Identify the most appropriate test for at least two example clinical presentations (diabetes, acute kidney injury, acute coronary syndrome) – C1

DIABETES

Patients with diabetes mellitus (DM) suffer from either lack of insulin production (type I) or insulin resistance (type II) both leading to high blood glucose levels after meals. Without treatment, the kidneys attempt removal of the excess glucose from the circulation giving rise to dehydration, thirst, and frequent urination. In addition, cells are not receiving adequate amounts of glucose and result in utilising fats as a source of energy. High levels of toxic acids build up the cells, causing the fatal disease ketoacidosis (DKA).

Type I Diabetes

DKA is more likely as initial presentation of the autoimmune type I diabetes which typically affects children or young adults, has acute onset, results in weight loss, and is ketosis-prone. These patients are treated with insulin injections to replace the lack of insulin production by the pancreas. Hypoglycaemia is a risk to the patient if an excess of insulin is taken and the patient may enter a coma; the brain needs a constant supply of glucose to function.

Type II Diabetes

Clinical presentation of type II diabetes typically includes symptoms of polydipsia and polyuria, fatigue, nausea, vomiting, numbness or pain in the feet, and blurred vision. The major characteristics of a patient with type II diabetes are middle-aged to elderly, overweight or obese, with gradual onset of symptoms, and possible family history of diabetes. Over time the disease can cause damage to the back of the eye causing blindness, and also to the kidneys causing kidney failure.

Laboratory Analysis

Testing for fasting plasma glucose is the most common test used for initial presentation of diabetes. The reference range for serum fasting glucose is 3.0 – 6.0 mmol/L. A random glucose greater or equal to 11.1 mmol/L (or fasting sample greater or equal to 7.0 mmol/L) with clinical features of diabetes is adequate for a diagnosis. In cases of doubt or absence of clinical features it may be necessary to perform an oral glucose tolerance test (OGTT).

Analysis of glucose requires venous blood with anticoagulant fluoride oxalate; the fluoride prevents glycolysis enzymes from metabolising glucose in the sample. The test is performed on the Olympus AU analysers, where hexokinase is utilised to convert glucose to glucose-6-phosphate, and the coupled reaction exploits the absorbance difference of NAD+ and NADH:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{Glucose-6-Phosphate} + NAD^+ \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADH} + H^+$$

Further tests such as HbA1c or the OGTT may be required if diabetes is suspected from the results.

Oral Glucose Tolerance Test (oGTT)

This is the gold standard for diagnosing DM, and results can distinguish between impaired fasting glycaemia and impaired glucose tolerance, as well as DM. The patient is required to drink 75g of glucose within 15 minutes (after a 12 hour fast) and then remain seated with no more food or drink for 2 hours. A venous blood sample is taken just before ingestion and at 2 hours post-glucose with samples labelled as such.

For a venous plasma sample the results can be interpreted as follows:

	Fasting glucose (mmol/L)		**2-hour glucose (mmol/L)**
Normal	< 6.1	*and*	< 7.8
Impaired fasting glycaemia (IFG)	≥ 6.1 and < 7.0	*and*	< 7.8
Impaired glucose tolerance (IGT)	< 7.0	*and*	≥ 7.8 and < 11.1
Diabetes mellitus	≥ 7.0	*and/or*	≥ 11.1

A diagnosis of DM can be made if the fasting sample or two hour glucose sample is above 7.0mmol/L and 11.1mmol/L respectively. Alternatively, impaired fasting glycaemia is suggested if the fasting glucose result is 6.1≤x<7.0mmol/L and the two hour glucose is below 7.8mmol/L. Equally, impaired glucose tolerance is implied with a fasting glucose below 7.0mmol/L and two hour glucose between 7.8 and 11.1mmol/L.

Any results indicative of DM should have another diagnostic result to confirm, unless the patient is symptomatic.

