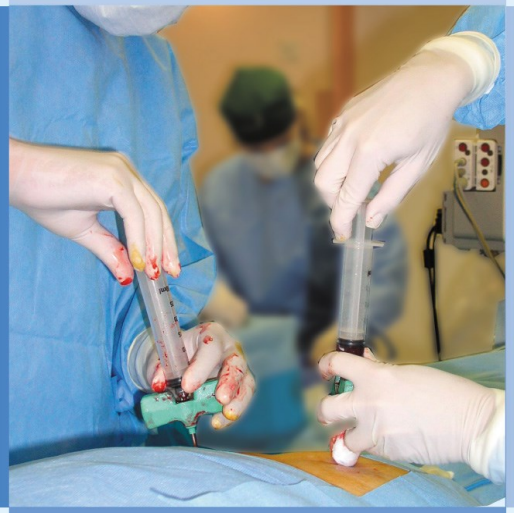
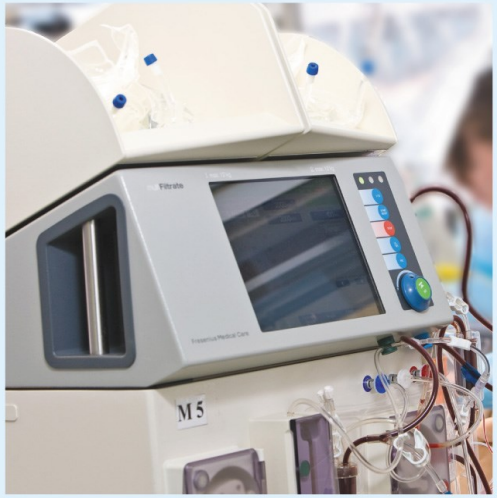
Michal Kaščák
Department of Hematooncology, University Hospital Ostrava



Modern diagnostic set and differential diagnostic issue - what you should know about Morbus Waldenström



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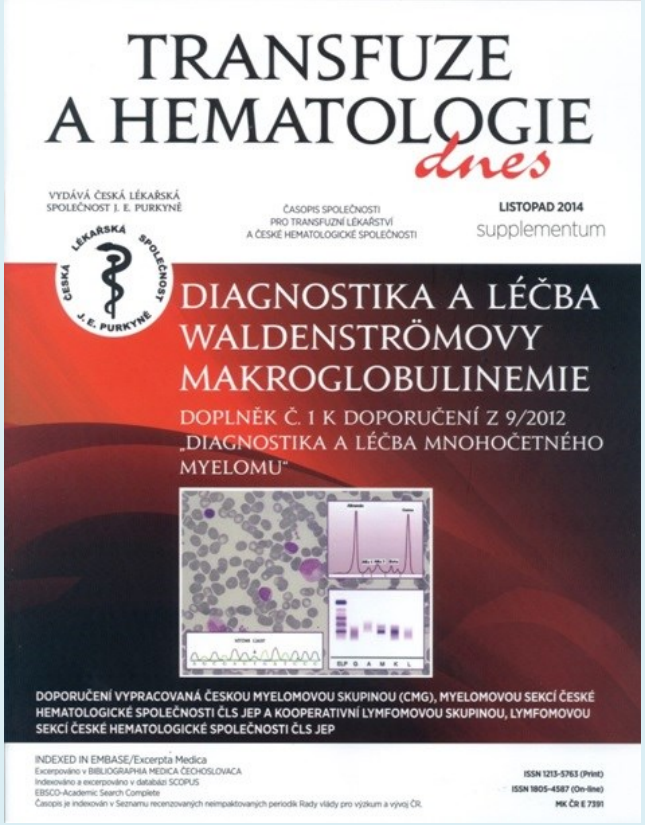
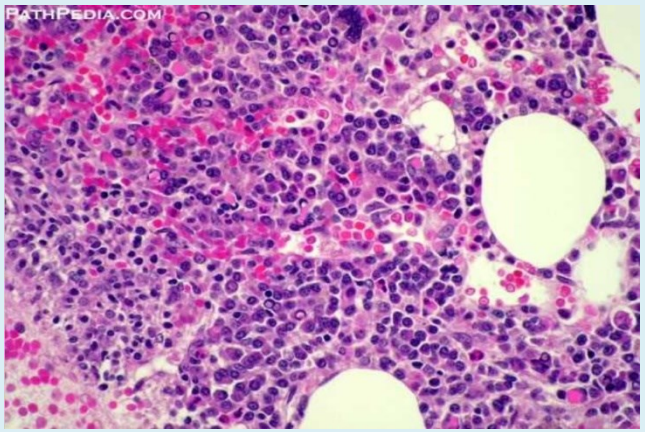
Outline

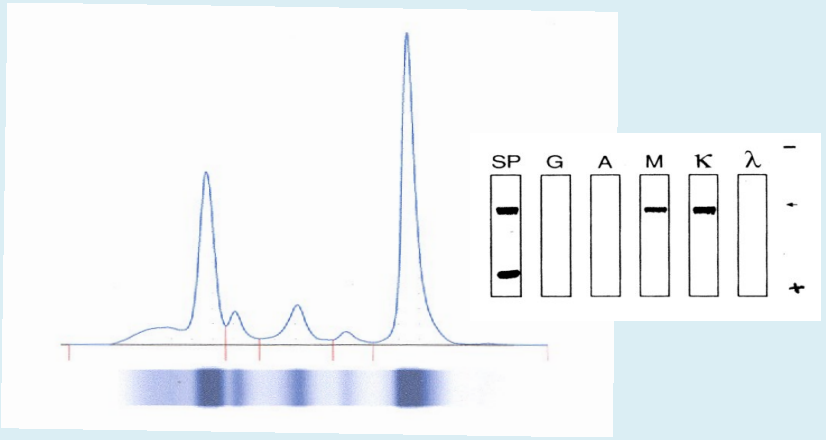
- Present definition and criteria
- Diagnostic workup by CMG 2014
  - Bone marrow examination
  - Immunophenotypic studies
  - Cytogenetic and molecular-genetic analysis
  - Laboratory assesment
  - Imaging
  - Risk assesment
- Differential diagnostic issues



