Hematology:

Understanding the Immunoglobulins and Bone Marrow Diseases

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Disclosures

- **Presenter: Dr. Andrea Lee**

- **Relationships with commercial interests:**
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  - **Other:** None

- **Potential for conflict(s) of interest: None**

Objectives

- Know the indications for ordering a serum protein electrophoresis (SPEP)
- Learn to interpret the SPEP
  - Understanding polyclonal versus monoclonal gammopathy
- Develop an approach to investigating a monoclonal protein (MCP)
- Be aware of the diseases associated with MCPs
- Know when to refer to hematology

Case 1

- 60 F postmenopausal
- Hx of hypertension, dyslipidemia, DM2
- BMD shows osteoporosis - no back pain or fractures
- Basic investigations
  - Complete blood count (CBC) – normal
  - Creatinine, Ca^{2+}, Phos, Mg^{2+} normal
  - Liver function tests normal, TSH normal
  - 25(OH)D low, PTH normal
- Your colleague suggests order a SPEP but you're not sure it is required….

Interactive Question

- In the work-up of post-menopausal osteoporosis, a serum protein electrophoresis is….

A. Always indicated
B. Sometimes indicated in selected patients
C. Never Indicated

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Primary Care Testing for SPEP

- No indication for routine testing on PHE
- Common indications[1]:
  - Suspected immunodeficiency
  - Chronic inflammatory conditions (RA, SLE) or chronic infections (e.g.. hepatitis C, HIV).

1. O'Connell TX, et al. Am Fam Physician. 2005 Jan 1;71(1):105-12.

Primary Care Testing for SPEP

- Symptoms suspicious for multiple myeloma, Waldenström's macroglobulinemia, amyloidosis
  - New-onset anemia + renal failure + bone pain
  - Rouleaux formation on peripheral blood smear
  - Back pain
  - Unexplained pathologic fracture or lytic lesion
  - Hypercalcemia
  - Renal insufficiency
  - Proteinuria
  - Unexplained peripheral neuropathy
  - Osteoporosis work up in selected patients

SPEP in Osteoporosis

- Prevalence of secondary osteoporosis is unknown
  - 26 – 27% of women and men > age 50 yr
  - as high as 60% in one study on men alone [2,3]
- SPEP recommended in SELECTED patients
  - Canadian: vertebral or atypical fractures[4]
  - National Osteoporosis Foundation - SPEP, SFLCR and IFE [5]
  - North American Menopause Society 2010 [6]
  - Am Assoc of Clinical Endocrinologists – SPEP, SFLCR [7]
  - Endocrine society (men) [8]

SPEP in Osteoporosis

- “Selected” subject to interpretation
  - Fractures, osteopenia, osteoporosis age <65 years
  - Symptom directed testing
  - Fracture history
- Monoclonal protein found in 2.1% of patients screened[1]

1. O’Connell TX, et al. Am Fam Physician. 2005 Jan 1;71(1):105-12.

Case 1: Now what?

- You order a SPEP and find a monoclonal protein of 2 g/L
- What should you do?
  A. Refer to hematology
  B. Forget about the result, it isn't significant
  C. Repeat testing in 6 months
  D. Order more tests to see if this is something significant
  E. Both C and D

Case 1: Now what?

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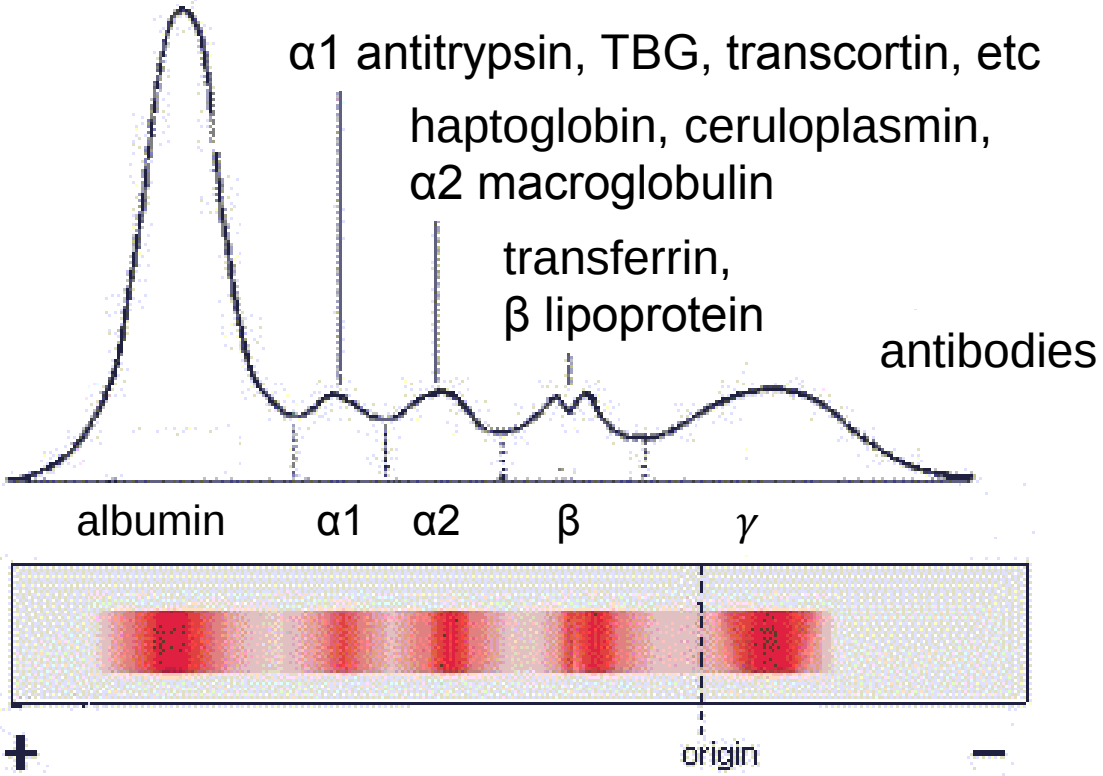
Understanding the SPEP



