MAYO CLINIC

Body fluid flow cytometric immunophenotyping

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Disclosures

- None

Objectives

- Utility of flow cytometric immunophenotyping
- Challenges encountered, and how to addressed
- Triaging body fluid specimens for flow cytometry

Which body fluids?

- Cerebrospinal fluid (CSF) and vitreous
- Serous effusion (pleural, pericardial, peritoneal / ascitic):
  - Transudate (ultrafiltrate of plasma) e.g. CHF, cirrhosis
  - Exudate (rich protein and WBC) e.g. infection, malignancy
- Acquired fluid collections e.g. breast implant-associated

Why perform flow cytometry?

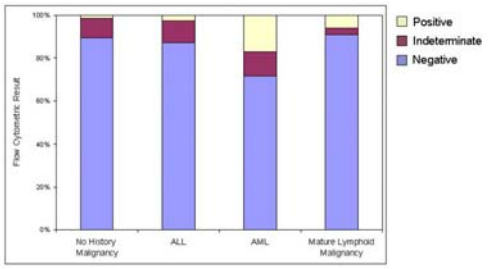
Hematolymphoid malignancy:

- Detection:
  - Immunoglobulin light chain restriction
  - Aberrant immunophenotype
- Classification:
  - Mature / Immature
  - B-cell, T-cell, myelomonocytic

Indications for CSF flow cytometry

- NCCN guidelines for the diagnosis of CNS lymphoma
- Staging hematolymphoid neoplasms:
  - Patients with CNS disease may be asymptomatic
  - WBC, RBC, protein and LDH of limited value
  - Flow more sensitive / specific than cytology

Hegde et al., *Blood* 2005: 105(2); 496-502
Bromberg et al., *Neurology* 2007: 68; 1674-1679
Quijano et al., *JCO* 2009:27(9); 1462-1469

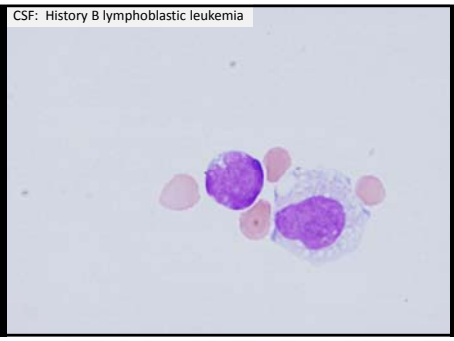


Craig, at al., *Am J Clin Pathol* 2011: 135(1); 22-34

What's the problem?

- Paucity of cells, especially CSF and vitreous
- Non-specific staining, especially serous effusions
- Flow cytometric findings may not be sufficient

CSF: History B lymphoblastic leukemia



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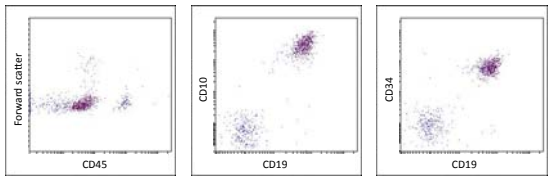


Paucity of cells

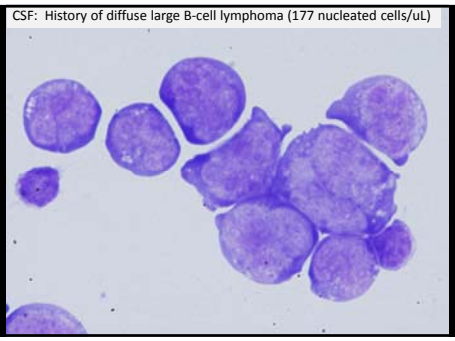
- Minimize cell loss through:
  - Degeneration
  - Allocation for other studies
  - Specimen processing
- Maximize information from each cell
- Avoid confounding artifacts

