and TFPI.
* The most important function of the fibrinolytic system is the degradation and dissolution of formed fibrin within the circulation

Venous Thrombosis

Prof Mike Laffan

# Learning Objectives

* Understand why some people get thrombosis in certain circumstances
* Be able to identify high risk individuals and high risk circumstances
* Understand the principles of prevention and treatment of thrombosis.
* Be able to choose appropriate therapeutic agents for prevention and treatment
* Therapeutic aims: understand how to achieve the optimum balance between risk of thrombosis and risk of bleeding for each patient.

# Why do (some) people get thrombosis?

There are several ways of answering this question. Firstly, from a pathological point of view we still rely on Virchow’s triad, which says that thrombosis arises as a result of factors that can be considered in three groups:

1. **The vessel wall.**

The vessel wall normally presents an anticoagulant surface to the blood. This is because it expresses numerous molecules that participate in anticoagulant mechanisms:

|  |  |
| --- | --- |
| **Molecule** | **Function** |
| Thrombomodulin (TM) | Directs thrombin to activate protein C |
| Endothelial protein C receptor (EPCR) | Presents PC to Thrombomodulin |
| Tissue factor pathway inhibitor (TFPI) | Inhibits Tissue factor |
| Prostacyclin (PGI2) | Inhibits platelet activation |
| Nitirc Oxide (NO) | Inhibits platelet activation |

Similarly, it does not normally express Tissue factor, thromboxane and adhesion molecules, all of which tend to favour coagulation.

This pattern is reversed by **inflammation** (ie thrombomodulin is down regulated and adhesion molecules are expressed)**.** Inflammation is the principal change in the endothelium that increases the likelihood of thrombosis. It may result from infection, trauma or inflammatory disorders such as autoimmune vasculitides or malignancy.

1. **The blood**

We know most about the blood because it is obviously easiest to study, but even here we tend to ignore the roles of platelets and leucocytes about which we know relatively little. However elevated platelet counts do increase the risk of thrombosis.

We usually regard plasma as existing in a balance between pro and anticoagulant forces. This allows us to understand that an increased risk of thrombosis arises from either:

* **A *reduced* level of *anti*coagulant factors eg:**
* Protein C
* Protein S
* Antithrombin

OR

……..

* **An *increased* level of *pro*coagulant factors eg:**
* Factor VIII
* Prothrombin
* Fibrinogen

Another important plasma variation causing and increased risk of thrombosis is Factor V Leiden. This polymorphic variant of Factor V is resistant to the action of activated Protein C and so results in an increase in procoagulant activity. In general the potency of these traits is in inverse proportion to their prevalence. Factor V Leiden is common, affecting 2-5% of the white population but is quite mild, increasing thrombotic risk by a factor of approximately 5.

The lupus anticoagulant is another plasma factor which causes an increased risk of thrombosis by a mechanism believed to involve endothelial activation.

1. **Flow**

Reduced blood flow due to compression or immobility, increases the risk of thrombosis via:

* Accumulation of activated coagulation factors
* Enhanced platelet adhesion.
* Increased endothelial activation both directly and secondary to hypoxia

**However**, it is sometimes useful to divide these contributory factors into 1) Patient related factors (eg Factor V Leiden or malignancy) and 2) those arising from circumstance (eg the type of surgery or pregnancy)

# The sum of thrombotic risk.

We can use the information from Virchow’s triad to sum up congenital, acquired and circumstantial factors and thus identify people at high risk of thrombosis. Another extremely important acquired risk factor not obvious from the above is age: the risk of thrombosis increases steeply in the elderly.

# Why are we interested in factors affecting thrombotic risk?

1. Thrombosis has serious sequelae
   1. Approximately 5% of events are fatal
   2. The recurrence rate is approximately 4% per annum
   3. Over 20% get severe thrombophlebitic syndrome (chronic swelling, pain and ulceration)
   4. 4% will get symptomatic pulmonary hypertension after 2 years
2. They help us to intervene with anticoagulants to prevent thrombosis at times of high thrombotic risk or in individuals whose individual risk is high.
3. They help us decide when long term anticoagulation is indicated.

# Anticoagulation

The principal anticoagulants in hospital practice remain heparin and warfarin. Their properties are contrasted below.

|  |  |  |
| --- | --- | --- |
|  | **Heparin** | **Warfarin** |
| Onset | Immediate | Delayed |
| Action | Cofactor for AT | Vitamin K antagonist |
| Administration | Parenteral | Oral |
| Monitoring | LMWH – none (or anti Xa)  UFH – APTT | INR |
| Pregnancy | Safe | Teratogenic |
| Half life | 1-2 hours | >24 hours |
| Reversal | LMWH – protamine (partial)  UFH – protamine | Vitamin K  Factor concentrates |

Thus their properties are complementary: heparin is used for immediate effect and warfarin for outpatient control in the longer term. Their principal side effect is the predictable increase in haemorrhagic risk. Heparin has the additional problems of Heparin Induced Thrombocytopenia (HIT) and osteoporosis.

New anticoagulants that are active orally and which do not require monitoring are now becoming available. Two of these, Rivaroxaban – a direct inhibitor of Factor Xa and Dabigatran – a direct inhibitor of thrombin, are now licensed for prevention of thrombosis after hip and knee replacement. They are likely to be more widely used over the next few years.

# Management

Of a thrombotic event:

* Immediate anticoagulation is essential. Therefore treatment is begun with heparin (preferably low molecular weight heparin: LMWH). Warfarin can be started simultaneously but takes several days to take effect at which time the heparin can be stopped. Warfarin anticoagulation effect is measured using the INR and needs regular monitoring. Warfarin is continued usually for 3-6 months but three months may be sufficient for post surgical thrombosis.

Prevention of thrombosis:

* New guidelines demand that all patients admitted to hospital (including day cases) are assessed for their risk of thrombosis.
* Patients at increased risk of thrombosis should receive chemical and/or mechanical prophylaxis. Mechanical prophylaxis alone (TED stocking or intermittent compression devices) can be used in patients who also have an increased risk of bleeding. In our Trust the chemical prophylaxis is Tinzaparin 4500u sc od.

In both cases we balance haemorrhagic risk of therapy against morbidity and mortality of thrombosis.

Blood Transfusion

Dr Megan Rowley  
(original handout © Dr Fiona Regan)

**Objectives:**

1. Explain Haemolytic Disease of Newborn & anti-D
2. Describe Adverse Reactions to Transfusion and the role of the patient information leaflet on Transfusion

**1. Haemolytic Disease of the Newborn (HDN) and the use of anti-D**

A person may form red cell antibodies either through blood transfusion or if fetal red cells enter a woman’s circulation during pregnancy or at the time of delivery (not uncommon). Only IgG antibodies can cross the placenta. If the maternal antibody level is high, it can destroy fetal red cells if they have the corresponding blood group antigen. This causes the fetus to be anaemic and red cell destruction causes a build up of bilirubin (jaundice). This is called Haemolytic Disease of the Newborn. The antibody most often responsible for serious HDN is anti-D - hence the reason for always transfusing Rh negative blood to Rh negative females of childbearing age (ie: <50 years). So now, anti-D is usually due to RhD negative women carrying an RhD positive fetus.

Rh D is the most important cause of severe HDN and is also the only one for which we have preventative treatment. Other blood groups on fetal cells can also cause HDN eg: anti-c (another Rh antibody) and anti-K (antibody to the K blood group antigen). IgG ABO antibodies can also cause HDN but this is usually not severe.

**Prevention of anti-D formation**

Sensitisation of the mother can be prevented by giving her intra-muscular anti-D immunoglobulin (anti-D Ig) injections at times when she is at risk of a feto-maternal haemorrhage

1) at delivery if the baby is RhD positive. RhD positive (fetal) red cells become coated with anti-D Ig and are then removed by the reticulo-endothelial system of the spleen, before they can sensitise the mother to produce anti-D antibodies. For it to be effective, the anti-D Ig must be given within 72 hours of the sensitising ‘event’. Anti-D immunoglobulin only works if the mother is not already sensitised.

1. 500iu of anti-D is capable of preventing sensitisation from 4 ml of Rh D positive red cells (8 ml of blood) that enter the Rh D negative woman’s circulation. A larger anti-D Ig dose may be required when a larger fetal bleed is demonstrated. So a Kleihauer test (which tests for fetal haemoglobin-containing red cells) is undertaken after delivery to check whether the fetal bleed was >4ml or red cells.

2) Anti-D should be given during pregnancy to RhD negative women, if any of these sensitising events occur:

* therapeutic abortions at any time during pregnancy
* spontaneous miscarriages that require medical/surgical evacuation of uterus any time during pregnancy.
* other sensitising events eg: amniocentesis, external version, abdominal trauma and stillbirths.

Dose: 250 iu anti D if <20 weeks ‘gestation’; 500 iu and Kleihauer test (to see if more needed) if > 20 weeks gestation.

3) Routine Antenatal Prophylaxis - anti-D Ig given at 28 and 34 weeks gestation has been shown to significantly reduce the sensitisation, due to ‘silent’ bleeds in the 3rd trimester, but not all hospitals do this yet.

All pregnant women are tested early in pregnancy for ABO and RhD blood group and antibodies (eg: anti D). This is repeated at 28 weeks gestation, to see if new antibodies have developed during pregnancy. If antibodies present, levels are monitored regularly and the fetus is monitored by scanning, to look for anaemia. Intra-uterine transfusions can be given to the fetus at regular intervals if necessary (but this is risky).

**2. Adverse Reactions to Transfusion**

**Administering Blood Transfusion** - Follow written procedures ON WARDS – for checking blood before connecting up to patient.

All blood products must be administered using a special blood giving set which incorporates a filter, to ensure that fibrin strands and large white cell aggregates are not infused.

**No drugs or other infusion solutions should be added to any blood component as haemolysis or drug interactions may occur.**

**Monitoring of patients during transfusion**

Before starting the transfusion, the patient’s temperature, pulse and blood pressure should be recorded then repeated within 15 minutes of starting the transfusion, then hourly after.

Throughout the transfusion, the patient should be observed for any signs of a reaction, such as fever, rigors, flushing, urticaria, vomiting, itching, dyspnoea, headache, pain at or near the transfusion site or circulatory failure. If these occur, the transfusion must be stopped immediately and a haematology doctor informed or local transfusion policy followed. Generally, the IV line should be kept open with crystalloid to maintain BP and renal function. If it is proved that the signs and symptoms are not due to the blood, transfusion can be restarted. If the signs or symptoms are mild, such as urticaria, transfusion can be resumed after the side effects have been treated, e.g. with antihistamines.

