The “APC” of Coag
Back to Basics Troubleshooting

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Overview of Presentation

• Collection and transport of specimens
• Pre-analytical checks for coagulation
• Reviewing clinical information/previous results
• Analyser and water bath testing
• Optical Analysers
  - Error flags
  - Clotting curve data
• Mechanical Analysers
  - Error flags
• Case studies - APTT and PT
  - Basic result patterns
  - Troubleshooting hints

Collection

• Collection is a critical step as the coagulation factors can be easily activated or denatured.
• Patient should be calm and at rest i.e. no stress
• Venous blood samples, atraumatic collection, release of tourniquet to prevent haemoconcentration.
• Tissue thromboplastin release can cause spurious results.
• Haemolysis - difficult collects, syringe collects.
• Collection from heparinised lines.
• Avoid drip arms.
• Well mixed, immediately, invert at least 4 times

Collection continued.

• 3.2% Trisodium citrate (109mM) - reference ranges established based on tube type/reagents/analysers.
• Lithium heparin and EDTA interfere with assays.
• Order of draw important.
• In date collection tubes - vacuum may be compromised
• Correct ratio blood to anticoagulant 9:1
• Under filled or over filled tubes are not acceptable.
  • CLSI guidelines >10% under or over not acceptable
• Hct > 0.55 may need to adjust citrate volume in tube.
Transport

- Transport of specimens should be at room temperature.
- Delays in testing then specimens should be double centrifuged, plasma removed and frozen.
- Centrifugation to produce platelet poor plasma (<10)
- 3000g for 10 minutes.
  - INR, D Dimer 24 hours
  - Coag Profile, Factor assays, Thrombophilia assays 4 hours
  - APTT/Heparin Assays 2 hours

Pre-analytical Checks

- Under filled or over filled specimens should be checked for clots prior to testing.
- Reject samples underfilled or overfilled by 10%
- Haemolysis
  - Comply with organisational guidelines
    - Path Qld if more than 2+ then NOTEST
- Intravascular haemolysis tested.
- Optical end point analysers - affected by lipaemia and bilirubin.
  - Clarify sample using ultracentrifugation OR manual testing may be required.

Analyser Errors and Water bath testing

- Know the limitations of your analyser
- Recognise errors and what they mean in terms of reporting of results
- Optical analysers - haemolysis, lipaemia, bilirubin, low fibrinogen, haemophiliacs
- Confidence with water bath testing
- When was the last time you performed a manual tilt tube PT or APTT?
  - Allows clot visualisation
- Investigate machine errors by testing patient samples manually and verify abnormal results
  - Use all data available including clot curves, delta values, error messages

Clinical Notes/Previous Results

- When investigating abnormal results any clinical information provided on the request form or from the clinician is helpful.
  - sometimes need to be intuitive
- Previous coagulation and other pathology results may also assist in interpretation of abnormal results.
What is a clot curve?

- Graphical representation of a clotting reaction run on optical endpoint coagulation analysers
- Generated from the optical data recorded during the clotting test
  - Absorbance or light transmission or light scatter points
  - Plotted versus time

What does a Clot Curve look like?

- Y-axis: change in absorbance
- X-axis: acquisition time in seconds
- Stable Baseline
  - Sample + reagents
- Acceleration phase
  - Rapid Fibrin formation
- Deceleration phase
  - Reaction slows as fibrinogen is depleted
- Stable endpoint
  - Data acquisition ends

How is the clotting time determined?

- Sysmex CA analysers
  - Clot time set at 50% change in light transmission

- ACL TOP
  - Clot time set at maximum speed
  - First or second derivative
Clot Curves - Troubleshooting Tool

- Starting Absorbance
- Shape
- Slope
- Amplitude
- Baseline biphasic (DIC pattern)
- Oscillations 2nd deriv - low fibrinogen

Clot Curves - Troubleshooting Tool

- High starting absorbance
- Rising baseline and end stage
- Lipaemia
- Ultracentrifugation of sample (removal of lipid) fixes atypical features
- Note clot time is slightly lower

Failed to clot

OK, acquisition extended

Clot Curves - Troubleshooting Tool

- Pooled plasmas fib 2-3g/L
- Defibrinate portion - incubate @ 56°C 15mins, centrifuge, remove supernatent
- Dilute pool, obtain PT, APTT and FIB and collate results (CA560, CA1500, ACL TOP)
- Thrombored S - PT
- TriniCLOT aPTT HS - APTT

