Hepatic Lymphoma Diagnosis

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General Comments

Systemic hematopoietic disorders may first present on liver biopsy and rare cases of primary hepatic lymphoma may be encountered. For this reason, a liver pathologist must be familiar with the full spectrum of hematopoietic disorders involving the liver and be prepared to exclude benign mimics.

Hematopathology is a rapidly evolving subspecialty in which diagnosis often involves integration of morphology, immunophenotyping, cytogenetics, and molecular data. Many of the recent changes are highlighted in the 2016 revision to the 4th edition of the *WHO classification of tumours of hematopoietic and lymphoid tissue*. Lineage (i.e. B-cell, T-cell, NK-cell) and maturation (immature vs. mature) still form the basis for lymphoproliferative disorder classification; immunophenotyping is important for classification and is essentially always required for classification of lymphoma on liver biopsy. Immunophenotypic variation is expected and must be interpreted in the context of morphology and clinical presentation. Clonality assessment can be helpful in select cases, but clonality is not synonymous with malignancy and genetic changes cannot be used in isolation for diagnosis.

Thin sections from well-fixed tissue along with adequate clinical history are important for accurate diagnosis.

Initial Evaluation

Initial evaluation of lymph node for lymphoma involves assessment of architectural effacement and cell size; while these are both useful morphologic criteria to apply to the liver, some hepatic cases will present with subtle findings in which cells are small or intermediate size, and/or there is no effacement of liver architecture, and thus lymphoma may be easily missed. Therefore, when considering a hepatic lymphoid infiltrate, one must first determine a minimum threshold for when to initiate further work-up, which requires clinicopathologic correlation. Is the infiltrate out of proportion to an established etiology for liver injury (e.g. HCV)? Is there overt cytologic atypia (large cells, Dutcher bodies, nuclear irregularity, trinucleate plasma cells, prominent mitotic activity or nucleoli)? Hemophagocytosis? Are bone marrow elements present (e.g. extramedullary hematopoiesis)? Are there granulomas or duct loss (i.e. vanishing bile duct syndrome, which can be associated with classical Hodgkin lymphoma, even without direct liver involvement by the lymphoma)? Is there clinical suspicion for lymphoma? HIV status? Post-transplant? Is LDH elevated? Once convinced that the case needs to be worked up for lymphoma, I find it most helpful to categorize the infiltrate into effacing/portal or sinusoidal predominant (Table 1).

Table 1. Select lymphomas that may be encountered on liver biopsy (2016 revision terminology). In my experience, those that may have a significant sinusoidal component are highlighted in bold italics.

Mature B-cell neoplasms	Mature T- and NK-cell neoplasms
Burkitt lymphoma*	Adult T-cell leukemia/lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	Aggressive NK cell leukemia
B-cell prolymphocytic leukemia	Anaplastic large cell lymphoma, ALK positive
CLL/SLL	Anaplastic large cell lymphoma, ALK negative
Diffuse large B-cell lymphoma NOS (Germinal-center type or Activated B-cell/non-germinal-center type)	Angioimmunoblastic T-cell lymphoma
EBV positive DLBCL, NOS	Extranodal NK/T-cell lymphoma, nasal type
Extranodal Marginal zone lymphoma of mucosa associated lymphoid tissue (MALT lymphoma)	Hepatosplenic T-cell lymphoma
Follicular lymphoma	Other cytotoxic T-cell lymphomas*
Hairy cell leukemia**	Mycosis fungoides/Sezary syndrome
High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements	Peripheral T-cell lymphoma, NOS
High grade B-cell lymphoma, NOS	PTLD
Hodgkin's lymphoma (NLPHL and CHL)	Systemic EBV+ T-cell lymphoma of childhood
Intravascular large B-cell lymphoma	T-cell LGL leukemia
Lymphomatoid granulomatosis	
Lymphoplasmacytic lymphoma	"Chronic active EBV infection"
Mantle cell lymphoma	
Plasmablastic lymphoma	
Plasma cell myeloma	
PTLD	
T-cell histiocyte rich large B-cell lymphoma	

*Note provisional entitiy "Burkitt lymphoma with 11q aberration."
** Note provisional entity "Hairy cell leukemia variant."

* e.g. Hydroa-vacciniforme-like lymphoproliferative disorder, enteropathy-associated T-cell lymphoma, monomorphic epitheliotropic intestinal T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma in the appropriate clinical scenario

Cell size is also helpful in narrowing the differential diagnosis (e.g. Table 2) and in selecting appropriate immunohistochemical stains. Since definite lineage/maturation may not be apparent on H&E stained sections, an initial selection of immunohistochemical stains to establish lineage (e.g. PAX5, CD3, CD5, CD56, EBV ISH; also a TdT immunostain can be helpful, if lymphoblastic lymphoma is a consideration) may be considered (with unstained slides cut at this time to preserve tissue for further evaluation), which can then be followed by a second round of stains targeted to a more specific differential diagnosis. If fresh tissue was saved at the time of biopsy, then flow cytometric evaluation can be helpful.

