Imperial College London

School of Medicine

Year 2 - Summer term 2013

Theme Leaders:
Dr Gareth Barnes & Dr Mike Wilson

Course leaders: all can be contacted via Blackboard
http://bb.imperial.ac.uk

Cardiovascular	Dr Philip Kilner
Physiology of Infection	Prof Shiranee Sriskandan
Critical appraisal of science and patient data	Dr Mike Wilson
Water and Electrolytes	Dr Damien Ashby
Drugs and the Hospitalised patient	Dr Gareth Barnes
Nutrition	Dr Lina Johansson

Science and Patient

Course guide

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SOLE Feedback – Science and Patient

We cannot overstress the importance of doing your SOLE feedback. Changes are made in response to your views. Additionally if there are aspects of the course that you find particularly enjoyable we are keen to learn about this too.

This year we have changed the order, content, lecturers and length of the modules based on last year's feedback. Please take the time to do this for the sake of next year's course.

We have changed the format of feedback this year and this greatly reduces the amount of feedback we ask you to give. Feedback will be on a per theme basis, rather than for every lecture. The relevant SOLE will "open" as the theme has completed.

Please use the following templates to record your thoughts as the course proceeds. At the end of the course you can enter your views onto SOLE. Thank you.

GB/MW

Please answer all questions by selecting the response which best reflects your view. After the questions there is an opportunity to comment on any aspects about which you feel strongly.

	N/A	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. By the end of the course, I think the aims and objectives will have been met.	☐	☐	☐	☐	☐	☐
2. Teaching and learning opportunities (e.g. lectures, small groups, practical) for this course are suitable.	☐	☐	☐	☐	☐	☐
3. Appropriate resources (e.g. books, computers, lab equipment etc.) are available for this course.	☐	☐	☐	☐	☐	☐
4. Appropriate support materials (e.g. handouts, web pages, problem sheets) are available for this course.	☐	☐	☐	☐	☐	☐
5. I receive sufficient guidance and feedback.	☐	☐	☐	☐	☐	☐
6. The workload on this course is manageable.	☐	☐	☐	☐	☐	☐
7. Overall I am satisfied with this course.	☐	☐	☐	☐	☐	☐

8. If you wish to make further comments about this course, please use the space below.

Introduction

Science and Patient started in the Autumn term, with the main focus on critical appraisal and reviewing scientific publications. The Summer term represents the main body of Science and Patient. The themes covered over the next seven weeks are:

Cardiovascular
Physiology of infection
Critical appraisal of science and patient data
Water and electrolytes
Drugs and the hospitalised patient
Normal and abnormal nutrition

While we have tried as far as possible to keep all the teaching for the individual themes together, occasionally we have had to move things around to accommodate the schedules of the excellent teachers we have lined up for you. The 'Critical appraisal' theme will take the form of a practical that you will largely design collectively as a year, more information about which can be found later in this guide under 'In-course assessment'.

Historically students find S&P a tough part of their undergraduate life and some even question the point of this course. We agree that it can be challenging, as we are starting to introduce a different style of learning, which is very much to help prepare you for your future studies. Much of your learning thus far has been didactic, with emphasis on learning and retaining factual information. This is an essential part of your studies and entirely required to work as a doctor.

However we want the focus of the next few weeks to change this. S&P serves as a link between years 2 and 3, when self-direction becomes really important. Therefore, in addition to directed teaching, the timetable includes what is known as 'white-space'. This is time in the timetable that apparently has nothing in it. The ideal use of white space time is for you to decide what you need to work on and then spend that time working on it. Read round subjects that you have encountered and found interesting. Over the next few years there will be many times on wards that you find yourself with time to spare and no direct teaching; making the most of this time is essential.

We have tried to include as many tutorials as possible to allow discussions rather than entirely lecture based themes. We hope that you prepare effectively ("white space learning") for this and contribute to the discussions. As with all such things, the more you put in, the more you get out.

Some of the content may seem esoteric and not immediately obvious. In part that may be true; why focus on one particular disease or problem when there are so many? However, part of the aim of this course is to develop skills, so certain topics are used to teach these skills, rather than deliver particular facts. Other sections reflect an understanding by theme leaders of what topics you will encounter next year and this will give you some foundation.

One of the most important elements of this course is application of knowledge that you have acquired in the previous two years and throughout Science and Patient. We want you to integrate what you know and apply it to what you are being asked; be inquisitive, do think laterally and critically appraise everything you read, both scientific and clinical. This reflects most accurately what you will encounter in your everyday life as a doctor.

You will also complete a BSc in year four. There are direct links from Science and Patient to the BSc year and this course will help in your preparation. You will be expected to read research articles, attend journal clubs, appraise content and become relatively expert in the subject of your choice. These skills have been introduced already and you should maintain and develop them through Science and Patient and the years ahead.

Learning outcomes

The learning outcomes for Science and Patient include both 'global' outcomes from the course as a whole, and more specific learning outcomes from each of the themes within it. By the end of the course you should be able to:

1. **Demonstrate** the ability to add to the taught material of the course through self directed learning
2. **Integrate** knowledge gained across the areas of the course and integrate them into the healthy and sick person
3. **Demonstrate** how defects in one system may lead to defects in others

You should also be able to **demonstrate** an in depth knowledge of each of the areas covered in the course:

	Topic	Overview
1	Cardiovascular	This module is designed to help prepare you to understand, recognise and investigate some of the more common cardiovascular pathologies.
2	Physiology of Infection	You need to be able to explain how bacteria and other pathogens stimulate the immune system to produce either a protective or a harmful response in the host. Lectures will link the immunological changes that occur following infection with development of multi-organ failure. Emphasis will be on pathogenesis rather than management.
3	Critical appraisal of science and patient data	It is important that you realise that publications have flaws, and that "publication bias" affects both science and clinical research. Reviewing publications and designing and writing up your own experiment and using a reference program (refworks) is essential.
4	Water and electrolytes	How is water and electrolyte balance regulated in healthy people? You will explore some of the more important ways in which homeostasis in these areas can be compromised in disease.
5	Drugs and the hospitalised patient	How do we choose the right drug for the right patient? We will follow the patterns of medication likely to be encountered in patients admitted with acute medical problems. We will also consider various aspects of making the right choice, including drug interactions, drug discovery and healthcare management.
6	Nutrition	What are our nutritional needs throughout life? Specific focus will be given to how appetite is controlled, the relationship of this to the rising incidence of obesity and how obesity predisposes to serious disorders such as type 2 diabetes.

You will additionally find more detailed learning outcomes for each of these themes within their respective sections in this guide.

Assessment

Main exam assessment

Since you sat Papers 2, 3 and 4 of Part 2 at the beginning of the summer term, the only exam at the end of this term is Paper 1, a 105-minute written exam covering Science and the Patient. This will take place on the morning of **25th June. You have several clear days between the end of the last lecture and the exam – so plenty of time for revision.**
There is plenty of time for white space learning, and we hope you will attend ALL the timetabled sessions.

Additionally, there is a strong focus through the modules on discussing cases and small group learning.

The exam paper consists of two sections. **Section A** (allocated 50 marks) comprises short answer questions (SAQs). **Section B** (allocated 50 marks) consists of a single essay from a choice of two. **The exam will constitute 80% of the assessment for S&P.**

In the essay we will test the ability to think across everything you have learned in the last two years, using the taught parts of Science and Patient as the basis, and are looking for integration of your knowledge.

Resources are available throughout the course, which will help with answering the essay questions. Tutorials with case histories will assist with understanding the complexities of clinical problem solving and the need to take information from more than one area.

There are also sample essays and old exam papers available on Blackboard to illustrate the breadth and depth of knowledge required. We will additionally be holding some 'mock exam' sessions based on the papers given at the back of this guide.

In course assessment

20% of the marks for S&P are allocated based on the write-up of a practical.

You have already started reviews of journals and know what papers are like. One of the most important tasks we will all do together is to design an experiment, plan its implementation, and then actually undertake it on 20th or 21st May.

We will give you some indication of what apparatus is available and a very, very broad outline of a protocol. However you as the year group will retain as much ownership as possible. There will be sessions throughout the course discussing the practical and Blackboard will generate plenty of activity around this. The primary goal is not necessarily to produce groundbreaking data, but by going through the process of designing an experiment, you will gain a much better idea of scientific method and the pros and cons of various experimental approaches.

You then have some white space to write up this experiment as if you were going to submit it to a journal. You MUST be able to use REFWORKS, and you need to have signed up for an account before you attend the sessions. There are library based practical sessions for ALL of you to ensure that you can actually login to the system and collect references to use in your submission. Attendance at these will be monitored.

You will need to write the publication to include all the standard sections of a paper, similar to the ones you read in the journal clubs in November. Aim, introduction (including a discussion of the literature so far), methods (that we will plan together before the practical), statistical methods, results, discussion and conclusion are essential. Carrying out statistical tests on your

own data is without any doubt the best way to begin to understand what can otherwise be a rather esoteric subject, and to help you with this Tom Sensky is going to hold a session on statistics with the practical in mind, just after you have run the experiment.

The deadline for us receiving this, **via Blackboard, is 23:59 on Monday 27th May** (so you have the bank holiday to finalise it!), and there are penalties for late submission.

Note: there will be a 5% contribution from the Year 2 Science & Patient assessment score to your BSc degree classification in Year 4.

Interactive Online Learning

Part of your course has been converted into online interactive content, which will be delivered using Blackboard. You will also need to use to Blackboard to submit your practical write-up, so ensure that you are familiar with the system well in advance.

You will be able to navigate within Blackboard in a very similar way to browsing the Internet and the Intranet.

There are tutorial MCQs linked to the 'normal and abnormal nutrition' theme of the course. Blackboard will enable you to look at questions at your leisure and obtain answers directly. Instructions as to how to use these quizzes are given below:

- Click on the Blackboard icon on a College PC or bb.imperial.ac.uk
- Login to Blackboard using your username and password
- Click on My Courses, Year 2 – Science & Patient
- Click on Learning Materials and this will open links for all the Discussions and Quizzes.
- **The ECG** and CXR section is under "clinical skills", which is posted on the Science and Patient intranet site (Friday 10th May)

*****This will also contain the link for submitting your in-course assessment*****

If you are experiencing problems logging into Blackboard or viewing content, please follow the instructions https://education.med.imperial.ac.uk/ITprobs.html
Contact us with queries and comments by email to webmaster.feo@imperial.ac.uk

Communication with theme leader or module organizers:
This must all be via Blackboard – there will be a discussion forum for the whole course and for each module. The module leaders will monitor the discussion threads and correct errors and answer questions where necessary. However, the best discussions are those where students help each other – do look each day and contribute.

PLEASE DO NOT EMAIL MODULE ORGANISERS OR INDIVIDUAL LECTURERS
- all communication must be via Blackboard except for clearly personal issues such as sickness. Queries about the course must be via Blackboard. We hope you enjoy the course – you should come away with an appetite for seeing patients and starting to apply all your basic science knowledge to real clinical problems in an integrated way.

Finally please note that we expect all students to behave in a professional manner during sessions of all types, including lectures and tutorials.

GB MW
Theme leaders

Cardiovascular module

Course leader: Dr Philip Kilner

This module aims to help prepare you to understand, recognise and investigate some of the more common cardiovascular pathologies. Examples include ischaemic heart disease, hypertrophic or dilated cardiomyopathies, disease of heart valves and common arrhythmias, notably atrial fibrillation.

Aspects of the complexity of the cardiovascular system will be illustrated. Please come willing to reconsider how you can observe, question and discover for yourselves. What is represented by images, dataset or explanations is always incomplete. These always fall short of representing the complex, multi-scale, ever-changing reality of a human organism in social and environmental contexts. Each approach can nevertheless give insight of a certain kind. Each should be used with awareness of its limitations, and with awareness of alternative approaches.

Analytical investigative approaches alone generally fail to deliver meaningful views of the processes of life, illness and recovery. Analysis should ideally be balanced by *integrative* and *contextual* awareness. This is important, although not easy to teach and hard to evaluate through exam questions. The habit of thinking contextually as well as analytically is something you should strive for and practice individually.

Individual Lectures:

Monday May 7th

9.20 Philip Kilner. 'Cardiovascular module: introduction'.
In this opening session, the plan of the module will be outlined and the nature of the cardiovascular system introduced. The forms, movements and flows of the healthy heart and circulation will be illustrated across a range of spatial and temporal scales. Aspects of its complexity and continuity will be considered, with glimpses back to the time scales of vertebrate evolution and the processes of embryonic formation.

Learning objectives
At the end of this lecture you should:

- Have gained confidence to engage your own faculties of observation and inquiry.
- Have shared an attempt to approach a subject (the cardiovascular system) contextually as well as analytically.
- Have a clearer appreciation of the fluent, interconnecting movements of blood through the heart, vessels and microvascular networks of the circulation.

10.00 Peter Kohl. 'Systems Biology of the Heart: Hope or Hype?'

Background: What is systems biology? How does it differ from classic biomedical research? Do we need it? What is the role of computer models in this context? Can they be relied on? Can computer simulations replace experiments on living animals? When will we have an all-inclusive model of a patient? These and other questions are often raised in the context of post-genomic research. This lecture will address the above questions, using illustrations from heart research conducted.

Aim: provide conceptual background, introduce definitions, and provide examples of the systems research approach to integration and reduction of cardiac structure and function data.

Objective: highlight the need for multi-level (from ion channel to ECG) and multi-type (e.g. including experimental and theoretical investigations) model systems for quantitative projection between basic science and clinical relevance.

Take home skills: at the end of the lecture, students will be expected to be able to:

- Explain their understanding of 'systems biology',
- Define the term 'model',
- Illustrate the concepts associated with these terms on at least one example from bio-medical research.

Thursday May 9th

09.00 Philip Kilner. 'Cardiovascular imaging and reality'.

The hardware, principles, strengths and limitations of x-ray, computed tomography, ultrasound and MRI approaches to imaging will be illustrated and considered in relation to cardiovascular imaging.

Learning objectives

At the end of this lecture students should:

- Have a basic appreciation of the principles and the relative strengths and limitations of x-ray, computed tomography, ultrasound and MRI approaches to imaging, particularly of the heart.
- Be ready to approach any medical image (or measurement or trace) with sense of inquisitiveness: 'what can I see and discover?', 'what *can't* I see through this approach?', 'why?'…. i.e. ignore any sense of ignorance and *engage your observant, inquisitive and critical faculties*.
- To recognize key imaging features of the cardiovascular pathologies illustrated.

10.00 Eliana Reyes. 'Ischaemic heart disease'

The initial assessment of patients with symptoms of suspected myocardial ischaemia relies on a detailed clinical history that emphasises the identification of cardiovascular risk factors, and a thorough physical exam. In some patients, further investigation may be needed. There are several methods available to assist clinicians in making the diagnosis of ischaemic heart disease including exercise ECG and non-invasive cardiac imaging (stress echocardiography, myocardial perfusion scintigraphy, cardiovascular magnetic resonance imaging, and computed tomography of the heart). Although they have similar diagnostic accuracies, each method has its own advantages and limitations and these may influence clinicians to choose one method over the others. Once the diagnosis of ischaemic heart disease is made, therapy is aimed at improving symptoms and prognosis. There are currently several therapeutic strategies, which may involve lifestyle changes, medication such as statins, anti-platelet drugs and beta-adrenergic receptor antagonists, and invasive intervention including percutaneous coronary revascularisation and coronary artery bypass grafting surgery.

Learning Objectives

At the end of this lecture students should be able to:

- Understand the importance of the clinical history and physical examination in the initial assessment of patients with suspected ischaemic heart disease
- State the clinical indications for exercise ECG, non-invasive cardiac imaging and invasive coronary angiography
- Describe the methods available for the investigation of chest pain of possible cardiovascular origin, and for the detection of myocardial ischaemia

- Understand the role of risk factor modification and pharmacological therapy in the management of patients with stable angina

11.00 John-Paul Carpenter. 'Cardiovascular case presentations'

Cases, illustrated by relevant imaging, include coronary artery disease, valve disease, heart muscle disorders and other causes of heart failure or breathlessness. You are invited to engage in the deductive process through which investigations are chosen and interpreted towards the diagnosis.

Learning objectives
At the end of this lecture students should be able to:

- Understand the rationale for choosing appropriate investigations in relation to the conditions covered
- Recognize key imaging features of the cardiovascular pathologies illustrated.

2.00 Aamir Ali. 'Clinical cardiology'

This lecture will provide an overview of the diagnosis and management of common cardiac presentations seen on the ward to include: chest pain, atrial fibrillation and cardiomyopathies.

Learning objectives
At the end of this lecture students should be able to:

- Characterise different types of chest pain and outline other symptoms that may be present.
- List common causes for each category of chest pain and associated features: cardiac, pleuritic, musculoskeletal, upper GI.
- Outline the common first line investigations for chest pain including: ECG, echo, coronary angiogram and CT coronary angiogram.
- Describe the different types of cardiomyopathy including first line investigations: echo, MRI
- Describe clinical features of atrial fibrillation, diagnosis and management including embolic risk stratification and rhythm vs. rate control
- Describe the treatment options for cardiomyopathy including medical therapy, implantable cardioverter defibrillators and bi-ventricular pacing.

3.15 Paul Thompson. 'Extreme physiology – methodology and science behind elite rowers'.

As a coach I apply physiological principles and training methods to get athletes to peak fitness and performance in a physiologically demanding race. I will talk about how we set about training, and how we monitor and test its progression. These principles were applied in the preparation of GB Rowers at the London Olympics.

For revision:

Friday 10th May, 9am till noon.
Opportunity for self directed learning, and clinical examination videos in cardiology.
ECG interpretation online and understanding the mechanism of heart sounds and murmurs (online youtube videos on the intranet).

Besides material in the lectures, the 'Cardiovascular medicine' section in the Oxford Handbook of Clinical Medicine is recommended.

Physiology of Infection

Course leader: Prof Shiranee Sriskandan

The Physiology of Infection module will comprise a series of lectures and teaching sessions that attempt to bring together aspects of microbiology, immunology, inflammation, and cardiovascular physiology which are traditionally taught separately.

Learning objectives
At the end of the course students should be able to:

- Demonstrate an understanding of the pathogenesis of infection
- List the common pathogens infectious to man and major virulence attributes thereof
- Explain how bacteria and other pathogens stimulate the immune system
- Demonstrate an understanding of innate immune response to microbial challenge
- Explain how the innate and adaptive immune responses to infection work together
- Explain how the immune response can be protective or harmful, and how host based factors may alter the efficiency of the immune response
- Describe the links between immune responses to pathogens and effects on vascular endothelium, and other organ systems
- Explain the pathophysiology of sepsis in the critically ill

Lectures will link the immunological changes that occur following infection with development of multi-organ failure. Emphasis will be on pathogenesis rather than management. It is hoped that students will take time to read up on specific areas highlighted for private study, in order to get a clear idea of how sepsis and septic shock might develop. None of the areas being taught are completely new, hence some may appear as revision; however, the key ability will be to integrate this knowledge with other knowledge relevant to infection. At the end of the Science and Patient course, it is hoped that students will be able to integrate their approach to the physiology of infection with approaches learned in the other Science and Patient modules (e.g. salt and water balance, pharmacology, nutrition)

Small group teaching and self-directed learning

To crystallise these ideas, students will be given a set of case scenarios at the start of the module, which they should discuss in small study groups during the week. Each study group will be expected to give a short presentation on their case scenario (one for each of the 2 small group teaching sessions), focused around specific questions. Suggested articles relevant to some or all of the case scenarios are listed over the subsequent pages and should be used as an additional resource for the small group discussions to supplement material given in the lectures. Events leading from infection to resultant clinical improvement or deterioration will be discussed, building on the information learned during the course. All students are expected to attend and participate in the preparation for these sessions as they provide useful strategies for tackling complex problem-based cases.

Tutorial groups for this module are on the electronic timetable. There are **3 cases plus a journal club paper** to be discussed in the morning session and **3 cases plus a journal club paper** to be discussed in the afternoon session. Each study group will take on one case/paper to work on and present to the rest of the tutorial group in the morning; this is then repeated with the different cases in the afternoon. Please ensure that the same group do not end up doing both journal club papers!

EACH TUTORIAL GROUP (e.g. E1+E2) NEEDS TO SPLIT INTO A TOTAL OF 4 STUDY GROUPS. This is simplest if E1 splits in two, and E2 splits in two, as an example.

e.g. in the **morning session**: case 1 and case 2 will be done by the students in E1, while case 3 and the journal club paper will be presented by students in E2. **Importantly ALL students must have read the journal club paper and answered the questions on the sheet in the guide**. In the **afternoon session**, the journal club paper and case 4 will be done by students in E1 while case 5 and case 6 will be done by students in E2.

The case scenarios in the morning relate to important disease pathogens, and will focus on specific features that allow these pathogens to survive, infect, and cause certain types of disease. Each small study group will report back to the group as a whole on their analysis of their case scenario, using appropriate media (acetates, white-board etc). The discussion will be facilitated by a nominated faculty member (who will be either an infectious diseases physician or microbiologist). It will be important for each group to stick to time when discussing their case; suggested time for presentation is 10-15 minutes max. **Groups are asked to focus on the questions posed by the case rather than reciting from the review papers listed to help research the case. Only some of the information in the reviews will be relevant to the case. Students are encouraged to summarise the pertinent information from the review for their fellow students in a way that is easy to understand and present.**

The case scenarios in the afternoon pertain to interactions of pathogens with the host response, and will focus on aspects of the innate immune response, cytokines, bacterial clearance, and influence of the adaptive response. Cardiovascular responses will also be discussed where appropriate; it will again be important for each group to stick to time when presenting their case and **to focus on the questions posed by the case**.

Discussion of clinical cases in a logical fashion, bringing together different areas of clinical science, will provide an important learning platform for the final examination where cases require discussion in the context of an essay

Tutors for the groups will be drawn from the following infectious diseases and microbiology clinical staff:

Dr. Sally Curtis	Dr. Lucy Lamb
Dr Hugo Donaldson	Dr Annette Jepson
Dr. Eimear Brannigan	Dr Marianne Nolan
Dr. Claire Thomas	Dr Kathy Bamford
Dr Dunisha Samarasinghe	Dr Lionel Tan
Prof Jon Friedland	Dr Darius Armstrong-James
Prof Sunil Shaunak	Dr Graham Cooke
Prof Shiranee Sriskandan	Prof Alison Holmes
Dr. Hema Sharma	Dr Catherine Ong

Cases for MORNING sessions (Pathogen Factors)

Case 1

A 36 year old injecting drug user was admitted with a massive swelling over the anterior part of the left upper thigh; the patient was profoundly hypotensive and hypothermic. The leg was oedematous and swollen with blue/black necrotic discolouration in one area and erythema elsewhere. The swelling extended over the perineum and into the scrotal area. He reported injecting heroin regularly into the left femoral vessels and had recently obtained a new supply. En route to theatre for surgical debridement, the patient deteriorated further with severe acidosis and cardiac arrest. Samples taken in the emergency department from fluid oozing from the leg were culture negative. However tissue post mortem grew Bacillus anthracis.

1. What are the ways in which B. anthracis infects humans?

2. What are the key virulence factors that lead to this fulminant course of infection?

3. How is management affected by these virulence factors and what could assist in treatment?

4. What are the public health implications of this case and what other infections might this case mimic?

Case 2

A 35-year-old man presents with shortness of breath and a suppurating wound infection overlying a recent insect bite sustained during a 4 week holiday in Thailand, where he was staying in a shared house with 4 friends. He gives a history of a similar lesion that burst some 5 days previously yielding pus. A doctor in Thailand had prescribed flucloxacillin but the lesion had not improved. A chest x-ray shows shadows in the left upper zone that appear to be cavitating. Cultures of blood and pus from the arm lesion yield methicillin resistant Staphylococcus aureus. MRSA swabs taken routinely on admission are also positive.

1. What are the bacterial factors which have allowed this infection to start and progress (bacterial resistance to immune response)?

2. Why were the original antibiotics insufficient and how might we treat him now?

3. What is the epidemiology of this type of infection in the UK and elsewhere, and how might it have spread to our patient?

Case 3

A patient with chronic lung disease (who was being given alternate day azithromycin to reduce infective exacerbations) syndrome has been intubated and ventilated on an ICU in a different hospital for 9 weeks following community-acquired pneumonia, for which they were originally treated with amoxicillin and clarithromycin, followed by tazocin for 2 weeks. The patient has been transferred to our own ICU with a tracheostomy and nursing staff report aspirating thick green secretions from the ET tube. This morning the patient became acutely unstable with high temperature, falling oxygen saturations, and tachycardia. The infection team commences therapy with meropenem (a carbapenem) and amikacin (an aminoglycoside) to cover Pseudomonas spp. as well as other organisms, in addition to basic resuscitation including fluids and increased oxygen therapy. Blood cultures yield a Pseudomonas aeruginosa sensitive to ciprofloxacin (a quinolone), meropenem, and amikacin but resistant to tazocin. The team continue meropenem and stop amikacin because of concerns about toxicity. The patient makes a dramatic improvement but after 7 d a new tracheal aspirate yields a Pseudomonas resistant to meropenem.

1. What are the features of Pseudomonas that enable it to survive in the lung/bronchial tree (biofilm and virulence factors - what other bacteria are notorious for biofilm)?

2. What are the specific reasons why the antibiogram changed? Why are they using more than one antibiotic? When else are multiple antibiotics used?

3. What are the mechanisms by which the patient suddenly became unwell?

Suggested reading for CASES 1-3 and journal club paper 1

All articles should be available either directly via PubMed using a College computer OR via the College electronic journal listing (accessed via the library website). Please notify the module leader if you cannot access an article ***only*** after trying both routes (check with colleagues first). As this is a rapidly moving subject and the course brings together several classically 'separate' fields there is no current textbook which is recommended.

Bacillus anthracis (groups presenting case 1)

Dixon TC, Meselson M, Guillemin J, Hanna PC. Anthrax. N Engl J Med. 1999 Sep 9;341(11):815-26

Koehler TM. Bacillus anthracis physiology and genetics. Mol Aspects Med. 2009 Dec;30(6):386-96.

Moayeri M, Leppla SH. The roles of anthrax toxin in pathogenesis. Curr Opin Microbiol. 2004 Feb;7(1):19-24.

Migone TS, Subramanian GM, Zhong J, Healey LM, Corey A, Devalaraja M, Lo L, Ullrich S, Zimmerman J, Chen A, Lewis M, Meister G, Gillum K, Sanford D, Mott J, Bolmer SD. Raxibacumab for the treatment of inhalational anthrax. N Engl J Med. 2009 Jul 9;361(2):135-44.

HPA website (see under anthrax outbreak 2010) and also
http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1265637164350

Community-acquired Staphylococcus aureus (groups presenting case 2)

http://www.hpa.org.uk/hpr/archives/2011/news0711.htm#pvl

http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1218699411960
(WARNING this is a long PDF- pick out the relevant bits and summarise!)

Nizet V. Understanding how leading bacterial pathogens subvert innate immunity to reveal novel therapeutic targets. J Allergy Clin Immunol. 2007 Jul;120(1):13-22. Review.

Otto M. Basis of virulence in community-associated methicillin-resistant Staphylococcus aureus. Annu Rev Microbiol. 2010;64:143-62. (WARNING! This is a long review article- Pick out the sections that are relevant)

Pseudomonas aeruginosa (groups presenting case 3)

Bleves S, Viarre V, Salacha R, Michel GP, Filloux A, Voulhoux R. Protein secretion systems in Pseudomonas aeruginosa: A wealth of pathogenic weapons. Int J Med Microbiol. 2010 Dec;300(8):534-43.

Maciá MD, Blanquer D, Togores B, Sauleda J, Pérez JL, Oliver A. Hypermutation is a key factor in development of multiple-antimicrobial resistance in Pseudomonas aeruginosa strains causing chronic lung infections. Antimicrob Agents Chemother. 2005 Aug;49(8):3382-6.

Veesenmeyer JL, Hauser AR, Lisboa T, Rello J. Pseudomonas aeruginosa virulence and therapy: evolving translational strategies. Crit Care Med. 2009 May;37(5):1777-86.

Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. Cell Microbiol. 2009 Jul;11(7):1034-43

Journal Club paper 1 (4th group)

WHOLE YEAR SHOULD LOOK AT THIS PAPER BEFORE THE TUTORIAL

Host imprints on bacterial genomes--rapid, divergent evolution in individual patients.

Zdziarski J, Brzuszkiewicz E, Wullt B, Liesegang H, Biran D, Voigt B, Grönberg-Hernandez J, Ragnarsdottir B, Hecker M, Ron EZ, Daniel R, Gottschalk G, Hacker J, Svanborg C, Dobrindt U.

PLoS Pathog. 2010 Aug 26;6(8):e1001078.

For the group presenting the paper please try to address as many of the following questions during your presentation:-

a. What was the rationale for the study?
b. Describe the type of study: what were the controls?
c. What were the ethical issues if any?
d. What was the intervention and what was being measured or monitored?
e. What were the techniques used by the investigators to monitor these patients? Can you (briefly!) explain the technique of whole genome sequencing.
f. What were the major findings?
g. How did the investigators corroborate the relevance of their sequencing findings?
h. Describe the immunological studies undertaken and rationale.
i. Is there anything else you think they could have done to add to the study?
j. What do you think (if any) the clinical relevance of this study is? Are there similar findings from other bacteria?

Papers on urosepsis and E. coli (groups presenting journal club paper 1)

Seed PC, Hultgren SJ. Blueprinting the regulatory response of Escherichia coli to the urinary tract. Trends Microbiol. 2005 Jun;13(6):246-8.

Roos V, Ulett GC, Schembri MA, Klemm P. The asymptomatic bacteriuria Escherichia coli strain 83972 outcompetes uropathogenic E. coli strains in human urine. Infect Immun. 2006 Jan;74(1):615-24. *Use of this strain of E. coli to combat infections*

Tobe T.The roles of two-component systems in virulence of pathogenic Escherichia coli and Shigella spp. Adv Exp Med Biol. 2008;631:189-99. doi: 10.1007/978-0-387-78885-2_13. Review of gene regulation in E coli. *Principle of two component regulators (if interested!)*

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PLoS PATHOGENS

Host Imprints on Bacterial Genomes—Rapid, Divergent Evolution in Individual Patients

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Abstract

Bacteria lose or gain genetic material and through selection, new variants become fixed in the population. Here we provide the first, genome-wide example of a single bacterial strain's evolution in different deliberately colonized patients and the surprising insight that hosts appear to personalize their microflora. By first obtaining the complete genome sequence of the prototype asymptomatic bacteriuria strain *E. coli* 83972 and then resequencing its descendants after therapeutic bladder colonization of different patients, we identified 34 mutations, which affected metabolic and virulence-related genes. Further transcriptome and proteome analysis proved that these genome changes altered bacterial gene expression resulting in unique adaptation patterns in each patient. Our results provide evidence that, in addition to stochastic events, adaptive bacterial evolution is driven by individual host environments. Ongoing loss of gene function supports the hypothesis that evolution towards commensalism rather than virulence is favored during asymptomatic bladder colonization.