Recommendations:
- CA560: err2, err4 OK if dh>20, but err 32, dh <20 usually fib <1.2g/L, perform manual tilt tube
- CA1500: valid results fib ≥ 0.6g/L, err 2, err4 dh <10 results OK, but err 32, scrutinise results & confirm with manual tilt tube
Atypical derivative plots - ACL TOP

Normals, LA Neg, Heparin (Absent) LA Pos, factor VIII, factor IX (Present)

Atypical plots not present

TC 108 (True Negative) 3 (False Negative) NPV = (108/111)*100 = 97.3%

Atypical plots present

TC 6 (False Positive) 58 (True Positive) PPV = (58/61)*100 = 90.6%

TC Specificity = (108/114)*100 = 94.7%

Sensitivity = (58/61)*100 = 95.1%

SP 109 5 NPV = 95.6%

SP Specificity = 95.6%

Sensitivity = 91.8%

FSL 56 9 NPV = 90.3%

FSL Specificity = 49.1%

Sensitivity = 85.2%

SF 30 0 NPV = 100%

SF Specificity = 26.3%

Sensitivity = 100%

Table 3 - Sensitivity and Specificity calculations for all reagents. Data from normal volunteer plasmas, LA negative plasmas and heparin plasmas were combined for the “absent” condition. LA positive plasmas, factor VIII and factor IX deficient plasma data were combined for the “present” condition.

Activated Partial Thromboplastin Time - APTT

- PL (no TF) + Contact Activator
- Calcium
- hHMWK, PK, XII, XI, VIII, IX def
- X, V, II, fibrinogen def
- Heparins, Hirudine
- Inhibitors
  - Factor VIII, IX, V
  - Lupus Anticoagulant
- Sample quality issues
  - activated, aged, diluted,
  - volume, contaminated, pH

Case #1-3

INR 1.1
PT 11s
APTT 48s (rr 24-39s)
FIBD 4.2g/L

Prev results
Clin notes
Determine follow up

Scenario #1 Renal patient,
- prev APTT OK,
- Check for UFH (PSO4, polybrene)

Scenario #2,
- Orthopaedic case, prev APTT OK,
- APTT +Protamine (APTTP) = 32s,
- Thrombin Time >80s, TCTP = 14s
- Specimen contains heparin.

Scenario #2,
- Orthopaedic case, prev APTT OK,
- APTTT = 42s,
- Thrombin Time = 14s, not UFH, but
- Phone ward, enquire about other anticoagulants
- Typical of Clexane, (also s.c.UFH)
  - antiXa activity = 0.95
UFH vs LMWH

- Still common in tertiary referral hospitals - i.v. Rx (monitored) and s.c prophylaxis (not monitored)
- Anti IIa activity: Anti Xa Activity = 1:1
- PT usually normal
- APTT elevated
- Protamine correction diagnostic
  - correction of abn APTT (ATCT)
  - despite any info to the contrary
- check patient anticoagulant history
  - coag prof/ps should not be requested for monitoring heparin
  - may also be a contaminant when samples drawn non-peripherally
- Commonly used prophylactically & Rx - NOT routinely monitored
  - Exceptions: renal patients, paediatric patients, obese or low body weight patients
  - anti-Xa activity assay
  - AntiXa activity >>> AntiIIa activity
  - PT is usually normal
  - APTT usually slightly elevated, but may be normal
  - TCT normal or slightly elevated
  - Max effect, 4 hrs post injection
  - Clearance APTT effect not corrected by protamine
  - the abn TCT will be corrected by protamine
  - check patient anticoagulant history

Lupus Anticoagulants

- The LA is a member of the anti-phospholipid autoantibody (aPLA) family.
  - aPLA are quite heterogeneous & include AntiCardiolipin & B2Glycoprotein1 aPLA
- They are associated with arterial & venous thromboembolic disease, recurrent miscarriages and thrombocytopenia.
  - also occur in various autoimmune diseases, infections, malignancies, with certain drug exposures or in patients with no underlying disorder
- Lab diagnosis involves a panel of tests to characterise the phospholipid dependent qualities of the inhibitor.
- Persistent aPLAs = Anti Phospholipid Syndrome
  - Often anticoagulated, monitoring UFH need antiXa activity assay
  - Often require maintenance at higher INR
  - Occasional catastrophic clinical complications

Case #1-3

INR 1.1
PT 11s
APTT 48s (r = 24-39s)
FIBD 4.2

Scenario #3

PreOp, No prev results, Name search
Repeat APTT, check for heparin, APTTP:49s
Thrombin Time = 16s
Phone requesting doctor, confirm no other anticoagulants, then APTTX = 45s,
indicates an inhibitor
Double-spin and refer for LA tests.
Typically the APTT is elevated:
PT is usually normal, but mildly abnormal if strong inhibitor present
No lab evidence of UFH:
  - TCT is normal
  - confirm no LMWH Rx, (result pattern similar)
  - No correction with Normal Pooled Plasma: indicative of an inhibitor
  - most common type is the lupus anticoagulant
Specific tests needed to confirm LA.

