Investigations in Medicine
Haematology

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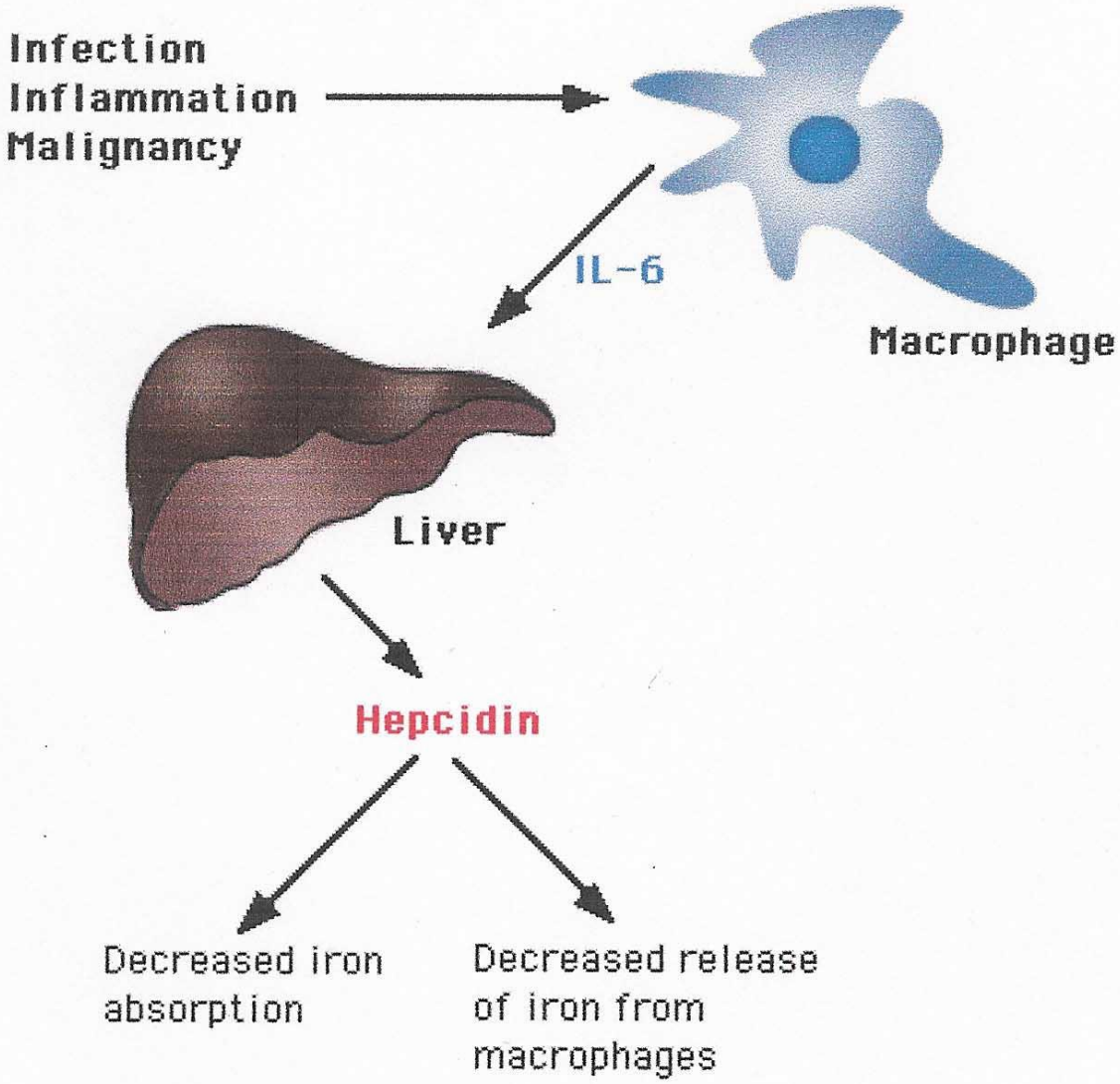
Haematologist

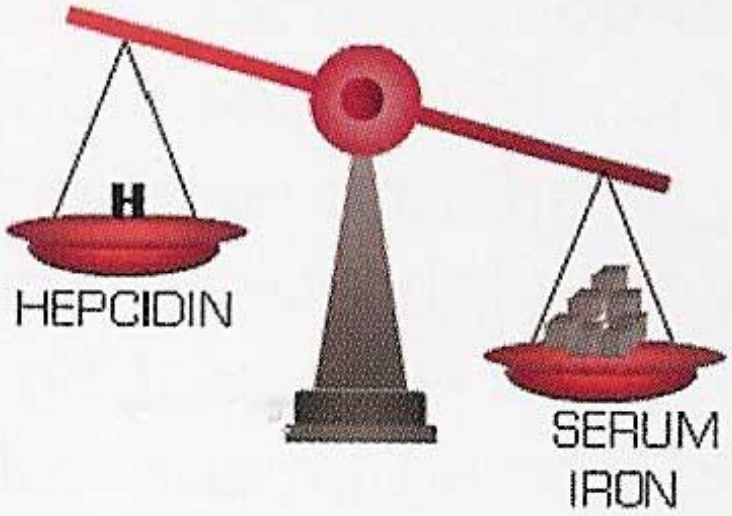
Reticulocyte counts

- Larger bluish (polychromatic) red cells (RNA)
- Increased with regenerating marrow
  - blood loss
  - haemolysis
  - post chemotherapy
- Reduced in
  - anaemia of chronic disease
  - deficiencies -B_{12}, folate, Fe
  - significant marrow disorders eg aplasia, MDS

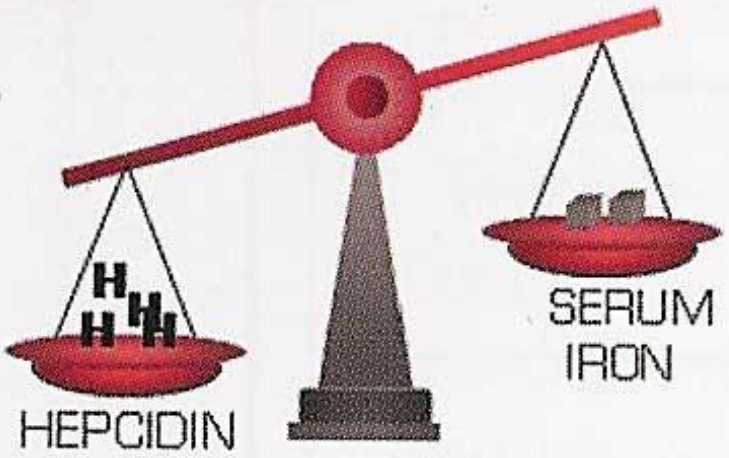
Anaemia of chronic disease

- Low Hb usually >90g/l, low reticulocyte count
- Unable to utilise iron
- Normal or low MCV
- High ferritin (acute phase/not utilised)
- High CRP
- May have low EPO, but often normal, few respond
- Mediated by TNF, γIFN, hepcidin
- If iron deficient %sat low, ferritin low-normal
- Trial of oral iron often indicated - will need investigation
- Anaemia of chronic disease may respond to iron infusion


Infection
Inflammation
Malignancy
IL-6
Macrophage
Liver
Hepcidin
Decreased iron absorption
Decreased release of iron from macrophages



Genetic hemochromatosis



Anemia of inflammation

Macrocytosis

- Liver disease
- Alcohol
- B_{12}/folate deficiency
- Drugs affecting DNA eg MTX, HU
- Hypoxia
- Reticulocytosis (gross)
- Myelodysplasia
- Others - hypothyroidism, cold aggs, aplasia, EPO

