

Trick or Treat?

Test Results
what they mean, and when to
worry.

We will cover....

- Urine tests
- Haematology tests
- A bit of genetics
- Biochemistry tests including eGFR and CKD
- But no radiology!

Urine tests

- Protein measurement
- Urinalysis
 - A chemical based test for a variety of substances, particularly for albumen/protein, glucose, haemoglobin and WBC.
- MSU
 - *This is a type of sample, not a test*
 - The doctor means a sample to test for MC&S, i.e. Microscopy, Culture and Sensitivity
 - We mean a sample to test for Microscopy and Urinalysis

Mid Stream Urine – was it?

- Should written instructions be issued?
 - Second sample of the day is best
 - Cells die in overnight urine
 - And urine is more acid
 - Needs to be a clean catch
 - Epithelial cells are a warning
 - Needs to be freshly examined
 - Cells lyse in dilute and in alkaline urine, resulting in positive stick test and negative microscopy

Urinalysis

- A stick with chemicals that react to substances in urine, which cause a colour change which develops over time
 - Read visually by comparison with a chart
 - Read by an automated machine
- Subject to errors
 - Out of date or poorly kept strips
 - Colour blindness
 - Incorrect timing
 - Aged sample (ideally <1 hour old!)

Urinalysis tests for.....

- Haemoglobin,
either from haemolysed RBC's (in vivo haemolysis or haemolysis in an aged, alkaline, or dilute sample)
or from intact RBC's
 - False negatives from Vit C
 - False positives from lots of bacteria, or from myoglobin
- Glucose
 - False negatives from Vit C and UTI
 - False positives from low renal threshold
 - FBG will be normal at the same time as positive glycosuria

Urinalysis tests for.....

- Protein
 - Usually $<150\text{mg/day}$
 - Made up of mucoprotein, blood group proteins, albumen and globulins
 - Increased loss due to
 - glomerular leak,
 - decreased tubular reabsorption, eg in Chronic Interstitial Nephritis
 - overflow, eg in myeloma
 - benign, eg fever, orthostatic proteinuria

Stick inaccuracy.....

- False negatives
 - Sticks test for albumen, not myeloma or tubular proteins
 - Not accurate at low albumen concentration eg due to a dilute urine
 - Not accurate beyond pH range of 5-7
- False positives
 - In alkaline urine, ie potentially in aged sample
 - From concentrated urine

Urinalysis also tests for....

- WBC's
 - tests for leucocyte esterase from lysed WBC's, so in very alkaline or very dilute or aged urine, the test may be positive when microscopy is negative.
- Nitrites
 - which are produced by bacteria, ie suggest infection, or an aged sample
- Ketones
 - which indicate fasting, starvation, or ketoacidosis in diabetics.

MSU - MC&S

- Microscopy
 - Traditional light microscopy, results per HPF
 - Errors due to observer error, and aged sample
 - Automated, results per unit of volume.
 - Errors due to aged sample, incorrect interpretation
 - Normal ranges, and conversion factors to “per HPF” vary from lab to lab
- Culture and Sensitivity

Cells counted by....

- Microscopy – per hpf
- Machine – per cumm or per microlitre
- To convert cumm or microlitre to hpf, divide by 5.5 or whatever your lab recommends

RBC's may be from....

- The urinary tract
 - The area of interest for a Urologist
- The glomerulus or tubules
 - The area of interest of a nephrologist
- Contamination by genital secretions (menses)
- So how do we tell?
 - By further Ix, but also by.....

