GP Education
Haematology laboratory abnormalities- when to refer?

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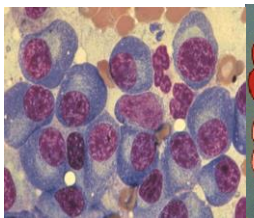
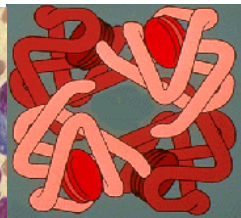
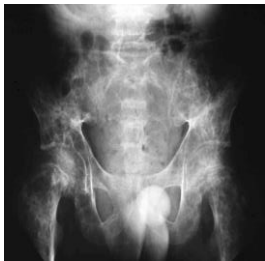




Topics for discussion

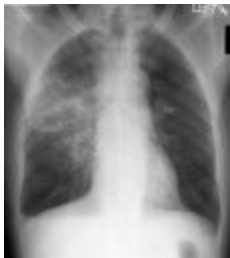
- Abnormal FBCs
  - Lymphocytosis
  - high and low platelets
  - cytopenias
  - raised MCV and its causes
- Blood tests for anaemias
- Polycythaemia
  - Relevant investigations and treatment
- Raised viscosity & monoclonal proteins
  - MGUS, what are these and how should we monitor?
  - When to refer?
- Familial haemachromatosis,
  - Symptoms and testing



Case 1

77 year old female with chest infection

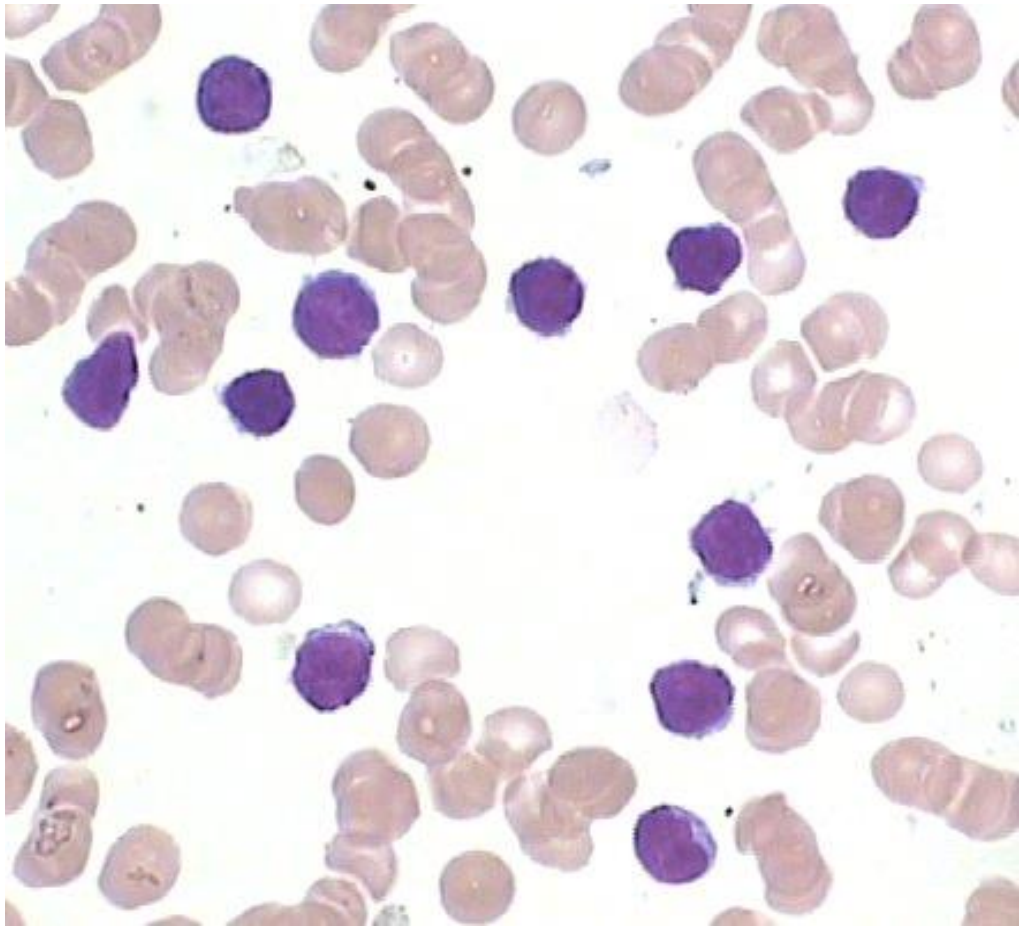
Hb 12.3g/dL	WBC 77.5 X10^9/L	Platelets 210 X 10^9/L
RBC: 3.64 x 10^9/L Hct: 0.38 MCV: 104fl MCH: 32pg	Neut 1.5 x 10^9/L Lymph 76 x 10^9/L Monos 0.1 x 10^9/L Blasts 0.0 x 10^9/L	



Questions

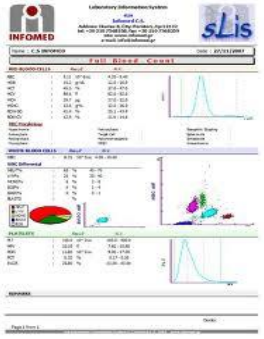
- What is the likely diagnosis?
- How would you confirm this?
- What action would you take?

