

Sorting out the what, why and when of blood tests in lymphoma

Lymphoma Support Group

May 3, 2022

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"Hold still, Mrs. Brown while I draw your blood."

Frequently asked questions

- 1) What are you looking for with these blood tests?
- 2) Can you tell I have lymphoma/can you tell if it has recurred with the blood tests?
- 3) What blood values do I need done before my chemotherapy treatments and why?
- 4) Does it matter where I do the blood tests?
- 5) Can I have my results?
- 6) Is it really necessary? My family doctor just did blood work ...

Diseases where the blood tests are crucial in documenting disease status:

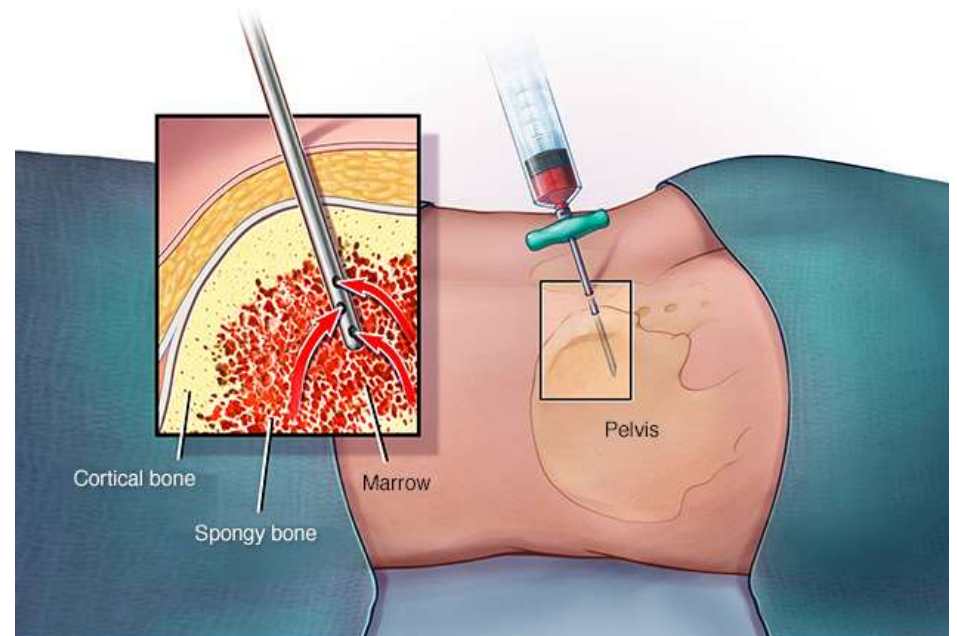
- 1) Chronic lymphocytic leukemia
- 2) Waldenstrom's macroglobulinemia (indolent B cell NHL)

Normal Blood Counts

Hemoglobin	Male: 135-170 g/L Female 115-155 g/L
White blood cell count	3.5-10.5 x10⁹/L
Platelet count	150-400 x10⁹/L
Neutrophil count	2-8 x10⁹/L
Lymphocyte count	1-4 x10⁹/L

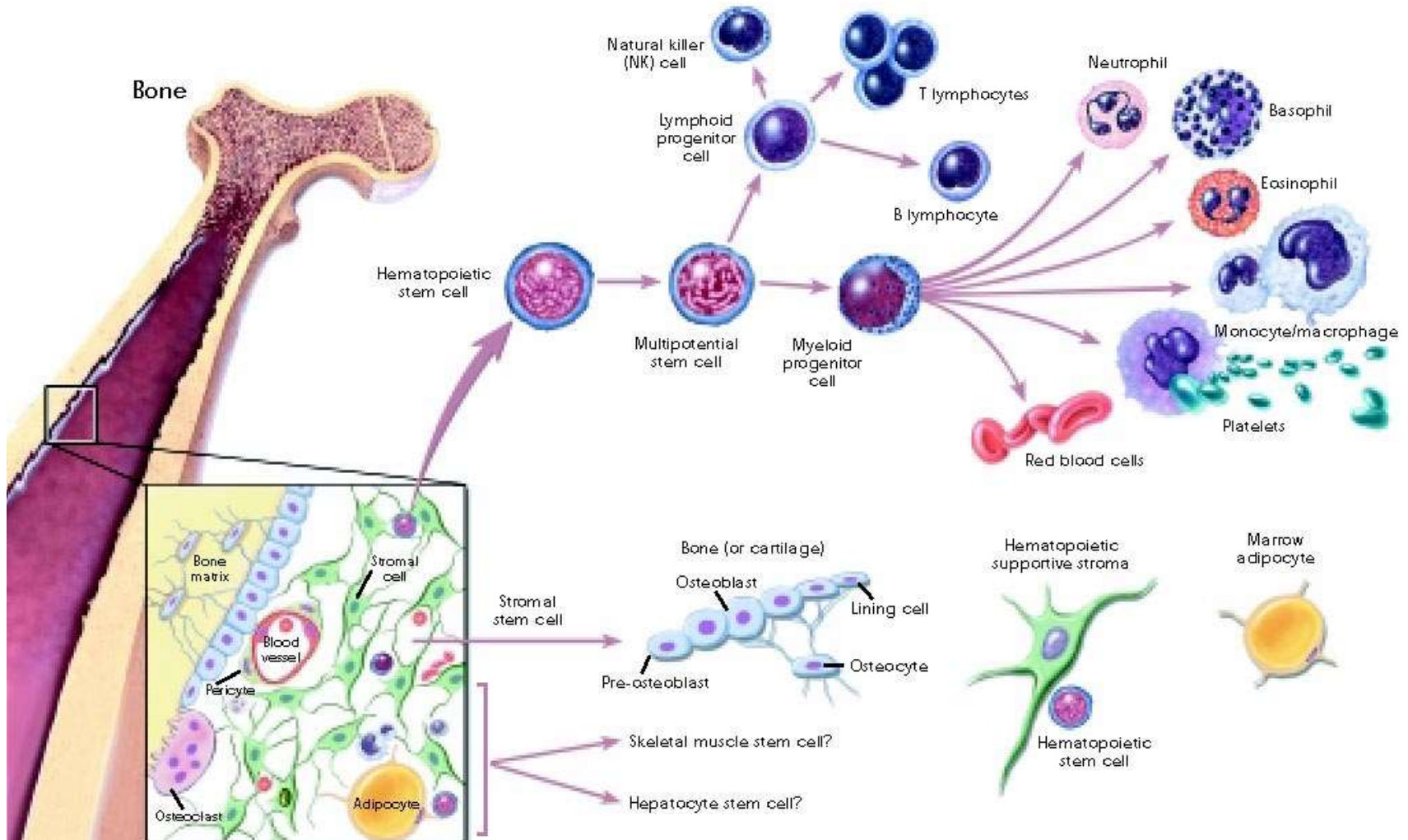
Bone Marrow

- Normal marrow
 - Hematopoiesis confined to the marrow in adults
 - Bone: cortex and medulla
 - Cortex; strong outer layer of compact bone
 - Medulla; cancellous bone
 - In adults, hematopoietic marrow is in the skull, vertebrae, ribs, clavicles, sternum, pelvis



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Normal bone marrow



Bone Marrow

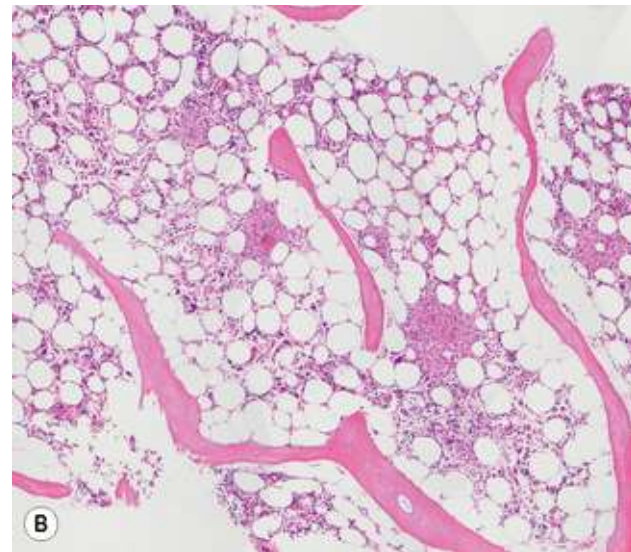
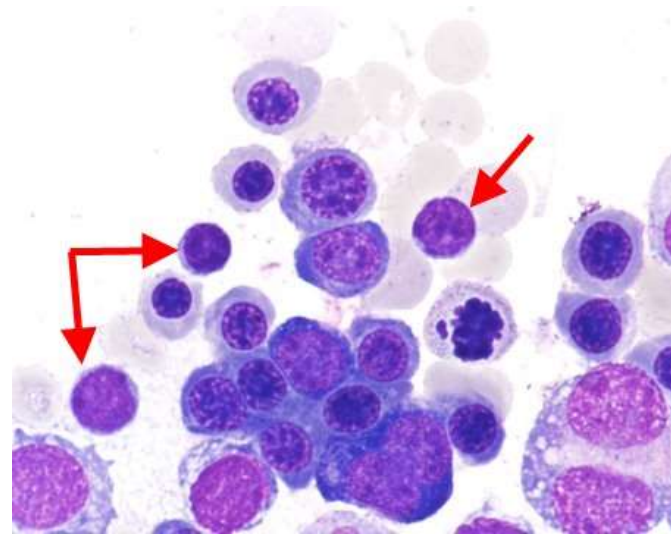
- Bone marrow aspirate
 - Iliac crest or sternum
 - Morphologic assessment
 - Flow cytometry
 - Cytogenetics/FISH
 - Molecular studies
 - Bone marrow culture