Distinct pathological entity

- Clinicopathological definition of WM:
  - Pathological diagnosis of **lymphoplasmacytic lymphoma** using REAL/WHO criteria with **any BM involvement**
  - Presence of monoclonal IgM protein **of any level**





Owen et al., Semin Oncol 2003, IWWM2, Athens 2002

Diagnostic criteria of Waldenström´s macroglobulinemia IWWM 2002, Owen et al. 2003

1.) IgM monoclonal protein of any concentration

2.) Bone marrow infiltration by small lymphocytes showing plasmocytoid/plasma cell diferentiation

3.) Intertracecular pattern of bone marrow infiltration*

4.) Surface IgM+,CD5+/-, CD10-, CD19+, CD20+,CD22+,CD23-,CD25+, CD27+,FMC7+,CD103-, CD138- immunophenotype*

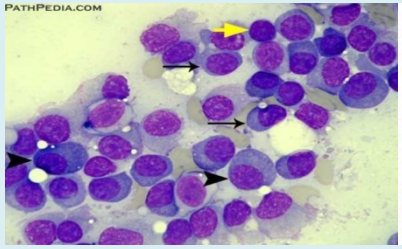
Criteria 3 and 4 are supportive of but not necessary

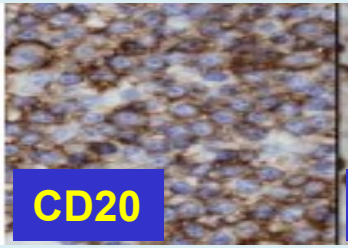
Diagnosis and treatment of WM Guidelines, Adam et al. 2014

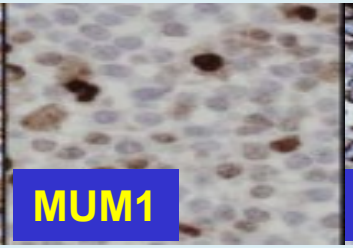
Diagnostic workup	Facultative assessment
Bone marrow aspirate and trephine biopsy **Immunophenotypic studies** (sIg kappa/lambda, cIg kappa/lambda, sIgM, CD19, CD20, CD22, CD23, CD5, CD10, CD25, CD27, FMC7, CD38, CD56, CD103, CD138)	**Cytogenetic analysis by FISH** **Testing MyD88 L265P by AS-PCR in BM and/or PB**
Concentration IgG, IgA, IgM **Testing for Cryoglobulins** **Monoclonal IgM assess. by SPEP and immunofixation** **Quantitative analysis by densitometry**	**FLC and HLC assay if standart IgM monitornig not possible**
CBC, Blood clotting tests	
Biochemical analysis including LDH, TP, albumin, B2M	
Direct antiglobulin test	
Fundoscopic examination if TP over 100g/L	Plasma viscosity testing
Hepatitis screening HAV,HBV,HCV	
Anti-MAG , Anti-GM serology and conduction studies EMG if neuropathy	
Computerized (CT) scans at baseline (chest,abdomen and pelvis) in symptomatic disease	Radiographic skeletal survey if bone disease suspected **PET/CT optional**
Risk assessment by ISSWM	

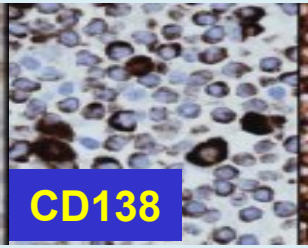
Bone marrow examination - Important BM features

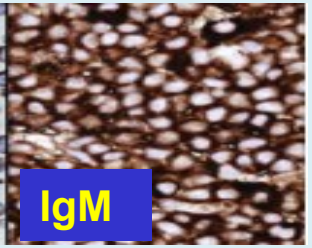
- **Lymphoplasmocytoid cells** 100% of cases[1] (n=59, p<0,001)



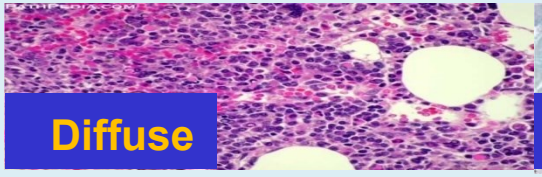




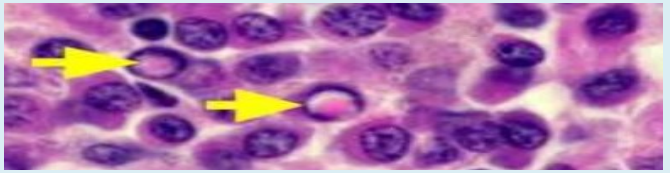




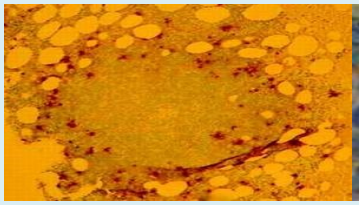
- **Pattern of infiltration:** interstitial, nodular or diffuse. Purely paratrabecular unusual **BUT focal paratrabecular involvement** in 57% of cases[1,2] (n=59, p<0,001)

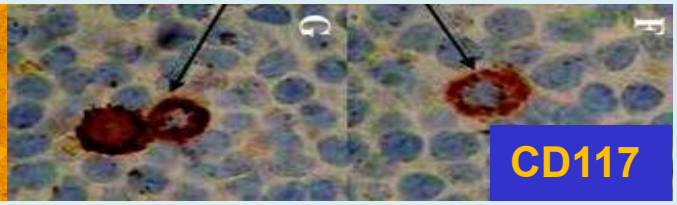


- **Dutcher bodies** 90% of cases[1] (n=59, p<0,001)



- **Increased mast cells** 87% of cases[1] (n=59 p<0,001)

