

Hypercoagulation

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Overview of Hypercoagulation (thrombophilia)

- ❑ Hypercoagulation: poorly understood phenomena
- ❑ No definite cause is identified in > 40% of cases
- ❑ Three major factors in thrombus formation (Rudolf Virchow, 1845): decreased blood flow; changes in the circulating blood (coagulation factors & inhibitors); changes in the vessel wall

Hypercoagulation Disorders

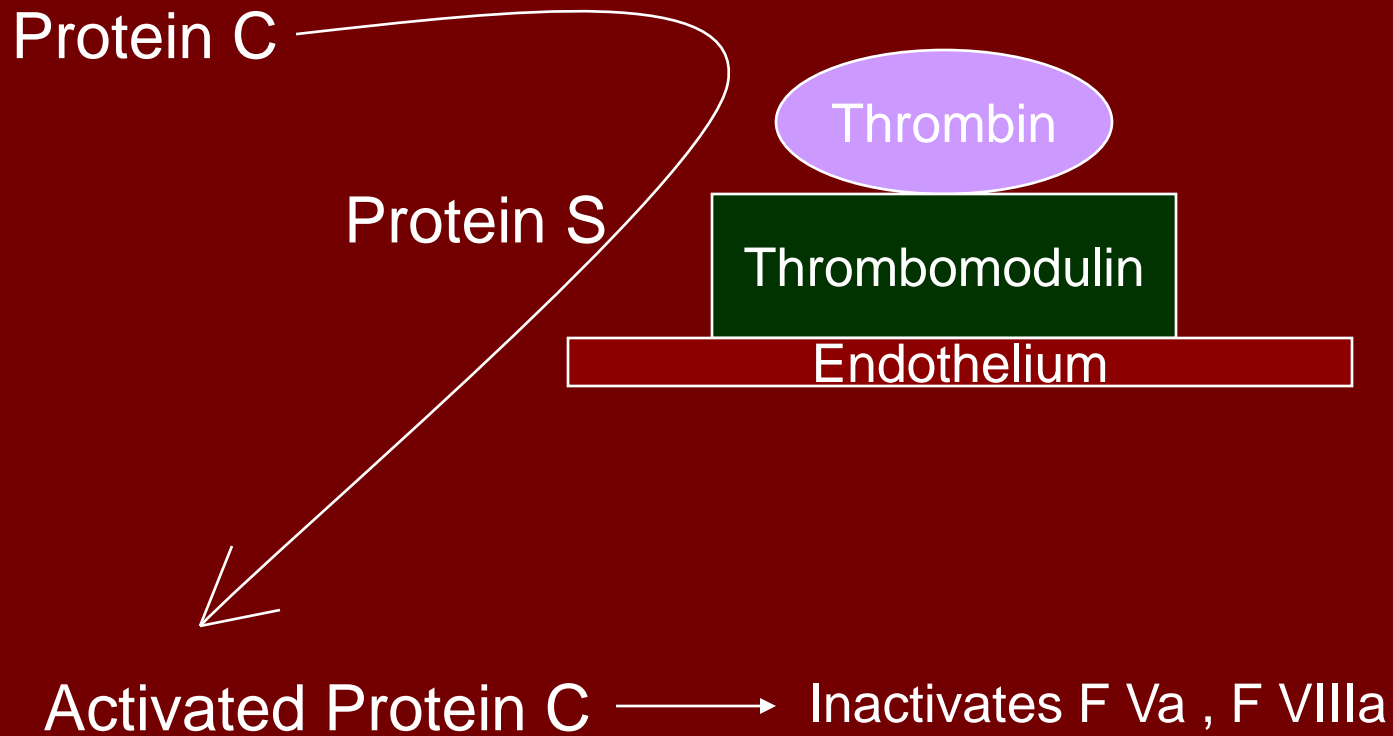
- Factor V Leiden (12-40% of hypercoagulation cases), V
- Prothrombin gene mutation (6-18%), V
- Protein C deficiency (6-10%), V
- Protein S deficiency (5-10%), V
- Antithrombin III (AT III) deficiency (5-10%), V
- Lupus anticoagulant (LA) (10-20%), A+V
- Anticardiolipin antibodies (ACA) (5-10%), A+V
- Heparin-induced thrombocytopenia, A+V
- Hyperhomocysteinemia (10-20%), A+V
- Hypercoagulation in COVID19 infection, A

- ▣ Legends: A (arterial thrombosis), V (venous thrombosis)

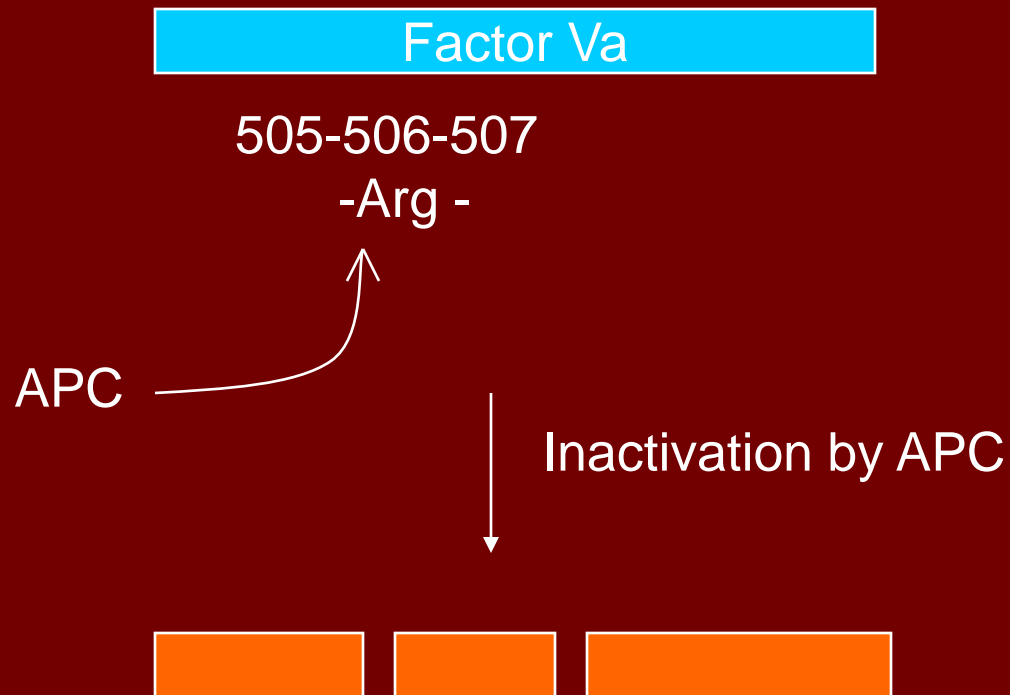
F V Leiden: INTRODUCTION

- Dahlback described an inherited (autosomal dominant) disorder associated with venous hypercoagulation (1993).
- This disorder is due to a mutation in Factor V gene on chromosome 1 (the mutated gene is called Factor V Leiden). Mutation at nucleotide 1691: Guanine-> Adenine, causing substitution at position 506: Arginine-> Glutamine [V506Q]
- Note: FV HR2 haplotype (A4070G, His199Arg) has unknown risk

