Hematopathology
Case #11

Dan Boyer, MD, PhD
11/1/2019
Clinical case

• 82-yo man with history of HTN, OSA and prostate cancer
• Chief complaint: word-finding difficulties and nonsensical speech
• Brain MRI: 2.3-cm enhancing mass lesion in the left parietal lobe with surrounding edema
• Lumbar puncture: Clear, colorless CSF. 4 WBC/μL, 1 RBC/μL, protein 18 mg/dL, glucose 65 mg/dL
CSF cytospin (Wright-Giemsa stain)
Differential diagnosis

• Metastatic carcinoma
• Lymphoma
• Acute leukemia
• Glioblastoma
Flow cytometric immunophenotyping of CSF

[Lymph] CD3-A750 / CD19-APC

[T cells]

[B cells]

[B cells]

[SS INT / FS INT]
Diagnosis

• Cytology + flow = large B-cell lymphoma
  • Primary vs secondary?
• Radiology: intraparenchymal brain mass and no evidence of extracranial involvement
• Final diagnosis: Primary diffuse large B-cell lymphoma of the CNS
Primary CNS lymphoma – clinical presentation

- 4% of intracranial neoplasms
- 1500 new patients/year in the US
- M ≈ F
- Adults over 40
- Median age used to be 56, but recently increasing with peak 70-79
- Most present with focal neurologic deficits, but some have nonspecific behavioral or neurocognitive changes
- Vast majority have parenchymal brain lesions
  - Solitary or multifocal
  - Usually involve supratentorial white matter
  - Often periventricular (including centrum semiovale, corpus callosum, basal ganglia)
- Lymphoma cells detected in CSF in ~25%
- Isolated leptomeningeal or spinal cord involvement is very rare
- 20-25% have ocular involvement at diagnosis
- Involvement of peripheral nerves (neurolymphomatosis) is rare

Grommes et al. Neuro-Oncology 2018;21:296-305
Primary CNS lymphoma - pathology

• 90% are diffuse large B-cell lymphomas
• Remainder are Burkitt lymphoma, T-cell lymphoma, or low-grade B-cell lymphomas (marginal zone lymphoma, follicular lymphoma)
• In some usage, the term “primary CNS lymphoma” implies a diagnosis of diffuse large B-cell lymphoma, but it’s preferable to explicitly state the type of lymphoma
• WHO terminology: “Primary diffuse large B-cell lymphoma of the CNS”
Primary DLBCL of the CNS

- >80% are activated B-cell type (ABC) DLBCL
  - 90% MUM1+
  - 60-80% BCL6+
  - <10% CD10+
- Prominent perivascular infiltration is common
- Most frequently mutated genes are MYD88, CD79B, CDKN2A, PIM1, CARD11 and TNFAIP3
- 9p24.1 amplification -> increased PD-L1 and PD-L2
- Median OS: 26 months (US SEER database)
- 10-15% primary refractory
- Up to 50% relapse
- Systemic spread is rare; tends to involve breast or testis
- Treatment with systemic chemotherapy +/- whole-brain radiation
Secondary CNS involvement by systemic DLBCL

• 2-4% of systemic DLBCL spread to the CNS
• Usually identified within 6 months of initial DLBCL diagnosis
• Median survival 2-5 months after dx of CNS involvement
• Brain parenchymal lesions more common than leptomeningeal
• Occult leptomeningeal involvement at diagnosis in ~10% of high-risk DLBCL

Risk factors
  • High IPI score (12%)
  • Testicular, renal or adrenal involvement (up to 25%)
  • MYC and BCL2/BCL6 double or triple hit (up to 50%)
  • ABC-DLBCL with MYC/BCL2 double-expression (15%)

Qualls and Abramson. Haematologica 2019;104:25-34
Morphologic pitfalls of DLBCL in CSF

• Overlapping features with other malignancies
• Overlapping features with reactive lymphocytes and monocytes
• Suboptimal specimen preparation
• Paucity of malignant cells in CSF
Variable morphology of DLBCL in CSF
Metastatic carcinoma
Metastatic carcinoma
Acute myeloid leukemia

AML with Auer rods

AML with monocytic differentiation
Acute lymphoblastic leukemia

B-ALL with small blasts

B-ALL with large pleomorphic blasts
Glioblastoma
Medulloblastoma
Pitfall: reactive lymphs and monos in encephalitis or meningitis
Reactive histiocytosis
Pitfall: suboptimal specimen preparation

Liquid Based Preparation:
ThinPrep with Pap stain at 40x

Cytospin Preparation:
Wright-Giemsa stain at 40x
Pitfall: Rare large atypical lymphocytes
Flow cytometry identified a clonal B-cell population
Correlation with MRI

• Multiple foci of abnormal enhancement:
  • Subependymal lesions adjacent to lateral ventricles
  • Bilateral fornical columns
  • Hippocampal commissure
• The combined radiology, cytology and flow cytometry findings support diagnosis of primary DLBCL of the CNS
• Patient was treated with high-dose methotrexate and rituximab
• Follow-up MRI showed resolution of lesions
• Patient’s mental status improved
Pitfalls: false-positive flow cytometry

• Peripheral blood contamination
• Monoclonal B-lymphocytosis
• Multiple sclerosis
Peripheral blood contamination
The patient’s peripheral blood contains a higher proportion of CLL cells.
Lambda-skewed B-cells in CSF
Similar kappa/lambda ratio in peripheral blood with CD5+,lambda-restricted B-cell population – Dx: Monoclonal B-lymphocytosis
Kappa-restricted B-cells with increased side scatter
Clinical correlation