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Introduction

Microbes have adapted many fascinating strategies to co-evolve with their hosts. The specific immune response to surface antigens drives the structural changes in influenza virus hemagglutinin and serotype [1], the antigenic drift in trypanosomes [2] and the immune evasion mechanisms in malaria [3]. Similar mechanisms operate in bacteria, forcing them to vary their surface antigens and to maintain critical functions encoded by those genes, even in the presence of a fully functional immune response [4]. While such host-modulated microbial elements have been extensively studied, less is known about microbial adaptation to environmental signals inside individual patients. Most importantly, a host-specific approach to the analysis of genome-wide alterations has not been taken.

Urinary tract infections (UTIs) present an interesting and highly relevant model for studying microbial adaptation. After establishing significant numbers, the bacteria either cause severe and potentially life threatening disease, or an asymptomatic carrier state resembling the normal flora at other mucosal sites. Patients with asymptomatic bacteriuria (ABU) may carry the same strain for months or years and this outcome is advantageous for the microbe as it can persist in a favored niche with little microbial competition. ABU is also favorable for the host who may be protected from re-infection if the carrier strain outcompetes new invaders [5,6]. In our previous work, we reported that at least 50% of ABU strains have evolved from virulent uropathogenic *E. coli* (UPEC) strains by genome reduction, i.e. inactivation of genes encoding virulence-associated factors, either by the accumulation of point mutations or by deletions [7,8]. These observations suggest that bacteria adapt to the urinary tract environment and that this human host niche is suitable for understanding the mechanisms involved. The determinants of long-term bacterial persistence and adaptation to the host environment are, however, still poorly understood. For these reasons, we looked at real-time evolution by sequencing the progenitor strain *E. coli* 83972 and then analyzing its re-isolates from several patients.

Author Summary

Bacterial virulence results from the interaction between bacteria and their hosts. This interaction provides selection pressure for bacterial adaptation towards increased fitness or virulence. Basic mechanisms involved in bacterial adaptation at the genetic level are point mutations and recombination. As bacterial genome plasticity is higher *in vivo* than *in vitro*, host-pathogen interaction may facilitate bacterial adaptation. Comparative genomics has so far been almost entirely focused on genomic changes upon prolonged bacterial growth *in vitro*. To achieve a better comprehension of bacterial genome plasticity and the capacity to adapt in response to their host, we studied bacterial genome evolution *in vivo*. We analyzed the impact of individual hosts on genome-wide bacterial adaptation under controlled conditions, by administration of asymptomatic bacteriuria *E. coli* isolate 83972 to several hosts. Interestingly, the different hosts appeared to personalize their microflora. Adaptation at the genomic level included point mutations in several metabolic and virulence-related genes, often affecting pleiotropic regulators, but re-isolates from each patient showed a distinct pattern of genetic alterations in addition to random changes. Our results provide new insights into bacterial traits under selection during *E. coli in vivo* growth, further explaining the mechanisms of bacterial adaptation to specific host environments.

The prototypic ABU *E. coli* strain 83972 has been extensively used for therapeutic urinary bladder colonization in patients with chronic UTI. After intravesical inoculation, the strain establishes ABU and this approach has proven to be safe and to protect the patient from super-infection with more virulent strains [6,9]. Here, we compare the genomes, transcriptomes and proteomes of *E. coli* 83972 to re-isolates from patients deliberately colonized with this strain. We provide evidence that the pattern of genetic and phenotypic changes was distinct for each host and that it involves a limited number of genes, including regulators, metabolic genes and virulence factors.

Results

Complete genome sequence of the protype ABU *E. coli* 83972

To characterize the prototype ABU *E. coli* 83972, we solved the chromosomal DNA sequence and compared it to genomes from other UPEC strains (CFT073, UTI89, 536), enterohemorrhagic *E. coli* (EHEC) strain O157:H7 Sakai and *E. coli* K-12 strain MG1655. The *E. coli* 83972 genome, which was originally isolated from the urinary tract of a schoolgirl [5], comprises a 5,131,397-bp chromosome and a small 1,565-bp cryptic plasmid (Figure 1). According to the genome sequence, *E. coli* 83972 was most closely related to the UPEC strains in particular to CFT073, sharing four chromosomal regions with only this strain (Figure 1, Table 1). Notably, large parts of region 2 and 4 are identical to genomic islands I and II of non-pathogenic *E. coli* strain Nissle 1917, a close relative of UPEC strain CFT073 that evolved by reductive evolution [10]. Six other islands were also shared with other UPEC, but not with EHEC or *E. coli* K-12 (Figure 1, Table 1). These genomic regions encode virulence and fitness-associated factors, including iron-uptake systems, adhesins, toxins, the K5 capsule, different secretion systems, as well as metabolic traits and transporters (Table 1). Other island-encoded traits shared with UPEC and EHEC included type 1 fimbriae, mannonate hydrolase (required for hexuronate degradation) and a C4-dicarboxylate transporter.

Six prophages were identified which were unique in type or chromosomal localization for *E. coli* 83972. Two of these are of particular interest. We found that prophage 4 was similar to prophages so far only described in the genomes of UPEC strain IAI39 (accession no. CU928164) or *Salmonella enterica* serovar Typhi (accession no. AE014613 or AL627270). In strain 83972, it was inserted into the *rstB* gene which encodes for the sensor histidine kinase RstB of the RstAB two-component system. The RstAB system controls the expression of genes involved in diverse processes relevant for bladder colonization, such as acid tolerance, curli formation and anaerobic respiration [11,12]. Prophage 2 was similar to EHEC prophages, disrupting *focD* and thus the F1C fimbrial determinant in *E. coli* 83972.

Human colonization and the *in vitro* continuous culture

Here, we have established asymptomatic carriage of a single bacterial strain in different human hosts and then, using re-isolates obtained from these individuals, studied the host-specific genome-wide changes. Therapeutic bacteriuria was established in six patients by intravesical inoculation of *E. coli* 83972 (Figure 2A). Afterwards, re-isolates obtained from each host at different times (*in vivo* re-isolates) were subjected to genetic and phenotypic analyses (Figure 2B). This was possible as *E. coli* 83972 establishes a monoculture in the human urinary tract and because bacteriuria often lasts for months or years. To distinguish genetic changes driven by the host environment from random events, we cultured *E. coli* 83972 *in vitro* in pooled human urine for more than 2000 generations and included corresponding isolates in the analysis.

Genome structure of re-isolates

By pulsed-field gel electrophoresis (PFGE), we observed alterations in overall genome structure in 31% (5/16) of individual *in vivo* re-isolates. The exhibited restriction pattern alterations differed in comparison to the progenitor strain and also among themselves (Figure S1A). In contrast, 17 independent isolates from long-term *in vitro* cultivation showed no change in genome structure, indicating that genomic alterations depended on individual hosts rather than on preexisting hot spots of genomic variability (Figure S1B). Larger changes in the genome size of *in vivo* re-isolates were not observed, as analyzed by PFGE following I-*Ceu*I digestion, with the exception of strain PII-4 displaying a reduction in genome size (Figure S2A). Analysis of multiple colonies from the corresponding urine samples confirmed that the genome variations were representative for each host and time of sampling (Figure S2B).

Sequencing of re-isolates from inoculated patients and *in vitro* control cultures

From the above candidates, we chose for genome sequencing three re-isolates with altered PFGE pattern from three patients and one randomly chosen *in vitro* propagated 83972 variant (*E. coli* 83972-4.9). Complete genome coverage was obtained and raw sequences were mapped on the chromosome of the progenitor strain *E. coli* 83972. After verification by single locus Sanger sequencing, 37 loci in the four sequenced re-isolates were confirmed to be polymorphic as compared to the parent strain. We found that genomic alterations occurred within conserved and flexible parts of the bacterial chromosome (Figure 3), and with only three exceptions, these affected coding regions. The majority of the alterations were single nucleotide polymorphisms (SNPs) (2 synonymous vs. 27 non-synonymous substitutions), but one

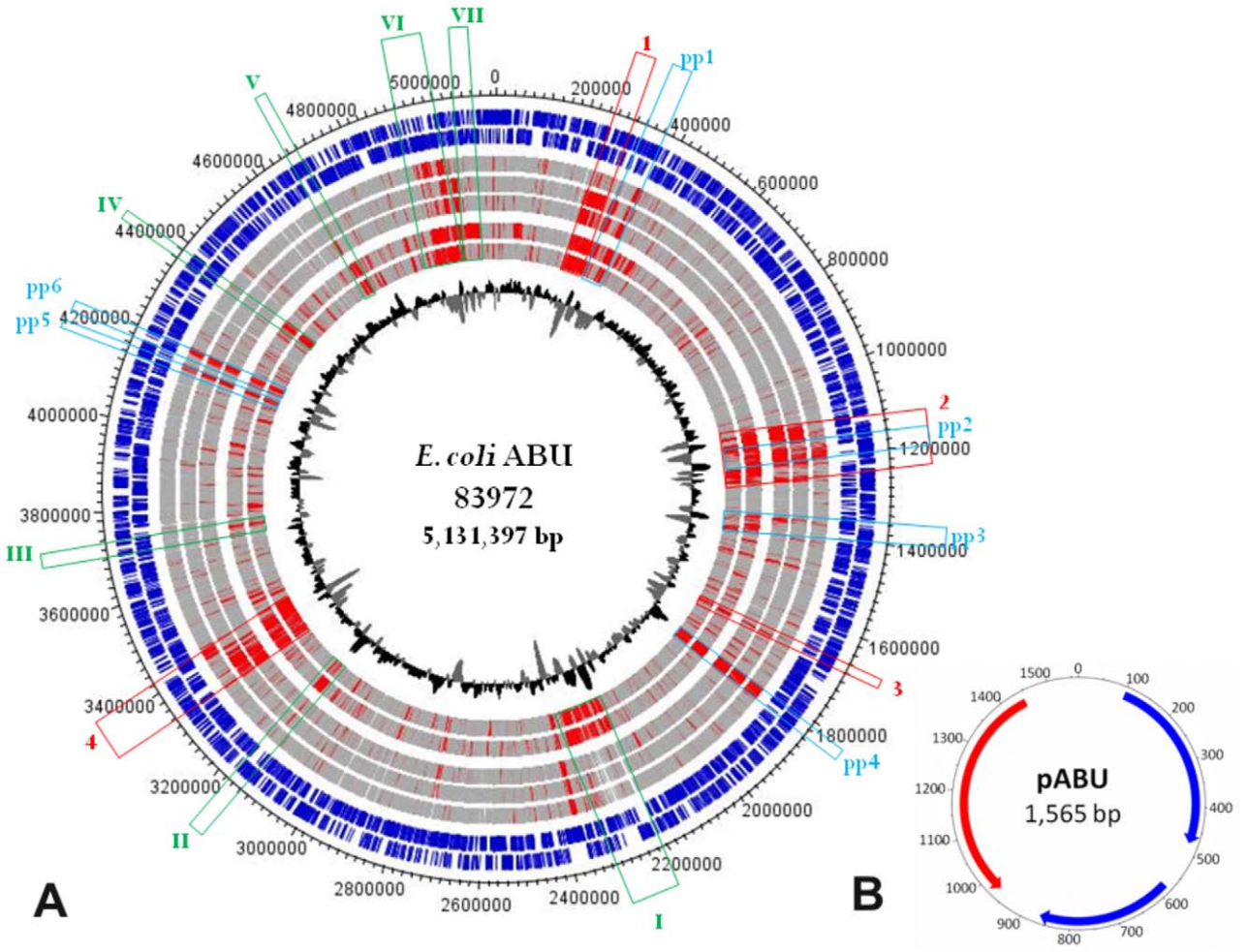

Figure 1. Genetic map of the *E. coli* 83972 chromosome and the small plasmid pABU. Nucleotide sequence analysis of the *E. coli* 83972 chromosome a): The two most outer circles represent all putative open reading frames (ORFs), depending on ORF orientation. The following five circles report the results of a two-way genome comparison between *E. coli* 83972 and one of the following *E. coli* strains: CFT073 (UPEC), 536 (UPEC), UTI89 (UPEC), MG1655 (K-12) and Sakai (EHEC O157:H7). Genes shared between the strain pair compared are indicated in grey and variable genome regions are indicated in red. The innermost circle represents the G+C distribution. Genomic regions only present in strains ABU83972 and CFT073 are framed in red. Chromosomal segments framed in green or blue are only present in the ABU isolate and pathogenic *E. coli* or represent bacteriophage-related DNA, respectively. Details on the gene content of these regions are compiled in Table 1. UPEC, uropathogenic *E. coli*; EHEC, enterohemorrhagic *E. coli*. Nucleotide sequence analysis of plasmid pABU b): putative predicted ORFs have been indicated.
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inversion of 1,731-bp, one large 27-kb deletion and four small deletions of 1, 5, 12 or 165 bp were also detected. Many altered genes encoding proteins with regulatory functions (Figure 3, Table S1) were independently acquired in multiple individual re-isolates but not after *in vitro* culture and thus seemed to represent adaptational hotspots *in vivo*. They included the BarA/UvrY two-component system that controls a global regulatory network affecting a multitude of cellular functions and that has been proposed as a virulence trait in UTI [13], and *mdoH* encoding a glycosyl transferase involved in osmoregulated periplasmic glucan synthesis [14] as well as genes involved in oxidative stress responses (*frmR*) [15].

In re-isolate PI-2, we found that nineteen different genomic loci were mutated relative to the progenitor strain, and 89% of these resulted in an altered amino acid sequence of the encoded proteins. Interestingly, 35% of the above mutations were stop codons and frame shifts. Furthermore, many of the mutations impacted pleiotropic regulatory genes involved in adaptation to different stress conditions including oxidative stress and/or resistance to antibiotics (*frmR*, *marR*, *oxyR*) [16]. Osmolarity, and virulence- or fitness-associated traits were also affected (*barA*, *ompR*, *ompC*, *mdoH*). The genes *barA* and *ompR* are part of the two-component systems OmpR/EnvZ and BarA/UvrY which regulate flagella and adhesin expression, biofilm formation, and glycolytic or gluconeogenic utilization of different carbon sources [17,18].

In re-isolate PII-4, we found nine genomic alterations including five non-synonymous SNPs, a frame shift in the gene encoding for cellulose synthase *bcsA*, as well as huge deletion and one mutation in a non-coding region. Most intriguingly, the last two mutations affected iron uptake systems: aerobactin (*iuc*) and the ferric citrate uptake system (*fec*). The aerobactin gene cluster was lost due to a 27-kb partial deletion of a pathogenicity island (Figure S2C) and the *fecI* upstream region required for ferric citrate uptake was polymorphic (T to C substitution). In addition, we detected sequence alterations in genes encoding the transcriptional repressor of ribonucleoside metabolism (*cytR*) and the transcriptional repressor of ribose catabolism (*rpiR*).

Table 1. Genomic islands and prophages in the *E. coli* 83972 genome.

Genomic region	Position in the genome	Encoded traits
Region 1[a]	ECABU_c02290-ECABU_c03230	Hemolysin expression modulating protein, put. iron transporter (absent in CFT073), put. PTS system, IgA-specific serine endopeptidase, HlyD family secretion protein, put. oligogalacturonide transporter
Region 2[a]	ECABU_c10540-ECABU_c12460	Tagatose utilization, hemagglutinin-related protein (frame shift), microcin V, F1C fimbriae (inactivated due to prophage 2 insertion), salmochelin, antigen 43
Region 3[a]	ECABU_c16830-ECABU_c16980	Vgr-like proteins and hypothetical proteins (type VI secretion system)
Region 4[a]	ECABU_c32560-ECABU_c33710	ShiA-like protein, aerobactin, Sat autotransporter protease, antigen 43, K5 capsule, general secretion pathway, glycolate utilization (*glc* operon)
Region I[b]	ECABU_c22350-ECABU_c23330	Yersiniabactin biosynthesis (high pathogenicity island, HPI), colibactin polyketide biosynthesis
Region II[b]	ECABU_c30880-ECABU_c31120	Vgr-related protein and hypothetical proteins (type VI secretion system)
Region III[b]	ECABU_c36660-ECABU_c36730	Ribose ABC transporter
Region IV[b]	ECABU_c43120-ECABU_c43350	PTS system, glucose-specific IIBC component, transketolase, transcriptional regulator, permease, glutamyl-tRNA(Gln) amidotransferase subunit A, isochorismatase family protein, dienelactone hydrolase family protein, uridine phosphorylase, 2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase, 2-dehydro-3-deoxygalactonokinase
Region V[b]	ECABU_c45860-ECABU_c45960	Alanine racemase, aromatic amino acid aminotransferase, 2-oxoglutarate DH, C4-dicarboxylate transport transcriptional regulatory protein
Region VI[b]	ECABU_c48360-ECABU_c49500	P fimbriae, F17-like fimbriae, cytotoxic necrotizing factor 1, α-haemolysin (internal stop codon), Fec siderophore system
Region VII[b]	ECABU_c49540-ECABU_c49920	Type 1 fimbriae (*fim*) determinant (truncated), mannonate hydrolase *(uxuABR)*, type I restriction-modification system, C4-dicarboxylate transporter, Na+/H+ antiporter (island also present in EHEC Sakai strain)
Prophage 1[c]	ECABU_c03450-ECABU_c03720	*E. coli* 83972-specific prophage with IgA-specific serine endopeptidase determinant
Prophage 2[c]	ECABU_c11290-ECABU_c11990	Inserted into the *focD* gene
Prophage 3[c]	ECABU_c13520-ECABU_c14200	Iron/manganese transport system (Sit)
Prophage 4[c]	ECABU_c18060-ECABU_c18600	Inserted into the sensor histidine protein kinase gene *rstB*; similar to bacteriophage of UPEC isolate IAI39 or *Salmonella enterica* sv. Typhi
Prophage 5[c]	ECABU_c40840-ECABU_c41030	*E. coli* 83972-specific prophage
Prophage 6[c]	ECABU_c41260-ECABU_c41440	*E. coli* 83972-specific prophage

[a]Region present only in ABU83972 and CFT073, indicated by red in Fig. 1.
[b]Region present only in ABU83972 and pathogenic *E. coli* (UPEC and EHEC), indicated by green in Fig. 1.
[c]Prophages of ABU83972, indicated by blue in Fig. 1.
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In re-isolate PIII-4, we also observed mutations in *barA* and *frmR*. In this strain, all six mutations affected coding sequences of housekeeping genes, four of which were non-synonymous, one nonsense mutation, and one was an internal deletion. Surprisingly, we found SNPs in *rpoC* and *gyrA*, which was consistent with previous studies of long-term *in vitro* experimental evolution [19,20].

In contrast to the *in vivo* re-isolates, the *in vitro*-propagated strain 4.9 showed only three genomic alterations: one predicted diguanylate cyclase (*yfiN*) and in two phage-related genes (Figure 3; Table S1).

Genomic alterations in re-isolates obtained after a second inoculation of each patient

To address the hypothesis that the host selects specific mutants or 'imprints' the pathogen during bladder colonization, we sequenced selected genomic regions of the *E. coli* 83972 genome in re-isolates from a second, independent inoculation of each patient. Therapeutic inoculations were repeated for medical reasons, urine cultures were obtained at monthly intervals and five independent bacterial colonies from the last sampling time point were subjected to Sanger sequencing. Specifically, we examined chromosomal loci, which were altered in *E. coli* 83972 re-isolates from the first inoculation event in PI-2, PII-4 and PIII-4.

Several loci were repeatedly altered in re-isolates of strain 83972 from the same host (Table S2). This included the *fecIR* promoter region where the re-isolate of the second bladder colonization of patient PII carried a point mutation 23 nucleotides upstream of the SNP previously detected in strain PII-4. Re-isolates from the first and second inoculation in patients PI and PIII had different point mutations in the *frmR* gene. The *mdoH* gene was mutated in isolates PI-2 and PII-4 from the first inoculation and mutations were detected in re-isolates from the second inoculation in all three patients. In contrast, these genomic alterations did not occur in five isolates from two independent *in vitro* urine cultures of *E. coli* 83972, further suggesting that the host environment may drive seletion of these genomic changes.

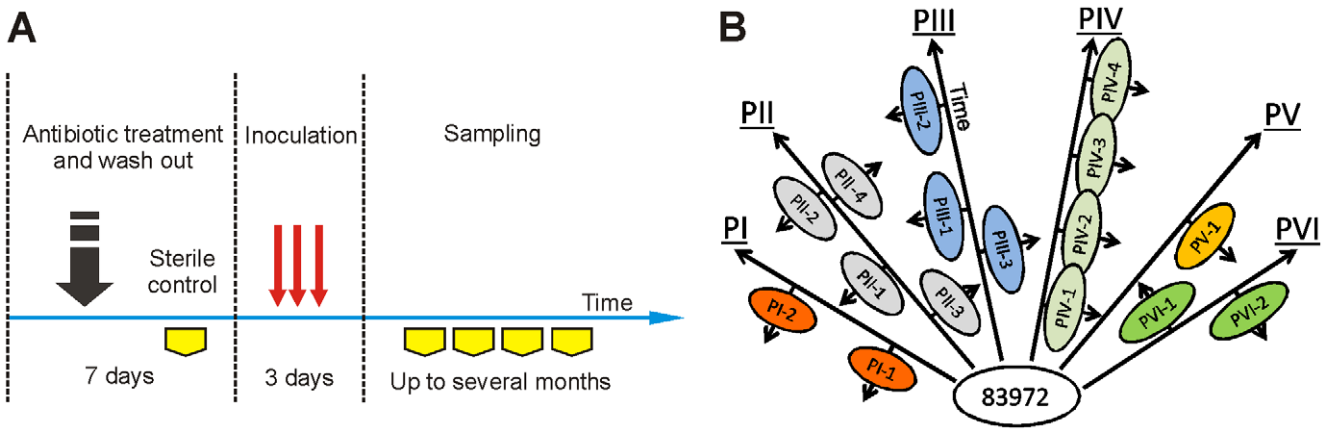

Figure 2. Therapeutic urinary tract inoculation with *E. coli* 83972. (A) Colonization scheme. Six patients received *E. coli* 83972 on three consecutive days and bacteriuria was established. Re-isolates from urine were obtained at different time points after inoculation. (B) Schematic representation of the sampling during human colonization. Arrows illustrate the time of colonization. Re-isolates obtained from different inoculations of the same patient are represented on opposite sides of an arrow.
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Stability of genomic alterations, examined in repeat re-isolates

To examine if the genetic alterations might represent adaptive changes that are cyclic in nature and that, in different patients, the re-isolates were picked at different cycles, we obtained *E. coli* 83972 re-isolates at a time point distant from that of PI-2 and PII-4 and subjected them to single locus Sanger sequencing. Most of the SNPs (17/19), in isolate PI-2 were still present in its progeny after an additional 126 days of bladder colonization. In descendants of PII-4, 4 out of 9 genomic changes (*mdoH*, *rpiR*, *fecI*, *yejM*) remained after an additional 125 days propagation time. Interestingly, all detected alterations in the later re-isolates were identical to those found in re-isolates PI-2 or PII-4. Isolate PIII-4 was the last sequential isolate derived from the inoculation of patient PIII and comparisons could not be performed.

Characterization of individual bacterial adaptation by transcriptome and proteome analysis of *E. coli* 83972 re-isolates

By comparing the three individual *in vivo* re-isolates and the *in vitro*-propagated variant 4.9 to the progenitor *E. coli* 83872, we observed differences in the respective phenotypes. Although

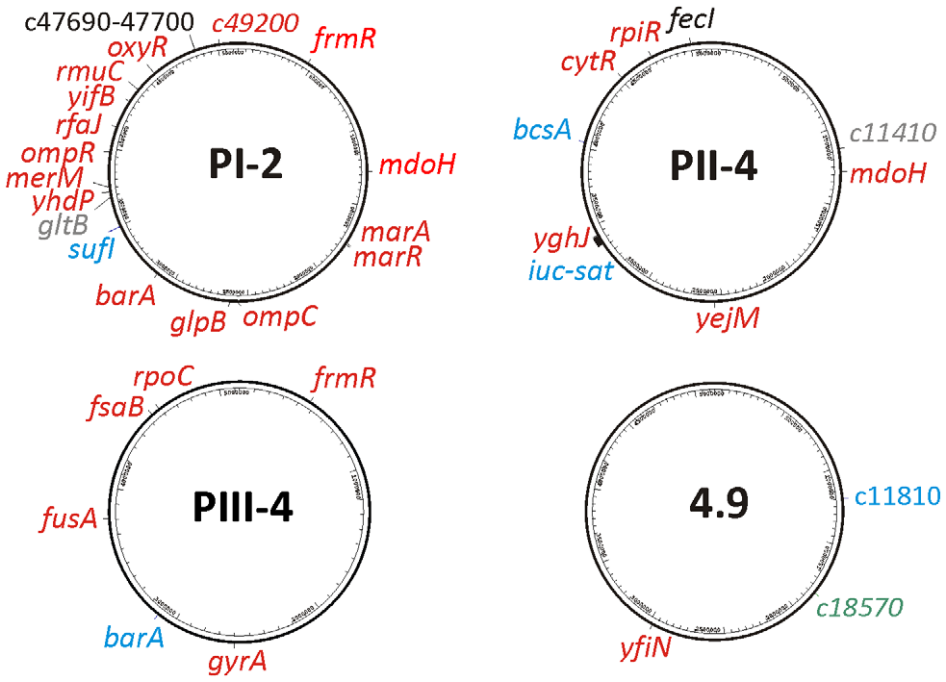

Figure 3. Localization of genomic alterations within the re-isolates' genomes relative to parent *E. coli* 83972 as revealed by whole genome sequencing. The nature of mutation is indicated by color: red- non-synonymous, grey- synonymous, black- intergenic, blue- deletion, green- inversion.
doi:10.1371/journal.ppat.1001078.g003

Table 2. Summary of global genomic alterations upon prolonged *in vivo* or *in vitro* growth of *E. coli* 83972.

Characteristics	PI-2	PII-4	PIII-4	4.9
Source	*in vivo*	*in vivo*	*in vivo*	*in vitro*
Propagation time [days]	214	155	54	68
Normalized time factor	3.1	2.3	0.8	1
No. of de-regulated genes	87	85	271	13
No. of genomic changes	19	9	6	3
Individual number of mutations (per normalized propagation time)	6.1	3.9	7.5	3
Up-regulated outer membrane proteins	-	Tsx, FecA, IroN, FepA	IutA, FliC	N/A
Down-regulated outer membrane proteins	-	Iha, IutA	Imp, YeaT, IroN, FhuAE, FepA	N/A

N/A, not analyzed.
doi:10.1371/journal.ppat.1001078.t002

growth characteristics in pooled human urine did not reveal major variations between re-isolates (Figure S3A), these strains differed in both motility and biofilm formation. The re-isolate PIII-4 was more motile than the parent strain (Figure S3B). Regarding biofilm formation, PI-2 formed significantly less biofilm than the parent strain while PII-4 showed significantly more (Figure S3C).

To further determine whether stable genomic changes of sequenced re-isolates (see previous section) affected the gene and protein expression level, we subjected them to transcriptome and outer membrane proteome (OMP) analysis. For this reason, prior to either RNA or protein isolation bacteria were grown *in vitro* in pooled human urine. Overall, the number of de-regulated genes, as implicated by the transcriptome, was higher in the patient re-isolates than in the *in vitro*-propagated variant 4.9 (Table 2). In each strain, we identified distinct gene expression patterns matching the proteome and genome data (Figure 4).