HbA1c

A patient presenting with diabetes mellitus will need to be constantly monitoring their blood glucose levels for effective control of the disease. Haemoglobin A joins with circulating blood glucose becoming ‘glycated’ and so the concentration of glycated haemoglobin is proportional to concentration of sugar in the blood. The analysis of HbA1c is used for patients with diabetes to monitor the control of the condition; therefore, this test would only be required post-diagnosis. Whereas a blood glucose test analyses the glucose present in the blood at one point in time, measuring HbA1c gives an indicator of glucose control over the last 2 or 3 months due to RBC turnover, so provides a regular indication of whether the patient has been controlling their diet and medication.

HbA1c cut-off has been recommended by WHO to be 48 mmol/mol for diagnosing diabetes. However, the result may not be reliable in patients with haematological disorders, such as sickle cell disease, or haemolytic disease. Furthermore, patients with liver disease or renal failure may produce deceptive HbA1c results and are not appropriate for diagnosis in these cases.

ACUTE CORONARY SYNDROME

The primary cause of Acute Coronary Syndrome (ACS) or myocardial infarction (MI) is atherosclerosis, where build-up of an atherosclerotic plaque from cholesterol, triglycerides, platelets and white blood cells thickens the artery walls. Over time this process narrows and eventually obstructs coronary arteries that are essential for providing blood to the heart muscle. Without this blood supply, the heart muscle starts to die giving symptoms of a heart attack. A patient that is suspected to have had a heart attack (symptoms of severe chest pain and tightness, shortness of breath, lightheaded or fatigue) are tested for troponins (specifically troponin I) to suspect or rule out a heart attack.

Troponins are proteins involved in control of contraction of striated muscle. Troponin I inhibits actomyosin ATPase and one isoform of this subunit strictly works with cardiac muscle (cTnI) and therefore, due to the specificity of its location, it is a cardiac marker for myocardial infarction (MI). The release of cTnI into the bloodstream means that it can be detected within 3-6 hours after symptoms of MI, with raised levels continuing for around 5 to 9 days. Both TnT and TnI can indicate a suspected MI, however, TnI has shown to have more specificity for patients with CKD[2] (patients with CKD may have raised troponin) and more sensitivity than TnT for diagnosis after 10 hours of symptom onset[3]. Because of the delay after a heart attack for troponin levels to become detectable in the blood, it is advised that two intervals of troponin are tested for, with the second sample tested 10-12 hours after the first[4]. This allows the clinician to identify a changed level of troponin and therefore identify the incidence of an MI.

The TnI-Ultra™ assay is a three-site sandwich immunoassay performed on the ADVIA Centaur® analysers which quantitatively determine cardiac troponin I in serum, heparinized or EDTA plasma. The Lite Reagent comprises of an acridinium ester labelled polyclonal anti-troponin I antibody and two biotinylated monoclonal antitroponin I antibodies. The solid phase is streptavidin-labelled magnetic latex particles. The relative light units (RLUs) detected by the analyser are directly proportional to the concentration of troponin I. The reference range for this assay is 0-40 ng/L. Damage of the myocardial tissue is extremely unlikely if the result is within the reference range and the sample has been taken after 12 hours since the onset of chest pain.

ACUTE KIDNEY INJURY

Acute kidney injury (AKI) affects 13-18% of all patients admitted to hospital (NICE) and describes the rapid deterioration of kidney function over hours or days. The disease causes retention of urea, creatinine, hydrogen ions, and also usually causes oliguria alongside. AKI is diagnosed through measurement of creatinine in the serum and urine; a product from the metabolism of creatine in the muscle excreted almost exclusively by the kidneys. Therefore, the measurement of creatinine clearance is used as a general indicator of glomerular filtration rate.

Clinical presentation of AKI is similar symptoms to chronic kidney disease (fatigue, weight loss, nocturia) but over a much shorter timescale. AKI is commonly split into three categories to emphasise the area of disease: renal blood flow decrease, intrinsic damage to the kidney, or urinary tract obstruction (pre-renal, intrinsic, and post-renal respectively). AKI is common for patients in intensive care. If patients survive their illness, the degree of damage to the organ determines the necessary treatment required. A severe case may require renal replacement therapy (RRT). NICE recommends the continued monitoring of patients that feel unwell or who have risk factors for AKI.

These include liver disease, diabetes, chronic kidney disease, heart disease, dehydration and certain drugs.

