Antiphospholipid Antibody:

Limitations of testing and Challenging Cases

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Disclosures

Consultation:

Bayer Healthcare

- *Apixaban*

Bristol-Myers Squibb

- *Rivaroxaban*

Boehringer Ingelheim

- *Dabigatran*

Leo Pharma

- *Tinzaparin*

Pfizer Canada

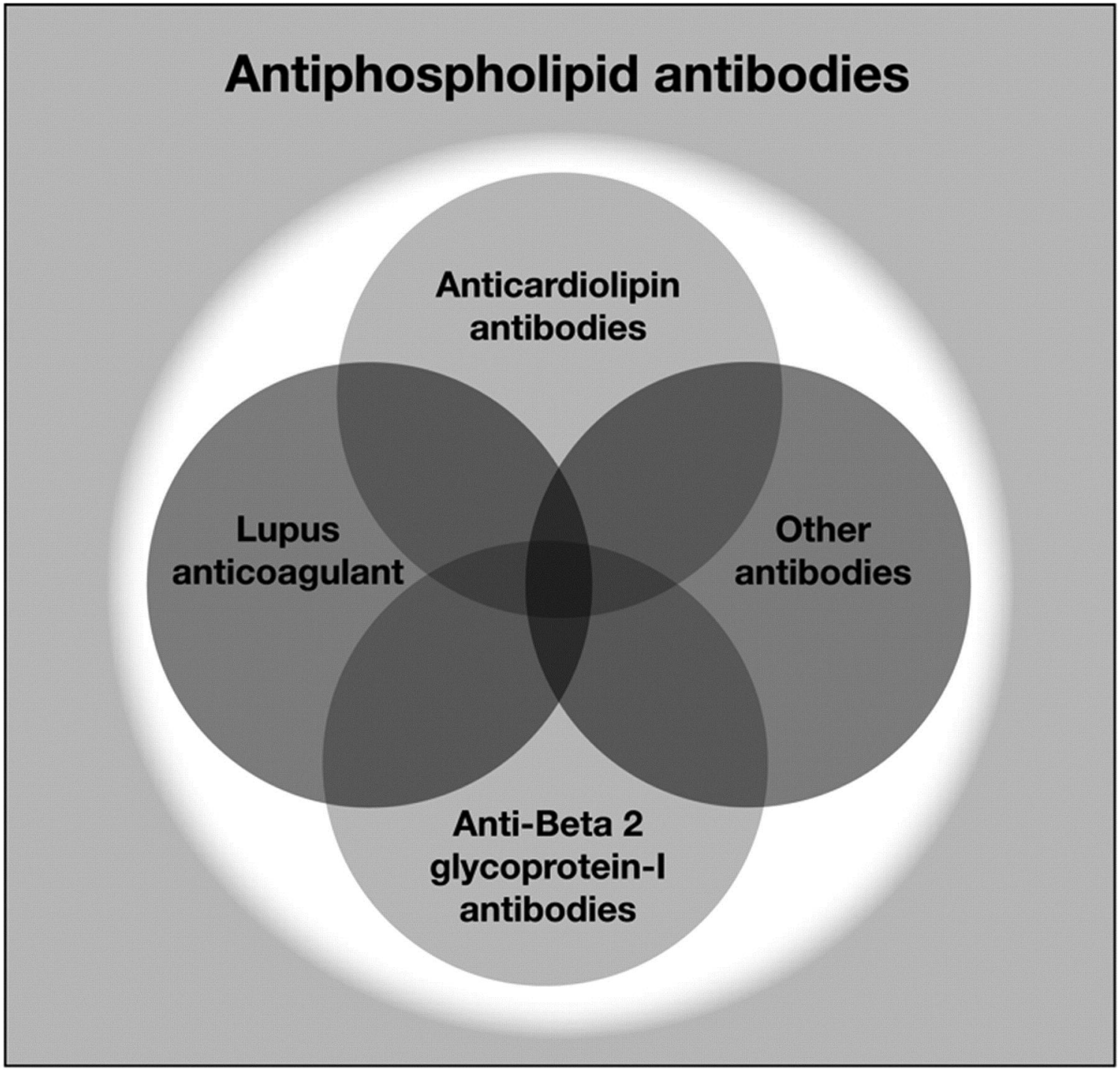
- *Dalteparin*

Sanofi

- *Enoxaparin*

Objectives

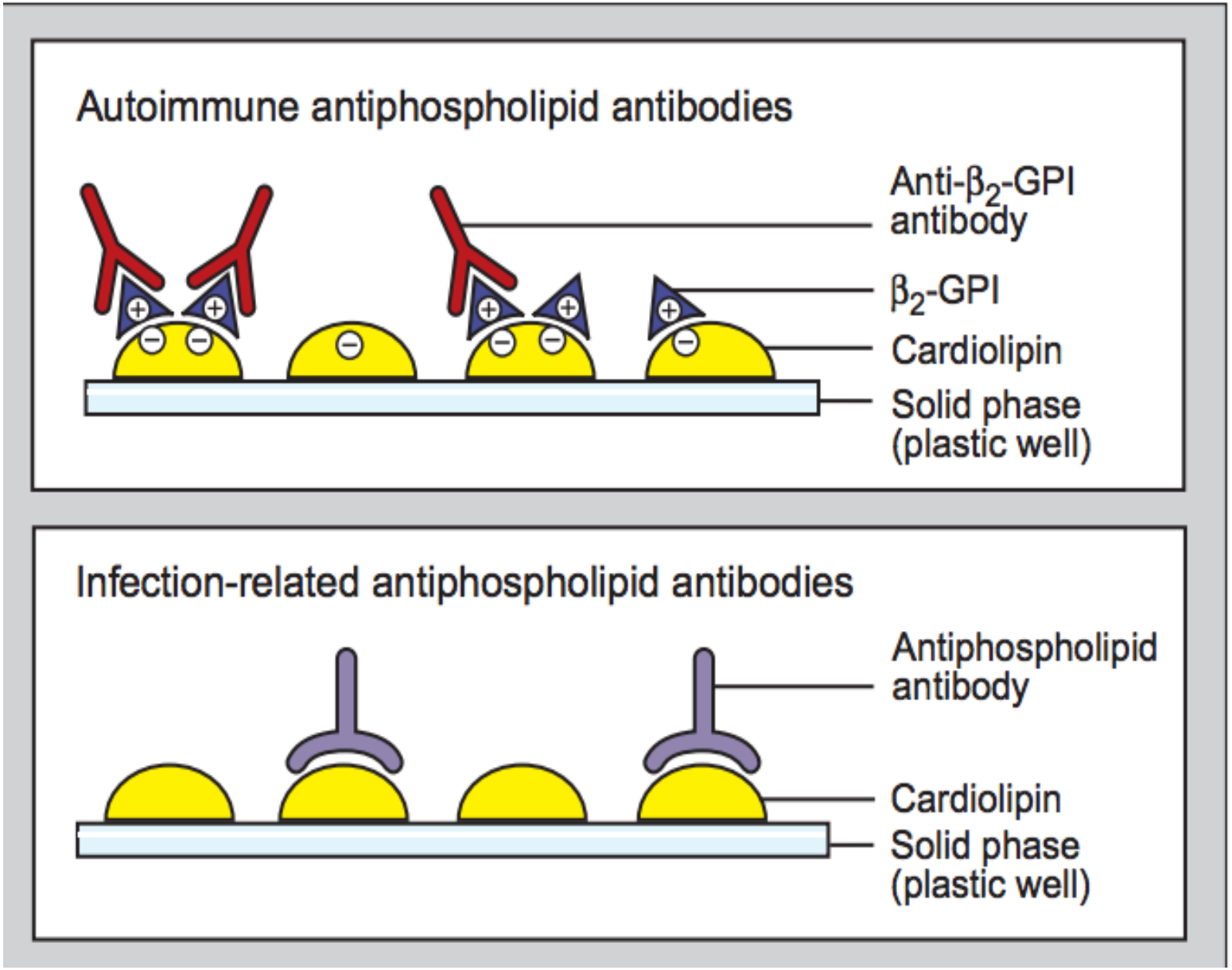
- Overview of Antiphospholipid Ab Syndrome (APS)
- Cases
- Management According to Clinical Pattern
  - Incidence
