Chronic Lymphocytic Leukemia: New Approaches for a Common Disease

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Disclosure

- None

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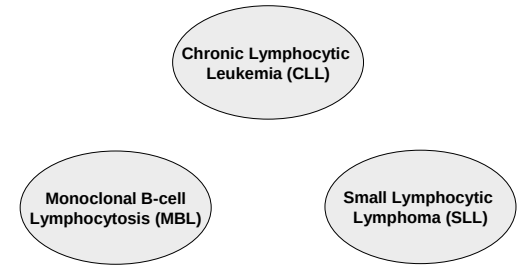
Goals Today:

- Distinguish Chronic Lymphocytic Leukemia (CLL) from other B-cell chronic lymphoproliferative disorders
- Understand the definition of the term monoclonal B-cell lymphocytosis (MBL)
- Realize the issues associated with minimal residual disease (MRD) detection in CLL
- *Future Hot Topics on CLL: "Risk Stratification in CLL – The Role of the Clinical Laboratory"*



CLL: Evolution of Diagnostic Criteria

- 1975 / Rai Staging:
  - $\geq$15 x 10^9/L ALC in peripheral blood
  - $\geq$30% lymphocytes in bone marrow aspirate
- 1988 & 1996 / NCI-WG:
  - $\geq$5 x 10^9/L ALC (flow cytometric detection of clonal B cells)
  - 1996: CLL immunophenotype necessary
- 2008 / IWCLL*:
  - B cells >5 x 10^9/L of at least 3 month duration
  - Clonality confirmed by flow cytometry; CLL immunophenotype
  - The presence of a cytopenia caused by a typical marrow infiltrate defines the diagnosis of CLL regardless of the number of B cells or nodal involvement

Hallek M, et al. Blood. 2008 Jun 15;111(12):5446-56

Chronic B-Cell Lymphoproliferative Disorders: Prototypic Immunophenotype

	sIg	CD20	CD5	CD23	CD10	CD103
CLL / SLL	Weak	Weak	+	+	–	–
Lymphoplasmacytic (LPL)	Mod	+	+/-	+/-	–	–
Mantle cell (MCL)	Mod	+	+	– (partial)	–	–
Marginal zone: Nodal / MALT	+	+	–	+/-	–	–
Splenic marginal zone (SMZL)	+	+	-/+	+/-	–	-/+
Follicular	+	+	–	+/-	+	–
Hairy cell	+	+	–	–	–	+

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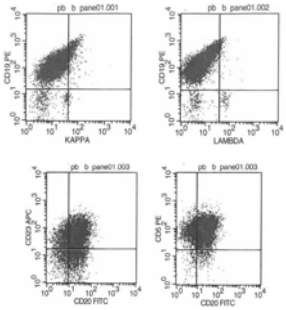
CLL: Dim sIg, dim CD20, CD5+ & CD23+



Chronic Lymphocytic Leukemia

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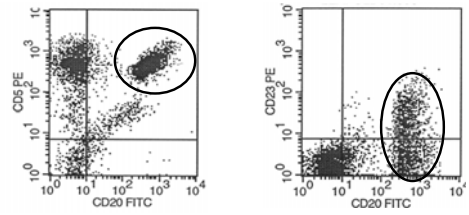
"Copycat": MCL with CD5+/partial CD23+



Chronic Lymphocytic Leukemia

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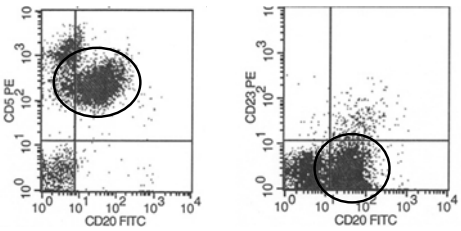
"Copycat": LPL with CD5+/CD23-