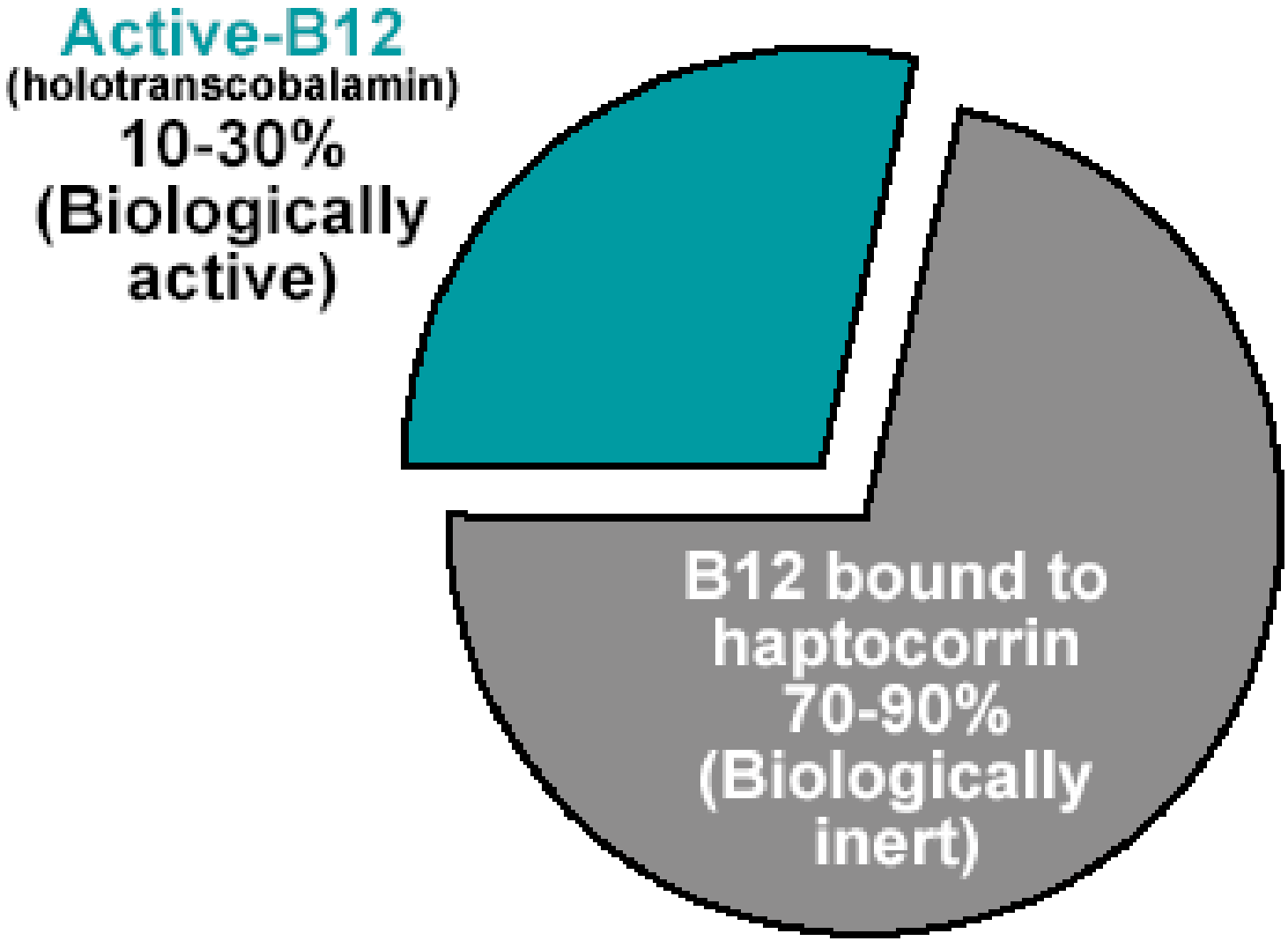
Low B_{12} levels

- 20% due to pernicious anaemia
- Elderly - atrophic gastritis, reduction in absorption of food bound B_{12}
- Gastrectomy
- Vegans - (some B_{12} on mushrooms)
- Terminal ileal disease
- Metformin
- Erroneous
- Low levels, but normal tissue stores

Pernicious anaemia

- Morphology
  - PB - RBC oval macrocytes, hypersegmented neutrophils, cytopenias
  - Marrow - megaloblastosis
- IF Abs in 50% (specific cf parietal cell)
- High serum gastrin
- High homocysteine levels (not specific)
- Schilling's test - rarely performed
- Urinary methylmalonic acid - not routine
- Holo TC II – recently introduced some labs

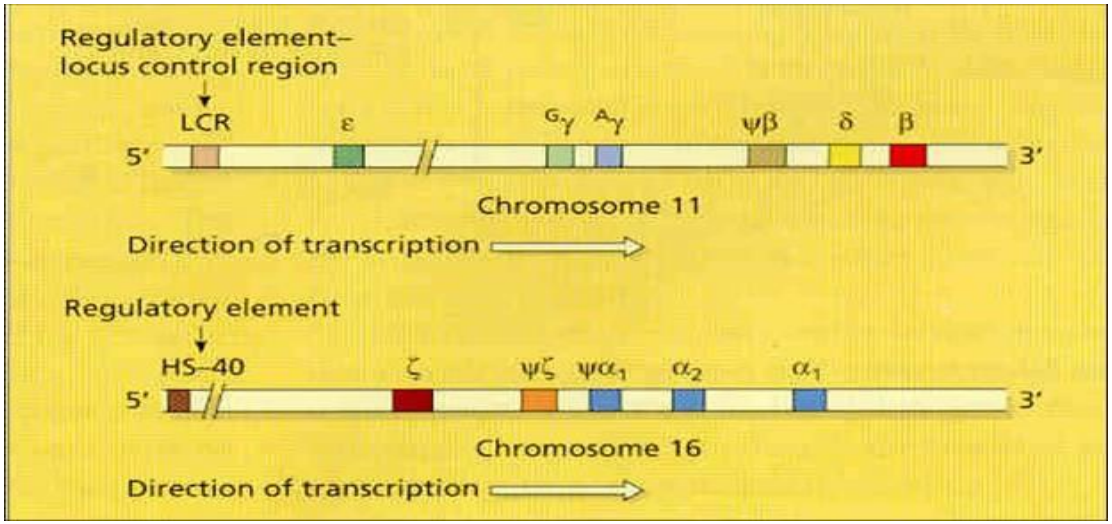
Holo-TC II

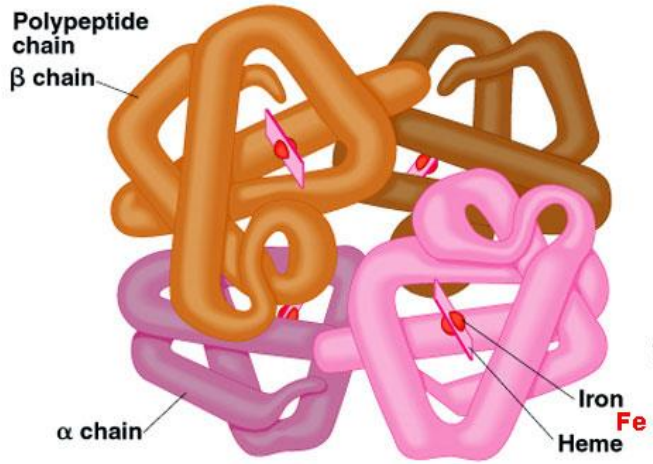

Active-B12
(holotranscobalamin)
10-30%
(Biologically active)
B12 bound to haptocorrin
70-90%
(Biologically inert)

Causes of low serum B_{12} (but normal stores)

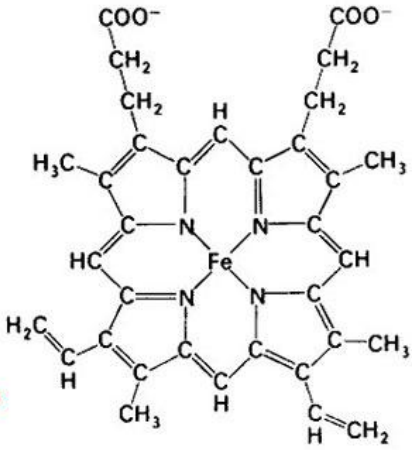
- Elderly
- Severe folate deficiency
- Pregnancy/OC
- Myeloma
- 10% of patients with carcinoma
- HIV
- Rare - TC 1 deficiency, Vitamin C overdose
- High CV of assay

Thalassaemias - globin chain production imbalance





Hemoglobin



Heme
(Fe-protoporphyrin IX)

ß thalassaemia trait

- ß thalassaemia - over 100 mutations, usually nucleotide substitutions (2 beta genes)
- Normal haemoglobin (lower limit)
- Low MCV (constant) – hypochromic, microcytic red cells, target cells, stippling
- High HbA_2 and HbF (check not iron deficient)
- Hb electrophoresis no abnormal bands unless haemoglobinopathies eg HbE

α thalassaemia

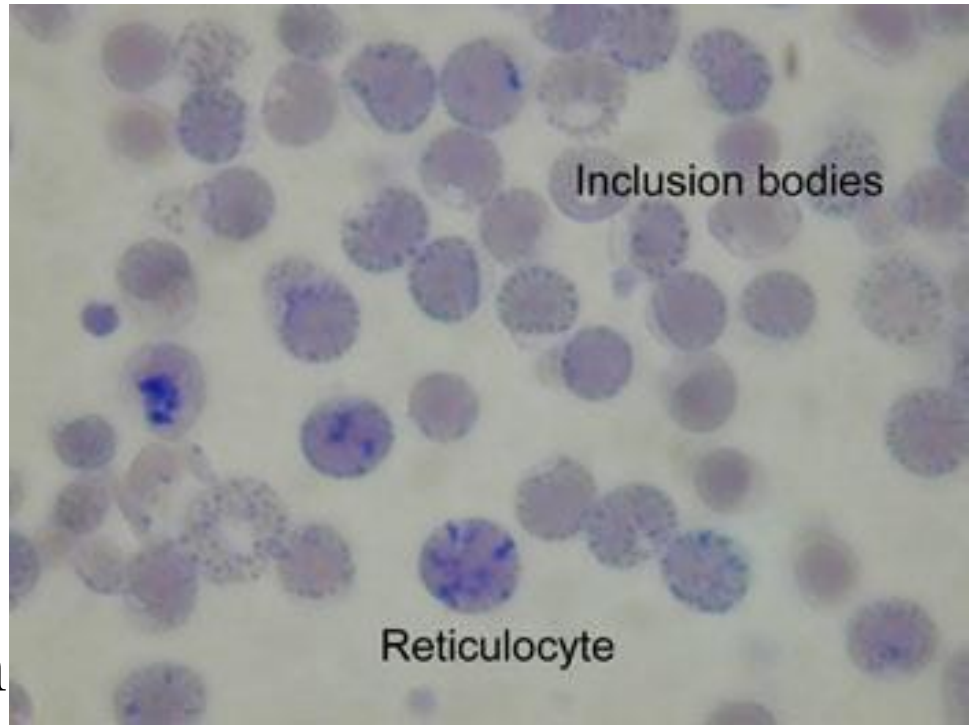
- α thalassaemia - usually large deletions of the alpha gene (4 alpha genes normally)
- Single gene mutation - low-normal MCV ($\alpha^+/\alpha\alpha$)
- Two gene deletions - low MCV, positive HbH bodies - on the same gene more significant ($\alpha^o/\alpha\alpha$ compared to α^+/α^+)
- Three gene deletions - HbH disease - life long anaemia, previously managed without Tx, splenomegaly
- Four gene deletions - hydrops foetalis - Hb Bart's (γ)

α thalassaemia

- Hb H preparation - 'golf balls' from precipitation of ß chains, not reliable, therefore genetic studies
- Hb A_2, Hb F levels,