Protein C Pathway

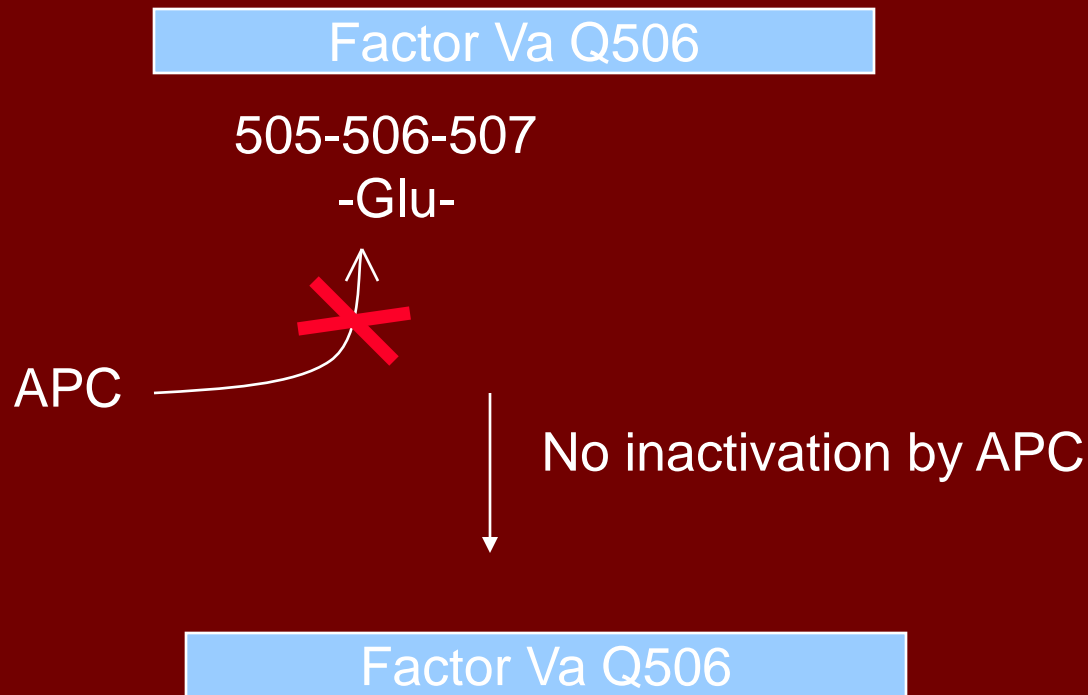


Cleavage Site on Factor V by APC: Inactivation of Factor V in normal patient



Cleavage Site on Factor V by APC:

No inactivation of Factor V in patient with Factor V Leiden (95% of APC resistance cases)



Two Forms of Factor V Leiden

- Heterozygous: 3-7% of general population, 3-5 fold increase in risk of deep vein thrombosis, 20% have thrombosis by 33 y/o (mean age of first thrombotic episode)
- Homozygous: 0.06-0.25% of general population, 50-100 fold increase in risk of deep vein thrombosis, 40% have thrombosis by 33 y/o

Other Relevant Information on Factor V Leiden

- Some patients do not have thrombosis unless exposed to hemostatic challenge
- Increased risk for hypercoagulation in combination with other risk factors (such as Lupus anticoagulant, Protein C or S deficiency)
- Factor V procoagulant activity is normal
- Treatment: heparin, coumadin

PCR Testing for F V Leiden

- Amplifies the mutated gene fragment. Results: negative, heterozygous, homozygous.
- Results not effected by factor deficiency, lupus anticoagulant, anticoagulant (as in clot-based tests)

Note: PCR testing cannot detect APC resistance that is not due to FV Leiden

PCR Testing (cont'd)

- Genomic DNA from lymphocytes
- DNA sequence flanking the mutation site is amplified by PCR, resultant product is analyzed by restriction enzyme digestion
- Normal (wild type): two normal FV alleles
Heterozygous: one abnormal allele
Homozygous: two abnormal alleles