  - Venous thromboembolic disease
  - Ischemic stroke
  - Obstetric Complications
  - Catastrophic APS
  - Refractory APS
- Possible role for newer anticoagulants (aka - NOACs)


Antiphospholipid antibodies
Anticardiolipin antibodies
Lupus anticoagulant
Other antibodies
Anti-Beta 2 glycoprotein-I antibodies

Nomenclature

- primary or secondary APS
- APS with or without associated rheumatic disease (preferred)

The Importance of Beta2GP1



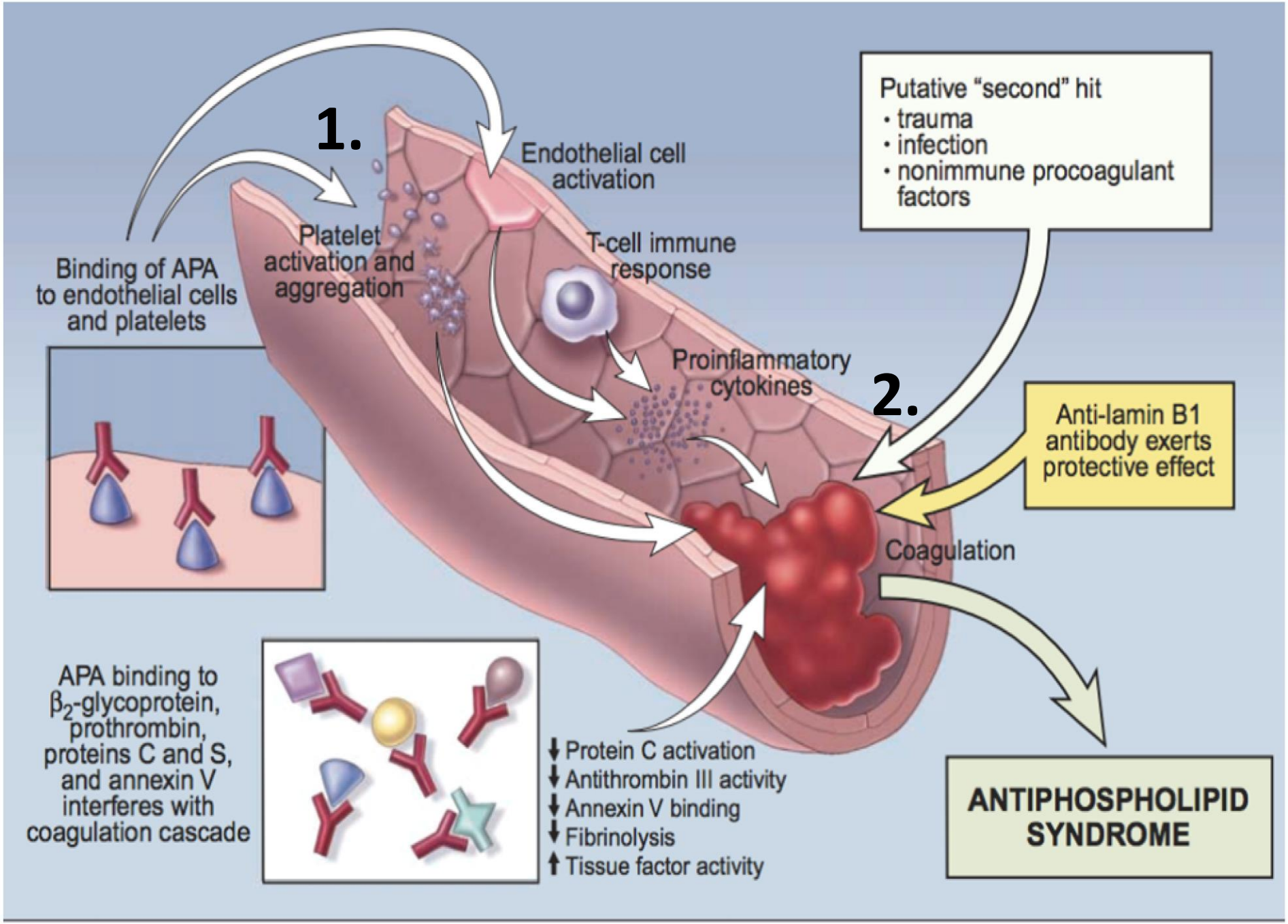


Fig. 3: Pathogenic mechanisms in antiphospholipid syndrome.