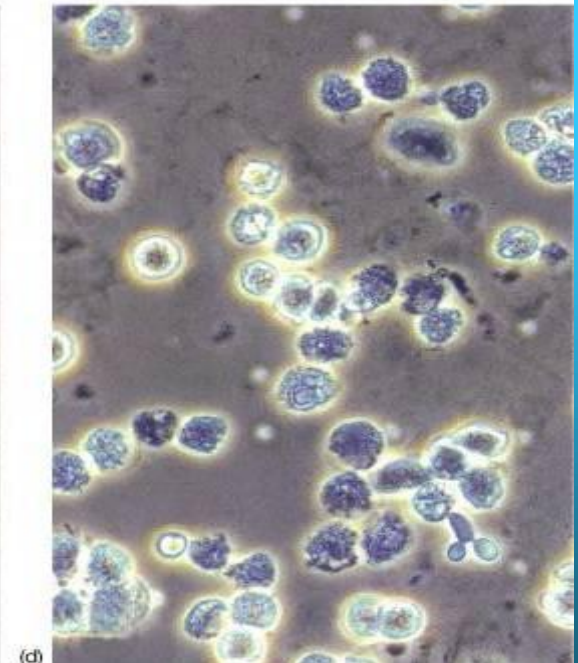
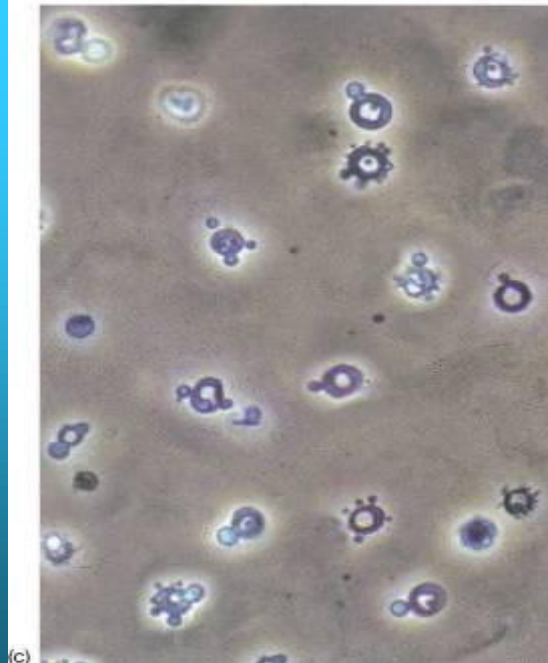
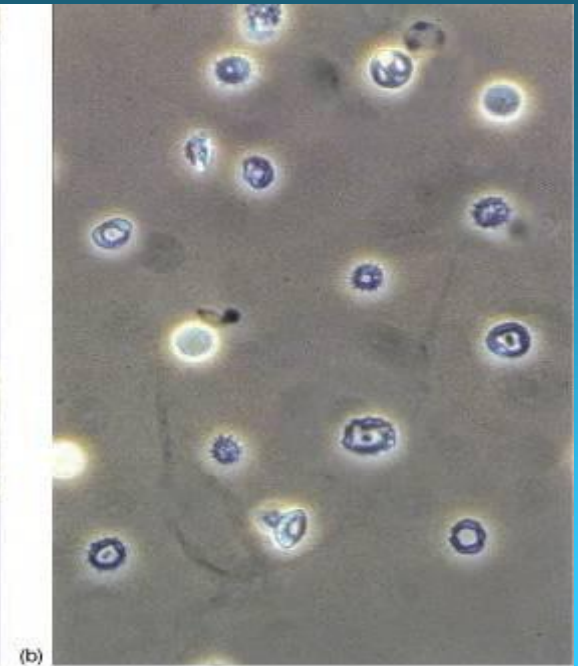
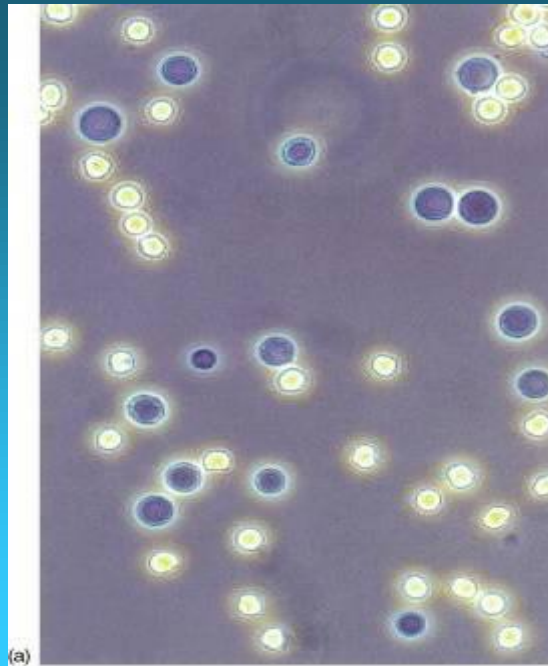
Cell Morphology

a – RBC's with loss of colour in dilute urine

b – differently shaped RBC's - glomerular

c – acanthocytes, very different shapes

d – WBC's



WBC's

- In UTI
- In Acute Glomerulonephritis
- In Chronic Interstitial Nephritis
- TB
- Rejection of transplanted kidney
- Contamination by genital secretions
 - And how do we tell?

Protein Measurement

- Why?
 - Often to test for leaking glomeruli - and if it is a leak you are measuring, you want to know the RATE of leakage
 - And for tubular damage
 - But present in genital secretions
- What?
 - Usually albumen as it passes through a damaged glomerulus
 - But other proteins sometimes
 - Secreted from the walls of the renal tubules
 - Also globulins that leak through the damaged glomerulus - “Highly Selective Proteinuria”
 - Genital secretions

How?