**NB:** The two main causes of an immediate severe reaction are: 1) Wrong (ABO incompatible) blood and   
2) Bacterial infection of the blood. So in such an event, always 1) check the patient’s details on their wristband against the ‘compatibility label’ on the blood bag and 2) check the patient for severe sepsis (temperature etc)

**Note:** to prevent bacterial infection from blood, before transfusion:

1. Check pack is intact (no tears or pin-holes)
2. Blood transfusion must be started within 30 minutes of removing packs from Blood Bank fridge – as blood warming up can allow bacterial multiplication. Blood should be removed from Blood Bank one unit at a time, if out of fridge for more than 30 minutes it must not be put back into Blood Bank to be used later.
3. Each red cell unit should be infused in less than 6 hours
4. Do not routinely warm units of blood before transfusion (if needed in massive transfusions, do so through a special blood warmer during the transfusion)
5. Examine blood packs for evidence of haemolysis (e.g. purple red cell mass, brown or red plasma) or clotting. If reaction due to bacteria is suspected, broad-spectrum antibiotics should be started and the transfusion lab alerted.

|  |  |  |
| --- | --- | --- |
|  | **Immediate** | **Delayed** |
| **Immune** | * ‘Wrong blood’ ABO incompatible * Febrile non-haemolytic * Allergic/ anaphylaxis * TRALI | * DHTR (red cell antibodies) |
| **Non-Immune** | * Bacterial infection | * Viral infections + other * Iron overload |

**A) Immediate Transfusion Reactions**

**1. Immediate haemolytic transfusion reactions due to ABO incompatible blood**

**Symptoms:** Restlessness, a feeling of oppression, chest pain, vomiting, abdominal or flank pain and facial flushing are common. Haemoglobinuria occurring during the transfusion indicate severe haemolysis. Bacterially contaminated blood has similar effects.

**2. Febrile non-haemolytic transfusion reactions** - due to white cell antibodies in the patient which can react with white cells in the donor's blood, causing fever and rigors. This is less common since leucodepletion of all donor blood.

**3. Allergic reactions:** range from urticaria to anaphylaxis - due to a reaction between a foreign protein (e.g. from pollen or milk) present in the donor's plasma and the corresponding antibody in the recipient. Urticaria is usually mild, but if severe the transfusion must be stopped and advice sought.

**4. Transfusion related acute lung injury (TRALI)** very rare and occurs if the donor's plasma contains potent white cell antibodies incompatible with the recipient's white cells, transfusion may cause a severe reaction characterised by chills, fever, a dry cough and breathlessness with cardiac failure.

TRALI is uncommon. Cardiac failure due to volume overload produces similar symptoms and is more common.

**Non-Immunological acute adverse effects of transfusion**

**5. Bacterial complications of transfusion** - these are relatively rare in the UK, but still more common than viral infections. Measures to minimise bacterial infection include the use of disposable collection sets, clean techniques, and processing in closed systems. Also citrate and the bactericidal powers of blood in addition to cold storage of red cells and plasma will destroy the vast majority of bacteria that may be introduced at collection. Bacterial contamination can be fatal.

**B) Delayed Transfusion Reactions**

**1. Delayed haemolytic transfusion reactions (DHTR)** - Many occur in patients who have antibodies (other than ABO), if specially selected blood is not given. Usually manifested by fever days to weeks after a transfusion, accompanied by a falling haemoglobin, and jaundice or haemoglobinuria.

**2. Iron overload** - each unit of blood has approximately 200mg of iron. Overload is a problem in patients requiring long term transfusions e.g. thalassaemia and aplastic anaemia.

**3. Infectious complications of blood transfusion** eg viral – hep B, CMV

1. Serious adverse events are reported to the SHOT scheme (Serious Hazards of Transfusion)
2. A Patient Information Leaflet can be used to inform patients of the risks and benefits of transfusion before they agree to have a transfusion

**Supplementary reading**

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

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

**Test Yourself**

1. What would you check on a patient’s wrist band to ensure correct patient identification when taking cross-match blood samples or before starting a blood transfusion?

Connective tissue diseases

Dr Mary Thompson

### Objectives

#### 1 To understand the term ‘connective tissue diseases’.

2 To know the clinical, immunological and pathological features of systemic lupus erythematosus and scleroderma.

3. To understand the difference between dermatomyositis and polymyositis

3. To know the classification of vasculitis and understand the term.

4. To know the main features of Kawasaki’s disease, polyarteritis nodosa and temporal arteritis.

5. To know the clinical, immunological and pathological features of sarcoidosis

## Multisystem diseases

## Defined by combination of clinical, pathological and serological criteria

## Autoimmune basis

# SYSTEMIC LUPUS ERYTHEMATOSUS

## Relatively common

## Young women (10:1)

## More common and more severe in afrocaribbeans

## Genetic factors

## Complement deficiences

## Drugs -hydrallazine, procainamide

## Immunology

## Immune complex disease

## Autoantibodies to: DNA histones nonhistone proteins bound to RNA nucleolar components phospholipid, smooth muscle, etc

## Complement activation

## B cell activation

# *Clinical*

## 4 of 11 criteria needed for diagnosis

## Skin rash, arthritis, serositis, renal involvement, heart, CNS, haemopoietic system

## Antiphospholipid antibodies -thromboses, recurrent miscarriages

# SCLERODERMA

## Systemic sclerosis

## M:F ratio1:3

## 20-50

## Unknown cause

## Immunology

## Antibodies to DNA topoisomerase (diffuse)

## Anticentromere antibody (CREST)

## Nucleolar pattern fluorescence

# *Pathology*

## Fibrosis

## ‘Onion skin’ intimal thickening of small arteries

## Two clinical groups: diffuse limited (CREST)

## Skin - Raynaud’s phenomenon, fibrosis

## GIT, joints, muscles, lungs, kidneys, heart

# OTHER CONNECTIVE TISSUE DISEASES

## Mixed connective tissue disease

## Polymyositis

## Dermatomyositis

# VASCULITIS

# Classification

## Giant cell arteritis

## Takayasu’s arteritis

## Polyarteritis nodosa

## Kawasaki’s disease

## Wegener’s granulomatosis

## Churg-Strauss syndrome

## Microscopic polyarteritis

## Henoch Schonlein purpura

## Cryoglobulinaemia

## Buerger’s disease

# immune mechanisms

## Immune complexes: DNA-antiDNA hepatitis B hepatitis C

## ANCA (antineutrophil cytoplasmic antibodies): C-ANCA P-ANCA

## ANCA titres useful indicator of activity

# KAWASAKI’S DISEASE

## Febrile disease of childhood ?viral

## Clinical

## Fever

## Erythema of palms and soles with later desquamation

## Non purulent conjunctivitis

## Rash

## Lymphadenopathy

# *Pathology*

## Vasculitis - microscopic polyangiitis

## Coronary arteries affected

## 0.5-1% die of myocardial infarct

# POLYARTERITIS NODOSA - CLASSICAL

## Males M:F 2:1

## 30% hepatitis B Ag +ve

## Affects medium to medium & small size arteries

## Any organ but most common are: kidneys (80%) heart (70%) liver (65%) GIT (50%)

# *Clinical features*

## PUO, weight loss

## Muscle aches

## Neuropathy

## Haematuria, renal failure

## Hypertension

## Abdominal pain, malaena

# *Radiology*

## Nodular appearance to medium sized vessels on angiography

# *Pathology*

## Microscopically necrotising arteritis with inflammation

## Polymorphs, lymphocytes, eosinophils

## Arteritis is focal and sharply demarcated

## Heals by fibrosis

# TEMPORAL ARTERITIS

## Elderly

## Commonest form of arteritis - 850/100,000 aged >80

## 50% have systemic involvement - polymyalgia rheumatica

## ESR usually markedly raised

## Danger of blindness - retinal artery

## Treatment is always urgent

# SARCOIDOSIS

## Multisystem disease of unknown cause characterised by non-caseating granulomas

## 10x more common in Afro-caribbeans and Africans

## CMI response to an unknown antigen

# *immune abnormalities*

## Raised CD4:CD8 cells in the lung ie actvation of T-helper cells

## Opposite in blood (T-helper cells reduced – may account for anergy in Heaf test)

## Lymphopenia

## Alveolar macrophages also in activated state resulting in hypercalcaemia and raised serum angiotensin converting enzyme

# *Clinical features 1*

## May be asymtomatic – X-ray diagnosis

## Weight loss, fatigue, fever

## Lungs – dry cough, dyspnoea

## Lymph nodes – hilar, head and neck

# *Clinical features 2*

## Skin – lupus pernio,

## subcutaneous,

## elevated erythematous plaques

## Eyes – iritis, iridocyclitis,

## choroiditis, retnitis, optic nerve

## Salivary glands – Mickulicz’s syndrome

# *Clinical course*

## 65 – 70% go into remission with minimal residual complications

## 20% permanent lung dysfunction or visual impairment

## 10 – 15% progressive lung fibrosis, cor pulmonale and death

# *Pathology*

## Non – caseating granuloma

## Langhans giant cells

## Schaumann bodies

## Asteroid bodies

## Epithelioid cells

## No necrosis usually

## Thin rim of lymphocytes – later fibrosis

# AMYLOID

## Deposition of an abnormal proteinaceous substance in non branching fibrils, 7.5-10nm diameter

## Beta-pleated sheet structure

## A variety of proteins can take on this conformation

## Resistant to enzymic degradation

# *Chemistry*

## May be: AA protein - derived from SAA (acute phase reactant) AL - derived from light chains Transthyretin Beta2-microglobulin Abeta2 protein - Alzheimer’s Insulin, calcitonin

## Always contains P component normal serum alpha-1 glycoprotein pentagonal structure only a minor constituent

## Stains with Congo red dye

## Shows apple green birefringence under polarised light

# *Clinical*

## Proteinuria, renal failure

## Restrictive cardiomyopathy, arrhythmias

## Autonomic neuropathy

## Carpal tunnel syndrome

## Macroglossia

## Bleeding on injury

## Also deposited in blood vessels, endocrine organs, liver, spleen

Neuropathology of Neurodegenerative Disorders   
and Multiple Sclerosis

Dr Federico Roncaroli

**Learning objectives**

1. Understanding of the basic pathologic reactions in neurodegenerative disorders
2. Understanding of principles of classification of neurodegenerative diseases
3. Understanding of principles of classification of demyelinating diseases
4. Knowledge of pathologic features of Multiple Sclerosis

**BASIC PATHOLOGIC REACTIONS OF THE CENTRAL NERVOUS SYSTEM**

**Axonal damage**: Axonal degeneration occurs as a consequence of direct insult, neuronal death or secondary to axonal transection. Primarily damaged axons appear to be wavy and swollen due to local accumulation of neurofilament protein. Axonal transport ceases leading to secondary death of the cell body. Pathological changes of axonal damage secondary to death of nerve cells are similar. Degeneration of distal axons when severed, called Wallerian degeneration starts within days after damage with breakdown of myelin followed by phagocytosis of myelin and axonal debris by macrophages.

**Demyelination:** Central myelin is an extension of the plasma membrane of oligodendrocytes. Myelin wraps axons to insulate them and allow depolarization to occur. Demyelination can be primary or secondary. Primary demyelination depends on genetic defects of myelin whereas secondary demyelination is caused by any inflammatory, toxic, metabolic cause that leads to breakdown of myelin sheaths.

**Gliosis**: Reactive gliosis is a non specific reaction of astrocytes to any parenchymal insult. When react, astrocytes increase their cytoplasm due to production of glial fibrillary acidic protein, the intermediate filament of glial cells. Reactive astrocytes appear a stellate cells or spindle cells with abundant fibrillary cytoplasm. This latter is called fibrillary gliosis.