Table 2. Mature B-cell neoplasms listed based on typical cell size

Small lymphoid cells	Intermediate-size lymphoid cells	Large lymphoid cells
B-cell prolymphocytic leukemia	Burkitt lymphoma	Diffuse large B-cell lymphoma NOS (Germinal-center type or Activated B-cell/non-germinal-center type)
CLL/SLL	High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (some cases have large cells)	EBV positive DLBCL, NOS
Extranodal Marginal zone lymphoma of mucosa associated lymphoid tissue (MALT lymphoma)	High grade B-cell lymphoma, NOS, (includes cases with blastoid large cells)	Intravascular large B-cell lymphoma
Follicular lymphoma	PTLD	Lymphomatoid granulomatosis
Hairy cell leukemia		Plasmablastic lymphoma
Lymphoplasmacytic lymphoma		T-cell histiocyte rich large B-cell lymphoma
Mantle cell lymphoma		PTLD
PTLD		
		Classical Hodgkin's lymphoma
		Nodular lymphocyte-predominant Hodgkin's lymphoma
		PTLD

A note about EBV

EBV typically infects B-cells, through the EBV receptor (CD21). In EBV hepatitis, liver biopsy most commonly shows a mild sinusoidal lymphohistiocytic infiltrate without piling up of the cells, hemophagocytosis, or overt atypia. There is usually no necrosis, but non-necrotizing granulomas (and rarely, fibrin ring granulomas) can be present. When I consider a diagnosis of EBV hepatitis, I find lineage markers helpful in excluding an EBV driven malignancy or chronic active EBV infection (which may involve B, T, or NK-cells). In EBV hepatitis, I expect to see a predominant population of T-cells (i.e. CD3 and CD5 positive small mature lymphocytes), with only occasional scattered B-cells (i.e. PAX5 positive small mature lymphocytes). The EBV in situ hybridization stain (EBER) will highlight a similar distribution of occasional positive cells as seen on the PAX5 stain. If a majority of cells, out or proportion to the number of PAX5 positive B-cells, are EBER positive, then EBV hepatitis is excluded. Note that NK-cells express cytoplasmic CD3 (i.e. CD3ε), so CD3 immunohistochemical staining of paraffin sections will label NK-cells (in addition to T-cells, which are more specifically identified by surface CD3 labeling in flow cytometric evaluation), and thus the EBER positive population in this scenario could be either T or NK (or NK/T) lineage and further evaluation is needed (NK-cell are CD5 negative, but so are some T-cell lymphomas, such as hepatosplenic T-cell lymphoma). Also, if a majority of cells are B-cell lineage and EBER positive, then an EBV driven B-cell neoplasm should be considered, in particular in the post-transplant setting.

Primary hepatic lymphoma

Primary hepatic lymphoma (PHL) is rare and data is usually presented in case report format. Our experience at UCSF, which we report as a series of 51 cases in abstract form at this meeting, is similar to other reports in which diffuse large B-cell lymphoma (DLBCL) is common (as well as post-transplant lymphoproliferative disorders (PTLD) in our series), usually occurring in older men, and usually as a solitary mass with longer survival than systemic lymphoma with liver involvement (SHL). A subset of PHL do not form a mass lesion and manifest with hepatomegaly with predominant sinusoidal involvement. Although some reports suggest an association with viral infection (e.g. HCV, HBV, or HIV), our data are similar to a recently published report that did not find a significant difference in viral infection between PHL and SHL (Peng Y, et al. Lymphoma of the liver: Clinicopathological features of 19 patients. Exp Mol Pathol. 2016 Apr;100(2):276-80), though there is a case report of response of a PHL (a "double hit lymphoma") to anti-viral therapy in the setting of HCV infection. Methotrexate (in the setting of rheumatoid arthritis) has also been implicated as a "reversible" cause of PHL. There is a report of primary biliary cholangitis (PBC) and PHL (MALT lymphoma). PBC patients may also develop large benign mass-like "nodular lymphoid lesions," (essentially a form of inflammatory pseudotumor; also noted in other settings, for example with HCV or HBV infection) that may require clonality testing to distinguish from primary hepatic MALT lymphoma. In our series we also noted that a majority of PTLD PHL were EBV positive and PTLD PHL was more common in the setting of liver allograft, than with other transplanted organs. PHL may mimic acute hepatitis and present with acute liver failure. On imaging, PHL can be mistaken for abscess or HCC, but plasma LDH is often elevated in the setting of lymphoma, including with PHL (though level may be lower than with SHL), and can raise clinical consideration for lymphoma, over another liver tumor, even when imaging is non-specific. Fever, night sweats, and/or weight loss may also suggest a lymphoma diagnosis, but are not always present in PHL. PHL has been reported as a collision tumor with hepatocellular carcinoma, further complicating imaging interpretation. Burkitt lymphoma represents a subset of PHL cases and has been reported as the initial finding that led to diagnosis of HIV infection in an adult. Primary follicular lymphoma and T-cell lymphomas have also been described as PHL.