  Hb EPG normal in adults

  if one or two gene deletion

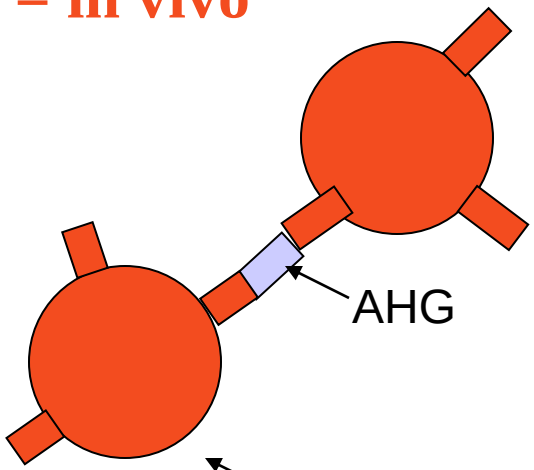


Tests that suggest haemolysis

- Fall in haemoglobin, increased bilirubin (uncong), urobilinogen on dipstick
- Increased reticulocytes, LDH, low haptoglobin
- Tests that suggest a cause of haemolysis
  - Blood film eg fragments, blister, sickle cells
  - DAT (Coomb's)
  - Urinary haemosiderin (IV haemolysis)
  - Family history, male gender for G6PD

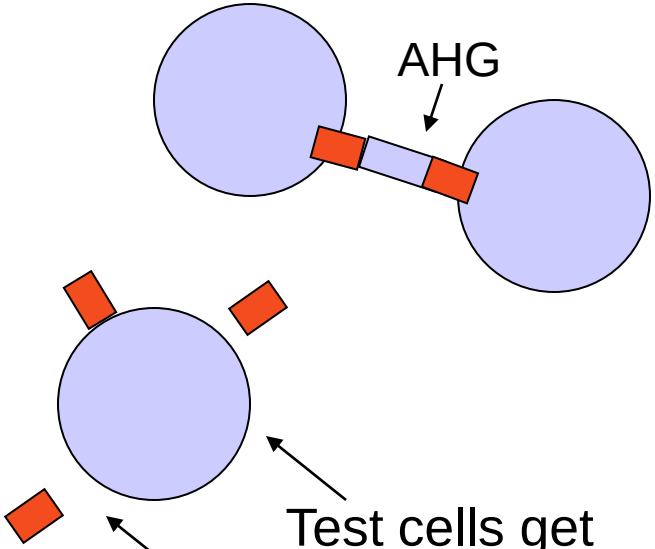
DAT

Red = in vivo



Patients cells coated with Antibody (IgG, IgA, IgM or C3) in vivo

IAT



Test cells get coated with antibody/C' from the patient's serum

Blood films in haemolysis

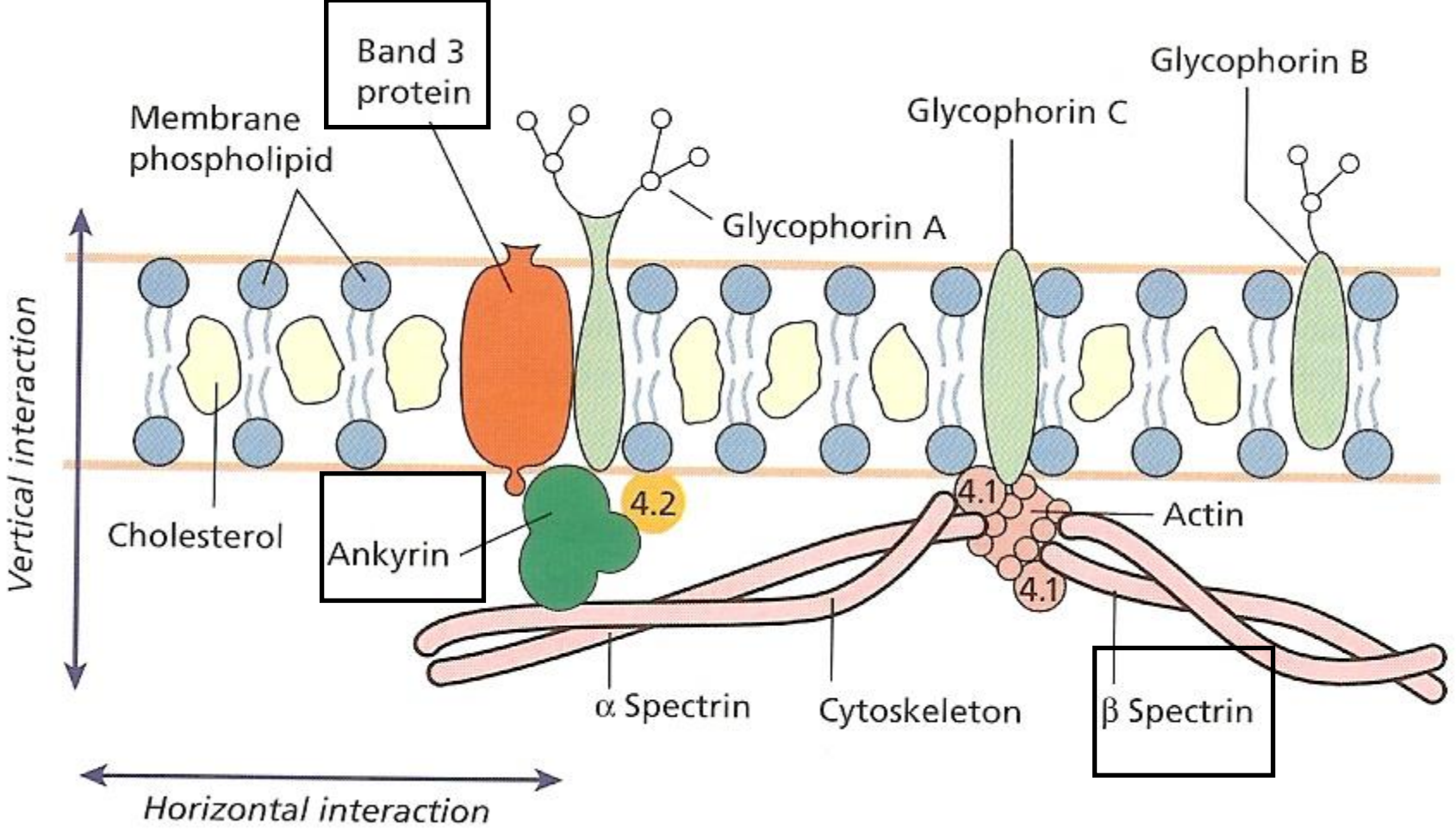
- Oxidant drugs and G-6PD deficiency - blister, bite cells
- Microangiopathy, valve haemolysis, TTP - fragments
- Hereditary spherocytosis, warm AIHA, burns - spherocytes
- Hb S - sickle cells
- Pyruvate kinase deficiency - spiny cells
- Cold AIHA - agglutinated cells

Further diagnostic tests for haemolysis

- G6PD deficiency and oxidative haemolysis
- Hereditary spherocytosis
- PNH
- PCH
- Cold agglutinins (titre)
- Hb S - sickling test, Hb EPG

Hereditary spherocytosis

- Anaemia, high MCHC, spherocytes >2%
- DAT negative
- Family history
- Increased osmotic fragility (no longer performed)
- Increased autohaemolysis, improved with glc
- Specialised - spectrin analysis, flow cytometry - eosin-5- maleimide



HS: *vertical*

Ankyrin: 50%	ANK1
Spectrin: 30%	SPTB
Band 3: 20%	EPB3
Pallidin / 4.2	

HE: *horizontal*

α or β spectrin dimer formation abn

α or β spectrin-ankryin assoc abn

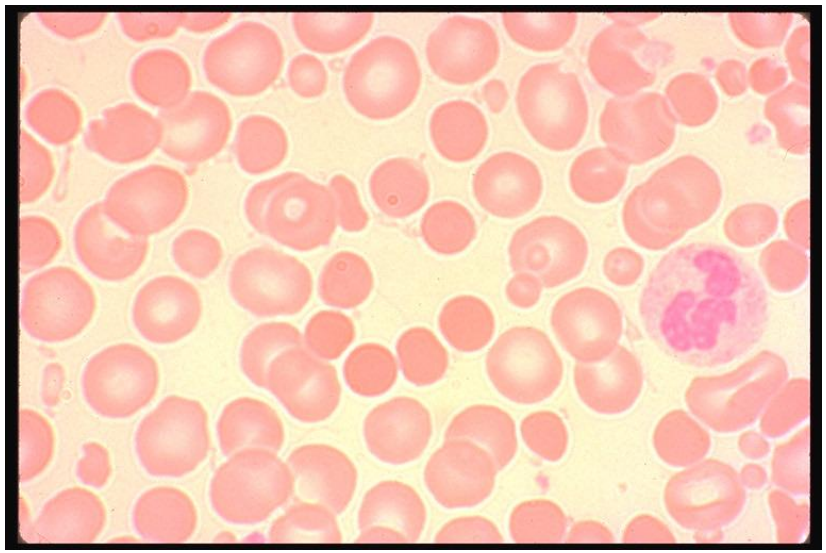
Protein 4.1 def/abn & Band 3 abn

SE Asian ovalocyte band 3 del

Warm autoimmune haemolytic anaemia

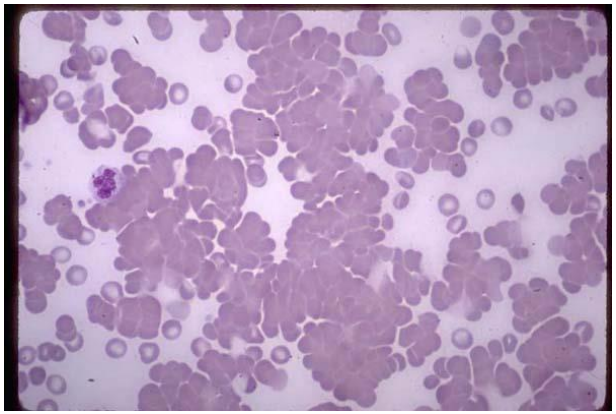
- Blood film, spherocytes, assoc with CLL/NHL