Case #4

INR 1.0
PT 11s
APTT 85s
FIBD 3.4g/L
F/34, Post Partum
Hb 68g/L,
Plt 232 x10^9/L

Occurred 3 mths later - another lab,
- APTT = ****, incorrectly reported as >200
- VIII deficiency or inhibitors, APTT 80-120s
- Flagged result – ascertain cause, use clot curve (if available), manual APTT to confirm
Acquired coagulation inhibitors

- Circulating antibodies - neutralise the activity of specific plasma clotting factors
  - Haemophiliac failing to show adequate clinical response to replacement therapy
  - Unexplained bleeding in the presence of a benign clinical history
  - Sudden onset of generalised ecchymoses &/or petechiae
  - Postpartum
  - Malignancy/lymphoma
  - Rheumatoid arthritis, SLE, Crohn's Disease or other autoimmune disorders
  - Drug reactions, eg. penicillin

- Rare - factor VIII inhibitors most common

Case #5-6

<table>
<thead>
<tr>
<th>Test</th>
<th>Value 1</th>
<th>Value 2</th>
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<tr>
<td>INR</td>
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<td>PT</td>
<td>10s</td>
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<td>APTT</td>
<td>30s</td>
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<td>FIBD</td>
<td>3.5g/L</td>
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<td>APTTP</td>
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Prev results
Why coags differ?

Scenario #1
- Oncology patient, no clinical notes
  - Check spec for clots
  - FBC no change, but citrate Hb not the same as EDTA
  - Phone ward, obtain collection info, suggest recollect

Scenario #2
- Medical patient, no clinical notes
  - Check spec for clots
  - WC 25 x 10^6/L, Plt 162 x 10^9/L
  - D Dimer 16.5 mg/L (RR <0.24)
  - Phone unexpected abnormal results

Case #7

<table>
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<tbody>
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<td>INR</td>
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<td>PT</td>
<td>13s</td>
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<tr>
<td>APTT</td>
<td>75s</td>
<td></td>
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<tr>
<td>FIBD</td>
<td>5.5g/L</td>
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<tr>
<td>M/20y</td>
<td>Haemoptysis, fever</td>
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No previous results

<table>
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<tr>
<td>APTTP</td>
<td>85s</td>
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<tr>
<td>Hb</td>
<td>135g/L</td>
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<tr>
<td>WC</td>
<td>13.9</td>
</tr>
<tr>
<td>Plt</td>
<td>303</td>
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3hrs later,
Repeat Coag = same,
Hb 100g/L, Plt 258
Now the panic!

- Urgent factor VIII, IX assays -
  - ? Haemophilia
  - VIII = 1.31, IX = 1.35 U/mL
  - Lymphs = 7.43, very reactive
  - APTX = 60s
  - LA tests POSITIVE
  - IM POSITIVE

- Be curious, embrace problems!
  - Consider all information
  - Haemophiliacs,
    - Joint bleeds, not mucosal bleeds
  - Practice every day!
Mechanical Clot Detection Errors
Stago STAR/ Compact

- V max error
- V min error
- QNS error
- Error
- Incorrect reagent identification when loading
- Analyser malfunction - pipetting etc
- Ball bearing missing from reaction cuvette
- Watch analyser perform testing to identify issue

Case DC and HC

- Two patients present with the following results:
  - INR: >10.0
  - PT: >100s
  - APTT: >200s
  - Fib: 1.0 g/L
  - APTTP: >200s
  - APTTX: >200s
  - ?Samples clotted
  - What to do?
  - Are the patients bleeding? DIC

Case HC

- Coagulation results collected yesterday were normal.
  - Plt. count: 200.
- Phone ward to check clinical condition of patient - patient well.
- Recollection suggested - normal results.
- Nurse admitted that blood collected through heparinised line.
- Gross heparin contamination.
- Reptilase time may be useful in this case.
- However patient DC who has similar results is not the same.