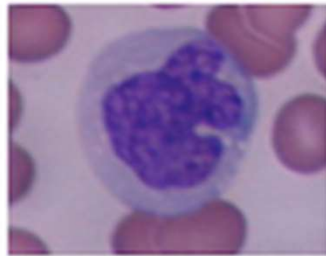
- Bone marrow biopsy
 - Iliac crest
 - Assessment of marrow architecture
 - Pattern of infiltrate

Hematopoietic Cells

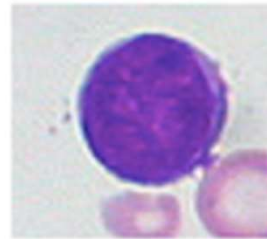
- Lymphopoiesis
 - Both B and T cells lymphocytes in marrow
 - More mature T cells and more immature B cells
 - Majority are CD8+ T cells
 - In marrow biopsies, can have both interstitial lymphocytes and lymphocyte nodules/aggregates



Types of White Blood Cells



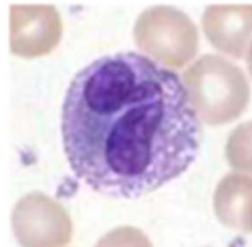
Monocyte



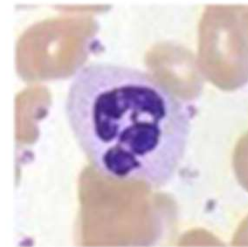
Lymphocyte



Basophil



Eosinophil



Neutrophil

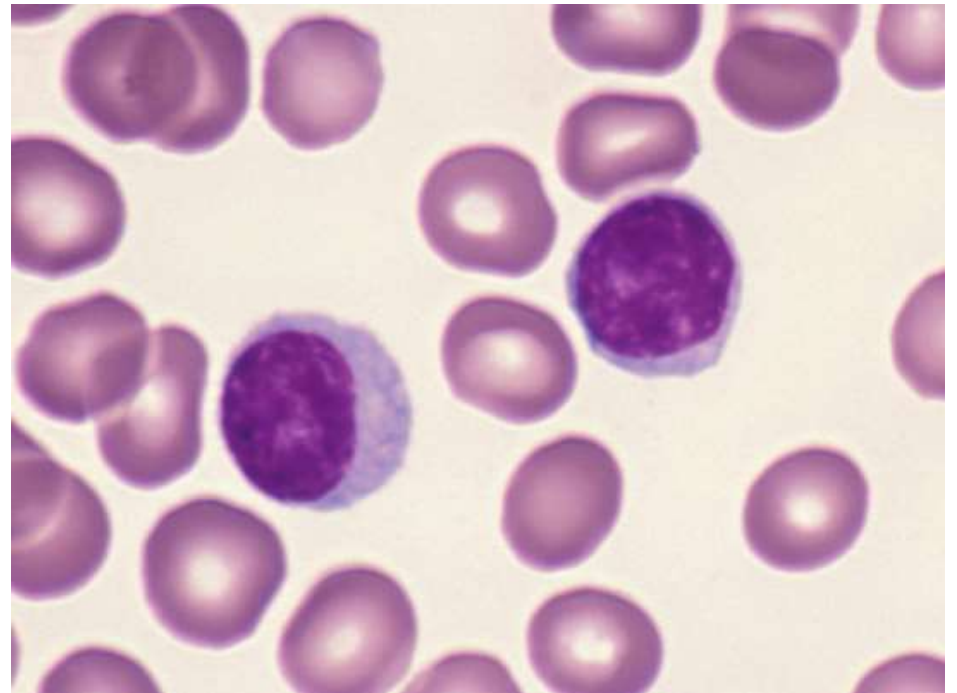
Neutrophils

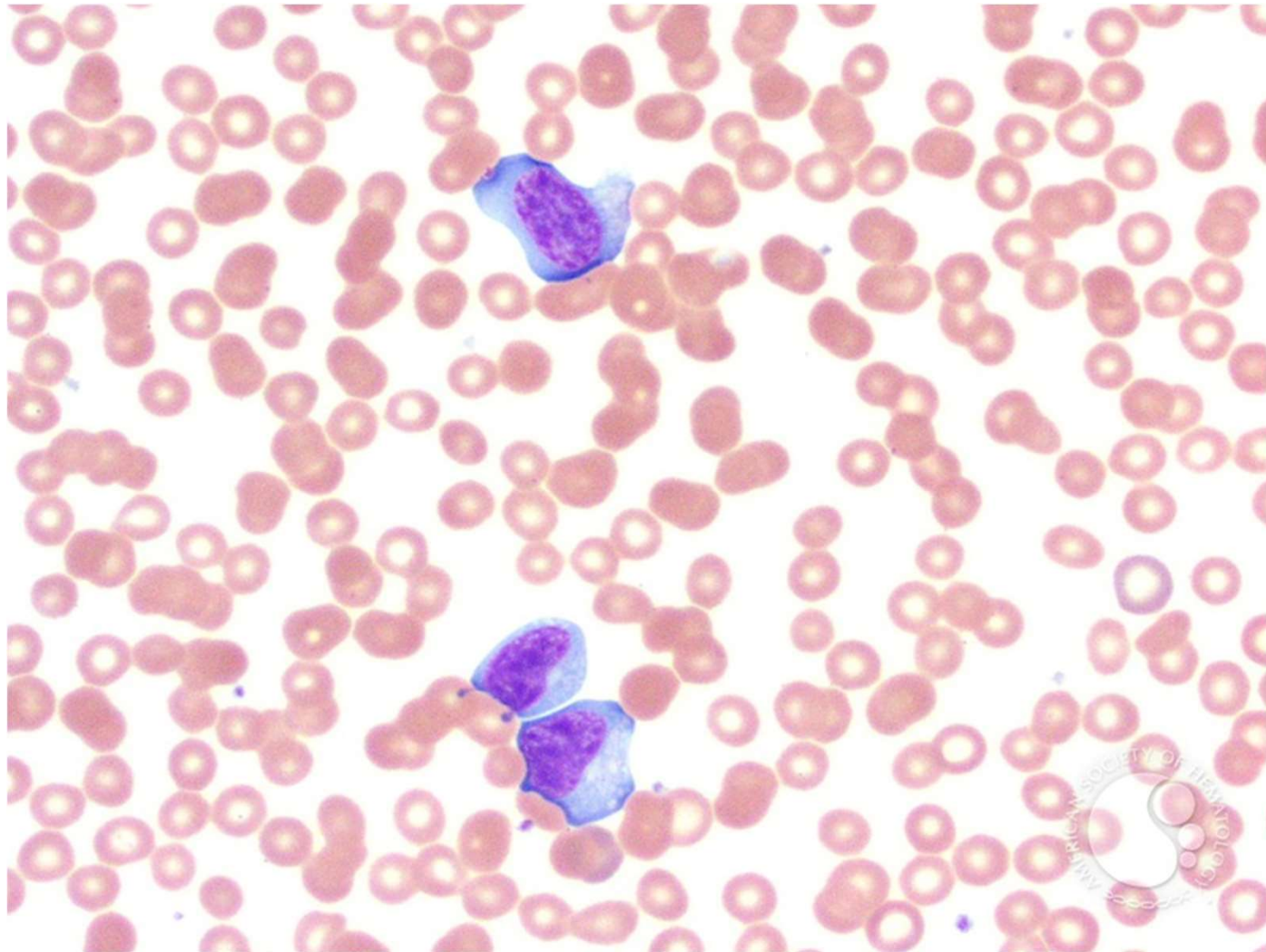
- 12-16 um
- Pink cytoplasm with granules
- Nucleus has clumped chromatin in 3-5 lobes