- Usually the concentration of protein in a sample of urine
- But a SAMPLE will be affected by the degree of concentration of that sample
 - Hence a protein/creatinine ratio or a microalbumin/creatinine ratio, expressed as mg/mM (never as mg/l, or g/l)
- And the amount of protein lost from the kidney varies throughout the 24 hours
 - So collected at a standard time, usually first specimen of the day
 - Or best of all, **a 24 hour collection** if done accurately

- So, a **24 hour urine protein** collection is best,
- A **timed collection** is next best,
- A **protein/creatinine ratio** is next best, eg a ratio of 100mg/mMol is equivalent to 1g protein/24 hours,
- A **measured concentration** on a random sample is next,
- A **stick tested sample** is least accurate

Haematology

- MCV - Mean Corpuscular Volume
- Hct or PCV - The total volume of all the cells
- MCH - Mean Corpuscular Haemoglobin
- MCHC - Mean CH Concentration

Raised MCV

- An aged sample
 - How do you tell?
 - As cells run out of oxygen and glucose, they start to die, and as they do so, they swell, so.....
 - MCV up, Hct up, MCH the same, MCHC down
- Deficiency of B12, Folate
 - Whereas iron deficiency and beta-thalassaemia give small cells.
- Alcohol – harmful use.
 - This is the cause in 35% of cases

Raised MCV

- Haemolytic anaemia
- Blood loss
- Myelodysplasias
- Hypothyroidism – in 2% of cases

Serology

- An ANTIGEN is a substance, usually a protein, that induces the production of an ANTIBODY
- An ANTIBODY is a substance which reacts against an ANTIGEN
- Four main types of Antibodies
 - IgM, the first to be produced, which disappears as IgG arrives
 - IgG, which remains to give lasting immunity
 - IgE, involved in asthma, though evolved to fight parasites
 - IgA, involved in mucosal defence, eg gut, bronchi, sinuses

Hepatitis Serology

- As a general rule, if you have the ANTIGEN, you will not have the ANTIBODY
- So, Anti-HepB sAg antibody positive means you are.....

HepB sAg negative

Hepatitis Serology

- So, HBeAg positive will be
Anti HBeAg negative, and vice versa
- HepB core antigen can be taken to be the same as Hep e antigen
- Anti HepB sAg antibody can be written as anti-HBs
- Anti-HepB sAg antibody positive and.....
Anti-HepB sAg IgM antibody negative means.....

A infection caught more than 3-6 months ago

Hepatitis Serology

- Occasionally you will see Hep B DNA test results
- A low level of HepB DNA is compatible with Hep B S-Ag positivity and e-Ag negativity
 - 300 or so is common in Hep B S-AG positive but e-Ag negative patients
 - 1000 is the level at which the NHS worker would be debarred
 - 10,000 would indicate active infection

Hepatitis Serology

- But for HepC, there is no Antigen test, but PCR is used instead
 - PCR is nature's DNA or RNA photocopier
 - Used in forensic medicine
 - Will hugely increase the amount of DNA or RNA from undetectable levels to levels that can be measured
 - And the person can be Anti-HepC positive AND PCR positive
 - And to be sure that the virus has stopped multiplying, at least TWO negative PCR's are needed.

Genetics

- The term usually applies to chromosome tests as well as the gene tests
- Genetic tests may be predictive or diagnostic
- **The ABI Moratorium applies only to predictive tests**

Biochemistry

Raised Bilirubin

- What is Gilbert's Disease?
 - Raised Unconjugated Bilirubin
 - What is that?
 - Other causes include haemolytic anaemia (Hb down, MCV up)
 - Other LFT's must be normal
 - Men > women, about 5:1, and about 5% of population
 - Chronic though fluctuating; goes up with fever and illness
 - Lower risk of CV disease especially in diabetics

Other causes of raised bilirubin

- Liver Disease
 - Almost always raised Conjugated BR
 - Usually with other LFT abnormalities
 - But established cirrhosis can be accompanied by isolated raised BR – but conjugated
- Haemolytic anaemia
- Rare and serious defects of conjugation

Alkaline Phosphatase

- Found in bone and in liver
- Is it really raised? If in doubt, find out
- If raised in isolation, more likely to be from bone
- If raised due to liver disease, GGT will usually be up as well