Case DC

- Clinical notes indicate pneumonia, patient unwell.
- Previous results from 2 days ago, post FFP as INR: 6.5
  - PT 27s INR: 2.5
  - APTT 74 s
  - Fib: 4.1 g/L
  - Plts: 96 x10^9/L
- Clinical notes, warfarin therapy, SLE positive lupus anticoagulant
- So what has happened today?
- Recollection 2 hours later showed similar results.
  - PT: >100s INR: >10.0
  - APTT: >200s APTTP: >200s
  - APTTX: 82 s
  - Fib: <1.0 g/L
- D-dimer added: 1.79 mg/L (RR<0.28)
- Diagnosis DIC due to sepsis.
  - Complicated by lupus anticoagulant and warfarin therapy.
DIC

- Acquired syndrome characterised by intravascular activation of coagulation and deposition of fibrin within the microvasculature, leading to organ ischaemia and infarction. Bleeding/thrombosis.
- Acute DIC - infection, extensive tissue trauma, pregnancy complications.
- Chronic DIC - malignancies, aortic aneurysms.
- Persistent oozing from venepuncture sites in a septic patient or massive bleeding in obstetric patient.
- Low fibrinogen in acute DIC but may be normal or elevated in chronic DIC.
- Treatment directed at the underlying cause.

Prothrombin Time

- Tissue factor and calcium
- Warfarin therapy
- Liver disease, particularly obstructive
- Vitamin K deficiency
- Factor deficiency - extrinsic pathway

Investigation prolonged PT

- PT mixing studies with normal pooled plasma(1:1) to see if the PT will correct with FFP
- Echis time (used in Path Qld) - snake venom that acts directly on prothrombin (FII) to form thrombin and is unaffected by the descarboxy forms of FII.
  - Echis time normal then warfarin/ Vitamin K deficiency.
  - Echis time prolonged indicates factor deficiency (FII, liver function impairment).

Case Study - WT and MT

- WT - Adult male presents with the following results:
  - INR 2.5 and PT: 27 s
  - APTT: 45 s (RR 25-38 secs)
  - Fibrinogen: 2.5g/L
  - Clinical notes: Atrial fibrillation
  - No previous results.

- MT - Adult male presents with the following:
  - INR 4.1 and PT: 46s
  - APTT: 87s
  - Patient on marevan. Mixing studies performed show APTT does not correct with NPP.
Warfarin Therapy

- Warfarin inhibits the enzymatic reduction of Vitamin K. This prevents the carboxylation of FII, VII, IX and X which is essential for their role in coagulation cascade.
- Clinical conditions in which warfarin is prescribed ie. Mechanical heart valves, atrial fibrillation, DVT/PE.
- Previous results indicating warfarin therapy.
- Other names for warfarin ie marevan, coumarin
- APTT may be affected the higher the INR
- Be aware of markedly prolonged APTT's on patients on warfarin therapy, may be on heparin therapy or have a co-existing LA

Case study - VK (1)

- A 21 week old male inpatient
  - INR: 2.0 PT: 25 s  (RR 12-16)
  - APTT: 42 s  (RR 29-50)
  - Fibrinogen: 3.9 g/L  (RR 1.7-4.2)
- Results on an admission one month earlier had been normal.
  - PT mixing studies with NPP: 16 s
  - Echis time: 14s  (RR 8-16 secs)
  - Clinical history of liver mass ? Hepatoblastoma

Vitamin K

- Vitamin K obtained from diet, green leafy vegetables and produced by gut flora.
- Vitamin K given at birth, parental consent required to prevent HDNB.
- Vitamin K absorption affected by diarrhoea, biliary tract obstruction liver, gut obstructions, long term antibiotic use, patients nil by mouth.
- Vitamin K required for gamma carboxylation of II, VII, IX and X.
- Deficiency causes prolonged PT in the early stages however APTT will be raised as well in chronic cases.

Case Study - LD

- Female patient presents with:
  - PT: 20 s INR: 1.8, APTT: 52 s, Fib: 1.7 g/L
  - Repeat collection 2 hours later
    - PT: 30 s INR: 2.6, APTT: 70s, Fib: 1.0 g/L,
    - D-Dimer added and is elevated, Platelet count dropping.
  - Liver function tests rapidly deteriorating.
  - Clinical notes ?paracetomol OD
  - Patient coagulation tests worsen as paracetomol in large does is toxic to the liver can cause irreversible damage.
Liver Disease

- Coagulation changes in liver disease are complex and often arise from several different mechanisms.
- Liver is principle site for synthesis and clearance of coagulation factors.
- Coagulation changes are dependent on the pathogenesis and rate of onset of the underlying liver disease.
  - Acute hepatitis characterised by a consumptive coagulopathy.
  - Cirrhosis in which there is decreased production of clotting factors.
  - Biliary obstruction, which leads to Vit.K deficiency.
  - Tumours, which arise in cirrhosis and may be associated with a dysfunctional fibrinogen.