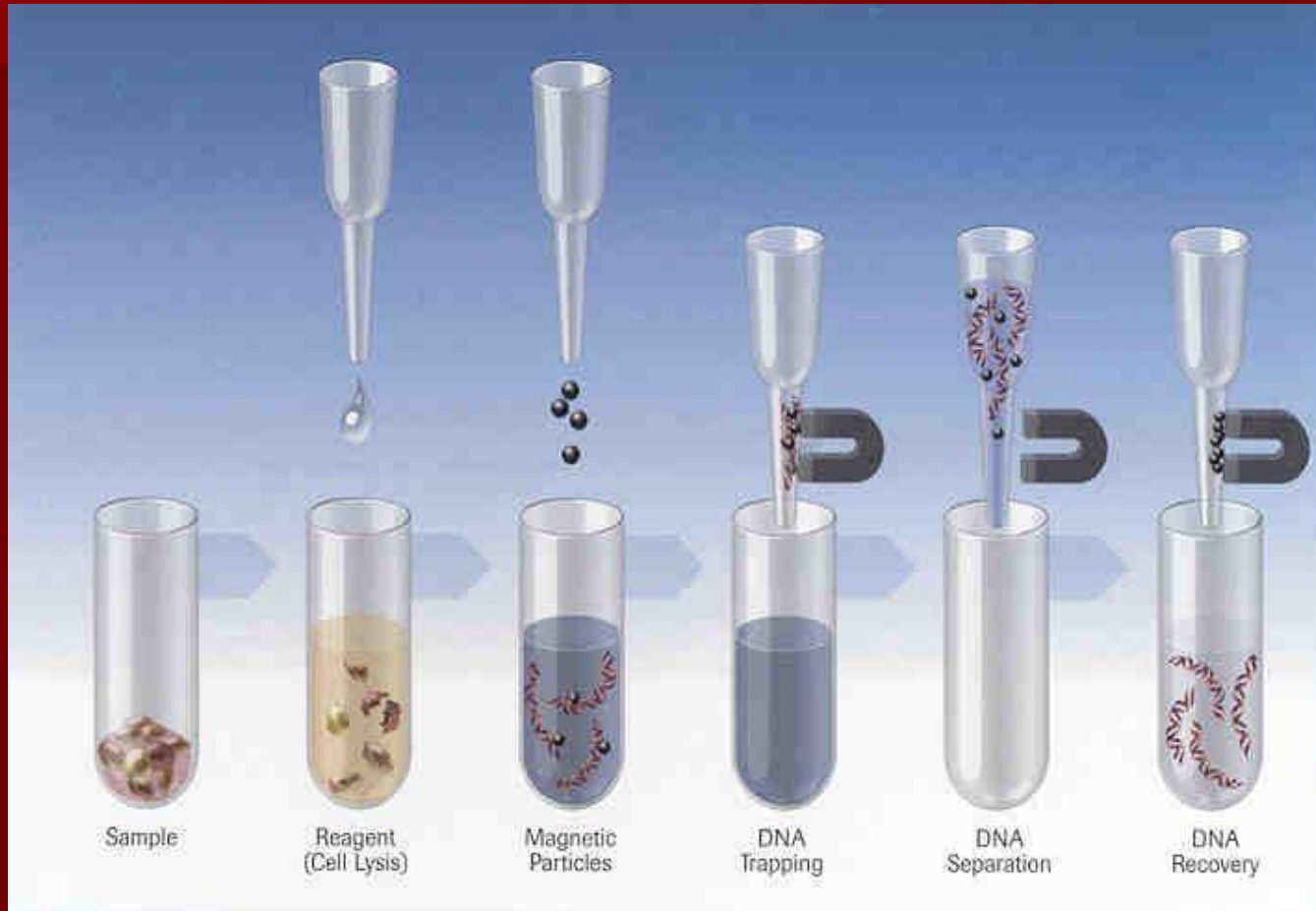
New PCR Testing: LightCycler (Roche)

- Melting curve analysis method
- “Real time” analysis
- 35 thermal cycles in 25 min, followed by melting curve analysis in 5 min -> results in 30 min
- Batch of 32 samples
- Designed for clinical lab setting
- Optional module for automated DNA extraction (60 min for 32 sample extraction)