LFT patterns

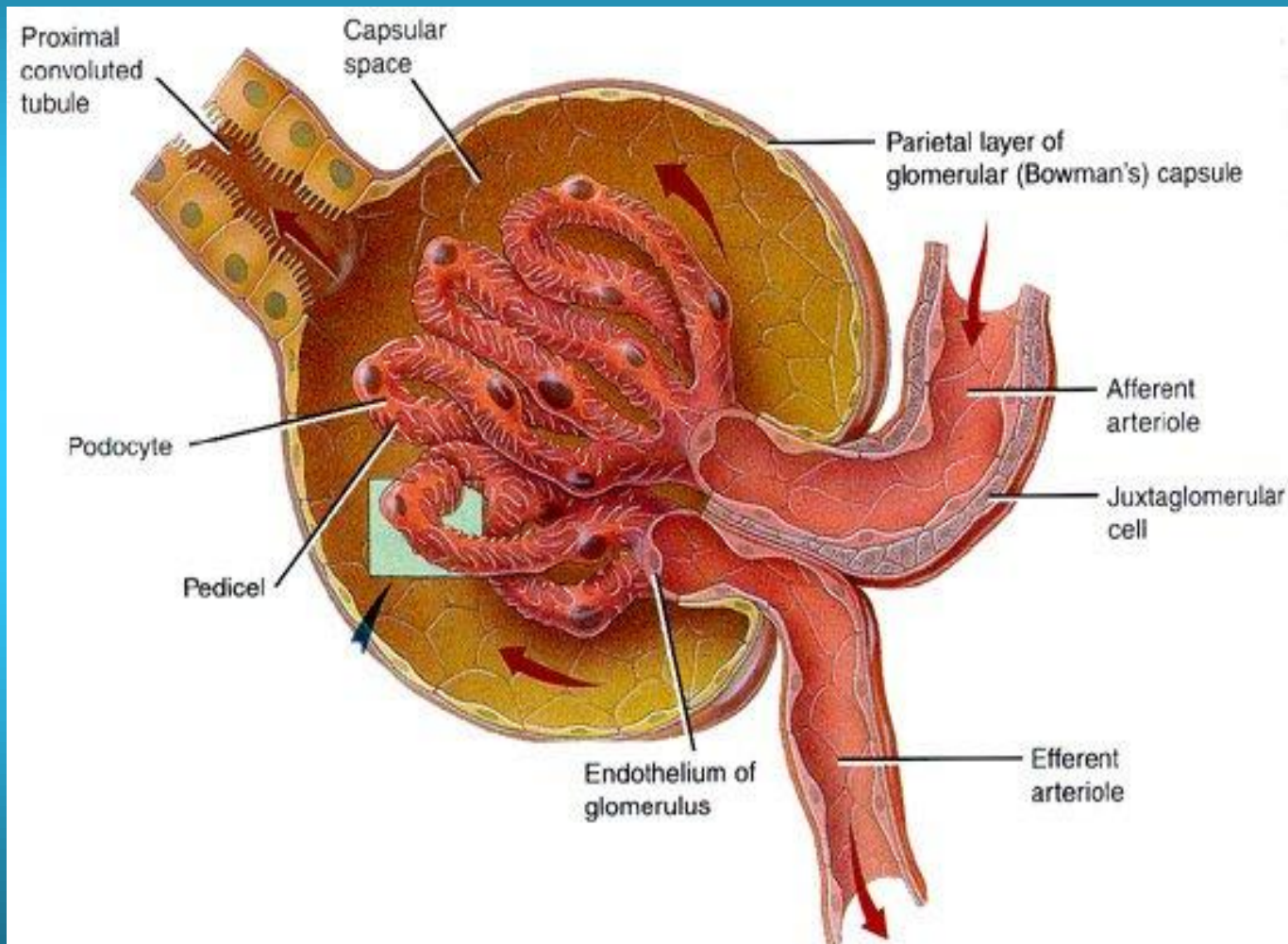
- Obstructive pattern - fibrosis, cirrhosis, stone or cancer
 - Predominantly BR, GGT and Alk P
 - Less so AST, ALT
- Inflammation of liver cells – a hepatitic pattern
 - Predominantly AST, ALT
 - Less so GGT
 - Typically not BR, Alk P

Renal Function Tests

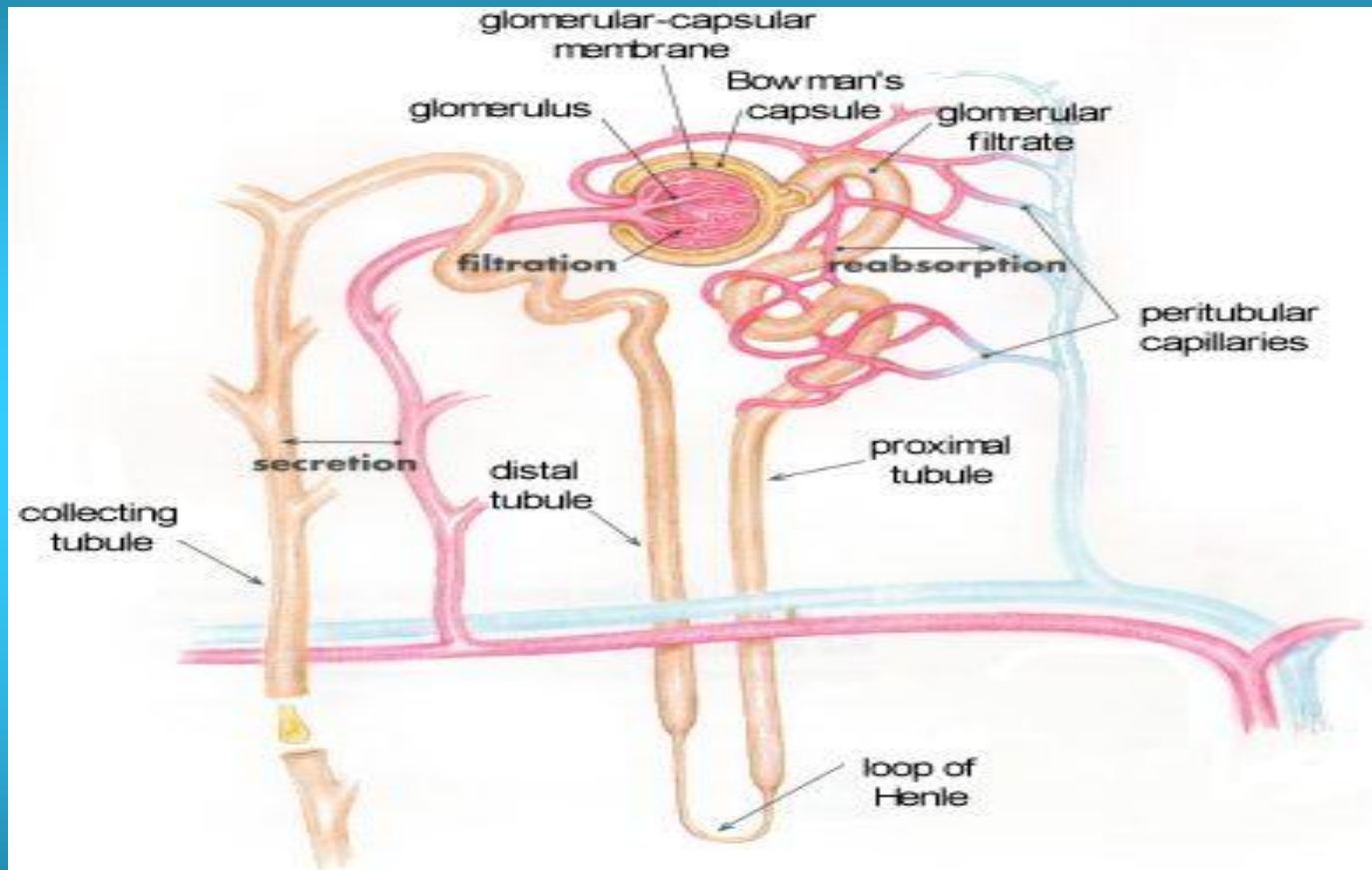
So how do we measure renal function?