- Proteins migrate in the electrical field according to charge, size, and shape
- Densitometric scan of the gel separation

9. Image: https://pbrainmd.wordpress.com/2013/05/27/spep-serum-protein-electrophoresis/.

Interpreting the SPEP

Fraction	Increased	Decreased
Albumin	Dehydration	Protein loss • Protein-losing enteropathy • Nephrotic syndrome • Hemorrhage, burns • Malnutrition • liver disease
Alpha 1	Estrogen effect (Pregnancy) Acute phase reactant: infection, injury, trauma	Alpha1-antitrypsin deficiency
Alpha 2	Acute / chronic inflammation Estrogen effect nephrotic syndrome steroid use, hyperthyroidism	Malnutrition, Megaloblastic anemia, Protein-losing enteropathies Hemolysis, liver disease

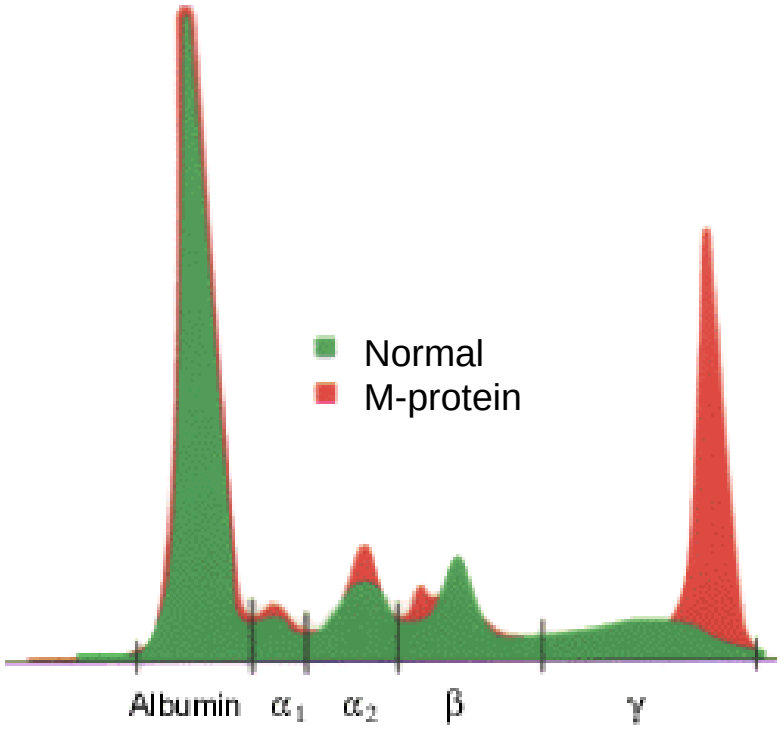
10. Keren, David F. Protein Electrophoresis in Clinical Diagnosis. Great Britain: Hodder Arnold; 2003: 109-137.

Interpreting the SPEP

Fraction	Increased	Decreased
Beta	Hyperlipidemia, iron-deficiency anemia	Protein malnutrition
Gamma	Polyclonal gammopathy • Chronic infections • Malignancy • Cirrhosis • CTD Monoclonal gammopathy (IFE) • MGUS • Multiple Myeloma, amyloidosis, Waldenstrom's Macroglobulinemia	Agammaglobulinemia Hypogammaglobulinemia

10. Keren, David F. Protein Electrophoresis in Clinical Diagnosis. Great Britain: Hodder Arnold; 2003: 109-137.

Abnormal SPEP: Monoclonal Protein



AKA: M-protein, M-spike, paraprotein

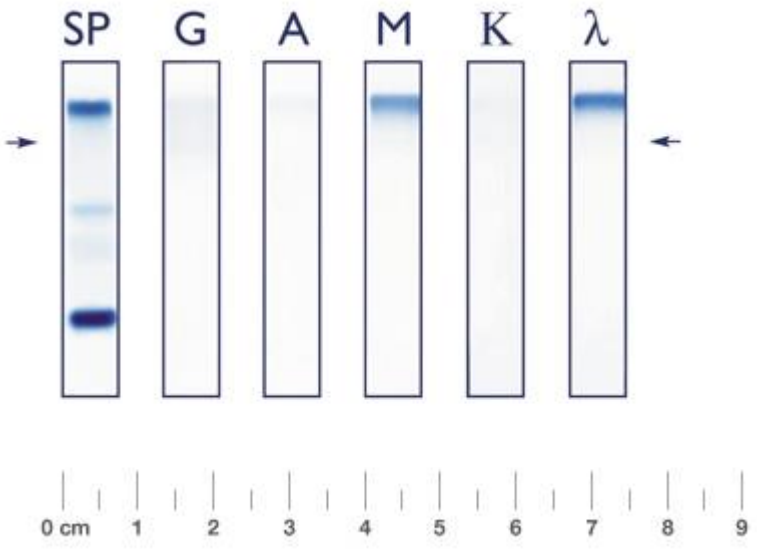
- Single class of immunoglobulins secreted by abnormally expanded plasma cell clone
- Uses:
  - Screens and quantifies MCP
- Limitations:
  - Insensitive to small MCP
  - Does not subtype MCP (e.g. IgG λ etc)

11. Image: Krafts, K. http://www.pathologystudent.com/?p=5282. 27 Feb 2012. Accessed 13 Nov 2016.

Additional Tests

- Quantitative immunoglobulins
  - ⬇ in immunodeficiency
  - ⬆ in polyclonal vs monoclonal gammopathy
- Immunofixation
  - Typing of MCP
  - Differentiates between monoclonal vs polyclonal gammopathy
  - Doesn't quantify protein → must order SPEP

SPEP and IFE alone will miss ~15% of plasma cell dyscrasias [1]



12. Katzmann JA, et al. *Clinical chemistry*. 2009;55(8):1517-1522.

Additional Testing

- 24 hour urine for Urine PEP and immunofixation
  - Bence Jones protein = monoclonal protein or light chain found in the urine
  - Limitations[12]:
    - Not sensitive to small M-proteins
    - Cumbersome to perform
    - Difficult to interpret with concentrated samples or heavy proteinuria containing polyclonal proteins
- Combined serum and urine PEP and IFE studies 97% sensitivity for plasma cell dyscrasia[13]

12. Jenner, E. Clin Chim Acta. 2014 Jan 1; 427: 15–20.
13. Katzmann JA, et al. *Clinical chemistry*. 2009;55(8):1517-1522.