When kidney function starts to decline, plasma creatinine levels will rise as the kidney starts to lose the ability to filter small molecules such as creatinine into the urine. Therefore, inadequate clearance of creatinine by the kidney can be measured by the rise in plasma creatinine (see figure below). As is shown by the graph, serum creatinine measurement is not sensitive enough to detect early renal damage, as the creatinine clearance has to reduce to below 40 mL/min before exponential increase in plasma creatinine outside of the reference range. However, it is a useful measurement to monitor the progression of the disease, evaluation of dialysis, and initial diagnosis.

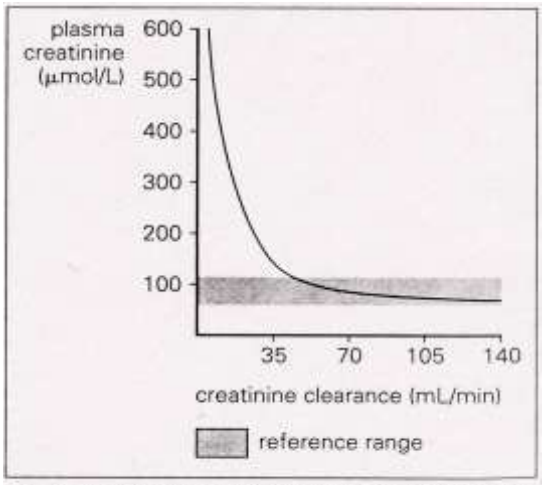

Fig. 4.3 Relationship between creatinine clearance and plasma creatinine concentration.

Analysis of creatinine is measured in serum using the Jaffe method on the Olympus AU analysers. Creatinine clearance can be calculated when a 24 hour urine sample is available. In alkaline solution, creatinine forms a yellow-orange complex with picric acid:

$$\text{Creatinine} + \text{Picric Acid} \xrightarrow{\text{Alkaline}} \text{Complex (Yellow/orange)}$$

The reference range here is 70–120 μmol/L. If the patient sample is icteric then the creatinine results will be invalid as the complex absorbs light at a similar wavelength to bilirubin. There is no significant interference with bilirubin levels up to 20 mg/dl but significant icteric levels will cause negative interference with this method.

AKI is divided into three stages depending on the severity of the disease. These can be defined biochemically by serum creatinine increase when compared to a baseline. The baseline is the last serum creatinine measured before hospital admissions, and if no baseline is available then 100 μmol/L should be cautiously assumed. See the table below for levels of creatinine increase defined for each stage of AKI.

AKIN Classification		
Stage	**Serum Creatinine**	**Urine Output**
1	1.5 – 2 times baseline (or ≥ 26µmol/L increase)	<0.5ml/kg/hr for 6 – 12 hours
2	2 – 3 times baseline	<0.5ml/kg/h for ≥12 hours
3	> 3 times baseline or acute rise of > 50µmol/L where baseline is > 300 or Initiation of renal replacement therapy	<0.3ml/kg/h for ≥24 hours OR anuria for ≥12 hours

Table 1: AKI Classification taken from the NUH trust website. Three stages of AKI are described by the serum creatinine increase or the quantity of urine output.

References

1. NICE guidelines [CG169], (2013) 'Acute kidney injury: prevention, detection and management'.
2. Roppolo L. *et al.,* (1999) 'A comparison of troponin T and troponin I as predictors of cardiac events in patients undergoing chronic dialysis at a Veteran's Hospital: a pilot study', *Journal of the American College of Cardiology*. 34(2):448-454.
3. Maynard S., Menown I., Adgey A., (2000) 'Troponin T or troponin I as cardiac markers in ischaemic heart disease', *Heart.* 83:371-373.
4. NICE diagnostics guidance [DG15], (2014). 'Myocardial infarction (acute): Early rule out using high-sensitivity troponin tests (Elecsys Troponin T high-sensitive, ARCHITECT STAT High Sensitive Troponin-I and AccuTnI+3 assays)'.
5. Nottingham University Hospitals NHS Trust, (2011) 'Acute Kidney Injury (AKI) Guideline: Early Identification and Management'.