- By measuring the clearance of waste, the filtration rate
- By measuring the effectiveness of the filter – proteinuria
- By measuring BP
- By measuring sodium and potassium
- By measuring Hb
- By measuring Calcium, Phosphate
- Indirectly, by imaging – IVP, USS etc

The glomerulus – the filter



The renal tubule



The kidneys filter approximately 100mls/min, or 150 litres per day (the GFR). 99mls is reabsorbed in the nephron, so that only about 1ml/min finally passes into urine

Some measures of waste clearance.....

- Urea
 - a break down product of protein
 - affected by protein intake, fluid intake, diuretics and muscle breakdown in illness
- Creatinine
 - a metabolic by-product of muscle metabolism
 - affected by meat intake, muscle mass
 - age of sample

Creatinine measurement

- probably the most widely used indirect measure of glomerular filtration rate
- easy and inexpensive to measure
- very insensitive to even substantial declines in renal function
- glomerular filtration rate may be reduced by up to 50% before serum creatinine becomes elevated

Factors affecting creatinine levels

Factor	Effect on Serum Creatinine
Aging	Decreased
Female sex	Decreased
Race or ethnic group†	
Black	Increased
Hispanic	Decreased
Asian	Decreased
Body habitus	
Muscular	Increased
Amputation	Decreased
Obesity	No change
Chronic illness	
Malnutrition, inflammation, deconditioning (e.g., cancer, severe cardiovascular disease, hospitalized patients)	Decreased
Neuromuscular diseases	Decreased
Diet	
Vegetarian diet	Decreased
Ingestion of cooked meat	Increased