Serum Free Light Chains (SFLC)

- Normal kappa : lambda RATIO is 2:1
  - Highly abnormal RATIOS → monoclonal gammopathy
  - Borderline abnormal ratio → interpret with caution, can be due to polyclonal gammopathy and renal impairment [12]
- Uses:
  - Dx of non or oligo secretory MM, amyloidosis
  - Predicting progression for MGUS, smoldering MM, plasmacytoma
  - Monitoring residual disease

12. Jenner, E. Clin Chim Acta. 2014 Jan 1; 427: 15–20.

Serum Free Light Chains (SFLC)

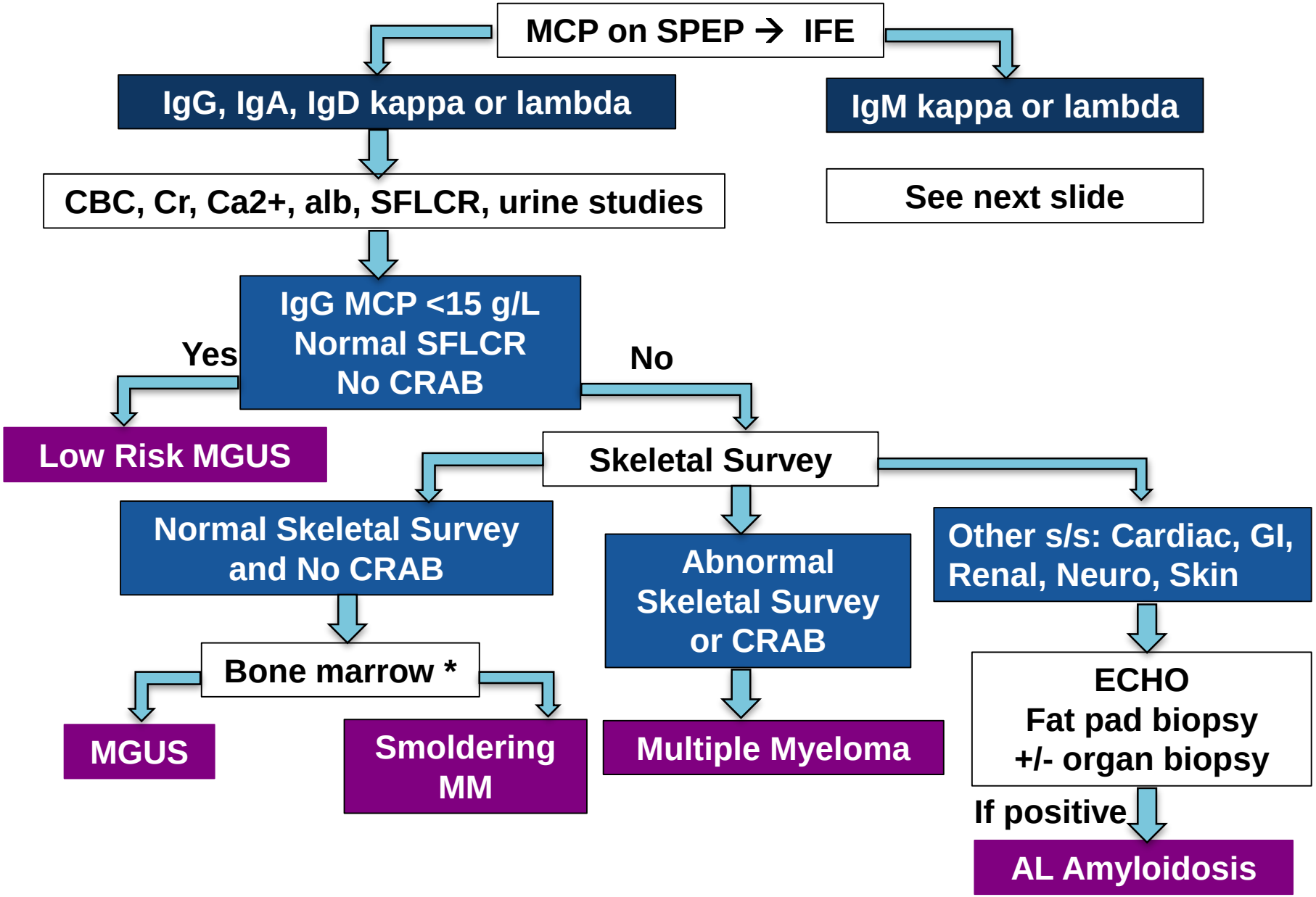
- Limitations:
  - imprecision, especially with different lots of FLC reagent [14]
- Sensitivity of SPEP + IFE + SFLCR approximately 97.4% for all PCD[13]
  - international guidelines recommend that SFLC testing replace urine electrophoresis in the diagnosis of monoclonal gammopathies

12. Jenner, E. Clin Chim Acta. 2014 Jan 1; 427: 15–20.
13. Katzmann JA, et al. *Clinical chemistry*. 2009;55(8):1517-1522.
14. Tate J, et al. Clin Biochem Rev. 2009 Aug 1;30(3):131-40.