- DAT - most likely positive in warm h'lysis
  - Ig G+/-C3d
- Otherwise associated with ANA positivity, drugs or idiopathic

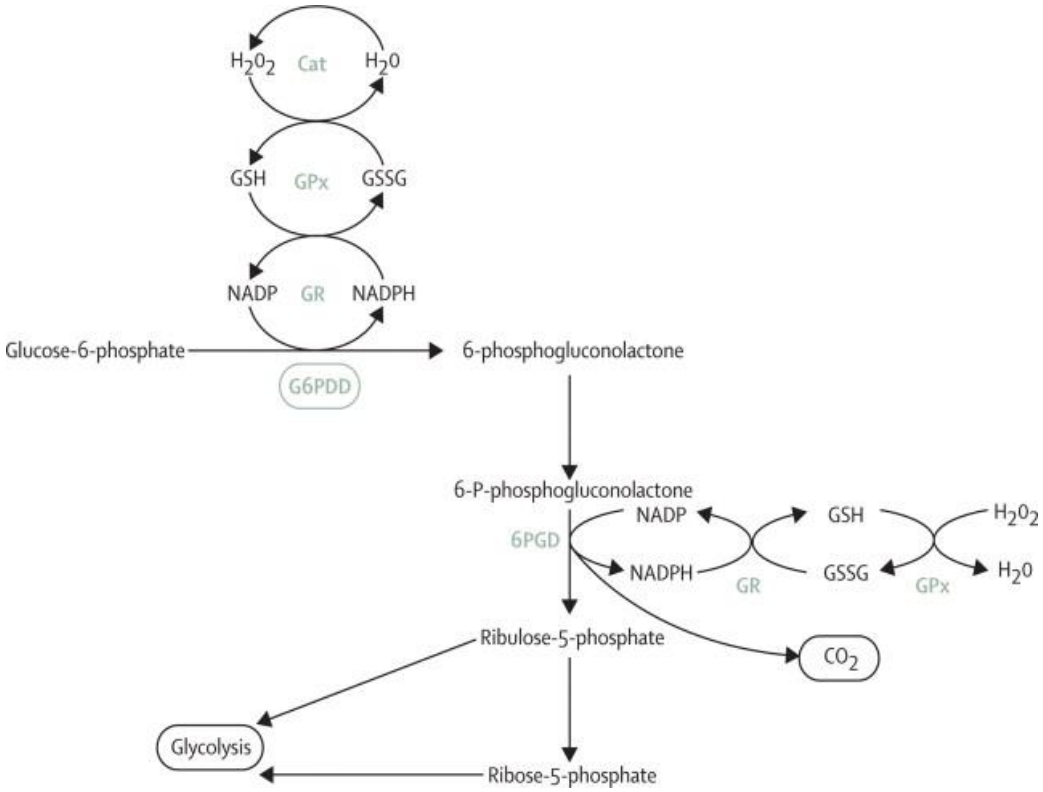


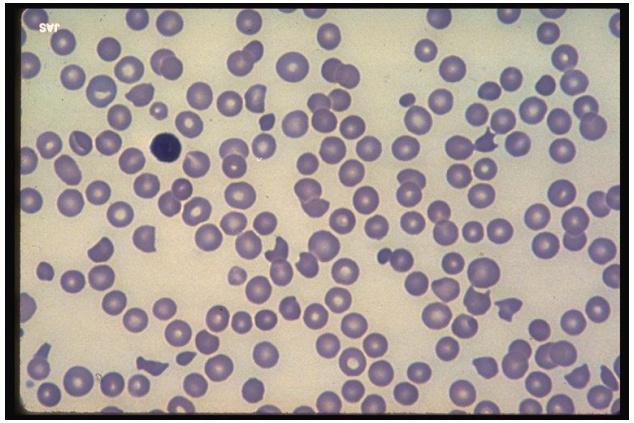
Cold autoimmune haemolytic anaemia

- Blood film - spherocytes, cold agglutinins,
  high MCV, high MCHC
  - may see atypical or malignant lymphocytes
- Cold haemolysis due to IgM (to I or i)
- DAT - if positive - C3d only
- EBV & Mycoplasma serology
- PCH - cold reactive IgG anti-P,
  Donath-Landsteiner test (rare)

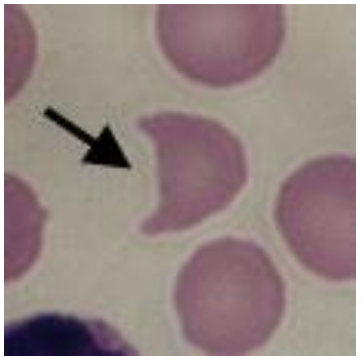


Oxidative haemolysis eg G6PD def





Glucose-6-phosphate dehydrogenase deficiency

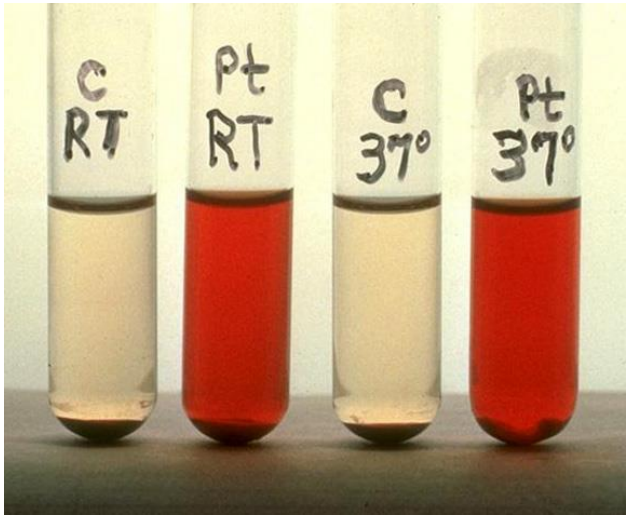


- Pentose phosphate pathway, X linked
- No haemolysis between episodes
- Precipitated by fava (broad) beans, sepsis and oxidant drugs
  e.g. dapsone, salazopyrine, cotrimoxazole, glibenclamide, primaquine, chloroquine, Fansidar, Maloprim
- Screen those at risk e.g. malaria, neonatal jaundice
- Reticulocytosis, helmet (arrow) and blister cells
- Heinz body preparation - denatured haemoglobin – few labs performing now
- Fluorescent spot screening or G6PD enzyme assay

Paroxysmal Nocturnal Haemoglobinuria

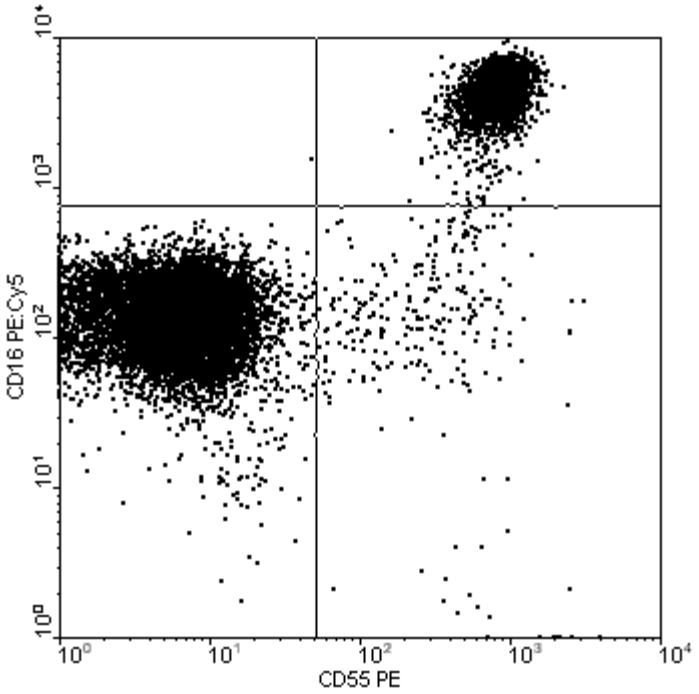
- Cytopenias, iron deficiency, thromboses (unusual sites) and intermittent dark urine (IV haemolysis)
- Marrow may be aplastic, Hb in urine
- Obsolete tests

 - sucrose lysis, Ham's test



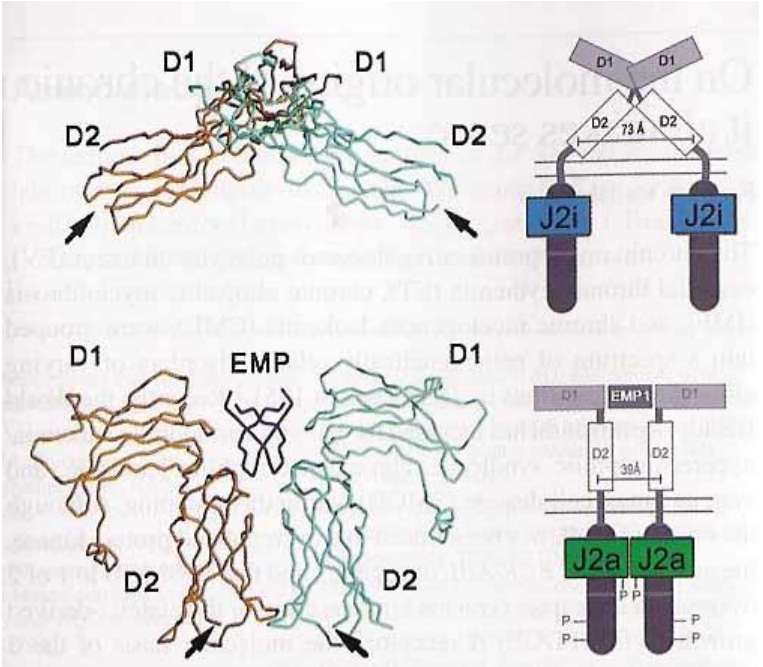
PNH – flow cytometry

- Reduced glycosophospha-tidylinositol (GPI) anchor on haemopoietic cells, due to mutations in the PIG-A gene
- Less inhibition of the complement cascade-lysis
  - DAF - CD55
  - MIRL - CD59* - flow cytometry of red/white cells