**Senile plaques:** Senile plaques are complex spherical structures ranging in size between 4 to 200 microns involving the grey matter. They are sub-classified in neuritic and diffuse. Neuritic plaques consist of clusters of radially orientated abnormal axons and dendrites and often contain a core of amyloid. Astrocyte and microglia are often seen around plaques. Diffuse plaques are more amorphous and lack thickened neurites and amyloid cores.

**Neuronal inclusions:** Nuclear or cytoplasmic inclusions can be seen in physiologic and pathologic conditions. Marinesco bodies are small spherical nuclear inclusions seen in pigmented nerve cells of the substantia nigra. They more often occur in patients with liver insufficiency. In ageing brains, normal nerve cells contain lipofuscins, Hirano bodies, rod-like inclusions. Bunina bodies are the pathologic inclusions seen in Motor Neuron Diseases, Lewy bodies are seen in Parkinson’s disease and Dementia with Lewy bodies, Pick’s bodies are characteristic of Pick’s dementia, neurofibrillary tangles are predominantly observed in ageing, Alzheimer’s disease, progressive supranuclear palsy.

**Neurofibrillary tangles**: Neurofibrillary tangles are intracellular structures composed of two filaments wounded in a double helix. The major antigenic component is phosphorylated tau. Morphologically, NFTs occupy cells body and the apical dendrite and vary in shape depending of shape of nerve cells.

**Neuropil threads:** Accumulation of proteins such as tau or alpha-synuclein within axons appears with the formation of thread-like structures.

**Hypoxic cell changes:** Nerve cells are vulnerable to hypoxia. Neurons exposed to reduced oxygen supply undergo microvacuolation due to swelling of intracytoplasmic organelles, shrinkage of cell body with increase cytoplasmic acidophilia (red cells), condensation of chromatin and nuclear pyknosis, increased acidophilia of the nucleus. Particularly sensitive to hypoxia are pyramidal neurons of CA1 sector of hippocampus, pyramidal nerve cells of layers III and V of the neocortex and Purkinje cells in the cerebellar cortex. Hypoxic changes occur during ischaemia. The terms hypoxia and ischaemia describe different phenomena. Hypoxia, which means inadequate oxygenation, causes less damage of tissues than ischaemia because there is no reduction of metabolic substrates including glucose. Ischaemia is a condition where blood flow and therefore protein and glucose are inadequate to maintain normal cellular functions.

**NEURODEGENERATIVE DISEASES**

This is a complex and heterogeneous group of conditions that eventually lead to permanent and severe morbidity and pathologically characterised by loss of nerve cells accompanied by reactive astrocytosis and neuronal death often due to accumulation of abnormal proteins. Many neurodegenerative disorders lead to dementia.

**PROTEIN CAUSING DEGENERATION OF AFFECTED NEURONS**

**Tau protein:** Tau is a normal component of neuronal cytoskeleton. It is microtubule associated protein which stabilizes microtubules. Six isoforms have been identified. Function of tau is regulated by phophorylation. When unphosphorylated or hyperphosphorylated tau protein fails to bind microtubules favouring their aggregation in the cytoplasm. Mutations are reported in the chromosome 17-linked dementia.

**Beta-amyloid:** This polypeptide is derived from Amyloid Precursor Protein (APP). APP is a ubiquitously expressed membrane molecule, whose function still unknown. APP is cleaved by secretases, proteolytic enzymes regulated by presenilin. Amyloid accumulation results from defective cleavage of APP. For instance, is the fragment composed of 39-42 aa that accumulates in Alzheimer’s disease.

**Alpha-synuclein:** Alpha-synuclein is a small protein of 18 kDa, which is known to be associated to plasma membranes due to its ability for binding lipids. Function of alpha synuclein is unknown. Accumulation has toxic effect on plasma membranes Mutations reported in familial Parkinson’s disease.

**Ubiquitin:** Ubiquitin is a small conserved intracellular protein which binds other proteins targeting for degradation. After degradation ubiquitin is released and recycled.

**Prion protein**: Prion protein is a membrane associated glycoprotein whose function in neurons and astrocytes is sill unknown. Pathologic prion protein, called PrPsc represents the folded protein where tertiary structure is predominantly -sheet instead of -helic. Part of PrPsc is resistant to protease digestion and the 22 and 23-residue protease resistant polypeptides do accumulate causing death of nerve cells. Notably, PrPsc is able to induce conformation changes in normal PrP.

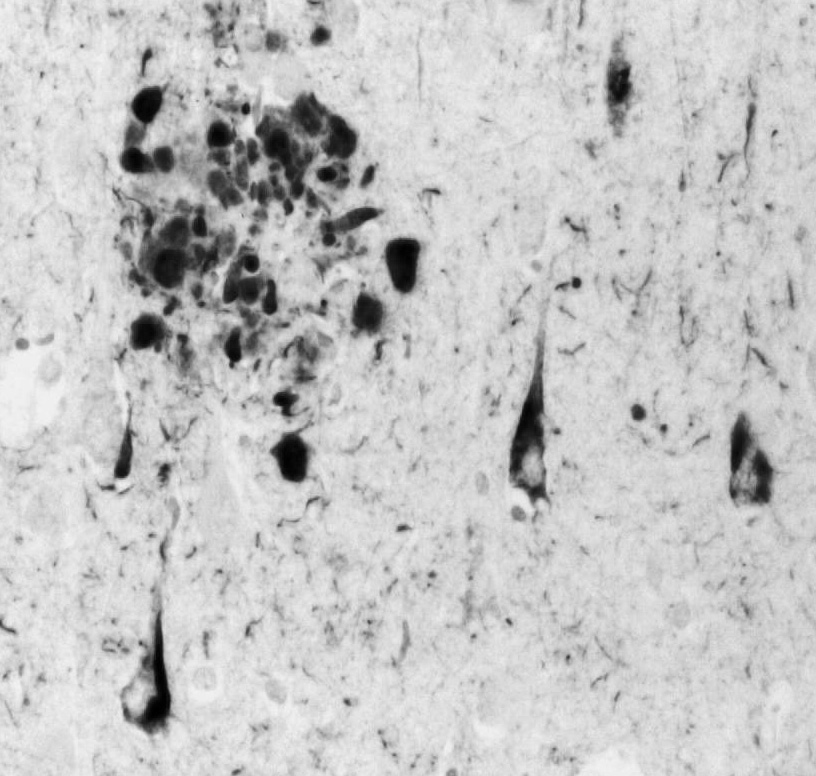
**NEURODEGENERATIVE DISEASE ASSOCIATED WITH DEMENTIA**

**DEFINITION OF DEMENTIA**: Dementia affects about 30% of individuals older than 80 years and 7% of the general population between 65 and 80 years. Clinically, dementia has been defined by the American Association of Pshychiatric as “development of multiple cognitive

deficits that include memory impairment and at least one of the following cognitive disturbances: aphasia, apraxia, agnosia or a disturbance in executive functioning. The cognitive deficit must be sufficiently severe to cause impairment in the occupational or social functioning and must represent a decline from a previously higher level of functioning”. In addition to neurodegenerative diseases, there are several other conditions including head injury, mass lesions, infective diseases, hydrocephalus, inherited or acquired disease of white matter, toxins, nutritional, or metabolic diseases that may cause dementia.

|  |  |
| --- | --- |
| **DISEASES CAUSING DEMENTIA** | **PATHOLOGIC PROTEIN** |
| Alzheimer’s disease | Tau, -amyloid |
| Dementia with Lewy bodies | -synuclein |
| Corticobasal degeneration | Tau |
| Frontotemporal dementia linked to Chr 17 | Tau |
| Pick’s disease | Tau |

**ALZHEIMER’S DISEASE (AD):** This disease has been described by Alois Alzheimer in 1906. Alzheimer’s disease accounts for 50-75% of all cases of dementia; it is usually sporadic and begins after 5th-6th decade. About 10% of patients have familial history. Pathogenesis of sporadic cases is unclear. In familial AD, mutations of presenilin gene have been identified. Characteristic pathological features are severe brain atrophy particularly prominent in hippocampus, loss of neurons, senile plaques, neurofibrillary tangles. Less commonly, there is deposition of amyloid in leptomeningeal and cortical arteries. Diagnosis of AD can be suspected clinically but differential diagnosis with other types of dementia particularly vascular dementia is difficult. A definite diagnosis of AD can be made at post-mortem examination.

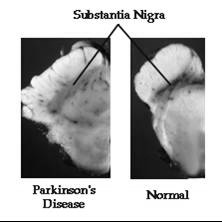
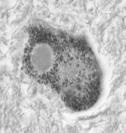
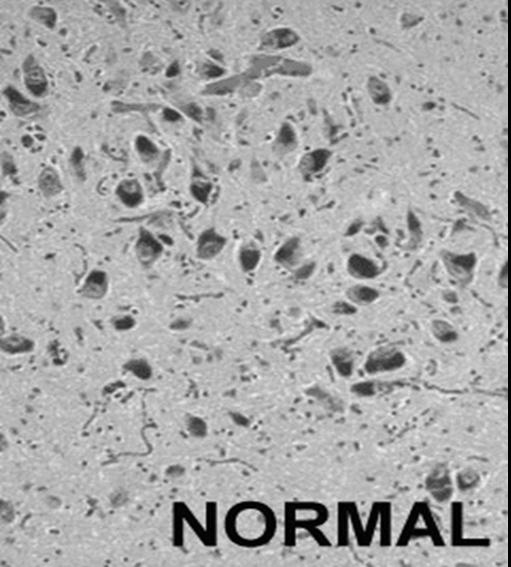
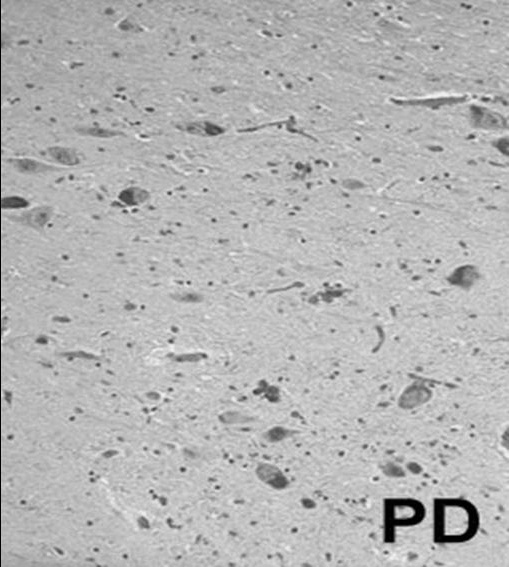


Case of Alzheimer’s disease. Immunolabelling for phophorylated tau demonstrates NFTs and a neuritic plaque

**NEURODEGERATIVE DISEASE WITH MOVEMENT DISORDERS**

The best known neurodegenerative disease causing movement disorders is idiopathic Parkinson’s disease (PD). Parkinson’s disease belongs to a group of movement disorders known as parkinsonian syndromes.