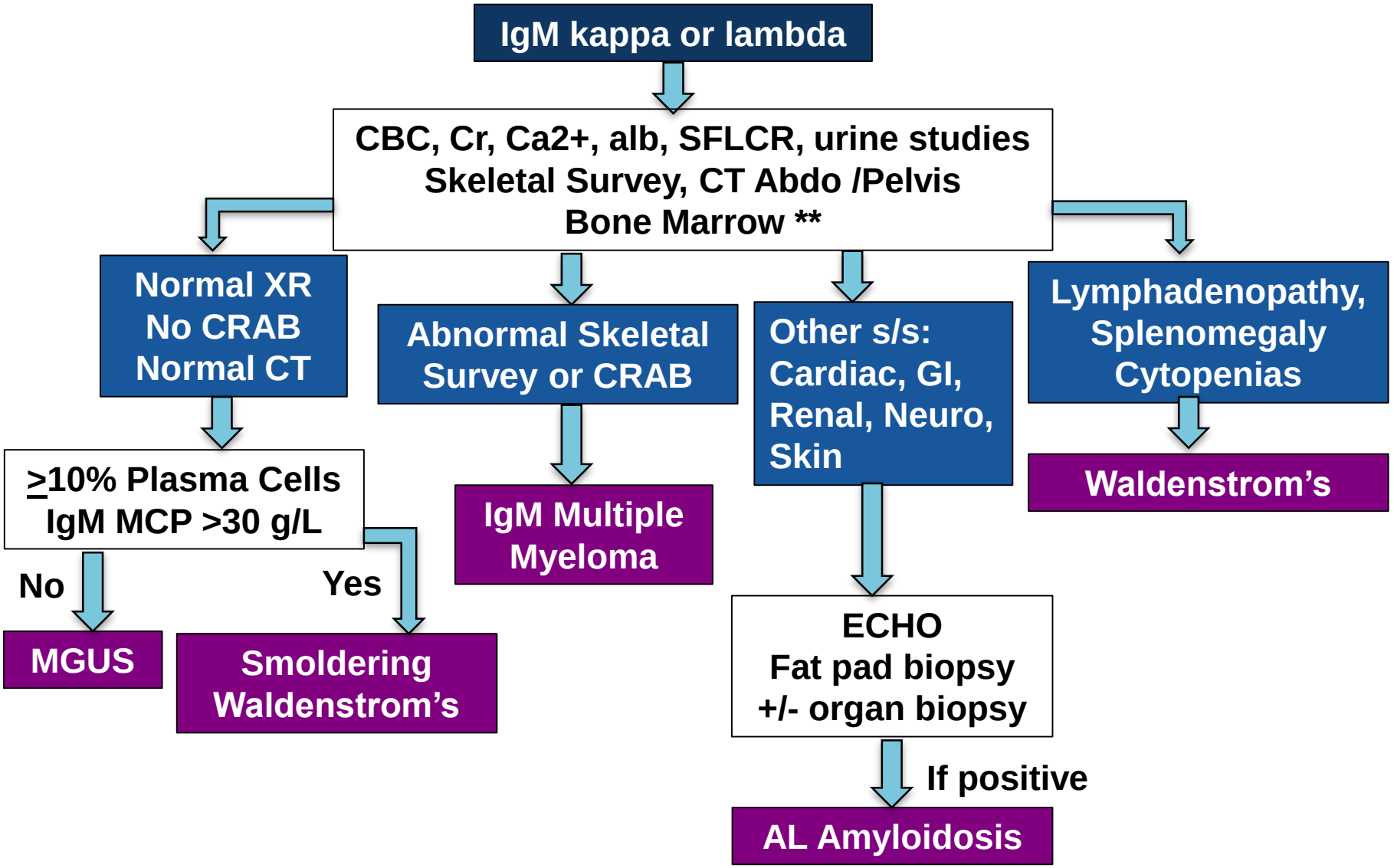
Imaging

- Skeletal survey:
  - Detects lytic bone lesions, osteopenia, fractures
  - Not routinely indicated if other investigations suggest MGUS
  - Nuclear bone scan not useful - lack of osteoblastic activity (i.e. can be normal)
- CT abdo/pelvis
  - To detect lymphadenopathy in patients in whom Waldenstrom’s is highly suspected or confirmed.

How to Investigate a MCP



How to Investigate a MCP



“CRAB” Symptoms

- C = Hypercalcemia: Ca^{2+} >2.8mmol/L
- R = Renal failure: Cr >177 or GFR <40ml/min
- A = Anemia: Hb<100 or > 20g below baseline
- B = Bony lesions (lytic lesions, plasmacytoma)

Case 1

Lab results

Measure	Results
Hb/ WBC/Plt	125/5.0/300
Creatinine	79
Calcium	2.30
Albumin	40
IgG	13.1 g/L
SPEP	M-protein 5g/L
Serum IFE	IgG kappa
Free kappa LC	18 mg/L
Free lambda LC	12.1 mg/L
SFLCR (range 0.26 – 1.65)	1.48

What is the most likely diagnosis?

1. Monoclonal protein of Undetermined Significance (MGUS)
2. Multiple myeloma
3. Amyloidosis
4. Waldenstrom's Macroglobulinemia
5. I have no idea