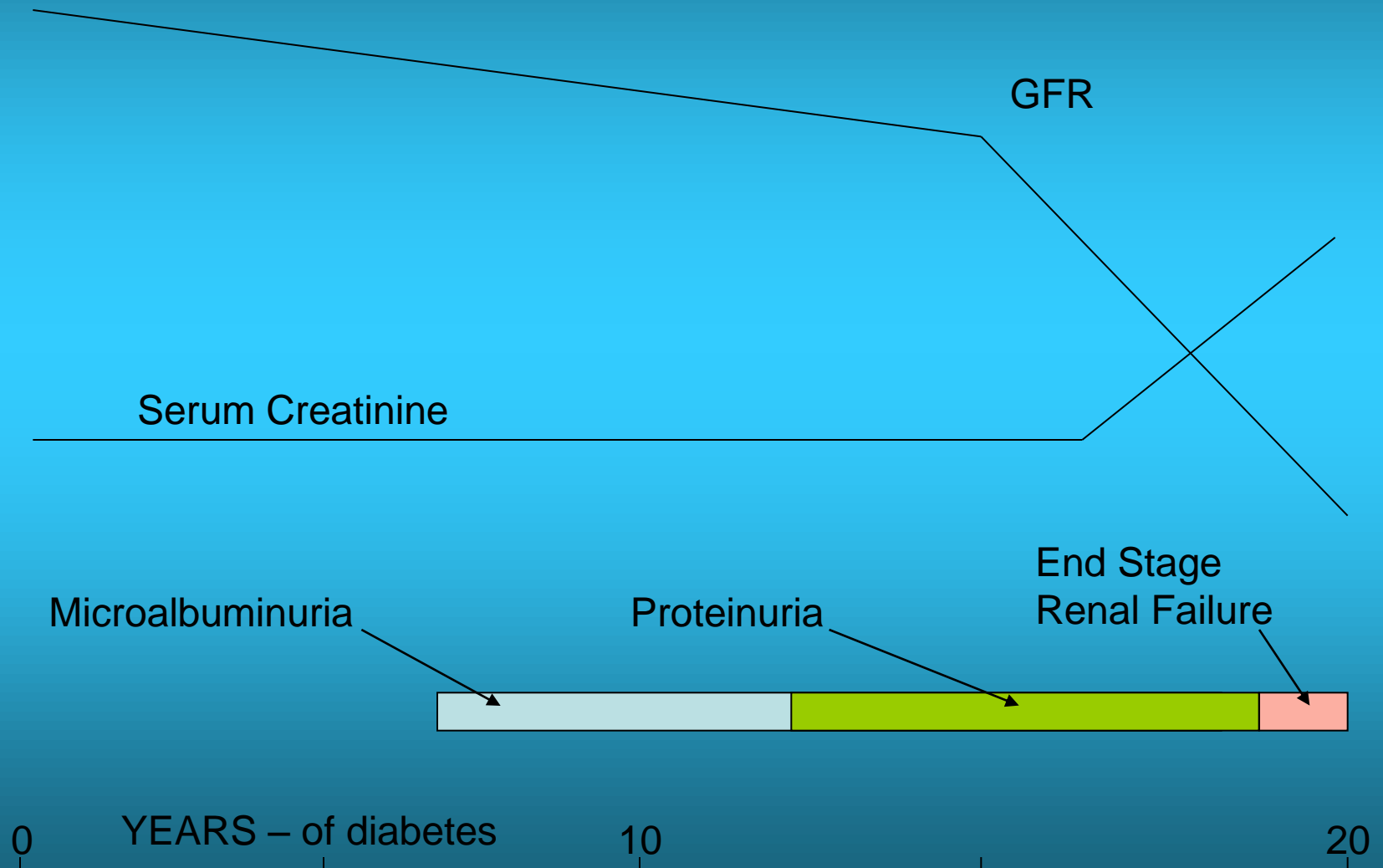
Creatinine affected by build



Normal renal function
Creat 60

Normal renal function
Creat 120

Relationship of creatinine, proteinuria and GFR



And remember....

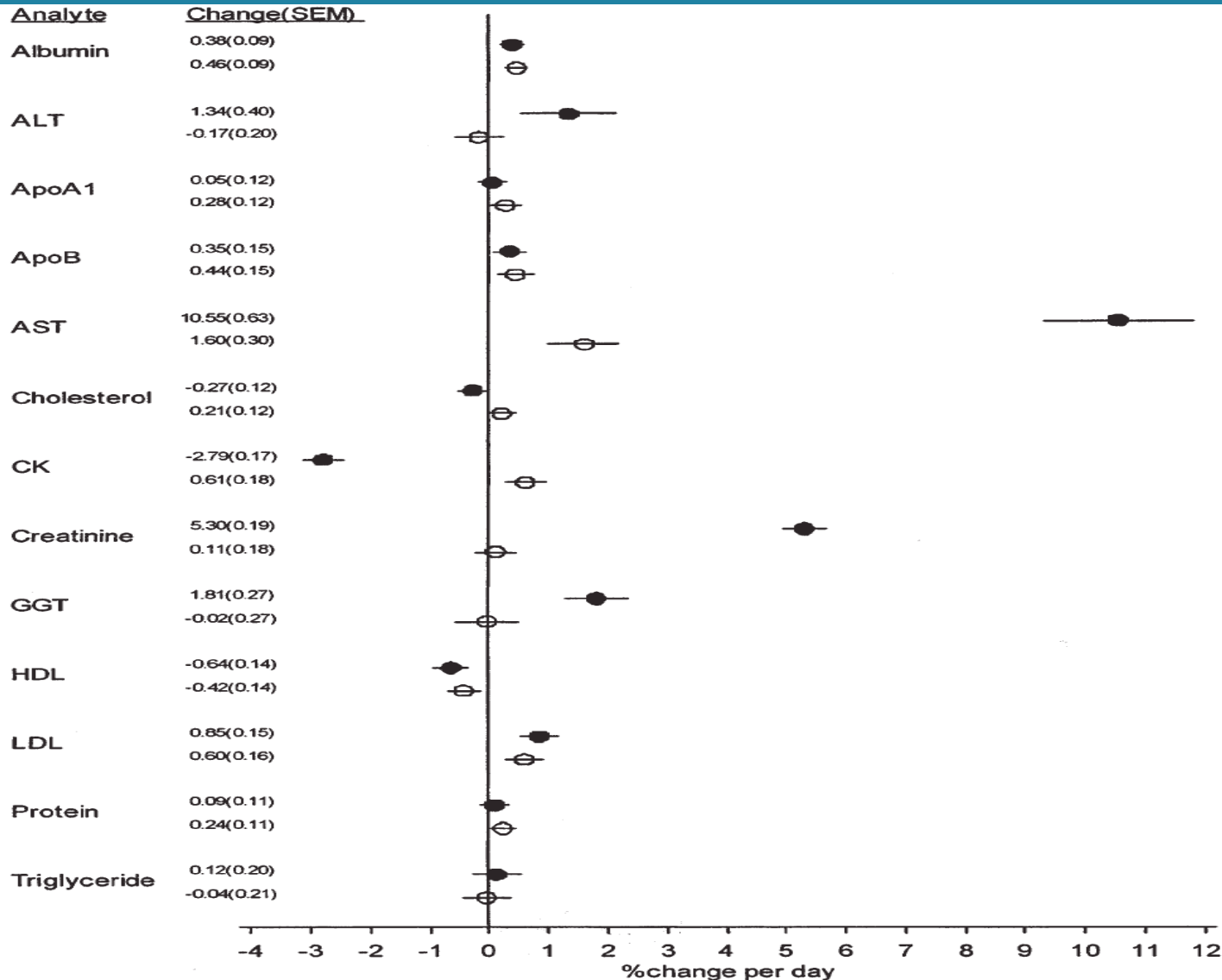
Creatinine level affected by TIME to
analysis