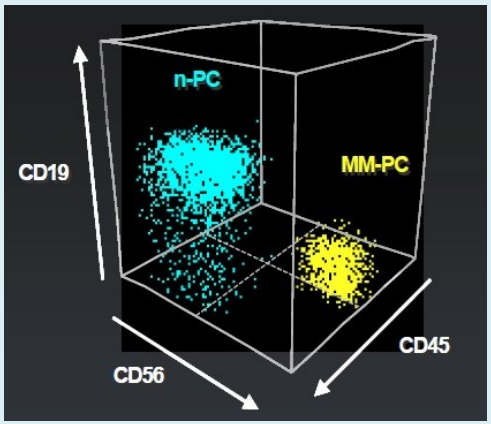




1) Assia Bassarova et al. 2014 2) Hamadeh F et al. 2014

Immunophenotypic studies

- **B-cell component - „standart“:** $CD19^{lo+}$,$CD20^{+}$,$CD22^{lo+}$,$CD27^{+}$, $CD25^{+}$,$CD79b^{+}$,$FMC7^{+}$,$CD27^{+}$,$PAX5^{+}$,$CD138^{-}$,$MUM1^{-}$, $sIgM^{+}$ (Owen 2001, Ocio 2005, Morice 2009, Paiva 2014)
  - If majority of B-cells are clonal - **„characteristic“ WM phenotype** (Paiva 2014) :
    - **$CD22^{low}/CD23^{-}/CD25^{+}/CD27^{+}/sIgM^{+}$**
    - **in >90%: $CD5^{-}$,$CD10^{-}$,$CD11c^{-}$,$CD103^{-}$**



- **PC component:** variably $CD19^{+}$, $CD20^{+}$, $CD138^{+}$,$CD45^{+}$,$CD56^{weak}$ (San Miguel 2003, Ocio 2005, Morice 2009, Paiva 2014)
  - **clearly different from myeloma (**$CD56^{high}$,$CD19^{-}$,$CD27^{-}$ $CD45^{-}$) (Paiva 2014)

Cytogenetic studies

- **Role in diferential diagnosis**
- **No disease defining abnormalities BUT *IGH* translocations are rare**

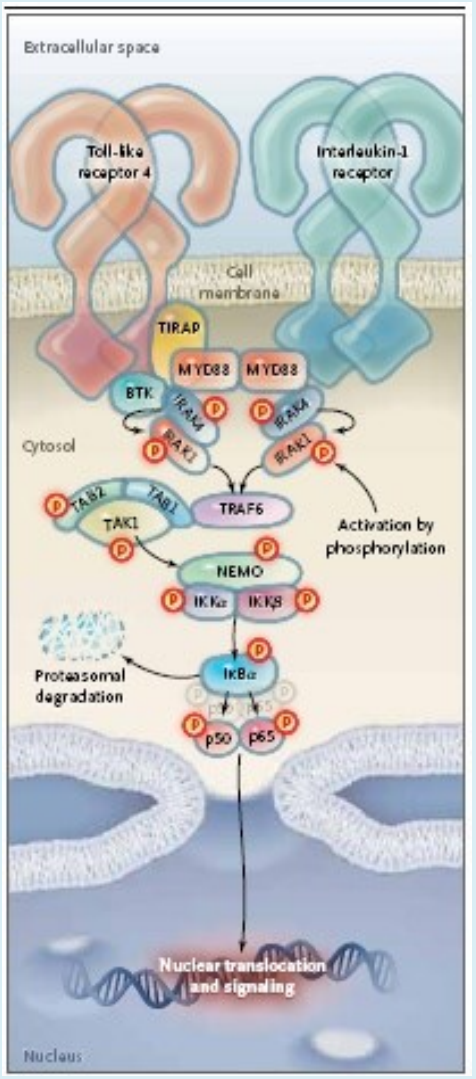
Cytogenetic findings in WM compared to other chronic B-LPD

	WM	CLL	MZL	MM
6q deletion	**++++ (30-50%)**	+	++	+/–
Trisomy 4	**++ (10%)**			
13q14 deletion	**++ (3-15%)**	++++	+	+++
Trisomy 12	**+ (4%)**	++	++	
IgH	**– (0-2%)**	+/–		t(11;14) t(4;14) t(14;16)
Miscellaneous	**- 17p13del (TP53) (5-8%)**		t(11;18) +3q, 7q, +18q, 8p	Hyper/Hypo

Nguyen-Khac 2013, Nguyen-Khac 2010 Fonseca 2004, San Miguel 2004

Molecular-genetics

- **MyD88 gene mutation (L265P)** is highly recurring mutation in WM.
- **In 90%** of WM pts.
- **High-concordance** between BM and PB ~ 90%
- **Absent or infrequently observed** (<10%) **in MZL,CLL, and MM**
- **Limitations:**
- **Not exclusive** for WM. In 10-15% SMZL
- **Risk of false negativity** if whole BM/PB testing (limitation of AS-PCR and/or interclonal heterogenity)



Treon et al. 2012, Xu et al. 2014, Chapman et al. 2011, Puente et al. 2011 , Ghobrial 2012, Martinez-Lopez 2015, Wang et al. 2014

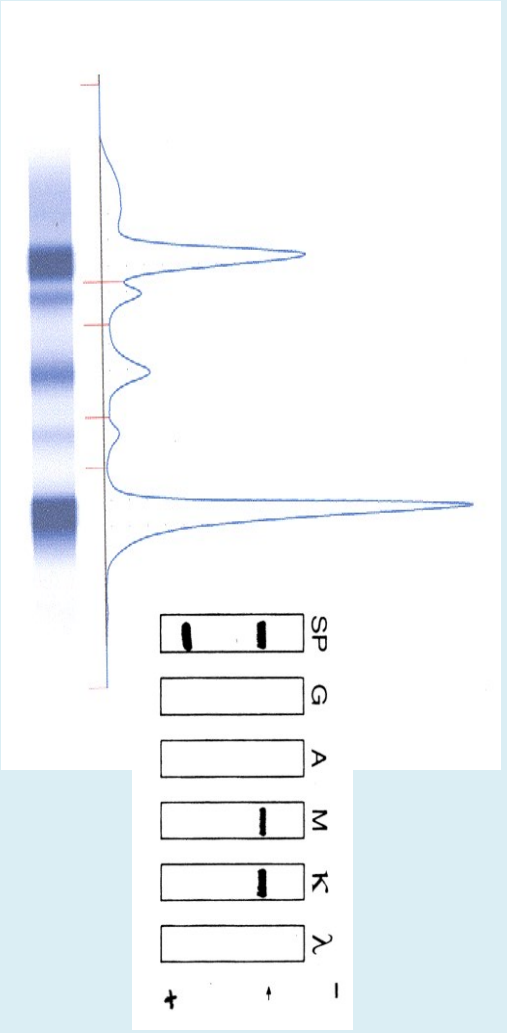
Monoclonal IgM

- **Monoclonal IgM component measurement** by SPEP with densitometry or nephelometry