CMAJ • JUNE 24, 2003; 168 (13)

How Common is APS?

- incidence 5 cases per 100,000 persons per year
- prevalence is approximately 40-50 cases per 100,000 persons

- About 30-50% of patients with SLE (lupus) will have aPL. (only 10% have APS)

- 30% of patients with HIV infection will also develop aPL
  - The infection associated aPL are not associated with thrombosis

- Up to 5% of healthy individuals are known to have aPL antibodies

Incidence aPL in Rheumatic disease

- Common autoimmune or rheumatic diseases and the percentage of affected patients with aPL antibodies **
  - SLE - 25-50%
  - Sjögren syndrome - 42%
  - Rheumatoid arthritis - 33%
  - Autoimmune thrombocytopenic purpura - 30%
  - Autoimmune hemolytic anemia - Unknown
  - Psoriatic arthritis - 28%
  - Systemic sclerosis - 25%
  - Mixed connective-tissue disease - 22%
  - Polymyalgia rheumatica or giant cell arteritis - 20%
  - Behçet syndrome - 20%

**Note that these represent percentages of patients with aPL antibodies rather than the clinical syndrome of APS

Other Factors in the development of Antiphospholipid Antibodies

- Genetic predisposition (?)

- Infections
  - Syphilis – History of aCL
  - Hepatitis C infection
  - HIV infection
  - Human T-cell lymphotrophic virus type 1 infection
  - Malaria
  - Bacterial septicemia

- Drugs
  - Cardiac - Procainamide, quinidine, propranolol, hydralazine
  - Neuroleptic or psychiatric - Phenytoin, chlorpromazine
  - Other - Interferon alpha, quinine, amoxicillin

DIAGNOSIS:
Sapporo Criteria (Updated)

International Consensus Statement on Classification Criteria for APS (2006).

Clinical criteria:

- Vascular thrombosis (venous or arterial).
- Pregnancy morbidity

Laboratory criteria:

- Lupus anticoagulant
- Anticardiolipin IgG or IgM antibody
- Anti-$\beta_2$glycoprotein-I IgG or IgM antibody

-Miyakis, *et al*., J.Thromb.Haemost., 2006; 4: 295-306.

(Revised Sapporo Criteria)

a) LAC	Positive on two or more occasions at least 12 weeks apart, detected according to the guidelines of ISTH.
b) aCL antibody	Positive for IgG or IgM isotype in serum or plasma, present in medium and high titer on two or more occasions, at least 12 weeks apart, measured by standardized ELISA.
c) Anti-β_2GPI antibody	Positive for IgG or IgM isotype, present in two or more occasions, at least 12 weeks apart measured by standardized ELISA.

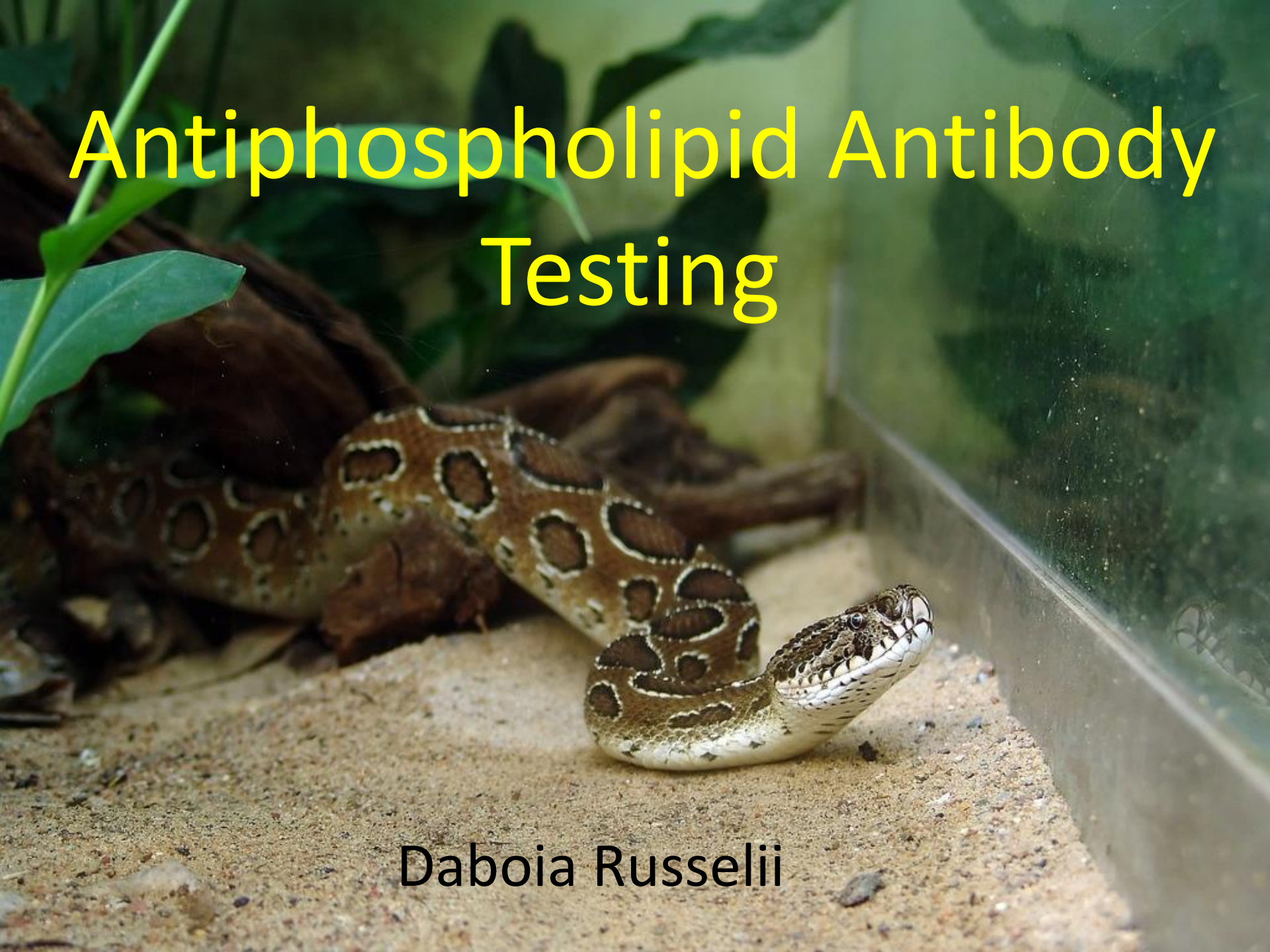
- Miyakis, *et al*., J.Thromb.Haemost., 2006; 4: 295-306.