Blood film



2 diagnoses

- CLL
- Macrocytosis ?cause

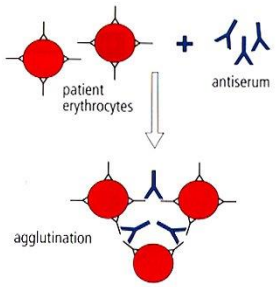


Tests to confirm a diagnosis of CLL

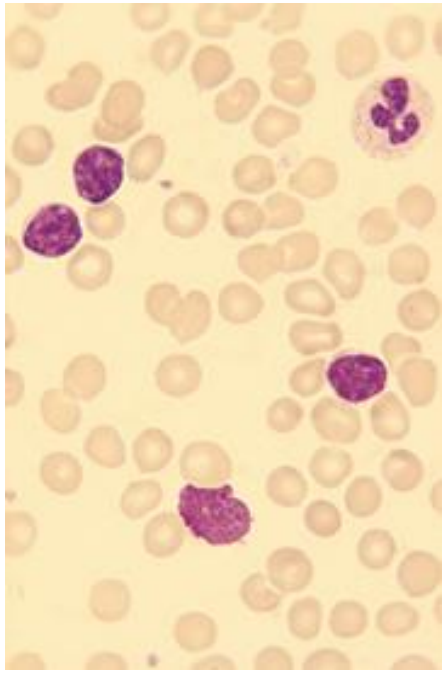
- Blood film morphology
- Peripheral blood immunophenotyping

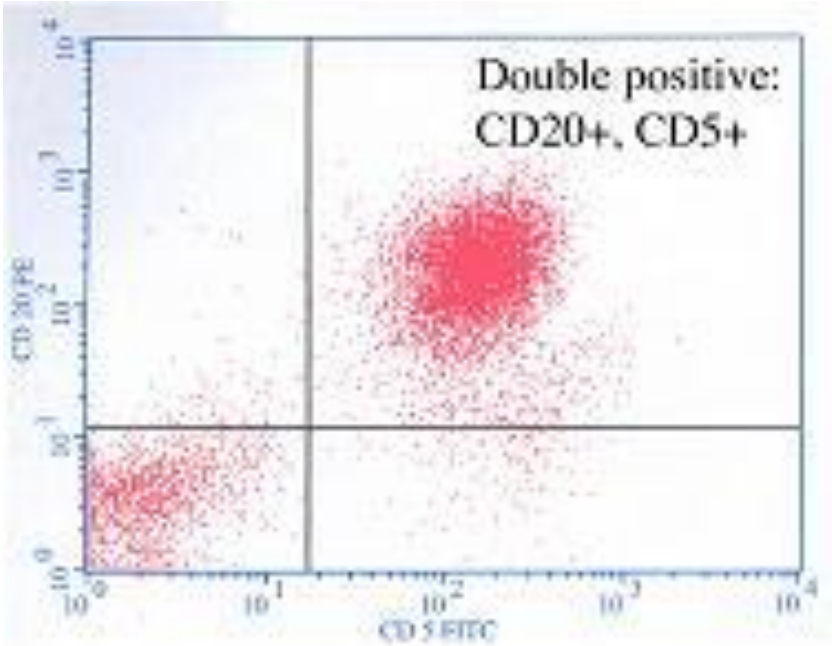
Additional tests

- direct antiglobulin test (DAT) (essential in all anaemic patients and before starting treatment)
- reticulocyte count
- renal and liver biochemistry (including urate levels)
- serum immunoglobulins
- chest X-ray /abdo ultrasound / CT scan
- bone marrow aspirate/trephine and/or lymph node biopsy



Chronic Lymphocytic Leukaemia
CLL





Lymphocytosis
BM failure
AIHA/ITP

Prognostic factors in chronic lymphocytic leukaemia

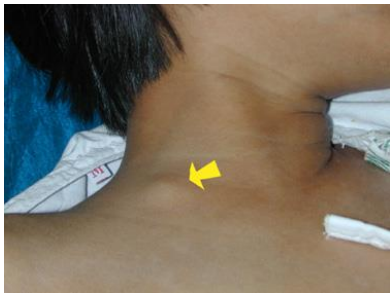
Factor	Low risk	High risk
Gender	Female	Male
Clinical stage	Binet A	Binet B or C
	Rai O,I	Rai II, III, IV
Lymphocyte morphology	Typical	Atypical
Pattern of marrow trephine infiltration	Non-diffuse	Diffuse
Lymphocyte doubling time	>12 months	<12 months
Serum markers*	Normal	Raised
CD38 expression	<20–30%	>20–30%
Genetic abnormalities	None	del 11q23
	del 13q (sole)	Loss/mutation of p53
IgVH gene status	Mutated	Unmutated

Indications for referral/follow up

- management of CLL requires a collaborative approach between primary care and haematology
- palliative care team may be valuable in the management of terminal drug resistant patients

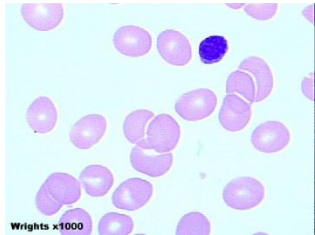
Indications for referral to a haematology department include:

- symptomatic disease
- the presence of lymphadenopathy or hepatosplenomegaly
- the investigation of a lymphocytosis, particularly if the lymphocyte count is high or there is anaemia or thrombocytopenia

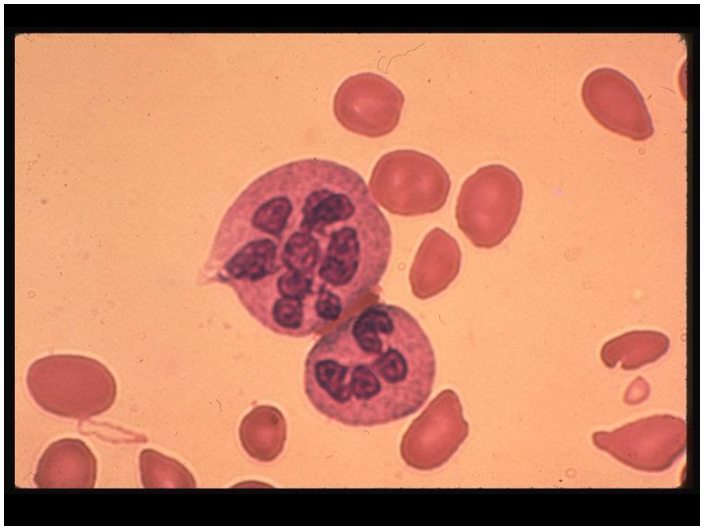


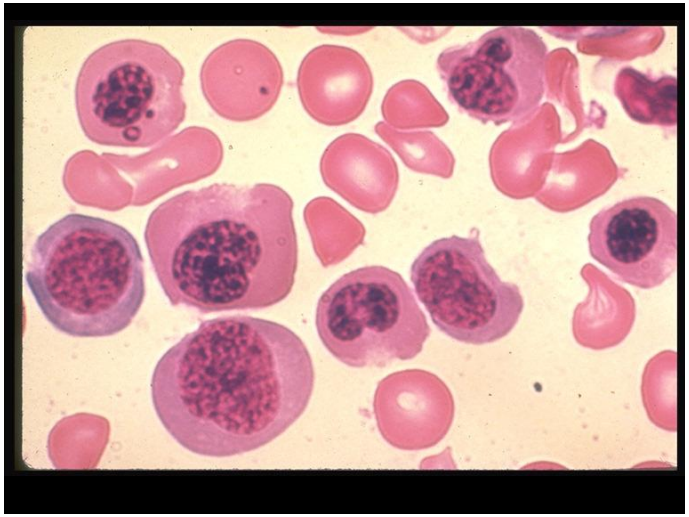
Macrocytosis

CAUSES	DIAGNOSTIC TESTS
B12/Folate deficiency	B12/Folate
Liver Disease	LFTs
Post-splenectomy	
Alcohol	γGT
Aplastic anaemia	FBC, retics
Myeloma	Igs/SPEP/urinary BJP
Myelodysplasia	blood film
Hypothyroidism	TFT
Reticulocytosis	DAT, retics, bili, LDH
Pregnancy	pregnancy test (!)
Drugs	drug history eg hydroxyurea
	anti-retroviral agents



Features of B12/folate deficiency





Macrocytic
Poikilocytosis
Neutrophil hypersegmentation
Raised bilirubin
Raised LDH

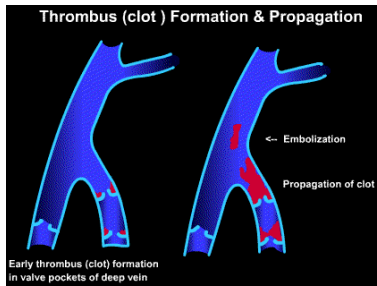
B12/folate assays
Intrinsic Factor Antibodies
Parietal cell antibodies

Megaloblastic bone marrow

Case 2: 55 year old man with hypertension and recent DVT

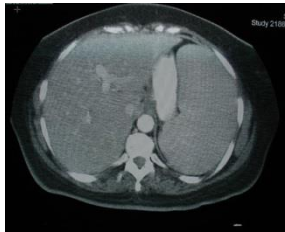
Hb 18.2g/dL	WBC 12.2 X 10^9/L	Platelets 804 X 10^9/L
RBC: 6.65 x 10^9/L Hct: 0.53 MCV: 80.6fl MCH: 30 pg	Neut 9.0 x 10^9/L Lymph 2.4 x 10^9/L Monos 0.8 x 10^9/L Blasts 0.0 x 10^9/L	