Studying re-isolate PIII-4, we found that its metabolism, motility and stress responses were affected when compared to the

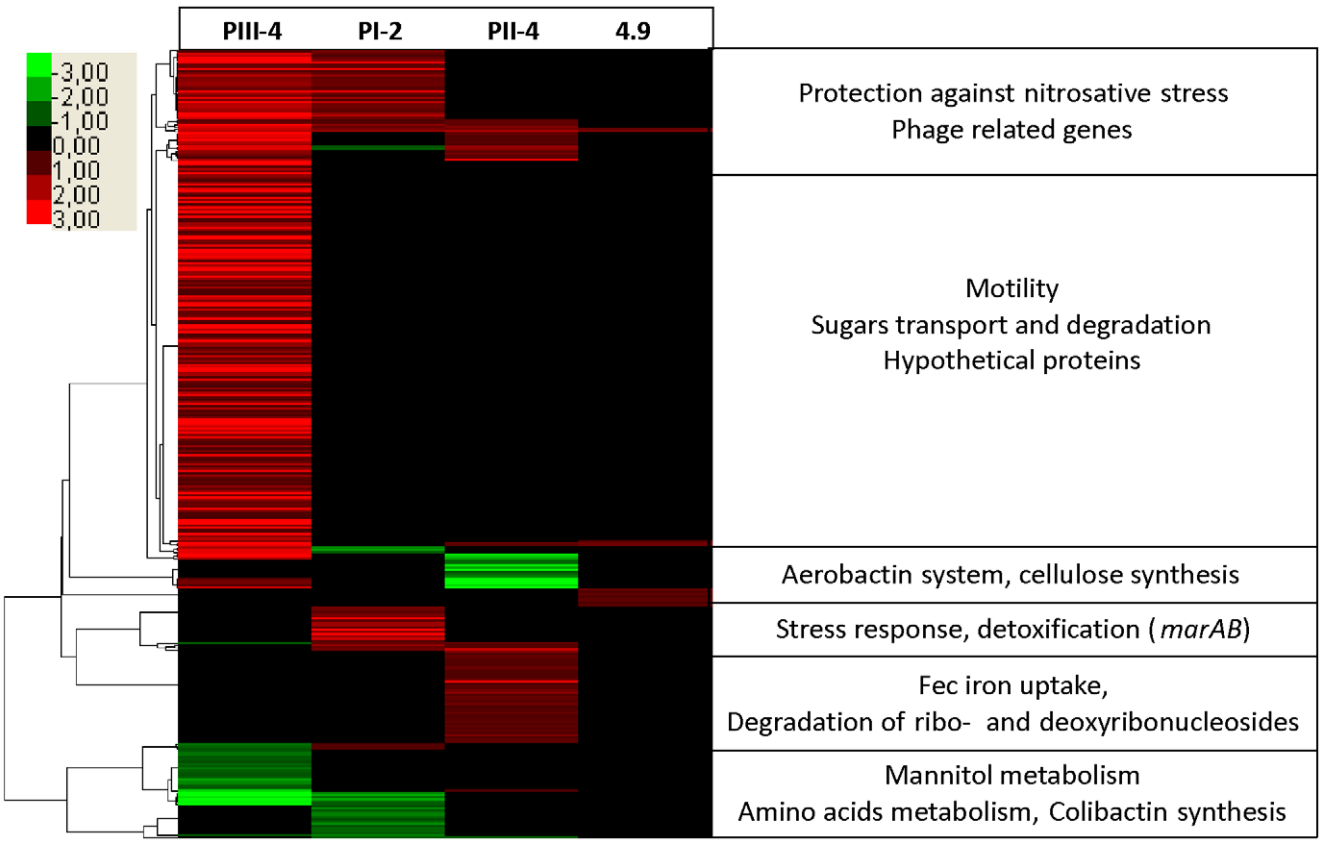

Figure 4. Host-specific changes in gene expression patterns of *E. coli* 83972 revealed by transcriptome analysis. Hierarchical clustering of de-regulated genes in *in vivo* re-isolates PI-2, PII-4 and PIII-4 and *in vitro* grown strain 4.9 relative to parent *E. coli* 83972 upon *in vitro* growth in pooled human urine. Each horizontal line represents one gene; expression is given relative to the intensity bar (log 2-fold, mean values of > three experiments). Unaffected genes are shown in black (p-value >0.09).
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progenitor strain 83972. As already indicated by phenotypic tests, this isolate showed increased motility. Indeed, flagellum and chemotaxis determinants made up 32 of the upregulated genes (Figure 4). OMP analysis further corroborated these results and FliC was the most upregulated protein on the bacterial surface (Figure 4). Against the background of generally impaired virulence gene expression in *E. coli* 83972 and its re-isolates, this is the first observation that expression of an immunogenic and functional virulence factor, i.e. flagella, is increased in *E. coli* 83972 upon prolonged *in vivo* growth. With regard to metabolic adaptations, we detected 68 upregulated genes involved in diverse processes, suggesting nutrition adaptation, e.g. sugar and sugar acid uptake fuelling glycolysis (galacturonate, glucuronate, sialic acid, arabinose and mannose), (see Figure S5A). Upregulated D-serine uptake and its deamination pathway in PIII-4, together with reduced glutamine uptake and degradation (downregulated *glnALG* and *glnHPQ* operons, see Figure S5B), mirror adaptation to urine as it is a nitrogen and D-serine-rich environment [21,22]. Utilization of the RNA degradation product pseudouridine, a nucleoside present in human urine [23], was also upregulated in strain PIII-4 as indicated by increased *yeiC* and *yeiN* gene expression. In addition to multiple metabolic alterations, we observed that genes *frmAB* were up-regulated when compared to the progenitor strain. Accordingly, genome analysis of this re-isolate demonstrated a corresponding point mutation in the *frmR* gene.

In the second re-isolate (PI-2), we found that the majority of the deregulated genes were also connected to growth and stress responses (Figure 4). Growth-related genes required for peptide/amino acid transport and utilization (*degP*, *metNIQ*, *pepD*, *oppD*, and *artJ*), that have been reported to be essential for bacterial multiplication in urine [24], were upregulated. As in the previous re-isolate (PIII-4), the genes *frmAB*, which were proposed to provide protection against oxidative or nitrosative stress [15], were upregulated. In addition, *marAB* expression was upregulated, corresponding to the genome sequence in which both *marR* and *marA* displayed point mutations (Table S1). This is important because the MarAB proteins are known to respond to oxidative/nitrosative stress as well as to antimicrobial peptides [25]. We also found that the expression of ribonucleotide-diphosphate reductase required for DNA synthesis, replication and repair was increased. It should be noted that expression of the ribonucleotide-diphosphate reductase 2-encoding *genes nrdHIEF*, which are increased by oxidative stress, is indirectly regulated by OxyR, and that *oxyR* was mutated in re-isolate PI-2 (Table S1).

In isolate PII-4 we mainly identified alterations in central intermediary metabolism and iron uptake, in contrast to the possible stress adaptations in previous re-isolates. Upregulation of the *tsx*, *cdd*, *udp* and *deoABCD* genes in re-isolate PII-4 (Figure 4, S7 and S8) indicated that ribo- and deoxyribonucleoside utilization was enhanced. Resulting ribose-5-phosphate or deoxyribose-5-phosphate could be channeled into the non-oxidative branch of the pentose phosphate or the TCA cycle, respectively. Derepression of this catabolic pathway was probably due to a SNP in *cytR* coding for a transcriptional repressor of the above-mentioned determinants (Table S1). It may be hypothesized that such adaptations could improve bacterial fitness as substantial amounts of nucleic acids are accessible in urine due to bacterial disintegration, exfoliation and lysis of bladder epithelial cells [26]. We also found that iron homeostasis was affected. Expression of ferric aerobactin receptor IutA was drastically reduced in re-isolate PII-4 (Figure 4) what could be explained by the loss of the aerobactin determinant through a 27-kb genomic deletion (Figure S2C). As IutA is highly immunogenic [27], this deletion may provide an adaptive advantage given the asymptomatic lifestyle of *E. coli* 83972. Moreover, transcriptome analysis indicated that *fec* transcript levels were significantly increased in this strain (Figure 4). This was further corroborated by OMP analysis showing that ferric dicitrate transporter FecA expression was upregulated relative to the parent strain (Figure S7). Comparing genomes of the 83972 progenitor and its descendant PII-4, we found a SNP in the putative binding site of the ferric uptake regulator (Fur) upstream of the *fecIR* regulatory genes (Table S1, Figure 3). Reporter gene assays with the wild type or the PII-4 *fecIR* upstream region that was fused with the promoterless luciferase gene cluster uncovered 8-fold increase of the re-isolate promoter activity (Figure 5A, S9). This result suggested differences in the binding efficiency of the Fur protein to the polymorphic *fecIR* promoter site of *E. coli* PII-4. To assess the molecular mechanisms underlying increased *fec* expression on the DNA/protein binding level, electrophoretic mobility shift assays (EMSA) were performed. We found that this point mutation decreases binding efficiency of Fur dimers to the altered Fur box on one DNA strand. Consequently, in the re-isolate PII-4 strong Fur tetramer-mediated repression of *fecIR* transcription was weakened resulting in upregulation of the ferric dicitrate uptake (Figure 5B and C). Interestingly, in the second inoculation re-isolate PII-B, we found another SNP again present within the Fur binding site (Figure 5D).

Relationship between duration of colonization and genomic alterations

The genomic analysis of re-isolates from the different time points of patient colonization indicated a positive correlation between the number of genetic changes and the colonization time. However, if one normalizes the propagation time of the *in vivo* re-isolates PI-2, PII-4 and PIII-4 to that of *in vitro* isolate 4.9, the propagation time of the *in vivo* re-isolates exceeds that of *E. coli* 4.9 by factor 3.1, 2.3 and 0.8, respectively. By dividing the number of genetic changes by the normalized propagation time of the isolate, we were able to assess the individual extent of genomic alterations upon *in vivo* and *in vitro* growth in urine (Table 2). Our data indicate that the number of mutations was markedly higher in re-isolates PI-2 and PIII-4 (2- and 2.5-fold, respectively) which, according to their gene expression profiles, were subjected to increased oxidative stress during bladder colonization (Figure 4). In contrast, the mutation rate of *E. coli* PII-4, which did not show adaptation to oxidative stress, was comparable to that of the *in vitro* isolate 4.9.

Bacterial adaptation and innate host response

To examine if the host immune status might influence bacterial adaptation, the innate immune response to inoculation was quantified on a monthly basis with regard to Interleukin 6 (IL-6) and Interleukin 8 (IL-8) concentrations and neutrophil infiltration. In addition, urine samples were subjected to extended cytokine/chemokine profiling (Figure 6).

Several interesting differences in the innate immune response profile were observed between the patients. PI, with the highest number of genomic alterations showed the highest IL-8 response over time and the strongest neutrophil recruitment (Figure 6A and B). In PIII neutrophil ($p<0.0001$) and IL-8 ($p<0.008$) responses were not detected, but this patient showed the highest IL-6 response and had very high concentrations of IL-1RA in urine, compared to PI and PII ($p<0.005$, Figure 6B and C). Some of the host response differences were reproduced during the second inoculation (Figure 6A). The results suggest that the patients activate different aspects of the innate immune response to infection.

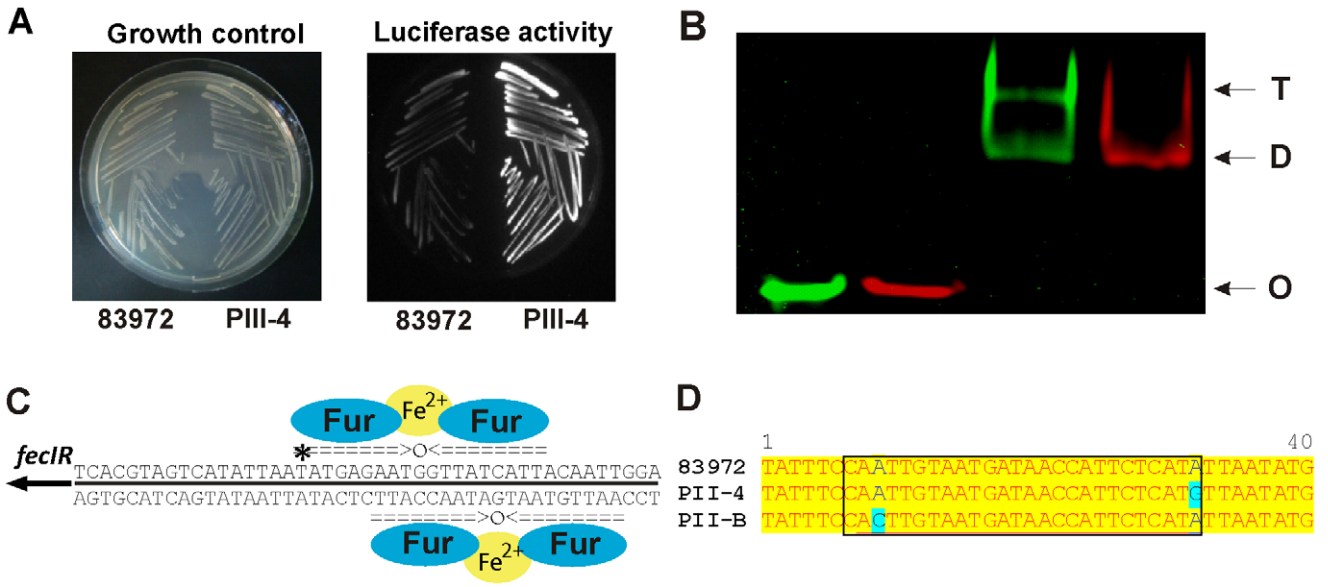


Figure 5. Increased *fecIR* expression due to a T → C transition in the upstream region of *fecIR* genes in re-isolate PII-4 relative to parent strain 83972. (A) Growth and luciferase activity of *E. coli* K-12 carrying pACYC184-based transcriptional reporter gene fusions of sequences upstream of *fecIR* from *E. coli* 83972 or PII-4, respectively, and the promoterless luciferase gene. (B) Electric mobility shift assay (EMSA) showing that the SNP in the *fecIR* upstream region of strain PII-4 abolishes tetramer formation of the Fur protein binding to the Fur box. Green, 83972; red, PII-4; D, Fur protein dimer; T, Fur protein tetramer; O, unbound Cy-3- or Cy-5-labeled DNA oligomer. (C) Model describing binding of the Fur protein to the upstream region of *fecIR*. The nucleotide sequence depicted corresponds to the 45-bp Cy-3- or Cy-5-labeled DNA oligomer comprising the Fur binding site upstream of *fecIR* used for electrophoretic mobility shift assays. The asterisk indicates the SNP in strain PII-4 relative to parent strain 83972. (D) Alignment of nucleotide sequences of the putative Fur binding site (region in black box) within the *fecIR* promoter from independent PII re-isolates. Letters in blue indicate two distinct point mutations acquired during independent colonization episodes.
doi:10.1371/journal.ppat.1001078.g005

Discussion

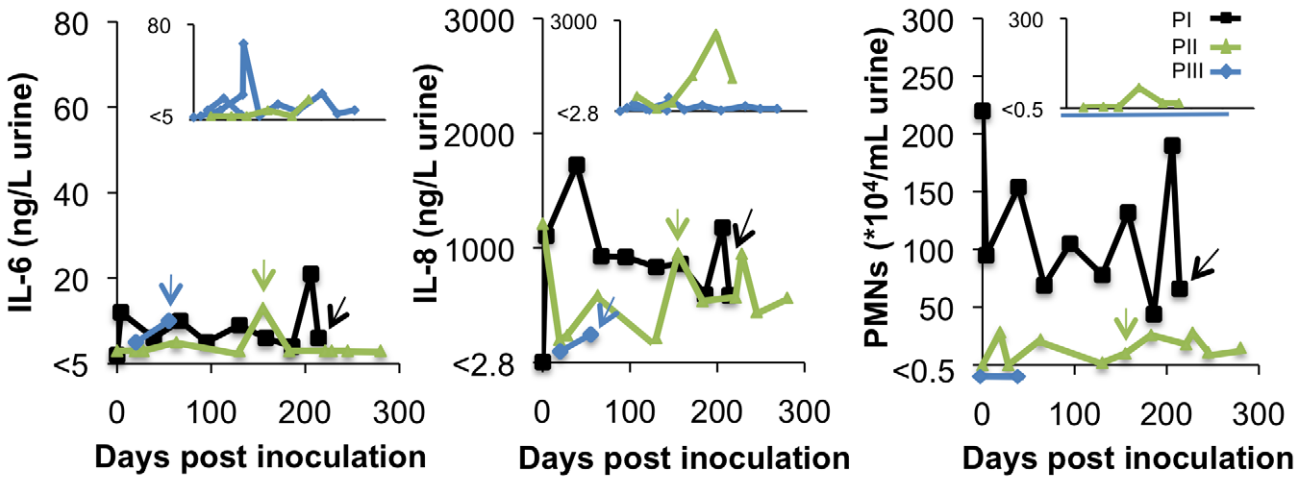
Single bacterial surface antigens or virulence factor profiles are known to vary under host immune pressure. For example, *E. coli* isolates from recurrent bacteremia or chronic UTI often lose the expression of long chain LPS, capsules or flagella [28,29] and enterohemorrhagic *E. coli* may lose major virulence determinants in the course of infection [30]. Data on genome-wide changes and adaptation during long-term growth of *E. coli in vitro* has only started to accumulate recently [31]. However, genomic alterations involved in bacterial adaptation to individual human host environments have largely not been studied. In this context, only a few studies focused on analyses of sequential isolates obtained from hosts persistently infected with *Pseudomonas aeruginosa* or *Helicobacter pylori* [32,33]. They reported a loss of virulence due to successive alterations in genome content and gene expression, but the extent to which different human hosts modify single bacterial genomes has not been investigated.

In our study, we have examined to which extent host imprinting guides the evolution of adaptive genomic modifications during asymptomatic bacterial carriage by comparing whole genomes, transcriptomes and proteomes of the prototype ABU strain *E. coli* 83972 before therapeutic inoculation and after re-isolation from several human hosts. The urinary tract inoculation protocol is a safe and efficient way to prevent symptomatic infections in certain patient groups [9] and allowed us to administer the same bacterial strain to multiple hosts rather than relying on natural infections of different hosts with different strains. We also controlled the time of bacterial carriage, thus ensuring that the *in vivo* adaptation of the bacterial genome was followed from the onset of establishment in each host.

We identified potential molecular adaptation mechanisms based on a limited number of point mutations and small deletions that frequently altered the coding regions (Figure 3, Table S1). Strikingly, some of these adaptation mechanisms appeared to be unique for each host, suggesting that the genomic identity of a bacterial isolate is flexible and relevant in a given host niche. Sequencing of the re-isolates enabled us to analyze the genome-wide extent of bacterial adaptation. As the *E. coli* strain 83972 was isolated from a young girl, who was colonized for more than three years [5], it was expected to be well-adapted to growth in urine. We observed that the number of genomic alterations increased with prolonged colonization time of the patients, as displayed by the number of mutations as a function of time (Table 2). Suboptimal fitness in the new hosts was apparently tailored by targeting regulators of bacterial metabolism. In consequence, each of the re-sequenced isolates demonstrated unique adaptations potentially resulting in growth advantages in their growth environment (Figure 7). It still remains to be elucidated to what extend growth conditions in the individual hosts contributed to this divergent evolution.

Adaptation patterns of the *in vivo* re-isolates supported the hypothesis that evolution in individual hosts was driven by positive selection of genetic variants which are better suited to the particular host and to some extend probably also by genetic drift. The results suggest that the genome of prototype ABU isolate *E. coli* 83972 is relatively stable as only 34 mutations were detected after bladder colonization for 423 patient days. To distinguish host imprinting from stochastic events, we sequenced the polymorphic positions in re-isolates from repeat inoculation events in each patient. The reproducibility of some genetic changes indicates that host-driven genetic change may play an important role in bacterial microevolution. Certain genetic alterations were detected in re-isolates from several hosts or from the same host, after independent inoculations, but not in bacteria propagated *in vitro*.

A



B

Cytokine secretion (median) during *E. coli* 83972 colonisation

	Samples				Samples						
Patient	(n)	IL-6	IL-8	PMNs*10^4	(n)	IL-1RA	MCP-1	IL-1α	GRO-α	IP-10	sIL-2Rα
PI	10	6	891.5	100	9	3.1	156.3	3.5	875.8	40.8	29.8
PII	16	3	551.5	15.8	14	0.1	155.5	7.6	386.7	190.9	460.5
PIII	16	8	90.0	0.5	13	160.2	394.5	6.6	188.2	86.7	503.1
P-value		0.0779	0.0008	0.0001		0.0050	0.0158	0.0521	0.0103	0.0060	0.0158

*Friedman test with Dunn´s post test was used

C

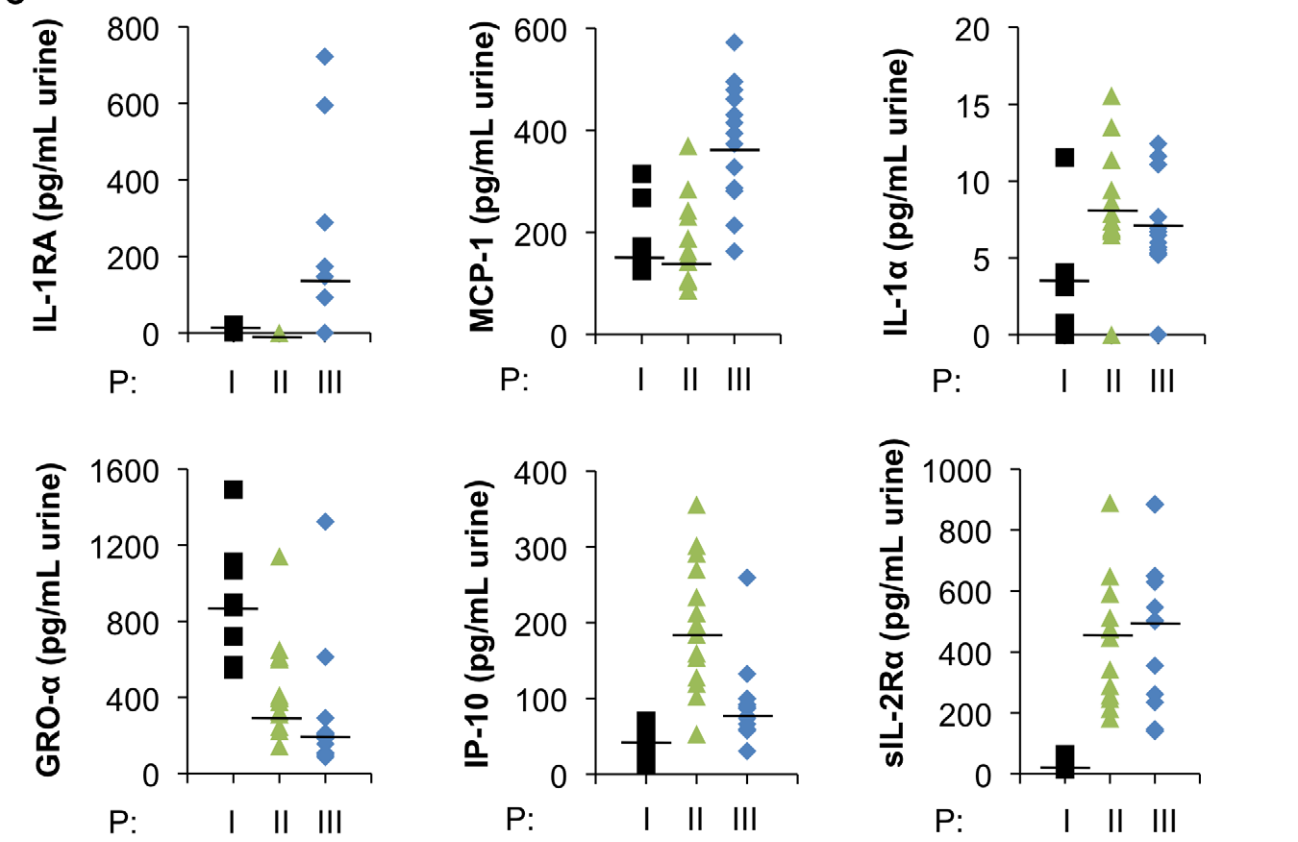


Figure 6. Innate immune response to inoculation with *E. coli* 83972. (A) IL-6 and IL-8 concentrations and neutrophil numbers were quantified in urine samples obtained from the three patients throughout the colonization period. Kinetics of the host response and time of collection of re-isolates PI-2, PII-4 and PIII-4. Inset diagrams present the host response parameters to re-inoculations of PII and PIII with *E. coli* 83972. (B) Median host responses for cytokines/chemokines in urine. (C) Extended cytokine/chemokine analysis, showing significant differences bwetween the three patients, except for IL-1α.
doi:10.1371/journal.ppat.1001078.g006

The number of non-redundant genetic changes observed after repeated inoculations might on the other hand be explained by random mutagenesis. We also examined if the adaptive changes might be cyclic in nature and if, in different patients, the re-isolates were picked at different cycles. In two of the patients, who carried *E. coli* 83972 for more than a hundred days after the initial re-isolate, we obtained repeat re-isolates and evidence that several genomic changes were stable in the population.

The impact of host-dependent selection of specific mutants ("genomic imprinting") versus random selection remains to be defined. Non-synonymous mutations were mainly detected suggesting that positive selection for structural changes over silent ones was favored during bladder colonization. Based on the genomic profile and on mechanisms of susceptibility in human hosts, several classes of host molecules may be discussed. Mutations reducing the sensitivity to stress [34,35] or changing metabolism pointed to specific host processes, as did genes that became redundant and were lost in the new environment [36,37]. In re-isolates PI-2 and PIII-4, whose gene expression profiles and genomic alterations indicate adaptation to oxidative stress (Figure 4 and 7), the mutation rate was markedly higher than in the *in vitro* propagated strain 4.9 (Table 2), suggesting that in these cases host response mechanisms, i.e. release of reactive oxygen species may have triggered bacterial adaptation. In line with this, the analysis of re-isolate PII-4 did not point towards pronounced adaptation to oxidative stress and its mutation rate was comparable relative to the *in vitro*-propagated *E. coli* 4.9.

Host resistance to UTI is controlled by innate immunity and there are genetic differences in innate immune responses between patients prone to severe, symptomatic infections and those who

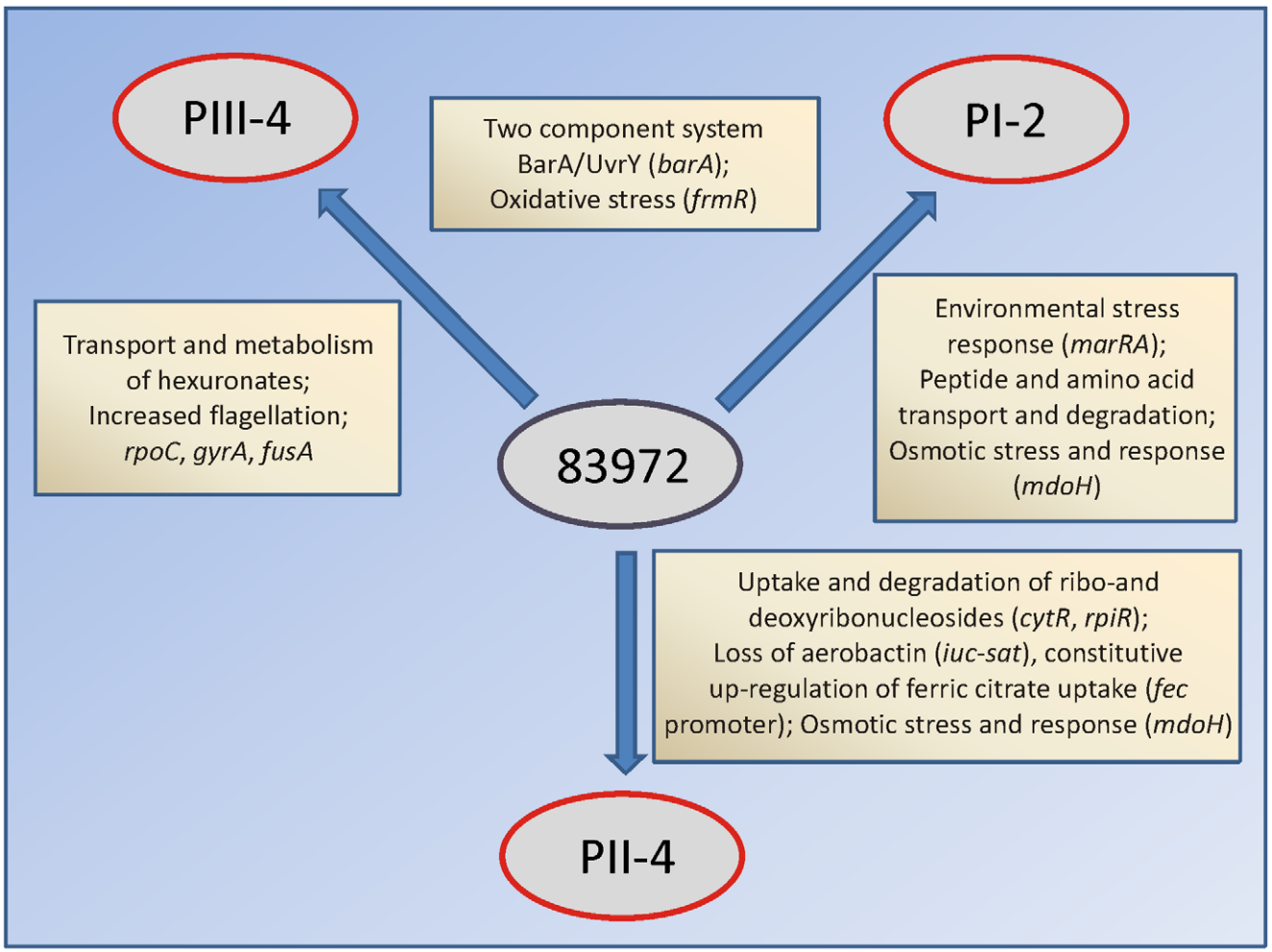


Figure 7. Different adaptational strategies of *E. coli* 83972 upon prolonged growth in the urinary bladder of human hosts. Adaptational strategies were deduced from genomic, transcriptomic and proteomic alterations in re-isolates PI-2, PII-4 and PIII-4. Genes in brackets are mutated in re-isolates relative to their parent *E. coli* 83972. Adaptation to individual hosts included different metabolic pathways, i.e. utilization of amino acids, hexuronates or (deoxy-) ribonucleosides; iron uptake and stress protection systems.
doi:10.1371/journal.ppat.1001078.g007

develop ABU, affecting the IL-8 receptor CXCR1, the IRF3 transcription factor and in TLR4 promoter sequences [38,39,40]. Such differences influence the efficiency of bacterial clearance and the extent of tissue damage, thus limiting or promoting the antibacterial host environment [38,41,42,43]. In this study, differences in innate immune responses to inoculation were detected, influencing IL-8 secretion and thus the CXCR1-mediated innate immune response. A second, differentially regulated pathway reflected events downstream of TRIF and IRF3, modifying the IL-1/IL-6 signaling pathways. The results suggest that the patients activate different aspects of the innate immune response to infection and are consistent with such responses driving bacterial adaptation. To understand this complexity is immensely challenging, but our findings illustrate the need to study microbial interactions within individual hosts in symptomatic infections versus asymptomatic carriage. It may be speculated that long-term asymptomatic carriage in a low responder host combined with attenuation of virulence might be an excellent mutual strategy.

In ABU patients, bacteria persist as a privileged monoculture, resembling the normal flora but without the complex microbial competition characteristic of other mucosal sites. Most ABU *E. coli* strains arise from virulent variants by gene loss, suggesting that attenuation may constitute a survival mechanism for mucosal pathogens [8,44]. This evolution of commensalism is interesting, as based on early predictions by Haldane [45], microbial populations evolve towards virulence. In this proposal, symptoms caused by the virulent organisms would promote transmission and the resulting increase in host number would be the most successful survival mechanism. The present study suggests that ABU bacteria may evolve towards commensalism rather than virulence, thereby achieving long-term carriage in individual hosts. While it is possible that ABU may favor between-host transmission, such consequences remain to be investigated.

The definition of commensalism has long been debated, and it is unclear if the relationship identified as commensalism is more likely to be slightly symbiotic or parasitic. The gut flora (''true commensals'') uses nutrients ingested by the host, indicating a slightly parasitic situation but may outcompete possible pathogens, indicating symbiosis. It may also be debated whether asymptomatic carriage of *E. coli* in the urinary tract should be considered as an infection as it represents the establishment of bacteria at a normally sterile site, or as a condition moving towards symbiosis/commensalism. The term asymptomatic bacteriuria is generally used to distinguish colonization from infection and to emphasize that the presence of bacteria at mucosal surfaces does not always cause symptoms and tissue damage. We have proposed asymptomatic bacteriuria as a model to study mechanisms underlying the development of commensalism. In the gut, a complex bacterial flora makes it technically difficult or impossible to study *de novo* responses of microbes to the host environment, unless germ free mice are used; in itself an artificial situation. As commensalism is defined as a relationship in which one symbiont, the commensal, benefits while the other (host) is neither harmed nor helped, asymptomatic bacteriuria clearly fulfills the definition in many individuals, while in others the asymptomatic carriage will be beneficial to both partners, thereby perhaps indicating a more symbiotic relationship.