"Non-criteria" APS findings

- Thrombocytopenia/hemolytic anemia.
- Transverse myelopathy or myelitis
- Non-thrombotic neurologic manifestations
  - multiple sclerosis-like syndrome
  - chorea
- Dermatologic – ie. Livedo reticularis
- Valvular disease
  - Separate from Libman-Sacks endocarditis – true valvulitis - immune deposition
- Nephropathy
- Abdominal
- Endocrine – adrenal insufficiency

-- Miyakis, *et al*., J.Thromb.Haemost., 2006; 4: 295-306.

Antiphospholipid Antibody Testing



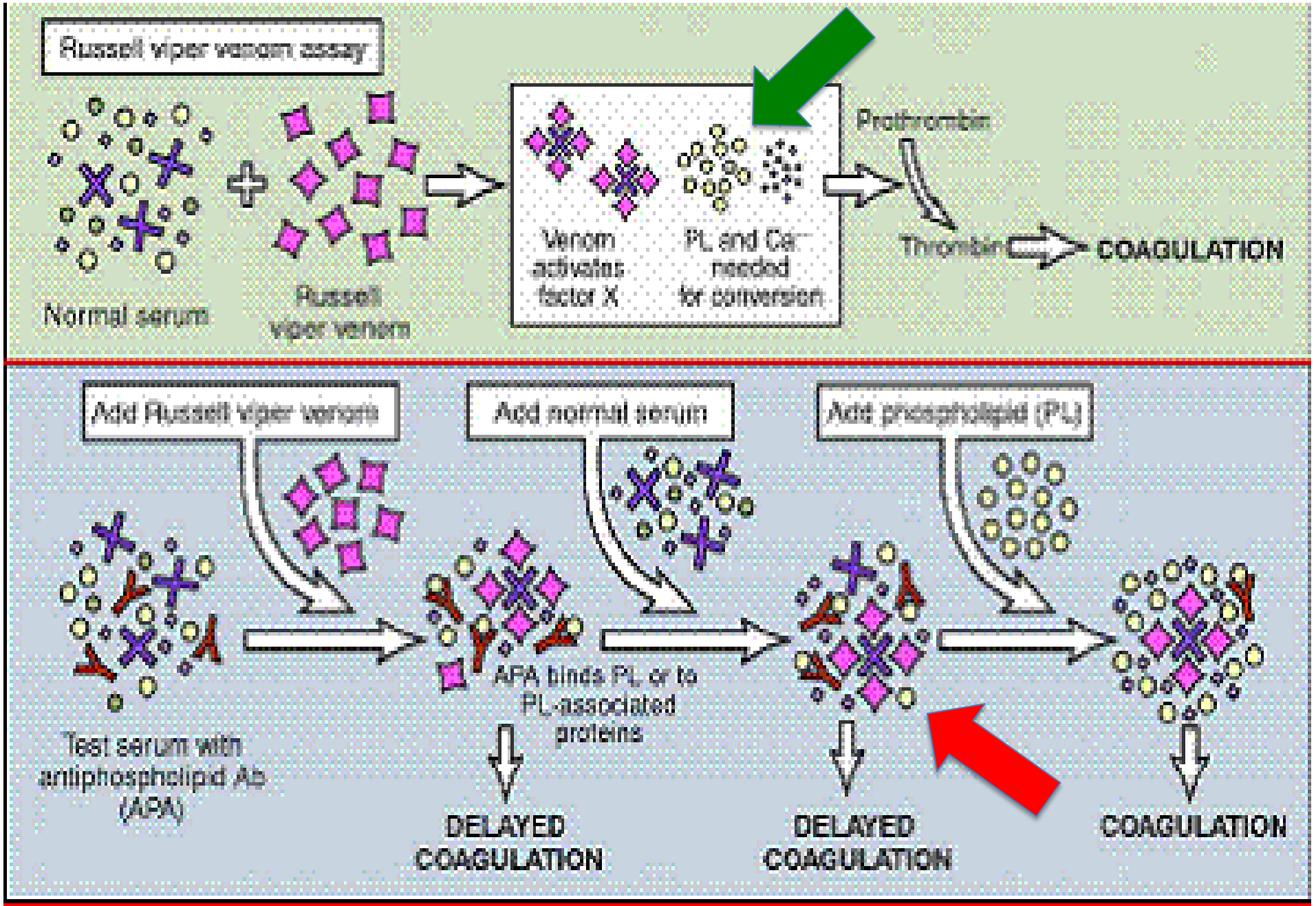
Lupus Anticoagulant (Nonspecific inhibitor)

- interferes with ***in vitro*** phospholipid-dependent coagulation tests (prolonged aPTT and PT)

- antibodies are “nonspecific”
  - not directed to phospholipid directly
  - They do no inhibit a specific coagulation factor

- LAC antibodies target neo-antigenic epitopes of phospholipid-binding plasma proteins
  - beta-2 glycoprotein I
  - prothrombin (coagulation factor II)
  - Because of the involvement of phospholipids, LACs are usually grouped in the antiphospholipid antibody family.