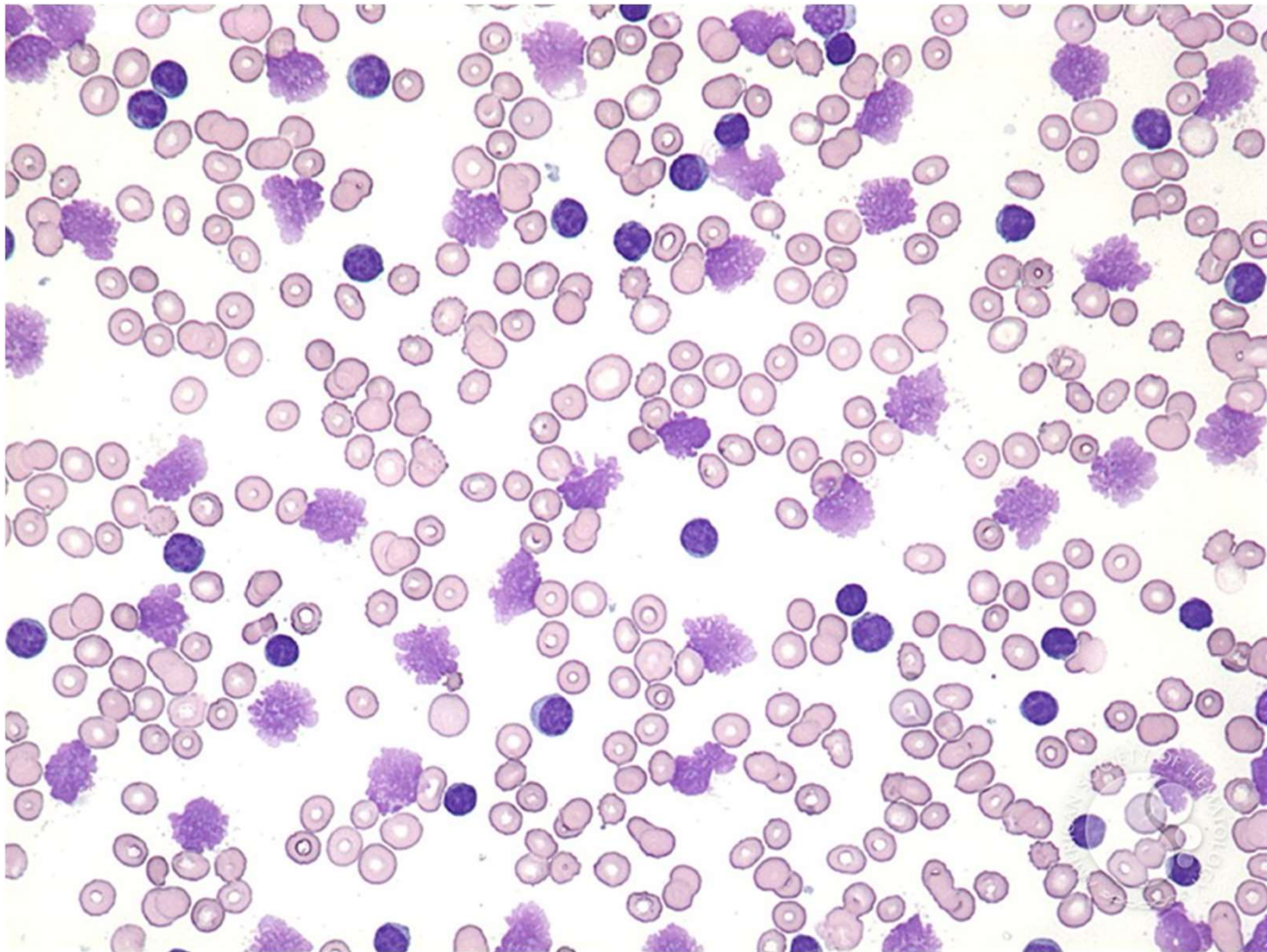
Lymphocytes

- 10-12 μm
- 12-16 μm if atypical/reactive
- Cytoplasm scant and blue
- Mononuclear
 - Mature; very clumped chromatin
 - Reactive may have less clumping and nucleoli





Smudge cells in CLL



Basic bloodwork for all patients with lymphoma at diagnosis

- Complete blood count
- Serum creatinine (renal/kidney function)
- Liver function (eg bilirubin, ALT, AST, ALP)
- LDH or LD (lactate dehydrogenase)
- Blood glucose
- Calcium
- Hepatitis B serology (if starting Rituxan-based treatment)



REQUISITION-ANALYSE (BLOOD ROUTINE-ROUTINE DU SANG) Biochemistry-Biochimie / Hematology-Hématologie

HEMATOLOGY-HÉMATOLOGIE (300)	
1 small EDTA tube this column (unless otherwise indicated)	
0000	<input checked="" type="checkbox"/> CBC-FSC
3745	<input checked="" type="checkbox"/> Blood film-Frottis sanguin
3510	<input checked="" type="checkbox"/> DIFF-Différentielle
4080	<input type="checkbox"/> Retic Count-Réticulocytes
4180	<input checked="" type="checkbox"/> ESR-VSE
5170	<input type="checkbox"/> Sickle cell screen-Test de falciformation (HbS)
5230	<input type="checkbox"/> Hemoglobinopathy Investigation Hémoglobinopathie
5190	<input type="checkbox"/> G6PD screen-Dépistage
5310	<input type="checkbox"/> Kleihauer-Test de Kleihauer
5160	<input type="checkbox"/> Infectious Mononucleosis screen-Dépistage de Mononucléose infectieuse

COAGULATION (300)	
4185	<input type="checkbox"/> INR
4200	<input type="checkbox"/> PTT
4220	<input type="checkbox"/> Thrombin Time-Temps
4230	<input type="checkbox"/> Fibrinogen(e)
4240	<input type="checkbox"/> D-Dimer(e)
Anti-Xa testing must be submitted using the STAT requisition. Les examens d'Anti-Xa doivent être soumis en utilisant la demande STAT.	

**MUST SPECIFY ANTICOAGULANT
PRÉCISER L'ANTICOAGULANT**

Std Hep
 Oral Anticoag(s) Oraux
 LMWH-HBPM: _____
 None-Aucun

3 tubes (for any or all of the following-pour un ou tous les tests suivants):

Factor assays-Dosage de facteurs			
5470	<input type="checkbox"/> FII	5560	<input type="checkbox"/> FVIII
5500	<input type="checkbox"/> FV	5620	<input type="checkbox"/> FIX
5530	<input type="checkbox"/> FVII	5650	<input type="checkbox"/> FX
5585	<input type="checkbox"/> Von Willebrand Assessment-Évaluation (Includes FVIII, VWAg, Ristocetin cofactor Incluant FVIII, VWAg, Activité cofacteur)		
6230	<input type="checkbox"/> Antithrombin(e)		
6260	<input type="checkbox"/> Protein(e)C	6270	<input type="checkbox"/> Protein(e)S (PRS)
6315	<input type="checkbox"/> Activated protein C Resist. à la protéine C activée		
5940	<input type="checkbox"/> Lupus Anticoagulant Lupique		
Factor inhibitors-Inhibiteurs de facteurs			
5860	<input type="checkbox"/> INHFVIII	5910	<input type="checkbox"/> INHFIX
6375	<input type="checkbox"/> HIT-TIH (info required-requise) 📞		

BIOCHEMISTRY-BIOCHIMIE (100)																	
1 PST tube this column (unless otherwise indicated)																	
1480	<input type="checkbox"/> Electrolytes (Na, K, Cl, tCO ₂)																
3670	<input type="checkbox"/> Sodium-Na ⁺																
3170	<input type="checkbox"/> Potassium-K ⁺																
0925	<input type="checkbox"/> Chloride-Chlorure-Cl ⁻																
0780	<input type="checkbox"/> Total CO ₂ total																
Glucose tolerance on reverse-Glycémie provoquée à l'endos																	
1869	<input checked="" type="checkbox"/> Glucose Random-Aléatoire																
1868	<input type="checkbox"/> Fasting-À jeun																
1862	<input type="checkbox"/> 2 h PC																
4070	<input type="checkbox"/> Urea-Urée																
1180	<input checked="" type="checkbox"/> Creatinine <input type="checkbox"/> eGFR																
0685	<input type="checkbox"/> Calcium-Ca ²⁺																
0085	<input checked="" type="checkbox"/> Albumin(e)																
2660	<input type="checkbox"/> Magnesium Mg ²⁺																
2940	<input type="checkbox"/> Phosphate PO ₄																
3975	<input type="checkbox"/> Urate-Acide urique																
0895	<input type="checkbox"/> CK																
3932	<input type="checkbox"/> Troponin(e) I																
0620	<input type="checkbox"/> Billirubin(e) <input type="checkbox"/> Total(e)																
0610	<input type="checkbox"/> Direct(e)																
0205	<input type="checkbox"/> ALP																
0490	<input type="checkbox"/> AST																
0260	<input type="checkbox"/> ALT																
1845	<input type="checkbox"/> GGT																
2400	<input checked="" type="checkbox"/> LD																
2475	<input type="checkbox"/> Lipase																
2504	<input checked="" type="checkbox"/> LFT-Foie (ALP, AST, ALT, GGT, T.Bill)																
3470	<input type="checkbox"/> Total protein(e)s totales																
0820	<input type="checkbox"/> Cholesterol																
3915	<input type="checkbox"/> Triglycerides																
2010	<input type="checkbox"/> HDL - Cholesterol																
2425	<input type="checkbox"/> LDL - Cholesterol (Calculated-Calculé)																
2305	<input type="checkbox"/> Iron and IBC-Fer et capacité de fixation																
THERAPEUTIC DRUGS-DROGUES THÉRAPEUTIQUES																	
0770	<input type="checkbox"/> Carbamazepine (Tegretol)																
1435	<input type="checkbox"/> Digoxin(e)																
2925	<input type="checkbox"/> Phenobarbital																
2935	<input type="checkbox"/> Phenytoin(e) (Dilantin)																
3785	<input type="checkbox"/> Theophylline																
4320	<input type="checkbox"/> Valproate-Acide valp (Depakene)																
2455	<input type="checkbox"/> Lithium (Li) (SST only-seulement)																
<table border="1"> <thead> <tr> <th></th> <th>PRE</th> <th>POST</th> <th>RDM-ALÉ.</th> </tr> </thead> <tbody> <tr> <td>Gentamicin(e)</td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>Tobramycin(e)</td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>Vancomycin(e)</td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </tbody> </table>			PRE	POST	RDM-ALÉ.	Gentamicin(e)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Tobramycin(e)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Vancomycin(e)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	PRE	POST	RDM-ALÉ.														
Gentamicin(e)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>														
Tobramycin(e)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>														
Vancomycin(e)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>														

Initial blood tests:

- Non Hodgkin lymphoma ✖
- Hodgkin lymphoma ✖
- Waldenstrom's macroglobulinemia ✖