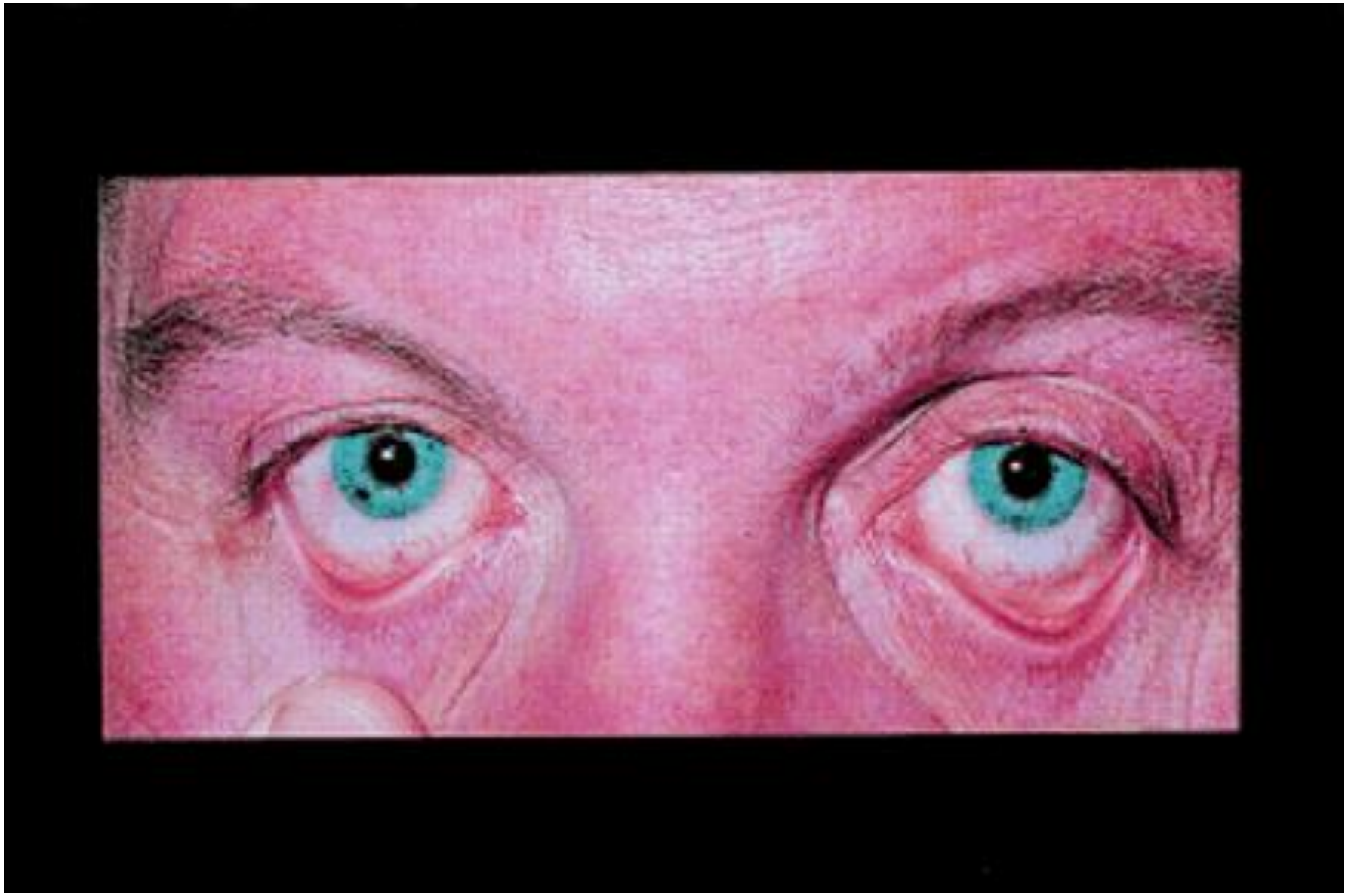
What is the likely diagnosis?
What are the risks associated with this?



Symptoms and signs of primary polycythaemia

- facial plethora
- headache
- mental clouding
- pruritis
- hypertension
- splenomegaly
- gout
- occlusive vascular lesions eg stroke, transient ischaemic attacks, digital ischaemia
- bleeding





Proposed revised WHO criteria for polycythemia vera

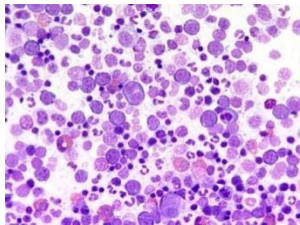
Major criteria

1. Haemoglobin 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of *JAK2*617VF or other functionally similar mutation such as *JAK2* exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth proliferation
2. Serum erythropoietin level below the normal reference range
3. Endogenous erythroid colony formation in vitro

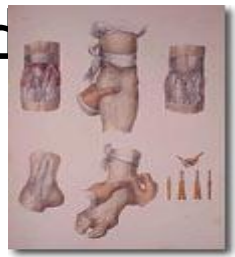
Diagnosis requires the presence of both major criteria and 1 minor criterion or the presence of the first major criterion together with 2 minor criteria



Treatment

1. Repeated venesections to maintain a PCV of < 0.45
2. Hydroxyurea if there is also thrombocytosis
3. Low dose aspirin (if there are no bleeding manifestations)
4. JAK2 inhibitors
5. Radioactive phosphorous

   Single dose controls disorder for 12 – 18 months but associated with increased risk of leukaemia. Reserved for elderly frail patients. Rarely used

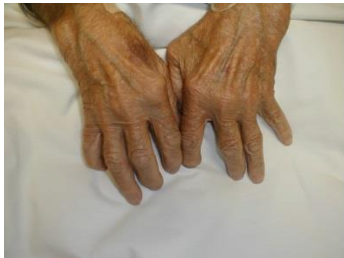


Case 3: A 69 year old lady with rheumatoid arthritis

Hb 9.2g/dL	WBC 10.0 X 10^9/L	Platelets 570 X 10^9/L
RBC: 3.91 x 10^9/L	Neut 7.0 x 10^9/L	
Hct: 0.32	Lymph 2.2 x 10^9/L	
MCV: 78fl	Monos 0.8 x 10^9/L	
MCH: 25pg	Blasts 0.0 x 10^9/L	

What tests are needed?