Polycythaemia

- Primary - itch, splenomegaly, panmyelosis
  - Marrow - hypercellular with inc reticulum
  - 50% splenomegaly on ultrasound
- Secondary -
  - lung disease ?sleep apnoea
  - congenital heart disease
  - Renal – PCKs & carcinoma
- pO_2 helpful, EPO not as helpful
- JAK2 mutation in > 95% PRV
- Other MPDs – CALR mutn~ 30%
  MPL <10%



EPO receptor – Jak-2 mutation

Causes of a long PT

- Liver disease
- Vitamin K deficiency
- Warfarin (INR allows laboratory comparison)
- Erroneous specimen
- (specific factor deficiency – rare)

- With prolonged APTT
  - Excess heparin
  - DIC, severe hypofibrinogenaemia
  - Lupus inhibitor (rarely effects PT)
  - Extreme of the above

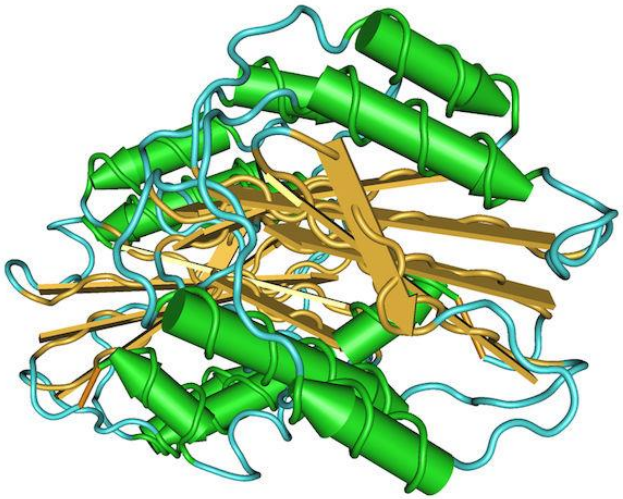
Causes of a prolonged APTT

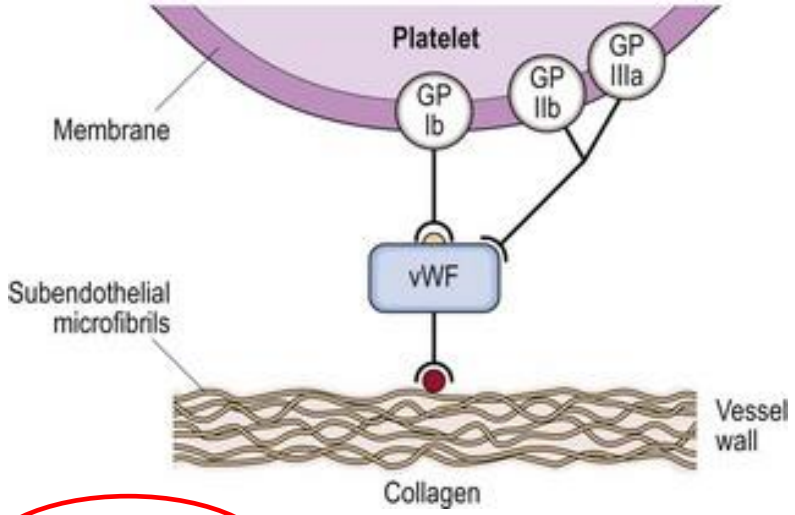
- Unfractionated heparin (line contamination)
- Lupus anticoagulant (inhibitor)
- Coagulation deficiency
  - Haemophilia A & B, von Willebrand disease
  - Acquired haemophilia (inhibitor – rare), but important not to miss
- Factors affecting both or common pathways
  - e.g low fibrinogen, DIC
- Incorrectly collected specimen

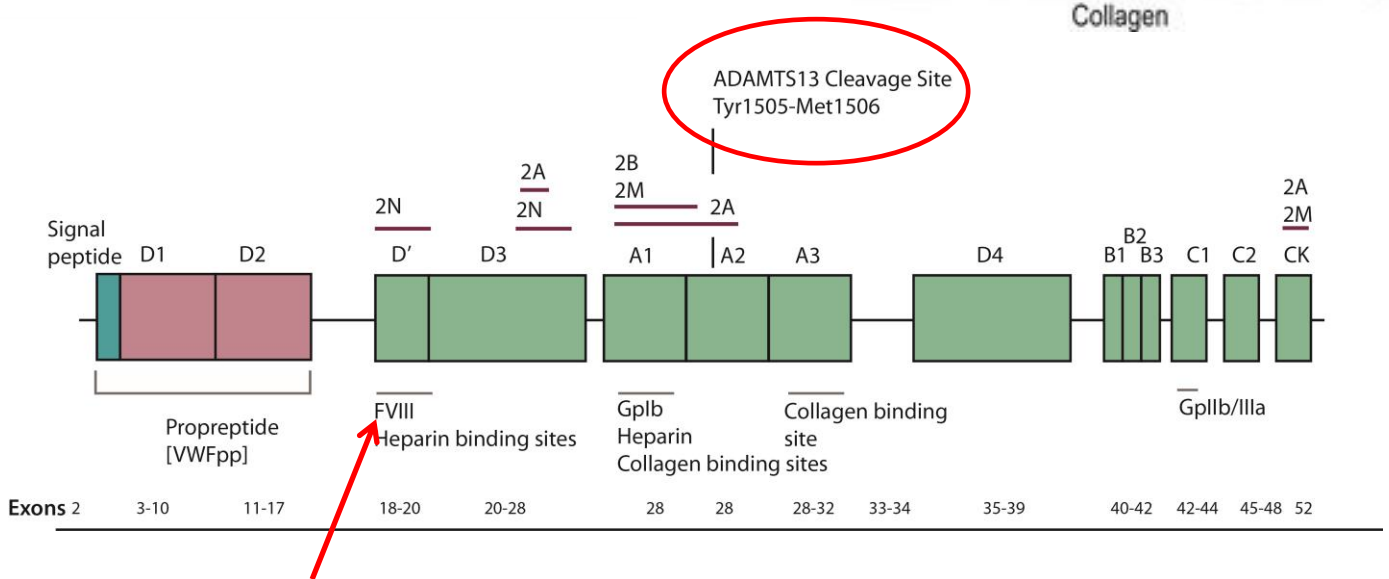
Coagulation tests and Novel ACs

- Monitoring not routine
- Dabigatran – inc aPPT, PT but not linear, inc TCT but too sensitive.
  - Hemoclot or dilute TCT
- Rivaroxaban – inc PT, but not reliable for monitoring
  - Specific anti-Xa assay
- Apixaban – only mildly elevated PT
  - Specific anti-Xa assay

von Willebrand Factor







Diagnosis of von Willebrand disorder

- **Family history** of mucosal bleeding - autosomal dominant (common forms)
- Prolonged APTT (may require repeat test, often N)
- vWF:Ag or Glycoprotein 1b receptor binding assay
- Factor VIII:C level
- Functional assay
  - ristocetin cofactor (vWF:RCo)
  - and/or collagen binding assay (vWF:CBA)
- Diagnosis can be difficult, mutational analysis not routine

Thrombocytopenia

- ?decreased production or increased destruction
  - with other cytopenias - as for neutropenia
- Severe sepsis +/- DIC
- ITP
- Viral infections (usually mild e.g hep C, rarely IM is associated with severe ITP, also HIV)
- Drugs
  - immune - heparin, quinine, thiazides
  - suppressive - cytotoxics, alcohol

Diagnosis of ITP

- Low platelet platelet count confirmed on blood film and rebleed, blood film otherwise NAD
- Marrow showing inc megakaryocytes
  - now not routine in young adults
- ANA, B_{12}, folate, HIV, hepatitis B and C, TFTs (?helicobacter)
- Platelet antibodies - not specific
- Normal spleen size
- Exclude drug cause and associated lymphoproliferative disorder

Thrombosis - consideration for further investigation

- Patient < 45 years and unprovoked
- Second or subsequent thrombosis
- Positive family history
- Unusual site
  - Consider whether will alter mgt or adversely affect family members
  - 50% of inherited thrombophilias have a thrombosis secondary to immobility or pregnancy