Cell degeneration

- CSF is toxic, with cell loss within 30 minutes
- Granulocytes and monocytes > lymphocytes
- CSF cell loss minimized by:
  - Quick specimen delivery and keeping cool
  - Adding stabilization medium e.g. RPMI +/- FBS, Transfix™

Greig et al., *Cytometry Part B* 2014;86B:135-8
De Graaf et al., *Cytometry Part B* 2011:80B;43-50

CSF: History of diffuse large B-cell lymphoma (177 nucleated cells/uL)



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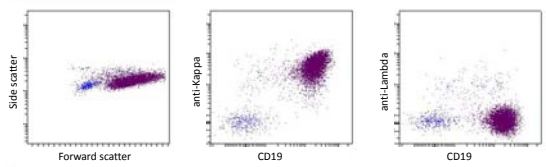
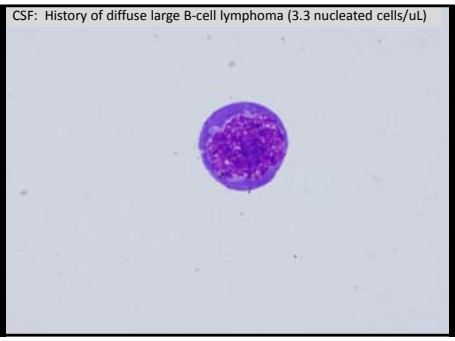


CSF: History of diffuse large B-cell lymphoma (3.3 nucleated cells/uL)



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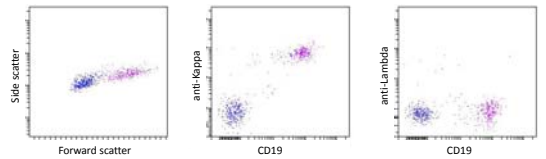
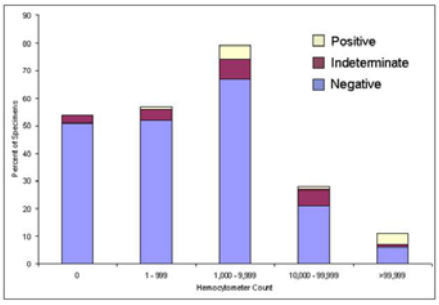


Handling paucicellular specimens

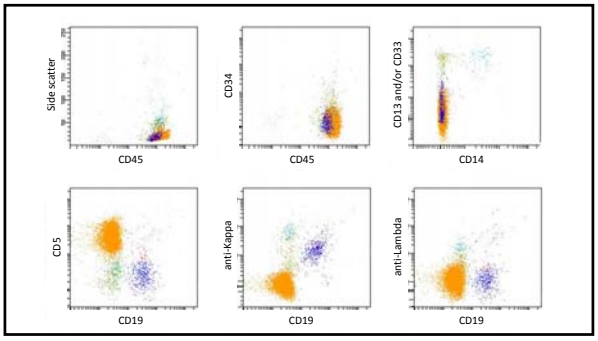
- Prioritize testing, depending on indication
- Consider eliminating count and seting up 1 flow tube
- Minimize centrifugation and pipette off supernatant
- Avoid intracytoplasmic staining / permeabilization



Maximize information from each cell

- Focus on disease(s) of interest (previous specimens, clinical history / clinical concern)
- Address realistic question(s) e.g. new diagnosis of T-cell lymphoma is unlikely from 1 paucicellular tube
- Higher color flow cytometry (8, 10, 12…) often helps
- Consider more than one antibody per fluorochrome

De Graaf et al., *Cytometry Part B* 2011:80B;43-50

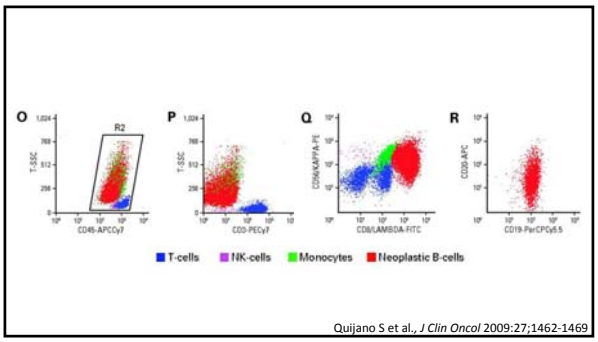


Maximize information from each cell

6-color, 11-parameter CSF flow cytometry for aggressive B-cell lymphoma:

- CD3 (PE-Cy7)
- CD20 (APC)
- CD8 and sIg lambda (FITC)
- CD56 and sIg kappa (PE)
- CD4 and CD19 (PerCP-Cy5.5)
- CD45 (APC-Cy7)

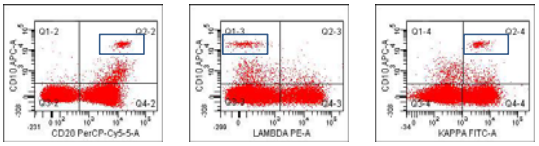
Quijano S et al., J Clin Oncol 2009:27;1462-1469



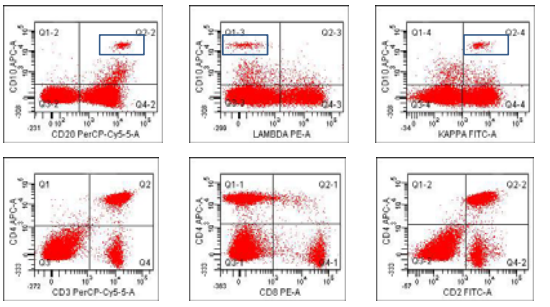
Quijano S et al., *J Clin Oncol* 2009:27;1462-1469

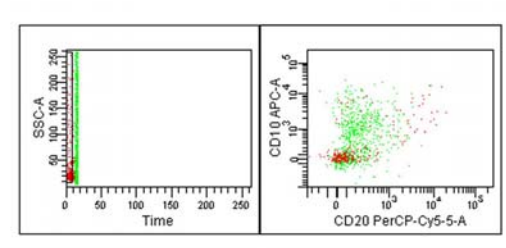
Avoiding confounding artifacts

- Use validated reagent cocktails, particularly because lack of internal control staining
- Avoid carryover (keep tubes capped and acquire blank tube first)
- Record acquisition "Time" as a parameter to gate out periods of unstable data acquisition e.g. aspirated air
- Gain familiarity with expected findings



Carryover?



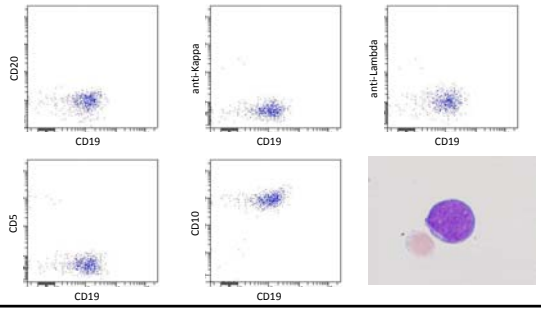


Craig, at al., *Am J Clin Pathol* 2011: 135(1); 22-34

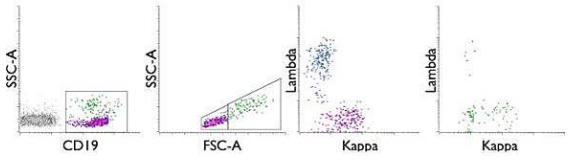
Familiarity with expected findings

Disease	WBC Count	Predominant cell type	Flow Cytometric Findings
None	0 – 5/mL	Lymphocyte	Predominantly CD4+ T-cells B-cells < 1%
Acute bacterial meningitis	100 - 100,000/mL	Neutrophils	Granulocytes
Viral meningitis	10 – 1,000/mL	Lymphocytes	Predominantly T-cells
Multiple sclerosis	often < 5/mL	Lymphocytes	Increased CD4+ T-cells, B-cells, and plasmacytoid dendritic cells
Guillain-Barré syndrome	often < 5/mL	Lymphocytes	? Increased CD8+ T-cells
Para-neoplastic neurological syndromes	Variable	Lymphocytes	Some increase T-cells (x3) Marked increase B-cells (x20)