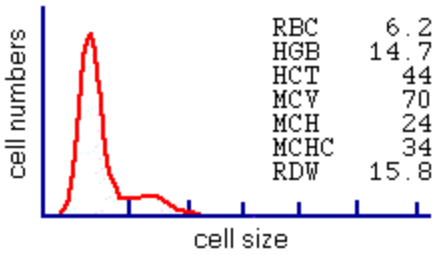
Why does she have a raised platelet count?



ANAEMIA

Normal ranges for red cell parameters:

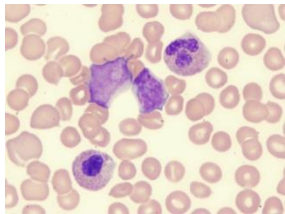
	Female	Male
Hb: g/dL	11.5 – 15.5	13.0 – 17.0
RBC x 10^{12}/L	3.8 – 5.3	4.5 – 6.0
Haematocrit	0.37 – 0.45	0.40 – 0.52
Mean cell volume (fl)	83 – 96	83 - 96
Mean cell haemoglobin (pg)	27 – 32	27 - 32



Anaemia

Microcytic	Normocytic	Macrocytic
Iron deficiency	Acute blood loss	B12/ folate deficiency
Anaemia of chronic disorder	Anaemia of chronic disorder	Hypothyroidism
Thalassaemias	Marrow infiltration	Liver disease
		Alcohol excess
	Haemolysis*	Myelodysplasia

*may be associated with mild macrocytosis due to reticulocytosis

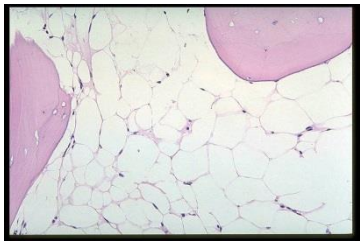


Investigation of anaemia: history

- Source of bleeding
- History of chronic illness/renal disease
- Country of origin/family history
- Dietary history
- Drug history
- Surgical history
- Foreign travel

Investigation of anaemia: laboratory findings (1)

- Red cell indices
  - important to distinguish between microcytic, macrocytic and normocytic and direct subsequent investigations
- White cell and platelet counts
  - helps to distinguish 'pure' anaemia from pancytopenia secondary to a marrow defect



Investigation of anaemia: laboratory findings (2)

- Haematinic assays - ferritin, B12 and folate
- Reticulocyte count (normal 0.5-2%)
  - should rise in response to anaemia
- Blood film
  - ESSENTIAL!
  - Look for abnormal red or white cell morphology, red cell inclusions or dimorphic picture
- Bone marrow examination
  - cell development, % cell lines and abnormal cells

Investigation of this patient

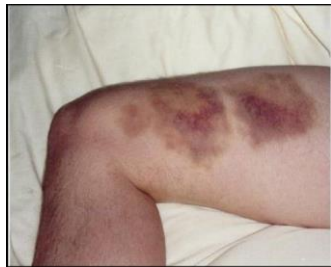
- Blood film
- Haematinics
  - ferritin, B12 and folate
  - Other measures of iron metabolism
- Measure of inflammation eg. CRP

Anaemia of chronic disease (ACD)

- The most frequent anaemia among hospitalized patients
- Caused by
  - chronic inflammatory disorders eg chronic infections, cancer, autoimmune diseases
- Causes
  - diversion of iron traffic and diminished erythropoiesis
  - blunted response to erythropoietin, erythrophagocytosis
  - bone marrow invasion by tumour cells and pathogens
- Diagnosis of ACD can be assessed by examination of changes in serum iron parameters
  - low to normal serum iron, TIBC
  - normal to increased ferritin, ZPP high in IDA
- Therapy of ACD includes the cure of the underlying the disease.
  - transfusions for rapid correction of haemoglobin levels
  - human recombinant erythropoietin but response rates are sometimes low.
  - Iron alone should be avoided

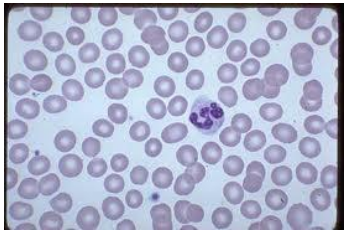
Case 4: A 26 year old male with spontaneous bruising

Hb 15.0g/dL	WBC 9.3 x 10^9/L	Platelets 10 x 10^9/L
RBC: 5.22 x 10^9/L	Neut	6.3 x 10^9/L
Hct: 0.44	Lymph	2.2 x 10^9/L
MCV: 88fl	Monos	0.8 x 10^9/L
MCH: 30pg	Blasts	0.0 x 10^9/L



Causes of thrombocytopenia

- **False thrombocytopenia**
  - Clot in the sample.
  - Platelets clumped (citrate effect)
- **Congenital thrombocytopenia**
  - Rare inherited disorders (eg May Hegglin anomaly, Bernard Soulier syndrome).
- **Defective platelet production**
  - Bone marrow aplasia (failure).
  - Metabolic disorders, eg kidney failure, alcohol.
  - Abnormal platelet precursors: viral infections, inherited abnormalities.
  - Bone marrow infiltration, eg leukaemia, lymphoma.
- **Diminished platelet survival**
  - Antibodies in response to drugs, blood transfusion or another disease, eg glandular fever, malaria, HIV, SLE
  - Unknown cause (ITP).
  - Clotting disorder (DIC).
  - Blood disorder (TTP).
- **Loss of platelets from the circulation**
  - Massive blood transfusion or exchange.
  - Enlarged spleen