Liver Disease Summary

<table>
<thead>
<tr>
<th></th>
<th>PT</th>
<th>APTT</th>
<th>Fibrinogen</th>
<th>Platelets</th>
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</thead>
<tbody>
<tr>
<td>Acute Hepatitis without LF</td>
<td>N or incr.</td>
<td>N</td>
<td>N or incr. +</td>
<td>N</td>
</tr>
<tr>
<td>Acute Hepatitis with LF</td>
<td>Incr. ++</td>
<td>Incr.+</td>
<td>Decr. ++</td>
<td>N or decr. +</td>
</tr>
<tr>
<td>Liver Cirrhosis</td>
<td>Incr. +</td>
<td>N or incr.+</td>
<td>N or decr.+</td>
<td>N or decr. +</td>
</tr>
<tr>
<td>Biliary Obstruction</td>
<td>Incr. ++</td>
<td>Incr.+ or N</td>
<td>Incr.+ or N</td>
<td>N</td>
</tr>
</tbody>
</table>

Case Studies – Chronic Liver Disease

- Male patient 35 year old presents with cirrhosis due to alcohol and end stage liver disease:
  - PT: 32 s INR: 3.3, APTT: 66 s, Fib: 0.4 g/L
  - LFTs - Albumin 26 g/L, Bilirubin and Bili(conj) increased Normal ALT and elevated AST.
  - Platelet count 50.

- Male patient 42 year old, admission for GI Bleed, Hb 86 g/L.
  - PT: 23 s INR 2.4, APTT: 50 s, Fib: 1.0 g/L
  - LFTs - Albumin decr < 15 g/L, Bilirubin and Bili (conj) both incr. Normal ALT and elevated AST

Case Studies – Acute Liver Failure

- Male patient 20 year old presents with acute liver failure ? cause:
  - PT: 39 s INR: 4.0, APTT: 65 s, Fib: 1.1 g/L
  - LFT’s Albumin 29 g/L,
  - Bilirubin 460 umol/L (RR <20),
  - Bili(conj) 233 umol/L (RR <4).
  - elevated ALT 871 U/mL (RR <34)
  - elevated AST 698 U/mL (RR < 31)
  - Elevated ALP and gamma GT
Case JF

- Geriatric Ward, M/74y, AF on warfarin
- INR >10.0, PT >120s
- Previously quite stable,
  - INR 2.9, PT 33s, INR 3.0, PT 34s
  - Specimen quality checks OK
- PT-PS >120s, REPT 14s
  - Recollect INR 2.5, PT 28s
  - No i.v.s, samples collected peripherally, where did it come from?
- Li 0.7mmol/L initial sample vs <0.1 for the recollect
  - ? Citrate topped up from LiHep tube

Case AC vs Case DW

- Casualty patient, NIDDM, on warfarin
  - PT 28s, INR 2.6, APTT 71s, FIBD 6.0g/L, TCT 16s
  - No prev results
  - Not the typical warfarin pattern!
    - APTT too high
  - PTX 12s, APTTX 55s
- Typical LA Pos on warfarin pattern
- Pre-Admission, MV repair
  - PT 28s, INR 2.5,
  - APTT 71s, FIBD 3.2,
  - TCT 14s
  - No prev results, advised no warfarin, no bleeding Hx, no vit K deficiency
  - PTX 12s, APTTX 32s
  - FII 95%, FX 89%, FV 3%
    - Rare 1:1,000,000
    - FV Inhibitor Neg
    - FVIII 79%
  - FV Deficiency pattern
Conclusion

• Importance of correct collection technique.
• Pre-analytical checks on specimen.
• Know your analyser limitations and errors,
  • view the clot curves.
• Water bath testing to visualise clot and confirm
  abnormal analyser results.
• Recollection of specimen is useful to confirm results.
• Review of previous results and clinical information –
  Is the patient bleeding or are they well?
• Medications or recent procedures.
• Discuss results with clinicians.

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