Here we present for the first time the complete genome sequence of an asymptomatic bacteriuria *E. coli* isolate and the analysis of bacterial microevolution in the human urinary tract. We demonstrate that upon prolonged bladder colonization metabolism, preferentially the exploitation of suitable carbon- and nitrogen sources in urine, iron uptake and stress resistance of *E. coli* 83972 was affected depending on the colonized host. Future work will analyze the biological relevance of the genomic alterations observed in this study and show if this knowledge can help us to identify potential drug targets to decrease bacterial fitness during symptomatic infections.

Materials and Methods

Ethics statement

The deliberate colonization study has been approved after written informed consent from the patients by the Medical Ethics committee, University of Lund, Sweden (Approval no. LU 742-01/2001).

Patients

Patients with lower urinary tract dysfunctions and recurrent lower UTI ($\geq$3 UTI/year, for two years) were invited to participate in the study [9]. Their UTI history was confirmed by the use of interviews and patient records, and patients with a history of acute pyelonephritis, urological malignancies or corticosteroid treatment were excluded. Enrolled patients underwent renal function tests, upper urinary tract imaging and cystoscopy to exclude renal disease or stone formation. All patients could not completely empty their bladder upon voiding (residual urine $\geq$100 ml). Bacterial culture records were consulted to acertain that the UTI episodes were accompanied by significant bacteriuria ($\geq 10^5$ cfu/ml) and that the patient experienced improvement after antibiotic therapy. Before inoculation, patients were treated with appropriate antibiotics to sterilize the urine and after an antibiotic free interval, the patients were catheterized. After emptying the bladder, 30 ml of *E. coli* 83972 (10^5 cfu/ml) was instilled and the patients were followed according to a defined study protocol [9]. *E. coli* 83972 was originally isolated from a girl with asymptomatic bacteriuria [5] and its ability to cause long term bacteriuria in patients with dysfunctional voiding is well documented. The inoculated patients developed long-term, asymptomatic bacteriuria, experiencing no discomfort, except for the first 24 hours after catheterization. In a standardized questionnaire addressing symptoms and need for therapeutic intervention, no significant events were recorded [6,9].

Throughout the colonization period, monthly urine samples were collected and analyzed for IL-6 and IL-8 as well as neutrophil infiltration. For each urine sample urine proteome array analysis was performed to study the specific host response. Bacteria from each urine sample were verified by PCR for presence of a kryptic plasmid unique for strain 83972 and one chromosomal marker (4.7-kb deletion in strain 83972 in the type 1 fimbrial gene cluster). For further analysis, five independent colonies per urine sample were used.

Genome sequencing, assembly and gap closure

Total genomic DNA of *E.coli* 83972 was mechanically sheared (HydroShear, GeneMachines) for a Sanger sequencing approach. A shotgun library based on pCR4.1-TOPO (Invitrogen) was constructed with the 1.5- to 3-kb size fraction of DNA fragments. Recombinant plasmids inserts were sequenced using dye terminator chemistry and ABI Prism 3730XL DNA sequencers (Applied Biosystems). Sequences were processed with Phred and assembled with the Phrap assembly tool (www.phrap.org). Additionally, genomic DNA of *E.coli* 83972 and its re-isolates was pyrosequenced using a 454 Life Sciences GS-FLX sequencer (Roche). The 454 reads were assembled using Newbler (Roche). Sequence editing of shotgun and 454 sequences was done with GAP4 [46]. For correction of misassembled regions and gap

closure, PCR or combinatorial multiplex PCR using the Extender System polymerase (5 Prime) or the TempliPhi Sequence Resolver kit (GE Healthcare), and primer walking with recombinant plasmids were applied. For the validation of genetic differences between the re-isolates and the ancestor strain, single locus sequencing (Sanger) was performed.

Open reading frames (ORFs) were predicted with YACOP [47]. For annotation, all proteins were screened against Swiss-Prot data and publicly available protein sequences from other completed genomes. All predictions were then verified and manually modified using the ERGO software package (Integrated Genomics) [48]. Complete genome comparisons were done with ACT [49] based on replicon-specific nucleotide BLAST [50] and with protein based BiBlast comparisons to selected *E.coli* genomes (Wollherr 2009, personal communication). The 83972 genome sequence reported in this paper has been deposited in the GenBank database (accession number CP001671).

Strain cultivation

E.coli strain 83972 was routinely grown *in vitro* in pooled sterile human urine at 37°C without agitation. For long-term propagation *in vitro*, the strain was grown as independent cultures for 68 days (>2000 generations) in pooled sterile human urine at 37°C in a continous culture.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE was done as previously described (Zdziarski *et. al*, 2007).

Total RNA isolation

Bacteria were harvested from mid-log phase cultures. Samples were treated with RNAprotect (Qiagen) and extracted using the RNeasy mini kit (Qiagen). DNA traces were removed by RNase-free DNase I (New England Biolabs).

Array hybridization and data processing

For expression profiling, custom-tailored oligonucleotide microarrays (Operon Biotechnologies) were used. The custom array contained 10,816 longmer oligonucleotide probes covering the complete genomes of six *E.coli* strains (non-pathogenic *E.coli* K-12 strain MG1655, EHEC O157:H7 strains EDL933 and Sakai, UPEC strains CFT073, 536 and UTI89, pOSAK1, pO157_Sakai, pO157_EDL933 and pUTI89).

10 µg of total RNA were reverse transcribed (SuperScript III, Invitrogen) with direct incorporation of fluorescently labelled (Cy3- or Cy5-) dCTP (GE Healthcare). 160 pmol of each Cy-3 and Cy-5 labelled probe were used for hybridisation. For each experiment, at least three independent hybridizations were performed. Hybridized and washed slides were scanned using a GenePix 4000B Microarray Scanner (GE Healthcare) with a resolution of 10 µm pixel size.

Outer membrane protein isolation

Outer membrane protein (OMP) preparations from bacteria were performed as described previously [27].

Two-dimensional protein gel electrophoresis

Proteome analysis was performed with 300 µg OMP samples as described previously [27]. Coomassie G-250-stained gels were scanned and analyzed with the Delta-2D Software (http://www.decodon.com).

Mass spectrometry

Protein spots were excised from stained 2-D gels. Following tryptic digestion, MALDI-TOF measurement was carried out with the 4800 MALDI TOF/TOF Analyzer (Applied Biosystems). The Mascot search engine version 2.1 (Matrix Science Ltd, London, UK) was used for data base search with a specific *E. coli* sequence database.

Luciferase measurements

The 485-bp upstream region of *fecIR* was fused with the promoterless luciferase gene cluster *luxABCDE* in pACYC184. Plasmids with the transcriptional reporter gene fusions were transformed into *E. coli* strain DH5α and grown at 37°C in Luria broth. 100 µl samples were withdrawn after 3 hours of growth and light emission was recorded with a luminometer (Berthold). To test the luciferase activity directly on LB agar plates, bacterial luminescence was recorded with the ChemiLux photoimager (Intas).

E. coli Fur expression and purification

E. coli Fur protein was purified with the IMPACT protein purification system (New England Biolabs) according to the manufacturer's instructions. The *fur* sequence was amplified using primers Fur_up_NdeI (5′-GGTGGTCATATGACTGATAA-CAATACCGCCC-3′) and Fur_down_SapI (5′-GGTGGTTG-CTCTTCCGCATTTGCCTTCGTGCGCGTGCTC-3′).

Electrophoretic Mobility Shift Assay (EMSA)

A 45-bp Cy3- or Cy5-labeled DNA oligomer (Operon) comprising the Fur binding site upstream of *fecIR* (tccaattgtaatga-taaccattctcatattaatatgactacgtga-Cy3 – 83972; tccaattgtaatgataac-cattctcatgttaatatgactacgtga-Cy5 – PII-4) was annealed with an unlabeled complementary 45-bp oligomer in annealing buffer (10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA; 95°C–5 min, 67°C–20 min, 30°C–1 h). EMSAs were performed as previously described [52]. Gels were subsequently scanned on a Typhoon variable mode imager (Molecular Dynamics).

Innate immune response parameters in urine

Neutrophil numbers were counted in un-centrifuged fresh urine using a Bürker chamber [53]. IL-6 and IL-8 concentrations in fresh urine samples were determined in the Lund University hospital routine lab using an Immulite 1000 (Siemens). The detection limits were 2.8 pg/ml (IL-6) and 5 ng/ml (IL-8). Samples with undetectable cytokine concentrations were assigned the value of lower detection limit. For extended cytokine/chemokine profiling, we used the MILLIPLEX MAP Human Cytokine/Chemokine Panel to detect IL-1RA, MCP-1, IL-1α, GRO-α, IP-10 and sIL-2Rα. The analysis was according to the manufacturer's protocol and measurements were in duplicates on a Luminex 200 instrument (Luminex Corp.).

Statistics

The Freidman test with Dunn's post test was used for comparisons of innate host responses.

Supporting Information

Figure S1 Genomic fingerprints of *E. coli* 83972 re-isolates. Pulsed field gel electrophoresis patterns of consecutive *in vivo* (A) as well as of *in vitro*-propagated isolates (B) of *E. coli* 83972 are shown. Arabic numbers indicate the order of sampling time points of consecutive *in vivo* re-isolates. *in vitro*: 17 independent colonies were picked after more than 2000 generations of continuous culture in pooled human urine. The genome structure was assessed by PFGE following *Xba*I (left panel) and *Avr*II (right panel) digestion.
Found at: doi:10.1371/journal.ppat.1001078.s001 (1.11 MB PDF)

Figure S2 Genotypic characterization of *E. coli* 83972 re-isolates. (A) Genome structure analysis of different clones from the same urine sample, by PFGE following *Avr*II digestion. With one exception (PIII-4_2), all clones exhibited the same restriction pattern, therefore re-isolates PI-2, PII-4 and PIII-4 are the major fraction of analyzed urine samples. The restriction patterns of parent strain 83972 and re-isolate PIII-4_2 were identical. (B) Genome sizes of *E. coli* 83972 re-isolates, analyzed by PFGE following I-*Ceu*I digestion. Only one re-isolate, PII-4, had a reduced genome size relative to parent strain 83972. (C) Genome size reduction in re-isolate PII-4 due to loss of the *iucABCD*, *iutA* and *sat* genes by partial deletion (27 kb) of a pathogenicity island during *in vivo* growth.
Found at: doi:10.1371/journal.ppat.1001078.s002 (0.14 MB PDF)

Figure S3 Phenotypic traits of *E. coli* 83972 re-isolates. (A) Identical growth rates of *E. coli* 83972 and its *in vivo* and *in vitro* re-isolates in pooled human urine (mean values of > three experiment). (B) Isolate PIII-4 shows increased motility in soft agar with pooled human urine. (C) Reduced biofilm formation in pooled human urine of strains PI-2 and PIII-4 relative to 83972. Mean values of > three experiments. Bonferroni's Multiple Comparison Test was used for statistical analysis.
Found at: doi:10.1371/journal.ppat.1001078.s003 (0.08 MB PDF)

Figure S4 Outer membrane proteome comparison of *E. coli* 83972 (green) and re-isolate PIII-4 (red) upon *in vitro* growth in pooled human urine. Proteins with similar expression level are indicated in yellow.
Found at: doi:10.1371/journal.ppat.1001078.s004 (1.39 MB PDF)

Figure S5 Different nutritional strategies of *in vivo* re-isolate PIII-4. (A) Altered expression of sugar transport and degradation pathways in the re-isolate PIII-4. (B) Adaptation of D-serine catabolism and nitrogen assimilation to growth in urine in re-isolate PIII-4. Red and black arrows indicate up-regulated and down-regulated genes, respectively, of re-isolate relative to parent strain 83972 during *in vitro* growth in pooled human urine.
Found at: doi:10.1371/journal.ppat.1001078.s005 (1.39 MB PDF)

Figure S6 Outer membrane proteome comparison of *E. coli* 83972 (green) and re-isolate PI-2 (red) upon *in vitro* growth in pooled human urine. Proteins with similar expression level are indicated in yellow.
Found at: doi:10.1371/journal.ppat.1001078.s006 (1.72 MB PDF)

Figure S7 Outer membrane proteome comparison of *E. coli* 83972 (green) and re-isolate PII-4 (red) upon *in vitro* growth in pooled human urine. Proteins with similar expression level are indicated in yellow.
Found at: doi:10.1371/journal.ppat.1001078.s007 (0.73 MB PDF)

Figure S8 Different nutritional strategies of *in vivo* re-isolate PII-4. (A) Adaptation of the ribonucleoside degradation pathway in re-isolate PII-4. (B) Adaptation of the deoxy-ribonucleoside degradation pathway in re-isolate PII-4. Red arrows indicate up-regulated genes of re-isolate relative to parent strain 83972 during *in vitro* growth in pooled human urine.
Found at: doi:10.1371/journal.ppat.1001078.s008 (0.07 MB PDF)

Figure S9 Eight-fold increase in luciferase activity upon fusion of *fecIR* upstream region of re-isolate PII-4 with the promoterless luciferase genes relative to the *fecIR* upstream region of parent strain 83972. Paired *t* test was performed for statistical analysis.
Found at: doi:10.1371/journal.ppat.1001078.s009 (0.05 MB PDF)

Table S1 Genomic alterations in in vivo and in vitro re-isolates relative to parent strain 83972.
Found at: doi:10.1371/journal.ppat.1001078.s010 (0.02 MB PDF)

Table S2 Summary of SNPs detected in candidate genes of re-isolates from two independent bladder colonization.
Found at: doi:10.1371/journal.ppat.1001078.s011 (0.01 MB PDF)

Author Contributions

Conceived and designed the experiments: JZ EB BW CS UD. Performed the experiments: JZ EB BW DB BV BR. Analyzed the data: JZ EB HL BV JGH BR MH EZR RD GG JH UD. Contributed reagents/materials/analysis tools: BW CS. Wrote the paper: JZ EB CS UD.

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Cases for AFTERNOON sessions (Host Responses)

Case 4

A 71-year old man is admitted in a semi-conscious state with a history of not having been seen by friends for two days. Examination reveals a high fever, tachycardia, low blood pressure, and stiff neck (meningism). The patient is very thin, and disorientated. Friends say he drinks a lot and never sees his GP. Blood tests show a neutrophil leukocytosis (high neutrophil count) and raised CRP. He is commenced on broad spectrum antibiotics (ceftriaxone for suspected severe sepsis) and a lumbar puncture is performed that reveals Gram positive cocci in pairs and a marked infiltrate of neutrophils confirming meningitis. On culture, the Gram positive cocci are subsequently identified as Streptococcus pneumoniae.

1. What are the mechanisms by which this organism has reached the brain? Where is it normally found as a colonizing bacterium, and what prevents it from becoming invasive?

2. This man was more prone than others to this infection- what is the relevance of his weight and alcohol history?

3. What else could his GP have done to protect him from this infection? Explain how this would have helped. What are the other causes of meningitis in adults?

Case 5

A young 22 year old South African woman is admitted complaining of an influenza-like illness for days. She reports that far from getting better, she now feels much worse with fevers of 40C and shortness of breath. On examination she is tachycardic, BP 85/60, Temperature 39.9C, and audible creptitations at the right base of the lung. Sputum samples are sent that yield "3+ Gram positive cocci in clumps". Her skin appears very sunburned, and blanches on pressure though she does not report recent sun exposure. Swabs for influenza A/H1N1 are positive and she is moved into a sideroom, but also given antibiotics for chest infection (augmentin). Her condition deteriorates and her BP falls further. She is clearly distressed and her lactate levels rise, so she is moved to the ICU. The sputum culture yields Staphylococcus aureus (methicillin sensitive) and PCR demonstrates it has the genes for staphylococcal enterotoxin C (SEC).

1. What is this syndrome commonly known as and when else might it present? How does the toxin trigger profound shock?

2. What is the relevance of the antecedent influenza infection and what are the other common linked secondary infections? How would the influenza have affected this?

3. What additional host factors might predispose to this condition?

4. What else could the team do to abrogate the effects of the toxin?

Case 6

An elderly woman is admitted from a residential home with severe abdominal pain of one day duration, on a background of a five week history of constipation and weight loss. On examination, she is profoundly cachectic with peritonism, and has a low blood pressure (80/55) with fast heart and respiratory rate (120 bpm; 36 resps/min); she is barely conscious though has no meningism. An erect chest xray shows gas under the diaphragm, while a CT scan confirms a perforation of the large bowel. Her blood tests reveal a CRP of 460 and a raised white cell count of 33 x 109/L. She looks clinically dehydrated but has an empty bladder when catheterized. The surgical team and intensive care feel that operative intervention is too risky at present due to her frail condition and advise that she should be managed medically at present. However her prothrombin time and partial thomboplastin time start to rise, and, with falling platelets, a diagnosis of disseminated intravascular coagulation is made.

1. What are the possible reasons for this patient's bowel perforation?

2. Why are her physiological measures (heart rate, BP, resp rate) as reported and what else should be measured?

3. What types of treatment options might be used when she collapses with regarding to managing her perforation 'medically'?

4. What bacteria might be present in the peritoneum and might require treatment? Why would she have deteriorated to such a degree and why are the surgeons reluctant to operate? Why does she have disseminated intravascular coagulation?

Suggested reading for CASES 4-6 and journal club paper 2

All articles should be available either directly via PubMed using a College computer OR via the College electronic journal listing (accessed via the library website). Please notify the module leader if you cannot access an article ***only*** after trying both routes (check with colleagues first) As this is a rapidly moving subject and the course brings together several classically 'separate' fields there is no current textbook which is recommended.

S. pneumoniae infection (groups doing case 4)

Gillespie SH, Balakrishnan I. Pathogenesis of pneumococcal infection. J Med Microbiol. 2000 Dec;49(12):1057-67. Review

Kadioglu et al. The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. Nature Reviews Microbiology. 2008; 6:288-299

Friedman H, Pross S, Klein TW. Addictive drugs and their relationship with infectious diseases. FEMS Immunol Med Microbiol. 2006 Aug;47(3):330-42. Review

Chandra, RK. Impact of nutritional status and nutrient supplements on immune responses and incidence of infection in older individuals. Ageing Res Rev. 2004 Jan;3(1):91-104.

Dorshkind K, Montecino-Rodriguez E, Signer RA. Nat Rev Immunol. 2009 Jan;9(1):57-62. The ageing immune system: is it ever too old to become young again?

Staphylococcal shock (groups doing case 5)

Ballinger and Standiford. Postinfluenza Bacterial Pneumonia: Host Defenses Gone Awry. JOURNAL OF INTERFERON & CYTOKINE RESEARCH Volume 30, Number 9, 2010

Centers for Disease Control and Prevention (CDC).Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - United States, May-August 2009. MMWR Morb Mortal Wkly Rep. 2009 Oct 2;58(38):1071-4.

Sriskandan S, Altmann DM. The immunology of sepsis. J Pathol. 2008 Jan;214(2):211-23.

Lappin E, Ferguson AJ. Gram-positive toxic shock syndromes. Lancet Infect Dis. 2009 May;9(5):281-90. Review.

Bowel perforation and septic shock (groups presenting case 6)

Daniela Weiskopf, Birgit Weinberger, Beatrix Grubeck-Loebenstein The ageing of the immune system. Transplant International Volume 22, Issue 11, pages 1041–1050, November 2009.

Dellinger RP, Levy MM, Carlet JM, et al Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med. 2008 Jan;36(1):296-327.

Levy MM, Dellinger RP, Townsend et al. Surviving Sepsis Campaign. The Surviving Sepsis Campaign: results of an international guideline-based performance improvement program targeting severe sepsis. Crit Care Med. 2010 Feb;38(2):367-74.

Zeerleder S, Hack CE, Wuillemin WA. Disseminated intravascular coagulation in sepsis. Chest. 2005 Oct;128(4):2864-75. Review.

Journal Club paper 2

WHOLE YEAR SHOULD LOOK AT THIS PAPER BEFORE THE TUTORIAL

Cytomegalovirus reactivation and associated outcome of critically ill patients with severe sepsis

Alexandra Heininger, Helene Haeberle, Imma Fischer, Robert Beck, Reimer Riessen, Frank Rohde, Christoph Meisner, Gerhard Jahn, Alfred Koenigsrainer, Klaus Unertl, Klaus Hamprecht

Crit Care. 2011; 15(2): R77

For the group presenting this paper please try and address the following:

a. What was the aim of this study?
b. What type of study was it?
c. What were the parameters measured and what were the key findings?
d. How does this compare with the findings of others?
e. Do the findings unequivocally support a role for CMV in poor outcome in ICU?
f. What are the ways in which the role of CMV could be more directly addressed? Has this been undertaken?
g. What are the possible mechanisms that link CMV with adverse outcome in severe sepsis or critical illness?
h. CMV reactivation is normally considered a problem in those who are actively immunosuppressed eg transplant recipients or those with HIV. What are the manifestations of this compared with primary acute CMV? (briefly!)
i. Are there other groups of non-immunosuppressed patients where CMV reactivation might explain susceptibility to infection?

Papers on CMV reactivation in critically ill sepsis patients (groups presenting journal club paper 2)

Kalil AC, Florescu DF.Is cytomegalovirus reactivation increasing the mortality of patients with severe sepsis? Crit Care. 2011;15(2):138.

Limaye AP, Kirby KA, Rubenfeld GD, Leisenring WM, Bulger EM, Neff MJ, Gibran NS, Huang ML, Santo Hayes TK, Corey L, Boeckh M. Cytomegalovirus reactivation in critically ill immunocompetent patients. JAMA. 2008 Jul 23;300(4):413-22.

Limaye AP, Boeckh M. CMV in critically ill patients: pathogen or bystander? Rev Med Virol. 2010 Nov;20(6):372-9. doi: 10.1002/rmv.664.

CRITICAL CARE

RESEARCH Open Access

Cytomegalovirus reactivation and associated outcome of critically ill patients with severe sepsis

Alexandra Heininger[1], Helene Haeberle[1*], Imma Fischer[2,3], Robert Beck[4], Reimer Riessen[5], Frank Rohde[6], Christoph Meisner[3], Gerhard Jahn[4], Alfred Koenigsrainer[7], Klaus Unertl[1] and Klaus Hamprecht[4]

Abstract

Introduction: Sepsis has been identified as a risk factor for human cytomegalovirus (CMV) reactivation in critically ill patients. However, the contribution of CMV reactivation on morbidity and mortality is still controversial. Therefore, we analyzed the incidence and impact of CMV reactivation on outcome in patients with severe sepsis.

Methods: In a prospective longitudinal double-blinded observational study, 97 adult nonimmunosuppressed CMV-seropositive patients with new onset of severe sepsis were included. Leukocytes, plasma and tracheal secretions were examined weekly for CMV-DNA by PCR. Tracheal secretions were additionally tested for HSV (Herpes Simplex Virus)-DNA. The influence of CMV-reactivation on the endpoints was analysed by Cox proportional-hazard regression analysis. Time-dependency was evaluated by landmark analysis.

Results: Six out 97 died and five were discharged from the hospital within 72 hours and were excluded of the analysis. CMV reactivation occurred in 35 of the 86 (40.69%) analysed patients. HSV infection occurred in 23 of the 35 (65.7%) CMV reactivators. In 10 patients CMV-plasma-DNAemia appeared with a DNA-content below 600 copies/ml in four cases and a peak amount of 2,830 copies/ml on average. In patients with and without CMV reactivation mortality rates were similar (37.1% vs. 35.3%, $P = 0.861$), respectively. However, in the multivariate COX regression analyses CMV reactivation was independently associated with increased length of stay in the ICU (30.0, interquartile range 14 to 48 vs. 12.0, interquartile range 7 to 19 days; HR (hazard ratio) 3.365; 95% CI (confidence interval) 1.233 to 9.183, $P = 0.018$) and in the hospital (33.0, interquartile range 24 to 62 vs. 16.0, interquartile range 10 to 24 days, HR 3.3, 95% CI 1.78 to 6.25, $P < 0.001$) as well as prolonged mechanical ventilation (22.0, interquartile range 6 to 36 vs. 7.5, interquartile range 5 to 15.5 days; HR 2.6,CI 95% 1.39 to 4.94; $P < 0.001$) and impaired pulmonary gas exchange (six days, interquartile range 1 to 17, vs. three, interquartile range 1 to 7, days in reactivators vs. non-reactivators, $P = 0.038$). HSV reactivation proved not to be a risk factor for these adverse effects.

Conclusions: These data indicate an independent correlation between CMV reactivation and increased morbidity in the well-defined group of nonimmunosuppressed patients with severe sepsis, but CMV reactivation had no impact on mortality in this group with low CMV-DNA plasma levels. Thus, the potential harms and benefits of antiviral treatment have to be weighed cautiously in patients with severe sepsis or septic shock.

Introduction

Human cytomegalovirus (CMV) is widely recognized as the most serious viral pathogen in immunosuppressed patients, such as solid organ transplant recipients or those with malignant haematologic disorders or human immunodeficiency virus (HIV) infection [1-6]. Like other herpesviruses, CMV persists in the host after primary infection, usually remaining in a latent state for the rest of the host's life [2]. Disturbances in the balance between the host`s immune defenses and the non-active virus are thought to trigger CMV reactivation, which may result in CMV disease being associated with high morbidity and mortality in immunosuppressed patients [2,7].

Generally, critically ill patients in the intensive care unit (ICU) without exogenous immunosuppression are not thought to be endangered by CMV reactivation. However, in the last 10 years CMV reactivation rates close to those found after kidney transplantation have

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been observed in CMV-seropositive ICU patients, although the typical mechanisms of immunosuppression were absent [8-14]. In addition, there is a growing body of evidence that not only CMV but also herpes simplex virus (HSV) infections might have been considerably underestimated in critically ill patients [11,15]. The reactivation of both viruses is frequently observed in the respiratory tract, but there are few systematic studies investigating the reactivation of either CMV or HSV in respiratory tract specimens [11,16,17].

Single studies in different types of various ICU populations suggest that CMV reactivation might adversely affect the outcome of critically ill CMV-seropositive patients, independently of the occurrence of CMV disease, and in a similar fashion HSV infections might have negative effects on intensive care patients [9,13,17,18].

Bacterial sepsis has been identified as an independent risk factor for CMV reactivation in the heterogeneous population of critically ill patients [8,9]. Therefore, the question arises whether CMV infection contributes to increased morbidity and mortality to an extent warranting antiviral strategies in the risk group of patients with severe sepsis. To our knowledge, until now only one prospective study has addressed this issue in men [16], but statistical analysis could not be performed due to the limited collective of only 25 patients. Moreover, the role of coinfection with HSV in this context still remains to be elucidated. Therefore, we performed a prospective, blinded study monitoring nonimmunosuppressed, critically ill patients with severe sepsis for CMV reactivation in blood and also in respiratory secretions. Active HSV infection was evaluated as a potential cofactor of CMV infection. The aim of this investigation was to assess the impact of active CMV infection on survival, length of ICU and hospital stay as well as on duration of mechanical ventilation of non immunosuppressed patients with severe sepsis.

Materials and methods

Patients

This prospective observational study was performed in the surgical and the medical ICUs of the University Hospital Tübingen between February 2004 and September 2006. All adult patients of the two ICUs were daily screened for enrolment. The inclusion criteria were the presence of severe sepsis as defined by the consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [19] and CMV seropositivity.

Exclusion criteria were the following: age younger than 18 years, pregnancy or breast feeding, duration of severe sepsis for longer than 72 hours, antiviral treatment with ganciclovir, valaciclovir, cidofovir, or foscarnet in the previous 7 days, and manifest immunosuppression because of HIV infection, congenital defects, leukopenia <2,000/μl, radiation or treatment with immunosuppressive substances within the last 6 months including prednisone, rituximab, alemtuzumab, tacrolimus, sirolimus, ciclosporin, mycophenolic acid, azathioprine, anti-lymphocytic or anti-IL6 antibodies.

Study protocol

The investigation was approved by the local ethics committee of the Faculty of Medicine, which waived the need for informed consent. As soon as a patient fulfilled the inclusion criterion of severe sepsis and had no exclusion criterion present the first set of virological examinations including CMV serology was performed within the next three days

Patients having a positive anti-CMV IgG titer were enrolled and further monitored for CMV reactivation once a week until discharge from the University Hospital or death.

Clinicians were not aware of the virological results, since they were assessed in a specific internal database applied for scientific purposes only. Ordering examinations to look for CMV disease as well as the initiation of antiviral treatment was left to the decision of the clinician, independently of the study.

The following data were evaluated at enrolment: age, gender, underlying disease requiring ICU treatment, the type of infection and the organ dysfunction constituting severe sepsis, presence of septic shock, the length of stay in the ICU, duration of mechanical ventilation and severity of illness and organ dysfunction as indicated by the Simplified Acute Physiology Score (SAPS) II [20] and the Sequential Organ Failure Assessment (SOFA) scores. Additionally, the records of each patient were reviewed for the presence of malignant disease, the number of surgical procedures, and the number of red blood cell units transfused during the current hospital stay before enrolment. After enrolment it was registered whether a CMV disease was diagnosed by the responsible clinicians. The study nurses collecting clinical data were blinded for virological findings with the exception of CMV serology, which was reported immediately.

The consequences of active CMV infection were longitudinally examined from enrolment until discharge or death by assessing in-hospital mortality, length of stay (LOS) in the ICU and in the hospital as well as time on mechanical ventilator.

Virological assays

Samples were processed in the virological laboratory each Monday and Thursday independently from the day severe sepsis was diagnosed. The personnel performing the virological examinations had no contact with patients and no insight into clinical data. All data were fed into an

internal database for scientific purposes to ensure mutual blinding. Longitudinal CMV monitoring included virus culture (human foreskin fibroblast monolayers) from tracheal secretions, qualitative nested PCR targeting the CMV IE1-Ex4 region [21] from leukocytes, plasma and tracheal secretions, and quantification of CMV-DNA (COBAS Amplicor CMV Monitor™ test, Roche Diagnostics, Mannheim, Germany) from qualitative PCR-positive plasma and tracheal secretion specimens. Experimental details are given elsewhere [22,23]. In the first virological examination CMV serology (anti-CMV IgG, anti-CMV IgM enzyme immunoassays from Medac, Wedel, Germany) was also assessed.

Moreover, respiratory secretions were examined by real time PCR using primers and hybridization probes derived from the DNA polymerase gene of HSV [24]. Vero cell monolayers were used to isolate HSV by cell culture. All CMV and HSV strains isolated from microculture were cryopreserved.