Case 1

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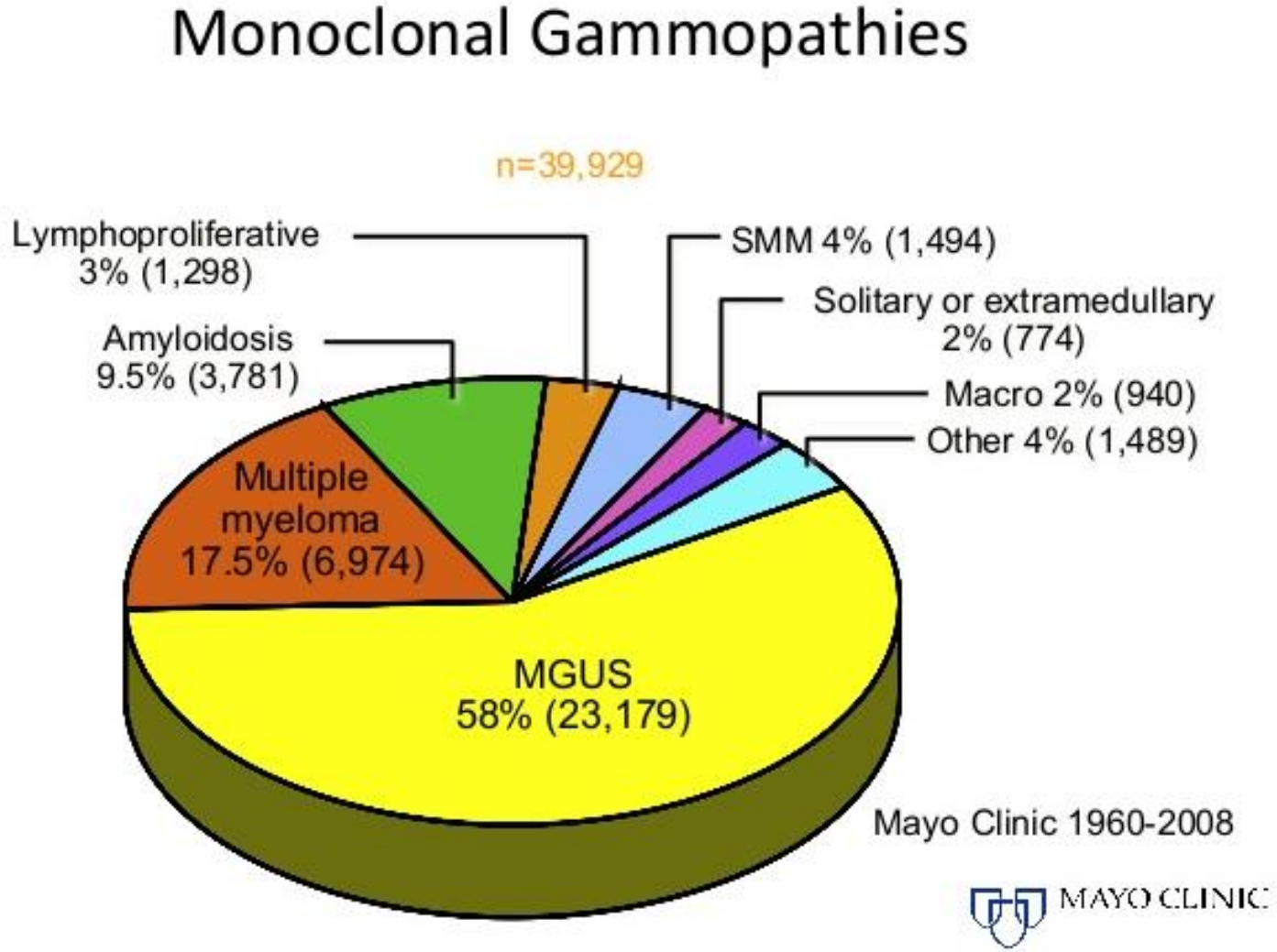
DDx Monoclonal Gammopathies

- Plasma Cell Disorders
  - Solitary plasmacytoma
  - Multiple myeloma
  - POEMS
- Lymphocytic Disorders
  - Waldenstrom's Macroglobulinemia
  - Heavy-Chain Diseases
- Infiltrative and Deposition Diseases
  - AL amyloidosis
  - Immunoglobulin Deposition Disease
- Miscellaneous
  - MGUS
  - Transplant related Monoclonal Gammopathy

DDx Monoclonal Gammopathies



15. Kumar, Shaji. http://www.slideshare.net/orthoprinciples/management-of-multiple-myeloma. Accessed 24 Oct 2016.

Diagnostic Criteria

MGUS	SMM	MM
MCP <30g/L **AND** Clonal BMPC <10% **AND** No “CRAB” or amyloidosis or end-organ damage	MCP (IgG or IgA) ≥30g/L **OR** Urinary MCP ≥500mg/24h **AND/OR** Clonal BMPC 10-60% **AND** No “CRAB” or amyloidosis	Clonal BMPC ≥10% with any level M-protein **OR** Plasmacytoma **AND** “CRAB” **OR Any 1 of** • ≥ 60% clonal plasma cells on bone marrow • Serum iFLC/uFLC >100 (level iFLC is at least 100mg/L) • >1 focal lesion on MRI at least 5mm in size.

16. Rajkumar et al. 2014 Lancet Oncology; 15:e538-.-48

MGUS

- Prevalence
  - 1.3% of healthy blood donors, 3% of adults >50 years old, 8% of adults >85 years old.[18,19]
  - 2-3 x more common in African Americans [20]
- Disease associations [21]
  - Plasma cell disorders, amyloidosis, LPD
  - CIDP
  - Kidney/liver transplant