Dilute Russell Viper Venom time - (dRVVT)



Criteria for Testing

- Prolongation of a phospholipid-dependent clotting time
  - **Dilute Russell's viper venom time (dRVVT)**
  - **Lupus sensitive Activated partial thromboplastin time (LS aPTT)** – silica as activator
  - Two or more tests with different assay principles should be performed for screening

- Inhibition of the prolongation must be demonstrated via a mixing test
  - Mixing of patient plasma with normal plasma does not correct the prolonged clotting time

- Phospholipid dependence of the prolongation and inhibition must be demonstrated
  - **dRVVT test**
  - **aPTT-based hexagonal phase phospholipid neutralization test**
  - aPTT-based platelet neutralization procedure

Limitations of Antiphopholipid Ab Testing

- LAC - Functional assays have limitations
  - dRVVT and Lupus sensitive aPTT – reported positive/negative (only need **ONE** to be positive)
  - Evaluate for confounding coagulopathies (drug and factor deficiencies)
- Cardiolipin antibodies
  - reported titre (clinically significant = mod to **HIGH**)
  - aCL antibodies directed against other proteins other than Beta 2GPI are without clinical importance
- Beta-2-glycoprotein-I antibodies
  - unavailable at UHN – ?clinically relevant Ab

CLINICAL CASES

Case 1 – Mr R.C.

Thrombotic events

- Venous Thrombosis
  - DVT/PE – most common presentation
- Arterial Thrombosis
  - less common than venous thrombosis
  - most commonly present with transient ischemic attack or stroke (50%) or myocardial infarction (23%)
  - The presence of aCL is considered to be a risk factor for first stroke
  - involve large and small vessels

Case 1 – R.C.

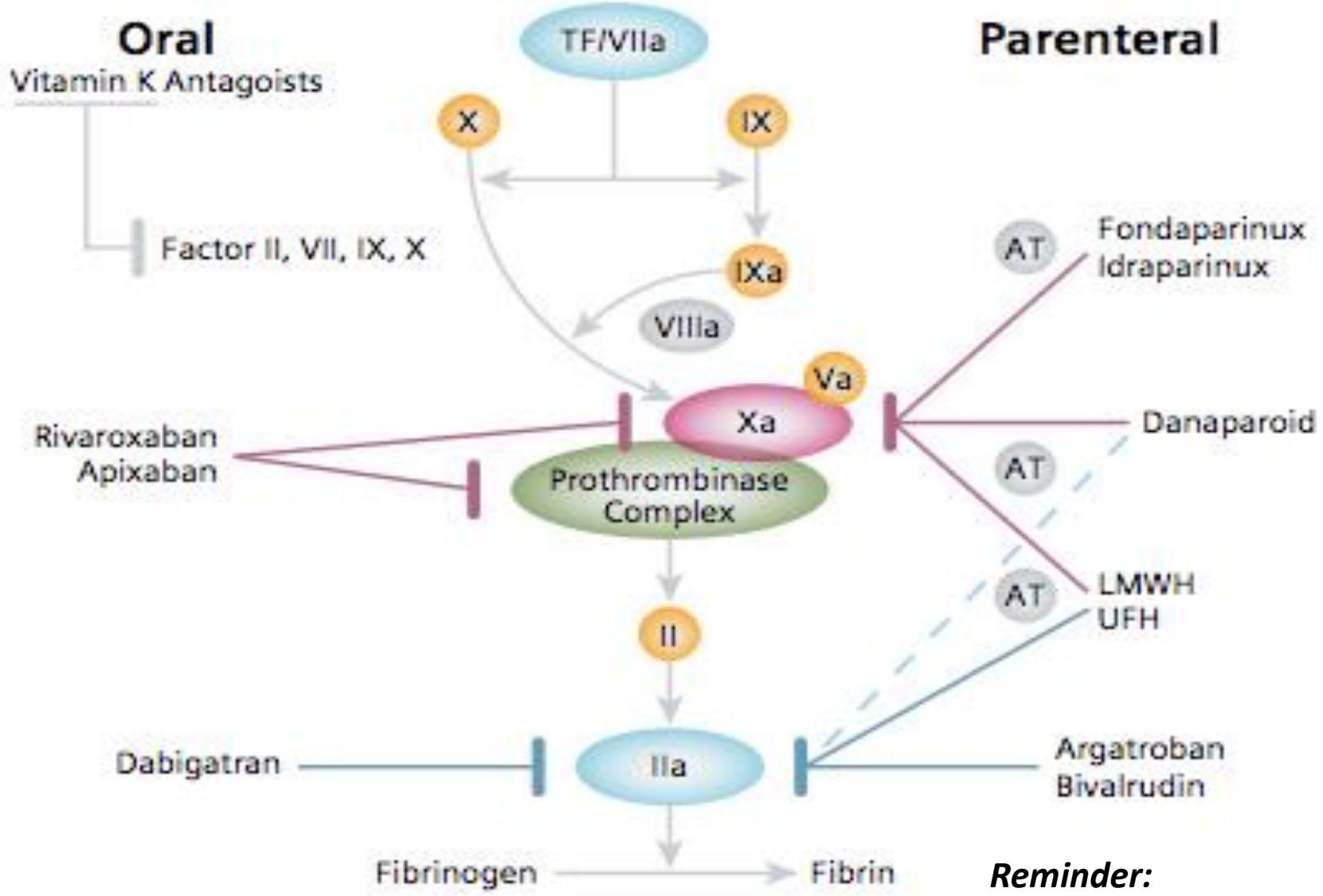
- 32 yo M
- Unprovoked DVT 6 years ago – wanted NOAC, unstable INR (outside of target > 60% - weekly monitoring)
- aCL testing sent October 20/14
  - Titre > 160 GPL-units/ml (ref range: positive >19), ANA borderline positive, dsDNA neg
  - Significant family Hx VTE and autoimmune disease
- Trial Rivaroxaban
  - broke through – extensive right leg DVT and PE – placed back on Warfarin
  - Moderate thrombocytopenia at D/C
  - Treated temporarily with LMWH given post-phlebotic syndrome symptoms
  - Re-presented to ER 2 days later – skin manifestation face
  - further drop in platelet count