CSF: High-grade B-cell lymphoma with rearrangement of BCL-2 and MYC



Non-neoplastic light chain restriction



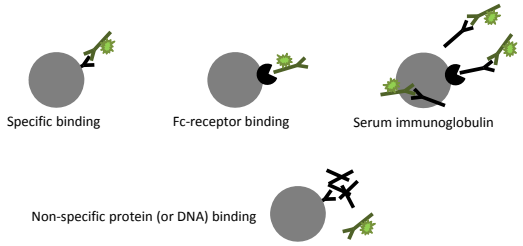
Vafaii P & DiGiuseppi JA. *Cytometry Part B* 2014; 86B:106-110

Non-specific, or no, staining

- Relatively frequent in serous fluid specimens
- Potential causes:
  - Reagent antibodies bind to Fc receptors
  - Reagent antibodies bind to serum immunoglobulin, which may be bound to Fc receptors or antigens
  - Bound proteins block reagent antibody binding

Horna P et al., *Am J Clin Pathol* 2011 136(6):954-9
Hulspas R. et al., *Cytometry, Part B* 2009;76B: 355-364

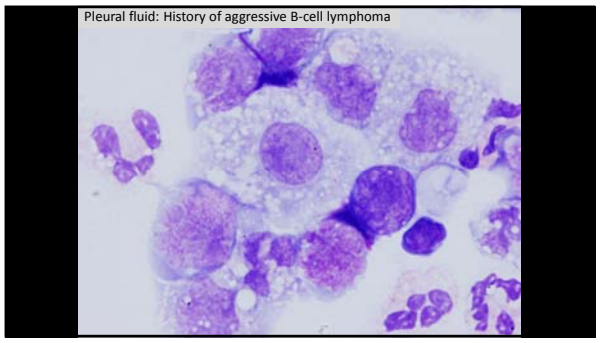
Non-specific, or no, staining

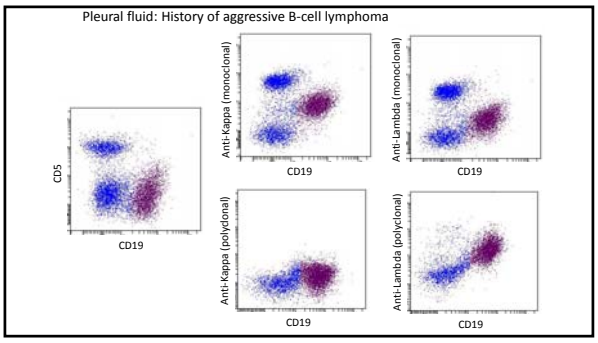


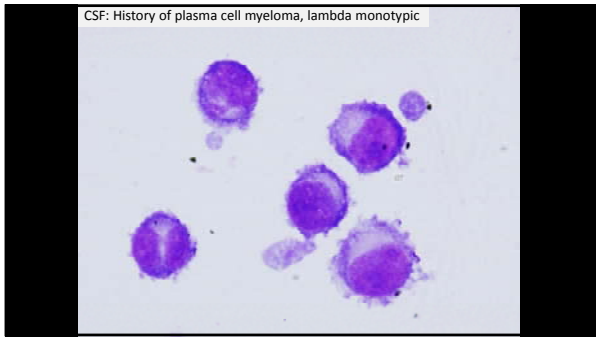
Non-specific, or no, staining

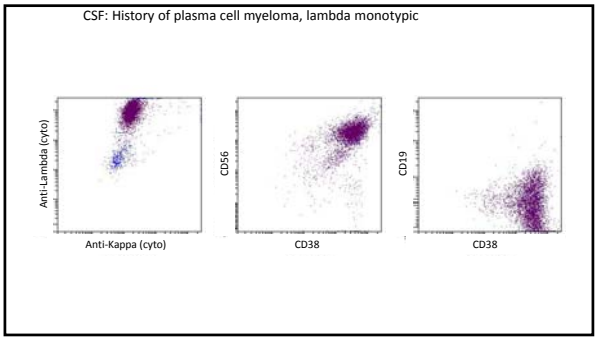
Potential solutions:

- Gate on cells of interest i.e. eliminate monocytes, NK
- Wash, +/- warm to remove serum immunoglobulin and other bound proteins
- Try monoclonal and polyclonal reagent antibodies
- Incubate with immune rabbit serum to block Fc receptors and displace bound serum
- Evaluate cytoplasmic antigens





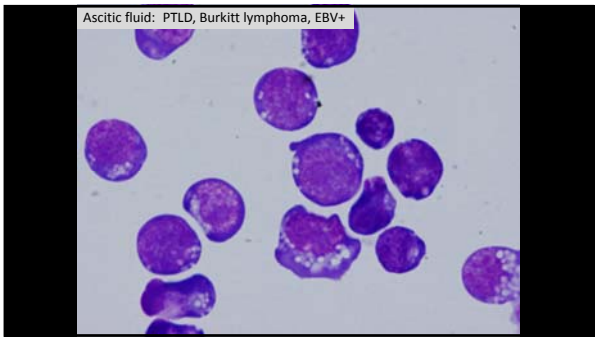


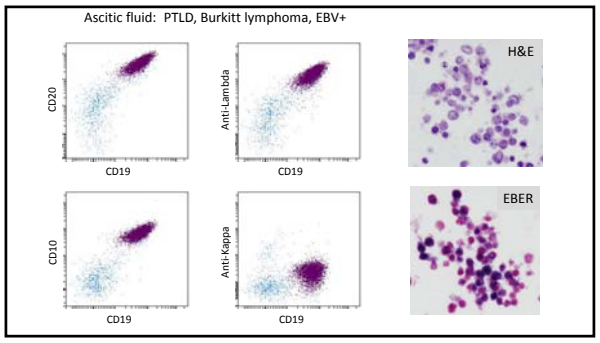


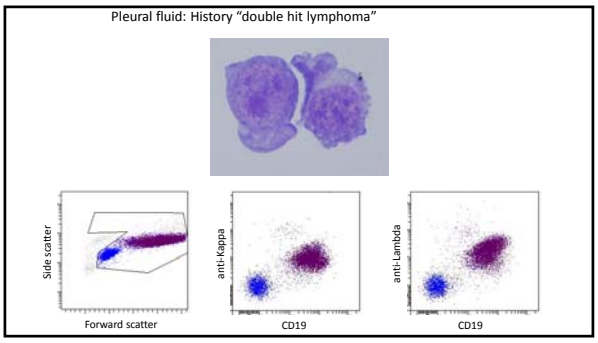
Flow findings not sufficient

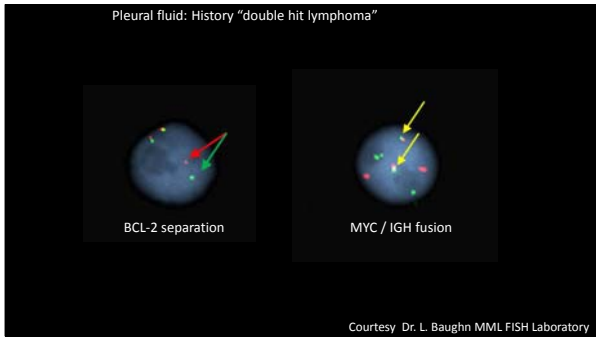
- Flow cytometric findings limited by paucicellular specimen or non-specific staining (if all else fails, consider other modalities e.g. FISH, IHC, molecular)
- Disease-related challenges e.g. limited antigen expression in 1° effusion lymphoma and anaplastic large cell lymphoma