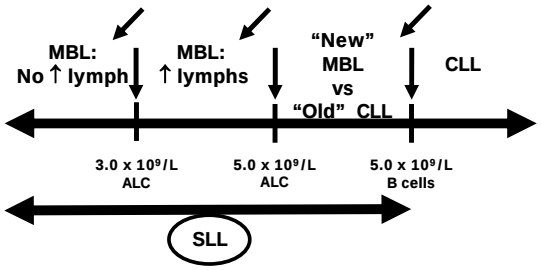


Chronic Lymphocytic Leukemia

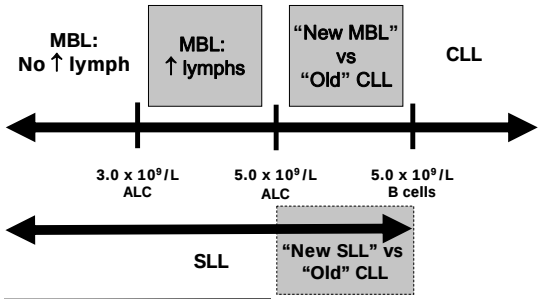
Diagnosis of MBL & SLL: IWCLL

- Monoclonal B-Cell Lymphocytosis (MBL):
  - B cells <5 x 10^9/L
  - Absence of lymphadenopathy / organomegaly (as defined by physical exam and CT scan)
  - Absence of cytopenias due to marrow involvement
  - Similar expression of genetic risk factors as compared to early stage CLL
- Small Lymphocytic Leukemia (SLL):
  - Lymphadenopathy and the absence of cytopenias caused by a clonal marrow infiltrate
  - B-cells should not exceed 5 x 10^9/L
  - Confirm by lymph node biopsy whenever possible

Diagnostic Criteria: CLL, MBL, and SLL



Diagnostic Criteria: CLL, MBL, and SLL



Identification of MBL

- General population screening
- Familial CLL
- Routine clinical practice

Need to keep in mind which group of patients we are talking about when we discuss MBL!

How will MBL be Recognized in Routine Clinical Practice?

- Lymphocytosis identified on CBC screening will be the most common way of identifying MBL in routine clinical practice

What is a lymphocytosis?

- Is it an ALC above ~3.0 x 10^9/L?
  - Labs with thorough normal value studies
  - Quantitative lymphocyte subsets → T-cell: 582 – 1992 + B-cell: 71 – 567 + NK-cell: 80 – 597
- Is it an ALC above ~5.0 x 10^9/L?
  - Textbooks (historic?): instrument manufacturers

How is MBL Recognized in Routine Clinical Practice?

- Incidental finding
  - Flow analysis of PB or BM for unrelated reasons
  - BM biopsy with a lymphoid infiltrate without PB lymphocytosis; followed by PB flow study
  - SLL identified at surgery in lymph node/tissue biopsy without associated lymphadenopathy, organomegaly, or PB lymphocytosis; followed by PB flow study

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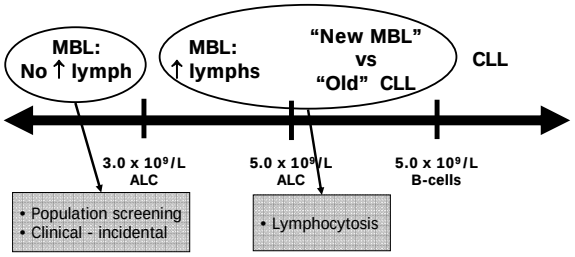
Diagnostic Criteria: MBL in Clinical Practice



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MBL: Prevalence and Progression

- Normal population
  - 3.5% will have a CLL phenotype
  - Another 1% will have a non-CLL phenotype
- Prevalence increases with age:
  - 2.1% (40 - 60 y.o.) to 8.0% (>70 y.o.)
- Low risk genetic factors (eg, mutated IgVH; 13q-)
- Progression rate to CLL uncertain
  - MBL identified via population studies: 1 to 3% per year
  - MBL identified in clinical practice: up to 40% per year

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MBL: Summary

- MBL identified through **population screening** may exhibit a different behavior than those identified through **routine clinical practice.**
- ~40% of new cases currently diagnosed as Rai Stage 0 CLL will be reclassified as MBL using IWCLL
- There is no standard method to measure MBL/CLL B-cell counts in the clinical flow cytometry laboratory
- Molecular prognostic factors will likely contribute to the risk of disease progression better than an arbitrary lymphocyte or B-cell count