Acquired thrombotic tendency

- Pregnancy, oral contraceptives
- Antiphospholipid antibodies
  - lupus AC, anticardiolipin, anti-β-2-glycoprotein Abs
- Malignancy - breast, mucin secreting
- Rarer
  - Myeloproliferative disorders
  - Nephrotic syndrome
  - Chronic haemolysis
  - PNH (very rare)

blood
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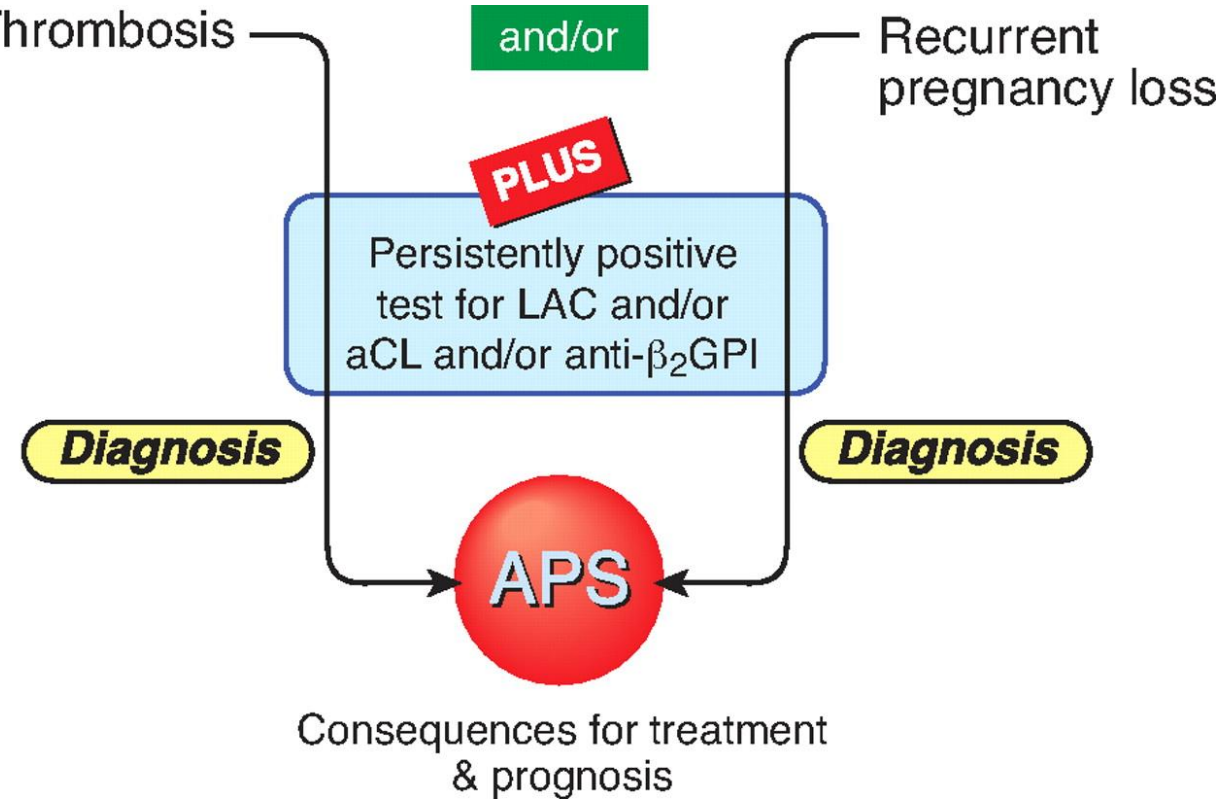
Diagnosis of antiphospholipid syndrome



Giannakopoulos, B. et al. Blood 2009;113:985-994

Diagnosis of lupus inhibitor

- Prolonged APTT (normal thrombin time)
- Mixing experiments consistent with an inhibitor
  - normal plasma does not correct APTT time
- Specific lupus AC testing
  - Kaolin Clotting Time
  - Dilute Russell Viper Venom Time
  - Correction of APTT with platelet phospholipid
- Associated antiphospholipid antibodies
  - e.g. ACL, anti$ß_2$GPI

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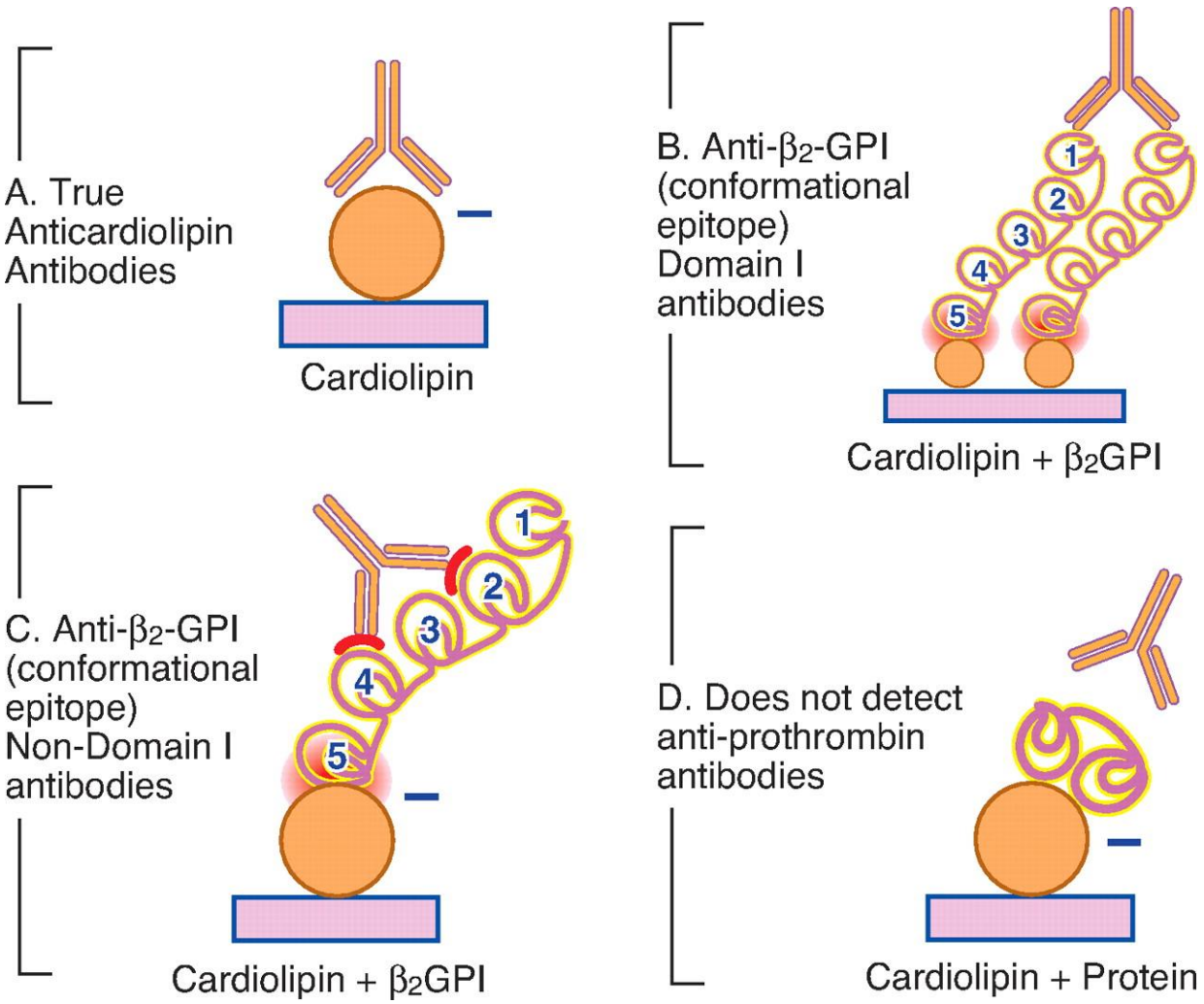
Figure 4



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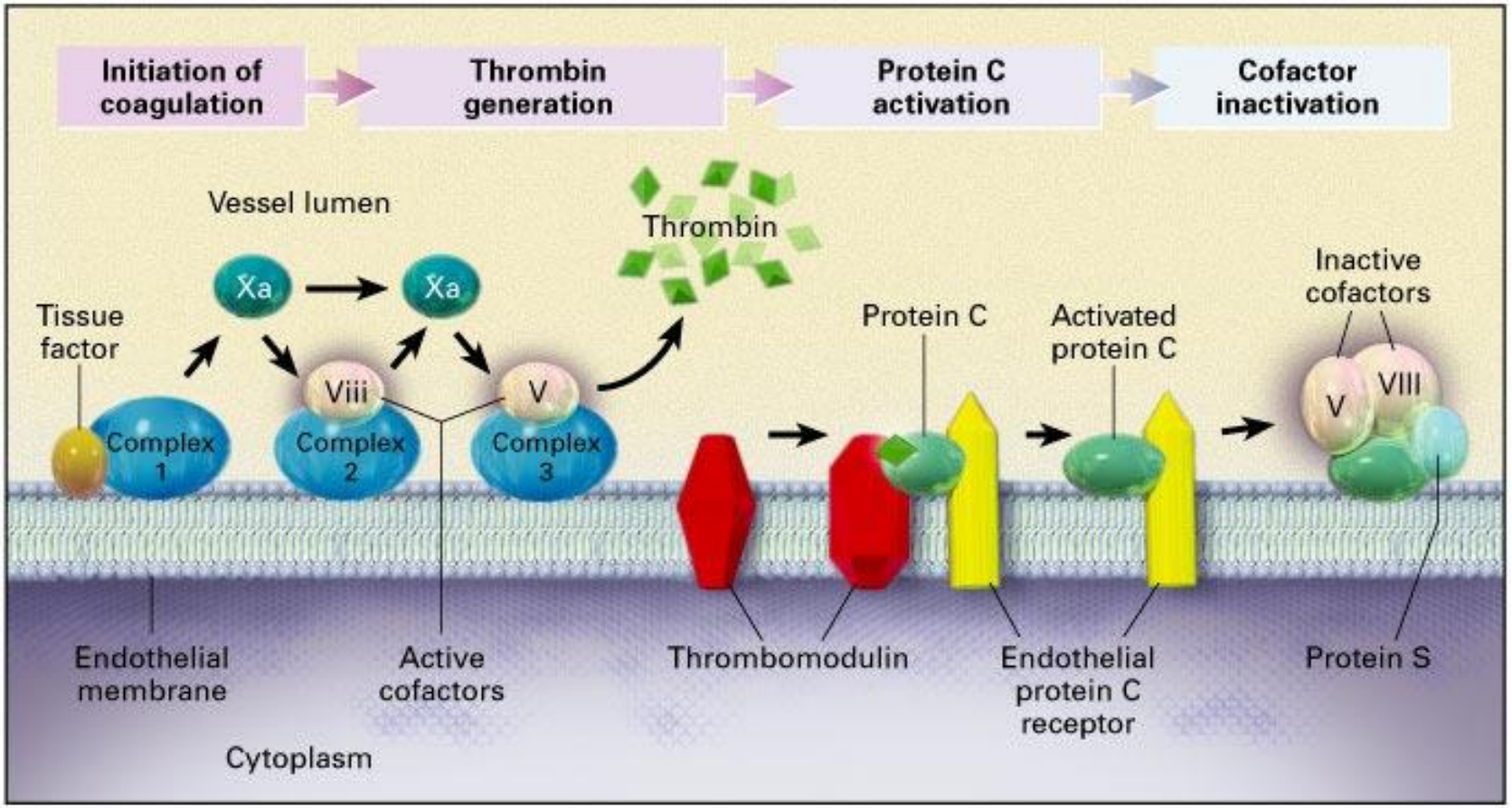


Figure 1. Endothelial Activation of Coagulation and the Protein C Pathway.
Coagulation is initiated by tissue factor and other coagulation-factor complexes on the surface of endothelial cells and monocytes. The activated factor X that is consequently generated requires activated cofactors V and VIII to produce thrombin, which in turn forms a complex with thrombomodulin. Protein C activation takes place by way of interaction between the thrombomodulin–thrombin complex and the endothelial protein C receptor. Activated protein C, together with its cofactor, protein S, inactivates factors V and VIII to provide negative feedback to the generation of thrombin. Complex 1 comprises tissue factor and coagulation factors VII, IX, and X; complex 2 comprises factors IX and X and cofactor VIII; and complex 3 comprises factor X, prothrombin, and cofactor V.