Creatinine increases by approximately
5% per day of delay.

But not just creatinine.....

% change after delayed testing

International Journal of Epidemiology 2003;32:125-130



More accurate measures of waste clearance.....

- The Glomerular Filtration Rate
but how do we measure that?
 - Isotope or Inulin Clearance
 - a research test
 - Creatinine Clearance
 - how many mls of blood are “cleared” of creatinine per minute
 - inconvenient, and inaccurate if not done properly
 - and so.....
 - eGFR
 - convenient and now in common use
 - Cockcroft-Gault or MDRD?

- **Creatinine Clearance** is a *measurement* of glomerular filtration rate
- **eGFR** is an *estimate* of glomerular filtration rate
- reflection of number of functioning nephrons and glomeruli
- and so a reflection of overall kidney function

How do we make the creatinine level more sensitive?

- Use it to calculate the Creatinine Clearance
- Use it to calculate the eGFR
 - by correcting for age, gender and weight
 - the Cockcroft Gault method
 - by correcting for age, gender and ethnicity
 - the MDRD method

eGFR calculation – the Cockcroft Gault method

Cockcroft-Gault Calculator (with SI Units)

Plasma creatinine
mg/dL / umol/L

Weight
kilograms / pounds

Gender

☐ Male ☐ Female

Age

Creatinine Clearance

But the UK and USA use the MDRD calculator.....

Using the four-variable MDRD, the eGFR
(mL/min/1.73 m²) =

175 x [serum creatinine (umol/L) x 0.011312] -1.154 x

[age] - 0.203 x

[1.212 if black] x

[0.742 if female]

Got that?!

Online eGFR calculator

eGFR calculator

Enter details below to calculate an eGFR

Calculate GFR here:

Creatinine micromol/l

Age years

Sex: Male ☐ Female ☐

Race: Black ☐ All others ☒

Press

This calculator uses the abbreviated MDRD equation to estimate GFR. You will see further information when you click on the 'submit' button, but you can also read [more detailed information about eGFR](#) (estimated GFR).

Estimated GFR problems

- **Accuracy** - even for the MDRD equation, which is the best there is, the confidence limits are wide. 90% of values are within 30% of the true value; 98% within 50%
- **Extremes** - none of the methods for estimating GFR are likely to be accurate in extreme examples of low muscle mass, or other unusual circumstances
- **Stability** - for all methods, creatinine must not be changing quickly
- **Systematic errors** - MDRD is better at low than at near normal GFRs
- **Age** - the MDRD should not be used in children.

So what is CKD?

- Chronic Kidney Disease
 - *not so much a diagnosis; more a marker of the possibility or probability of an underlying chronic renal disorder*
- Developed by the National Kidney Foundation in the USA in 2000
- Now an internationally accepted standard
- Came to the forefront in the UK when incorporated in GP Quality Points framework in 2004

CKD and eGFR

<u>GFR>90</u>	Normal or Stage 1 CKD
<u>GFR 60-89</u>	Normal or Stage 2 CKD
<u>GFR 30-59</u>	Stage 3 CKD
<u>GFR<30</u>	Stages 4+5 CKD

Important Notes:

Many labs do not report 60-89 – simply >60 mls/min/1.73m²

Stage 1 or 2 CKD must have associated abnormalities – urinalysis, imaging, genetic

Should be diagnosed, 'labelled' only if at least two readings performed

Prevalence levels of CKD

Stage	GFR (ml/min)	Number	Pop. prevalence
1	≥ 90	8,745	
2	60-89	25,323	
3	30-59	6884	4.2%
4	15-29	280	0.17%
5	<15	64	0.04%

Stevens et al, EDTA-ERA Lisbon 2004

So why do we worry?