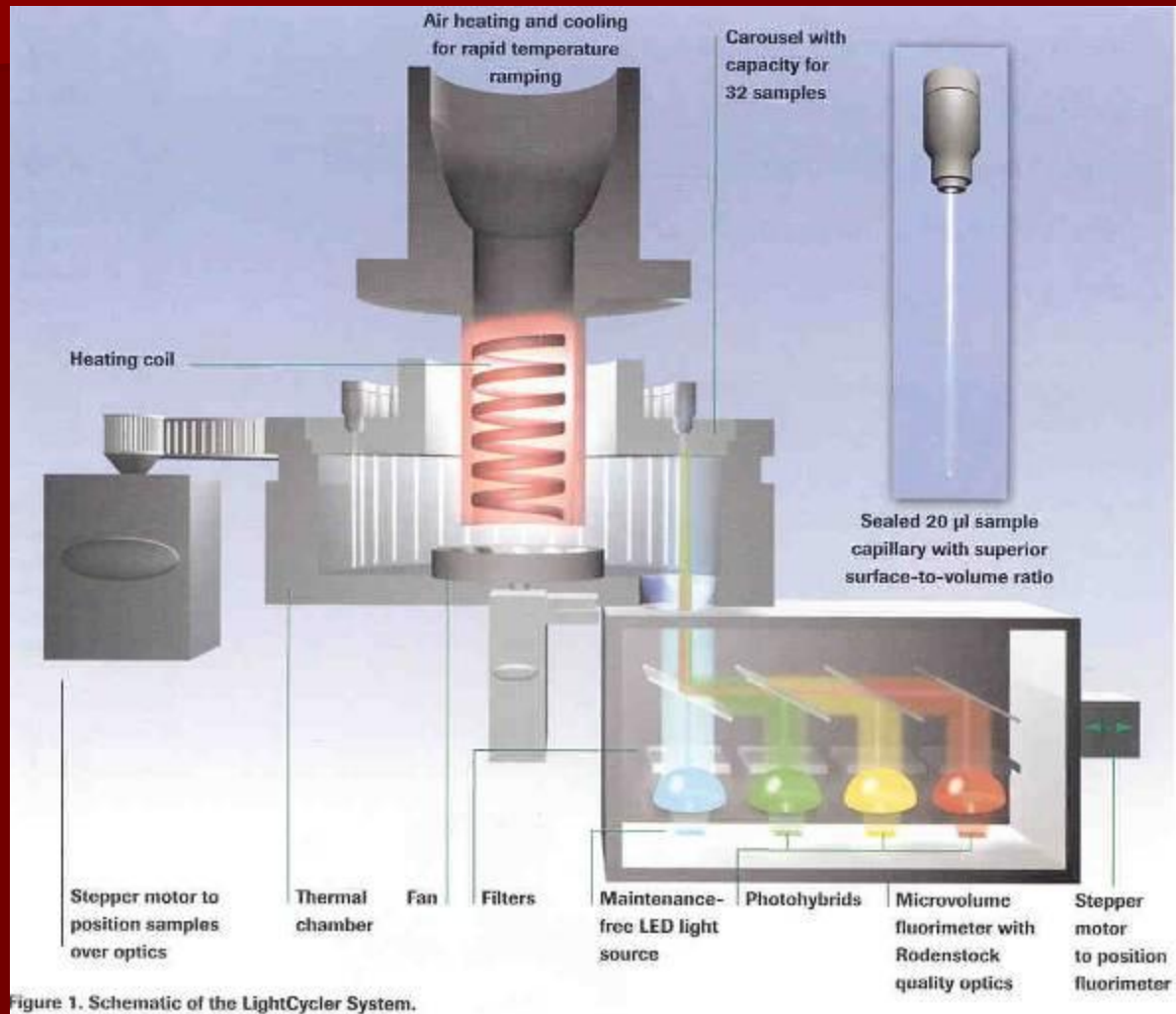
MagNA Pure LC and LightCycler



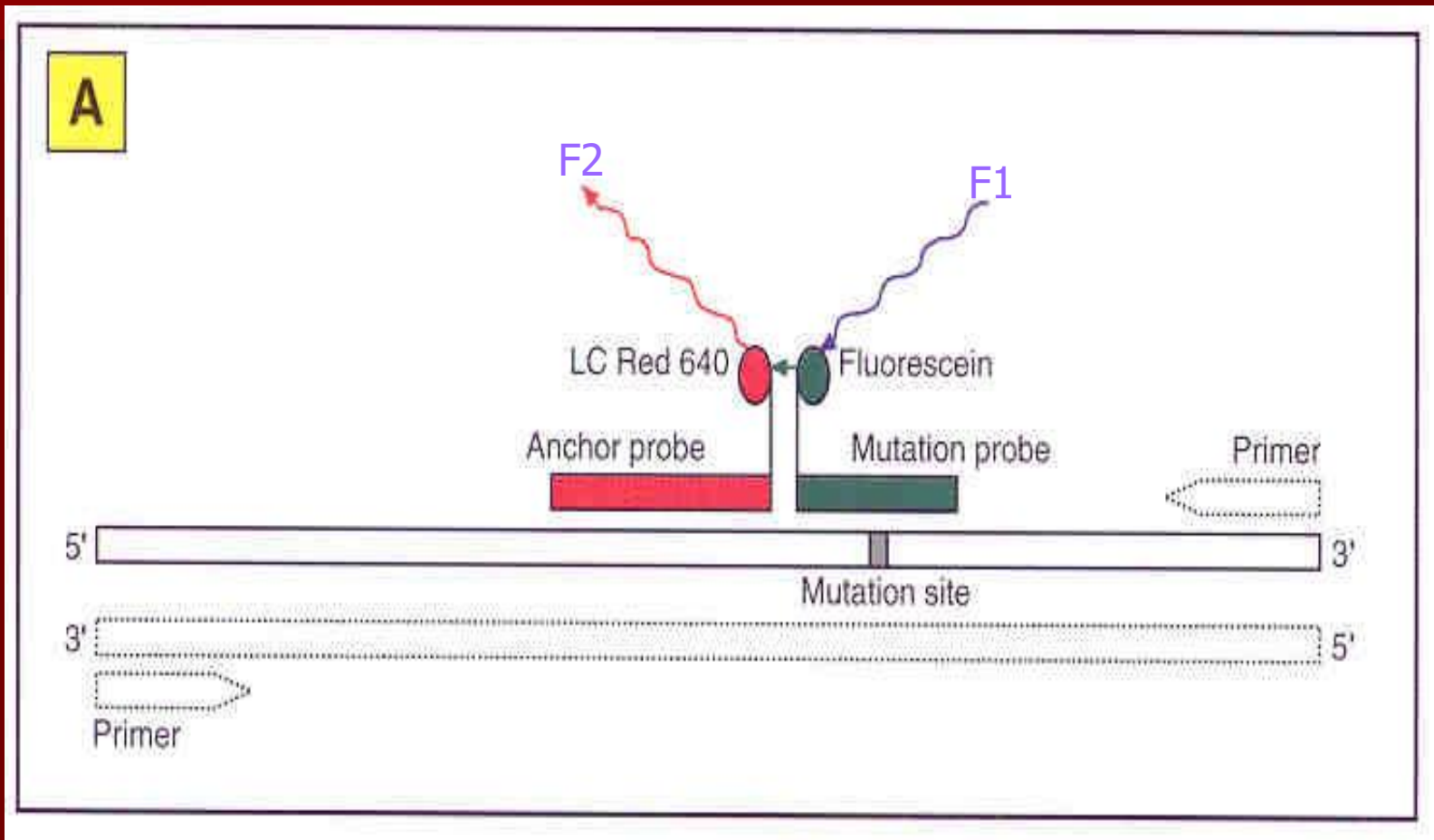
Magnetic Bead Technology for DNA Extraction