Drugs associated with thrombocytopenia (1)

- Quinine/Quinidine group
- Heparin
- Gold salts
- Antimicrobials
  - Cephalosporins
    - Cephamandazole
    - Cefotetan
    - Ceftazidime
    - Cephalothin
  - Ciprofloxacin
  - Clarithromycin
  - Fluconazole
  - Fusidic acid
  - Gentamicin
  - Nilidixic acid
  - Penicillins
    - Ampicillin
    - Methicillin
    - Penicillin
    - Piperacillin
  - Pentamidine
  - Rifampin
  - Sulpha group
    - Sulfamethoxazole
    - Sulfamethoxypyridazine
    - Sulfisoxazole
  - Vancomycin
- Anti-inflammatory drugs
  - Acetaminophen
  - Salicylates
    - Aspirin
    - Sulfasalazine
  - Diclofenac
  - Ibuprofen
  - Indomethacin
  - Mefanamic acid
  - Naproxen
  - Oxyphebutazone
  - Phenylbutazone
  - Piroxicam
  - Sulindac
- Cardiac medications and diuretics
  - Digoxin
  - Amiodarone
  - Procainamide
  - Oxprenolol
  - Captopril
  - Diazoxide
  - Alpha-methyldopa
  - Acetazolamide
  - Chlorothiazide
  - Furosemide
  - Hydrochlorothiazide
  - Sprinolactone

Drugs associated with thrombocytopenia (2)

- **Benzodiazepines**
  - **Diazepam**
- **Anti-epileptic drugs**
  - **Carbamazepine**
  - **Phenytoin**
  - **Valproic acid**
- **H2-antagonists**
  - **Cimetidine**
  - **Ranitidine**
- **Sulfonylurea drugs**
  - **Chlorpropamide**
  - **Glibenclamide**
- **Iodinated contrast agents**
- **Retinoids**
  - **Isotretinoin**
  - **Etretinate**
- **Anti-histamines**
  - **Antazoline**
  - **Chlorpheniramine**
- **Illicit drugs**
  - **Cocaine**
  - **Heroin**
- **Antidepressants**
  - **Amitriptyline**
  - **Desipramine**
  - **Doxepin**
  - **Imipramine**
  - **Mianserine**
- **Miscellaneous drugs**
  - **Tamoxifen**
  - **Actinomycin-D**
  - **Aminoglutethimide**
  - **Danazole**
  - **Desferrioxamine**
  - **Levamizole**
  - **Lidocaine**
  - **Morphine**
  - **Papaverine**
  - **Ticlopidine**

Approach to diagnosis

- history of symptoms, signs of bleeding or bruising, other medical problems, recent infections and medications.
- Repeat full blood count
- Blood film
- Indications for referral

What is haemochromatosis?

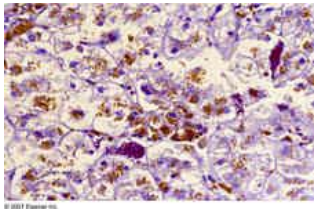
the clinical condition of iron overload

Investigation of patients with a raised serum ferritin

- **Chronic inflammation/infection**
  - may be associated with an anaemia of chronic disorder
  - Suggested by raised plasma viscosity and/or CRP
  - ferritin is an acute phase reactant and does not necessarily reflect iron stores
- **Liver disease**
  - Chronic liver disease (increased hepatic iron) or acute liver injury (liver-derived ferritin released by hepatocytes) - check LFT and γ GT.
  - Alcohol excess and non-alcoholic fatty liver disease are common causes
  - Consider referral to a hepatologist
- **Hereditary Haemochromatosis**
  - Request "Iron Studies" to determine transferrin saturation (> 50%) and HFE gene analysis Homozygous C282Y mutation is present in 90% of cases and 5% of patients are compound heterozygous for C282Y/H63D
  - 5% of cases have a normal genotype and referral to a hepatologist is appropriate if there is doubt about the diagnosis
- **Iatrogenic and iron self - medication**
- **Chronic anaemias associated with iron-loading eg.haemolytic anaemias and sideroblastic anaemia**
  - If patient is anaemic, request blood film and reticulocyte count

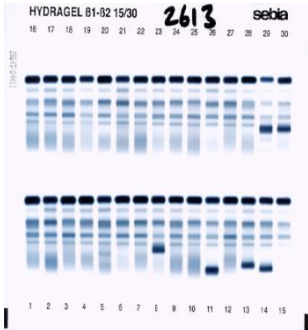
Classification of haemochromatosis

- **Genetic haemochromatosis**
  - iron accumulation in the body due to the inheritance of mutations in the HFE gene on both copies of chromosome 6
  - leads to excessive absorption of iron from food.
- **Juvenile haemochromatosis**
  - an inherited condition in which there is clinical onset in the 2nd or 3rd decade.
  - The gene responsible is probably located on chromosome 1
- **Secondary iron overload** (secondary haemochromatosis, haemosiderosis)
  - iron overload following chronic blood transfusion for haematological conditions, including thalassaemia major and aplastic anaemia
  - also includes conditions in which enhanced iron absorption is secondary to ineffective erythropoiesis with marrow hyperplasia eg.thalassaemia intermedia
- **Neonatal haemochromatosis**
  - condition of acute liver damage with iron accumulation