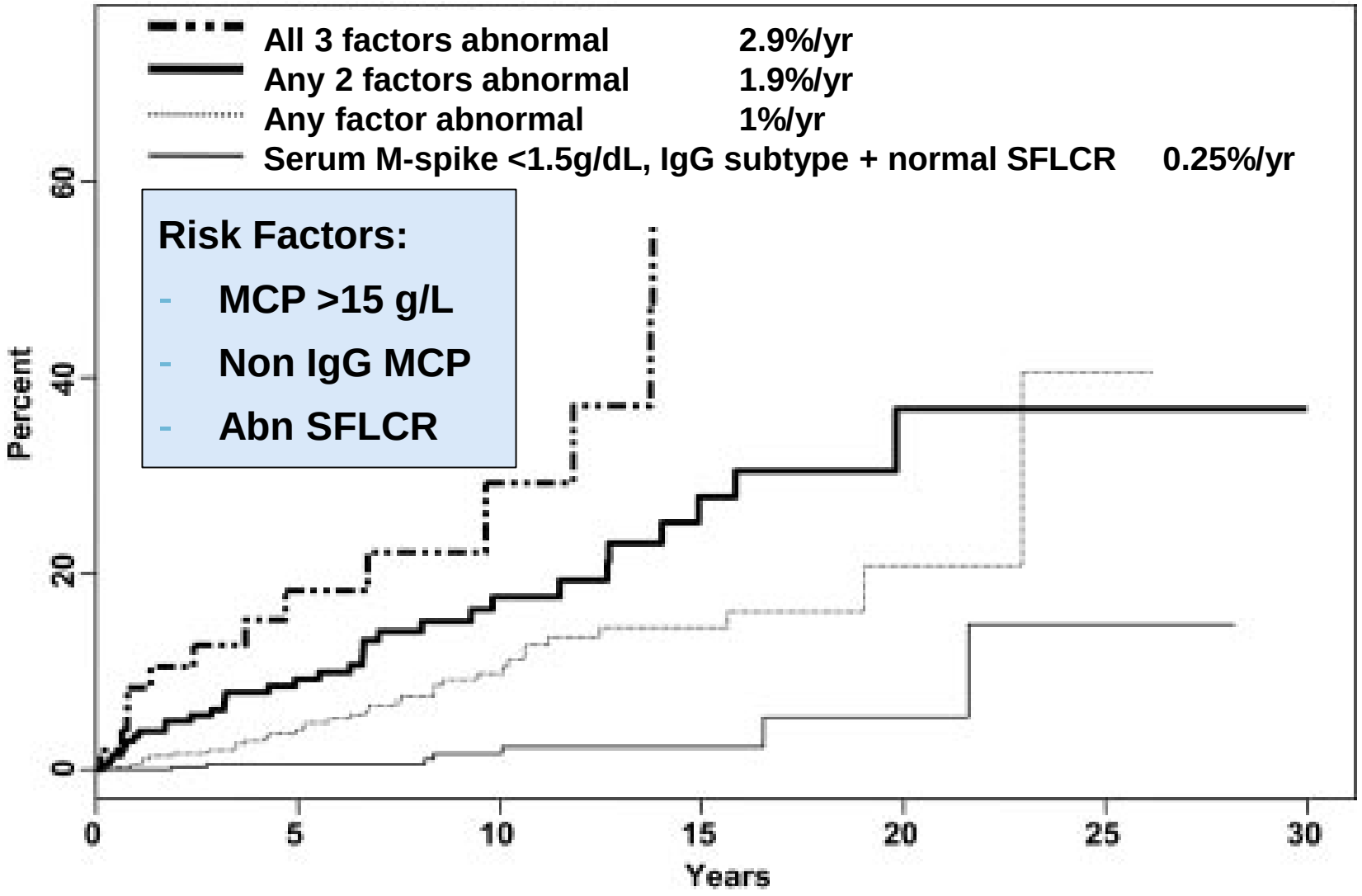
17. La Raja M, et al. Blood Transfusion. 2012;10(3):338-343.
18. Kyle RA, et al. Br J Haematol 2006; 134: 573–589.
19. Landgren O, et al. Blood. 2006; 107(3):904–906
20. Bida JP, et al. Mayo Clinic Proceedings. 2009;84(8):685-693.

MGUS

- Disease associations [21]
  - Osteoporosis and increased risk of fractures
    - Increased pro-osteoclast and anti-osteoblast cytokines
    - Possible compromised bone microarchitecture and strength [22]
    - Level of the paraprotein in the blood does not seem to correlate with this increased risk [3]
  - Possible associations:
    - Chronic infection, CTD, Thrombophlebitis

20. Bida JP, et al. Mayo Clinic Proceedings. 2009;84(8):685-693.
21. Farr JN, et al. Blood. 2014 Jan 30;123(5):647-9.

Risk of Progression MGUS



22. Rajkumar SV, et al. Blood. 2005 Aug 1;106(3):812-7.

MGUS – IMWG Guidelines

MGUS Risk Category	Follow-up
Low – 0 risk factors	▪ Baseline BM or skeletal survey not routinely indicated if other investigations suggest MGUS ▪ Repeat SPEP in 6 months ▪ If stable, repeat every 2–3 years or when symptoms of arise
Intermediate - High	▪ Baseline bone marrow * ▪ CT abdomen if IgM MCP ▪ If still MGUS, repeat CBC/SPEP in 6 months then yearly for life ▪ Reassess earlier if symptoms arise

22. Rajkumar SV, et al. Blood. 2005 Aug 1;106(3):812-7.
23. Kyle, RA, et al. Leukemia. 2010 Jun;24(6):1121-7.

MGUS: Other considerations

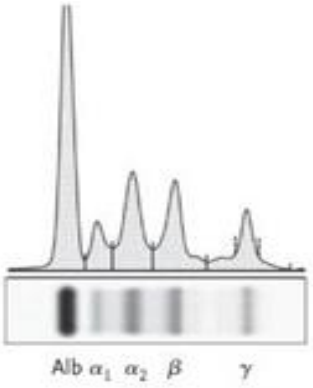
- Consider DXA to assess BMD given association with osteopenia/osteoporosis [24]
- Optimize Vitamin D and calcium doses
- If osteoporosis or osteopenia identified → consider therapy with bisphosphonates [25,26]
- If fractures develop [24]
  - Refer to bone specialist
  - Consider kyphoplasty for treating symptomatic vertebral compression fractures

24. Berenson JR, et al. Br J Haematol. 2010 Jul;150(1):28-38.
25. Berenson JR, et al. Clin Cancer Res. 2008 Oct 1;14(19):6289-95
26. Pepe J, et al. Calcif Tissue Int. 2008 Jun;82(6):418-26.