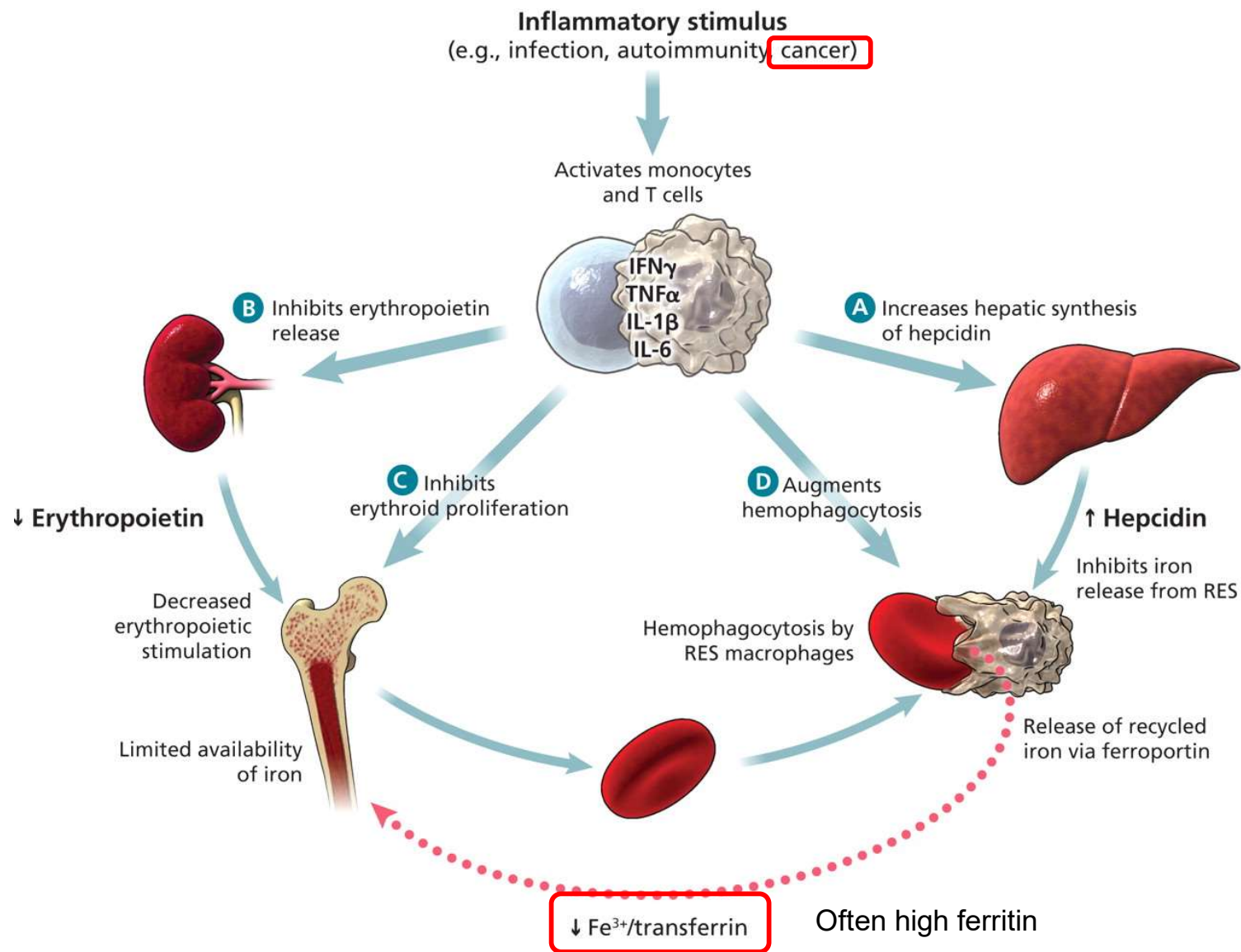
Location-Lieu

Physician last name-Nom de famille du médecin		First name-Prénom		Init.
CC to-à	Collection by-Échantillon par	Date	Time-Heure	
PROTEIN(E)S/IMMUNOGLOBULIN(E)S (100)		HORMONES (100)		
3595	<input type="checkbox"/> Rheumatoid factor-Facteur rhumatoïde	Maximum 4 tests / tube		
1005	<input type="checkbox"/> Complement, C3 + C4	2005	<input type="checkbox"/> βhCG	
0235	<input type="checkbox"/> Alpha 1 Antitrypsin(e)	3445	<input type="checkbox"/> Progesterone	
0810	<input type="checkbox"/> Ceruloplasmin(e)	1585	<input type="checkbox"/> Estradiol-Oestradiol	
1305	<input type="checkbox"/> C-reactive Protein(e) (CRP)	1690	<input type="checkbox"/> FSH	
1953	<input type="checkbox"/> Haptoglobin(e)	2450	<input type="checkbox"/> LH	
2245	<input type="checkbox"/> IgG	3450	<input type="checkbox"/> Prolactin(e)	
2235	<input type="checkbox"/> IgA	1125	<input type="checkbox"/> Cortisol	
2250	<input checked="" type="checkbox"/> IgM	1405	<input type="checkbox"/> DHEAS	
2255	<input type="checkbox"/> Immunoglobulin(e)s (G, A, M)	3780	<input type="checkbox"/> Testosterone, Total(e)	
3900	<input type="checkbox"/> Transferrin(e)	3645	<input type="checkbox"/> SHBG	
Separate tube for each test		3773	<input type="checkbox"/> TestoProfile (Tot., calc, Free, SHBG, Bioavail)	
1965	<input type="checkbox"/> HbA _{1c} -Hémoglobine A _{1c}	3805	<input type="checkbox"/> T ₄ free-libre 3925 <input type="checkbox"/> T ₃ free-libre	
3765	<input type="checkbox"/> Tacrolimus (Prograf)	3800	<input type="checkbox"/> TSH	
3627	<input type="checkbox"/> Sirolimus (Rapamune)	1645	<input type="checkbox"/> Ferritin(e)	
Cyclosporine		4370	<input type="checkbox"/> Vitamin(e) B12	
1340 C0 (pre)	<input type="checkbox"/>	1685	<input type="checkbox"/> Serum Folate sérique	
1341 C2 (2h post)	<input type="checkbox"/>	1852	<input type="checkbox"/> Growth Hormone de croissance	
Separate tube for each test		3545	<input type="checkbox"/> PTH (intact)	
0310	<input type="checkbox"/> Ammonia-Ammoniaque @	Other tests/Comments Autres tests/Commentaires		
0705	<input type="checkbox"/> Calcium, ionized-ionisé [I] *			
2795	<input type="checkbox"/> Osmolality-Osmolalité			
2350	<input type="checkbox"/> Lactate *			
3465	<input type="checkbox"/> Protein electrophoresis Électrophorèse des protéines			

Why are some lymphoma patients anemic at diagnosis?

- Often more than one cause
- May be due to bone marrow involvement
- Often due to “**anemia of chronic disease**”
- Less often due to hemolysis (red cells destroyed by autoimmune antibodies)
- Not often due to iron deficiency, poor diet or blood loss

Anemia of Chronic Disease (inflammation)