Genetic haemochromatosis

- In the UK over 90% of patients with genetic haemochromatosis are homozygous for the C282Y mutation of the HFE gene and another 4% are compound heterozygotes (C282Y/H63D).
- There are other rarer forms of inherited haemochromatosis where patients have ‘classical’ clinical features of haemochromatosis but lack mutations in the HFE gene



Presenting symptoms in patients with haemochromatosis

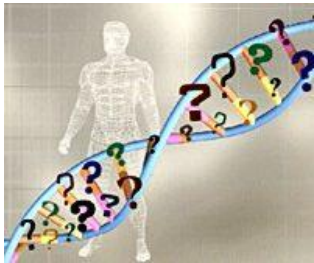
	% of patients	
Symptom or physical finding	**1**	**2**
Weakness or fatigue	52	82
Pigmentation	47	72
Arthralgia	32	44
Impotence (% of males)	40*	36
Cirrhosis	27	57
Diabetes mellitus	15	48
Cardiac disease	10	12†

Study1- 277 patients presenting in Rennes (Brittany) and London (Ontario) between 1962 and1995. The incidence of symptoms was lower in family members tested after the diagnosis was made in the proband. *All patients – including family members

Study 2 - 251 patients presenting in Düsseldorf and Bad Kissinger (Germany) from 1947–1991. 8% of these were identified through family screening. †Dyspnoea on exertion.

Diagnosis

- Early diagnosis is not easy
- symptoms with which patients present are relatively common and non-specific
- Raised ferritin concentrations are common in hospital patients
- genetic testing offers the best approach to early detection



Management guidelines

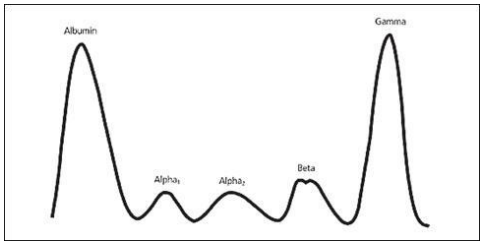
http://www.bcshguidelines.com/documents/haemochromotosis_2000.pdf

Monoclonal gammopathy of undetermined significance (MGUS)

- Definition

  the presence of a monoclonal protein in the serum or urine of an individual with no evidence of multiple myeloma, AL amyloidosis, Waldenstrom's macroglobulinaemia or other related disorders.

  (Kyle, Mayo Clinic 1978)

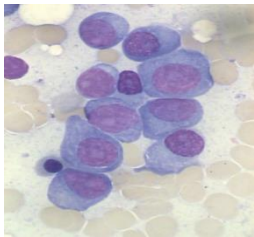


Incidence of M-proteins

- Varies greatly with age.
  - 1 - 2% of people in their 6th decade
  - 2- 4% in their 7th decade
  - 4-5% in their 8th decade
    - *694 out of 21,463 in a normal Minnesota population > 50 years. (Kyle et al 2006)*
  - 14% over the age of 90 *Crawford et al, 1987*
- Twice as common in black people as white people

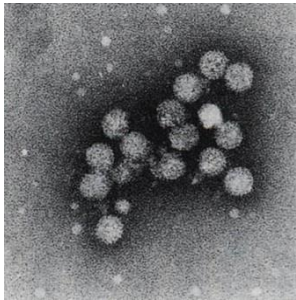
Monoclonal gammopathies include:

- Monoclonal gammopathy of undetermined significance (MGUS)
- Multiple myeloma
- Solitary plasmacytoma (skeletal or extra-medullary)
- AL amyloidosis
- Waldenstrom's macroglobulinaemia
- Low grade non-Hodgkin's lymphoma and other lymphoproliferative disorders
- Other M-protein related disorders



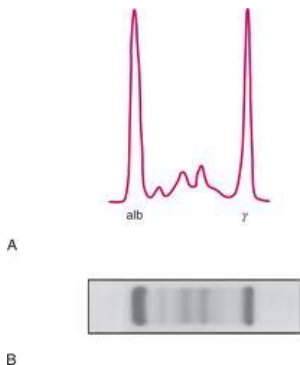
M-proteins may occur in association with:

- Connective tissue disorders
  - such as rheumatoid arthritis (RA) systemic lupus erythematosis, scleroderma, polymyositis and ankylosing spondylitis.
- Skin disorders
- Infections
  - hepatitis C virus (HCV)-related chronic liver disease (may be accompanied by mixed cryoglobulinaemia)
  - HIV
  - Helicobacter pylori



Why is guidance on newly diagnosed M-proteins needed?

- M-proteins are common;
  - Overall occur in about 1% of the population
    - *in population-based studies in Europe and North America*
  - 200 paraproteins a year found in UK DGH serving 300-400k population
    - St Helier unpublished study



What should happen when M-proteins are found?