**IDIOPATHIC PARKINSON’S DISEASE:** Idiopathic Parkinson’s disease was first described by James Parkinson in 1817 in his “Essay on the Shaking Palsy”. It is a progressive condition presenting clinically with the characteristic triad resting tremor, bradykinesia and rigidity. Mean age at onset is about 60 years. The juvenile form usually occurs before 21 years and the young onset occurs between 21 and 40 years. The average duration of symptoms is about 13 years. About 95% of cases are sporadic and 5% have familial history for PD. These latter are due to mutations of alpha-synuclein. Pathological features include reduction of pigmented nerve cells in the substantia nigra (pars compacta), of Lewy bodies in spared pigmented neurons of substantia nigra and locus coeruleus, alpha-synuclein accumulation in nucleus of Meynert, basal ganglia, hypothalamus, and to lesser extent in the neocortex.

**Lewy body**

**DEMYELINATING DISORDERS**

**CAUSES OF DEMYELINATION**

* Viral infections (PML, HIV)
* Genetic (Leukodystrophies)
* Autoimmune (MS, acute haemorrhagic encephalomyelitis, acute disseminated encephalomyelitis)
* Nutritional/metabolic (central pontine myelinolysis, B12 deficiency)
* Toxic (radiotherapy, chemical agents)

**MULTIPLE SCLEROSIS (MS):** This disease represents the leading cause of disability in Western countries among young individuals. The peak of age is between 20 and 40 years and there is female predominance. Pathogenesis is unknown. There are different clinical forms including Classic Charcot’s type; Devic’s optic neuromyelitis; Marburg type; Concentric sclerosis of Balo; Schilder type. Classic MS may be primary progressive disease (no recovery after episodes of demyelination), relapsing-remitting disease (recovery between relapses) and secondary progressive (evolution of relapsing-remitting).

Pathologically, the hallmark of MS is the plaque. Plaques are tissue area of loss of myelin; sharp margin, slightly depressed, single or confluent; variable size (most 0.2–1 cm). Their number ranges from a few to several hundreds. Plaques are usually centred by a vein and have sharp margins. Axons are often preserved in early plaques but usually damaged in chronic inactive plaques. Characteristically, plaques throughout the brain show different stages of activation. According to activity, plaques can be classified as follows: acute: minor changes (oedema), difficult to recognize; early chronic active: Oedema, macrophages, myelin breakdown, some myelin sheaths still preserved, reactive astrocytosis, perivascular inflammation. Late chronic active: Complete loss of myelin, some macrophages containin myelin debris, very mild perivascular inflammation, enlaged perivascular spaces; Inactive: Complete loss of myelin and no macrophages; Shadow: Nearly complete remyelination, thin myelin, scattered macrophages laft, mild microglial up-regulation, corpora amylacea, very difficult to see.

|  |  |  |
| --- | --- | --- |
| Picture2The whole coronal section shows diffuse bilateral demyelination of centrum semiovale (a, Luxol Fast blue); |  | Picture1 Inactive plaques present as tissue areas of demyelination centred by a vein and showing sharp margins (b, Luxol Fast blue) |

Sexually Transmitted Diseases

Dr Linda Greene

**BACTERIAL STIs**

***Chlamydia***

**Epidemiology**: Worldwide distribution. In UK, 10% of under 25s affected.

**Clinical**: Asymptomatic in 50% men and 80% women. Can present as urethritis (dysuria/discharge) in men, cervicitis in women. Complications are pelvic inflammatory disease, infertility and ectopic pregnancy in women; epididymitis and orchitis in men.

**Bacteriology**: Chlamydia Trachomatis (serovars D to K) is an obligate intracellular bacterium with complex life cycle. Infectious “elementary bodies” are extracellular and metabolically inactive. Once in the cell, they form “reticulate particles” which are metabolically inactive. Chlamydiae are potent inducers of inflammation, which can cause scarring/fibrosis hence the complications.

**Diagnosis**: Obligate intracellular bacterium, can’t be cultured easily. PCR based methods used which are highly sensitive and specific, plus can be used on non-invasive specimens.

***Lymphogranuloma venereum (Chlamydia trachomatis serovars L1,L2,L3)***

**Epidemiology**: Tropical STI but in last 5 years an ongoing outbreak identified in European MSM.

**Clinical**: Classically see a genital ulcer +/-bubo. This progresses to lymphoedema and deformity in some cases. Current UK epidemic in MSM characterised by painful proctitis, with some progressing to bowel strictures if not picked up.

**Bacteriology**: as above, but maybe more propensity for scarring with LGV serovars.

**Diagnosis**: PCR as above, followed by specific LGV serovar PCR for cases where suspected, done at reference lab.

***Gonorrhoea***

**Epidemiology**: Worldwide distribution, in UK particularly seen in core groups (MSM, young people of black ethnicity).

**Clinical**: Urethral discharge in men, more rarely discharge in women. Asymptomatic in many women. Complications: PID, epididymo-orchitis. Can disseminate and cause rash.

**Bacteriology**: Neisseria Gonnorrhoeae, gram negative diplococci

**Diagnosis**: seen on smear from urethral or cervical discharge. Can be cultured on specific media (fastidious organism). PCR based techniques increasingly used.

***Syphilis***

**Epidemiology**: world wide distribution. Almost disappeared in UK but reappeared in late 1990s in MSM and now endemic again.

**Clinical**: Primary-painless ulcer “chancre”. Secondary-disseminated infection, with rash, systemic symptoms, alopecia, uveitis, hearing loss, lymphadenopathy, snail track ulcers, condlyomata acuminata. Tertiary- years later. Cardiovascular, neurological and/or skin manifestations.

**Bacteriology**: Treponema pallidum, spirochaete. Obligate human parasite.

**Diagnosis**: Can’t be cultured. Specific tests: Dark ground microscopy of ulcers/skin lesions can reveal spirochaete. On blood, EIA for antibody, TPHA/TPPA haemagglutinin assays. PCR recently available for ulcers. Non-specific tests: RPR (VDRL in past) used to show activity but may be increased in other conditions.

***Others (rare in UK)***

Chancroid is caused by *Haemophilus ducreyi* and diagnosed by microscopy, culture or PCR. It is seen in Sub-saharan Africa but incidence has decreased in recent years.

Donovanosis (Granuloma inguinale) is caused by *Klebsiella granulomatis* and is found in Australian aboriginal populations and some other tropical regions. Diagnosis is via visualisation of Donovan bodies on Giemsa staining of a tissue smear, crush preparation or biopsy.

**VIRAL STIs**

***Herpes***

**Epidemiology**: Worldwide, particularly developing countries. In UK, increasing proportion of genital Herpes caused by HSV-1.

**Clinical**: painful, usually multiple, ulcers on genitals or elsewhere. Primary episode often associated with systemic symptoms. Recurrences less severe.

**Virology**: Herpes simplex types 1 and 2, members of Herpes virus family. DNA viruses, become latent in host.

**Diagnosis**: Culture in cells now largely superceded by PCR methods. Serology may be helpful in specific clinical scenarios.

**Treatment**: No curative treatment available. Severe/primary episodes should be treated with oral acyclovir or equivalent. Frequent episodes can be suppressed with daily acyclovir treatment.

***HPV***

**Epidemiology**: worldwide. 2/3 of UK adults exposed to genital wart types by mid 30s.

Clinical: often asymptomatic. May produce lumps (warts) on genital skin, which can recur after treatment. Oncogenic types can cause dysplasia leading to neoplasia (cervical, anal, genital skin, head and neck).

**Virology** Serotypes 6 and 11 cause genital warts. Types 16 and 18 cause cervical and anal dysplasia and cancer.

**Diagnosis**: clinical diagnosis genital warts, biopsy if atypical. PCR available to identify infection and serotype but mainly a research tool. Cervical cytology to identify dysplasia.

**Treatment**: Cosmetic/destructive. No curative treatment for virus. Cryotherapy or podophyllotoxin cream/lotion are first line. Vaccine now available for prevention.

***Molluscum contagiosum***

**Epidemiology**: worldwide.

**Clinical**: Commonly seen in children as lesions on hands/body. In adults often presents with lesions on genitals. Spread via skin to skin contact. Self limiting. Can cause giant lesions in immunosuppressed. Facial lesions in adults are highly suspicious of HIV infection.

**Virology**: Pox virus. Diagnosis: clinical. Treatment: Cryotherapy.

***Other viruses*** which are STIs but covered elsewhere: Hepatitis A, B and C, HIV.

**OTHER STIs**

Pubic lice (*Pthirus Pubis*)

Epidemiology: worldwide. Fall in recent years in UK.

Clinical: presents with itching or with patient noticing lice.

Treatment: Topical e.g. Malathion lotion, Permethrin lotion, applied to whole body as per instructions. Partners will also need treatment.

**SEX ASSOCIATED CONDITIONS**

**Bacterial vaginosis**

**Epidemiology**: associated with sex, also with hygiene practices such as douching.

Polymicrobial, altered vaginal flora with raised vaginal pH. Causes discharge with offensive odour. Diagnosed on gram stain of discharge. Treatment with oral metronidazole.

Vaginal candidiasis

Usually candida albicans, rarely others. Presents with itch, soreness and white, thick discharge. Diagnosed by culture or direct gram stain for spores/hyphae. Treatment: topical (pessary) antifungals e.g. clotrimazole or oral e.g. fluconazole.

Respiratory disease

Dr Alex Rice

Microbiology of Respiratory Tract Infections

(modified from previous lecture by Marianne Nolan)

**Learning objectives:**

To familiarise the student with:

* The normal flora and defences of the respiratory tract.
* The main clinical syndromes of respiratory tract infection and common aetiological agents.
* The role of the microbiology laboratory in diagnosis of respiratory tract infections.
* Treatment and prevention of respiratory tract infections.

**The normal flora and defences of the respiratory tract.**

The upper respiratory tract is lined by mucous membranes and covered with bacteria.   
The commonest colonising bacteria are alpha-haemolytic streptococci (viridans-type). The lower respiratory tract is normally sterile even though it is in direct continuity with the heavily colonised upper respiratory tract. This is achieved by a variety of mechanisms: epiglottis; cough reflex; ciliated respiratory mucosa; mucus layer; and pulmonary macrophages.   
Any defects in these defences will render that person more likely to develop chest infections – e.g. poor swallow post CVA (stroke) may lead to aspiration of mouth contents; impaired mucociliary clearance in cystic fibrosis; damaged respiratory endothelium due to cigarette smoking or viral infection etc. On the other hand, certain bacteria can behave in a particularly virulent fashion and invade those with previously normal host defences.

**The main types of lower respiratory tract infection (LRTI) are bronchitis and pneumonia.**

**1. Bronchitis:** Inflammation of the medium sized airways of the lower respiratory tract.

Clinical symptoms: fever, cough, increased sputum production and increasing shortness of breath. Bronchitis may be an acute illness in otherwise healthy people but usually associated with cigarette induced damage. No clinical signs audible.

*S. pneumoniae, H. influenzae* or *M. catarrhalis* are isolated most commonly. However, many cases are viral and the presence of these bacteria in sputum is not proof that they are clinically significant. Therapy directed at dealing with airways obstruction (e.g. bronchodilator) and mobilisation of secretions from the lower respiratory tract (e.g. physiotherapy) are probably more important than antibacterial agents.

**2. Pneumonia:** Inflammation of the lung alveoli.

Clinical symptoms: Generally sicker than bronchitis. Fever (or hypothermia), cough, pleuritic chest pain, shortness of breath. Localised clinical signs (e.g. crackles; bronchial breathing etc.) may be present. The CXR is usually abnormal – the pattern seen is often used to describe the pneumonia e.g. lobar (involving one lobe only) etc.