Zarychanski R , Houston D S CMAJ 2008;179:333-337

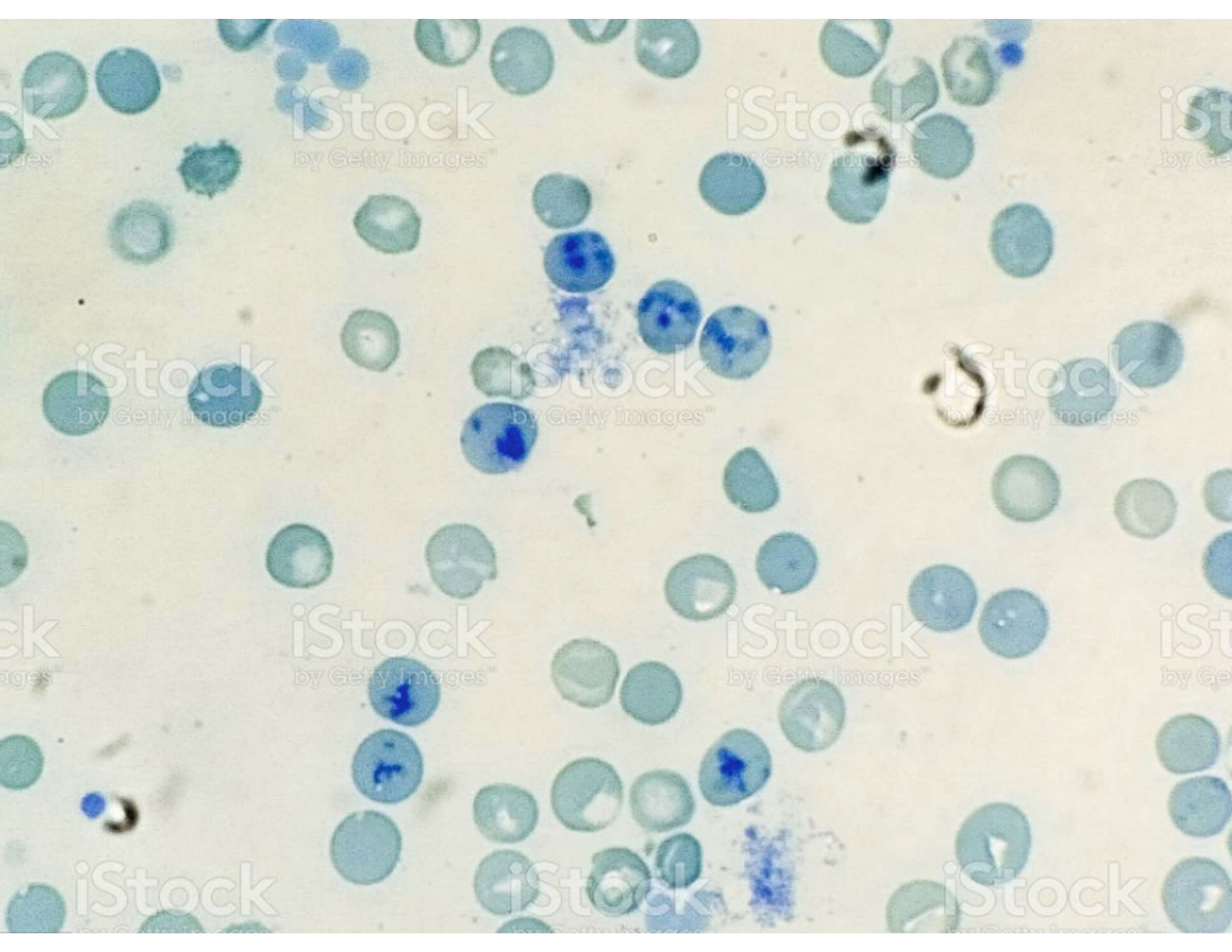


Reticulocyte Count

HEMATOLOGY
| C3 COMPLEMENT
| C4 COMPLEMENT
| BRUNNENGLERIN 10
| BRUNNENGLERIN 100

10120 | WBC
10120 | PLATELETS
10120 | VON
10120 | BRUNNENGLERIN 100

URINE & BODY FLUIDS
| URINE
| URINE
| URINE



Causes of Abnormal Reticulocyte Count

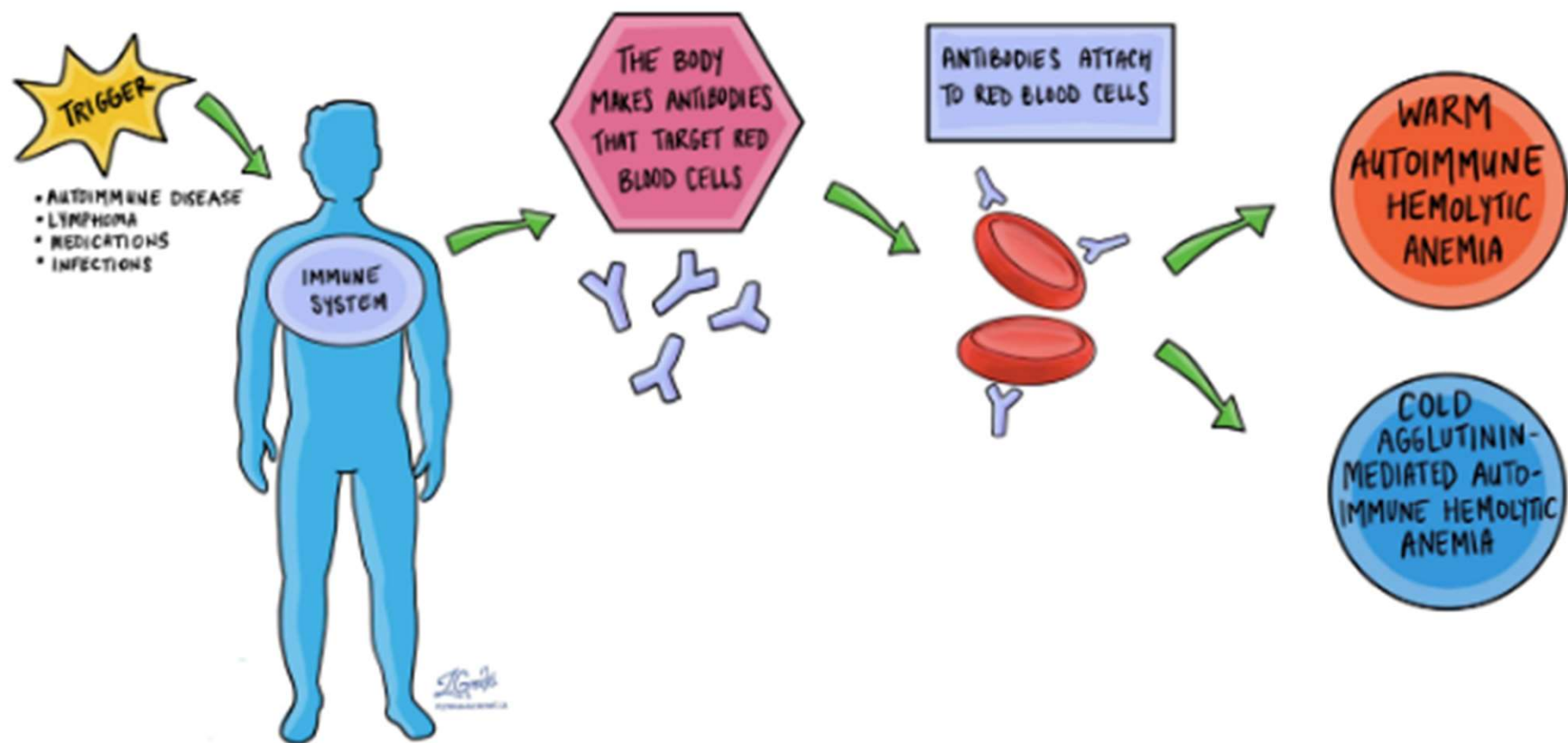
Decreased reticulocyte count

Bone marrow failure
Chronic disease
Folate deficiency
Infection
Iron deficiency
Liver disease
Malignancy
Pernicious anemia
Vitamin B₁₂ deficiency

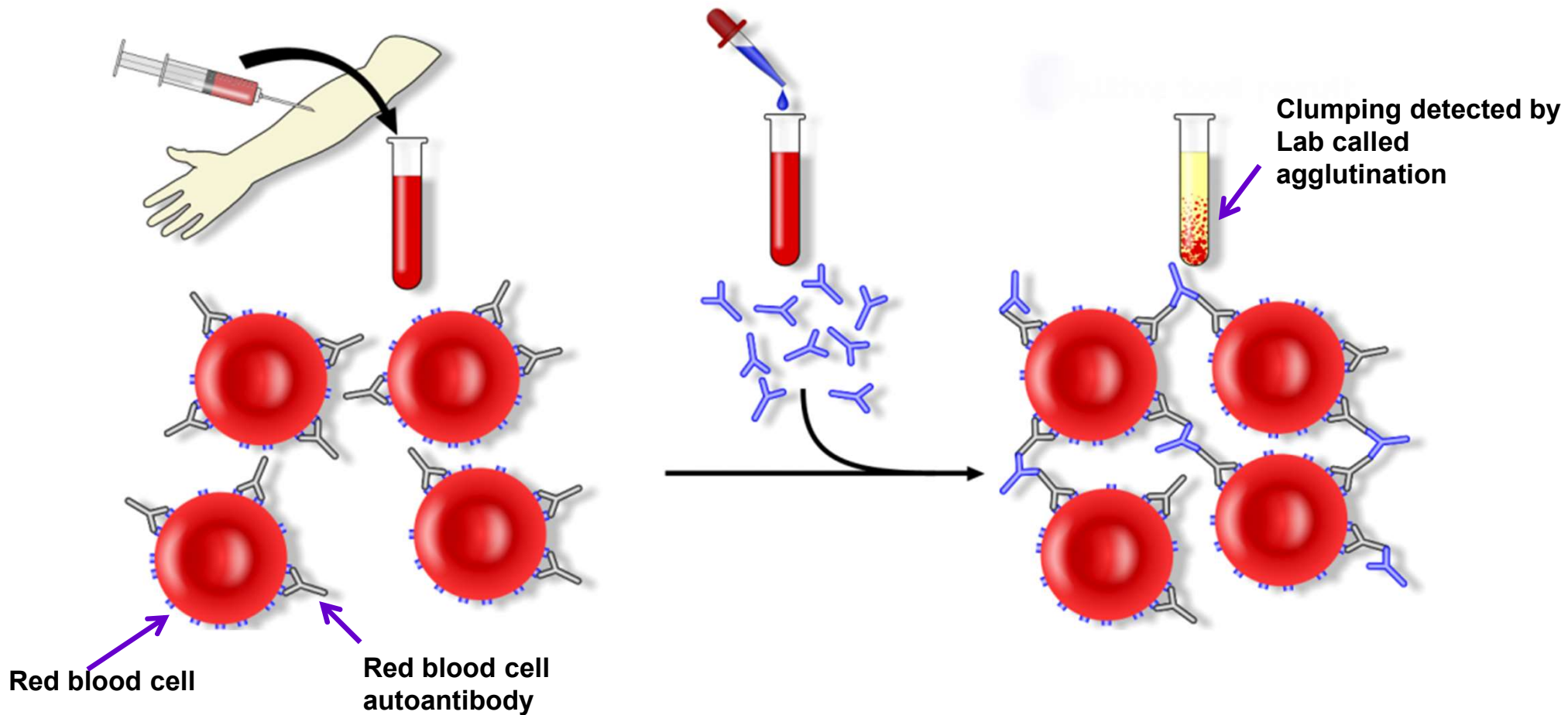
Increased reticulocyte count

Hemolysis
Hemorrhage
Leukemia
Pregnancy
Recovery from vitamin B₁₂,
folate, or iron deficiency
Sickle cell anemia