A status of viral latency was assigned if anti-CMV immunoglobulin G (IgG) was present but the virus could not be detected otherwise. Since earlier investigations had shown that healthy seropositive blood donors deliver negative CMV PCR results from leukocytes and plasma [25], CMV-DNA detection in plasma, leukocytes or respiratory secretions or positive virus isolation was defined as CMV reactivation.

Statistics

Baseline patient characteristics were summarized using absolute frequencies and percentages with 95% confidence interval (CI 95%) for nominal data, and median (interquartile range (IQR)) for continuous data. The baseline characteristics were compared between the groups of patients with and without CMV reactivation using Fisher's Exact Test or Chi-Squared Test for nominal variables and Wilcoxon-Test for continuous variables. Patients who died or were discharged within the first 72 hours after study enrolment, were excluded from data analysis. The two primary endpoints were the rate of in-hospital mortality and length of stay in the ICU, defined as days from study enrolment to death or discharge from ICU. Secondary endpoints were duration of hospital treatment and length of mechanical ventilation defined accordingly. To evaluate the influence of CMV-reactivation on these endpoints we conducted uni- and multivariate Cox proportional-hazard regression analyses adjusting for confounding factors. The analyses regarding duration of hospital treatment and time on mechanical ventilation (secondary endpoints) were based on the data of the 55 surviving patients considering the following variables: SAPS II at inclusion (Score points), ICU stay before enrolment (days), septic shock at enrolment (yes/no) and HSV detection (duration of hospital treatment) and SAPS II, paO2/fiO2 ratio and presence of pneumonia causing sepsis at enrolment (yes/no) as well as duration of mechanical ventilation before inclusion (time on mechanical ventilation). Continuous variables were generally used as linear factors, all others were used as dichotomous factors in the regression models. Univariate hazard ratios were calculated with 95% CI (not shown in the tables) applying the Cox proportional-hazards model. The modelling included testing for co-linearity, interactions with the factor CMV reactivation, and proportional hazard assumption for the risk factors. In a first step the multivariate model considered all relevant risk factors, which were in a second step optimized keeping only CMV reactivation and those factors with a $P < 0.05$. Incidence figures were created using the Kaplan-Meier estimates. All P-values are two-sided. To additionally consider time-dependency a landmark analysis was performed at the time point 0, Day 7 and Day 14 based on the Cox-Regression. All statistical analyses were performed with SAS System version 9.1 for Windows (SAS Institute, Cary, NC, USA), and incidence figures were created with SPSS version 17.0 for Windows (SPSS Inc. Chicago, Illinois 60606, USA).

Results

Study population

A total of 129 patients were screened initially; 28 of them were excluded because of negative CMV IgG serology, 2 suffered from lymphoma, and 1 had to be excluded due to immunosuppressive chemotherapy. One patient was excluded because of missing data. Thus a total of 97 patients were enrolled for further CMV monitoring. Since six of them died and five were discharged from the hospital within 72 hours, the data of 86 patients were analysed; the majority of them ($n = 64$) were treated in the two surgical ICUs. Baseline demographic characteristics and clinical data of the 86 patients at enrolment are presented in Table 1.

Virological examination results

In the 86 study patients on average (median) four sets of samples for virological examination could be taken; 3.0 of them were collected during ICU stay, 3.0 on the ward.

In 77 of the 86 patients both blood and tracheal secretions could be obtained for virological testing; 9 patients delivered only blood samples. Parameters of CMV reactivation were found in 35 of the 86 patients (40.7%, CI 95%: 30.2 to 51.8) with severe sepsis. The distribution of positive PCR results in the different compartments is presented in Figure 1, indicating that in 13 of the 35 cases CMV reactivation was detected exclusively in the lungs. On average (median) CMV reactivation occurred 21 days after enrolment into the study, becoming

Table 1 Demography and underlying conditions of included patients (*n* = 86)

	All patients	Active CMV infection		*P*-value
		Yes	No	
	n = 86 (100%)	*n* = 35 (40.69%)	*n* = 51 (59.31%)	
Demographic data				
Age (years)[a]	68.0 (59 to 76)	68.0 (52 to 73)	69.0 (59 to 76)	0.237
Male sex (n (%))	67 (77.9)	27 (77.1)	40 (78.4)	0.888
Surgical interventions requiring intensive care (n)				
Neurosurgery	4	2	2	
Abdominal surgery	33	14	19	
Cardiovascular surgery	18	7	11	
Other surgical procedures	9	6	3	
All surgical interventions	64	29	35	
Medical diseases requiring intensive care (n)				
Liver disease	1	1	0	
Coronary heart disease	2	0	2	
Infections in the internal ICU	16	4	12	
Other	3	1	2	
All medical diseases	22	6	16	0.137
Infection causing severe sepsis (n (%))[b]				
Pneumonia	22 (25.9%)	11 (31.4%)	11 (22.0%)	0.329
Peritonitis	34 (40.5%)	12 (35.3%)	22 (44.0%)	0.425
Urinary tract infection	7 (8.5%)	4 (12.5%)	3 (6.0%)	0.263*
Catheter-associated bacteremia	6 (7.3%)	3 (9.4%)	3 (6.0%)	0.435*
Other	38 (55.1%)	18 (66.7%)	20 (47.6%)	0.121
Severity of illness at enrolment				
Duration of ICU stay (days) [c,d]	4.0 (2 to 9)	5.0 (2 to 11)	4.0 (2 to 9)	0.245
SAPS II Score[a]	43.0 (36 to 51)	43.0 (33 to 47)	44.0 (37 to 33)	0.150
SOFA Score[a]	8.0 (7 to 11)	8.0 (6 to 10)	9.0 (7 to 12)	0.060
Septic Shock present (n (%))	55 (65.5%)	19 (57.6%)	36 (70.6%)	0.221
Transfusion of packed red cells[a,d]	2.0 (0 to 6)	2.0 (0 to 5)	3.0 (0 to 8.5)	0.561
Duration of ventilation (days) [c,d]	4.0 (2 to 9)	5.0 (2 to 11)	3.0 (2 to 9)	0.157
Surgical interventions [a,d]	1.0 (1 to 2)	1.0 (1 to 2)	1.0 (1 to 2)	0.586
Horowitz index (paO_2/fiO_2) <200 (n (%))	56 (65.9)	23 (67.7)	33 (64.7)	0.779

[a]Median (interquartile range), [b]All infections sum up to more than 100%, because more than one infection could be noted in one patient, [c]Median (interquartile range), [d]During the actual hospital stay until enrolment into the study * Fisher's exact test.

CMV, cytomegalovirus; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment score.

obvious earlier in tracheal secretions (median 14 days, range 0 to 77 days) than in blood (median 24.5 days, range 0 to 49 days), as shown in Figure 2. Interestingly, HSV-DNA appeared even more frequently and mostly earlier than CMV in respiratory secretions (Figures 1 and 2), yielding a positive PCR in 44 of the 86 study patients. In patients with CMV reactivation (n = 35) the rate of HSV detection added up to 65.7% (23 of 35) compared to 41.2% (21 of 51) in the group where CMV remained in the latent state (P = 0.025). Quantification of CMV-DNA was performed in the 10 patients who were tested positive in plasma by qualitative PCR. Four of them showed plasma DNA levels beyond the detection limit of the COBAS Amplicor® PCR system (Roche) (600 copies/ml); in the other six patients the CMV-DNA content in plasma was low with a peak amount of 2,830 copies/ml on average (minimum 600, maximum 1,608 copies/ml). CMV-DNA in leukocytes was detected in 22 cases.

Consequences of CMV reactivation

The in-hospital mortality of all enrolled patients was 36.1% (31 of 86) without any relevant difference between those who showed CMV reactivation (37.1%, that is, 13 of 35; CI 95% 21.5 to 55.1) and those who did not (35.3%, that is, 18 of 51, CI 95% 22.4 to 50.0; P = 0.861) (Table 2). No CMV disease was diagnosed by the responsible clinicians and thus no treatment was initiated. Even when adjusted for severity of illness, presence of septic shock, duration of ICU stay before study enrolment and HSV reactivation, in-hospital mortality of patients with CMV reactivation was not increased (HR: 0.369, 95% CI: 0.136 to 1.005, P =

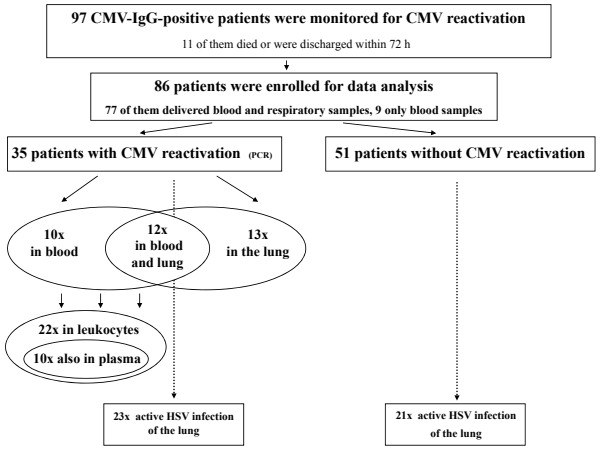

Figure 1 Positive CMV and HSV PCR results in leukocytes, plasma, respiratory secretions of 97 patients.

0.051 Table 3). To light up time-dependency of the CMV effect on in-hospital mortality, we applied Cox regression modelling at days 0, 7 and 14 (landmark analysis) considering the same factors thereby including HSV detection according to its occurrence at the three time points. At each time point, interaction between the tested factors was proven to be not statistically significant. Results of the optimized models are shown in Table 3. These data confirm that only SAPS II at inclusion influenced the in-hospital mortality.

Focussing on increased morbidity an association with CMV reactivation was observed. The LOS in the ICU (30.0, interquartile range 14 to 48 vs. 12, interquartile range 7 to 19 days; $P < 0.001$) as well as the duration of hospital treatment (33.0, interquartile range 24 to 62 vs. 16.0 days, interquartile range 10 to 24; $P < 0.001$) and the time on mechanical ventilation (22.0, interquartile range 6 to 36 vs. 7.5 days, interquartile range 5 to 15.5; $P = 0.003$) were significantly longer in patients with CMV reactivation than in those without (Table 2).

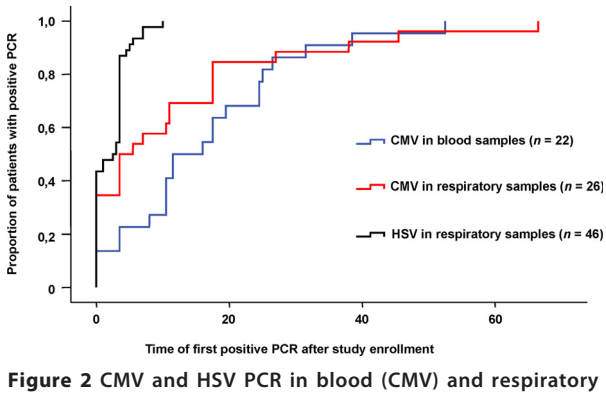

Figure 2 CMV and HSV PCR in blood (CMV) and respiratory secretions against time after study enrolment.

The impact of CMV reactivation on the LOS in the ICU was further elucidated by Cox regression again considering the factors mentioned above (Table 4). This Cox model showed that CMV reactivation was clearly associated with a longer ICU stay (HR 3.365, CI 95% 1.233 to 9.183; $P = 0.018$ and HR 2.441, CI 95% 1.011 to 5.897, $P = 0.047$, respectively, according to the optimized model). Moreover, as for in-hospital mortality a landmark analysis was performed based on the same risk factors looking forward on the length of ICU stay following days 0, 7 and 14 after enrolment, respectively (Table 4). The SAPS II at inclusion became statistically significant at all three time points and proved to be the most important risk factor for prolonged ICU treatment. In this landmark analysis only CMV reactivation at Day 7 was identified as a second risk factor with an independent impact on the length of ICU stay following Day 7 after study inclusion (HR 2.853, CI 95% 1.003 to 8.117, $P = 0.049$).

Moreover the surviving patients with CMV reactivation were at significantly higher risk for prolonged in-hospital treatment as shown in Figure 3 (HR 3.3; CI 95% 1.78 to 6.25; $P < 0.001$). The adjusted proportional hazard ratio for prolonged mechanical ventilation was 2.6 times higher in CMV reactivating patients than in those who remained in a latent state (CI 95% 1.39 to 4.94, $P < 0.001$; optimized model; Figure 3). The increased time of mechanical ventilation went along with a significantly compromised pulmonary function in patients with CMV reactivation. The Horowitz index (paO_2/fiO_2 ratio) remained below 200 for six days (interquartile range 1 to 17) in CMV reactivators compared with three days in non-reactivators (interquartile range 1 to 7, $P = 0.038$).

Discussion

This prospective, observational study demonstrated CMV reactivation in 40.69% (35 of 86) of patients with severe sepsis, despite the absence of other factors causing immunosuppression. This incidence of CMV reactivation is amazingly consistent with the results of two previous small German studies [12,16] and a more recent retrospective investigation [26]. These authors calculated a CMV reactivation rate of 45% in patients with systemic inflammatory response syndrome or sepsis [12], of 32% in patients with septic shock [16] and of 35% in cryopreserved plasma samples of long-term ICU patients [26]. Thus, as proposed in the review by Osawa and Singh [27], our prospectively assessed data clearly identify septic patients as a defined subgroup in the ICU population being at high risk for CMV reactivation.

A study examining 120 CMV-seropositive patients in six US ICUs also revealed CMV reactivation in approximately one-third of the study group [13]. There is,

Table 2 Outcomes of included patients with and without CMV reactivation (*n* = 86)

		All patients	Active CMV infection		*P*-value
			Yes	No	
Parameter	**n**	***n* = 86**	***n* = 35**	***n* = 51**	
Mortality (n (%))*	86	31 (36.1)	13 (37.1)	18 (35.3)	0.861
Length of stay in the ICU (days)[a, b] *	86	16.5 (7 to 29)	30.0 (14 to 48)	12.0 (7 to 19)	<0.001
Length of hospital stay (days)[a, b] **	86	22.5 (13 to 38)	33.0 (24 to 62)	16.0 (10 to 24)	<0.001
Duration of mechanical ventilation (days)[a, b] **	82	12.0 (6 to 23)	22.0 (6 to 36)	7.5 (5 to 15.5)	0.003

[a]Median (interquartile range); [b]Defined as days after study enrolment.
*Primary endpoints; ** Secondary endpoints. CMV, cytomegalovirus.

however, an important difference; whereas Limaye and co-workers [13] defined CMV reactivation exclusively on the basis of findings in plasma, our study additionally considered findings in leukocytes and respiratory secretions. Referred to positive PCR results in plasma only, the CMV reactivation rate in our study group was clearly lower (11.6%) than in Limaye's population.

A recent French investigation of 242 patients in a medical ICU [9], which evaluated both blood and respiratory samples indicated active CMV infection in 16% of the patients, which is less than half of the CMV reactivation rate observed in our patient population. This discrepancy can be attributed to differing CMV detection methods, since the French results rely on virus isolation from respiratory secretions. This technique has been shown to be less sensitive than PCR-based methods [18,27] as used in our study. Another explanation might be a lower CMV risk of medical compared with surgical ICU patients in general [14,27]. This difference was also reflected in our own study group with a CMV reactivation rate of 27.3% versus 45.3% (*P* = 0.137) in medical and surgical patients, respectively.

In general any comparison of incidence rates for CMV reactivation in critically ill patients is still compromised by differences in the examined materials, by the use of various virological methods [14,26] and most importantly, by differences between patient populations, which are not reflected by usual scores as for example SAPS II or SOFA.

Our finding that HSV reactivation appeared in nearly half of the patients and was thus clearly more frequent than CMV infection (Figure 2) agrees closely with the data of Cook *et al*., who reported positive HSV and CMV cultures in 23% and 15% of critically ill surgical patients, respectively [11]. The frequent coincidence of both herpes virus infections appeared quite similarly in a small study group of 25 septic patients, where 6 of the 8 CMV-reactivating patients showed active HSV infection as well [16].

Table 3 Cox regression analyses of factors associated with in-hospital mortality of the 86 included patients

	Univariable analysis		Multivariable analysis		
Factor	**HR**	***P*-value**	**HR**	**95% CI**	***P*-value**
CMV reactivation	0.410	0.029	0.369	0.136 to 1.005	0.051
SAPS II at inclusion	1.062	<0.001	1.047	1.012 to 1.082	0.008
Septic Shock present	2.193	0.081	1.470	0.555 to 3.896	0.438
ICU stay before enrolment	1.010	0.298	1.022	0.999 to 1.046	0.058
HSV detection in respiratory secretions	1.539	0.268	1.546	0.687 to 3.480	0.292
Optimized model					
CMV reactivation			0.496	0.215 to 1.145	0.101
SAPS II at inclusion			1.056	1.024 to 1.089	<0.001
Landmark analysis for Day 0, Day 7 and Day 14					
Day 0 (86 patients, 12 of them with CMV reactivation)					
CMV reactivation	0.983	0.974	1.005	0.347 to 2.911	0.993
SAPS II at inclusion	1.062	<0.001	1.062	1.030 to 1.096	<0.001
Day 7 (75 patients, 18 of them with CMV reactivation)					
CMV reactivation	0.559	0.259	0.486	0.173 to 1.370	0.172
SAPS II at inclusion	1.063	0.001	1.064	1.027 to 1.102	<0.001
Day 14 (62 patients, 20 of them with CMV reactivation)					
CMV reactivation	0.707	0.492	0.561	0.204 to 1.544	0.263
SAPS II at inclusion	1.065	0.003	1.067	1.026 to 1.111	0.001

CMV, cytomegalovirus; HR, hazard ratio; HSV, herpes simplex virus; SAPS, Sequential Organ Failure Assessment score.

Table 4 Cox regression analyses of factors associated with LOS in the ICU of the 86 included patients

	Univariable analysis		Multivariable analysis		
Factor	**HR**	***P*-value**	**HR**	**95% CI**	***P*-value**
CMV reactivation	3.101	0.009	3.365	1.233 to 9.183	0.018
SAPS II at inclusion	1.065	<0.001	1.045	1.008 to 1.083	0.016
Septic Shock present	2.282	0.081	1.486	0.535 to 4.127	0.447
ICU stay before enrolment	1.006	0.577	1.020	0.996 to 1.044	0.104
HSV detection in respiratory secretions	1.302	0.501	1.338	0.595 to 3.013	0.481
Optimized model					
CMV reactivation			2.441	1.011 to 5.897	0.047
SAPS II at inclusion			1.055	1.020 to 1.091	0.002
Landmark analysis for Day 0, Day 7 and Day 14					
Day 0 (86 patients, 12 of them with CMV reactivation)					
CMV reactivation	1.017	0.975	1.061	0.367 to 3.068	0.913
SAPS II at inclusion	1.065	<0.001	1.065	1.030 to 1.101	<0.001
Day 7 (64 patients, 16 of them with CMV reactivation)					
CMV reactivation	2.274	0.112	2.853	1.003 to 8.117	0.049
SAPS II at inclusion	1.071	0.001	1.074	1.033 to 1.116	<0.001
Day 14 (47 patients, 17 of them with CMV reactivation)					
CMV reactivation	2.109	0.164	2.538	0.861 to 7.479	0.091
SAPS II at inclusion	1.065	0.010	1.069	1.021 to 1.120	0.004

CI, confidence interval; CMV, cytomegalovirus; HR, hazard ratio; HSV, herpes simplex virus; LOS, length of stay; SAPS, Sequential Organ Failure Assessment score.

Mortality rates did not differ between patients with and without CMV reactivation in our study group. Slight differences between the two patient groups at baseline regarding SAPS II, presence of septic shock and ICU stay before enrolment suggest that selection bias might have contributed to this finding. This limitation due to the observational design of our study has to be taken into account. To address this problem a Cox regression adjusting for other potential risk factors was conducted. But even this adjusted analysis showed no impact of CMV reactivation on mortality (Table 3). This finding may surprise at the first glance, because recent results obtained in patients of French and US ICUs [9,13] as well as our own earlier findings in surgical ICU patients [8] suggested a higher mortality rate in CMV reactivators. The main reason for this discrepancy might be the difference between the homogenous group of patients with severe sepsis or septic shock presented here and the more heterogeneous cohorts of ICU patients enrolled in the other studies. This assumption is strongly corroborated, when our own previous results [8] are compared with the actual findings. Although identical methods were applied in the same setting, a remarkable effect of CMV reactivation on mortality appeared in the mixed group of seropositive surgical ICU patients of our former study, but not in the current one exclusively focussing on patients with severe sepsis. In this patient population, severity and treatment of sepsis might be the most crucial prognostic factors overriding potential effects of CMV reactivation. Beyond this, the extent of the viral load might strongly determine the effects on patient outcome, as suggested by Limaye's data [13] and also by the observations of Linssen and coworkers [28] in ICU patients with HSV detection in respiratory secretions. This assumption is strongly corroborated by the comparison of our findings with Limaye's results. Indeed, quantitative PCR examinations delivered >1,000 copies per ml plasma in 8% of our patients but in 20% of Limaye's patients. The higher incidence of plasma-DNAemia as well as the higher level of plasma DNAemia observed by Limaye *et al*. indicates that their patients developed a more serious pattern of CMV reactivation than ours. Unequal treatment modalities, such as transfusion of leukocyte-depleted versus non-depleted blood products or different catecholamine use [29] might have contributed to the different severity of CMV reactivation and, thereby, to different effects on in-hospital mortality.

Finally, it has to be mentioned, that a small effect of CMV reactivation on mortality could have been overseen in our study due to the restricted number of examined patients. This is a major limitation of our study, delivering a statistical power of less than 20% to detect a 10% mortality difference between CMV reactivators and non-reactivators.

CMV reactivation in our patients with severe sepsis was accompanied by an increased LOS in the ICU (30.0, interquartile range 14 to 48 vs. 12, interquartile range 7 to 19 days; $P < 0.001$), an extended time of in-hospital treatment (33.0, interquartile range 24 to 62, vs. 16.0, interquartile range 10 to 24 days; $P < 0.001$), and longer

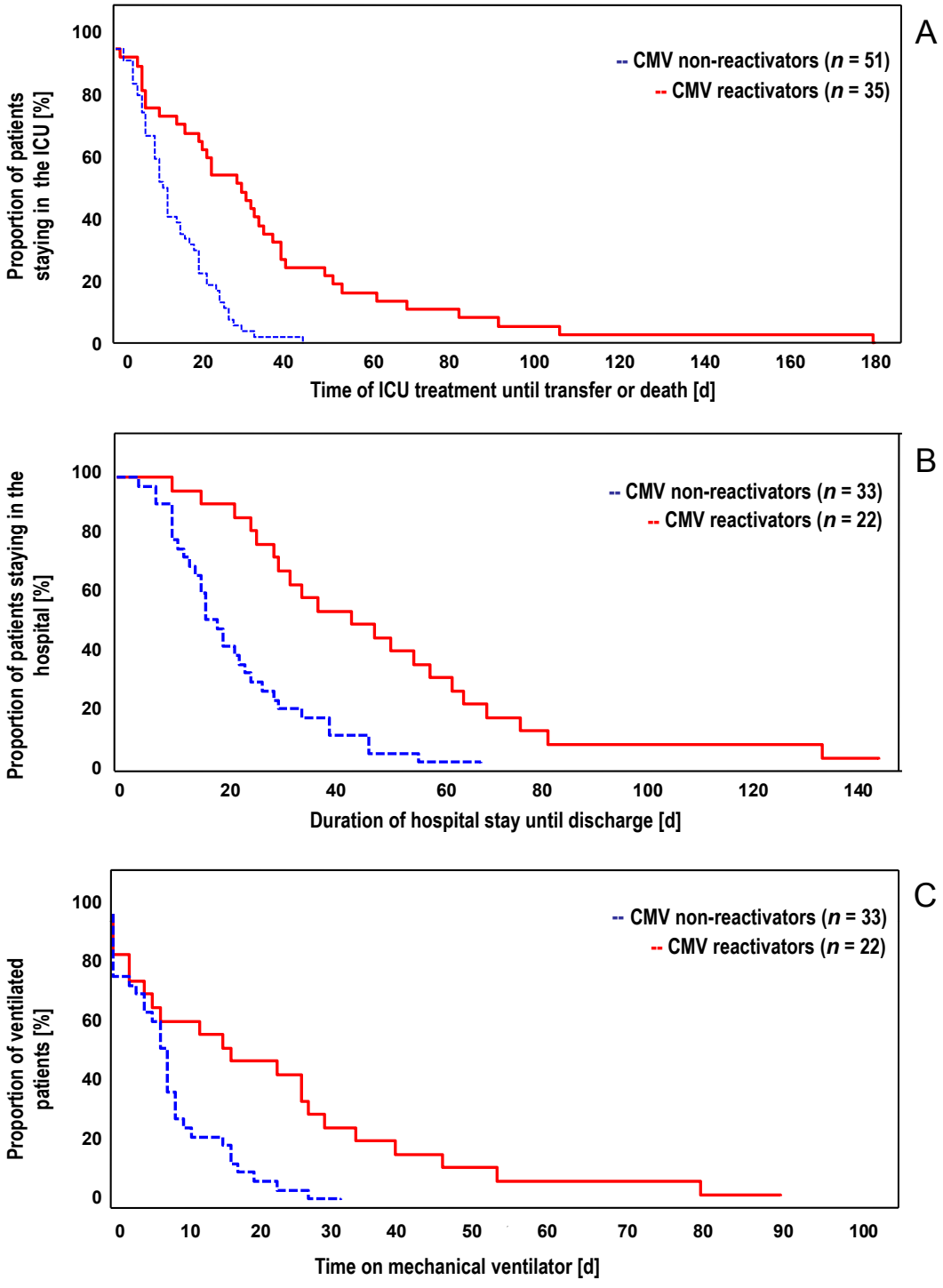

Figure 3 A: Patients leaving ICU due to transfer or death against days after study enrolment. **B**: Surviving patients discharged from hospital against days after study enrolment. **C**: Patients on mechanical ventilation against days after study enrolment.

time on the ventilator (22.0, interquartile range 6 to 36, vs. 7.5 interquartile range 5 to 15.5 days; P = 0.003) (Table 2). However, these data alone cannot give a clue on the causality of CMV reactivation. The first reason is that other risk factors rather than CMV reactivation might have led to these enhanced treatment requirements. To address this point, we adjusted for the most probably relevant factors using Cox regression (Table 4)

but the number of included factors had to be limited to four (beyond CMV reactivation) in order to avoid overfitting of the model [30], which is a limitation due to sample size in our study. Nevertheless, the adjusted analysis confirmed the significant impact of CMV reactivation on the LOS in the ICU (primary endpoint) as well as on the duration of in-hospital treatment and time on mechanical ventilator (secondary endpoints). The other result of the Cox regression, delineating HSV in contrast to CMV not as a risk factor for prolonged ICU stay, fits well with an earlier finding of Tuxen *et al*. [31], who found no positive effect of acyclovir prophylaxis on the LOS in ICU.

A second problem questioning the causative role of CMV reactivation for prolonged intensive care treatment is the potential confounding of LOS in the ICU with opportunity to detect CMV. Unfortunately, a major limitation of our study was that, due to logistical reasons, CMV monitoring could not be continued after hospital discharge of the patients. Therefore, in an attempt to address this important issue, a landmark analysis was also performed. This evaluation corroborated the association of CMV reactivation with prolonged ICU stay when we looked forward from Day 7 on the subsequent LOS. Since this statistical approach allows control for time-dependency of an effect it strengthened the assumption, that CMV reactivation might be a true causative factor contributing to extended treatment needs in patients with severe sepsis. This suggestion is corroborated by the findings in various mixed ICU populations [9,13,17,18] and in one small population of 25 septic patients [16].

In severely immunosuppressed patients like in stem cell transplantation, CMV pneumonia may lead to fatal outcome. CMV disease was reported in single cases of acutely ill, but otherwise immunocompetent, patients [32], but such cases are rare and were not observed in this study. Thus, other effects of CMV must be responsible for our findings. Although the incidence of acute respiratory distress syndromes was not specifically addressed in our study; impaired pulmonary function might be a possible explanation. In patients with CMV reactivation, impairment of the pulmonary gas exchange (paO2/fiO2 <200) persisted significantly longer than in non-reactivating patients (6.0, interquartile range 1 to 17 vs. 3.0, interquartile range 1 to 7 days, $P = 0.038$. This result corresponds well with the findings of Cook *et al*. [33] obtained in a mouse model of CMV reactivation due to sepsis.

Nevertheless, one must keep in mind that the above mentioned limitations of our study design do not allow us to unequivocally establish the causative role of CMV for extended treatment requirements. We cannot exclude the possibility that CMV reactivation could be a marker, rather than a cause, of serious illness.

As proposed by Osawa and Singh [27], a prospective randomised multicenter trial of prophylactic antiviral treatment might be the most goal-oriented method to establish the causative role of CMV in adverse outcomes. The fact that Limaye *et al*. observed a quantitative association between CMV reactivation in terms of plasma CMV-DNA levels and a combined endpoint (death or ICU stay beyond day 30), whereas survival was unaffected in our patients, who had much lower plasma DNA levels, corroborates the importance of quantitative examinations. A quantitative approach might offer the chance to optimize the balance of potential harms and benefits for participants of a randomized treatment trial.

Conclusions

In summary, our data indicate an independent correlation between CMV reactivation and increased morbidity in the well-defined group of nonimmunosuppressed patients with severe sepsis, but CMV reactivation had no impact on mortality in this group with low CMV-DNA plasma levels. Thus, the potential harms and benefits of antiviral treatment have to be weighed very cautiously in patients with severe sepsis or septic shock.

Key messages

- Cytomegalovirus reactivation occurs in 40% of non-immunosuppressed CMV-seropositive critically ill patients with severe sepsis.
- Cytomegalovirus reactivation had no relevant impact on mortality but was associated with increased length of stay in the ICU and in the hospital.
- Cytomegalovirus reactivation was accompanied by Herpes simplex infection in 65.7% of cases.
- Herpes simplex occurs earlier than CMV reactivation during severe sepsis.

Abbreviations

ACCP: American College of Chest Physicians; CI: confidence interval; CMV: cytomegalovirus; HR: hazard ratio; HSV: herpes simplex virus; LOS: length of stay; SAPS: Simplified Acute Physiology Score; SCCM: Society of Critical Care Medicine; SOFA: Simplified Organ Failure Assessment.