Classification: Many classifications, but the **main** considerations in diagnosis and empiric therapy are:

1. How sick is the patient? - severe Vs non-severe;

2. Where did they acquire it? – community/hospital;

3. Any underlying risk factors? e.g. travel; immunosuppression; alcoholism; smoking; pets; cystic fibrosis etc.;

4. Special considerations: e.g.Tuberculosis and other mycobacteria; lung abscess; empyema; unusual infections: e.g. nocardia; fungi; melioidosis; pertussis etc.

**Community Acquired Pneumonia:**

|  |  |  |
| --- | --- | --- |
| **“Typical” or classical pneumonia** (clinical signs on chest exam; abnormal CXR) | **Common pathogens**:   * + 1. **S. pneumoniae**   *H. influenzae*  *M. catarrhalis*  *S. aureus*   1. **K. pneumoniae** | * 1. **Considerations**   Often lobar. ? vaccinate.  Often associated with smoking.  Often associated with smoking.  +/- recent viral infection. +/- cavities on CXR.  Associated with alcoholism |
| **“Atypical”**  (clinical signs absent or not in keeping with CXR). | *L. pneumophila*   1. **M. pneumoniae**   *Chlamydia pneumoniae*  *Chlamydia psittaci* | Travel; Air-conditioning; Cooling towers  Systemic symptoms common.  “TWAR” agent.  Bird exposure |
| **Other causes of CAP:** | 1. **Bordetella pertussis** 2. **M. tuberculosis** | Whooping cough. Unvaccinated children. May be missed in adults.  Tuberculosis. Consider if poor response to conventional antibiotic therapy. |

**Hospital acquired pneumonia/Ventilator associated pneumonia:** May be difficult to differentiate infectious from non-infectious causes of abnormal respiratory function and abnormal CXR- e.g. pulmonary oedema/embolus; etc. May also be difficult to interpret significance of organisms grown from samples taken from the upper airways. For this reason, a BAL (bronchoalveolar lavage) is desirable both to elucidate and quantify the pathogens from the lower respiratory tract. Aerobic gram-negative rods tend to be common causes of hospital-acquired pneumonia, e.g. *P. aeruginosa, Klebsiella spp, Enterobacter spp., e*tc. Staphylocci, especially MRSA is another major pathogen. Anaerobic bacteria are a problem after aspiration.

**Immunosuppressed patients and LRTI:**

More susceptible to the main pathogens but other classical associations are:

Splenectomy: encapsulated organisms e.g. *S. pneumoniae; H. influenzae.*

Cystic fibrosis: *Pseudomonas aeruginosa; Burkholderia cepacia* – often resistant strains.

HIV: PCP – Pneumocyctis pneumonia (PCP, now *P. jiroveci*); TB and atypical mycobacteria; *Cryptococcus neoformans*.

Neutropenia/bone marrow or solid organ transplantation: fungi e.g. *Aspergillus spp*.

* + - 1. **The role of the laboratory in diagnosis of LRTI**
      2. **No bacteriological cause found in up to 50% of patients with LRTI**

Sputum C&S

Commonest sample sent in cases of ? LRTI.

Problem of interpretation of “normal flora” in upper respiratory tract.

Patient may not produce sputum – physiotherapy may help in this respect.

Most useful if isolate a pure growth of a definite pathogen e.g. *S. pneumoniae*.

In laboratory, we look for all the common pathogens by setting up various plates.

Induced sputum: Mostly for diagnosing PCP and TB, if BAL not available

Samples taken by Physiotherapists in negative pressure siderooms to minimise risk to other people.

Blood cultures: Only useful if patient is bacteraemic.

BAL (Bronchoalveolar lavage): Invasive, but yields samples representative of the lower respiratory tract rather than the mouth/upper airway. Especially useful for diagnosis of ventilator associated pneumonia (VAP), TB and occasionally for fungi e.g. aspergillus etc.

Antigen tests: Urine rapid antigen tests available for Legionella and Pneumococci.

Antibody tests: Occasionally look for paired serum (taken 10-14/7 apart) to establish the diagnosis of Legionella.

Immunofluorescence: PCP diagnosis. Also diagnosed by cytology or Silver stain.

**Treatment and prevention of respiratory tract infections.**

Community-acquired pneumonia:Mild-moderate: Amoxicillin or clarithromycin Moderate-severe: Coamoxiclav **AND** clarithromycin.

Hospital-acquired pneumonia: -varies with local policy.

**Prevention**: General: smoking etc.; Vaccination.

Auto-inflammatory and auto-immune diseases

Margaret Callan

***Objectives***

Understand the recently proposed classification of auto-inflammatory and auto-immune disease

Understand the concept of loss of tolerance in development of auto-immune disease

Understand the spectrum of auto-immune disease from organ-specific to non-organ-specific

Understand the conventional classification of auto-immune responses in terms of hypersensitivity reactions

Become familiar with clinical features of selected auto-inflammatory and auto-immune disease

Become familiar with immunological tests that contribute to diagnosis of auto-inflammatory and auto-immune disease

***Classification of auto-inflammatory and auto-immune disease***

Both auto-inflammatory and auto-immune disease are characterised by self-directed inflammation.

**Auto-inflammatory disease**: Local factors at sites predisposed to disease lead to activation of innate immune cells such as macrophages and neutrophils leading to tissue damage. Genetic polymorphisms affecting key cytokine pathways are common.

**Auto-immune disease**: Aberrant dendritic cell, B cell and T cell responses lead to breaking of tolerance and development of adaptive immune responses directed to self- antigens. These adaptive immune responses play a prominent role in disease pathogenesis. Auto-antibodies are often a feature and may predate clinical disease by years. Genetic polymorphisms affecting HLA molecules, T cell activation, pathways, regulatory T cells and T cell selection have been described.

Many diseases have both auto-inflammatory and auto-immune components and classification is predominantly determined by genetic basis if known

**Rare monogenic auto-inflammatory disorders**

E.g. Familial mediterranean fever, TRAPS

**Polygenic auto-inflammatory disorders**

E.g. Crohn’s disease, Ulcerative colitis, Osteoarthritis, Gout, Giant cell arteritis, Takayasu’s arteritis

**Mixed pattern disease**

E.g. Ankylosing spondylitis, Psoriatic arthritis, Behcet’s disease

**Polygenic auto-immune disease**

E.g. Rheumatoid arthritis, Systemic lupus erythematosus, Myaesthenia Gravis,

Primary biliary cirrhosis, Pernicious anaemia, Addison’s disease

**Rare monogenic auto-immune disease**

E.g. Autoimmune polyendocrine syndrome type I (APECED or APS-1), IPEX, Autoimmune lymphoproliferative syndrome (ALPS)

Genetic factors are important in pathogenesis of disease. However they may not be sufficient for development of disease and environmental factors such as infection, or other factors such as local trauma, may also be important.

***auto-immune disease and Failure of tolerance***

Auto-immune disease results from a failure in the acquired immune system leading to immune responses against host antigens. These responses lead to different pathologies depending on the nature of the antigen and the type of immune response involved. Immunopathologies range from tissue damage to metabolic dysfunction.

Autoimmune responses occur when the immune system has not been tolerised to, or is no longer tolerant to self antigens as a result of:

1. Effects on central tolerance: Failure to delete auto-reactive T-cells in the thymus or B cells in the bone marrow.
2. Effects on peripheral tolerance: Reactivation of weakly auto-reactive or cross-reactive T and B-cells in the periphery as a result of inflammation, infection or tissue damage. Tissue damage or infection at immunologically privileged sites.

***spectrum of auto-immune disease in terms of organ specificity***

The type, location and specificity of the immune response influence the autoimmune disease pathology. Auto-immune diseases can be classified into organ specific and non-organ specific according to the localisation of the disease pathology.



***HYPERSENSITIVITY REACTIONS***

The types of immune response that result in auto-immune disease have been classified according to the reactions originally described by Coombs and Gell for hypersensitivity. Note that these only include adaptive immune responses.

**Type II**

**IgG or IgM antibody** reactive with self antigen and resulting in tissue damage, receptor blockade or activation:

Found in Graves Disease, Goodpastures Syndrome, Auto-immune haemolytic anaemia.

**Type III**

IgG or IgM **Immune complex** mediated tissue damage:

Found in Systemic lupus erythematosis (SLE).

**Type IV**

Immune pathologies depending on **T-cell** responses:

Found in Rheumatoid arthritis (RA), Type 1 diabetes and Multiple sclerosis (MS)

*Note that Type I (IgE mediated, immediate) hypersensitivity reactions are not usually found in autoimmune disease*

*Note that autommune reactions often involve a combination of these mechanisms.*

***Investigations in autoimmune disease***

**Auto-antibodies**

The amount of antibody is measured either by titre (the minimal dilution at which the antibody can be detected) or by concentration in standardised units.

The normal range for a given population may vary depending on the method used, and results should always be interpreted with laboratory-derived normal values.

The usefulness of a specific test increases with prior probability, so the ‘shotgun approach’ of ordering a number of laboratory tests without reference to clinical context can lead to false positive results.

**Rheumatoid factor and anti-CCp antibodies**

A rheumatoid factor is an antibody directed against the common (Fc) region of human IgG.

Only 50% of patients with rheumatoid arthritis are positive for rheumatoid factor at the time of diagnosis; a further 25% will become seropositive in the first 2 years of disease. So the absence of RhF is insufficiently sensitive to rule out the diagnosis of rheumatoid arthritis.

RhF is associated with a wide variety of auto-immune and non-auto-immune conditions, and is a common finding in the elderly

Anti-cyclic citrullinated peptide (CCP) antibodies are as sensitive as RhF and are much more specific as a diagnostic test for rheumatoid arthritis.

**Anti-nuclear antibody**

Anti-nuclear antibodies (ANA) are a group of antibodies which bind to components of the nucleus. They are detected by staining cells (eg Hep2 cells) with a patient’s serum.

An antibody titre of > 1:80 is usually considered positive.

The pattern of immunofluorescence reflects binding to discrete nuclear components; specific patterns may be associated with clinical subgroups.

The major indication for ANA testing is the diagnosis of SLE, where it has a very high sensitivity (almost 100%). A negative ANA test virtually excludes the diagnosis of SLE. However, the specificity is low.

Repeating ANA tests is rarely useful, and there is no role for serial monitoring of ANA titre.

If an ANA test is positive, it is useful to establish which nuclear component is being recognised; this may be dsDNA or an ‘extractable nuclear antigen’ (ENA) such as Ro, La, Sm, RNP, Scl70, RNA polymerase or fibrillarin. Other antigens include histones or centromere. Cytoplasmic staining may also be seen (eg specificity for mitochondria or tRNA synthetase).

There is no value in testing for antibodies to dsDNA or extractable nuclear antigens if the ANA is negative.

**Anti-DNA antibodies**

*Anti-DNA antibodies bind to double stranded DNA. These antibodies*

Are highly specific for SLE (95%)

Occur in up to 60% of SLE patients at some time in their disease

Very high titres are often associated with more severe disease, including renal or central nervous system involvement.