Hepatosplenic T-cell lymphoma

Hepatosplenic T-cell lymphoma (HSTCL) is rare, <5% of peripheral T-cell lymphomas, and is more common in males (median age 35). HSTCL presents with non-specific systemic symptoms and hepatosplenomegaly, but no lymphadenopathy. Hemophagocytosis may be present and there may be an associated peliotic like change. Biopsy classically shows marked sinusoidal dilatation by medium size lymphoid cells (often you can find 3 or 4 lymphoma cells piling up in the sinusoids). Bone marrow is also involved (and may allow for more definitive diagnosis in some cases). A subset of HSTCL arise in the setting of chronic immune suppression or in patients taking azathioprine and infliximab for Crohns disease. Cells have a non-activated cytotoxic phenotype (i.e. TIA1+, granzyme B -). Most are derived from gamma/delta T cells. CD56 immunostain may be positive,

but EBER is most often negative (in contrast to aggressive NK-cell leukemia). On cytogenetic evaluation there is a recurrent cytogenetic abnormality (i7q), often with trisomy 8. HSTCL is an aggressive disease with early relapse and allogeneic bone marrow transplant may be considered.

Lymphoma specific comments/updates from the 2016 WHO revision relevant to hepatic lymphoma work up *(adapted from Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, Jaffe ES. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016 May19;127(20):2375-90).*

- **Hairy cell leukemia** – Specific *BRAF* V600E or *MAP2K1* mutations identified in most cases.
- **Lymphoplasmacytic lymphoma (LPL)** – *MYD88* L265P mutation identified in most cases; associated with IgM MGUS. Revised criteria indicate that LPL should consist of a monotonous lymphoplasmacytic proliferation (unless there is transformation), in which there may be complete architectural effacement or follicular colonization.
- **Mantle cell lymphoma** – Two variants recognized based on SOX11 staining. SOX11 positive classical variant (unmutated/minimally mutated IGHV that usually involves lymph nodes and extranodal sites, can become blastoid or pleomorphic, in which disease is even more aggressive) and SOX11 negative variant (mutated IGHV, usually presents as an indolent leukemic mantle cell lymphoma with peripheral blood, bone marrow, and splenic involvement in some cases, but without lymph node involvement; secondary mutations can lead to very aggressive disease). *CCND2* is rearranged in a significant proportion of cyclin D1 negative mantle cell lymphoma.
- **Diffuse large B-cell lymphoma (DLBCL), NOS** – Must distinguish between germinal center B-cell type and activated/non-germinal center B-cell type (use of immunohistochemistry to make this determination is acceptable, e.g. CD10, BCL6, and IRF4/MUM1 for the Hans algorithm), as this designation may affect therapy. MYC (>40%) and BCL-2 (>50%) co-expression on immunohistochemical stains ("double-expressor" lymphoma) is an adverse prognostic finding (though such tumors do not behave as aggressively as high grade B-cell lymphomas with rearrangements of *MYC* and *BCL2* and/or *BCL6*).
- **EBV+ DLBCL, NOS** – replaces EBV + DLBCL of the elderly, which is no longer considered age-specific.
- **Burkitt lymphoma (BL)** – Specific *TCF3* or *ID3* (negative regulator of *TCF3*) mutations are present in 70% of sporadic and endemic BL and in 40% of immunodeficiency related BL.
- **High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* translocations** – New category for all "double/triple-hit" lymphomas other than follicular lymphomas or lymphoblastic lymphomas and the morphology ranges from Burkitt-like (e.g. "starry sky" with tingible body macrophages), blastoid lymphoma similar in appearance to lymphoblastic lymphoma (but

TdT negative), and morphology similar to diffuse large B-cell lymphoma (may have prominent immunoblasts, usually germinal center B-cell type)

- **High grade B-cell lymphoma, NOS** – Together with the above category, this designation now encompasses the old category of "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma" and includes blastoid appearing large B-cell lymphomas (and cases intermediate between DLBCL and BL) which lack *MYC* and *BCL2* and/or *BCL6* rearrangement. There is no consensus as to which large B-cell lymphomas should have FISH testing for *MYC, BCL2*, and *BCL6*.
- **T-cell large granular lymphocyte leukemia** – *STAT3* and *STAT5B* mutations identified in most cases, with *STAT5B* mutation associated with more aggressive disease.
- **Systemic EBV+ T-cell lymphoma of childhood** – Name changed to "lymphoma" (from "lymphoproliferative disorder") due to its fulminant clinical course and to clearly distinguish from chronic active EBV infection.
- **Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL)** – It is now recognized that synchronous or metachronous NLPHL may present with morphology indistinguishable from T-cell histiocyte rich large B-cell lymphoma (THRLBCL), without a nodular component, and should be considered THRLBC-like transformation of NLPHL. Variant growth patterns (e.g. diffuse or with numerous T-cells) must be noted.

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