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Authors' contributions

AH designed and carried out the clinical study including data analysis, and was the responsible first author, with an unrestricted grant as mentioned above. HH recruited samples and is the corresponding author. IF was responsible for statistical analysis. RB performed the virological analysis (HSV PCR) and data analysis. RR recruited patients. FR was responsible for discussion and the literature search. CM was responsible for statistical analysis. GJ interpreted the data. AK and KU recruited patients and contributed to the discussion. KH co-designed the virological study and was responsible for virological monitoring, including the data analysis.

Competing interests

This study was sponsored in part by Roche Pharma AG, Grenzach-Wyhlen, Germany. One of the co-authors (FR) was formerly employed by Roche Pharma AG, whose product is active against CMV infection, which was studied in the present work. There are no other competing interests.

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doi:10.1186/cc10069
Cite this article as: Heininger *et al.*: Cytomegalovirus reactivation and associated outcome of critically ill patients with severe sepsis. *Critical Care* 2011 **15**:R77.

Additional reading relevant to whole infection course

Host genetic factors and infection
Holmes CL, Russell JA, Walley KR. Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. Chest. 2003 Sep;124(3):1103-15.

Innate immune response to infection/TLR
Akira S, Uematsu S, Takeuchi O.Pathogen recognition and innate immunity. Cell. 2006 Feb 24;124(4):783-801. Review.

Defensins/antimicrobial peptides
Ganz T. Nat Rev Immunol. 2003 Sep;3(9):710-20.

Complement
Gasque P. Mol Immunol. 2004 Nov;41(11):1089-98

Neutrophils and bacterial clearance
Nourshargh S, Marelli-Berg FM.Transmigration through venular walls: a key regulator of leukocyte phenotype and function.Trends Immunol. 2005 Mar;26(3):157-65. Review.

Segal AW.How neutrophils kill microbes. Annu Rev Immunol. 2005;23:197-223. Review.

Evasion of the innate host immune response by pathogens
Coombes BK. Curr Biol. 2004 Oct 5;14(19):R856-67.

Voyich. Microbes Infect. 2004 Oct;6(12):1117-23. Review

Water and Electrolytes

Course leader: Dr. Damien Ashby

How is water and electrolyte balance regulated in healthy people? During this theme you will explore some of the more important ways in which homeostasis in these areas can be compromised in disease.

The overall learning outcomes of the water and electrolytes theme may be separated into a number of (related) areas:

Water
By the end of the course students should be able:

- To appreciate the centrality of water in the control of cell volume, blood pressure and metabolism
- To understand the physiological implications of dehydration and how the body responds to it
- To understand the physiological implications of water loading states and how the body responds to them

Sodium and potassium
By the end of the course students should be able:

- To understand the intracellular and extracellular balance of these two ions including how and why the gradients are maintained
- To understand how the body responds to overload and deficiencies of each, including the pathological features found in each situation
- To be able to determine the implications of sodium and potassium abnormalities in a number of different clinical scenarios

Acid-base balance
By the end of the course students should be able:

- To understand why maintenance of appropriate pH is important physiologically
- To identify several clinical scenarios in which acid-base balance is disrupted
- To be able to describe how the body deals with acid-base abnormalities in a number of different situations

Renal failure
By the end of the course students should be able:

- To outline the principal causes of acute and chronic renal failure
- To appreciate the clinical features people may develop in those circumstances
- To outline the possible ways of managing these patients
- To appreciate the different modalities of renal replacement therapy

Renal physiology lectures
By the end of the course students should be able:

- To understand the mechanism of the control of plasma and urine osmolality
- To review a publication in the form of a journal club

A short list of background reading

Crash course: Renal and Urinary Systems by Mirpuri and Patel. Mosby

Principles of renal physiology by Lote, Christopher J. 4th ed.
Kluwer Academic Publishers, 2000

Advanced text: Clinical physiology of acid-base and electrolyte disorders
Burton David Rose and Theodore W. Post. 5th ed. McGraw-Hill, 2001

Drugs and the Hospitalised Patient

Course leader: Dr Gareth Barnes

Throughout this theme we will cover a range of aspects of pharmacology, from understanding the correct drugs to use in common medical conditions to the process of drug development and the challenges that this presents.

Some of activities planned for this week are designed to help you think laterally and allow you to integrate your knowledge from different aspects of this course in various clinical medical scenarios. This is particularly true of the tutorial sessions. Please complete all the questions prior to attending, as this will enhance the discussion within the group.

The lectures should be a mix of very practical subjects, which will be relevant to your early clinical years, and some thought provoking topics that will be more relevant to your BSc.

Learning objectives
By the end of the course, students will:

- Understand the process of drug discovery and the challenges of bring a new medication to market
- Understand the risks and challenges of investigating novel pharmacological molecules
- Know how to prescribe safely and the impact of illness on prescribing
- Understand evidence based prescribing, using heart failure and systemic hypertension and as models.
- Understand the drugs used, and their pharmacology, for all medical conditions discussed in this theme.
- Have experience of integrating their knowledge of pharmacology, physiology and pathophysiology in the context of complex clinical situations.
- Have had practice of relating basic biomedical science to clinical treatment and outcomes.
- Have completed all online material related to this theme; pharmacology and ECG quiz.

Individual Lectures:

Vincenzo Libri. Drug Discovery.
Fri 10th May CXH, Drewe LT 1400-1500

Aims
Understand how drugs are discovered
Understand phases of drug development
Business model of drug development
Study designs, volunteers, sample sizes and marker end points in early phase clinical trials

Martin Wilkins. Why drugs fail in clinical trials.
Fri 10th May CXH, Drewe LT 1500-1600

Aims
Why drugs fail to make it to market
Role of trial design to enhance success

Neil Chapman. Treatment of heart failure and hypertension.
Mon 3rd June, LT1, South Ken 0910-1000

Aims
Evidence based treatments used in systemic hypertension
Evidence based treatments used in heart failure
Pharmacology

Gareth Barnes. Acute Coronary Syndromes.
Mon 3rd June LT1, South Ken 1000-1100

Aims
Clinical features of acute coronary syndromes
ECG features of acute coronary syndromes
Evidence based pharmacological treatment in acute coronary syndromes

Rohini Sharma. Pain management.
Mon 3rd June LT1, South Ken 1100-1200

Aims
Common analgesiscs used, WHO pain ladder
Adjuvant drugs
Toxicity of analgesics

David Nutt. How can we get doctors to tell the truth about drugs?
Tues 4th June LT1, South Ken 1200-1300

Aims
Appreciate risk analysis

Gareth Barnes . Safe prescribing
Tues 4th June LT1, South Ken 1400-1500

Aims
Understand the need to prescribed medications correctly
The consequence of poor prescribing
Impact of illness of prescribing – renal and liver disease

Nicola Kalk. Overdose
Tues 4th June LT1, South Ken 1500-1600

Aims
General principles of management of intentional overdose – ABC, TOXBASE, supportive treatment, NB re half-life.
Specific treatments –paracetamol, heroin, bicarb and TCA's.
Risk assessment in OD

Tutorials

On the 4 and 5th June tutorials have been arranged and these will support the lectures. It is essential that you complete all questions in the guide prior to attending; you will get far more out of these sessions this way.

Please read through case 1 and 2 (following pages) for the sessions on the 4th June, and then work through cases 3 and 4 for the session on the 5th June.

Online learning

Questions will be posted on line for you to work through and these will supplement both the lectures and tutorials. Model answers are provided.

ECG's
There are a number of ECG's on line for you to analyse throughout this module

Case 1

Ms Kaye is a young lady with previous history of deep vein thrombosis and has been taking warfarin therapy to prevent recurrence. She presented to A&E as she was extremely short of breath.

Her friend who came to A&E with her commented that Ms Kaye had noticed that her leg was swollen. This was similar to her previous DVT but as she was taking warfarin she didn't go to see her GP. She had recently bought some over the counter medicine (OTC), as she was feeling low after a recent break-up.

Her oxygen saturations were 82% on room air. Blood pressure 135/82, HR 98, apyrexial.

Breath sounds are equal throughout both lung fields. Heart sounds are audible, with a murmur over her left sternal edge.

1. Integrate the case and provide a differential diagnosis, with investigations you would undertake

2. What are the risk factors for thrombosis?

3. How is warfarin metabolised and how does it interact with other medications?

4. What are the common indications for warfarin?

5. What are the common complications of warfarin?

Case 2

A 78 year old man arrives in A&E complaining of 3/52 history of increasing shortness of breath, productive cough and fever. He is a current smoker of long duration, around 45 pack years. He has a background of hypertension and diabetes, both of which are well controlled

His observations are; HR 128, irregular, low volume, oxygen saturations 93% on air, blood pressure 102/56mmHg. On examination his chest is hyper-inflated. Poor air entry through thorax with generalised expiratory wheeze. Heart sounds are audible, but soft. You cannot hear any murmurs given his rapid heart rate.

1. Integrate the case history for a likely diagnosis and what investigations you may plan and treatments

2. What treatments are given during an exacerbation of COPD?

3. How much oxygen would you prescribe?

4. How would you treat this gentleman's atrial fibrillation and what considerations are relevant to this particular case?

5. How would you reduce the risk of stroke in this man?

Case 3.

A 45 year old man attends A&E claiming to have taken 44 amitriptyline tablets (previously prescribed for chronic pain) in the last 12 hours. He has recently been made redundant and is worried about how he will manage to pay his mortgage. He has been treated for depression in the past, but this has not been a problem for several years.

His Glasgow Coma Scale is 7/15. His respiratory rate is 6 breaths/min and his pupils are dilated.

1. Describe the absorption, distribution, metabolism and excretion of Amitriptyline

2. What are the clinical features of TCA overdose and what are the underlying mechanisms of these features?

3. What are the immediate actions that you would perform in A&E and what is your management plan?

4. What features of the history would suggest that this was deliberate and what other questions would you ask?

5. What planning would you make before you discharge him?

Case 4

A drug company, Pharmatec, has been investigating new therapies for hypertension. One compound has had some very promising results in the initial phases of development and is now progressing to trials in patients.

Pharmatec has sought your expertise and recruited you to assist them with their research. They want advice about how to conduct medical trials as they have never been involved in patient studies before. They are not sure of the best way to judge if the new medication will be effective; equally there may be important side effects to record. They need your help to decide the best way to make these assessments.

1. What steps are required before embarking on this research protocol?

2. What is informed consent?

3. What outcome measures would you use and how would you assess them?

Normal and abnormal nutrition

Course leader: Dr Lina Johansson

This course aims to provide a sound grounding in normal and abnormal clinical nutrition that can be drawn on throughout your clinical experience.

The Nutrition course is composed of a series of lectures as well as two tutorials. All the material that is taught, as well as key references, are examinable.

Overall nutrition aims:

1. To provide an overview of the role of nutrition and dietetic intervention throughout human life (paediatric and elderly) as well as that in health and exercise.

2. To understand the nutritional implications in a range of chronic illnesses such as renal disease, diabetes, coronary heart disease and conditions such as obesity and cancer, as well when the body is subjected to extreme stresses such as that in critical illness.

3. To gain an understanding of the importance of identifying patients who are at risk of malnutrition and methods of improving this taking into consideration underlying clinical processes.

Overall learning objectives:
By the end of the course students should be able to:

- Demonstrate how nutritional requirements change throughout life.
- Integrate basic science of appetite regulation to the rise in obesity in the UK.
- Demonstrate an understanding of the problem of obesity in the UK and how this can be managed.
- Identify patients at risk of malnutrition.
- Describe ways of improving nutritional status.
- Explain the differences between starvation and trauma and the effects they have on nutritional status and re-feeding.
- Demonstrate why ageing has an effect on nutritional status and how this affects the hospitalised patient.
- Demonstrate how nutritional requirements change in different disease states, namely cardiovascular disease, renal disease, cancer and diabetes.

Each lecture will additionally come with its own set of learning objectives and references.

INDIVIDUAL LECTURE OUTLINES

Title of session	**Nutrition and Cardiovascular Disease**
Date	Tuesday 7th May 1500-1600
Lecturer	Prof Gary Frost, Chair in Nutrition and Dietetics, Imperial College
Content	The impact of nutrition intervention in coronary heart disease (CHD).
Objectives within session	• To have an understanding of the metabolic abnormalities in CHD. • To interpret biochemical parameters as a function of nutrition in CHD. • To understand the role of nutritional intervention in CHD to preventive morbidity and mortality • To be able to provide simple and practical dietary guidance to patients with CHD to improve risk profile
Private study objectives	• To have an understanding of the prevalence of CHD • To identify the lifestyle and risk factor targets for preventive cardiology
Recommended reading	NICE Guideline. Secondary prevention in primary and secondary care for patients following a myocardial infarction http://www.nice.org.uk/guidance/index.jsp?action=byID&o=11008 NICE Guideline. Cardiovascular risk assessment: the modification of blood lipids for the primary and secondary prevention of cardiovascular disease. http://guidance.nice.org.uk/CG67 Mead A. et al. Dietetic guidelines on food and nutrition in the secondary prevention of cardiovascular disease – evidence from systematic reviews of RCTs (second update, Jan 2006). *J.Hum.Nutr.Diet.* 2006;19:401-419. JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* 2005;91 Suppl 5:v1-52.Cardiac National Service Framework Department of Health WEB site Van Horn et al. The evidence for dietary prevention and treatment of CVD. *J Am Diet Assoc.* 2008;108:287-331

Title of session	Nutrition and the gut
Date	Tuesday 7th May 1600-1700
Lecturer	Dearbháile O'Hanlon, Specialist gastrointestinal dietitian
Content	Overview of dietary considerations in: • Inflammatory bowel disease • Irritable bowel syndrome • Coeliac disease • Short bowel syndrome
Objectives within session	• Identify factors that can affect nutritional status in inflammatory bowel disease. • Understand the potential of dietary treatment to manage symptoms of inflammatory bowel disease and as a treatment for Crohn's disease. • Understand the role of dietary manipulation as a treatment for irritable bowel syndrome • Identify nutritional consequences of short bowel syndrome and management options • Understand the pathophysiology of coeliac disease and the role of the gluten free diet
Recommended reading	Mowat C et al. Guidelines for the management of inflammatory bowel disease in adults. Gut 2011. May; 60(5): 571-607 **sections 4.1.1, 4.1.2, 7.6** Nightingale J, Woodward JM. Guidelines for management of patients with a short bowel. Gut 2006; 55(suppl IV). Doi:10.1136/gut.2006.091108

Title of Session	Infant Nutrition and Feeding
Date	Friday 17th May, 1400-1500
Lecturer	Caroline King, Paediatric Dietitian (Neonatal Specialist)
Content	Outline major differences between paediatric and adult nutrition with respect to nutrients required per kg and methods of obtaining nutrition along with a discussion of the implications of immaturity on nutritional management. A summary of the change in diet during first year will be given, including brief description of the major differences between breast vs. formula feeding. The process of weaning onto solid food will be covered and the implications for short and long term health discussed with respect to both type of milk feeding and timing of weaning.
Objectives Within Session	• To list main factors distinguishing child and adult nutrition. • To know how diet changes during first year with respect to milk feeding • To understand the benefits of breast milk • To appreciate the impact of weaning on a childs health & nutrition
Recommended reading	Clinical Paediatric Dietetics 3rd edition Eds Shaw & Lawson (Blackwell publishing), 4th edition due in 2013

Title of session	Common paediatric nutritional issues
Date	Friday 17th May, 1500-1600
Lecturer	Katie Elwig, Dietetic Clinical Lead for Paediatrics
Content	Paediatric Vitamin D deficiency, iron deficiency anaemia, allergy, obesity
Objectives within session	To have basic understanding of some of the more common paediatric nutritional disorders.
Recommended reading	Clinical Paediatric Dietetics 3rd edition Eds Shaw & Lawson (Blackwell publishing), 4th edition due in 2013 Paediatric medical text book Paediatric section in any general medicine textbook.

Title of Session	**Nutrition and the renal patient**
Date	Thursday 30th May 1500-1600
Lecturer	Nevine El-Sherbini, Clinical Specialist Renal Dietitian
Content	The metabolic abnormalities found in renal disease and the impact this has on nutrition in patients with renal failure.
Objectives within session	• To have an understanding of the metabolic abnormalities found in renal disease and to understand the nutritional implications. • To interpret biochemical parameters in relation to nutritional intake in chronic dialysis patients. • To be able to predict dietary modifications required in chronic kidney disease and renal replacement therapy. • To have an understanding of the aetiology of protein energy wasting in renal disease
Recommended reading	Nutritional Management of Renal Disease, Joel D. Kopple & Shaul G.Massry. 2nd Edition, 2003 Handbook of Nutrition & the Kidney; *William E Mitch, Saula Klahr.* 5th Edition, 2005 Oxford Handbook of Dialysis; Jeremy Levy, Julie Morgan & Edwina Brown. 2nd Edition, 2004 Kidney Disease Outcome Quality Initiative (K/DOQI) Clinical Practice Guidelines on Nutrition 2000: http://www.kidney.org/professionals/KDOQI/guidelines_updates/doqi_nut.html Renal Association Clinical Practice Guidelines Nutrition in Chronic Kidney Disease 5th Edition 2009-2010: http://www.renal.org/Libraries/Guidelines/Nutrition_in_CKD_-_Final_Version_-_17_March_2010.sflb.ashx European Best Practice Guideline on Nutrition http://ndt.oxfordjournals.org/content/22/suppl_2/ii45.full.pdf+html
Structure of Session	1 hour Lecture and tutorial

Title of Session	**Malnutrition in surgical, trauma, critical illness and cancer patients**
Date	Thursday 6th June 1300-1400
Session Coordinator	Ella Segaran (Specialist Dietitian for Critical Care) & Suzy Evans (Lead Dietitian – Surgery and Cancer)
Content	Overview of malnutrition in relation to surgery, trauma, critical illness and cancer patients
Objectives Within Session	• Recognise patients at risk from malnutrition • Identify metabolic changes that contribute to the development of malnutrition and cancer cachexia • Describe implications of malnutrition on patient morbidity and mortality • Discuss ways to correct pre-existing malnutrition and prevent development of malnutrition with appropriate nutritional support interventions
Private Study Objectives	• To identify the nutritional challenges that surgery, trauma, critical illness and cancer pose • Outline the factors that contribute to malnutrition • Highlight nutritional support strategies to prevent and treat these patients.
Recommended Reading	Bruera E BMJ 1997;315;1219-1222 ABC of Palliative Care. Anorexia, cachexia and nutrition Vets Study, NEJM,1991:315;525-532 Perioperative Total Parenteral Nutrition in Surgical Patients Baldwin C, Weekes CE: Dietary advice for illness-related malnutrition in adults. Cochrane Database of Systematic Reviews 2008, Issue 1. NICE guidelines for nutrition support in adults: http://www.nice.org.uk/nicemedia/pdf/cg032fullguideline.pdf
Structure of Session	30 minute lecture: Malnutrition and metabolism in surgical, trauma and critically ill patients 20 minute lecture: Malnutrition and metabolism in cancer and cancer cachexia patients Tutorial

Title of Session	Nutritional assessment and support
Date	Thursday 6th June 1400-1600
Lecturer	Jenni Foulds, Specialist Dietitian in Cardiothoracics
Content	Role of nutrition screening in identifying patients at risk of malnutrition with prevention/treatment of refeeding syndrome
Objectives Within Session	• On completion of this module students will be able to: • Use nutritional screening to identify patients at risk of malnutrition • Recognise patients at risk of malnutrition • Understand refeeding syndrome • Recognise patients who are at risk of developing refeeding syndrome and provide appropriate treatment
Recommended Reading	Mehanna HM, Moledinia J & Travis J. BMJ 2008 336: 1495 – 1498. Refeeding Syndrome: What it is and how to prevent and treat it. NICE guidelines for nutrition support in adults: http://www.nice.org.uk/nicemedia/pdf/cg032fullguideline.pdf
Structure of Session	1 hour lecture delivered back to back to half the year at a time

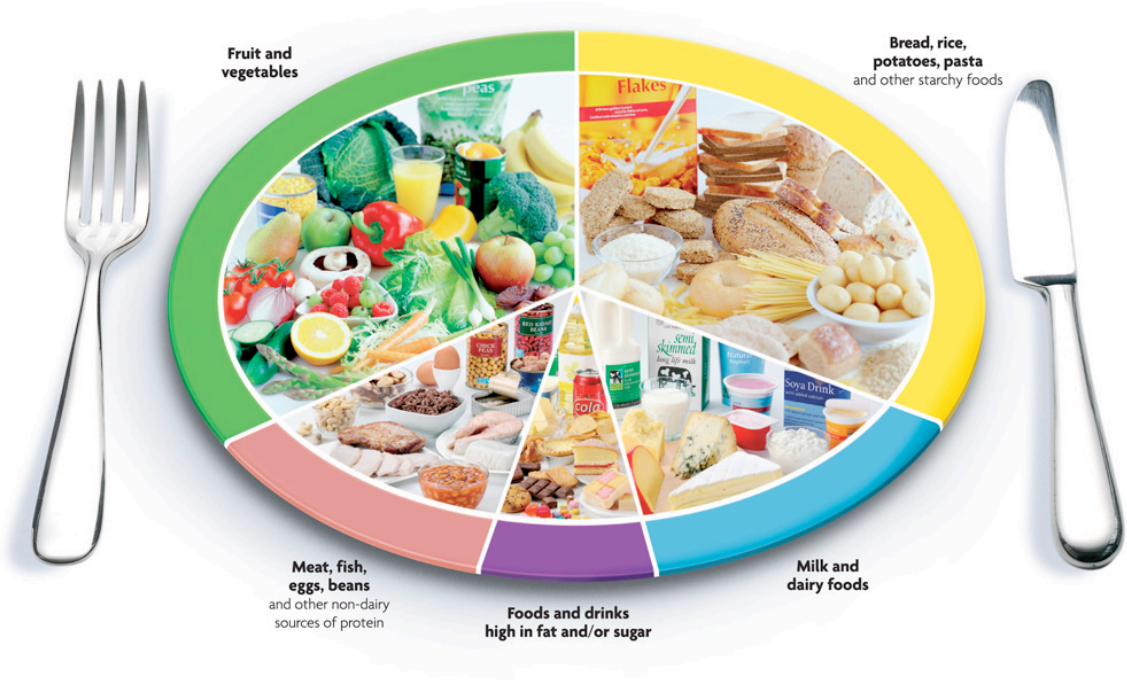
Title of Session	**Nutrition and Diabetes**
Date	Monday 10th June, 1300-1400
Lecturer	Sian Rilstone, Diabetes Specialist Dietitian
Content	• Prevention & nutritional management of Type 2 diabetes • Nutritional management of Type 1 diabetes
Objectives Within Session	• To understand the impact of diet and lifestyle on the prevalence, prevention and management of Type 2 diabetes and its co-morbidities • To understand the role of nutrition, including carbohydrate counting, in the management of Type 1 diabetes • To understand the differences between the nutritional management of Type 1 and Type 2 diabetes and how the focus of dietary advice may differ according to medical management plan
Private Study Objectives	• To find out more about the Glycaemic Index and its benefits in the prevention and management of diabetes and other health issues www.glycaemicindex.com (University of Sydney) • To be aware of the support offered nationally to people with diabetes www.diabetes.org.uk • To understand the principles of structured education for people with diabetes such as the BERTIE and DAFNE programmes • http://213.106.147.101/bdec2/bertie.shtml#bertie • http://www.dafne.uk.com/
Recommended reading	Diabetes UK Nutrition working group (2011) Evidence-based nutrition guidelines for the prevention and management of diabetes. Available online at: http://www.diabetes.org.uk/Professionals/Publications-reports-and-resources/Reports-statistics-and-case-studies/Reports/Evidence-based-nutrition-guidelines-for-the-prevention-and-management-of-diabetes-May-2011/ NICE (2008) Continuous subcutaneous insulin infusion for the treatment of diabetes mellitus Review of technology appraisal guidance 57. London: NICE. American Diabetes Association (2008). Nutrition recommendations and interventions for diabetes. A position statement of the American Diabetes Association *Diabetes Care* 31: S61-78. Frost G, Dornhorst A, Moses R (Eds.) (2003). *Nutritional Management of Diabetes Mellitus.* Wiley, West Sussex, England (ISBN: 0 471 49751 7).
Structure of Session	Lecture and tutorials

Title of session	**Healthy Eating – what does it all mean?**
Date	Monday 10th June, 1400-1500
Lecturer	Prof Gary Frost, Chair in Nutrition and Dietetics
Content	**What is healthy eating?** An optimal range of intake of nutrients needed to sustain the health of individuals of all ages. **Where did the concept come from?** It all started by the need to maintain health through rationing in the second world war. In the 1950s Keys *et al* linked heart disease to quality of dietary fat. In 1992 the first review of diet and health and the first national guidelines. In developing countries there is a struggle between under and over nutrition. **What are nutritional goals? :** Each goal is the national average intake of a particular nutrient that is needed to maintain optimal health. This may include both prevention of over and under nutrition. In the West all the healthy eating guidelines are aimed at prevention of chronic disease. This includes recommendations for energy, fat, carbohydrate and fruit & vegetables. **Implementing the guidelines:** There is evidence that there are changes but these are small and slow to change.
Objectives within session	To give an overview of healthy eating as a backbone to understanding the nutritional advice given to people with coronary disease, diabetes and obesity.
Private study objectives	To understand the impact of healthy eating on chronic disease
Recommended reading	http://www.bhf.org.uk/keeping_your_heart_healthy/healthy_eating/what_is_a_healthy_diet.aspx Human nutrition and dietetics Garrow, J. S.
Structure of session	1 hour lecture; 1 hour self-directed learning

The eatwell plate

Food Standards Agency
eatwell.gov.uk

Use the eatwell plate to help you get the balance right. It shows how much of what you eat should come from each food group.



Title of Session	**Obesity management and appetite regulation**
Date	Monday 10th June, 1500-1600
Lecturers	Gary Frost & Karim Meeran
Content	To cover epidemiology, personal and health service cost, energy expenditure, treatment webs and hormones controlling appetite and fat deposition.
Objectives Within Session	• To give basic understanding of obesity • To give an understanding of health cost of obesity • To give an understanding of the science behind obesity • To give an overview of the management of obesity
Private Study Objectives	Case study to be handed out. The answers to the questions will be posted on the WEB.
Recommended reading	Obesity Seminar in the Lancet 1997, 423-426. ABC of Obesity – BMJ, first article in the series: *BMJ* 2006; 333; 640-642. NICE clinical guideline 43: Obesity (2006) http://www.nice.org.uk/nicemedia/pdf/CG43NICEGuideline.pdf
Structure of Session	Lectures, tutorials and a quiz

Title of session	Diet and Exercise
Date	Wed 12 June 1000-1100
Lecturer	Claire Pettitt, Specialist Dietitian, PhD researcher
Content	The role of nutrition for exercise in varying conditions.
Objectives within session	• To have an understanding of the energy requirements in exercise. • To understand the different fuel sources for different exercise types. • To understand how exercise in extreme conditions impacts energy requirements. • To have an awareness of various supplements available and understand their uses and limitations.
Private study objectives	To have an understanding of the prevalence of CHD To identify the lifestyle and risk factor targets for preventive cardiology
Recommended reading	ACSM Position Stands Clinical Sports Nutrition, 4th Edition, Louise Burke (McGraw Hill Publishing) http://www.wada-ama.org/ http://www.ausport.gov.au/ **Baylis A, D. Cameron-Smith and L. Burke.** Inadvertent doping through supplement use by athletes: assessment and management of the risk in Australia. *International Journal Sport Nutrition and Exercise Metabolism* 11: 365-83, 2001.

Title of session	**Nutrition and the Elderly in Health & Illness**
Date	Wed 12th June 1100-1200
Lecturer	Mary Hickson, Therapy Research Lead
Content	Nutrition Aspects of Ageing Malnutrition, Nutritional assessment and Older people The Older person and artificial feeding
Objectives within session	• Describe what changes occur to body composition with ageing and explain the possible mechanisms behind these changes. • List risk factors (medical, psychological and social) that may affect nutrition in the older population and particularly while in hospital. • State the reasons for undertaking nutritional screening or assessment, and list the three main items of information that can provide an initial guide to nutritional risk. • Describe the appropriate use of nutritional supplements. • State what factors need to be considered when deciding if tube feeding and PEG feeding are appropriate
Private study objectives	To enable students to: • Describe the signs of deficiency of the most commonly deficient nutrients in the elderly • State what tests are available to diagnose each deficiency • Outline how each deficiency should be treated • List which foods are rich sources of each nutrient
Recommended reading	Royal College of Physicians (2002). Nutrition and Patients: A doctor's responsibility. See summary on web site: http://bookshop.rcplondon.ac.uk/details.aspx?e=133
References	Hickson M. Malnutrition and Ageing. *Postgraduate Medical Journal*. 2005. 82, 2-8. Schiffman SS (1997): Taste and smell losses in normal ageing and disease. *JAMA* 278, (16) 1357-1362. Hebuterne X, Bermon S and Schneider SM (2001): Ageing and muscle: the effects of malnutrition, re-nutrition, and physical exercise. *Curr Opin Clin Nutr Met Care* 4, (4) 295-300. Bates CJ, Prentice A, Cole TJ, van der Pols JC, Doyle W, Finch S, Smithers G, Clarke PC. Micronutrients: highlights and research challenges from the 1994-5 National Diet and Nutrition Survey of people aged 65 years and over. Br J Nutr. 1999 Jul;82(1):7-15.
Structure of session	1 hour lecture and 1 hour self-directed learning

Nutrition Support Tutorial
Date Thurs 6th June 1400-1600
Organiser: Jacqui O'Flynn and tutors

Case Study- Renal patient

Name:	*Mr Patel*
Age:	67
Occupation	*Retired*
Social History:	Married with 3 children.
PMH:	***ESRF* on haemodialysis (3x per week). He is anuric. Diabetes (on insulin), Hypertension.*** Mr Patel has had a poor appetite for weeks prior to his admission.
Admission:	Admitted with Stroke

Nutritional Assessment:	
Height (m):	1.72
Usual Weight (kg):	71
Usual BMI (kg/m^2):	24
Current weight(kg):	63 (Was at usual weight 2 months ago)
Current BMI (kg/m^2):	21
Alb (g/l) (33-47g/l):	17
CRP(mg/l) (<10mg/l):	58
K (mmol/l) (3.8-5.5mmol/l):	5.8
Ur (mmol/l) (2.5-8.0mmols/l)	25
Creat (60-125micromols/l)	332
Referral:	Nutrition assessment

*** ESRF – End Stage Renal Failure**

1. Calculate percentage weight loss. Is this clinically significant?

2. Is this patient malnourished? What factors would alert you to the fact that he might be malnourished?

3. This patient is dysphagic; what method of nutrition support would you recommend ie oral, nasogastric feeding, PEG feeding?