- 6 months of right upper extremity weakness and 4 days of right monocular vision loss and pain
- Brain MRI showed multiple foci of hyperintensity on T2/FLAIR in the bilateral periventricular white matter, suspicious for a demyelinating process
- CSF showed elevated IgG index and 11 oligoclonal bands (compared to 0 in serum)
- Dx: Multiple sclerosis
Monotypic B-cells in CSF of MS patients

- 3 patients with MS
- Kappa-restricted B cells with increased FS and SS
- Background of polytypic B cells with low FS and SS
- No atypical lymphocytes were identified by cytology
- No evidence lymphoma on imaging or clinical follow-up

Vafaii and DiGiuseppe. Cytometry Part B 2014;86B:106-110
Pitfalls: false-negative flow cytometry

- Large fragile lymphoma cells
- Normal kappa/lambda ratio because of reactive background
- Rapid deterioration of CSF cells *ex vivo*
Flow performed 24hrs after CSF collection
8 WBC/uL, 53% blasts
Flow performed 3 hrs after CSF collection
5 WBC/uL, 38% blasts
Mixing CSF with RPMI + 5%FBS decreases loss of WBCs

- CSF was split into tubes with or without RPMI at the time of LP
- Flow cytometry was performed at 30 min, 1hr and 5hrs
- No significant loss of lymphocytes or monocytes after 5hrs in RPMI + 5% FBS

De Graaf et al. J Neurol 2011;258:1507-12
TransFix improves stability of CSF held overnight

- CSF split into 3 tubes at time of collection: Transfix, RPMI+5%FBS or Empty tube
- 1 aliquot of each specimen run at 30 min
- Specimens stored overnight at 4°C
- 2nd aliquot run at 18 hrs
- Cell counts normalized to the result at 30 min with RPMI+5%FBS

De Jongste et al. Cytometry Part B 2014;86B:272-9
CSF stabilization summary

• CSF should be processed for flow cytometry ASAP

• If specimens cannot be processed immediately:
  • Addition of equal volume of RPMI recommended if specimen will be flowed later the same day
  • Use of Cytomark TransFix or Streck Cell Preservative recommended if specimen will be held or transported overnight
Comparisons of flow and cytology on CSF

- Because FC is significantly more expensive than cytology, there have been numerous studies to examine whether the addition of FC to cytologic evaluation is worthwhile.
- A recent review article identified 41 publications on this topic between 1998 – 2016.
- 6 studies of CSF for ALL staging (1722 specimens):
  - Positive FC and/or Cyto: 198 (11%)
  - FC+/Cyto-: 135 (8% of total, 68% of positive)
  - FC-/Cyto+: 6 (0.4% of total, 3% of positive)
- 15 studies of CSF for NHL staging (1216 specimens):
  - Positive FC and/or Cyto: 269 (22%)
  - FC+/Cyto-: 140 (12% of total, 52% of positive)
  - FC-/Cyto+: 14 (1.2% of total, 5% of positive)
- Note: Most of these studies considered an “atypical” cytology diagnosis as negative.

Canovi and Campioli. Diagn Cytopathol 2016;44:841-856
CSF analysis for primary CNS lymphoma

• Schroers et al studied 30 patients with biopsy proven primary CNS lymphoma
  • Positive FC and/or Cyto: 8 (27%)
  • FC+/Cyto-: 3 (10% of total, 38% of positive)
  • FC-/Cyto+: 1 (3% of total, 13% of positive)

• Armand et al studied 75 patients with PCNSL at diagnosis or relapse
  • Positive FC and/or Cyto: 18 (24%)
  • FC+/Cyto-: 11 (15% of total, 61% of positive)
  • FC-/Cyto+: 1 (1.3% of total, 6% of positive)

CSF analysis to identify PCNSL without prior diagnosis

• Collie et al. examined 323 CSFs from 254 patients with possible PCNSL but no prior diagnosis
  • 5 specimens were positive by FC (1.5%)
  • 1 FC-/Cyto+ (0.3%)
  • All 5 FC+ specimens had at least atypical Cyto result

• Kovach et al. examined 118 CSFs from 108 patients with possible PCNSL but no prior diagnosis
  • 1 specimen was positive by FC (0.8%) but negative by Cyto

• Craig et al. examined 71 CSFs from patients with possible PCNSL but no prior diagnosis
  • 1 specimen was positive by FC and Cyto (1.4%)

Optimal use of FC and cytology on CSF?

- The combination of FC and cytology for diagnosis of leukemia or lymphoma is more effective than either alone.
- The data of Collie et al. suggest that morphologic screening could save costs by selecting out only CSF specimens with atypical or suspicious cells to run flow cytometry.
- However, implementing pathologist slide review before running the CSF is challenging because of the importance of rapid processing to prevent loss of viable cells.
- Screening CSFs before running flow also requires clinician buy-in, because current clinical guidelines recommend flow cytometry whenever there is suspicion of PCNSL.
Main points

- CSF is positive for lymphoma in ~25% of patients with primary CNS lymphoma
- Secondary spread to the CNS occurs in 2-4% of patients with systemic DLBCL
- Features that portend high risk of CNS involvement in systemic DLBCL:
  - High IPI score
  - Testicular, renal or adrenal involvement
  - MYC and BCL2/BCL6 double or triple hit
  - ABC-DLBCL with MYC/BCL2 double-expression
- Flow cytometry of CSF helps to distinguish large cell lymphoma from other types of malignant cells and from reactive lymphocytes and monocytes
- Beware of peripheral blood contamination creating false-positive flow cytometry results in CSF specimens, especially if the findings are suggestive of a small cell lymphoma
- If CSF specimens cannot be processed for flow cytometry immediately, then use of a stabilizing solution is recommended, such as RPMI or TransFix
Acknowledgements

• Madelyn Lew
• Eileen Putnam