Inherited thrombotic tendency

- Factor V Leiden/ APCR - 20-50%
- Prothrombin 20210A - 3-5%
- Protein C deficiency - 5 %
- Protein S deficiency - 5%
- Antithrombin III deficiency - <5%
- ? Hyperhomocysteine deficiency (MTHFR)
- Rare - dysfibrinogenaemia
- Not-defined (50%) or ill defined
  - eg plasminogen activator deficiency

Factor V Leiden

- Activated protein C resistance (APCR)
  - screening test, PCR for genetic testing
- 1-7% of the Caucasian population
- Glu to Arg mutation in FV at nucleotide 1691
- Site where APC cleaves and inactivates FVa

Thrombotic risk - 10X for heterozygotes

- 80X for homozygotes

Compound heterozygotes with other thrombophilias

What tests should I do?

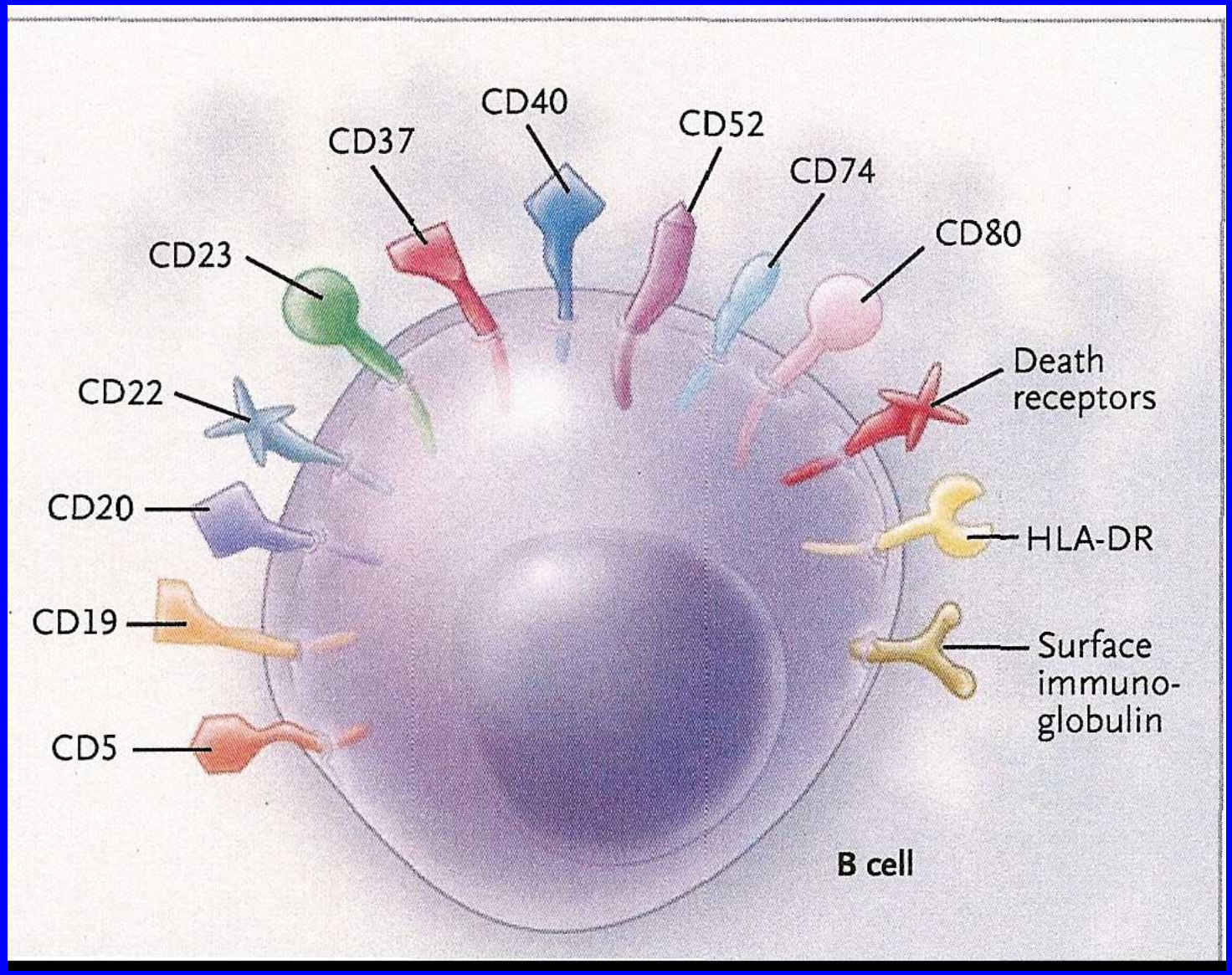
- Difficult! Not many! Will they alter management?

(Choosing Wisely)

  - FBE
  - Lupus AC
  - Congenital haemophilias – perhaps ATIII is the only one where a result may influence mgt
  - JAK2 mutation for MPD if unusual site e.g Budd Chiari

Flow cytometry

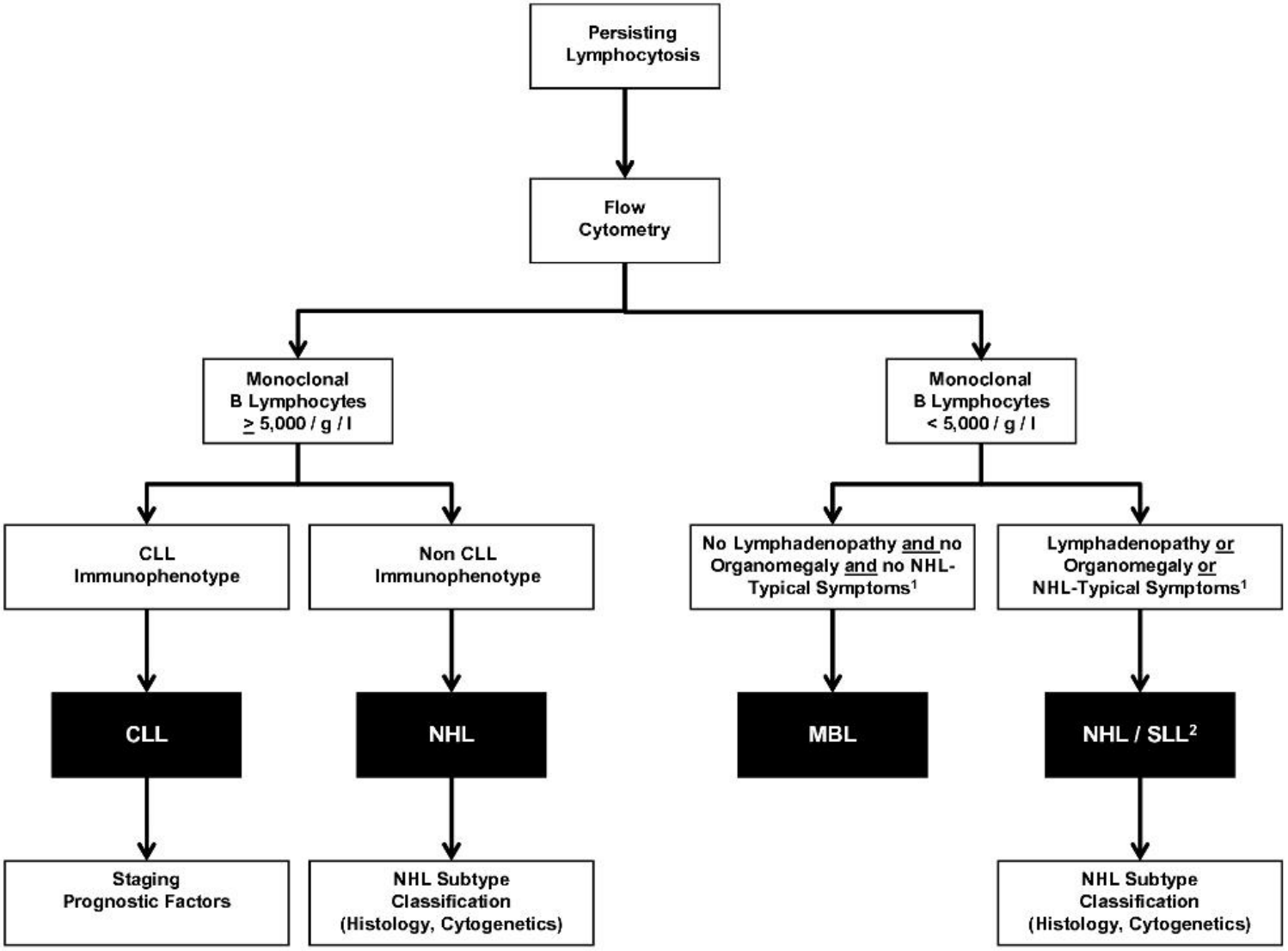
- Used for
  - lymphocyte analysis eg CD4 number
  - classification, diagnosis and detection of residual disease of lymphoproliferative disorders, acute leukaemias and myeloma
  - PNH, platelet Abs, foetomaternal haemorrhage
  - Stem cell enumeration of stem cell transplantation.
- PB, nodes and BM
- Aberrant or immature markers or
- Monoclonality of B cells (not T) by kappa/lamba ratio