4. As a doctor what would you hope a Dietitian would consider in terms of planning nutrition support for this gentleman?

Case Study- Oncology patient

Name:	*Mrs Smith*
Age:	51
Occupation	*Retired Teacher*
Social History:	Married. 3 Supportive children.
PMH:	***Ca Ovary. Hysterectomy-Bilateral salpingo-oophorectomy and adjuvant 6 cycles of chemotherapy completed 3 months ago.*** ***Currently having Radiotherapy for recurrence and peritoneal metastasis.***
Admission:	Admitted during radiotherapy with profuse diarrhoea and dehydration
Diagnosis:	Provisional diagnosis - Radiation enteritis

Nutritional Assessment:	
Height (m):	1.61m
Premorbid Weight (kg):	70
Premorbid BMI (kg/m^2):	27
Post chemo weight (kg):	62
Post chemo BMI (kg/m^2):	24
Current weight:	55
BMI:	21
Albumin (g/l) (33-47):	18
CRP(mg/l) (0-10):	76
Oral Intake:	Meeting less than a quarter of her requirements.

1. Calculate percentage weight loss since diagnosis when she was 70kg

2. Comment on her nutritional status giving reasons for your answer. What score would you calculate her to have on the nutrition risk assessment tool (see following pages for a copy of the tool)?

3. What would your dietetic/ medical management plan be?

4. This lady has been diagnosed with radiation enteritis. What ways would you consider to help this lady meet her nutritional requirements?

5. She has been on nasogastric feeds for 4 days and is having diarrhoea. What dietetic management could you suggest?

NUTRITION SCREENING TOOL

Initial assessment must be performed **within 6 hours of admission**
Height, weight, and BMI are recorded on page 2 of this document
Please refer to Dietitian if patient is on a **tube feed or texture -modified diet**

Is the patient's Body Mass Index (BMI) **less than 20.5** ?*	**YES**	**NO**
Has the patient lost any weight in the last three months?	**YES**	**NO**
Has the patient had a reduced dietary intake in the last week?	**YES**	**NO**
Is the patient severely ill? (e.g. in ITU, severe pneumonia etc.)	**YES**	**NO**

If **YES** to any question above, the patient has triggered – **perform the NUTRITION RISK ASSESSMENT overleaf**

If the answer to all questions is **NO**, the patient should be re -screened weekly and documented here

If the patient is scheduled for e.g. a major operation, a Nutrition Care Plan should still be considered

Date					Has the patient triggered?				
D D	M M	Y Y	**YES**	**NO**	Print name	Signature	Designation		
D D	M M	Y Y	**YES**	**NO**	Print name	Signature	Designation		
D D	M M	Y Y	**YES**	**NO**	Print name	Signature	Designation		
D D	M M	Y Y	**YES**	**NO**	Print name	Signature	Designation		
D D	M M	Y Y	**YES**	**NO**	Print name	Signature	Designation		

*This table gives the height/weight thresholds for a BMI of 20.5, e.g. a patient 1.4 metres tall will weigh 40 kg at BMI 20.5

BMI	20.5												
Height (m)	1.4	1.45	1.5	1.55	1.6	1.65	1.7	1.75	1.8	1.85	1.9	1.95	2.00
Weight (kg)	40	43	46	49	52	56	59	63	66	70	74	78	82

Height (feet and inches)

Weight (kg)	4'11"	5'	5'1"	5'2"	5'3"	5'4"	5'5"	5'6"	5'7"	5'8"	5'9"	5'10"	5'11"	6'	6'1"	6'2"	6'3"	6'4"	6'5"	6'6"	6'7"	
35	16	15	15	14	14	13	13	12	12	12	11	11	11	10	10	10	10	9	9	9	9	5 7
40	18	17	17	16	16	15	15	14	14	13	13	13	12	12	12	11	11	11	10	10	10	6 4
45	20	19	19	18	18	17	17	16	16	15	15	14	14	13	13	13	12	12	12	11	11	7 1
50	22	22	21	20	20	19	18	18	17	17	16	16	15	15	15	14	14	13	13	13	12	7 12
55	24	24	23	22	21	21	20	20	19	18	18	17	17	16	16	16	15	15	14	14	14	8 9
60	27	26	25	24	23	23	22	21	21	20	20	19	18	18	17	17	17	16	16	15	15	9 6
65	29	28	27	26	25	25	24	23	22	22	21	21	20	19	19	18	18	17	17	17	16	10 3
70	31	30	29	28	27	26	26	25	24	23	23	22	22	21	20	20	19	19	18	18	17	11
75	33	32	31	30	29	28	28	27	26	25	24	24	23	22	22	21	21	20	20	19	19	11 11
80	36	34	33	32	31	30	29	28	28	27	26	25	25	24	23	23	22	21	21	20	20	12 8
85	38	37	35	34	33	32	31	30	29	28	28	27	26	25	25	24	23	23	22	22	21	13 5
90	40	39	37	36	35	34	33	32	31	30	29	28	28	27	26	25	25	24	24	23	22	14 2
95	42	41	40	38	37	36	35	34	33	32	31	30	29	28	28	27	26	25	25	24	24	14 13
	1.50	1.52	1.55	1.57	1.60	1.63	1.65	1.68	1.70	1.73	1.75	1.78	1.80	1.83	1.85	1.88	1.91	1.93	1.96	1.98	2.01	

Height (metres)

NUTRITION RISK ASSESSMENT TOOL

Nutritional status		
Absent	Normal nutritional status	**0**
Mild	Weight loss > 5% * in last 3 months **OR** Food intake below 50 -70% of normal requirements in the last week	**1**
Moderate	Weight loss > 5% in last 2 months **OR** BMI 18.5 – 20.5 + impaired general condition **OR** Food intake 25 -60% of normal requirement in the last week	**2**
Severe	Weight loss > 5% in last 1 month **OR** BMI < 18.5 + impaired general condition **OR** Food intake 0 -25% of normal requirement in the last week	**3**

Nutritional requirements		
Absent	Normal nutritional requirements	**0**
Mild	e.g. Hip fracture, cirrhosis, COPD, diabetes, oncology, chronic haemodialysis *A patient with chronic disease admitted to hospital due to complications. The patient is weak but out of bed regularly.*	**1**
Moderate	e.g. Major abdominal surgery, stroke, severe pneumonia, haematologic malignancy *A patient confined to bed due to severity of illness.*	**2**
Severe	e.g. head injury, stem cell transplantation, ITU patients *A patient in intensive care with assisted ventilation etc.*	**3**

Add scores from nutritional status and nutritional requirements sections. Add 1 to the score if the patient is older than 70

D	D	M	M	Y	Y	Score:	Action:
D	D	M	M	Y	Y	Score:	Action:
D	D	M	M	Y	Y	Score:	Action:

If total score is **greater than or equal to** ***5***, commence **Nutrition Care Plan** and **refer to Dietitian** , stating the total score

If the total score is **greater than or equal to** ***3***, commence **Nutrition Care Plan** (in Nursing Care Plan booklet)

If the total score is less than 3, the patient should be re-screened **weekly** using the Nutrition Screening Tool

Original weight (kg)	90	88	86	84	82	80	78	76	74	72	70	68	66	64	62	60	58	56	54	52	50	48	46	44	42	40
5% of original weight (kg)	4.5	4.4	4.3	4.2	4.1	4	3.9	3.8	3.7	3.6	3.5	3.4	3.3	3.2	3.1	3	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.1	2
Current weight (kg)	85.5	83.6	81.7	79.8	77.9	76	74.1	72.2	70.3	68.4	66.5	64.6	62.7	60.8	58.9	57	55.1	53.2	51.3	49.4	47.5	45.6	43.7	41.8	39.9	38

Use the table above to estimate 5% weight loss. The top row shows the patient's original weight. The second row shows 5% of this, and the third row the patient's weight after 5% weight loss.

Imperial College Healthcare NHS
NHS Trust

FOOD INTAKE CHART
- ADULT INPATIENT

AFFIX PATIENT ID LABEL

Date:	¼	½	¾	**1**
Breakfast				
Snacks				
Lunch				
Snacks				
Evening meal				
Bedtime				

Date:	¼	½	¾	**1**
Breakfast				
Snacks				
Lunch				
Snacks				
Evening meal				
Bedtime				

Date:	¼	½	¾	**1**
Breakfast				
Snacks				
Lunch				
Snacks				
Evening meal				
Bedtime				

Obesity Tutorial
Date Tuesday 11th June 1000-1200
Organiser: Joanne Boyle and tutors

Case Study- Obesity

Name:	*Mrs A*
Age:	63
Ethnic background:	Caucasian
Occupation:	*Previously ward sister- very active job. Now working in a nursing home.*
Social History:	Enjoys socialising and entertaining
PMH:	*Type 2 DM 1993, started on insulin 1995* Obesity
Drug History:	*Novomix 30 24u am/ 22u pm* Metformin 1000mg Aspirin 75mg

Nutritional and clinical assessment:	
Height:	1.52m
Weight:	80 kg
Weight history:	43.6 kg as a young adult Gained 38kg from 30-60 yrs
Waist measurement:	107.8cm/ 42.5 inches
Blood pressure:	157/75 mmHg
Fasting glucose:	7.2mmol/L
HbA1C:	6.8% (51mmol/mol)
Triglycerides:	1.34mmol/L
Total cholesterol:	4.9mmol/L
LDL:	3.0mmol/L
HDL:	1.18mmol/L

Diet history	
Breakfast	Often skips breakfast Mid-morning snack e.g. biscuits or croissant or bagel
Lunch:	Tomato soup and white roll and butter Pear
Mid afternoon:	2 custard creams
Supper:	Smoked mackerel with salad with olive oil and lemon juice Boiled potatoes with butter Raspberries and cream 2 glasses of red wine.

Eating Behaviour
Denies eating large amounts
Frequently snacks between meals
Dinner parties frequently
Occasional emotional or comfort eating

Physical activity history
Drives to and from work each day
Sedentary job
Used to go for a walk after supper each day. Now stopped.

1. Calculate the patient's BMI

2. What weight category of the WHO classification does this fit into?

3. What waist circumference puts a woman and a man at a

i) Moderately increased CV risk?

ii) High CV risk?

Man	*Woman*
i)	*i)*
ii)	*ii)*

4. What other medical conditions (in addition to diabetes and heart disease) is this patient at risk of as a result of a high BMI?

5. List 5 possible reasons for her weight gain.

6. What dietary changes should this lady be recommended to help her lose weight?

7. What behavioural strategies could be suggested to aid weight loss? Give reasons.

8. What would be a realistic target weight for this lady? Explain your reasons.

9. What are the Department of Health's recommendations for physical activity?

10. What level of activity is suggested for those individuals who have lost weight in order to maintain their weight loss?

11. Do you think this lady would be eligible for bariatric surgery if she failed to lose weight over 6 months?

Mock Exams

Mock exam 1

Imperial College London

Undergraduate Medicine Office
Faculty of Medicine

Candidate Number: _____

Candidate Name: _____

MBBS/BSc Year 2 Examinations June 2004

Friday 11 June
3.00 pm – 4.45 pm

Paper 1: Integrated Body Function and Dysfunction

This examination is in TWO sections:

*Section A: **5** Short Answer Questions.*

It is suggested you spend around **50** minutes on this section in the real exam, which has 10 marks per section. Please be aware that the example below is approximately half of the length of the real exam.

1. Nutrition

A. List 3 potential advantages of breastfeeding (NB: for mother and child).

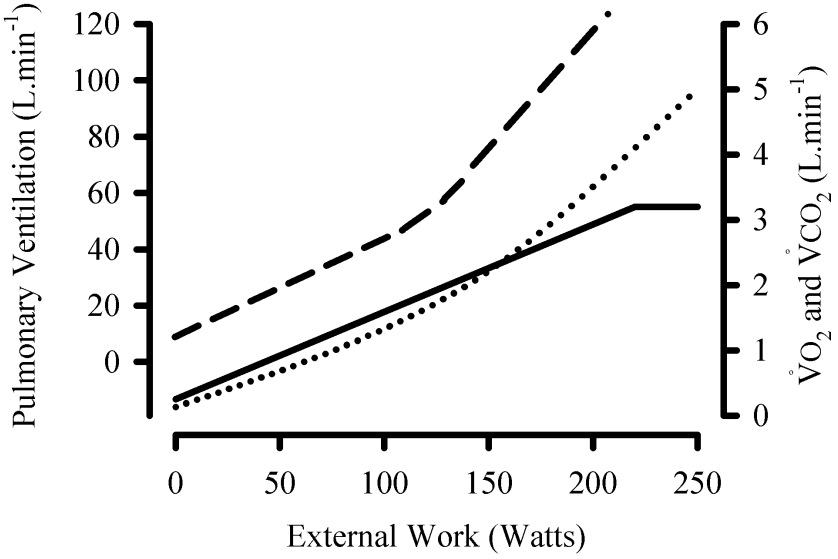
B. List 2 potential disadvantages of breastfeeding (NB: for mother and child).

2. Water and Electrolyte balance

1) In a partially compensated metabolic acidosis in someone who usually has normal lungs, the blood gases would expect to be:
   A. pO2
   B. pCO2
   C. pH
   D. bicarbonate (4 marks)

2) Give a clinical example of each of the following (1 mark each)
   A. Respiratory acidosis
   B. Respiratory alkalosis
   C. Metabolic acidosis

3. Exercise



The figure shows a schematic depiction of the changes in pulmonary ventilation ($\dot{V}E$), oxygen consumption ($\dot{V}O_2$) and CO_2 elimination ($\dot{V}CO_2$) in response to graded incremental exercise on a cycle ergometer by a healthy male subject.

A. Indicate which curve depicts $\dot{V}E$, which depicts $\dot{V}O_2$ and which depicts $\dot{V}CO_2$ (3 marks)

B. Estimate $\dot{V}O_2$ max from the information provided in the figure (1 mark)

C. Given that the subjects' arteriovenous difference is 150 ml/L at the end of exercise, calculate the subject's cardiac output at maximal exercise (2 marks)

4. Sepsis

In a patient with a wound infection due to *Staphylococcus aureus*, what are the bacterial components which can trigger an **appropriate response** in the patient? (2 marks)

What soluble factors enable neutrophils to get to the site of infection-what are the sources of these factors? (4 marks)

5. Drugs

1. Pre-medication is given to some patients prior to them being taken to the operating theatre and consists of treatment with a combination of drugs.

Complete the table below with the class of drug and an example or the reason for drug use as appropriate. [3 marks]

Reason	Drug

2. Why is the age of the patient a potential problem for the anaesthetist? [2 marks]

Imperial College London

Undergraduate Medicine Office
Faculty of Medicine

Candidate Number: _____

Candidate Name: _____

MBBS/BSc Year 2 Examinations June 2004

Friday 11 June
3.00 pm – 4.45 pm

Paper 1: Integrated Body Function and Dysfunction

Section B: Integrated Essay Questions

1. Write your Candidate Number and Name at the top of this page.

2. Choose **ONE** essay topic from the 2 provided below and write your answer in the separate booklet provided, clearly indicating on the front cover of the booklet the number of the essay question you have attempted.

3. **This question paper must not be removed from the exam hall - it must be left on your desk for collection at the end of the exam.**

Section B: **ONE** Essay Question from a choice of 2. It is suggested you spend around **55** minutes on this section. The choices below were identical to those that have come up in previous examinations.

Either:

1. You are an emergency doctor on call over a bank-holiday weekend; you see a 32-year old male who has amputated his left hand with a circular saw following a DIY accident. The bleeding has been controlled, but he has lost a lot of blood and he has severe tachycardia and hypotension.

Explain the major physiological implications for this patient and how they might impact upon his treatment.

Or:

2. Why do patients with chronic infection become malnourished? How does the malnutrition resulting from infection differ from someone who is starved?

Exam answers

SAQs

1. Nutrition

A. List 3 potential advantages of breastfeeding (NB: for mother and child).

Answer. Advantages: Correct temperature, decrease risk infections especially respiratory, GIT, EAR. Low solute load, high bioavailability, easily digested, increase IQ? Decrease risk IDDM & IBD, decrease risk maternal breast cancer, promotes 'normal' gut flora. (1 mark for each correct answer up to 3 marks)

B. List 2 potential disadvantages of breastfeeding (NB: for mother and child).

Answer. Disadvantages: Transfer of environmental pollutants mum has been exposed to, Transfer of HIV, CMV, hepatitis. (1 mark for each correct answer up to 2 marks)

2. Water and Electrolyte balance

1) In a partially compensated metabolic acidosis in someone who usually has normal lungs, the blood gases would expect to be:
   A. pO2
   B. pCO2
   C. pH
   D. bicarbonate (4 marks)

2) Give a clinical example of each of the following (1 mark each)
   A. Respiratory acidosis
   B. Respiratory alkalosis
   C. Metabolic acidosis

Answers

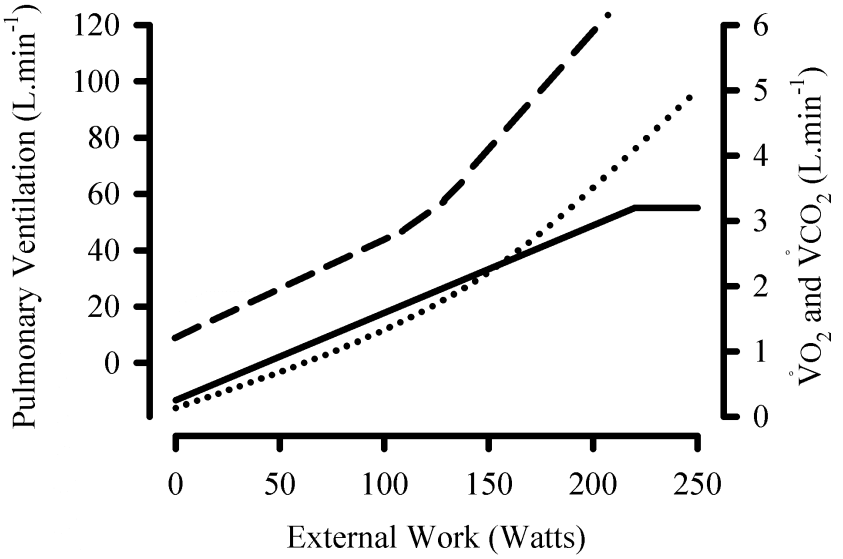
1A	Normal
1B	Low
1C	Low
1D	Low

2A: any cause of respiratory depression (underbreathing)

2B. Any cause of overbreathing (eg anxiety)

2C Ingestion of an acid or RTA, uraemia, lactic acidosis, etc

3. Exercise



The figure shows a schematic depiction of the changes in pulmonary ventilation ($\dot{V}E$),oxygen consumption ($\dot{V}O_2$) and CO_2 elimination ($\dot{V}CO_2$) in response to graded incremental exercise on a cycle ergometer by a healthy male subject.

A. Indicate which curve depicts $\dot{V}E$, which depicts $\dot{V}O_2$ and which depicts $\dot{V}CO_2$ (3 marks)

Answer *(Dashed line=$\dot{V}E$, Dotted line=$\dot{V}CO_2$ and $\dot{V}O_2$=Solid line; 1mark each)*

B. Estimate $\dot{V}O_2$ max from the information provided in the figure (1 mark)

Answer *(≈3.2 L.min^{-1})*

C. Given that the subjects' arteriovenous difference is 150 ml/L at the end of exercise, calculate the subject's cardiac output at maximal exercise (2 marks)

Answer *(C.O. = 3200/150 ≈ 21 L.min-1; Only 1 mark if no units given)*

4. Sepsis

In a patient with a wound infection due to *Staphylococcus aureus*, what are the bacterial components which can trigger an **appropriate response** in the patient? (2 marks)

Answers
Bacterial cell wall components:
Peptidoglycan
Lipoteichoic acid

What soluble factors enable neutrophils to get to the site of infection-what are the sources of these factors? (4 marks)

Answers
Epithelial cells
Chemokines **(IL-8)** and cytokines

Endothelial cells
Chemokines **(IL-8),** Integrin upregulation

Monocyte/macrophages
Cytokines-upregulate VSMCs -vasodilatation (via iNOS)

5. Drugs

1. Pre-medication is given to some patients prior to them being taken to the operating theatre and consists of treatment with a combination of drugs.

Answers: Complete the table below with the class of drug and an example or the reason for drug use as appropriate. [3 marks]

Reason	Drug
Analgesia	Opioid (Fentanyl) / NSAID's
Anxiolysis & Amnesia	Benzodiazepines (eg Diazepam / Lorazepam)
Antiemesis	Dopamine antagonist (Metoclopromide) $5HT_3$ antagonist (Ondansetron)
Thromboembolic prophylaxis	Low molecular weight Heparin
Reduction of parasympathetic bradycardia & secretions	Muscarinic receptor blocker (Hyoscine)
Relaxing narrow or irritable airways	α_2 adrenoceptor agonist (Salbutamol)

2. Why is the age of the patient a potential problem for the anaesthetist? [2 marks]

Answer
Every aspect of drug handling is different when comparing children – Adults – Elderly patients. For example in neonates renal function is markedly reduced and this can result in low clearance of drug & metabolites. In the elderly, in general, anaesthetic potency increases with age. The difficulty is to recognise that patient age may effect how the anaesthetic is absorbed, metabolised and cleared and to calculate an appropriate dose. [It is also a matter of altered responsiveness probably at receptor level]

Integrated essay questions 2004 - key points for your essays:

1. You are an emergency doctor on call over a bank-holiday weekend; you see a 32-year old male who has amputated his left hand with a circular saw following a DIY accident. The bleeding has been controlled, but he has lost a lot of blood and he has severe tachycardia and hypotension.

   Explain the major physiological implications for this patient and how they might impact upon his treatment.

Answer

The main problem with this patient is hypovolaemic shock. Shock is an acute state in which tissue perfusion is inadequate to maintain normal cell function.

- Stages of shock:
  - Compensatory:

    Body attempts to maintain perfusion by physiological adaptation.
  - Progressive:

    Compensatory mechanisms begin to fail and perfusion is inadequate, ultimately leading to multi organ failure.
  - Irreversible:

    If not corrected will progress to cell death and brain damage and eventually death.
- Patients symptoms suggest Class 3 blood loss with a loss of 30-40% blood volume
- Treatment:
  - Correcting underlying cause – haemostasis – surgical intervention
  - Optimising ventilation and oxygenation
  - Optimising cardiac out-put
  - Optimising intravascular volume
- Volume replacement – controversy as to whether rapid volume replacement helps or hinders recovery or whether low-volume fluid replacement should be used. Basically large volume infusions maintain volume but can dilute blood (depending on fluid used) and can inhibit clot formation and act against the body's natural physiological compensatory mechanisms. However, if no fluid is given the patient can progress to irreversible shock due to severe volume depletion. Blood products are better than salt solutions and low volume fluid resuscitation may be better than attempting aggressive fluid replacement.
- Correction of the initial problem may not cure the hypotension – vasodilatory shock can follow volume resuscitation as a result of inappropriate activation of vasodilator mechanisms and the failure of vasoconstrictor mechanisms.

There are other considerations that may also be discussed in this essay

- Pain relief – immediate & longer term
- Infection – antibiotics and tetanus
- Surgery – replantation (& possibly ethics of using "donor" tissue)
- Prosthetics – fitting & rehabilitation
- Psychiatric support

This is a wide essay title and good students will include a discussion of some or all of these further topics

2. Why do patients with chronic infection become malnourished? How does the malnutrition resulting from infection differ from someone who is starved?

Answer

Chronic infection results in a biochemical response similar to that seen in trauma, here the body pulls on all tissue reserves to meet its energy demands.

There is an increase in cytokine response (TNF alpha and IL6). This has a number effects perhaps the most important of which is on the insulin receptor causing insulin resistance and negatively affecting the anabolic effect of insulin. Also TNF alpha has a negative effect on appetite. Coupled with this is the textbook effect on hormonal status, an increase in epinephrine, norepinephrine, glucagon and cortisol and a relative insensitivity to insulin. The biochemical effect of this is negative nitrogen balance as well as a decrease in body fat/ increase in fat mobilisation.

Fat metabolism is elevated despite the reduced rate of ketogenesis. Instead, non-glycolytic tissues directly oxidise fatty acids as fuel.

Carbohydrate metabolism: Glucose metabolism is altered in chronic infection, the ability to oxidise glucose is reduced whilst the ability to store glucose as glycogen is unaffected.

Protein metabolism: As gluconeogenesis remains high, erosion of protein stores continues throughout chronic infection to provide the amino acid substrate.

Starvation is different, as it is a controlled response, where the body tries to maintain it's nutritional status. Its primary aim is to provide glucose for glycolytic tissues.

There is a relatively low level of insulin with raised glucagon. Low levels of circulating insulin stimulates fatty acid release. Raised glucagon stimulates gluconeogenesis in an attempt to raise circulating glucose concentrations.

Mobilisation of glycogen occurs to satisfy glucose requirements. Glycogen stores are rapidly depleted. When glycogen stores depleted, body resorts to exogenous sources. Gluconegenesis uses AA's and glycerol as substrates. Gluconeogenesis is an energy consuming process and energy is derived from fat.

Fat stores are thus mobilised. Tg's are broken down to glycerol which enters gluconegenesis pathways and fatty acids which can be partially oxidised in the liver to 'ketone bodies' or oxidised in places such as muscle, heart and kidneys. Ketone bodies become major fuel source as starvation progresses.

Protein stores are protected as much as possible, only being used to meet the demand of tissues, which can only use glucose as a fuel. The adaptation to conserve protein stores means that ketone bodies become the preferential substrate for glycolytic tissues i.e. glucose oxidation in brain is reduced and ketone body oxidation is increased. This shift in fuel source results in reduced gluconeogenesis activity leading to a reduction in the breaking down of protein. To maintain increased need for ketogenesis, fat mobilisation remains high throughout starvation.

There is no cytokine response.

Comparison:

Like acute starvation, gluconeogenesis activity is increased but following chronic infection, glucose requirements are hugely increased compared with starvation and these requirements remain high.

In starvation, glycogen stores are often depleted but in chronic infection, glycogen stores are often replete. Gluconeogenesis continues in the presence of high circulating levels of glucose.

Glucose requirements remain high following chronic infection because ketogenesis is significantly lower than in starvation and therefore glucose is needed to meet the demands of glycolytic tissues.

Muscle protein breakdown is greater in chronic infection compared with the breakdown seen in starvation.

The catabolism seen in chronic infection is due to dysregulation of hormonal control mechanisms, which are due to the absence of an adaptation mechanism seen in starvation.

Fat mobilisation is greater in starvation to accommodate increased fatty acid oxidation.

Information for Tutorials and mock exam 2

Appetite regulation tutorial

Case studies

A: A 5-year-old girl has a BMI of 45kg/m2. She has been progressively gaining weight from the time she was weaned, growing upwards through the percentiles for weight and is over the 95th centile for her height. She has an uncontrollable appetite and once starts eating will continue until she is sick. Both parents are obese with BMI above 35kg/m2 as were her grandparents

1. What is a possible explanation for this child's obesity? And why?

2. Explain how the answer relates to the homeostatic control of appetite in the hypothalamus ?

3. What phenotypic effect does the NPY knockout mouse show? And why?

4. How common is monogenic obesity?

B: At the age of 20 Mr Smith has a BMI of 23Kg/m2 and is fit and well. He walks the 1.5 miles to work and plays regular sport but by the age of 50 his BMI is 34Kg/m2 and has type 2 diabetes and a cholesterol of 6.0 mmol/l.

1. What is the likely cause of Mr Smith's weight gain?

2. What peripheral signals try and compensate for Mr Smith's change in lifestyle?

3. What will be the effect of over consumption on the appetite signalling in the hypothalamus?

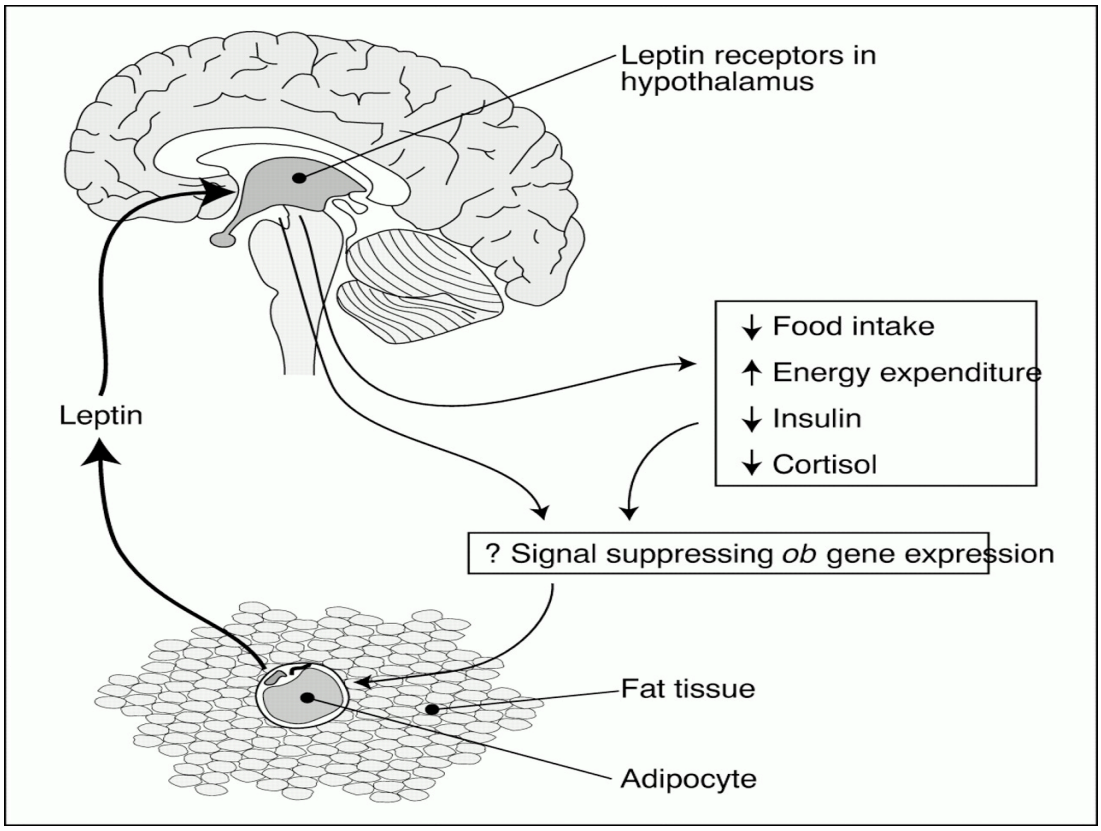
4. Why do humans continue to gain weight despite feedback?