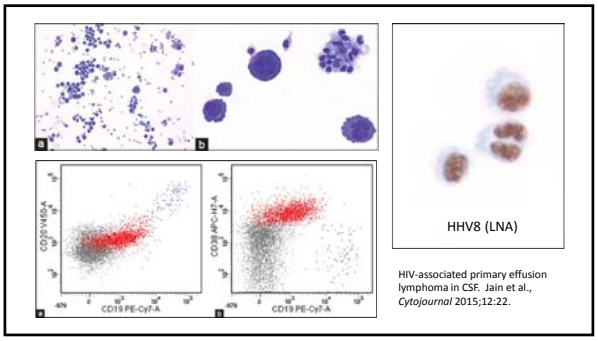
Nowakowski GS et al., *Cytometry Part B* 2005; 63B:23-27











HIV-associated primary effusion lymphoma in CSF. Jain et al., *Cytojournal* 2015;12:22.

Flow findings (often) not sufficient

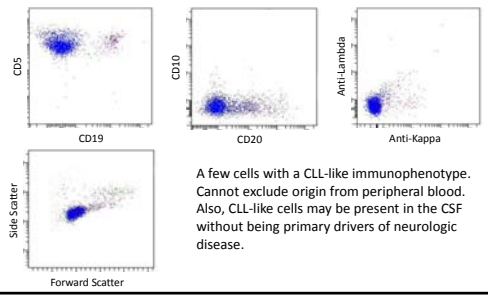
- Primary effusion lymphoma:
  - CD30(+), CD38(+), CD138(+), EMA(+), CD45(+)
  - CD19(-), CD20(-), CD79a(-), sIg(-), cIg(-)
- Breast implant-associated anaplastic large cell lymphoma:
  - CD30(+), CD4(+), CD43(+), incomplete pan-T-cells antigens
  - Alk-1(-), CD19(-), CD20(-), CD79a(-), sIg(-), cIg(-)

Flow findings not sufficient

- Contamination with peripheral blood, especially if:
  - WBC high e.g. chronic lymphocytic leukemia
  - Distinctive phenotype e.g. B lymphoblastic leukemia
- Abnormal cells, but not primary drivers disease e.g. CLL cells and inflammatory neurologic disease
- Non-neoplastic light chain restriction e.g. MS

Nowakowski GS et al., *Cytometry Part B* 2005; 63B:23-27

CSF: History of CLL 6.1/uL nucleated cells, 2.2/uL RBC



A few cells with a CLL-like immunophenotype. Cannot exclude origin from peripheral blood. Also, CLL-like cells may be present in the CSF without being primary drivers of neurologic disease.

Flow Cytometry on all CSF specimens?

- Craig FE et al., Am J Clin Pathol. 2011; 135:22-34:
  - Positive flow 4.8% (11 of 230 specimens)
  - All but 1 had a previous diagnosis of malignancy
- Collie AM et al., Am J Clin Pathol 2014; 141:515-21:
  - Positive flow 8.2% (41 of 501 specimens)
  - All had history hematologic malignancy or atypical morphology (atypical lymphocytes or blasts)

Indications for body fluid flow

- Demonstrated value in staging diagnosed disease
- Can eliminate need for surgery e.g. suspected T-lymphoblastic leukemia
- Controversy about use as a screening tool:
  - Low frequency positive results if no clinical suspicion hematolymphoid neoplasm
  - CSF monotypic populations in multiple sclerosis
- Work with clinical colleagues to determine algorithm

Body fluid flow cytometry

- Apply good flow cytometric practices
- Prevent cell loss and maximize information
- Reduce non-specific staining, if possible
- Gain familiarity with non-neoplastic disorders
- Consider contamination, and clinical significance, before reporting
- Discuss indications with clinical colleagues