- **Standard** for diagnosis, response assessment, prognostication

BUT

- Long IgM half life
- Interlaboratory differences
- Impaired by cryoglobulin activity (20%) and/or hyperviscosity



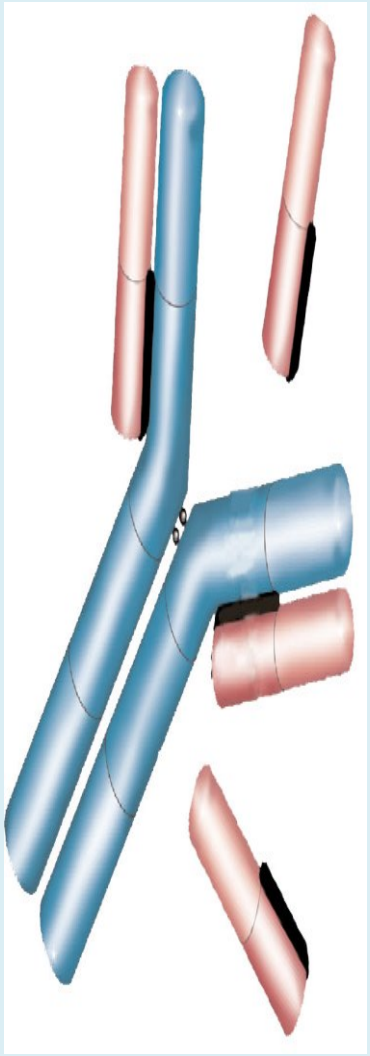
Ghobrial et al. 2004, Owen et al. 2013, Pattenden et al. 2009, Dimoupolous et al. 2009, Leleu et al. 2011

serum Free Light Chain (sFLC)

- Currently NOT essential for rutine assessment
- elevated levels in 80% WM with relatively low levels

BUT

- **Not impaired** by cryoglobulin activity or hyperviscosity
- **Predicts independenty time to treatment** if sFLC>60-80mg/L median to treatment 1year of dg.
- **Sensitive and early predictor of response** ~1month earlier than IgM
- **Early predictor of progresion** ~ 5months earlier



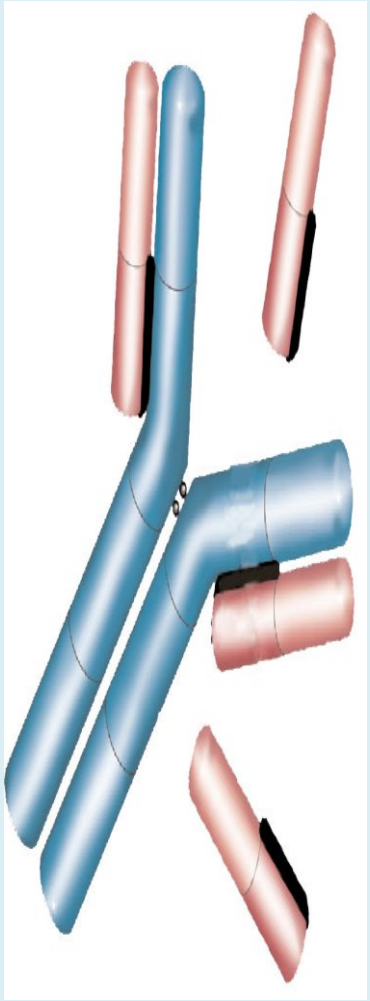
Itzykson et al. 2008, Leleu et al. 2008, Pattenden et al. 2009, Leleu et al. 2010, Maltezas et al. 2010, Keren et al. 2009

serum IgM Hevylite assay

- Currently NOT essential for rutine assessment

BUT

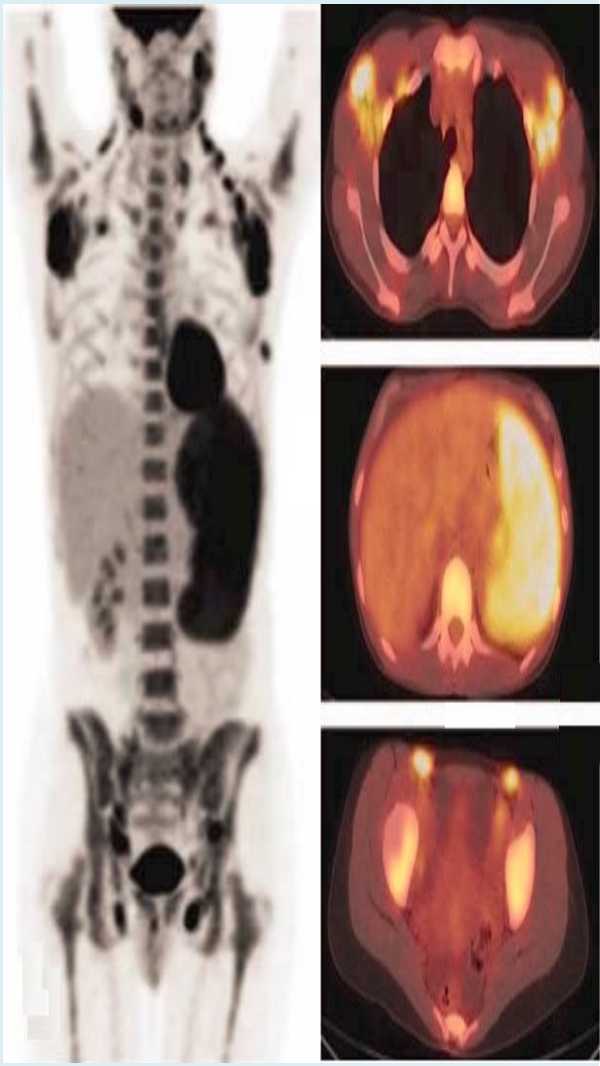
- Standardized and accurate
- Good correlation with SPEP
- Greater sensitivity to detect residual disease with IgMkappa/IgMlambda ratios compared to SPEP
- **Potential future reference to monitor IgM M-spike**