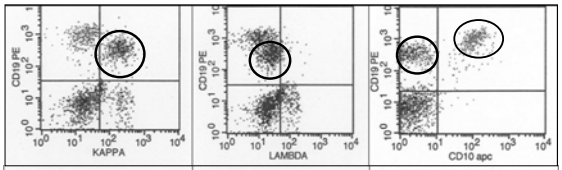
Familial CLL

- Families with known CLL patients have an increased risk of having MBL or CLL identified in other family members
- 12% to 18% of CLL patients have an extended family member with CLL or some other type of lymphoproliferative disorder
- Genetic factors remain uncertain

MBL Case: Clinical History

- 66 y.o. female
- Normal CBC; absolute lymphs = 1.2 x 10^9/L
- Normal blood smear
- No adenopathy or organomegaly
- "Only God knows why the flow study was ordered."
- Evaluated in Hematology; bone marrow performed

MBL Case: Peripheral Blood Immunophenotype

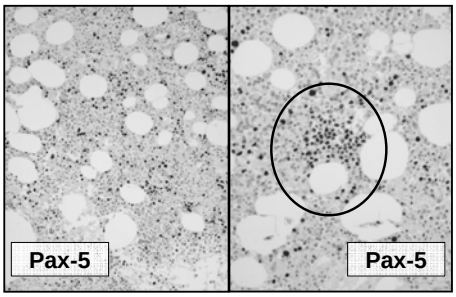


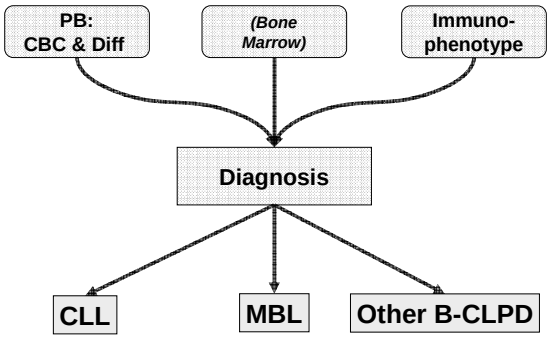
Quad	Events	% Gated	% Total
UL	415	21.52	0.52
UR	456	23.65	0.57
LL	824	42.74	1.04
LR	233	12.09	0.29

Quad	Events	% Gated	% Total
UL	1077	45.08	1.18
UR	57	2.39	0.06
LL	1015	42.49	1.12
LR	240	10.05	0.26

Quad	Events	% Gated	% Total
UL	463	24.01	0.58
UR	445	23.08	0.56
LL	1003	52.02	1.26
LR	17	0.88	0.02

MBL Case: Bone Marrow Biopsy





CLL and
Minimal Residual Disease (MRD)

- MRD eradication is goal of current therapies.
- Does absence of MRD improve overall survival?
- Does MRD detection predict early relapse?
- These questions have not been definitively answered. But laboratories are being asked to detect MRD in CLL patients.

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CLL and Minimal Residual Disease (MRD)

- Flow immunophenotyping studies: PB or BM?
  - PB is the preferred specimen
- To what detection level: 1% to 0.01%?
  - 0.01%; need to collect 200,000 to 500,000 events
- CD5/CD19 vs. 4-color vs. 6-color?
  - Multicolor adds specificity – not necessarily sensitivity.
  - Sensitivity is dependent on the cell mix and how many polyclonal B cells are present. Challenges arise when there is a mixture of monoclonal and polyclonal B-cells.

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CLL and Minimal Residual Disease (MRD)

- Does immunohistochemistry (IHC) have a role in bone marrow specimens?
  - Stains are often complementary to flow studies in BM, but are often hard to interpret in isolation.
  - T-cell nodules depleted of B cells may be identified post-Rituxan therapy and can be confused with CLL.
- What antibodies should be used for IHC?
  - No specific and easy answer. PAX-5, CD19, CD79b may all be used. CD20 has a minimal role (usually post-Rituxan). A pan-T cell marker (eg, CD3) is also necessary. However, CD5 may be hard to interpret.