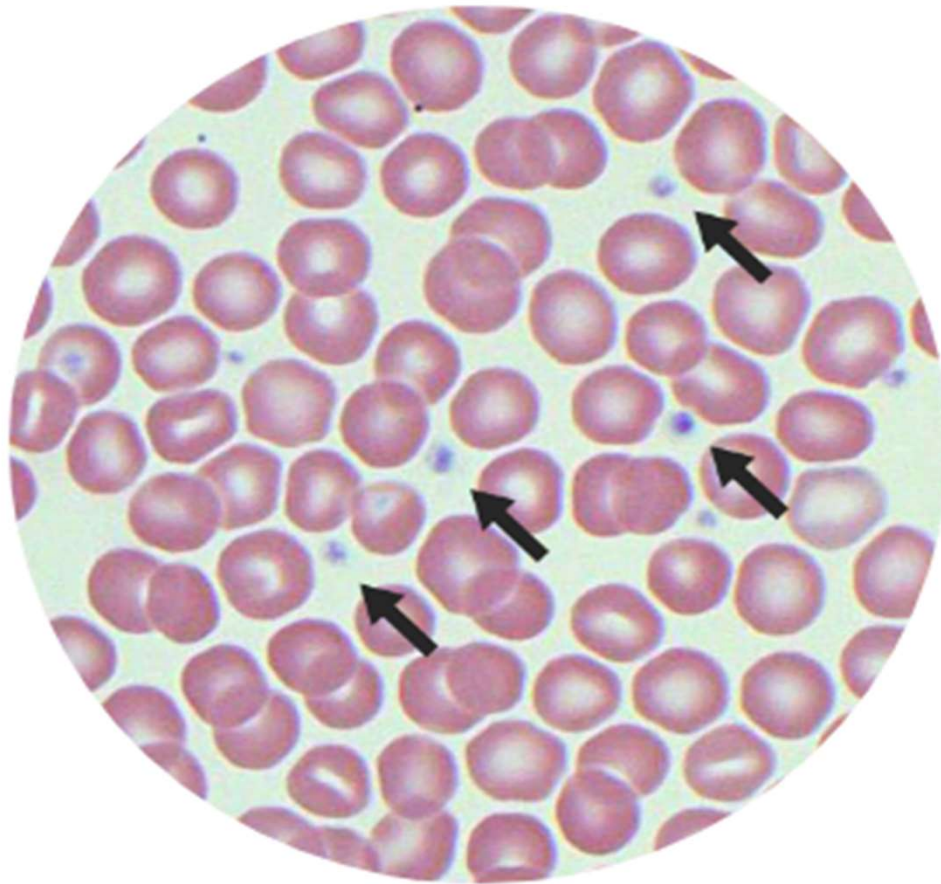
IMMUNE HEMOLYSIS



Direct antibody test (Coomb's test) done for hemolytic anemia



Platelets



Thrombocytosis=high platelet count

Can be caused by a variety of things:

- Inflammation (autoimmune disease, acute hemorrhage, burns, fractures, surgery, trauma)
- Cancer
- Iron deficiency
- Post-splenectomy
- Primary bone marrow disorder (myeloproliferative disorders)

Generally, you treat the underlying disorder and not the platelet count itself

Thrombocytopenia: low platelet count

- Mildly low platelet counts are very common and often not related to any kind of disorder
- Low folate or Vitamin B 12 can cause underproduction of platelets
- Enlarged spleen causes sequestration
- Increased destruction- immune thrombocytopenic purpura (ITP), often associated with CLL , sometimes lymphoma
- Marrow involvement with lymphoma
- Chemotherapy can temporarily lower platelets

Lactate Dehydrogenase (LDH)

- An enzyme found inside most body cells
- Circulates at low levels in the blood
- Any kind of tissue damage will increase LDH such as muscle damage, heart attack, liver inflammation, surgery, trauma.
- Not useful as a screening test in general, but may be important when diagnosing and monitoring lymphoma
- *** mild elevations of LDH are commonplace and benign ***

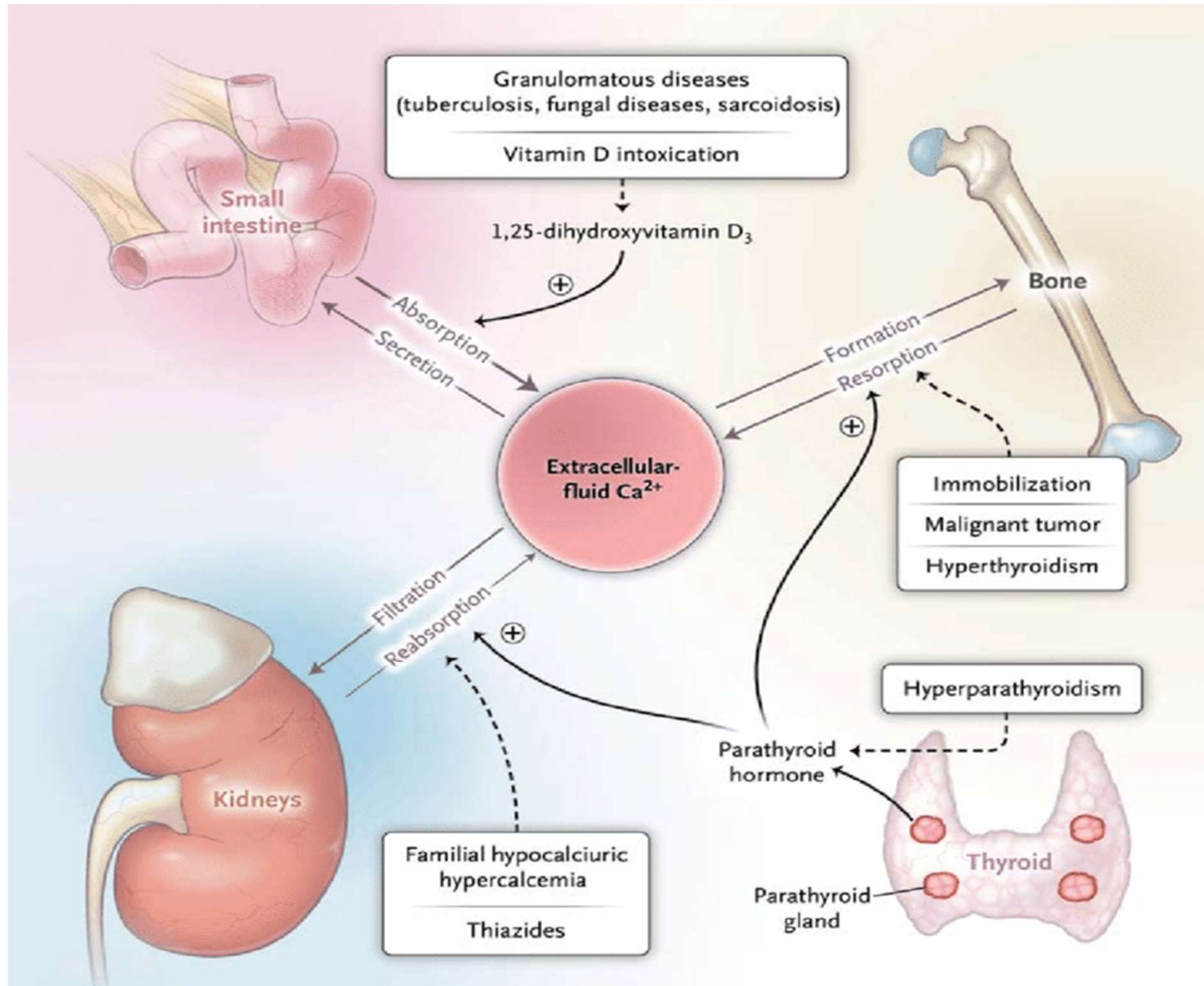
Special Bloodwork required for rituximab

Screen for hepatitis B

Hepatitis B virus reactivation can occur by rituximab in lymphoma patients with previously resolved hepatitis B infections

Rarely severe neutropenia can be seen during rituximab maintenance → CBC required pre-rituximab

Hypercalcemia



Monitoring during chemotherapy

CBC required before each chemotherapy treatment – within 48-72 hours

Special attention when neutrophil count too low (neutrophils need to be ≥ 1.0)

Hodgkin lymphoma is the exception to the neutrophil rule – we generally treat no matter how low the count

Post-Treatment Testing

- There are many guidelines with various recommendations based on little evidence
- Generally we do blood tests every 3 months for two years (CBC, LDH, creatinine)
- Then every 6-12 months for very stable patients in long term remission
- Some diseases require more monitoring
- **A blood test alone is generally insufficient to say you are 100% fine**

Follicular NHL

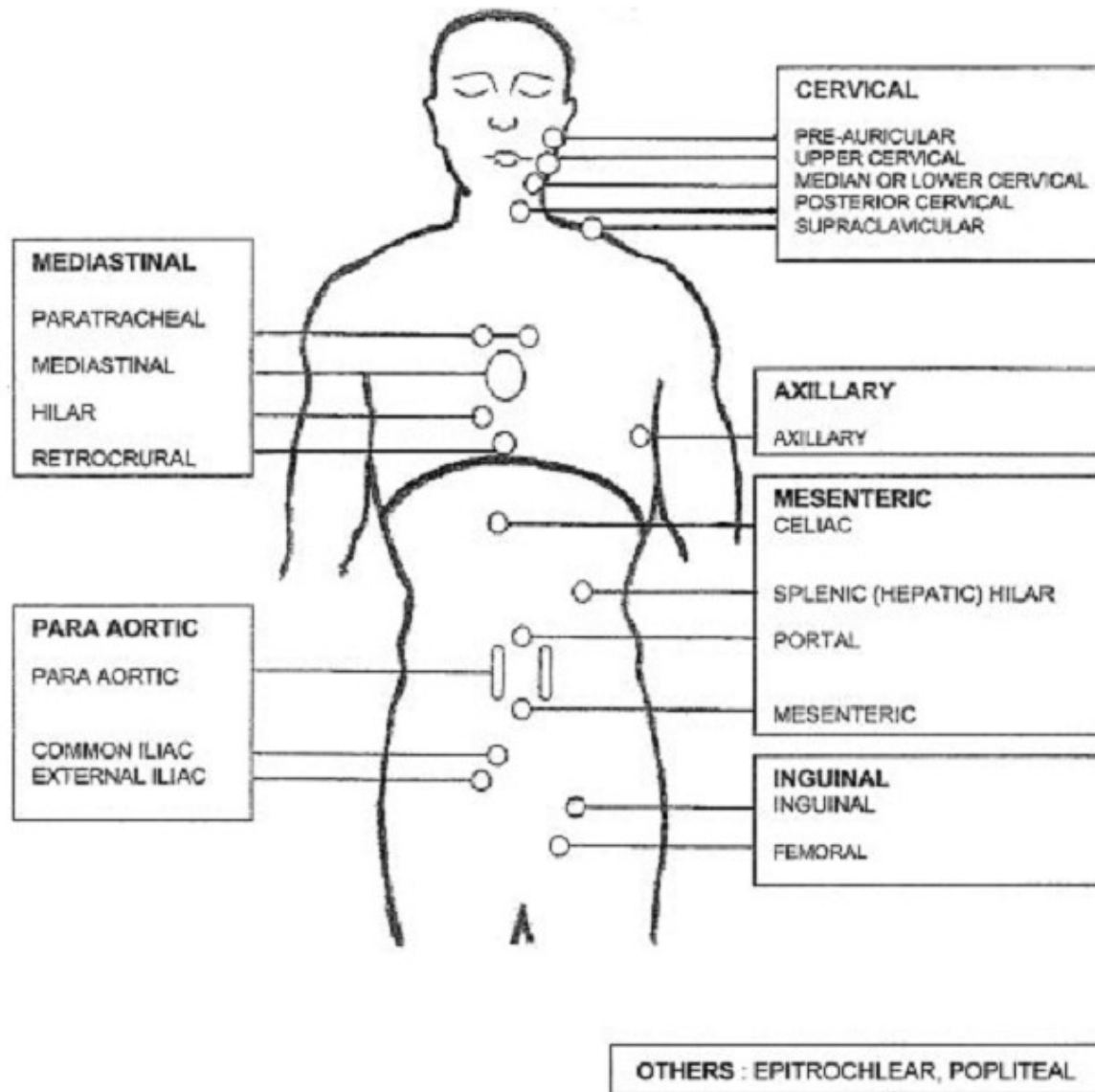
Prognostic score often used to determine need for treatment, predict severity of disease