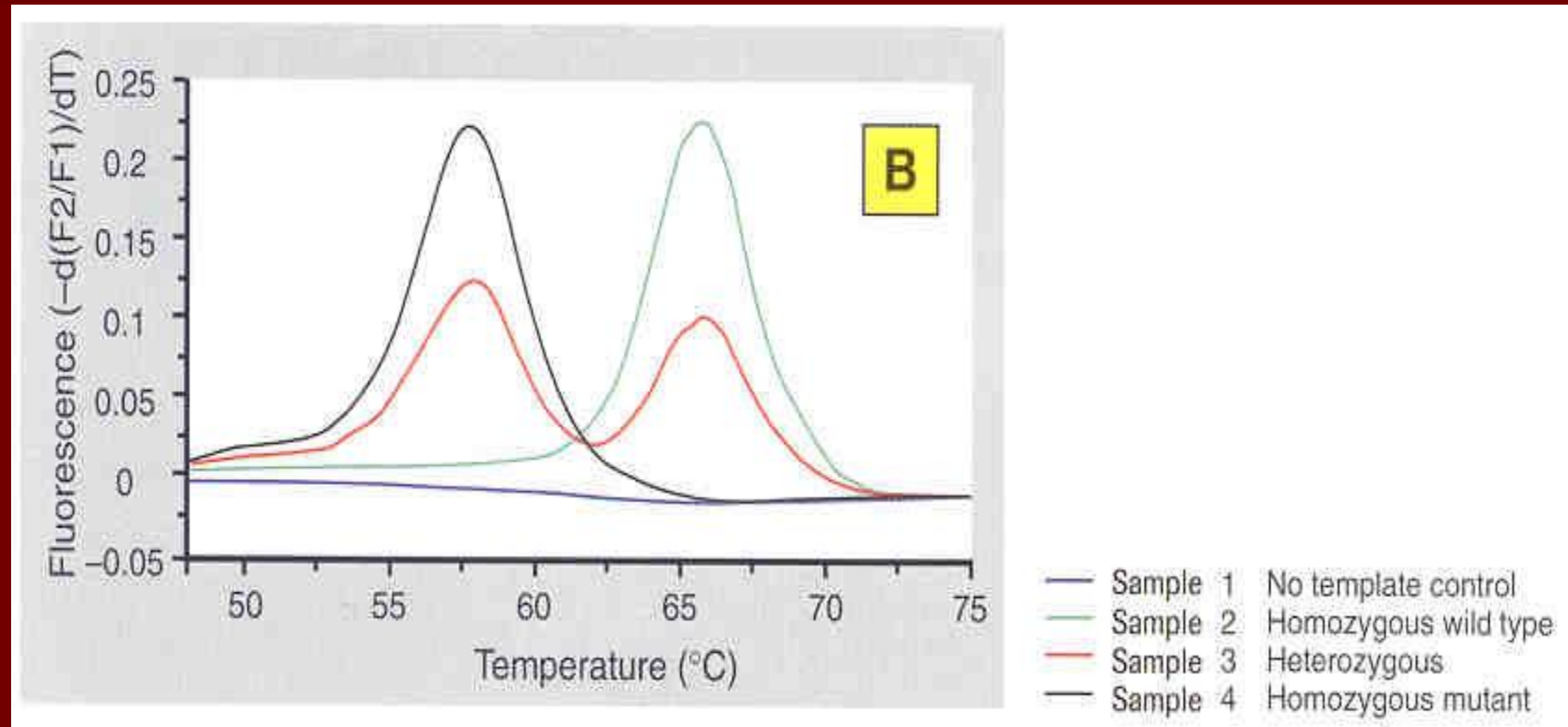
LightCycler Schematics



FV Leiden Mutation: Hybridization Probe with Fluorescence Resonance Energy Transfer (FRET)



FV Leiden Mutation: Melting Curve Analysis



Prothrombin Gene Mutation

- ▣ Single base pair substitution at nucleotide position 20210 in chromosome 11, guanine-> adenine (**G20210A**) . This results in relatively high prothrombin level with increased risk for venous thrombosis (132% vs 105% of normal)
- ▣ Autosomal dominant. 1-3% of Caucasian population; risk increased 3 fold (heterozygote)
- ▣ 5-18% of hypercoagulation cases. Tx: heparin, coumadin
- ▣ Laboratory: PCR testing for G20210A, Factor II assay (optional)

Protein C Deficiency

- ▣ Protein C: a vitamin K-dependent coagulation inhibitor; synthesized in the liver; inactivating F Va and F VIIIa
- ▣ Protein C deficiency: autosomal dominant; 0.14-0.5% of population; risk increased 6.5-8 fold; 6-10% of hypercoagulation cases
- ▣ Clinical manifestation: recurrent deep vein thrombosis, pulmonary embolism, neonatal purpura fulminans (in homozygote). Tx: heparin, Coumadin
- ▣ Laboratory: immunological, functional assays, no mutation testing (>160 mutations)

Protein S Deficiency

- ▣ Protein S: a vitamin K-dependent protein; synthesized in the liver and megakaryocytes; cofactor of protein C
- ▣ Protein S deficiency: autosomal dominant; 0.7% of population; risk increased 1.6-11.5 fold; 5-10% of hypercoagulation cases
- ▣ Clinical manifestation: recurrent deep vein thrombosis, pulmonary embolism, neonatal purpura fulminans. Tx: heparin, coumadin
- ▣ Laboratory: immunological assay, functional assay, no mutation testing (>70 mutations)

AT III Deficiency

- ▣ AT III: inactivates thrombin and other factors (Xa, IXa, XIa, XIIa, kallikrein); accelerated by heparin
- ▣ AT III deficiency: autosomal dominant; 0.17% of population; risk increased 5-8.1 fold; 5-10% of hypercoagulation cases
- ▣ Clinical manifestation: recurrent deep vein thrombosis, pulmonary embolism. Tx: AT III, heparin, coumadin
- ▣ Laboratory: functional assay (chromogenic), immunologic assay, no mutation testing (>250 mutations)

Lupus Anticoagulant

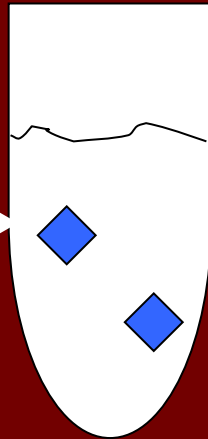
- ▣ Immunoglobulins that prolong in-vitro phospholipid-dependent clotting times
- ▣ Found in various conditions; 30% of patients have thrombosis ; 10-20% of hypercoagulation cases
- ▣ Antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibody, antiphosphatidyl serine, anti Beta 2 Glycoprotein I, etc): 1-2% of population, 50% of SLE patients
- ▣ Clinical manifestation: variety of thrombotic diseases. Tx: Heparin, Coumadin, Aspirin & prednisone (to prevent fetal demise)
- ▣ Laboratory: aPTT, dilute Russell Viper venom time (dRVVT), Hexagonal Phospholipid Neutralization, Platelet Neutralization Procedure

Evaluation of lupus anticoagulant

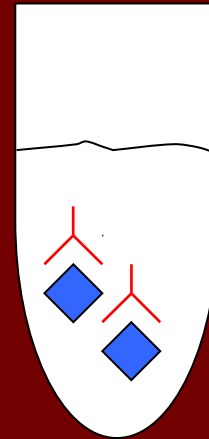
- Mixing studies
 - Mix equal parts patient and control plasma
 - aPTT will correct if prolongation due to factor deficiencies
 - If LA present will fail to correct aPTT
 - Usually immediate acting (before incubation)

Dilute Russell Viper Venom Time

Dilute
Phospholipid



Normal plasma
dRVVT 36-42 sec



Plasma with lupus anticoagulant
dRVVT > 43 sec

Neutralization Procedure

Neutralization study:

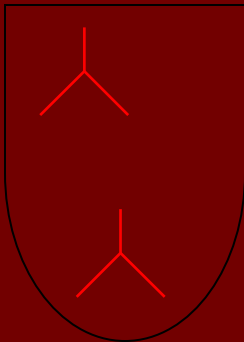
Addition of phospholipid will neutralize lupus anticoagulant

Two common neutralization tests:

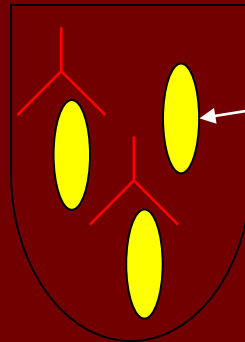
- Platelet neutralization:
lysates of frozen platelets-> thawed and washed before testing
- Hexagonal phase phospholipid neutralization:
hexagonal phospholipid with high affinity for lupus anticoagulant