Leleu et al. 2010, Manier et al. 2010, Boyle EM et al. 2014)

FDG-PET/CT (Banwait et al. 2011, Tamayo et al. 2014)

- NOT routinly recomended BUT
- **~ 70-80% positive PET/CT findings in symptomatic WM compared 20% CT positivity**
  - Lymph nodes ~ 60%
  - BM involvement ~ 40%
  - Extranodal involvement ~ 10%
  - Spleen ~ 10%
- **negative in smoldering WM** (~ 100%)
- **Potentional prognostic impact of PET/CT postivity on OS:**
  - **26 months vs. NR** (negative and focal lesions in BM) (p=0.16)



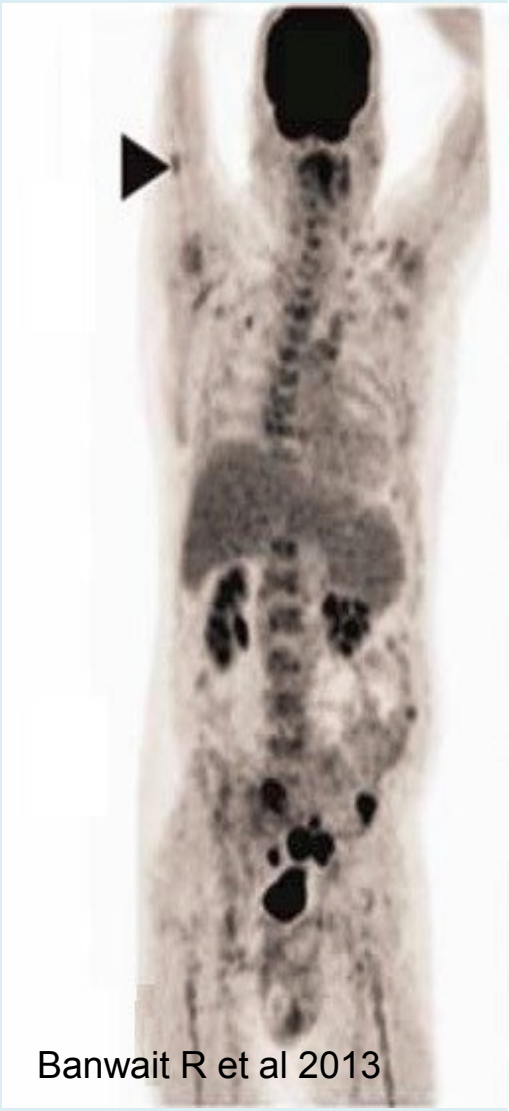
PET/CT diffuse BM,nodal,spleen involvement in WM case

Bone disease with FDG-PET/CT or NMRI (Banwait et al. 2013)

- **Bone disease (BD) in WM**
  - absence considered a differentiating feature from multiple myeloma
  - infrequently observed in case studies (Leb L et al. 1977, Krausz Y et al. 1977, Marks MA et al. 1985, Schlesinger N et al. 2000)

BUT

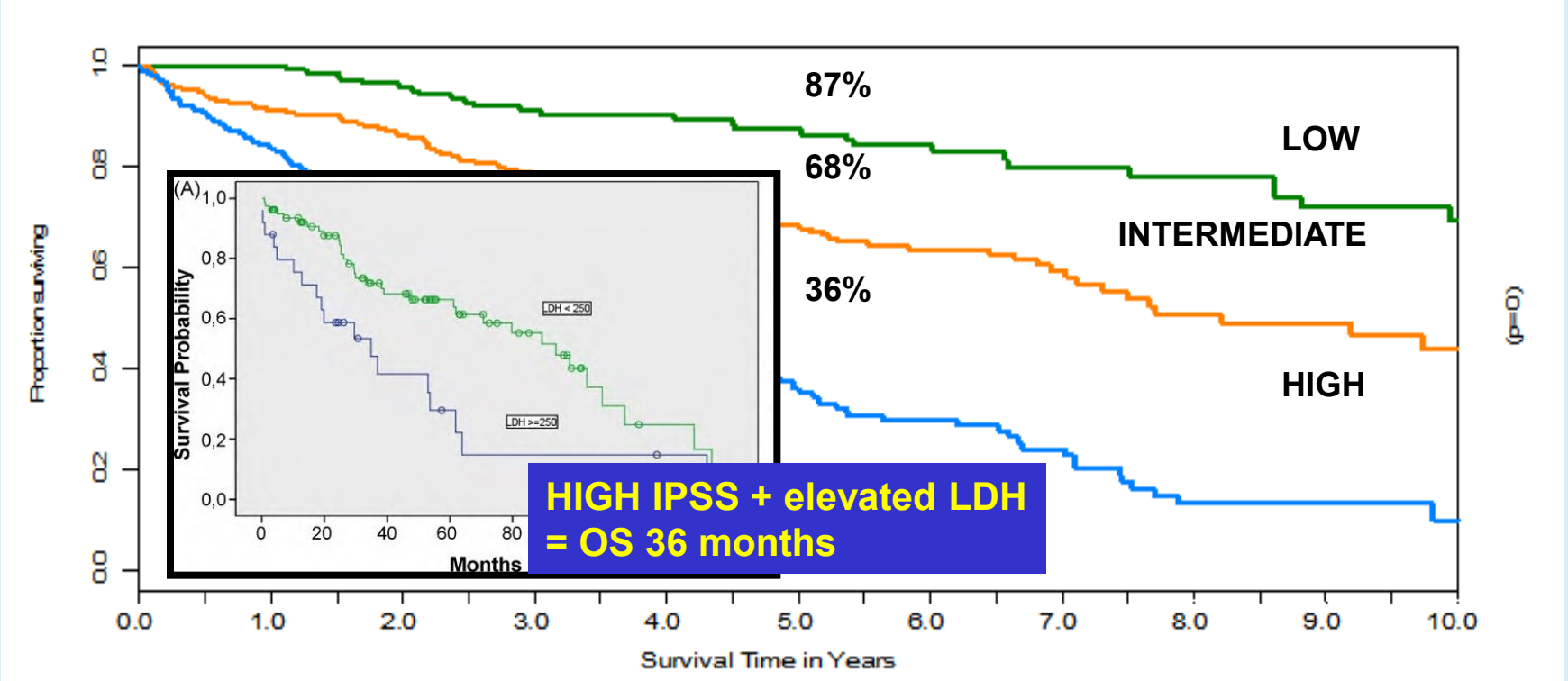
  - With use of sensitive detection methods (Papanicolau X et al. 2014) :
    - **BD (focal lesions) reported in about ~ 20% WM cases**
    - **Dominant localization in spine and limbs**
    - **Without PFS or OS impact or any other clinical parameter**