Are useful in disease monitoring - an increase in antibody titre is associated with disease activity and may precede disease relapse.

Antibodies to single stranded DNA are non-specific and have little clinical utility.

**Antibodies to extractable nuclear antigens**

|  |  |
| --- | --- |
| **DISEASES ASSOCIATED WITH ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS** | |
| **Antibody** | **Disease association** |
| **SLE and SJOGRENS**  Anti-Ro antibody (anti-SS-A)  Anti-La antibody (anti-SS-B)  Anti-Smith antibody (Anti-Sm)  Anti-ribonucleoprotein antibody (anti-RNP)  Anti-histone antibody  **SCLERODERMA**  Anti-topoisomerase I antibody (Anti-Scl70)  Anti-centromere antibody | SLE (associated with photosensitivity and subacute cutaneous SLE), Sjogren’s,  Maternal anti-Ro antibodies are associated with neonatal lupus and congenital heart block  SLE  Sjogrens  SLE  SLE, often with anti-Sm antibodies  MCTD  Drug-induced Lupus  Diffuse cutaneous variant of scleroderma (associated with pulmonary fibrosis and renal crisis)  Limited cutaneous (CREST) variant of scleroderma (sensitivity 60%, specificity 98%)  Primary raynauds (but may be pre-scleroderma) |
| Anti-RNA polymerase I, III  Anti-fibrillarin | Diffuse cutaneous scleroderma  Diffuse cutaneous scleroderma |
|  |  |
|  |  |

**ANA-cytoplasmic antibodies**

Reactivity with cytoplasmic antigens may be seen when performing an ANA test using Hep2 cells. These are reported as ANA-cytoplasmic and must not be confused with ANCA which are anti-neutrophil cytoplasmic antibodies.

Anti tRNA synthetase antibodies (eg anti-JO-1) give a speckled staining pattern in the cytoplasm and are associated with immune myositis

Anti-mitochondrial antibodies, associated with primary biliary cirrhosis, may be detected

**complement C3 and C4**

*Quantitation of complement components (C3 and C4) is useful in the evaluation of immune complex mediated diseases:*

(Unactivated) C3 and C4 levels inversely reflect the magnitude of immune complex deposition,

So classical complement pathway activation leads to a decrease in circulating C4, and is often also associated with decreased C3 levels.

Serial measurement of C3 and C4 is used in the routine monitoring of SLE as a surrogate marker of presence of circulating immune complexes

**Anti-phospholipid antibodies**

Anti-phospholipid antibodies are associated with the development of venous and arterial thrombosis and recurrent fetal loss

They may occur in isolation (primary anti-phospholipid syndrome) or as a complication of SLE (secondary anti-phospholipid syndrome).

Anti-phospholipid antibodies can also be detected in a wide variety of rheumatic, infectious and malignant conditions - in these situations they are not usually associated with thrombosis.

*There are two major ways of measuring antiphospholipid antibodies*

Anti-cardiolipin antibodies

Lupus anticoagulant

These antibodies are discordant in up to 40% of cases – if there is a clinical suspicion of the antiphospholipid syndrome, both tests should be performed.

**Anti-neutrophil cytoplasmic antibodies**

*ANCA antibodies are associated with a subset of the systemic vasculitides:*

c-ANCA is associated with antibodies to Proteinase 3 and occurs in most patients with Wegener’s granulomatosis, especially where there is renal involvement

p-ANCA is associated with antibodies to myeloperoxidase and may be associated with microscopic polyarteritis

Serial measurement of anti-PR3 or anti-MPO antibodies may be useful for disease monitoring

Therapeutic Drug Monitoring

Dr Jaimini Cegla

Learning objectives

* Understand the situations in which measurement of a drug level may be used to provide useful clinical information.
* Be aware of the factors affecting serum levels for a given dose (pharmacokinetics) and those factors affecting response to a drug at its active site (pharmacodynamics)
* Understand the concept of a therapeutic range and be aware of its limitations.
* Be able to cite examples e.g. anticonvulsants, lithium, digoxin and theophylline.

**INTRODUCTION**

* Where possible, response to treatment is best assessed using clinical or laboratory measurements of effect, e.g. blood pressure for antihypertensives and INR for anticoagulants
* Where this is not applicable Therapeutic Drug Monitoring (TDM) may be an aid for the clinician in adjusting dosage to provide optimum treatment and avoid iatrogenic toxicity.
* It is only helpful for a limited number of drugs.

For TDM to be clinically worthwhile, the following criteria should be fulfilled:

1. Established relationship between plasma drug concentration and therapeutic response and/or toxicity;

2. Poor relationship between plasma concentration and drug dosage;

3. A good clinical indication for the test such as: no response to treatment; suspected non-compliance; signs of toxicity;

**FACTORS AFFECTING DRUG LEVELS**

**Compliance**

Failure to take a drug as prescribed is common. This may be a particular problem in chronic and relatively asymptomatic conditions. However variable compliance may be difficult to detect by TDM.

# PHARMACOKINETIC FACTORS

# *Absorption/bioavailability*

#### Absorption of a drug may be variable after oral administration

# *Distribution*

May vary depending on weight of patient, amount of adipose tissue, and presence of disease (e.g. ascites).

***Metabolism***

Many drugs are metabolised in the liver in two phases (oxidation and conjugation)

Metabolism may be affected by factors such as   
 Genetic variation, Age, Nutritional status, Disease, Smoking, Alcohol and other Drugs

# *Excretion*

Most drugs and their metabolites are largely excreted by the kidney. If renal function is impaired reduced doses of water soluble drugs e.g. digoxin, lithium, and aminoglycosides are advised.

Drugs may also be excreted in bile, faeces, saliva and expired air

## FACTORS AFFECTING DRUG EFFECTS - PHARMACODYNAMIC FACTORS

Pharmacodynamic factors may affect the response to a drug at its site of action   
(e.g. hypokalaemia, competition and tolerance).

## INTERPRETATION - TARGET or THERAPEUTIC RANGE

The concentration range over which a drug exhibits therapeutic benefit, without toxicity, in the majority of patients.

The "target" or "therapeutic" range is only a guide to proper dosing of the patient. Aim to treat the whole patient rather than the drug concentration.

## FACTORS AFFECTING INTERPRETATION OF DRUG LEVELS

## Timing of Sample

4-5 half-lives are needed to reach a steady state.

Levels are not constant particularly if the drug has a short half life.

Some drugs have a significant initial distribution phase

## Active Metabolites

May not be measured by the assay

## Protein Binding

Many drugs are largely protein bound (e.g. phenytoin).

Routine assays measure?: *a). Total drug (free+bound).*

*b). Free drug.*

*c). Bound drug*

**EXAMPLES OF DRUGS REGULARLY MONITORED**

## PHENYTOIN

Difficult to assess the effect clinically so monitoring is essential as a guide to dosage adjustment, since phenytoin has a low therapeutic index and exhibits saturation kinetics (i.e. small increases in dose result in large increases in concentration).

Maintenance doses are normally between 200 and 600 mg/day. The main reason for this is the wide variation in the rate at which phenytoin is metabolised by the liver in different patients. In addition hepatic metabolism becomes saturated so that equal increments in dose produce bigger and bigger increases in drug level. It takes at least 2 weeks to reach a steady state after a dosage alteration.

Low albumin concentration or displacement by other drugs may lead to toxicity within the therapeutic range.

Some patients may require levels above the therapeutic range to achieve adequate control of seizures. Toxicity is a clinical diagnosis, a level above the quoted therapeutic range is not necessarily “toxic”.

## DIGOXIN

There is considerable overlap between therapeutic and toxic concentrations.

Factors increasing myocardial sensitivity include hypokalaemia, hypercalcaemia, hypomagnesia, hypoxia and hypothyroidism.

Excreted by kidneys so half life prolonged and danger of accumulation in renal impairment.

Digoxin levels in cardiac muscle are high, but there is a long distribution phase so that samples taken soon after a dose may give high levels which do not reflect tissue levels. Samples taken within 6 hours of a dose cannot be safely interpreted.

## LITHIUM

Excreted by kidneys. Large variations in renal clearance

Monitoring essential as there is a wide variation in levels produced by standard maintenance doses. Variations in sodium intake, fluid balance or renal function may affect levels. resulting from variations in absorption, renal clearance, volume of distribution and compliance. Toxicity is life threatening.

12 hours post-dose is the standardised time for monitoring lithium levels.

## THEOPHYLLINE

Plasma concentrations are related predictably to both bronchodilator and toxic effects. The wide variation in metabolism (increased in smokers, decreased in liver disease) and many drug interactions mean that without monitoring the majority of patients are undertreated

##### GENTAMICIN

Low therapeutic index. Excreted by kidney. Danger of ototoxicity and nephrotoxicity

Used to treat serious illness when adequate blood levels must be maintained.

Serious side effects are ototoxicity and nephrotoxicity

Metabolic Disorders and Screening 2

Dr Maggie Hancock

**Topics to address:**

* Hints to IMD
* First line tests for IMD
* Urea cycle defects (UCD)

7 defects of urea cycle + Lysinuric protein intolerance, Hyperornithinaemia-hyperammonaemia homocitrullinuria (HHH) and Citrullinaemia type II.

Associated with **encephalopathy, respiratory alkalosis** and irreversible neurological damage.

**Ammonia** is highly toxic, usual plasma level <50 μmol/l

Values >300 μmol/l may be lethal

Free flowing, venous sample, delivered to laboratory stat on ice

Plasma amino acids, urine amino and organic acids (+ urine orotic acid)

* Organic acidurias (OA)

The most important involve the complex metabolism of branched chain amino acids (leucine, isoleucine and valine). Truncal hypotonia /limb hypertonia, myoclonic jerks, **unusual odour**.

**Metabolic acidosis** with high anion gap (not lactate)

Hypo/hypercalcaemia

Hypoglycaemia

Hyperammonaemia

**Neutropenia**, thrombopenia, pancytopenia

Plasma amino acids

Urine organic acids– whilst symptomatic

* Fatty acid oxidation defects

13 recognised disorders including those involved with the carnitine transport system. Failure of β-oxidation leads to **hypoketotic hypoglycaemia** often with hepatomegaly and cardiomyopathy.

Urine organic acids – whilst symptomatic

Blood spot for carnitine profile

* Carbohydrate degradation (non-Glycogen Storage Disoder)

Of the 3 known disorders of galactose metabolism, galactosaemia due to deficiency of galactose-1-phosphate uridyl transferase (Gal-1-PUT OMIM 230400) is the most severe and the most common.

Galactose-1-phosphate is considered responsible for liver and kidney problems. Common neonatal presentation is **conjugated hyperbilirubinaemia**, hepatomegaly, **sepsis** and hypoglycaemia. Galactitol accumulation accounts for cataract formation.

**Conjugated hyperbilirubinaemia**

Urine reducing substances – shows huge preponderance of galactose

Red cell Gal-1-PUT

* Lysosomal storage diseases

Defects of lysosomal hydrolases lead to substrate accumulation within the organelles leading to **organomegally** (connective tissue, solid organs, cartilage, bone and, above all, nervous tissue).