- Future renal failure?
 - less important
- A marker for other risks
 - the main reason for concern

Impaired renal function

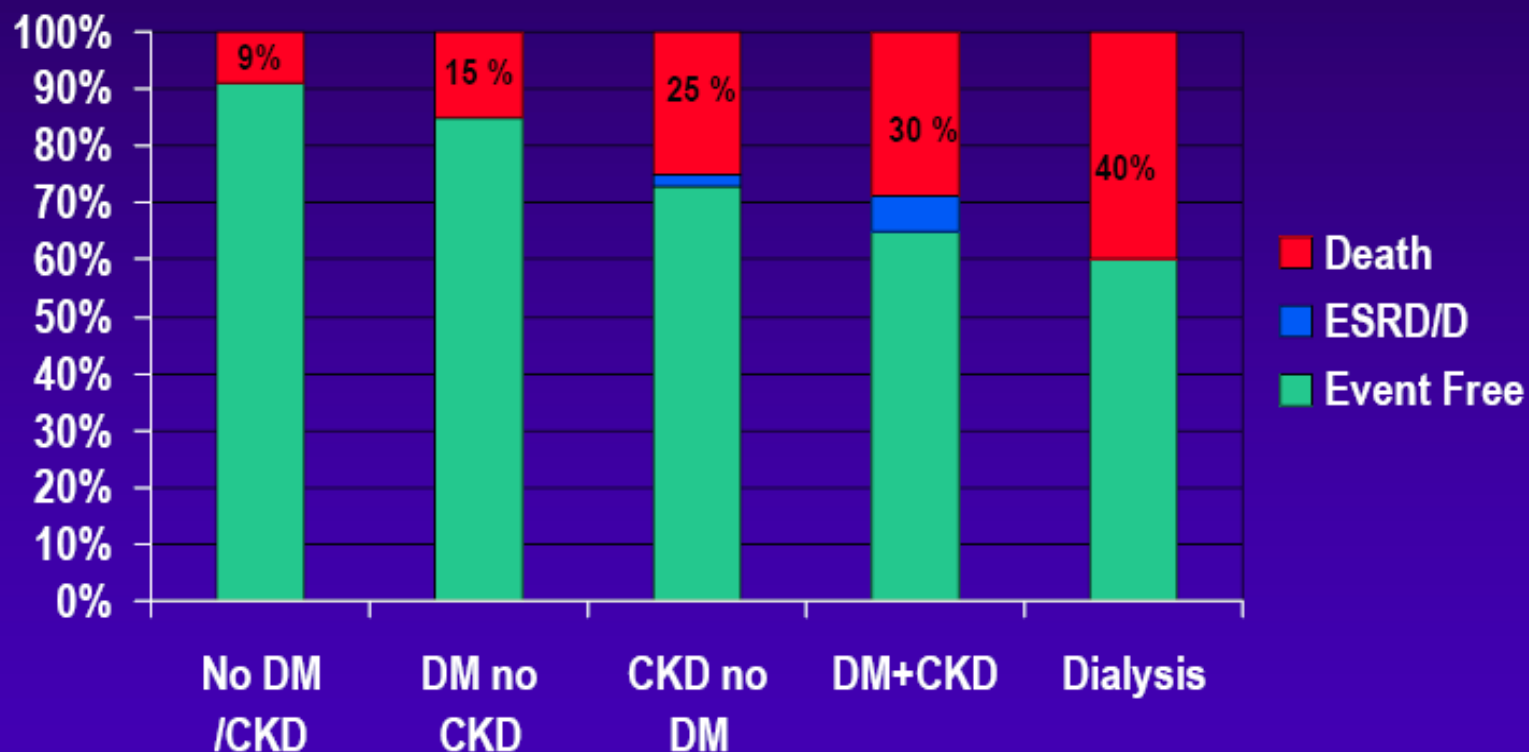
- Up to 10% population have reduced renal function
- Most are elderly and will not develop renal failure
- Dialysis usually starts when $\text{GFR} < 10 \text{ mls/min/1.73m}^2$

BUT.....

eGFR is a marker for Vascular Risk

Yes another!

Patients with CKD are more likely to die than go onto dialysis



Adapted from Collins, Adv Studies in Med, (3C) 2003, Medicare Cohort 1998-99

Example

- Male, age 69, Caucasian
- Creatinine 120,
- eGFR = 55 ml/min so Stage 3 CKD
 - Should we worry?
- CV risk is the main concern so think about BP, cholesterol, FH

Example

- Female, age 30, Caucasian
- Creatinine 120,
 - Should we worry?
- eGFR calculator gives 49 ml/min
- Underlying renal problem more likely at this age so think about urinalysis and BP

So the same creatinine level gives different
eGFR's

With different concerns

In conclusion.....

Results have to be interpreted in context

The effects of an aged sample need to be considered when interpreting results from our labs

The trick is to know which tests to ignore

The treat is to be able to rate in confidence.

If you want a copy of this
presentation, email me
at.....

petergroom@doctors.org.uk