Neutralization Procedure Tests

Platelet Neutralization



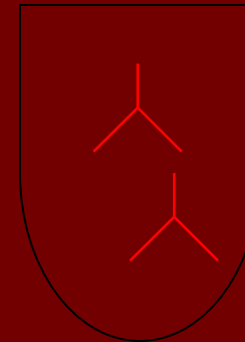
Prolonged aPTT



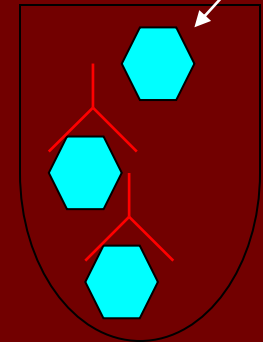
Platelets

Shortened aPTT

Sta Clot-LA



Prolonged aPTT



Hexagonal
PL

Shortened aPTT

Clotting time decreases for >10 seconds after addition of PL -> pos for LA

Anticardiolipin Antibodies

- ▣ ACA: IgG, IGM, IgA
- ▣ Found in various conditions; thrombotic manifestations; in 5-10% of hypercoagulation cases. Tx: not well worked out, including Heparin, coumadin, and steroid
- ▣ Laboratory: ACA by ELISA; high levels are associated with high risks of thrombosis

Anticardiolipin Antibodies (cont'd)

	Normal range	Clinically insignificant	Moderate risk	High risk
IgG	< 15 GPL	15-20	20-80	> 80
IgM	< 12.5 MPL	12.5-20	20-80	> 80
IgA	< 15 APL	15-20	20-80	> 80

Genetic and Environmental Factors in Thrombophilia

- ▣ Combination of risks: genetic abnormality + environment factor (trauma, surgery, immobility, pregnancy, oral contraceptive, etc.)
- ▣ Multiple genetic abnormalities lead to synergistic effect:
Example: Factor V Leiden heterozygote (risk 3-6 fold) + Prothrombin gene mutation heterozygote (risk 3 fold)-> risk 25 fold
- ▣ Hypercoagulation is relatively expensive since the panel includes all significant tests

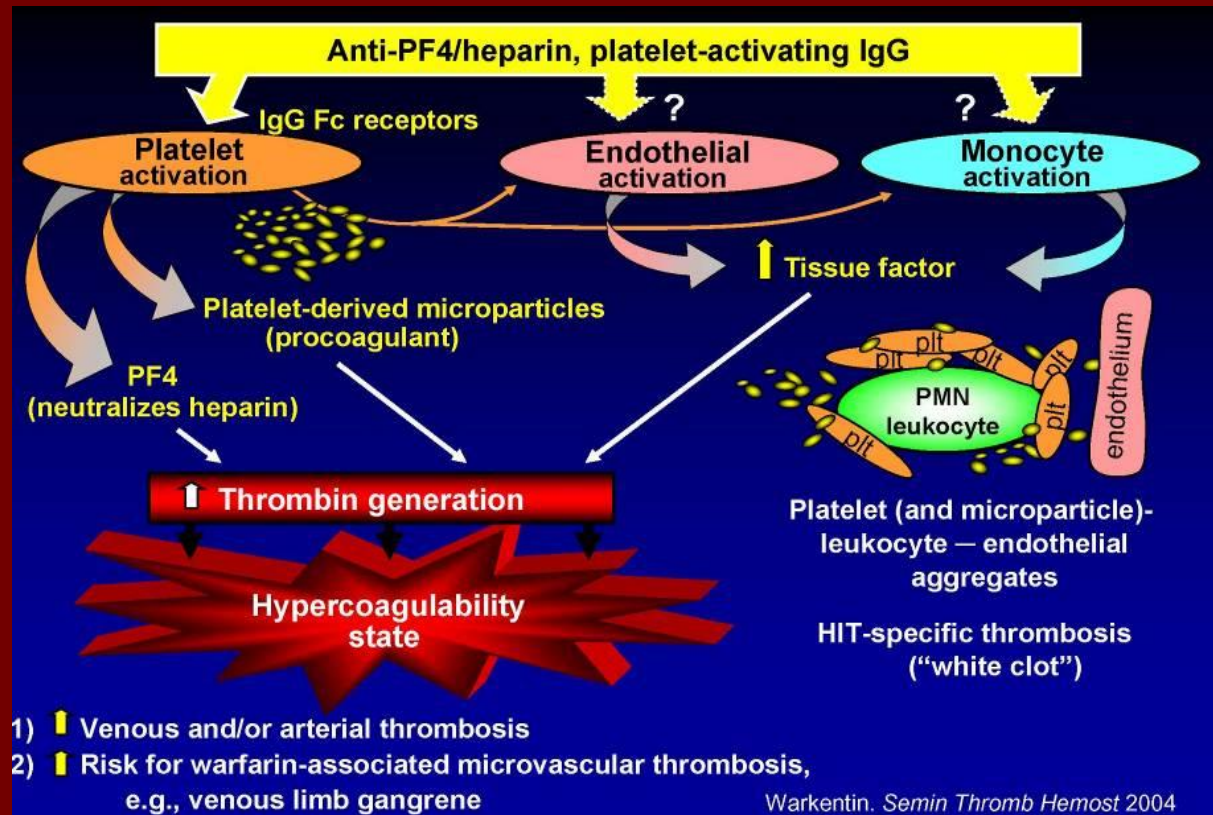
Interference in hypercoagulation tests

- Patients in active DVT/PE may have low levels of: AT, Protein C, protein S (normal levels are still useful)
- Heparin affects: lupus anticoagulant
- Coumadin affects: protein C, protein S

- For baseline testing of the above:
 - Get pre-anticoagulant samples
 - Test patients after discharge (follow-up visit)
 - Patients off heparin for one day
 - Patients off coumadin for 2 weeks