FLIPI SCORE (Follicular lymphoma international prognostic index)

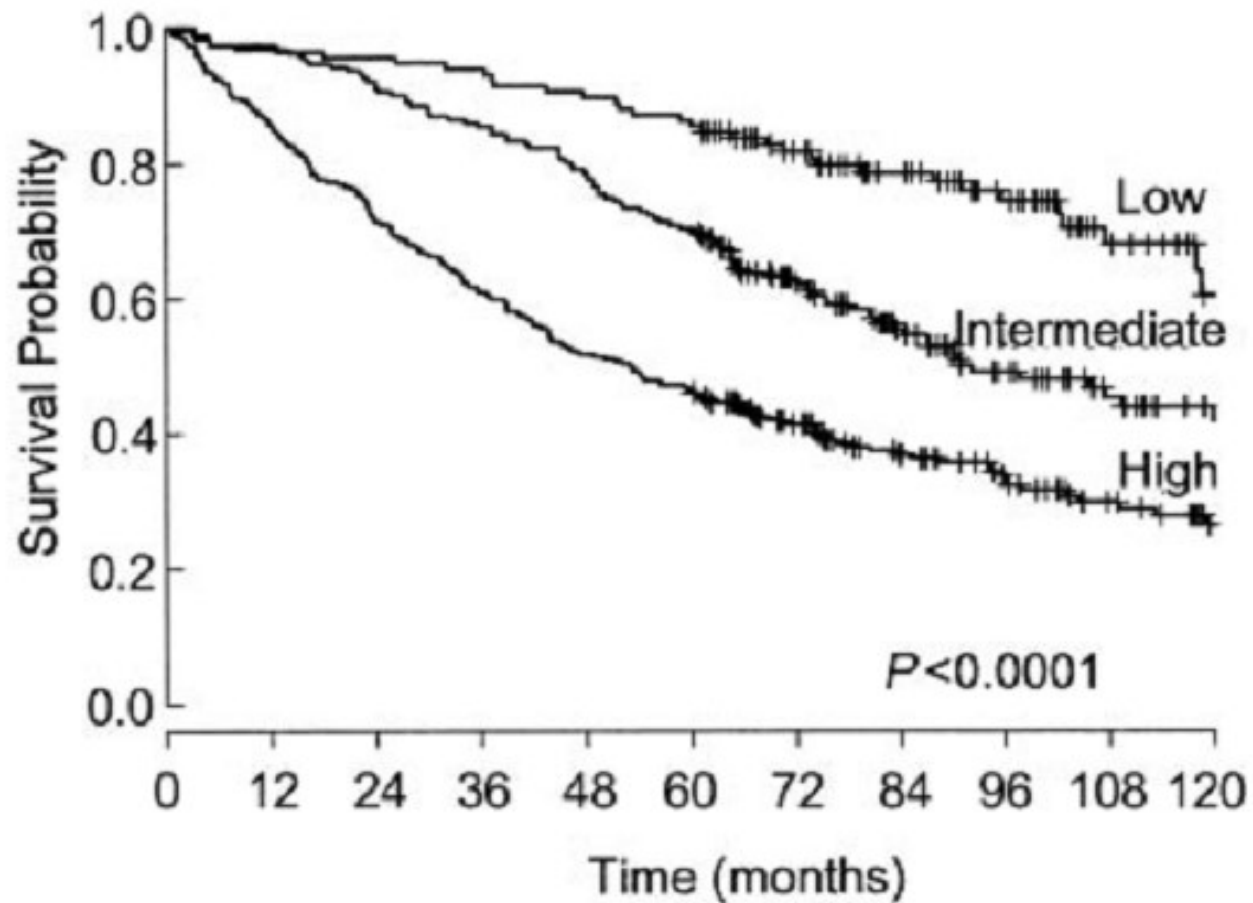
- 1) Hemoglobin < 120g/L*
- 2) LDH > normal*
- 3) Number of nodal sites >4
- 4) Stage III/IV
- 5) age >60 years

FLIPI 0-5

Counting the number of nodal sites



FLIPI Score for Follicular NHL



Case: Follicular NHL

- 75 year old female with 3 month history of progressive fatigue, 3 week history of being unwell, decreased appetite and 6 pound weight loss
- Seen by family doctor who sent her in to ER with severe anemia
- CT scans in hospital reveal enlarged abdominal and pelvic lymph nodes
- Core needle biopsy reveals follicular grade 1/2 NHL

Case: Follicular NHL

CBC: hemoglobin 60 g/L

platelets $115 \times 10^9/L$

WBC $3.9 \times 10^9/L$

LDH 463 (high) , reticulocyte count low

Why is she so anemic?

What should be done?

Any problems she may encounter during chemotherapy?

Bone Marrow Involvement by Follicular NHL



Paratrabecular involvement of Follicular NHL



Hodgkin Lymphoma Advanced Stage III/IV International Prognostic Index (IPI)

- 1) Age >45
- 2) Male sex
- 3) Stage IV
- 4) Albumin < 40 g/L*
- 5) Hemoglobin < 105 g/L*
- 6) White blood cell count > 15 x10⁹/L*
- 7) Lymphocyte count < 0.6 x10⁹/L*

IPI 0-7

Case: Hodgkin Lymphoma

- 18 year old male with 6 month history of waxing and waning enlarged nodal mass in left neck, associated with intermittent fevers
- Excisional lymph node biopsy of neck mass shows Hodgkin lymphoma, nodular sclerosing subtype
- **Stage 3B** based on symptoms and CT scans

Blood work:

- Hemoglobin 90 g/L*
- WBC $2.3 \times 10^9/L$
- Platelets $110 \times 10^9/L$
- Lymphocytes $0.4 \times 10^9/L$ *
- LDH 250 $\mu\text{mol/L}$
- Albumin 25 $\mu\text{mol/L}$ *

Anemia is usually due to chronic disease with Hodgkin lymphoma and improves with chemotherapy

