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Laboratory Testing of DOAC's When, Why & How.

Directorate of Laboratory Medicine

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Introduction

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- The new/noval oral anti-coagulants have now been in use for a number of years.
- They are called DOACs Direct oral anticoagulants as they act directly on the coagulation factors unlike warfarin which reduces the production of vitamin K.
- However as heparin has been used since 1930's and warfarin since 1960's many of us still think of them as new.

How they work

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There are two types of DOAC

- Those which act directly on factor II Direct Thrombin Inhibitor. – Dabigatran (brand name Pradaxa)
- Those which act directly on factor X – Rivaroxaban, apixaban and edoxaban (brand names Xarelto, Eliquis and Savaysa)
- These are licensed for the treatment of AF and VTE, they are contra indicated in patients with mechanical heart valve replacement due to adverse events during trials.

Why do we need them

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- Warfarin is long lasting, can be given orally and reversed with vitamin K but requires regular monitoring
- Heparin is short acting and can only be given by intravenous or intramuscular injection.
- The new anticoagulants can be given orally and do not require monitoring but occasionally require measuring.

When would we measure levels?

- In cases of prolonged or life threatening bleeding .
- Before emergency surgery when the patient has taken the drug in the last 24hrs or has reduced creatinine clearance
- Assessment of compliance
- Other drug interaction
- Extremes of body weight
- Deteriorating renal function
- Overdose

Why do we need specific assays?

Direct Thrombin Inhibitor

- PT is variable in its response to dabigatran.
- aPTT can be used to determine the degree of anticoagulation due to dabigatran but not the drug level.
- Thrombin time is too sensitive to determine degree of anticoagulation but a normal TT excludes the presence of dabigatran

Factor X inhibitors

- PT or aPTT can be used to determine the degree of anticoagulation due to rivaroxaban/apixaban/edoxaban but is inconsistent.
- PT is usually more sensitive but varies with reagents.
- No effect on the Thrombin time .

Dabigatran

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- Back in Sept 2011 we were tasked with setting up an assay for dabigatran measurement.
- Dabigatran is a direct thrombin inhibitor with a half life of 14-17 hours, it is renally excreted and its half life is prolonged in renal impairment.
- The recommended method is a calibrated dilute thrombin time.
- We chose the Hyphen Biomed Direct Thrombin Inhibitor assay with dabigatran calibrators and controls.

How the assay works

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- The Dabigatran in a sample inhibits the function of factor II to such a degree that a thrombin time will be >240secs at therapeutic levels of dabigatran.
- To overcome this the calibrator, control or patient plasmas are diluted.
- The diluted sample to be measured is mixed with normal pooled plasma and we measure the inhibitory affect of the dabigatran in the sample to prolong the thrombin time of the pooled plasma.
- Comparing control and patient clotting times to a calibration plasma gives us the level of circulating drug.

Case study 1 Patient AM

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- A 73yr old female with a new diagnosis of AF started on dabigatran
- She is seen in A&E with a diagnosis of unwell and abnormal clotting results, we queried the results and suggested repeat samples.
- Presents four days later with an acute GI bleed.

	29/11	4/12	5/12	6/12	7/12	11/12	18/12
PT secs	45.2	36.5	23.2	18.8	19.3	15.3	
APTT secs	90.4	74.8	60.5	46.9	35.9	32.1	
TT secs		>240	184.6	149.6	102.7	26.2	16.9
Dabig level ng/ml		317	157		10.6		
Red cells		3 units	2 units				
Plats		1 unit	1 unit				
PCC		3000iu	3000iu				

Rivoroxaban, Apixaban, Edoxaban

- These are a factor X inhibitors with a half life of 10-12hrs. In elderly patients, this may be prolonged. They are measured using an anti Xa method.
- Specific calibrators and controls are required but we use our “Hyphen Biomed liquid Xa kit”
- As this is on board our analysers 24/7 we are easily able to perform Rivaroxaban levels urgently.

How the assay works

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- The heparin anti-Xa assay is a chromogenic assay for the determination of anti-Xa activity in human plasma.
- The method is a one-step reaction based on a principle of competition; as soon as factor Xa is added to the plasma-substrate mixture, two reactions take place simultaneously, namely:
- – hydrolysis of the substrate by factor Xa
- – inhibition of factor Xa by rivaroxaban, apixaban or endoxaban.
- After the necessary period of time for the competitive reaction to reach equilibrium, the quantity of paranitroaniline (chomogenic substrate) that is released is inversely proportional to the concentration of anti-coagulant present in the sample or control.
- Specific calibrators are used to produce a calibration curve.

Patients

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- We have done a number of patients on rivaroxaban and there is no consistency in PT and APTT results. Hence the need for specific assays.

	Patient 1 pre (Lupus)	Patient 1 post (Lupus)	Patient 2 pre (VTE)	Patient 2 post (VTE)	Patient 3 pre (AF)	Patient 3 post (AF)
PT secs	17.2	33.9	14.0	39.0	14.1	18.8
APTT secs	47.6	81.3	23.8	30.5	34.6	46.9
Rivaroxaban ng/ml	37.5	249.8	40.7	698	34.0	229.2
Factor VIII			440u/dl			

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Case study 2
Patient AL

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- A 91yr old female with a diagnosis of AF on Rivaroxaban
- Admitted following a fall and needing to go to theatre # NOF.
- Readmitted 3 months later with further fracture.

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Patient AL	14/7	15/7	17/7		14/10	15/10 am	15/10 pm	16/10
PT secs	16.4	13.7	12.8		18.3			
APTT secs	29.4	31.6	31.7		41.2			
Rivaroxaban ng/ml	65.2	47.9	18		447.9	154.1	119.6	48.3
Creatinine umol/L	146	317	157		140	156		139

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Further information

- Due to renal impairment and recurrent fractures/falls the patient anti-coagulation was changed to warfarin.

Case study 3
Patient KM

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- 78yrs old female patient started on Apixaban following a DVT in February.
- Presented in May with a further query DVT.
- One week later admitted with GI bleeding.

Patient KM	22/5	24/5	25/5
PT secs	13.5	10.7	10.9
APTT secs	25.3	19.6	18.8
Apixaban ng/ml	>800		81.6
Creatinine umol/L	123	128	127
PCC	3000iu		

Case study 4
Patient MA

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- 91yrs old female patient on Rivaroxaban.
- Presented with a large haematoma and a haemoglobin of 44g/L

Patient MA	11/8	12/8	15/8
PT secs	13.6		10.5
APTT secs	40.7		23.9
Rivoroxaban ng/ml	443.8	46.4	12.4
Creatinine umol/L	163	156	
PCC	3000iu		
Red cell	5 units		

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Follow up

- Converted to LMWH
- Diagnosed with new DVT on 23/8
- Warfarin commenced.

The future of DOACs

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- There are more “new” anticoagulants awaiting approval and we may have to provide assays for them all!
- Some patient groups will remain on warfarin eg valve replacements.