Over investigation

Risk of causing patients unnecessary anxiety,

+

Risk of inappropriate use of resources

v

Under-investigation

Risk of failing to identify patients at risk of developing myeloma (and thus perhaps missing the opportunity of avoiding advanced renal and lytic bone disease), amyloid etc

In the future may be a place for using agents which may delay or prevent progression

Predicting Progression:

- 1384 patients, residents of Olmstead County followed up for total of 11900 person years
- Average follow-up was 15.4 years (range 0-35)
- Median age at diagnosis 72 years
  - 2% were younger than 40 years
  - 59% were over 70
- 8.9% (115) of the group developed multiple myeloma or other lymphoproliferative disorder
- Myeloma : 65% of the 115

Kyle et al, Mayo Clinic

Predicting Progression

- There were only 2 statistically significant risk factors for progression
  - **The concentration** of monoclonal protein
  - **The type** of monoclonal protein
    - IgA and IgM gammopathy more likely than IgG to progress
    - IgM rarely becoming myeloma

Predicting Progression

- **Not** predictive of progression were
  - Bence Jones Proteinuria
  - Immunosuppression
  - Age
  - Sex

The risks of progression at 20 years follow-up

M protein level	Risk of progression
5 g/l	14%,
10 g/l	16%,
15 g/l	25%,
20 g/l	41%
25 g/l	49%

The cumulative risk of progression

- 10% at 10 years
- 21% at 20 years
- 26% at 25 years

Overall risk : 1% per annum

  - Risk remained even after 25 years or more

Proposed risk stratification model

Risk Group	No. pts	Relative risk 95% CI	20 year risk of progression %	20 year risk accounting for death %
Low risk (serum M- protein < 15g/dl, IgG subtype, normal FLC ratio (normal range 0.26-0.65)	449	1	5	2
Low-intermediate risk (any 1 factor abnormal)	420	5.4	21	10
High-intermediate risk (any 2 factors abnormal)	226	10.1	37	18
High risk (all 3 factors abnormal)	53	20.8	58	27

Rajkumar et al, 2005

Recommendations for investigation of M-proteins in primary care

- The initial evaluation requires the following:
  - Detailed history and examination
    - Focusing on the possibility that the patient has a plasma cell or lympho-proliferative malignant disorder.
    - Identifying symptoms and signs and test results commonly associated with myeloma, lymphoma or AL amyloid
  - FBC and U and E and calcium
  - Definition of the immunoglobulin class of the M-protein
  - Serum immunoglobulin levels
  - Spot urine for urinary protein excretion and urinary protein electrophoresis

Indications for referral of a person with an M-protein to a haematologist

- **<u>All</u> patients with symptoms or physical signs suggestive of underlying myeloma, other lympho-proliferative disorder or AL amyloidosis**

- **M-proteins**
  - IgG M-proteins >15g/l;
  - IgA or IgM M-proteins >10g/l
  - Any IgD or E paraprotein irrespective of size

- **Significant Bence-Jones proteinuria (eg. >500mg/l)**

- **Unexplained abnormal investigation results even in absence of symptoms**
  - eg anaemia, renal impairment, hypercalcaemia
  - lytic lesions,

Monitoring of patients with MGUS

The purpose of monitoring is to try to identify disease at an early stage when there is no significant irreversible lytic bone disease, renal failure, or other disabling symptoms and at a stage when the patient is fit enough to benefit from increasingly effective treatments.

Evidence for the efficacy of monitoring

None!

General principles of monitoring

Clinicians responsible for monitoring patients should be aware that

- the risk of progression to myeloma or other lymphoproliferative disease remains lifelong

- that risk never disappears even if the M-protein remains stable

Monitoring patients with MGUS: General principles

- It is essential that patients should be monitored not only by laboratory testing but also clinically
  - Patients and practitioners should be aware of and report relevant new symptoms and signs particularly the development of new bone pain, weight loss, fatigue and other symptoms which might indicate progression to myeloma amyloid or other lymphoproliferative disease.
    .

Monitoring in the low risk group

- Low risk defined as
  - one in which
    - **IgG M-protein <15g/l**
    - **IgA or IgM M-protein <10g/l**
    - **Non IgD or IgE M-protein**
  - there are no symptoms, signs or results of initial investigations suggestive of myeloma, other lympho-proliferative disorder or AL amyloidosis

Monitoring in the low risk group

- This group forms the vast majority of M-proteins detected in routine practice.

- For example, 60 % of M-proteins found in the laboratory in one District General Hospital were below 5 g/ l and have a very low risk of progression

Frequency of follow-up

- It could reasonably be argued that in the people with a very short actuarial life expectancy (perhaps less than 5 years) and very small paraproteins (eg. below 5 g/l) **regular follow up is not required** once myeloma amyloid and LPD have been excluded.

- However it would not be unreasonable to measure the M-protein occasionally when the patient was having other monitoring blood tests

Blood tests at monitoring visits

- Quantitation of the M-protein and immunoglobulin levels

- FBC, creatinine, urea and electrolytes, corrected calcium

Criteria for re-referral

- If symptoms compatible with a diagnosis of myeloma or lymphoma develop
- If the size of the M-component increases by more than 25% (a minimum absolute increase of 5g/l)
- If unexplained anaemia, other cytopenias or abnormal renal function or hypercalcaemia develop

Monitoring in the higher risk group

- Overall this group of patients requires much more frequent follow up, usually under the care of a Consultant Haematologist.
- Anything less that than 4 monthly is likely to prove ineffective.
- Clinicians should be aware of the patterns of progression.

Thank you