Urine mucopolysaccharides / oligosaccharides

Leucocyte enzyme activities

* Peroxisomal diseases (PD)

Peroxisomes are responsible for the metabolism of very long chain fatty acids and the biosynthesis of complex phospholipids.

.

**Neonatal profile**: muscular **hypotonia**, seizures, hepatic dysfunction **dysmorphia**

**Infantile profile**: **retinopathy** often leading to early blindness, sensorineural deafness, hepatic dysfunction, mental deficiency, (often) ftt, **dysmorphic signs**.

Bony changes involve a large fontanel, osteopaenia of long bones, often with calcified stippling especially the patellar region.

Very long chain fatty acid profile

* Mitochondrial disorders

Defective oxidative phosphorylation (OXPHOS). Mitochondrial disorders can present in any organ, at any time with any form of inheritance! ‘Classic’ presentation is **chronic muscle weakness** with **hyperlactataemia**.

Elevated **lactate** (alanine) – after periods of fasting (e.g. overnight)

Elevated **creatine kinase**

CSF lactate / pyruvate – deproteinised at bedside

CSF protein (raised in Kearns-Sayre syndrome)

Mitochondrial DNA analysis

Muscle biopsy / ragged red fibres & complex activities

* Congenital disorders of glycosylation (CDG)

Multisystem disorders associated with **cardiomyopathy**, osteopaenia, hepatomegaly and (in some cases) facial dysmorphia, **abnormal fat distribution**.

Transferrin glycoforms (serum)

Interactive Haematology Cases

Dr Nina Salooja

Liver function tests and cases

Dr Jonathan Hoare

**Content of presentation**

* Basic liver functions
* Liver function tests
* Cases

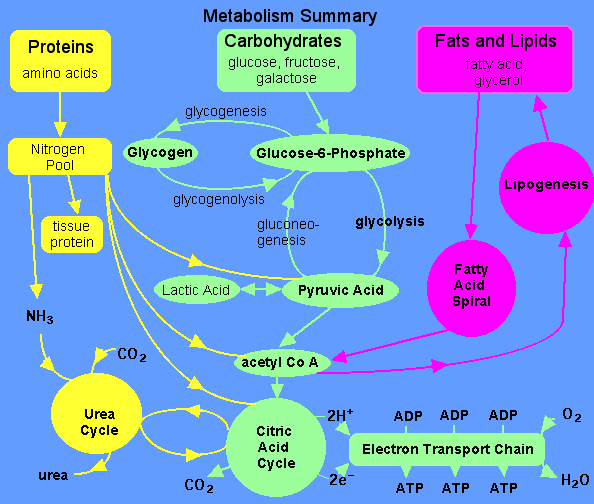
**NORMAL LIVER FUNCTION**

* Intermediary Metabolism
* Protein Synthesis
* Xenobiotic Metabolism
* Hormone Metabolism
* Bile Synthesis
* Reticulo-endothelial

**INTERMEDIARY METABOLISM**

* Glycolysis
* Glycogen storage
* Glucose synthesis
* Amino-acid synthesis
* Fatty acid synthesis
* Lipoprotein metabolism

*Enzyme-catalysed processes within cells that extract energy from nutrient molecules and use that energy to construct cellular components*



**PROTEINS SYNTHESIS IN THE LIVER**

|  |  |
| --- | --- |
|  | “Human Liver Proteome Project” 4975 unique proteins 2338 groups with 9245 proteins involved  Approx. 1200 plasma proteins, most synthesized by liver  Clotting factors / albumin |

**XENOBIOTIC METABOLISM (xeno – foreign)**

**Chemical Modification**

* P450 Enzyme System
* Acetylation / de-acetylation
* Oxidation / Reduction

**Conjugation**

glucuronate

sulphate

**Excretion**

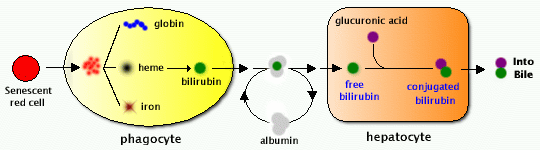
**HORMONE METABOLISM**

|  |  |  |
| --- | --- | --- |
| * **Vitamin D**    + hydroxylation   + absorption | * **Steroid Hormone**    + conjugation   + excretion | * **Peptide Hormone**   + Catabolism |

**BILE**

|  |  |
| --- | --- |
| ***Constituents***   * + Water   + Bile salts/acids   + Bilirubin   + Phospholipids   + Cholesterol   + Proteins   + Drugs and Metabolites | ***Function***   * + Excretion   + Micelle formation   + Digestion |

**BILIRUBIN METABOLISM AND TRANSPORT**



**RETICULOENDOTHELIAL FUNCTION**

|  |  |
| --- | --- |
| * Kupffer Cells   + Clearance of infection and LPS   + Antigen presentation   + Immune modulation     - Cytokines, etc. * Erythropoesis |  |

**LIVER FUNCTION TESTS “LFT’s”**

* What are LFT’s ?
* Do they test liver function?

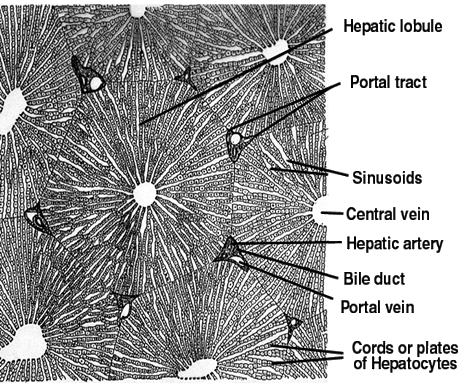
**LFT’s**

* Alanine Transaminase ALT
* Aspartate Transaminase AST
* Alkaline Phosphatase ALP
* Albumin
* Bilirubin
* Gamma glutamyl transferase γGT / GGT
* Clotting factors PT
  + Pro-thrombin time
* Alpha fetoprotein αFP /AFP

**Serum markers of   
liver cell damage**

|  |  |  |
| --- | --- | --- |
| * ALT * AST * ALP * GGT   Tumour marker **AFP** | Synthetic function | * Albumin * Pro-thrombin time (PT) |
|  | * Bilirubin |

**LIVER ARCHITECTURE**



**Alanine transferase (ALT) & aspartate transaminase (AST)**

* enzymes contained within cytoplasm of hepatocytes
* elevated when hepatocytes die
* “catalyze the transfer of the alpha-amino groups of alanine and aspartate, respectively, to the alpha-keto group of ketoglutarate, which results in the formation of pyruvate and oxaloacetate…..”
* present in other organs but in low amounts
  + muscle, kidney, brain, pancreas
  + AST rises more in alcohol and cirrhosis

**Gamma-glutaryl transpeptidase (GGT)**

“Gamma-glutamyl transpeptidase (GGT) catalyzes the transfer of the gamma-glutamyl group from gamma-glutamyl peptides such as glutathione to other peptides and to L-amino acids…”

* found in liver, kidney, pancreas, spleen, heart, brain, seminal vesicles
* in liver found in hepatocytes and epithelium of small bile ducts
* elevated in chronic alcohol use
* also raised in bile duct disease and hepatic metastasis

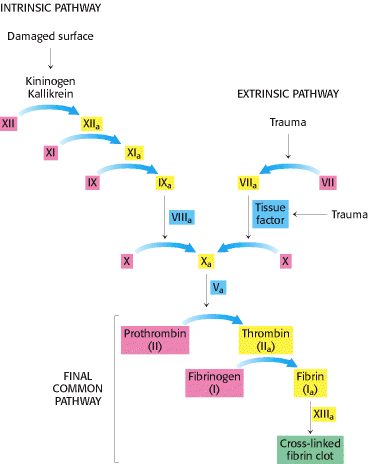
**Alkaline phosphatase**

* “..alkaline phosphatase refers to a group of enzymes that catalyze the hydrolysis of a large number of organic phosphate esters at an alkaline pH…. its precise function is not yet known !!
* liver isoenzyme located in sinusoidal and canalicular membranes
* liver isoenzyme markedly elevated if obstructive jaundice or bile duct damage
* less elevated in viral hepatitis or alcoholic liver disease i.e. hepatocyte damge
* other sources bone, small intestine, kidney, WBC’s, placenta etc
* other causes of a rise include bone disease (especially metastatic and pregnancy)

**Albumin**

* the major protein synthesised by the liver (8-14g/day)
* molecular mass 65,000
* half life 20 days
* contributes to oncotic pressure and binds steroids /drugs/bilirubin/calcium etc
* low in
  + low production (chronic liver disease, malnutrition)
  + loss (e.g. gut, kidney)
  + sepsis (“3rd spacing”)

**Clotting Cascade / PT**



**Alpha-feto protein (AFP)**

* glycoprotein MW 69 000 /albumin superfamily
* Fetal transport-immune regulation/tolerance
* in fetal life made by yolk sac, GI epithelium and liver
* in adult concentration low / no known function
* used in diagnosis of hepatocellular carcinoma (but may rise too late or not at all)
* also raised in hepatic damage/regeneration
* raised in pregnancy and testicular cancer

**Jaundice / “raised blood bilirubin”**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Unconjugated Bilirubin** | **Conjugated Bilirubin** |
| Pre-Hepatic Haemolysis | **+++** | **-** |
| Hepatic Genetic Hepatitis Drug reaction | **++** | **++** |
| Post-Hepatic Bile duct obstruction Drugs | **-** | **+++** |
|  |  |  | Conjugated bilirubin appears in urine |

**Urine Dipstick tests**

|  |  |
| --- | --- |
|  | **Bilirubin** – not normally detected in the urine. Large amounts can be detected with the naked eye.  **Urobilinogen** – normally detected in small amounts in urine but cannot be detected in extra-hepatic biliary obstruction.  **Pale stools / dark urine** - particularly in obstructive jaundice |

**Jaundice /abnormal LFTs - simple approach**

**Jaundice**

**ALT/AST**

Bilirubin **↑** Hepatocellular

Normal enzymes

Haemolysis Cholestatic Acute Chronic

Gilbert’s **ALP**

Dilated ducts Undilated ducts

i.e. obstruction drugs / PBC-PSC

gallstones/cancer etc. pregnancy, etc.

Clinically rarely measure unconj/conj bilirubin

In fact, LFT’s often a mixed picture, i.e. raised ALP and ALT



Gallstones, cancer, etc.

Dilated ducts

History LFT’s+USS

Not dilated other tests Think!   
“liver screen Biopsy

**Other Tests Used in Diagnosis**

* Viral serology
* Auto-antibodies
* Iron studies
* Copper studies
* Detailed drug history
* Radiological imaging – US and CT
* Histopathology
* ERCP

**Measuring Liver Function**

* Dye tests
  + Indocyanine green / Bromsulphalein
    - Measure excretory capacity of liver
    - Measure hepatic blood flow
* Breath tests
  + Aminopyrine / Galactose (carbon 14)
    - measure residual functioning liver cell mass
    - ? predict survival in alcoholic hepatitis
    - ? distinguish cirrhosis without biopsy (70-80%sensitivity)
* Serum bile acids
  + Elevated esp. in cholestasis
    - 10-100x in cholestasis of pregnancy
    - 25X in PBC/PSC

**Summary**

* “LFTs” are serum proteins produced by the liver
* ALT/AST “transaminases” / GGT / ALP
* not true tests of liver function
* help locate site/cause of inflammation/cell damage
* Albumin / pro-thrombin time-INR
* represent “synthetic” function in absence of confounders
* prognostically important in acute and chronic liver disease
* Bilirubin
* level is a test of liver function / urine and blood
* “split” can help determine underlying cause
* The LFT’s give you a probability of what the diagnosis is……..
* They need supporting evidence from the……..
* history
* examination
* radiology
* other blood tests
* occasionally liver biopsy

Allergic Disorders and C1 Inhibitor Deficiency

Dr Peter Kelleher

**Learning Objectives**

* Outline the epidemiology of allergic diseases
* Discuss the investigation and management of allergic disorders
* Describe the clinical features of the following conditions:
* Anaphylaxis,
* Allergic rhinitis,
* Food allergy,
* Chronic urticaria and Angioedema
* Outline the clinical features and laboratory findings in patients with C1 inhibitor deficiency.