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CLL MRD Case: Clinical History

- Female; 58 y.o.
- July 1999
  - WBC: 38.1 / Lymphs: 81%
  - Flow: sIg k (d), CD19, CD5, CD20 (d), CD23
  - Dx: CLL
  - No organomegaly
  - No cytopenias
  - Rai Stage 0
  - Observation; no Rx

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CLL MRD Case: Clinical History

- 1999 to 2008: Steady progression of disease
- 2007: Chemo – Pentostatin, Cytoxan, Rituxan
- Jan. 2008: minimal clinical disease
  - Anterior node (~1 cm.); no organomegaly
  - WBC: 6.9 / Lymphs: 11%
  - Hgb: 12.5 / MCV: 75.6
  - Plt: 346
- June 2008
  - No radiologic evidence of disease
  - Normal CBC (9% lymphs)

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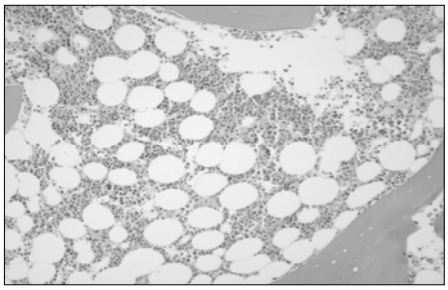
CLL MRD Case: Bone Marrow Biopsy



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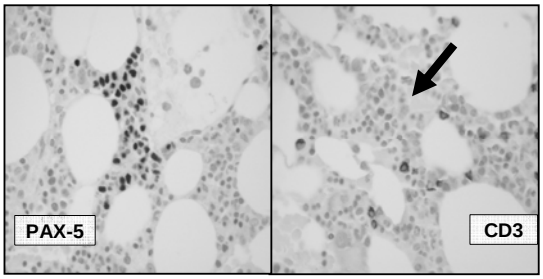
CLL MRD Case: Bone Marrow Biopsy



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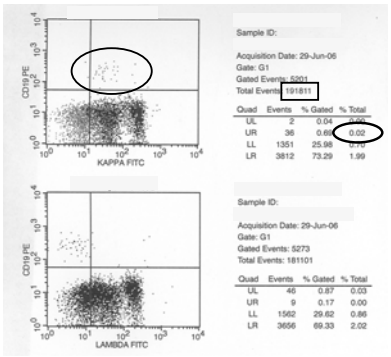
CLL MRD Case: PB Flow



Sample ID:
Acquisition Date: 29-Jun-06
Gate: G1
Gated Events: 5201
Total Events: 191811

Quad	Events	% Gated	% Total
UL	2	0.04	0.00
UR	36	0.69	0.02
LL	1351	25.98	0.70
LR	3812	73.29	1.99

Sample ID:
Acquisition Date: 29-Jun-06
Gate: G1
Gated Events: 5273
Total Events: 181101

Quad	Events	% Gated	% Total
UL	46	0.87	0.03
UR	9	0.17	0.00
LL	1562	29.62	0.86
LR	3656	69.33	2.02

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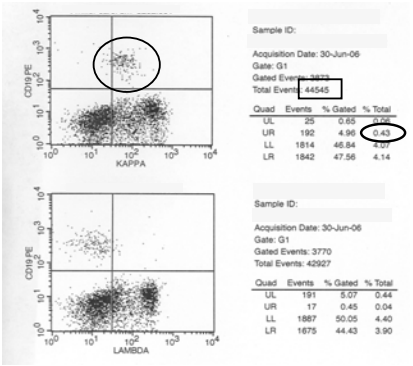
CLL MRD Case: BM Flow



Sample ID:
Acquisition Date: 30-Jun-06
Gate: G1
Gated Events: 3873
Total Events: 44545

Quad	Events	% Gated	% Total
UL	25	0.65	0.06
UR	192	4.96	0.43
LL	1814	46.84	4.07
LR	1842	47.56	4.14

Sample ID:
Acquisition Date: 30-Jun-06
Gate: G1
Gated Events: 3770
Total Events: 42927

Quad	Events	% Gated	% Total
UL	191	5.07	0.44
UR	17	0.45	0.04
LL	1887	50.05	4.40
LR	1675	44.43	3.90

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- Realize the issues associated with minimal residual disease (MRD) detection in CLL

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