Banwait R et al 2013

International prognostic scoring system for WM

Age, hemoglobin, platelet count, B2M, M-IgM



Stratum	Score
Low:	0 or 1 (except age)
Intermediate:	age or 2
High :	≥3

Morel et al Blood 2009

validated by Kastritis 2010 in rituximab era

Potential future prognostic markers in WM

	N P-value	% of asympt. WM	Time to progresion (median)	Overall survival (median)
Bone marrow infiltration > 10% by MFC [1]	N=93 0,08 univ. 0,016 univ. 0,22 mult.	34%	**47 months v.s. 145 m.**	**46months v.s. 78m.**
100% light chain restriction by MFC [1]	N=121 0,001 univ. 0.004 mult.	19%	**47 months v.s. 145 m.**	**44 months v.s. 78m** with High IPSS 16months
Serum FLC > median (60-80mg/L) [2,3,4]	N=42[2], N=44[3], N=59 0.006[2]/0,044[3]	NA	**~ 12 months** (Time to treatment)[4]	**138 months v.s. NR[3]**
IgMi HLC ratios > median (90-100) [4,5]	N=59[4], N=37[5] 0.014[4], 0.029[5]	NA	Shorter time to treatment NA	In stratification model with abnormal LDH and B2M>5,5 shorter OS
von Willebrand factor > 200U/L[6]	N=42[6] P<0,001	NA	**PFS 12 months v.s. 63m.**	**37 months v.s. >110 m.**

1)Paiva et al. 2014 , 2)Itzykson et al. 2008 3) Maltezas et al. 2010 4) Manier et al. 2010 5) Koulieris et al. 2010 6) Kastritis et al. 2013

Differential diagnostic issue. Why? Some resemblance.

B-CLL

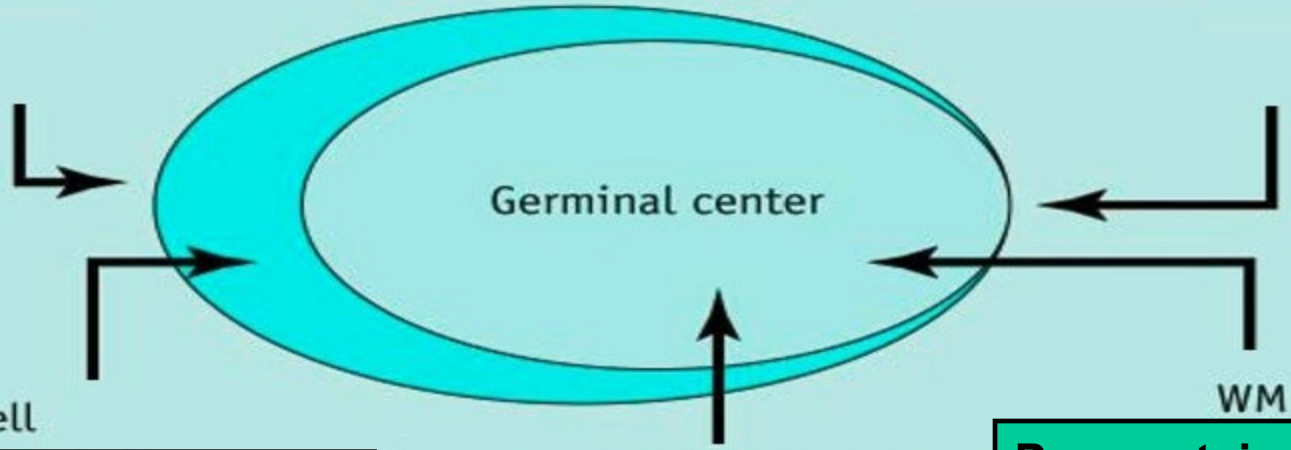
IgM paraprotein: 9-20%
PC differentiation: 20%