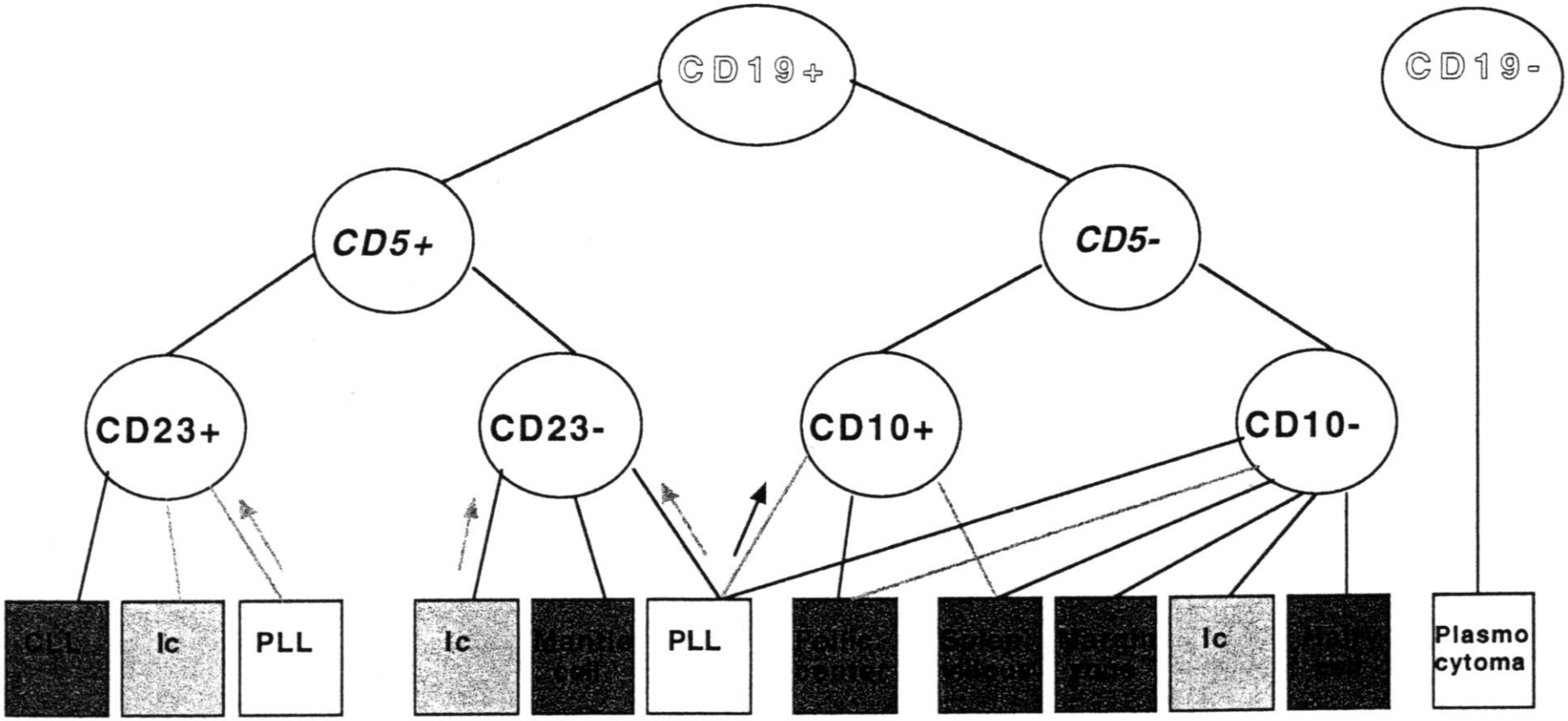

CD40
CD37
CD52
CD74
CD23
CD80
Death receptors
CD22
CD20
HLA-DR
CD19
Surface immuno-globulin
CD5
B cell

Flow cytometry (2)

- T cell markers - CD3
  - CD4 (T helpers) - low in HIV
  - CD8 (T suppressor/cytotoxic)
- B cell markers - CD19
  - kappa/lambda light chain ratio 2:1
- CLL - CD19/CD5 dual markers

Algorithm for B lymphocytosis




CD19+
CD19-
CD5+
CD5-
CD23+
CD23-
CD10+
CD10-
CLL
Ic
PLL
Ic
PLL
Ic
Plasmo cytoma
SIgM-/+ CIgM-/+ CD10- CD43+
SIgM+ CIgM+ CD10- CD43+/-
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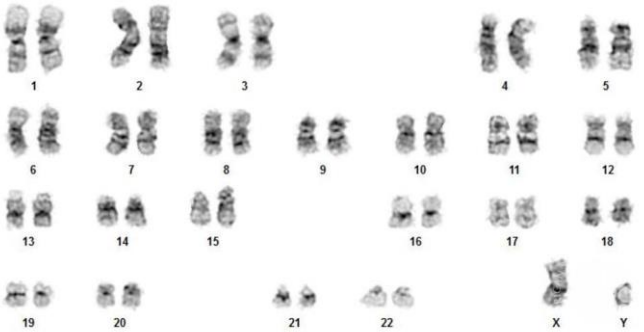
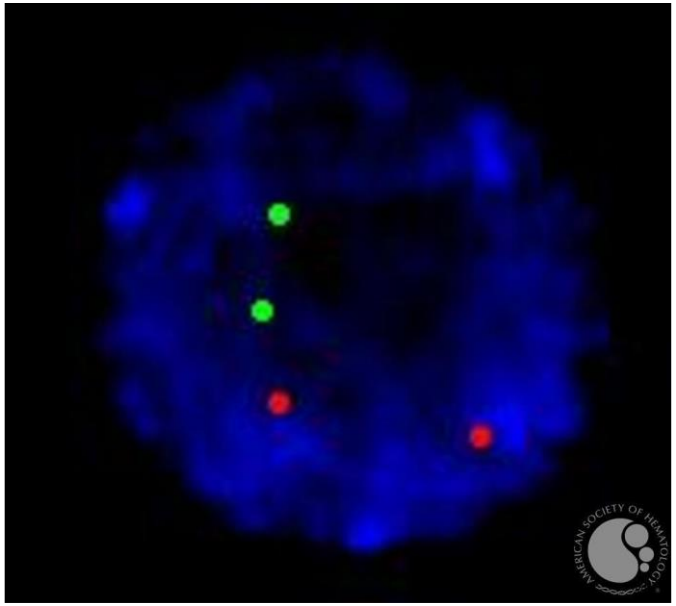
SIgM+ CIgM- CD43- CD5-/+ CD23-/+
SIgM+ CIgM+ 40% CD43+ CD5-/+ CD23+/- CD25-/+
SIgM+ CIgM+ 40% CD43-/+ CD23+/- CD25-
SIgM+ CIgM+ CD43+/- CD5-/+ CD23-
SIgM+ CIgM-/+ CD43+ C D23- CD25+ CD11c+ CD103+
SIgM- CIg++ G,A,M,D CD43+/-
CLL
ProLL
Mantle CL
ProLL
Follic L
Splenic villous
Marginal Zone L
Hairy Cell L
CD138 PlasmaCs

Cytogenetics/FISH

- Can be diagnostic, prognostic or direct treatment.
- Conventional cytogenetics in metaphase.
- Florescent in situ hybridization (FISH). Can be used in interphase, often used for lymphoid malignancies as often hard to induce cell division.





APML
Ash Image bank

Cytogenetics/FISH

- Examples of diagnostic are myelodysplasia in conjunction with morphology and subtypes of AML, e.g. APML, inv16
- Prognostic examples are numerous, but e.g. poor risk cytogenetics in AML may lead to allografting or treatment is not given in patients with high toxicity risk.
- Treatment altering e.g. ATRA for APML, 17p- in CLL (poor prognosis, respond to Bruton kinase inhibitors such as ibrutinib).

Examples of molecular tests other than for MPN fairly specific for diagnosis of haem malignancy

- bcr-abl - CML (Ph chromosome, t9:21)*
- PML-RARA - APML (t15:17)*
- c-myc - Burkitt and other high grade NHL
- cyclin D1 - mantle cell lymphoma (t11:14)
- bcl-2 - follicular lymphoma (t14:18)
- MYD88 - Waldenstrom's macroglobulinaemia (LPL)
- BRAF - hairy cell leukaemia
  - *Some molecular tests are quantitative and can be used monitor disease

Molecular testing and next generation sequencing (NGS)

- Tests a suite of genes quickly e.g. myeloid panel.
- Depending on institution can be very expensive.
- Can be used to test for JAK2/CALR/MPL at once rather than sequentially - in some institutions can be cost equivalent and efficient.
- Can’t always diagnose myelodysplasia with certainty as clonal haematopoiesis of indeterminate potential (CHIP) is very common.
- Requires lots of expert interpretation.

Minimal Residual Disease (MRD) evaluation and monitoring

- Associated with increased incidence of relapse in ALL and AML.
- Gives prognostic information in these and other disorders, often incurable such as CLL, myeloma and hopefully informs treatment decisions e.g. more intensive therapy, allografting.
- Performed by
  - flow cytometry (as discussed above)
  - immunoglobulin rearrangements
  - quantitative PCR e.g PML-RARa (APML), NPM1 (AML).