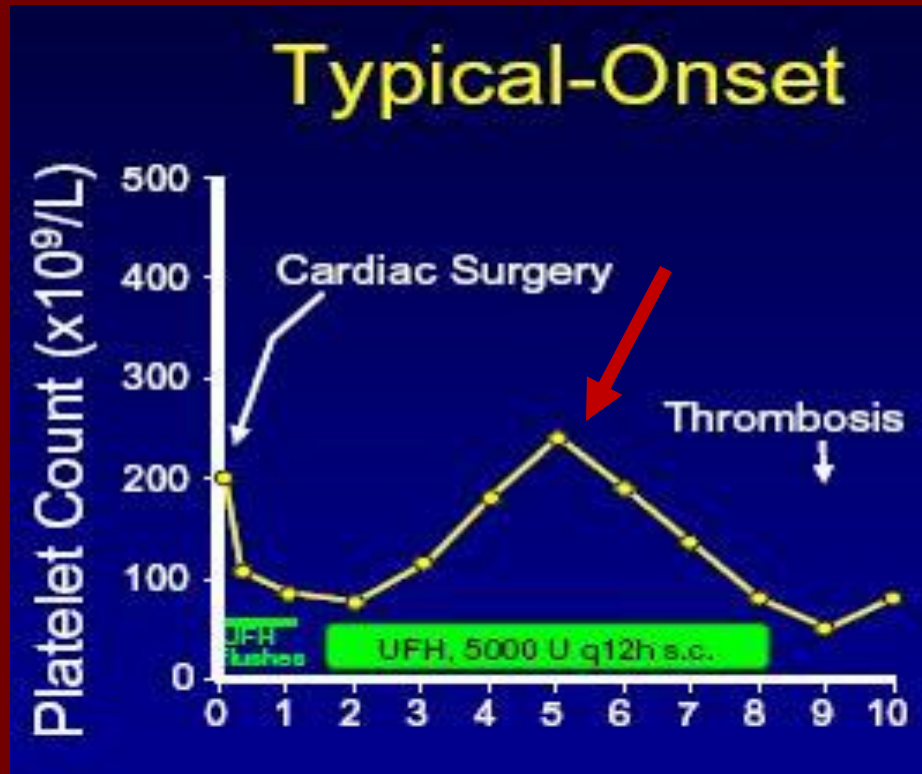
Heparin-induced thrombocytopenia (HIT)

- Upon exposure to heparin, the immune system may form antibody against heparin and platelet factor 4 (PF4)
- Upon re-exposure to heparin, the development of these antibodies takes about 5 days to activate platelets forming thrombosis and subsequent thrombocytopenia



HIT

- Risk of HIT is higher with UFH than with LMWH
- Typical onset: 5 days after heparin exposure



Example for cardiopulmonary bypass

Complications of HIT

- Venous thrombosis (50%)
- Arterial thrombosis (10-15%)