Marginal zone

IgM paraprotein: 3-7%
PC diff.: up to 30%

Multiple myeloma

IgM paraprotein: RARE
Purely PC infiltration of BM



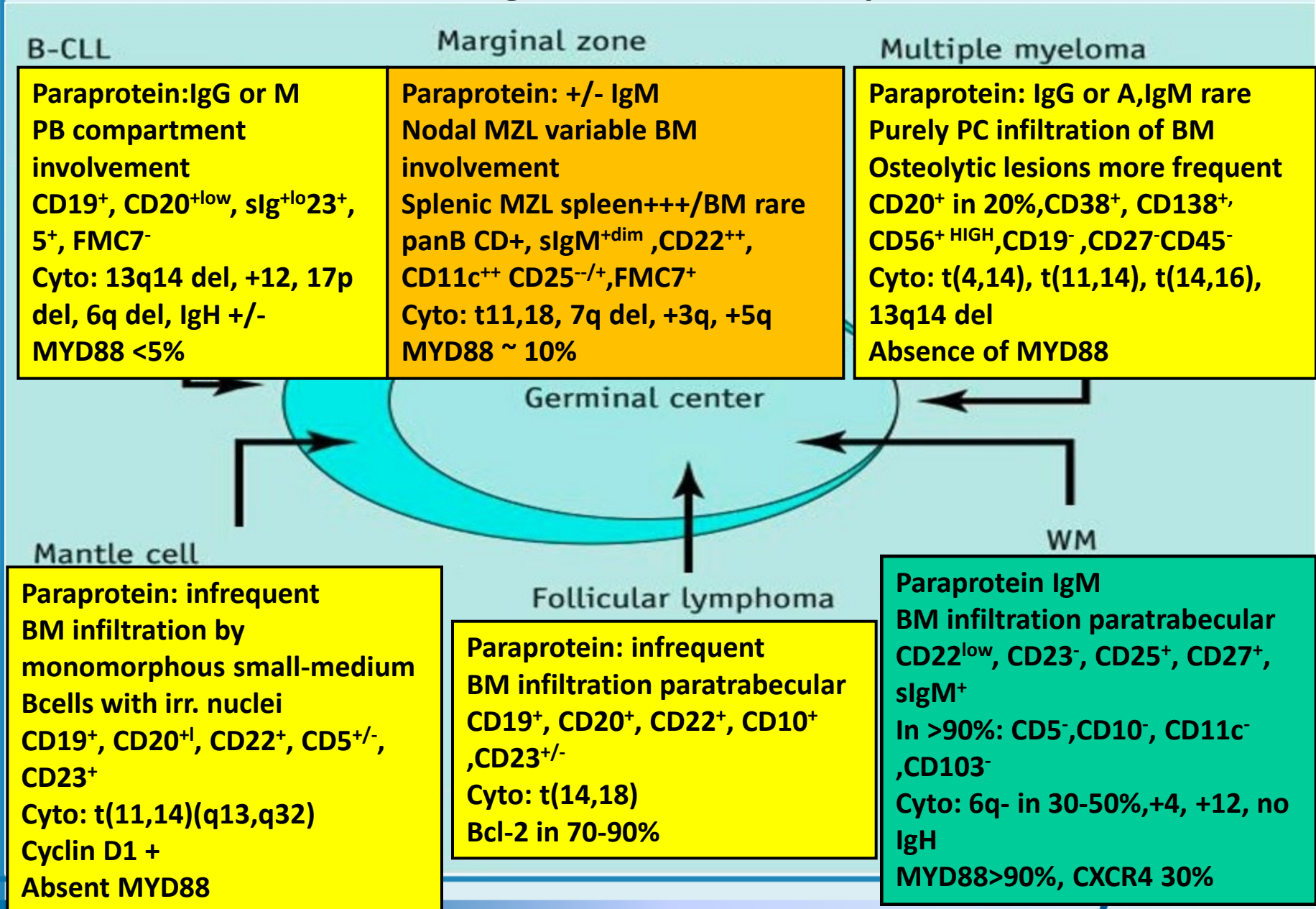
IgM paraprotein : 3%
PC differeniation: RARE

IgM paraprotein : 3-5%
PC differiantion: <5%
RARE

Paraprotein IgM
LPL BM infiltration

Owen et al. 2003, Lin et al. 2005, Kent et al. 2002, Bassarova et al. 2014, Roberts MJ et al. 2013, Yin C et al. 2005, Kieth TA et al. 1985 Lin et al. 2005, Young et al. 2006, Naushad et al. 2009, Visco et al. 2005, Evans et al. 2000, Lin P et al Am J Clin Pathol 2005 Preud'homme JL et al Blood 1997

Differential diagnostic issue . Complicated?



B-CLL

Paraprotein:IgG or M
PB compartment involvement
$CD19^{+}$, $CD20^{+low}$, $sIg^{+lo}23^{+}$, 5^{+}, $FMC7^{-}$
Cyto: 13q14 del, +12, 17p del, 6q del, IgH +/-
MYD88 <5%

Marginal zone

Paraprotein: +/- IgM
Nodal MZL variable BM involvement
Splenic MZL spleen+++/BM rare
panB CD+, $sIgM^{+dim}$,$CD22^{++}$, $CD11c^{++}$ $CD25^{--/+}$,$FMC7^{+}$
Cyto: t11,18, 7q del, +3q, +5q
MYD88 ~ 10%

Multiple myeloma

Paraprotein: IgG or A,IgM rare
Purely PC infiltration of BM
Osteolytic lesions more frequent
$CD20^{+}$ in 20%,$CD38^{+}$, $CD138^{+,}$ $CD56^{+\ HIGH}$,$CD19^{-}$,$CD27^{-}CD45^{-}$
Cyto: t(4,14), t(11,14), t(14,16), 13q14 del
Absence of MYD88

Mantle cell

Paraprotein: infrequent
BM infiltration by monomorphous small-medium Bcells with irr. nuclei
$CD19^{+}$, $CD20^{+l}$, $CD22^{+}$, $CD5^{+/-}$, $CD23^{+}$
Cyto: t(11,14)(q13,q32)
Cyclin D1 +
Absent MYD88