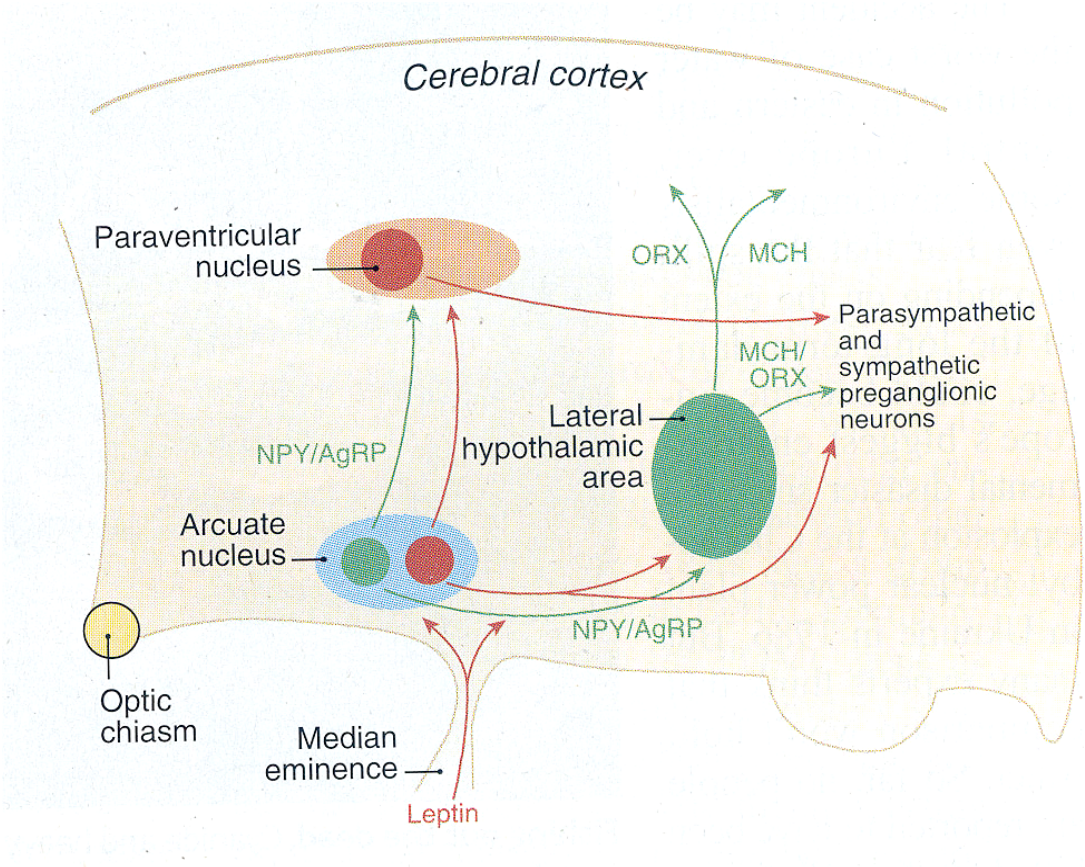
C: As part of the management for type 2 diabetes, Mr Smith successfully loses 5 kg in weight.

1. Explain the signalling involved as Mr Smith loses weight?

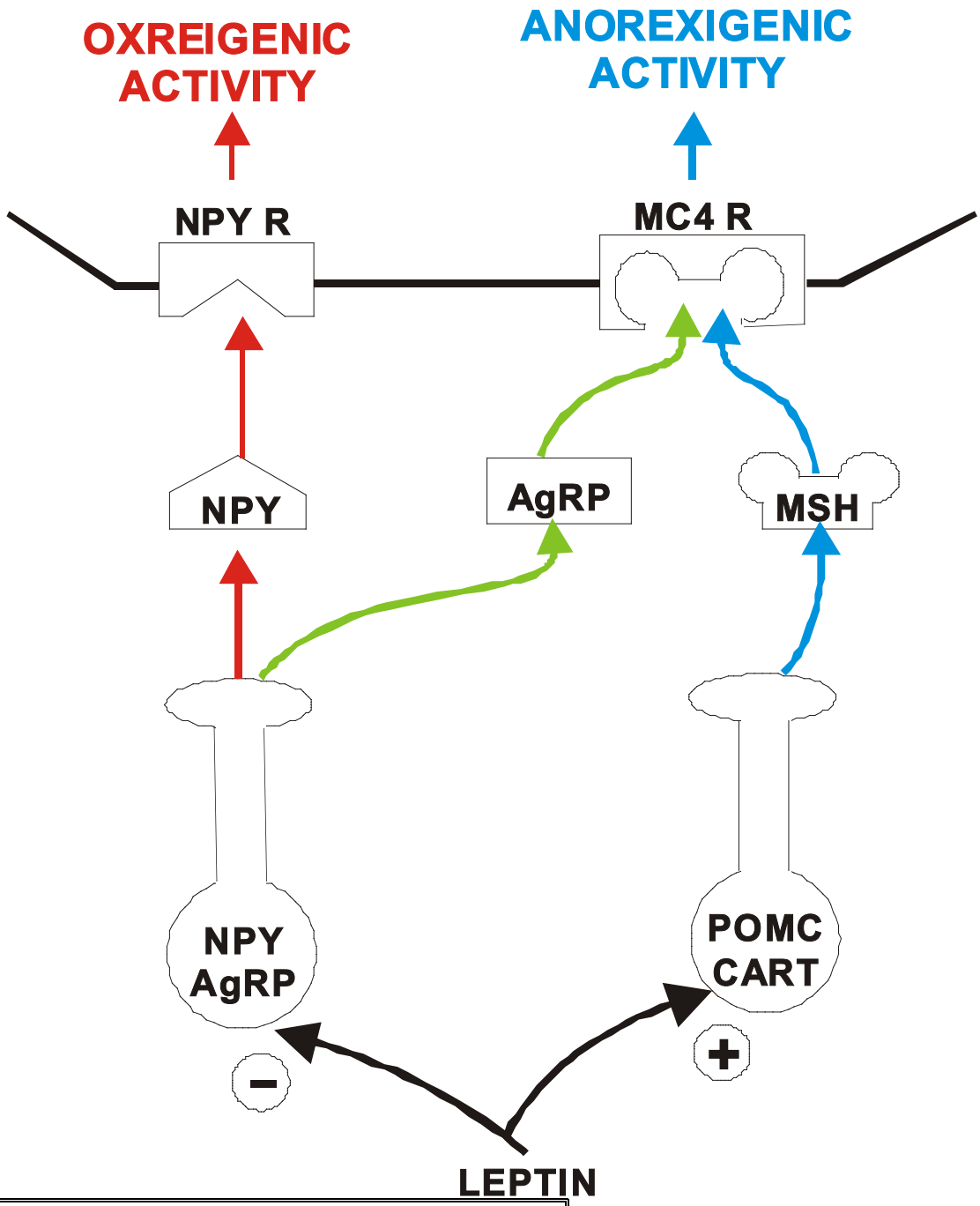
2. What in health benefits would be the effect of the weight loss?

Neural pathways





OREXIGENIC & ANOREXIGENIC ACTIVITY



KEY:
AgRP – agouri-related protein
CART – cocaine and amphetamine-related peptide
MC4 R – melanoelanocortin 4 receptor
MSH – ∝-melanocyte-stimulating hormone
NPY – orexigenic neuropeptide Y
NPY R – neuropeptide Y receptors
POMC – anorexigenic pro-opiomelanocortin

Mock exam 2: Integrated case study

An example of answering an integrated essay question

Admission detail
A 68 year old African Caribbean male is admitted with suspected pneumonia via A&E after being discovered in a poor state by his sister. On admission he is very confused with a fast respiratory rate. His health has declined over the last 2 weeks. The last week he has been bed bound with significant breathlessness. Over this period of time he has not eaten and drunk very little.

Admission biochemistry
Albumin 28g/l (normal range 35-30)
CRP 100 (normal range <1)
Pyrexial 39^{o}C

Social history
Lives by himself in a second floor flat

Past medical history
Obese - BMI 32
Type 2 diabetes – Diagnosed 10 years ago. Currently on oral hypoglycaemic agent.
Last HbA1c 10.5% (target level <5.5%), total cholesterol 5.8mmol/l.

1. What would be the initial action plan?

2. Explain the relationship between the pneumonia, diabetes and obesity

2b. What acid-base abnormality would you expect to see? Explain your answer.

3. How would you monitor progress?

4. What will be the effects of dehydration on admission?

5. What will be the effects of diabetes on your treatment plan?

6. What are the important considerations when planning rehabilitation?

7. What is the target level for his total cholesterol and why?

Mock Exam 3

Do this on Friday 7th June, and bring it in to mark on Monday 10th June 2013.

Imperial College London

Undergraduate Medicine Office
Faculty of Medicine

Candidate Number: _____

Candidate Name: _____

MBBS/BSc Year 2 Examinations June 2008

Paper 1: Integrated Body Function and Dysfunction

Section B: Integrated Essay Questions

1. Write your Candidate Number and Name at the top of this page.
2. Choose **ONE** essay topic from the 2 provided below and write your answer in the separate booklet provided, clearly indicating on the front cover of the booklet the number of the essay question you have attempted.
3. This question paper must not be removed from the exam hall - it must be left on your desk for collection at the end of the exam.

It is suggested you spend around **55** minutes on this section.

Essay 1: (Please do this essay on Friday 7th June (or over the weekend) at home under exam conditions.

1. A young mother gets distracted by her children whilst frying chips at home. The chip oil catches fire and whilst trying to pick up the burning pan she pours burning fat over herself. As a consequence she suffers 70% burns including to her face. She is admitted to intensive care and requires immediate ventilation.

   a. Describe the fluid balance problems she will face and how these should be corrected.

   b. She is at high risk of infections in the first few days and over subsequent weeks. What aspects of the host response to infection are damaged in this situation? What are the likely organisms that will cause infection in this setting?

   c. How best can the patient with severe burns be supported to provide adequate nutrition?

[50 marks]

Essay 2: NO ANSWER WILL BE SUPPLIED FOR THIS BUT IT MAY BE USEFUL FOR PRACTICE

2. A fit young man at the start of his training for the Tour de France undergoes a baseline medical assessment for fitness. Unfortunately, his assessment indicates that he has developed premature ischaemic heart disease as a result of familial hyperlipidaemia. He is excluded from the race and a few months later, following a large myocardial infarction, he goes into heart failure.

a. How would his physiological response to cycling have differed from normal at his baseline assessment?

b. What drugs are useful in the treatment of heart failure and why? Discuss fully the mechanisms underlying their use.

c. How would you advise him to modify his diet in order to minimise his symptoms of heart failure and to prevent further coronary artery disease?

[50 marks]

Assay Practical

Practical at South Ken for Thursday 6 June 2013.
(Revise "Measurement in Science" from 15 Nov 2012)

A Data Interpretation Exercise on Immunoradiometric assay for the pregnancy test and Radioimmunoassay for hCG of a choriocarcinoma.

BACKGROUND:
The technique of radioimmunoassay entered the diagnostic armoury less than four decades ago and revolutionised the ability to diagnose any condition where abnormal levels of proteins and peptides circulate in the blood. This initially applied to classical endocrine conditions where the hypo- and hyper-activity of the anterior pituitary gland or its peripheral target organs and tissues have the potential to disrupt metabolism, fertility, growth and even brain function. However, it soon became clear that certain tumours were associated with the production of abnormal forms of peptides and proteins and new forms of immunoassays were developed to distinguish these from the normal molecules, thereby aiding diagnosis by the relatively non-invasive means of taking a blood sample. To-day's over-the-counter dip-stick method for self-diagnosis of pregnancy represents the highly successful commercial exploitation of this technique. The ability to understand the basic principles of radioimmunoassay, and to interpret the results, is, therefore, a fundamental skill for every doctor.

AIM:

To illustrate the theoretical principles and clinical application of immunoradiometric assay and radioimmunoassay, as an illustration of the diagnostic use of antibodies.

OBJECTIVES:

1. to construct a standard curves describing the relationship between concentration of a peptide and its truncated forms viz: human chorionic gonadotrophin, HCG, and the specific binding of a constant amount of radioactive tracer/peptide to limiting amounts of antibody.
2. to be able to distinguish between the highly specific sandwich assay for detecting full-length HCG (a biomarker of early pregnancy) and conventional assays which are less specific and serve as biomarkers for tumours and ectopic pregnancy.
3. to use the standard curve to calculate the concentration of HCG-like immunoreactivity in the plasma of a patients who are pregnant (normal vs. ectopic pregnancy) or have cancer.
4. to differentiate between the plasma profiles of HCG in normal pregnancy, ectopic pregnancy or cancer.

CASE STUDIES

Two women attend the GP surgery for a pregnancy test. The samples come to your laboratory for analysis for a pregnancy test.

A third patient who had lost her pregnancy and who required repeated pregnancy tests to ensure that there were no retained products of conception, or possible hydatidiform mole (malignant product from a failed pregnancy). This third patient has a sample for a pregnancy test every week for 6 weeks.

You need to analyse the level of beta hCG in the first two patients to determine if they are pregnant, and ALL the samples from the third patient to ensure that the test becomes reliably negative and so that you can be sure that there are no retained products of conception.

METHODS

hCG IRMA standard curve

You started by creating standards by serial dilution of initial hCG of 100 mIU/ml as described in your previous lecture (done in November). You add the sample to the IRMA kit tubes, and then add the second radiolabelled antibody, and after washing the tubes, count the radiation in each of the tubes. Be aware that at very low abundance, that there is background radiation so that error bars should be plotted around all values. The radiation is quantified using a gamma-counter.

Concentration of standard mIU/ml	Counts per minute	Counts per minute	Counts per minute	Mean counts	Actual concentration
0.7	12233	4369	6222		0.7
1.5	14000	17323	12111		1.5
3.1	17211	12321	8232		3.1
6.25	25650	8761	13322		6.25
12.5	39302	25501	23211		12.5
25	82447	68274	69320		25
50	123455	108661	107223		50
100	161000	146142	157999		100
Patient 1	11777	26222	23999		
Patient 2	134223	97022	94222		

Patient 3 has samples each week, and the counts for the three measurements made are given in the table.

	Counts per minute	Counts per minute	Counts per minute	Mean counts	Actual (IU/ml) concentration
week 1	62000	58000	54300		
week 2	44000	63000	42004		
week 3	37000	39000	26999		
week 4	27000	24000	29004		
week 5	30778	33555	28556		
week 6	29555	32007	32002		

TASKS: To be completed on a separate sheet of paper.

1. Describe the main components of an immunoradiometric assay.

2. Plot out the standard curve for the beta hCG IRMA

3. Determine the concentration of hCG in patient 1 and in patient 2, together with a likely margin of error.

4. For patient 3, calculate the concentration of hCG each week, and plot a separate graph of the weekly hCG concentration over the 6 weeks. Are you confident that the levels fall to pre-pregnancy levels, and can you therefore reassure the patient that she has no retained products of conception?

5. If not, run a radioimmunoassay for these samples, and run the samples for patient 1 and patient 2, as well as all the samples for patient 3 on this curve, and determine the concentration of hCG in patients 1 and 2, and also for all six weeks on patient 3.

6. Describe the main components of a radioimmunoassay.

7. Construct a standard curve by plotting a graph of cpm vs. log concentration of standard amounts of hCG (you can either use semi-log paper, or take logs of the values and plot on normal graph paper). Note that for this project you started with a standard of 800 mIU/ml, and undertook serial dilution too.

Concentration of standard mIU/ml	Counts per minute	Counts per minute	Counts per minute	Mean counts
0.7	10093	10165	9988	
1.5	9997	10022	9989	
3.1	10078	10103	10119	
6.25	9946	10111	9966	
12.5	9333	9222	9200	
25	7890	7998	7818	
50	6320	6250	6330	
100	4711	4655	4740	
200	3422	3343	3450	
400	3240	3250	3359	
800	3331	3260	3399	

8. Use the standard curve to calculate the concentration of hCG-like immunoreactivity in the plasma of the patients and in patient 3 for each week.

Sample	Counts per minute	Counts per minute	Counts per minute	Mean counts	Actual concentration
patient 1	9450	9527	9541		
patient 2	6450	6555	6492		
patient 3 week 1	7666	7770	7679		
patient 3 week 2	7945	7932	7988		
patient 3 week 3	8240	8224	8286		
patient 3 week 4	8650	8555	8592		
patient 3 week 5	9090	9160	9056		
patient 3 week 6	9105	9240	9255		

Chapter 5: "In vivo and invitro methods for assessing neuroendocrine function." pp129-155. Cowell, Buckingham & Gillies

From: "Stress, stress hormones and the immune system." Ed. J.C. Buckingham, G.E. Gillies, A-M Cowell. John Wiley & Sons. 1997 ISBN 0-471-95886-7 (on following pages)

5.5.2 Measurement of Hormones and Neurotransmitters

Immunoassays and high performance liquid chromatography (HPLC) are the most commonly used methods for the detection of hormones and neurotransmitters of the hypothalamo–pituitary axes and their peripheral endocrine organs.

Immunoassays

Immunoassays involve the interaction of a specific antibody with the antigen (hormone) and can be divided into two main classes, namely radioisotope assays (e.g. radioimmunoassay and immunoradiometric assays) and non-isotope assays (e.g. enzyme-linked immunosorbent assays). In an assay it is essential that a standard curve is set up under conditions identical to those of the samples and that low, medium and high quality control samples are also included in the assay. Standards and samples should always be prepared at least in duplicate and parallelism should be demonstrated between dilutions of the standards and samples. One of the main problems with immunoassay is the potential cross-reactivity of the antibody with biologically inactive fragments or precursors of the hormone under investigation, resulting in marked dissociations between the data from biological and immunological assays. This problem is minimised by the use of two-site assays (see below). In addition, structural similarities between different hormones, for example GH and prolactin, may result in immunologic cross-reactivity. Care should also be taken to ensure that substances (e.g. drugs) present in the sample for analysis do not interfere with the assay.

Radioimmunoassay. The principle of radioimmunoassay (RIA) is illustrated in Fig. 5.1. The unlabelled antigen (i.e. standard or sample) is mixed with a finite amount of corresponding radiolabelled antigen and a highly specific antibody.

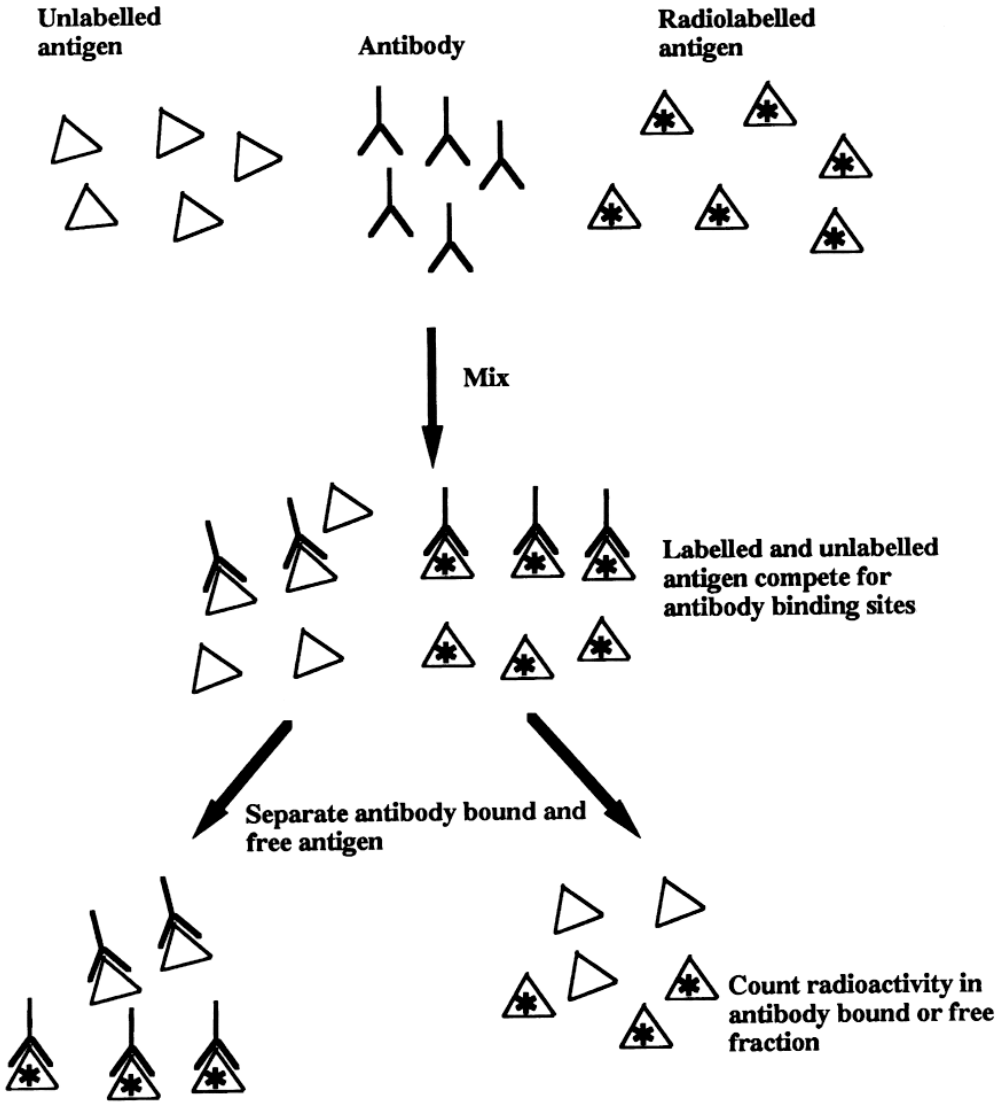


Figure 5.1 Principles of radioimmunoassay

The unlabelled and labelled antigen compete for the free binding sites on the antibody and soluble antigen-antibody complexes are formed. The amount of bound labelled antigen is inversely proportional to the amount of unlabelled antigen. Once equilibrium has been attained the antibody-bound and free antigen are separated and the radioactivity in one of the fractions is measured. Efficient separation of the bound and free radioactivity is essential. Separation methods include removal of free antigen by adsorption onto charcoal or silica, precipitation of antibody-bound using a second antibody capable of reacting specifically with the first antibody or non-specific precipitation of antibody-bound antigen with salt or organic solvent. The advantages and disadvantages of these separation techniques have been reviewed by Walker (39).

The advantages of RIA are that it is an extremely sensitive and specific method, with a precision that is comparable to that of other physio-chemical techniques and better than that of bioassays. In addition, RIA is highly automated and allows a large number of samples to be assayed with minimal handling. The major disadvantages of RIA are that the equipment and reagents are relatively expensive and the assays also usually take several days to complete. Moreover, operators are exposed to radiological hazards and since the antigens need labelling frequently due to the short half-lives (e.g. $t_{\frac{1}{2}}$ ^{131}I , 8 days; $t_{\frac{1}{2}}$ ^{125}I, 60 days) regular thyroid scans should be performed.

Immunoradiometric assay. Immunoradiometric assays (IRMAs) are used less frequently than RIAs but they have been successfully employed to measure hormones such as the hypothalamic hormone CRH. These assays have similar sensitivities to RIAs but require a much greater quantity of antibody (fiftyfold or more) than for the corresponding RIA. The principle of IRMA is similar to RIA but IRMAs differ from RIAs in that the antibody is radiolabelled rather than the antigen. The antigen (standard or sample) is incubated with an excess of radiolabelled antibody and the mixture is allowed to equilibrate. The subsequent separation of the immunocomplex from the free radiolabelled antibody is achieved by addition of an excess of immunoadsorbent which binds to any free labelled antibody. The antibody-immunoadsorbent complex is removed by centrifugation and the radioactivity in the supernatant, which is proportional to the amount of antigen in the standard or sample, is measured. Unless the antibody is effectively irreversible, removal of the free antibody from the initial equilibrium mixture will disrupt the equilibrium position and this assay is therefore potentially sensitive to timing and washing.

An extension of this assay is the two-site IRMA in which the major proviso is that the antigen has more than one immunological group available. The first step of this assay involves immobilisation of the target antigen by allowing it to bind to an unlabelled antibody which itself is bound to a solid phase (such as polyethylene centrifuge tubes). This complex is then reacted with a second labelled antibody which binds to a different site on the antigen. The free antibody is then decanted and the radioactivity of the solid phase measured. The main advantage of the two-site assay is that there is increased specificity and sensitivity compared to the one-site assay. Further details of these assays are provided by Mayer and Walker (40).

Enzyme-linked immunosorbent assay. Initially, enzyme-linked immunosorbent assays (ELISAs) were used in immunology to measure the titre and specificity of antisera but they have also been used for the detection and quantification of various antigens, including the anterior pituitary hormones prolactin, thyrotrophin and GH (41–43). The principle of ELISA is the same as that of RIA except that an enzyme, rather than a radioisotope, is used to label the antibody or

antigen. The enzyme is readily detected on addition of the appropriate substrate and can be quantified by measuring the absorbance of the colour produced. The concentration of the unknown is then determined by comparison with a standard curve. A number of ELISAs, including competitive, double antibody and indirect competitive assays, have been developed and the principle of each one is summarised in Fig. 5.2.

In the competitive ELISA, a specific antibody is bound to a solid phase such as cross-linked dextran, polyacrylamide beads, cellulose discs, polypropylene tubes or polystyrene microtitration plates, the last of which are particularly convenient for large numbers of samples. The solid phase antibody is then reacted with samples containing a known amount of enzyme-labelled antigen and a known (standard) or unknown (test) amount of unlabelled antigen. Following an appropriate incubation period the complex is washed, the enzyme substrate is added and a coloured precipitate becomes visible. The enzyme activity is determined by measuring the absorbance of the precipitate which is proportional to the ratio of labelled to unlabelled antigen in the sample. Thus a high concentration of unknown antigen in the sample will result in a low absorbance detected from the coloured products of the enzyme reaction.

The double antibody method also involves a specific antibody bound to a solid phase but in this instance a sample containing a known (standard) or unknown (test) amount of unlabelled antigen is allowed to react with the antibody. The

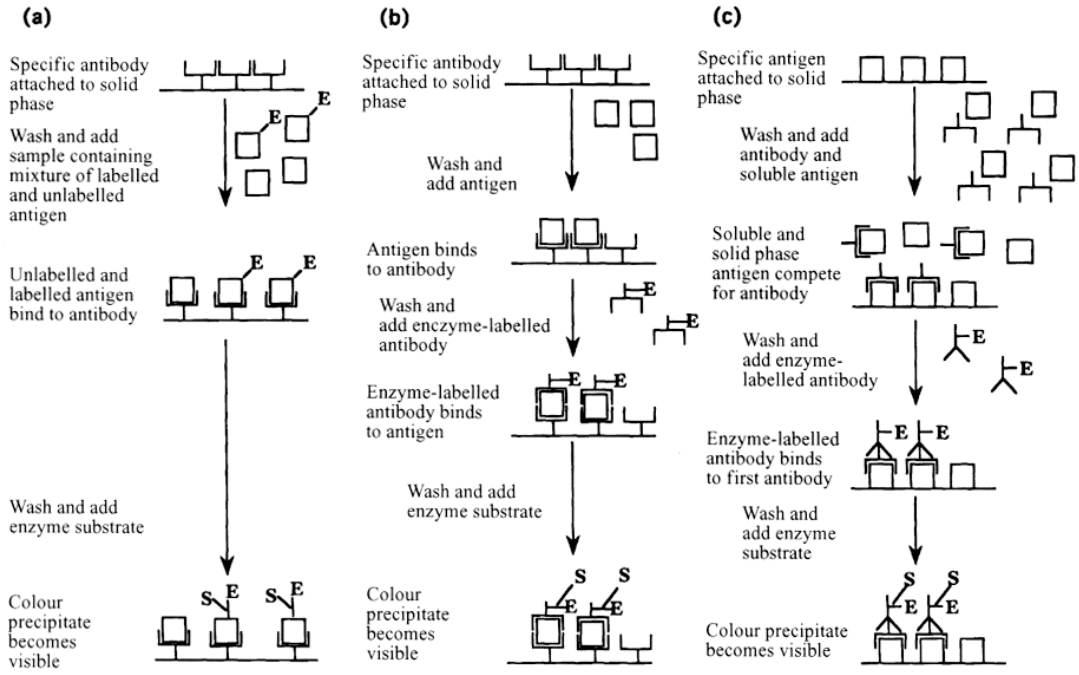


Figure 5.2 Principles of (a) competitive (b) double antibody and (c) indirect competitive enzyme-linked immunosorbent assays. E, enzyme; S, substrate

complex formed is then washed and an enzyme-labelled second antibody is added and allowed to react with it. After further washing the enzyme substrate is added and the amount of enzyme activity is determined as above. Under standard conditions this is directly proportional to the amount of antigen present in the sample.

More recently, indirect competitive ELISAs have been developed for the detection of anterior pituitary hormones (42, 43). In this approach the specific antigen is attached to the solid phase. A mixture of a known amount of first antibody and a known (standard) or unknown (test) amount of soluble antigen is added and the soluble and solid phase antigen compete for the antibody binding sites. Therefore a high concentration of soluble antigen in the sample results in a low concentration of antibody immobilised to the adsorbed antigen. The immobilised antigen–antibody complex is washed and exposed to an enzyme labelled anti-immunoglobulin antibody which binds to the primary antibody. This complex is then washed and the substrate for the enzyme added. The absorbance of the resulting coloured precipitate is indirectly proportional to the amount of antigen in the original sample.

As with RIA, a large number of samples can be tested at the same time in an ELISA. Moreover ELISAs have a number of advantages over RIAs, although the latter are extensively automated and sometimes more sensitive. Firstly, ELISAs are relatively cheap to operate, since the counting equipment is replaced by less expensive and less cumbersome absorbance detectors. Secondly, ELISAs can often be completed more quickly than RIAs and the analysis time is also considerably shorter since each ELISA plate takes only about 5 min to read. Thirdly, enzyme-conjugated reagents are stable upon storage for prolonged periods (i.e. they have a long shelf life) whereas radioactive isotopes of iodine have a short half-life. In addition, the reagents for ELISA are generally safe and the radiological hazards associated with RIA are eliminated. The main source of error is that in some ELISAs there is a large number of pipetting stages. Moreover, there are differences in absorbance values between well plates, although this discrepancy can be minimised by setting up a standard curve on each well plate within an assay. The perimeter wells also give a significantly higher absorbance value and for this reason only the inner wells of the well plate should be used.