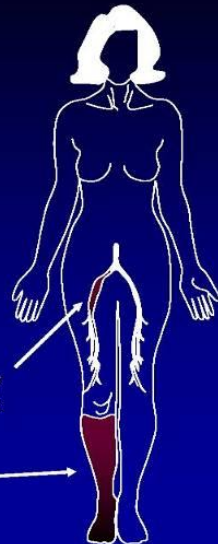
Ischemic Limb Syndromes in HIT



White clot syndrome

Limb artery thrombosis

Acral necrosis



Warkentin *J Crit Illn* 2002

Heparin-induced Skin Lesions



Necrotizing lesions

Erythematous plaques

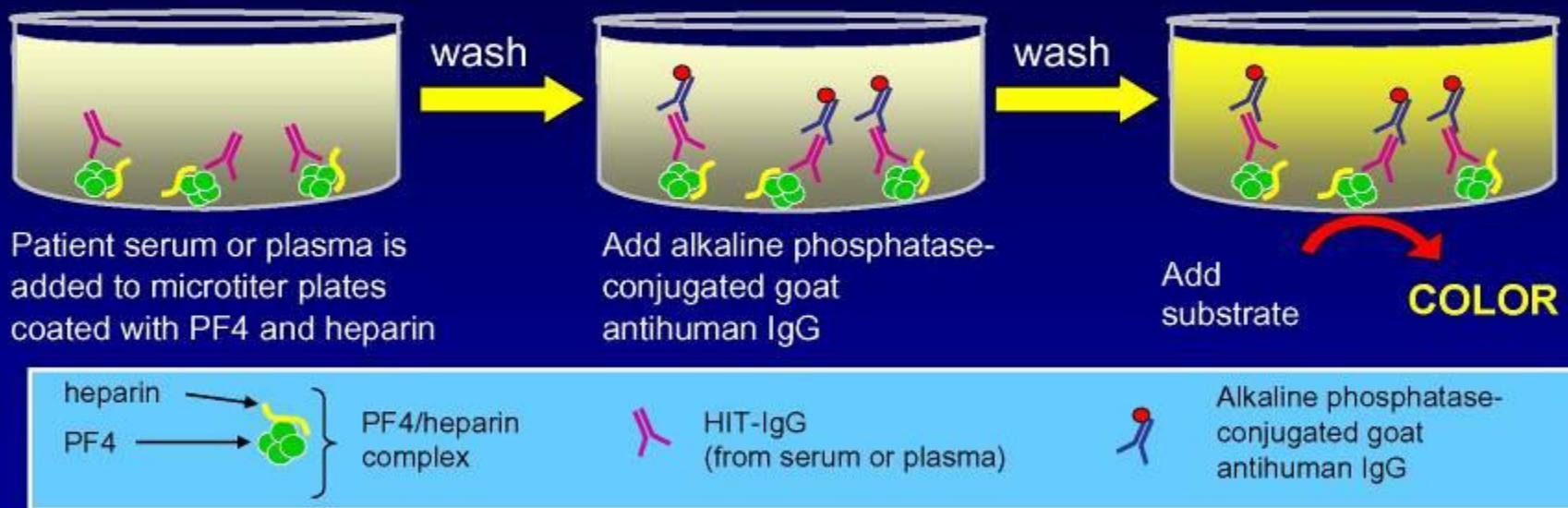
Warkentin *Br J Haematol* 1996

Testing for Heparin Antibody

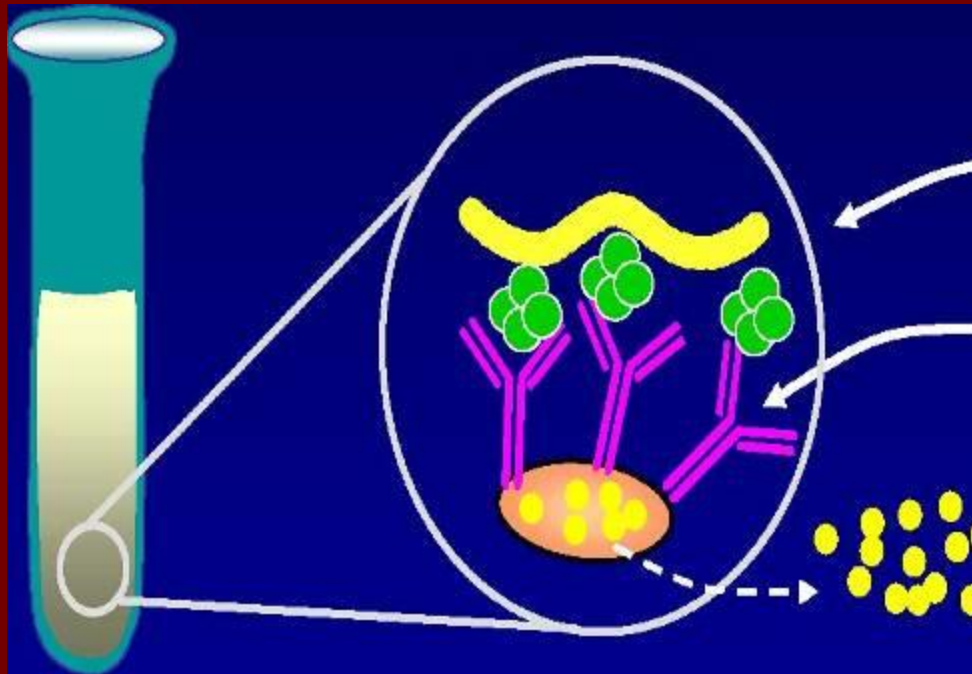
- Heparin-PF4 antibody (ELISA): sensitivity 82%, specificity 70%
- Serotonin release assay: sensitivity 80%, specificity 85%, limited use due to radioisotope (^{14}C)

Anti-PF4/heparin by ELISA

Solid-phase Anti-PF4/heparin-ELISA “Immunoassay”



Anti-PF4/heparin by Serotonin release assay



Heparin/PF4 complex

HIT IgG

Radiolabeled serotonin
released from platelets

HIT Treatment Principles

- Stop Heparin
- Switch to alternate anticoagulant
(Danaparoid, Lepirudin, Argatroban, Angiomax)
- No warfarin (which would decrease proteins C and S)
- No prophylactic platelets

Hypercoagulation in COVID19 Infection

- J Thrombosis Research (April 2020): 31% of 184 COVID19 patients suffered thrombotic complications
- New England Journal of Medicine (May 2020): young patients in the 30's and 40's with strokes
- The Lancet (April 2020): the virus enters cells via the angiotensin converting enzyme 2 (ACE2) receptors, which are most commonly found in the alveolar epithelial cells, followed by endothelial cells. When the virus binds to these cells, it may damage the blood vessel, especially the microcirculation of the small blood vessels, and thus activates platelet aggregation, leading to hypercoagulation.
- Pulmonary intravascular coagulopathy (Lancet Rheumatol 2020, May 7, 2020): elevated D-Dimer/fibrinogen, normal platelet count, elevated cardiac enzymes with pulmonary hypertension). Lungs filled with microclots helped explain why ventilators work poorly for patients with low blood oxygen. The microclots block circulation and blood is leaving the lungs with less oxygen.

Hypercoagulation in COVID19 Infection

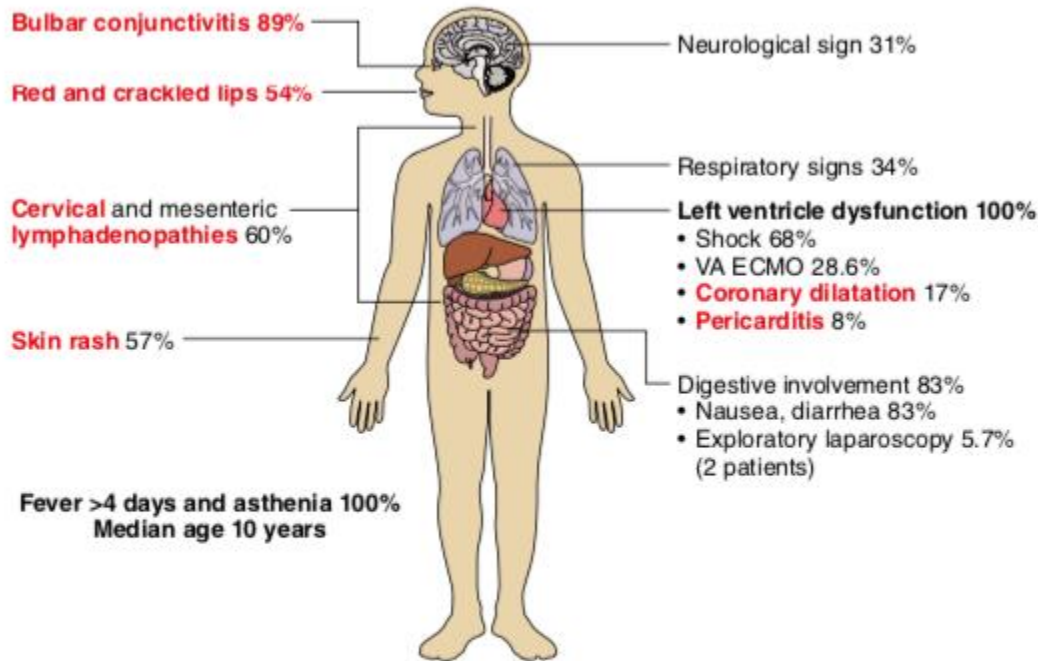
- The International Society on Thrombosis and Haemostasis (ISTH) recommended that all hospitalized COVID-19 patients, should get prophylactic-dose low molecular weight heparin (LMWH), unless they have contraindications (active bleeding and platelet count $<25 \times 10^3/\mu\text{L}$).
- Many institutions choose threshold values upon which to start systemic anticoagulation: D-dimer $>1,500$ ng/mL or fibrinogen >800 mg/mL

Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with COVID19

- While children are less likely to become severely ill than older adults, there are subpopulations of children with an increased risk for more significant illness (MIS-C).
- On April 26, 2020, reports in the United Kingdom (UK) of 8 previously healthy children presenting with a severe inflammatory syndrome with Kawasaki disease-like features: persistent fever and a constellation of symptoms including hypotension, multiorgan (e.g., cardiac, gastrointestinal, renal, hematologic, dermatologic and neurologic) involvement, and elevated inflammatory markers. As of May 12, 2020, the New York State Department of Health identified 102 patients with similar presentations
- Most patients responded well to intravenous immunoglobulin (IVIG) and high-dose aspirin (ASA) [Veena Jones et al, J Hospital Pediatrics 2020]

Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with COVID19

SARS-COV-2 related multisystem inflammation



Skin rash

Some of the patient presentations for MIS-C and findings from the Circulation study on multi-system inflammatory syndrome in children. Image courtesy of the American Heart Association. The findings suggest coronavirus is linked with this inflammatory disease in children.