Case 1 - Investigations

- aCL IgG >160 GPL-units/ml (Dec 30/14)
- HITT assay neg
- ANA screen repeatedly mild positive (titre 1:80)
- dsDNA, ENA, cryoglobulin and ANCA neg
- C3C4 normal, renal function normal, mild hepatocellular transaminitis, ASMA neg
- US liver – mild hepatomegally with fatty liver, normal spleen, no portal vein thrombosis
- Skin Bx
  - Denuded epidermis with thrombosed dermal vessels and mild inflammatory infiltrates
  - No definite evidence of vasculitis

MANGEMENT:

- bridged back to Warfarin – stabilized, rash resolved quickly, transaminitis resolved



Reminder:

<u>Vitamin K dependent:</u>

Factor X (36 hour half life)

Factor IX (24 hour half life)

Factor VII (6 hour half life)

Factor II (50-72 hour half life)

Treatment APLA Syndrome

- UFH/LMWH to Warfarin
  - target INR 2.0-3.0
  - Refractory cases target INR 3.0-4.0
  - Warfarin + ASA

- RAPS (Rivaroxaban in antiphospholipid syndrome) trial is currently ongoing
  - McMaster – Dr Mark Crowther and Dr Kim Legault
  - TBRHSC site – NOSM IM resident involvement – Dr Manoji Pereira

- Azathioprine
- Rituximab can be considered for recurrent thrombosis despite adequate anticoagulation
- Hydroxychloroquine

Case 2 – Mrs. C.R.

Neurologic - Stroke

- primary thrombosis of cerebral arteries
- embolic occlusion (valvular involvement)
- Recurrent small strokes may contribute to a picture of multiple-infarct dementia

Typically APS patients with stroke are relatively young and lack other classical risk factors of stroke

Case 2 – Mrs C.R.

- 68 yo F
- Rural community without CT scan
- Hx APS – 1998 (LAC) – unprovoked Lt DVT
  - 2012 - stop A/C given bleed risk with recurrent severe thrombocytopenia secondary to her low grade lymphoma – continued low dose ASA
  - No bleeding on Warfarin prior to 2012
- April 2014 - admitted with new Rt cerebellar infarct
  - Tia symptoms March on lone ASA
  - Restarted Warfarin without ASA, restart ASA q3days if digital ulcerations reoccur

Case 2 – Mrs C.R.

- 2 weeks Headache Dec 2014 – Ibuprofen in conjunction with Warfarin and low dose ASA
- Ct head- Dec 27/14 – extensive right subdural hematoma – warfarin reversed
- Warfarin restarted 3rd week January - Jan 28, 2015 – headaches – resolving subdural (warfarin reversed again)
- Repeated interruptions warfarin due to recurrent headaches
- Feb 2/15 – severe dyspnea - ? PE – CT Thorax – microthromboemboli vs NSIP
- restarted A/C IVH to Warfarin – CBC weekly to monitor plt count
  - aCL>160 in addition to persistent positive LAC
- Repeated CT head Jan to Feb for recurrent Headache, dyspnea improved with A/C therapy, new digital ulcerations
- March 2015 – presented with aphasia (resolved) – CT head acute on chronic subdural hematoma

Case 2 - Management

- March/15 - Started Fragmin 12,500 units s/c daily
- Azithioprine 100 mg po daily
  - Non-adherence – concerned about side effects and further drop in platelet count
  - platelet counts (22-60) – inadequate trial of immunosuppression
- Painful digital ulcers refractory to LMWH – May/15 addition of ASA q3days – helpful
- Plt count 28-40 - Declined Rituximab
- Repeat bone marrow confirmed minimal involvement of marginal zone B cell lymphoproliferative disorder – no progression to large B cell lymphoma

August/15 – after development ascites x 1 month – no new thrombosis – CT imaging peritoneal caking with elevated CA-125

Cardiac

- Arterial occlusion may be either thrombotic or embolic
- Premature atherosclerosis
- Valve involvement
  - thickening, vegetation, regurgitation
  - Mitral>aortic
  - Regurgitant abnormality NOT stenotic
  - Case 2 – Moderate Aortic regurgitation – no calcification
- pulmonary hypertension
  - Pulmonary emboli
  - Insitu thrombosis and chronic venous thromboembolic disease

For Recurrent Thrombotic Events - despite adequate therapy

- 13th Congress on aPL antibodies
- INR 3-4.5 vs 2-3 = 22% bleeding vs 4%
- INR 2-3 vs INR 2-3 plus low dose ASA has comparable bleeding risk

Suggest:

- High bleeding risk, low serological risk: use combination
- Low bleeding risk, high serological risk: use INR >3

13th International Congress on Antiphospholipid Antibodies (APLA 2010)
Lupus February 2011 20: 152

Additional Considerations

- Hydroxychloroquine
  - Reverse binding of anti-B2GPI complexes from phospholipid
  - anti-inflammatory and antithrombotic properties
  - known ability to reduce expression of GPIIb/IIIa on activated platelets
- ? Statins
  - Anti-inflammatory effects
- Aggressive management of CV risk factors
  - APL – cardiovascular risk factor

Case 3 – Ms K.G.

Case 3 – K.G.