**Reading List**

Ewan PW. ABC of allergies- Anaphylaxis. BMJ 1998;**316**:1442-1445.

Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2006;**117**:S470-475.

Greaves MW. Chronic idiopathic urticaria. Curr Opinion Allergy Clin Immunol 2003;**3**:363-368.

Fay A, Abunin M. Current management of hereditary angio-edema (C’1 esterase inhibitor defciiency) J Clin Pathol 2002;55:266-270.

**Internet list**

British Allergy Foundation [www.allergyfoundation.com](http://www.allergyfoundation.com)

Anaphylaxis Campaign [www.anaphylaxis.org.uk](http://www.anaphylaxis.org.uk)

**Theme: Allergy**

**OPTION LIST**

|  |  |  |  |
| --- | --- | --- | --- |
| A | Acute Urticaria | 4 | Oro-genital allergy |
| B | Allergic asthma | 5 | Panic attack |
| C | Allergic Rhinitis | 6 | Physical urticaria |
| D | Anaphylaxis |  |  |
| E | C1 inhibitor deficiency |  |  |
| 1 | Chronic Sinusitis |  |  |
| 2 | Idiopathic Angioedema |  |  |
| 3 | Latex allergy |  |  |

**For each scenario below, choose the most appropriate answer from the list above. Each option may be used once, more than once or not at all.**

1. A 20-year-old male presents to his GP with a two year history on nasal itch, sneezing and a clear discharge in the summer months. He also finds that his nose is usually blocked. He gets some symptomatic relief from an anti-histamine tablets which he buys in Boots.

2. A 40-year-old lady is admitted to A&E with swelling of her lips, tongue, throat and difficulty in breathing. Clinical examination reveals an urticarial skin rash and low blood pressure. She says she has been stung her by a wasp 30 minutes previously.

3. A 22-year-old medical student experiences an urticarial skin rash when he uses condoms for sexual intercourse. He also gives a history of lip irritation and swelling when he blows up balloons.

4. A 25 year old woman presents to her GP complaining of itchy, red wheals on her torso which have been present for 8 weeks. She can not remember how they started but she says that the skin rash is worse in the heat and when she exercises.

5. A 45 year old lady presents to A&E at 18.00 hours with a four history of increasing swelling of face and tongue. She states that she is now feeling it more difficult to breath. She has dental surgery in the morning. She also says that her sister also suffers from swelling of hands or her hands and feet.

ANSWERS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1. | 2. | 3. | 4. | 5. |

The Immunology of Transplantation

Dr Candice Roufosse

**Objectives:**

To understand the immunological mechanisms of graft rejection (solid organ transplantation) and graft-versus-host disease (haematopoietic stem cell transplantation)

To understand how the knowledge of these mechanisms is used to prevent and treat rejection:

* donor and recipient matching (pre-transplantation)
* immunological monitoring
* treatment strategies to prevent or treat organ rejection

**Solid organ transplant rejection**

Solid organ transplantation refers to transplantation of kidney, liver, pancreas, heart and lung.

Recognition of the graft as non-self is mainly mediated through disparities in blood group antigen (ABO) and in HLA (human leukocyte antigens) class I (A,B,C) and class II (DR,DQ,DP). Exposure to the foreign epitopes in the graft results in allo-specific T-cell and B-cell mediated responses. T-cell activation requires presentation of the foreign protein in the context of HLA molecule by an antigen-presenting cell.

In the effector phase of graft rejection, other elements of the immune system are recruited by the allospecific T and B cells. In T-cell mediated rejection, this takes the form of a hypersensitivity type IV response; in B-cell mediated rejection complement and endothelial cell damage play a central role.

Rejection can occur at different time-points after transplantation:

* Acute rejection - Most rejection happens within the 1st few weeks after transplantation, following exposure to the foreign donor antigens
* Hyperacute rejection - Some rejection occurs within minutes/hours of transplantation, when pre-formed antibodies to the donor antigens are present in the recipient’s serum
* Rejection can occur at any time post-transplant, particularly in the case of non-compliance with immunosuppressive drugs
* “Chronic rejection” may result from a low-level long term form of alloimmune reaction

Accomodation of the recipient to the grafted organ is the “holy grail” of transplantation research: some patients have long graft survival without immunosuppressive drugs.

Histological examination of a transplant biopsy is used when graft dysfunction develops in order to distinguish rejection from other causes of dysfunction such as poor perfusion, drug toxicity. There are defined histological criteria for diagnosing T-cell mediated rejection, antibody-mediated rejection, or a mixture of both. The type of rejection influences treatment and outcome.

**Prevention and treatment of solid organ transplant rejection**

Donor/recipient matching

This aims to maximize the similarity between donor and recipient to minimize rejection. ABO and HLA matching are part of organ allocation protocols for kidney transplantation. Live donation from genetically related family members is encouraged.

1. ABO blood groups: there are naturally occurring antibodies in the blood against A and B antigens. Patients are generally matched for ABO blood group.

2. HLA matching: antibodies against HLA antigens may be present before transplantation (due to exposure via previous organ transplants, blood transfusions, or pregnancies) or develop after transplantation. The difference between donor and recipient HLA type is expressed as the number of mismatches at the 3 most important sites: HLA-A, HLA-B, and HLA-DR. Outcome is related to the number of mismatches in kidney transplantation, although less so for other organs.

Through improvements in antibody-removal techniques, ABO-incompatible and positive cross-match (pre-formed antibodies against mismatched donor HLA) transplantation are now performed in some centres.

Immunological investigations before and after transplantation

There are no routine assays for allo-specific T-cell activation.

There are several techniques for detecting recipient antibodies against the graft.

The recipient serum is checked for antibodies against HLA epitopes in the work-up to transplantation (to inform organ allocation), and at the time of transplantation when a potential donor has been identified to double-check and avoid hyperacute rejection: the cross-match. This assay directly tests if serum from the recipient is able to bind and/or to kill the identified donor’s lymphocytes. A positive cross-match (+XM) is generally a contraindication to transplantation.

After transplantation, the recipient serum can be checked for antibodies; if antibodies develop against the graft, the patient is at higher risk of rejection and graft failure.

Immunosuppressive therapy

Modern era immunosuppression protocols usually involve an induction agent (pre-transplant: front-load) and one or several post-transplant agents taken as long as the graft is functional. When rejection develops, further drugs are given. The different types of drugs used are illustrated in lecture.

The need to prevent and treat rejection must always be balanced in the individual patient with the complications related to immunosuppressive drugs: malignancy, drug toxicity and infections.

**Haematopoietic stem cell transplantation (HSCT)**

Transplantation of bone marrow stem cells is used in the treatment of haematological and lymphoid cancers, some autoimmune diseases leading to loss of marrow cells, and acquired (autoimmune) or inherited deficiencies in marrow cells such as errors of metabolism or immunodeficiencies.

The host’s immune system is eradicated using total body irradiation and cytotoxic drugs, then replaced with patient’s own (autologous) or an HLA-matched donor (allogeneic) bone marrow. Allogeneic HSCT leads to reaction of donor lymphocytes against host tissues: graft-versus-host-disease (GVHD), which affects mainly the skin, gut and liver. The intensity of GVHD is related to degree of HLA-incompatibility. It is accompanied by a graft-versus-tumour effect. Immunosuppressive drugs are used to prevent and treat GVHD.

**Whoever though exams were a good idea? How to pass Year 6 exams and the educational background to what makes a good assessment**

**By Dr Paul Booton**

Human Prion Diseases

Dr Alex Everitt

|  |  |  |
| --- | --- | --- |
| **Table 1: Human Prion Diseases** | | |
| **Type** | **Clinical syndromes** | **Etiology** |
| **Acquired CJD** | Kuru  Iatrogenic CJD  Variant CJD (vCJD) | Exposure to human prions during cannibalistic feasts  Accidental inoculation with human prions  Exposure to Bovine spongiform encephalopathy (BSE)-like prion strain |
| **Sporadic CJD (sCJD)** | sCJD  Atypical CJD | Somatic PRNP (human prion protein gene) mutation or spontaneous conversion of PrPc to PrPsc |
| **Inherited** | Familial CJD, GSS, Fatal familial insomnia (FFI), various atypical dementias | Germline PRNP mutation |

|  |  |
| --- | --- |
| **Table 2: Diagnosis of human prion diseases** | |
| **Sporadic (classical) CJD** | * Serial EEG shows pseudoperiodic complexes in most cases * CSF 14-3-3 protein usually positive * CT and MRI normal, or atrophy, or abnormal signal in basal ganglia * PRNP analysis: no pathogenic mutations, most are 129 MM (VV and MV may be of longer duration, clinically atypical, and with EEG less often positive) * Brain biopsy in highly selected cases (to exclude treatable alternative diagnoses): PrP immunocytochemistry or western blot for PrPSc types 1–3 |
| **Iatrogenic CJD** | * EEG, CSF, and MRI generally less helpful than in sporadic cases * PRNP analysis: no pathogenic mutations, most are 129 homozygotes * Brain biopsy in highly selected cases (to exclude treatable alternative diagnoses): PrP immunocytochemistry or western blot for PrPSc types 1–3 |
| **vCJD (Human BSE)** | * EEG: non-specific slow waves * CSF 14-3-3 may be elevated or normal * MRI: most show high T2 weighted signal in posterior thalamus bilaterally * PRNP analysis: no mutations, all are 129 MM to date * Tonsil biopsy: characteristic PrP immunostaining and PrPSc on western blot (type 4t) |
| **Inherited prion disease** | * PRNP analysis: diagnostic, codon 129 genotype may predict age at onset in pre-symptomatic testing |

Renal Function, Acute Kidney and Chronic Kidney Disease

Dr Peter Choi

**Learning Objectives:**

* To understand the relative strengths of different methods of assessment of GFR
* To understand why serum creatinine alone is a poor marker of absolute kidney function, and that a normal serum creatinine does not mean that renal function is normal
* To appreciate the importance of other functional markers of kidney function, such as urinalysis, biochemistry of serum and urine, and imaging.
* To appreciate the underlying pathophysiological differences between acute and chronic renal disease.
* To understand the clinical syndromes and sequalae of acute and chronic renal disease, and how this determines management objectives.
* To learn how to distinguish between acute and chronic renal disease in practice.
* To understand the role of renal biopsy