Diffuse Large B Cell Lymphoma

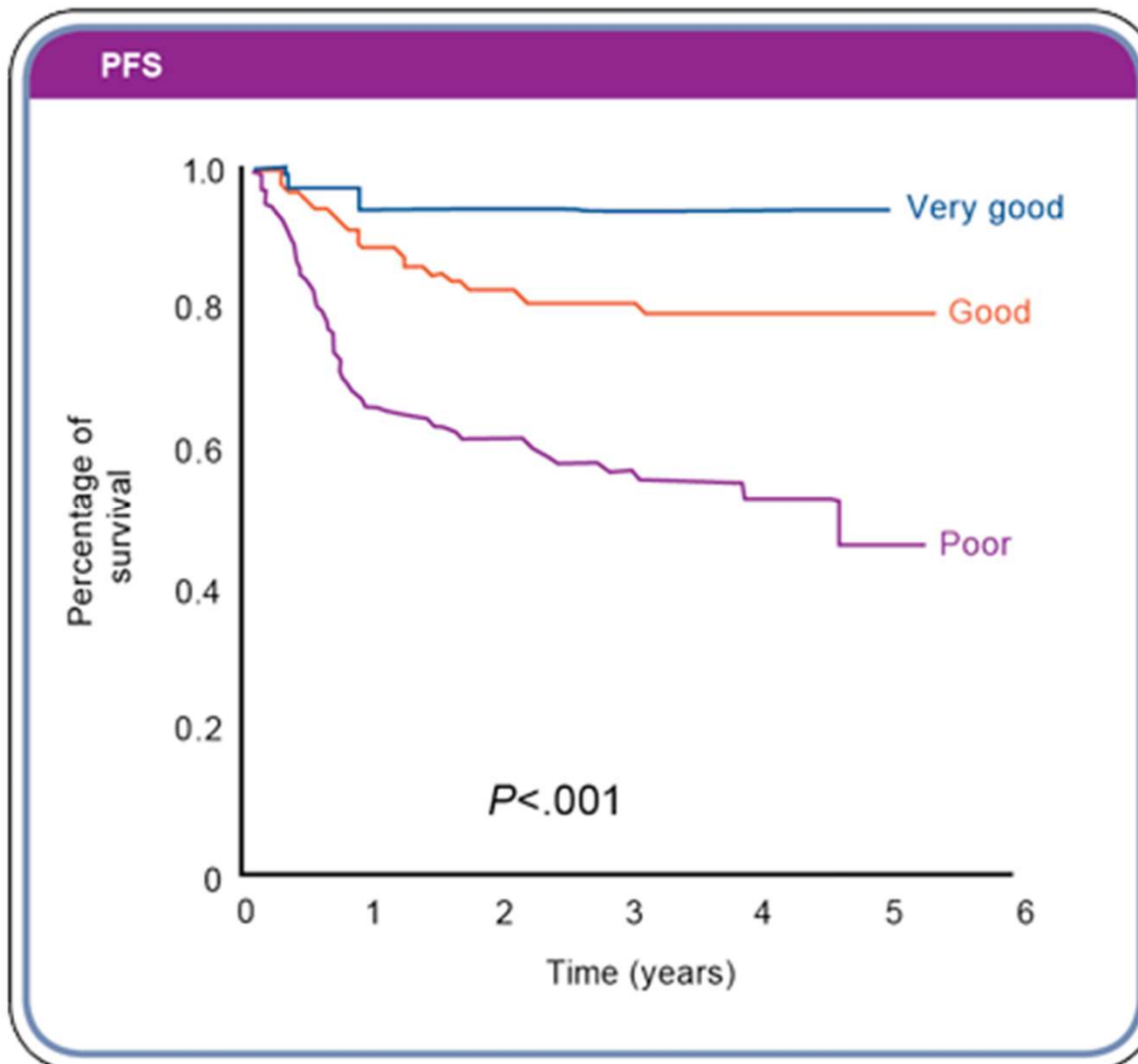
IPI – International Prognostic Index

- 1) Age > 60 years
- 2) Stage III/IV
- 3) More than 1 extranodal site
- 4) LDH above normal*
- 5) Performance status $\geq 2/5$

The Revised-IPI (R-IPI) (2005)

Risk group	No. of IPI factors	Percentage of patients	4-year PFS	4-year OS
Very good	0	10%	94%	94%
Good	1, 2	45%	80%	79%
Poor	3, 4, 5	45%	53%	55%

PFS according to the R-IPI



Case: DLBCL

53 year old woman with history of lupus, mild kidney disease, high BP

HPI: slightly low hemoglobin and fatigue x 1 year

Admitted to hospital from clinic with severe anemia, splenomegaly, large nodes in left parotid and axillary region

Lymph node biopsy → diffuse large B cell lymphoma

Blood work:

Hemoglobin 52 g/L, WBC 2.6, platelets 124

LDH elevated

Blood film – polychromasia (young “blue” red blood cells)

Reticulocyte count – elevated

Why is she so anemic?

What should be done next?

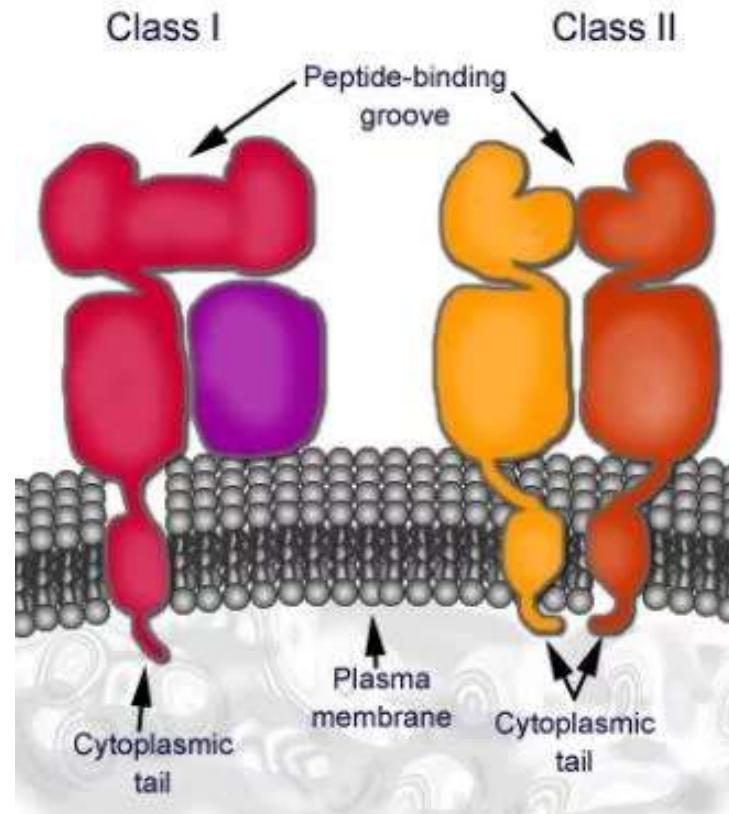
Blood work:

Transfusion medicine tests reveal presence of auto antibodies against red blood cells

This makes cross match for future blood transfusion difficult

Cause for anemia: autoimmune hemolytic anemia. Will resolve when the underlying lymphoma is treated. Short term treated with steroids (prednisone) and transfusions

Beta-2 microglobulin



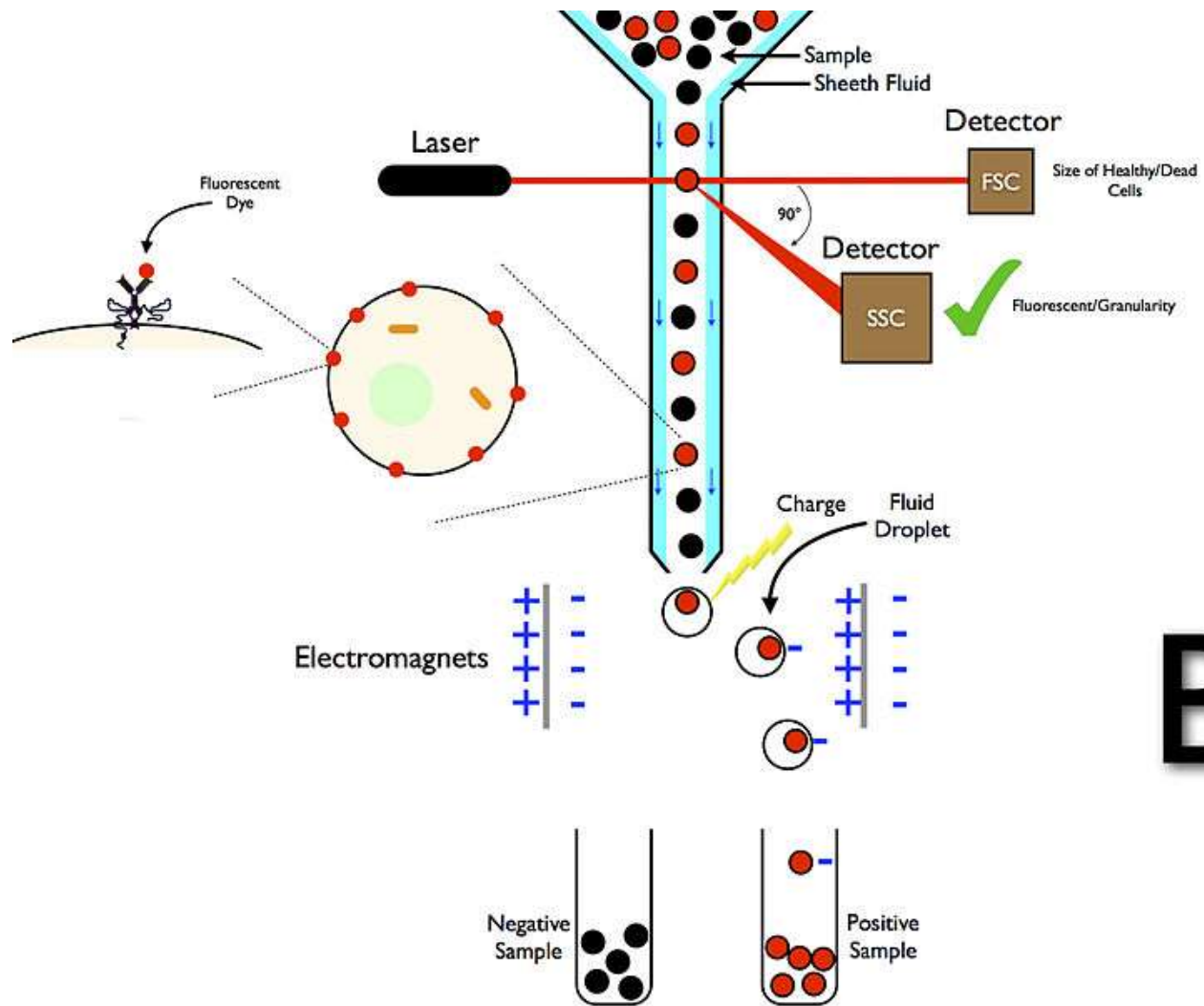
Beta-2 microglobulin

- beta-2 microglobulin (B2M) test is used as a tumor marker for multiple myeloma and sometimes for low grade NHL
- It is a blood test that detects the level of this protein that is found on the surface of most cells in your body, particularly some blood cells
- The blood test measures the amount of this protein that is shed into your blood

Waldenstrom's Macroglobulinemia International Prognostic Index

- 1) age >65 years;
- 2) hemoglobin ≤ 115 g/L*
- 3) platelet count $\leq 100 \times 10^9$ /L*
- 4) B2-microglobulin >3 mg/L*
- 5) Serum IgM concentration >70 g/L*

Flow cytometry



B

Flow cytometry test uses

- Used to diagnose CLL
- Done if there is a high lymphocyte count in the blood for any reason
- Is done on bone marrow cells as part of bone marrow test
- Can be done on any fluid, such as pleural (lung) fluid, spinal fluid
- Can be done on a node biopsy if sample appropriately prepared

Questions?