- 28yo F – recurrent miscarriage in context of IVF
- Unclear APLA testing – positive LAC by RVVT but unclear if on A/C at time of testing
- ASA and Fragmin 5,000 S/C daily at onset of estrogen therapies for pregnancy maintenence
- Successful IVF
- Watched closely for early pre-eclampsia and IUGR
- Delivered healthy twin girls October 2014 – evidence of placental insufficiency
- LMWH 6 weeks postpartum
- Repeat testing true positive LAC – patient elects to continue low dose ASA – weight loss, persistent HTN

Obstetrical Complications

- Recurrent early term pregnancy loss
- fetal demise
- Early pre-eclampsia and eclampsia
- intrauterine growth restriction
- HELLP syndrome
- premature birth
- VTE

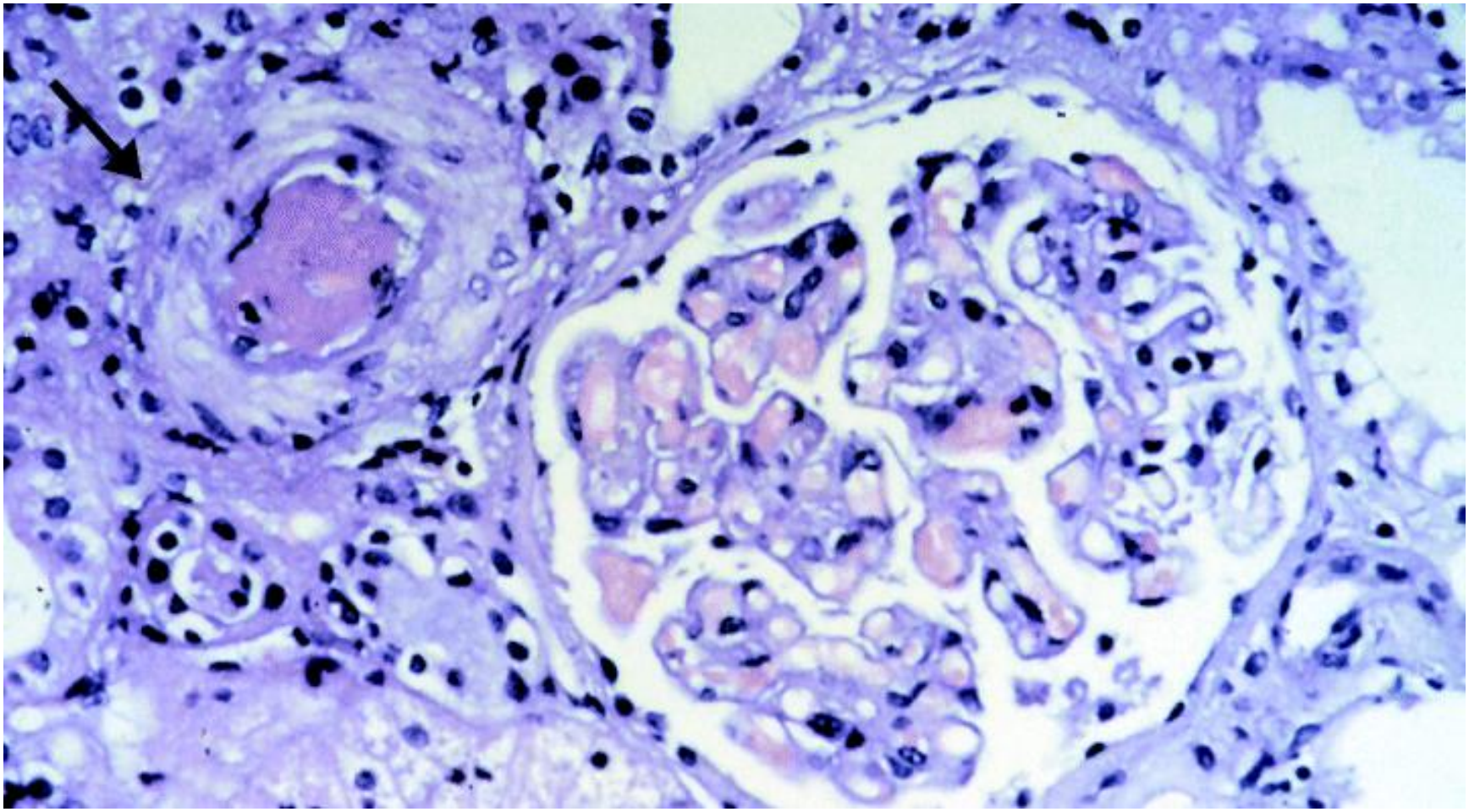
Remember…

- Post-partum thromboprophylaxis needed – intensely prothrombotic time
- Postpartum LMWH for 6 weeks (possibly warfarin)
- LMWH safe in breastfeeding – Mother risk – Sick kids