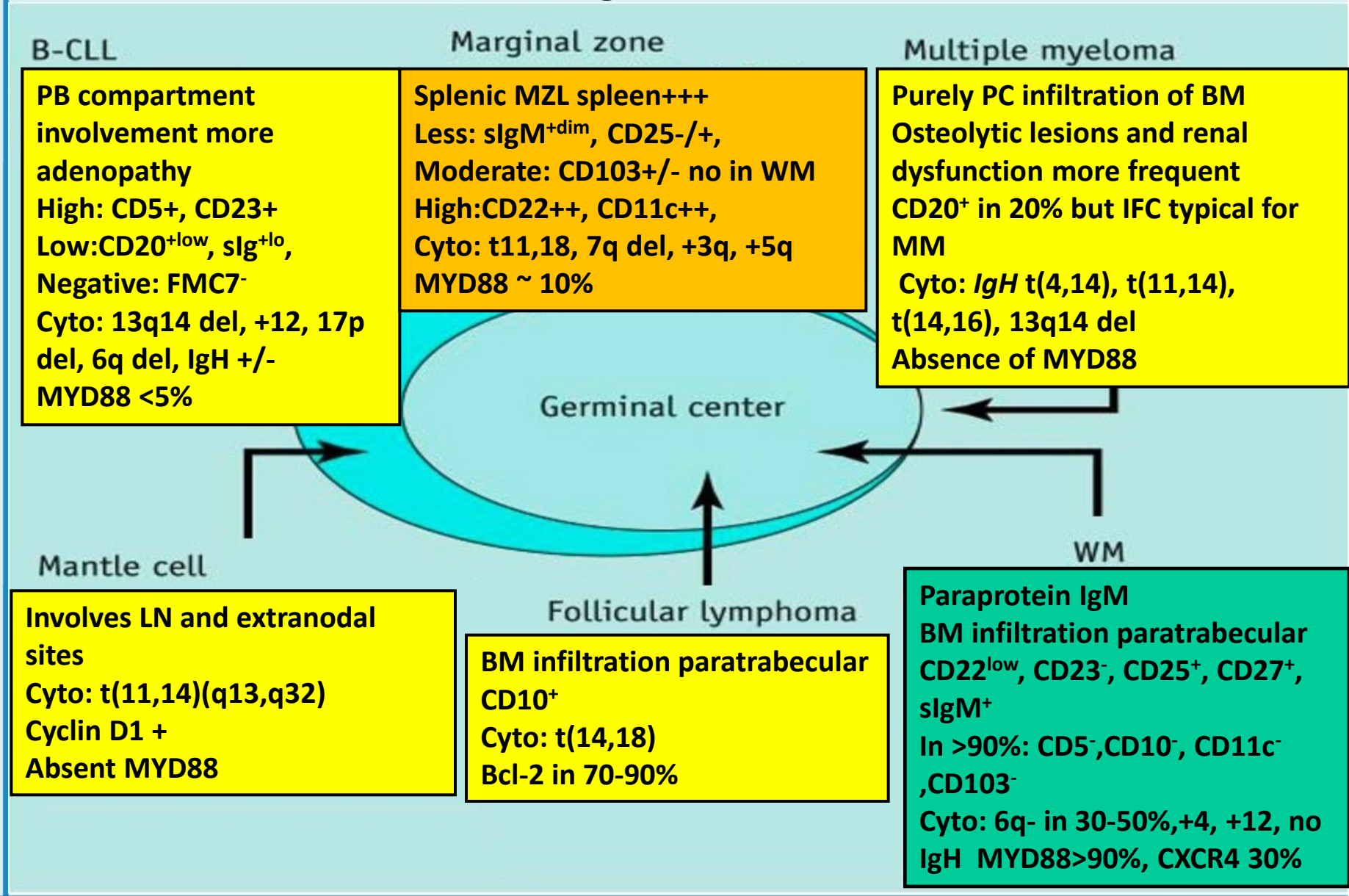
Follicular lymphoma

Paraprotein: infrequent
BM infiltration paratrabecular
$CD19^{+}$, $CD20^{+}$, $CD22^{+}$, $CD10^{+}$,$CD23^{+/-}$
Cyto: t(14,18)
Bcl-2 in 70-90%

WM

Paraprotein IgM
BM infiltration paratrabecular
$CD22^{low}$, $CD23^{-}$, $CD25^{+}$, $CD27^{+}$, $sIgM^{+}$
In >90%: $CD5^{-}$,$CD10^{-}$, $CD11c^{-}$,$CD103^{-}$
Cyto: 6q- in 30-50%,+4, +12, no IgH
MYD88>90%, CXCR4 30%

Differential diagnostic issue. Clues.



Conclusion

- Diagnostic, risk and response assessment of WM patient is complex
- Should be standardized and guided by current CMG GUIDELINES 2014
- In case of differential diagnostic issues, unusual clinical course, problems with IgM measurement and response assessment there´s appropriate to use necessary facilities and methods

Acknowledgment

Tým pro MDS a myeloproliferace
prof. MUDr. Elena Tóthová, CSc.
MUDr. Lukáš Stejskal
MUDr. Cecília Bodzásová
MUDr. Zuzana Kučerová
MUDr. Petra Richterová

Tým pro trombózu a hemostázu
prim. MUDr. Jaromír Gumulec
prim. MUDr. Milan Matuška, Ph.D.

Myelomový tým
prof. MUDr. Roman Hájek, CSc.
MUDr. Lenka Zahradová, Ph.D.
MUDr. Hana Plonková
MUDr. Tomáš Jelínek

ALA a WM tým
prof. MUDr. Roman Hájek, CSc.
MUDr. Lenka Zahradová, Ph.D.
MUDr. Hana Plonková
MUDr. Tomáš Jelínek
MUDr. Michal Kaščák

CLL tým
prof. MUDr. Roman Hájek, CSc.
MUDr. Jana Zuchnická

Tým pro ALL/AML
MUDr. Zdeněk Kořístek, Ph.D.
MUDr. Petra Richterová

Lymfomový tým
MUDr. Juraj Ďuraš
MUDr. Milan Navrátil
MUDr. Michal Kaščák

Ostatní lékaři KHO
MUDr. Jana Fečková – Mihályová

Výzkumný tým KHO
MUDr. Fedor Kryukov, Ph.D.
MUDr. Elena Kryukova, Ph.D.
Mgr. Zuzana Kufová
Bc. Jana Filipová

Laboratorní tým KHO
Mgr. Jana Smejkalová, Ph.D.
Mgr. Lucie Adamusová
Mgr. Lukáš Grebeníček
Bc. Petra Vrublová
Mgr. Hana Svěchová

Sesterský tým pod vedením:
Mgr. Kateřiny Hašové
Kateřiny Horákové
Mgr. Františky Vyhnánkové
Petry Ramikové

Datamanažerský tým pod vedením:
Mgr. Martiny Januškové