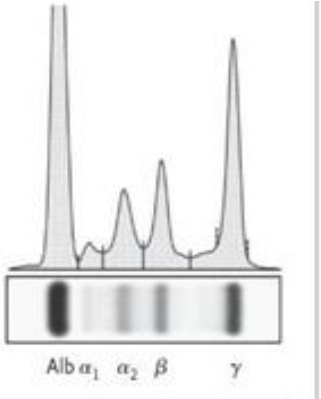
Case 1:

- You monitor the patient over 3 years and repeat BW shows …

2013



2016



Measure	2013	2016
Hb/ WBC/Plt	125/5.0/300	100/3.5/250
Creatinine	79	185
Calcium	2.30	2.42
Albumin	40	40
IgG	13.1 g/L	35 g/L
SPEP	M-protein 5g/L	24 g/L
Serum IFE	IgG kappa	IgG kappa
Free kappa LC	18 mg/L	356mg/L
Free lambda LC	12.1 mg/L	26 mg/L
SFLCR (range 0.26 – 1.65)	1.48	13.69

Case 1:

- What should you do?
  A. Repeat skeletal survey and if normal, re-evaluate in 12 months
  B. Repeat skeletal survey and if normal, re-evaluate in 6 months
  C. Repeat skeletal survey and if normal, re-evaluate in 3 months
  D. Refer to hematology

Case 1:

- What should you do?
  A. Repeat skeletal survey and if normal, re-evaluate in 12 months
  B. Repeat skeletal survey and if normal, re-evaluate in 6 months
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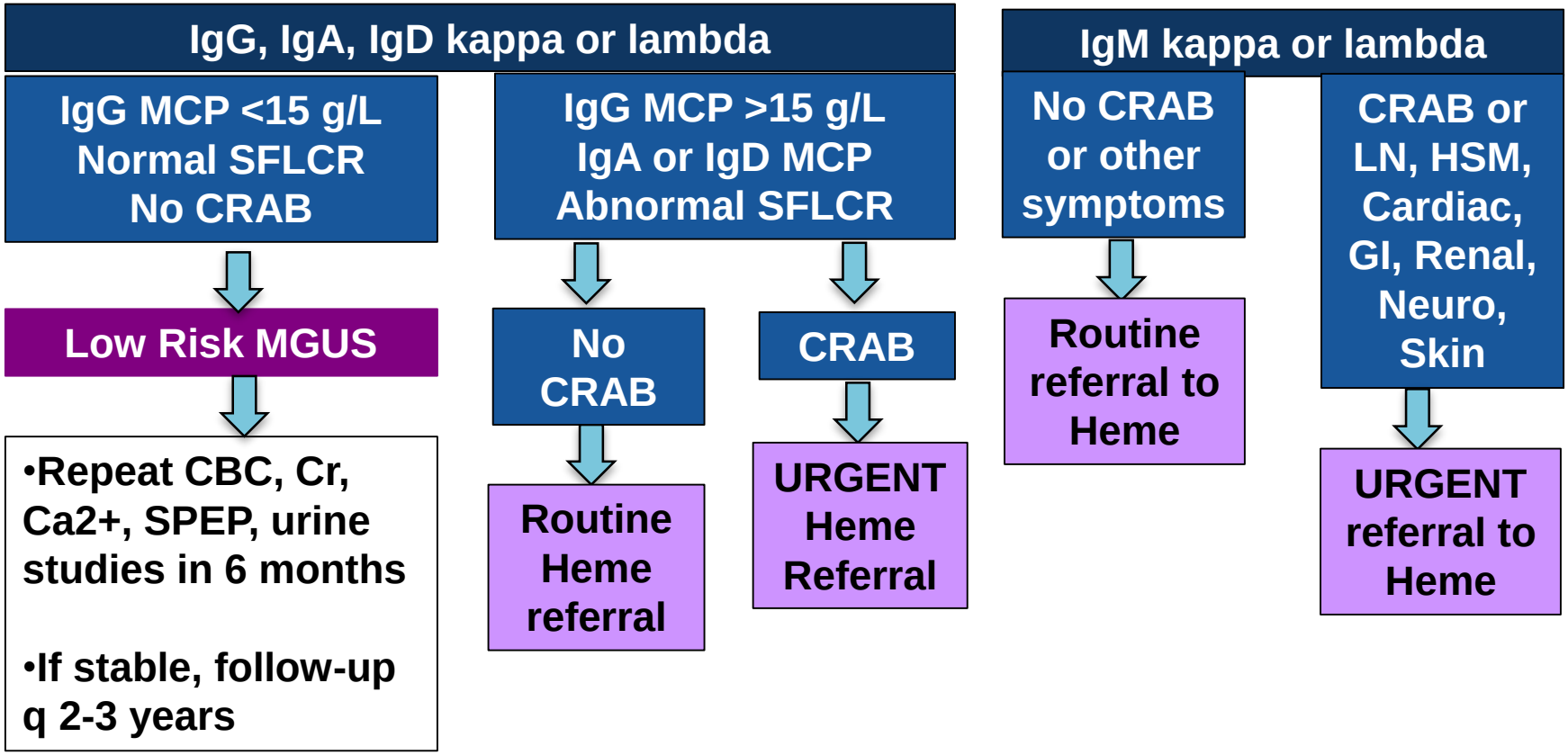
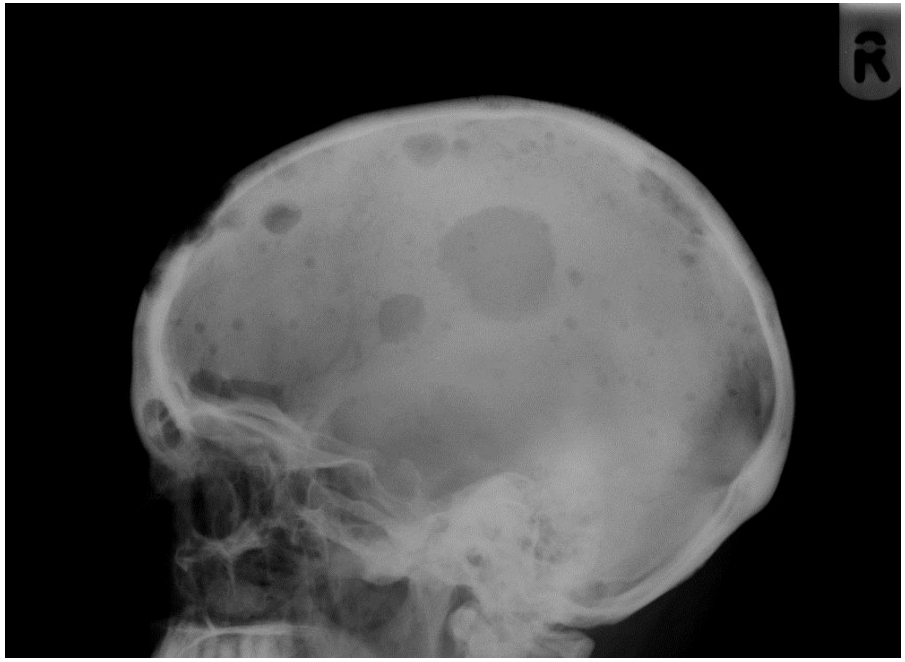
When to refer to:



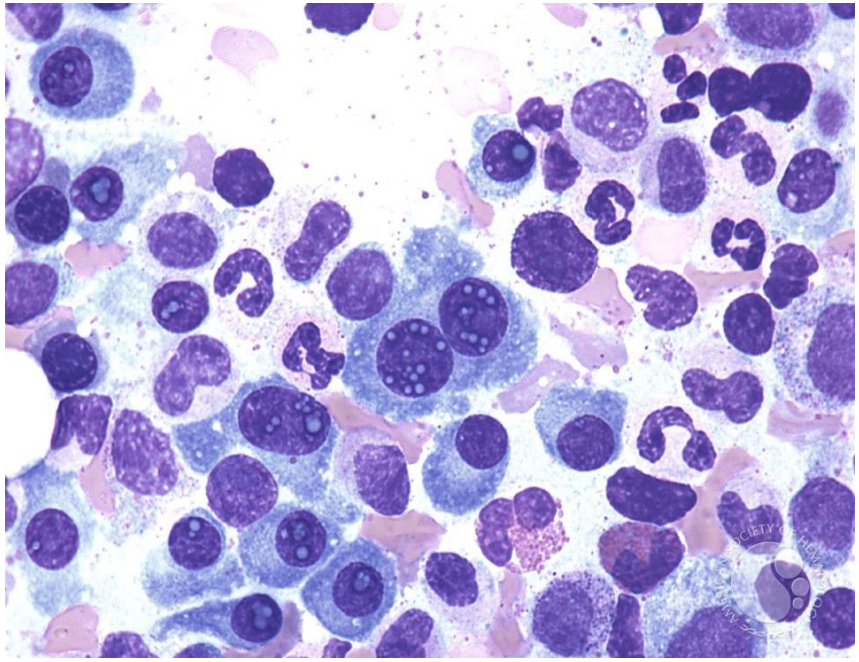
Case 1: Multiple Myeloma

Lytic Lesions on X-Ray



27. Nunn, Heidi. http://www.imageinterpretation.co.uk/tumour.php

Bone Marrow Biopsy



28. Maslak, Peter. ASH Image Bank

AL Amyloidosis

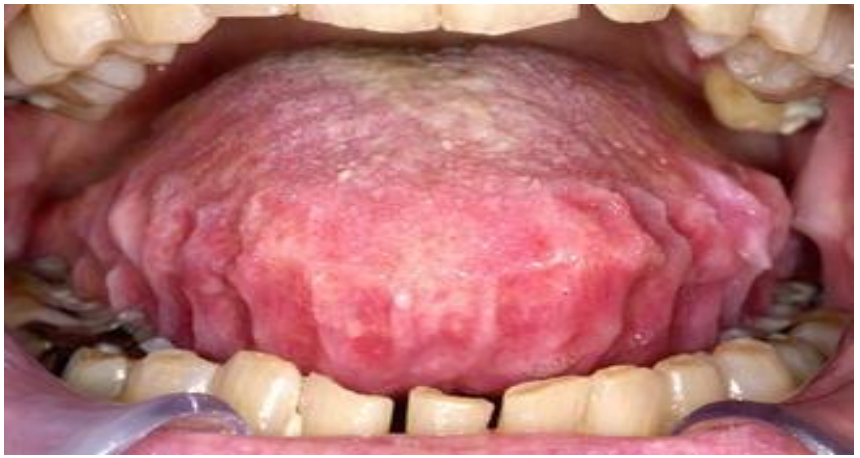
Symptoms:

- Cardiac: Arrhythmias, CHF, orthostatic hypotension, syncope
- Renal: Proteinuria, edema, renal failure
- GI: Nausea, diarrhea, constipation, loss of appetite, weight loss
- Other: bruising, skin lesions
- Neuro: Neuropathy

Periorbital Bruising

29. Silverstein, Sophie. *Dermatology Online Journal*. 2005; 11(1).

Macroglossia



30. Pocket Dentistry. http://pocketdentistry.com/diseases-of-the-tongue/#Fig1.

Take Home Points

- SPEP should be used in primary care to investigate:
  - Patients with symptoms suggestive of plasma cell disorders
  - Selected patients with osteoporosis
- SPEP and immunofixation help to differentiate between polyclonal versus monoclonal gammopathy
- Look for “CRAB” symptoms to differentiate between MGUS and more serious diseases

Take Home Points

- Low risk MGUS can be followed by primary care physicians
- Refer to hematology when:
  - MCP >15 g/L
  - Non IgG MCP
  - Abnormal light chain ratio
  - CRAB symptoms, cardiac, neurologic, GI, LN, HSM, skin or constitutional s/s

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6. Bonnick SL et al. Position statement: Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. Menopause 2010 Jan/Feb; 17:25.

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21. Farr JN, Zhang W, Kumar SK, et al. Altered cortical microarchitecture in patients with monoclonal gammopathy of undetermined significance. Blood. 2014 Jan 30;123(5):647-9.

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29. Silverstein, Sophie R MB. Primary, systemic amyloidosis and the dermatologist: Where classic skin lesions may provide the clue for early diagnosis. *Dermatology Online Journal*. 2005; 11(1).

30. Diseases of the Tongue. Pocket Dentistry. http://pocketdentistry.com/diseases-of-the-tongue/#Fig1. Accessed 24 Oct 2016.