Renal

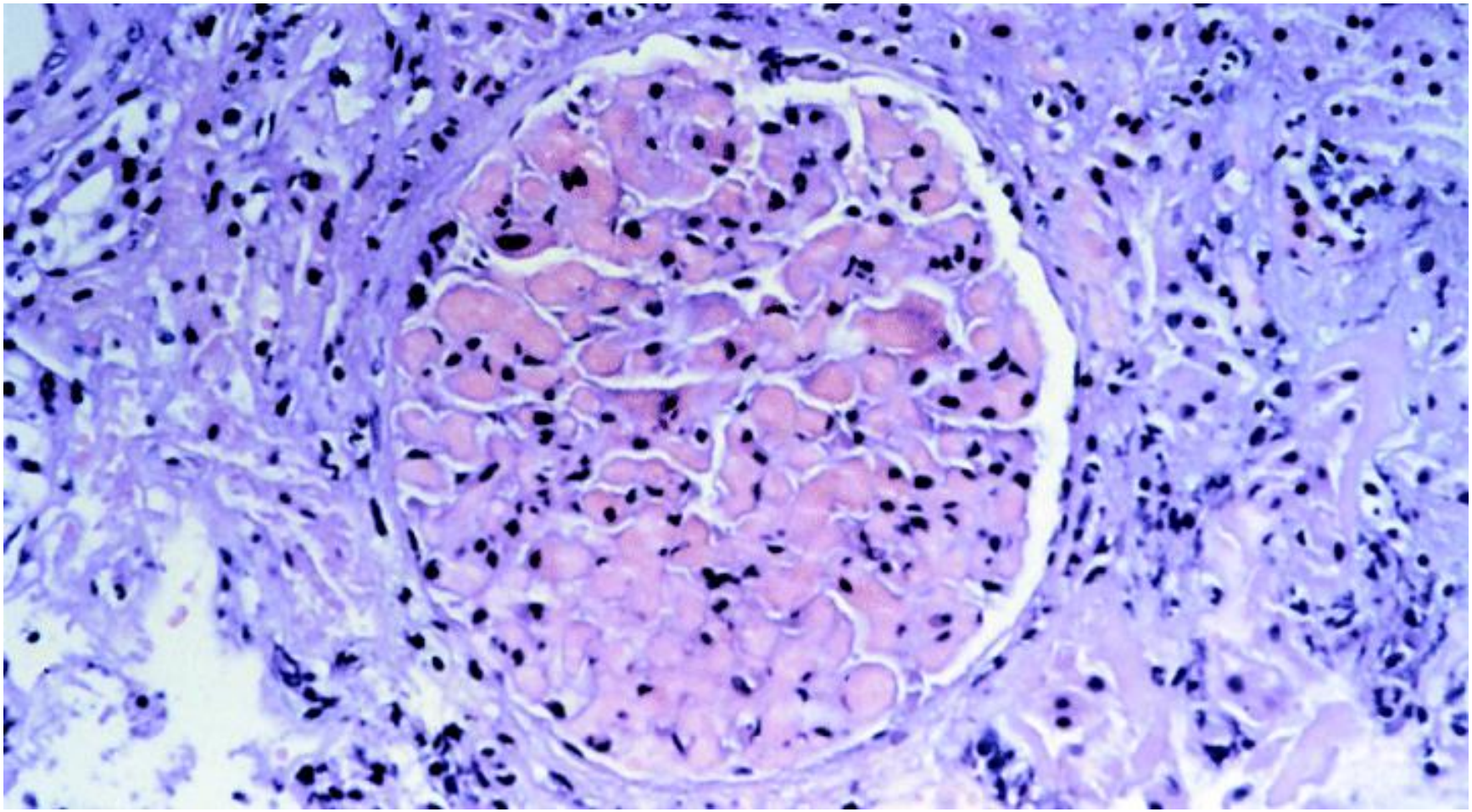
- Thrombosis can occur at any location within the renal
- vasculature.
  - renal artery stenosis
  - malignant hypertension
  - renal infarction
  - renal vein thrombosis
  - reduced survival of renal allografts
  - Glomerulonephritis
- kidney appears to be a major target organ in both primary and secondary APS

APLA Nephropathy



Kidney biopsy findings in 35-year-old woman with antiphospholipid antibody syndrome (APS) nephropathy and systemic lupus erythematosus (SLE) who developed rapidly progressive renal failure

APLA Nephropathy



Glomerular capillaries occluded by agglutinated red blood cells.

Catastrophic APS (CAPS)

- often fatal manifestation of APS
- mortality rate of approximately 50%
- Multi-organ infarctions over a period of days to weeks.
- Differential Dx
  - Disseminated Intravascular Coagulation
  - Infective Endocarditis
  - Thrombotic Thrombocytopenic Purpura

Treatment of Catastrophic APS

- Find and treat trigger – often infection
- Anticoagulation: heparin and warfarin
- Removal/inactivation of aPL
  - Plasmapheresis
  - IVIG
  - High dose steroid

Management Asymptomatic Antiphospholipid Ab

- reduce risk factors for cardiovascular disease
- high titers: avoid oral contraceptive/estrogen preparations
- Life style change: weight control, avoid smoking
- Lipid profile
- Hypertension management
- 2 HIT theory – Autoimmune inquiry/family Hx if unprovoked VTE/arterial event

Highest risk:

- "triple positivity" gives high risk for thrombosis and CV events
- LAC
- persistently high titers of aCL antibodies in IgG class

Recommend:

- prophylactic anticoagulation with LMWH in high risk situations
- Low dose ASA if warranted based on other CV risks

Recommendations for the asymptomatic individual with aPL

- “…a low threshold for the use of thromboprophylaxis at times of high risk is indicated.”
  - Greaves, *et al*. Br.J.Haematol.,2000; 109: 704.

- “In most instances there was consensus in adding low dose aspirin…”
  - Alarcon-Segovia, *et al*. Lupus,2003; 12: 499.

Following the INR

- Check baseline PT
  - If prolongation w/o coagulopathy then will need alternative PT reagent for monitoring

- Do not use point of care devices in APS
  - Inaccurate
  - Not validated

Summary

- Untreated APS
  - permanent disability
  - severe maternal and/or perinatal morbidity
  - Death

- Symptoms can occur in virtually all organ systems

- **Venous thrombosis** and **stroke** are the most common thrombotic manifestations

- In pregnancy the syndrome is associated with adverse maternal and fetal outcomes

- **dRVVT for LAC** is the most useful because positivity correlates strongly with clinical manifestations

- Cardiac valvular disease is an important clinical manifestation and may contribute to the risk of stroke

QUESTIONS?

References

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2. Punnialingam, Khamashta. Curr Rheumatol Rep 2013;15:318
3. Alijotas-Reig. Lupus 2013;22:6-17
4. Cervera et al. Antiphospholipid Antibody. Ann Rheum Dis 2009;68:1428
5. Pengo, V et al. (2009). Update of the guidelines for Lupus anticoagulant detection. Journal of Thrombosis and Hemostasis 7: 1737-1740
6. Meroni,P. Springer (2015). Antiphospholipid Antibody Syndrome – from bench to bedside

Testing available

- ***Coagulation Based Tests (ie RVVT):****
  - APLA react with phospholipid
  - PL provide surface for coagulation to occur
  - Hypothesis - antibodies binding to the phospholipid, it will interfere with the coagulation reaction and prolong the clotting time.

- ***Platelet Neutralization Test: ****
  - plasma which does not correct with a 50:50 mix and adds extracts of platelet. If it corrects to normal this is very specific for APLA.

- ***Hexagonal Phase Phospholipid:***
  - hexagonal phase phospholipids instead of adding normal plasma
  - only valid test for lupus inhibitors when patients are on anticoagulants.

- ***Anticardiolipin Antibodies:****
  - ELISA test for antibodies to cardiolipin.
  - can be performed on plasma which has been anticoagulated.