Lymphomas involving the spleen

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Abstract

Splenectomy is undertaken for diagnosis, and in later stages of management, of patients with a diverse range of lymphomas. High quality histological sections, requiring careful attention to tissue fixation, are needed to assess these lesions adequately. Increasing use of fine-needle aspiration, needle core biopsy and laparoscopic surgery add further diagnostic challenges. In addition to involvement by dissemination of lymphomas based primarily in lymph nodes, bone marrow or other tissues, spleen is the predominant site of disease in several distinctive types of lymphoma. In particular, splenic marginal zone B-cell lymphoma, hairy cell leukaemia and T-cell and macrophage-rich large B-cell lymphoma are recognized as clinicopathologically distinct entities. Research into the cellular and molecular origins of these lymphomas is ongoing; variants and new entities are becoming evident. Histological and immunohistochemical features of the spleen following treatment for lymphoma are complex and may cause diagnostic confusion. Inflammatory and reactive processes in the spleen can also provide clinical, radiological or pathological mimicry of lymphomatous involvement.

Keywords diffuse splenic red pulp B-cell lymphoma; hairy cell leukaemia; hairy cell leukaemia variant, hepatosplenic T-cell lymphoma; lymphoma; micronodular T-cell/histiocyte-rich large B-cell lymphoma; red pulp; spleen; splenic marginal zone B-cell lymphoma; white pulp

Introduction

The spleen may be involved by a wide variety of lymphomas. In some cases it is infiltrated as part of widespread dissemination of a node-based or other lymphoma but some lymphoma types predominantly involve splenic tissue. Even for lymphomas presenting with splenomegaly, and with no obvious disease elsewhere, exclusive splenic localization is extremely rare (if, indeed, it genuinely occurs). In most cases of "primary" splenic lymphoma, the bone marrow is also involved and there may be leukaemic spread of neoplastic cells in the absence of lymphadenopathy. Despite many years of research into the origins and characteristics of lymphomas favouring splenic involvement, the earliest type described preferentially involving this site, hairy cell leukaemia, remains mysterious. Splenic marginal zone B-cell lymphoma and its more recently described variants have been extensively investigated but their true relationship to normal B cells of the splenic marginal zone still remains controversial.

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General principles

Growth of lymphomas within the spleen

The cells of many lymphomas grow within the spleen by replacing or colonizing the compartments that their normal counterparts occupy. Most small B-cell lymphomas disseminate through the tissue in a miliary pattern, expanding and replacing normal white pulp B-cell follicles. The peri-arteriolar sheaths of the white pulp represent a T-cell compartment resembling nodal paracortex; they contain predominantly CD4-positive T-lymphocytes and are preferentially expanded by proliferations of small neoplastic CD4-positive T cells. The red pulp is a separate compartment, which contains mainly CD8-positive cells; lymphomas of CD8-positive T cells tend to infiltrate this compartment diffusely. Large B-cell lymphomas and Hodgkin lymphoma tend to cause single or multiple discrete tumour masses in the spleen, with the exception of micronodular T-cell/histiocyte-rich large B-cell lymphoma that shows miliary dissemination and mimics splenic marginal zone B-cell lymphoma. Lymphomas with a substantial or predominant leukaemic growth phase (such as chronic lymphocytic leukaemia, some examples of mantle cell lymphoma, B- and T-cell forms of prolymphocytic leukaemia and T- and natural killer cell variants of large granular lymphocytic leukaemia) show extensive, sometimes exclusive, diffuse red pulp infiltration, usually involving cords as well as sinusoidal lumens. A newly recognized entity, splenic diffuse red pulp B-cell lymphoma, also has an entirely diffuse pattern of infiltration.

Use of fine-needle aspiration (FNA) and radiologically guided needle biopsy for splenic diagnosis is increasing and these procedures are generally safe for localized solid tumour masses such as those formed by diffuse large B-cell or Hodgkin lymphomas. The needle biopsy approach is less successful for the small B-cell lymphomas and T-cell lymphomas that involve the spleen more diffusely although FNA immunophenotyping[1] can be valuable. With diffuse or miliary processes, the risk of haemorrhage is greater and the sample may be insufficient or unrepresentative. Needle biopsy is not advised in follow-up re-staging of residual tumour masses after treatment, since secondary inflammatory and reparative changes may obscure residual lymphoma or suggest misleading diagnoses.

Involvement of splenunculi by lymphoma generally mirrors the pattern of infiltration in the spleen itself; miliary and diffuse infiltration accompanying similar infiltrates in the spleen is typical. Involvement by those lymphomas which form focal tumour-like masses is rare.

Involvement of splenic hilar lymph nodes is usual in lymphomas whose pattern of spread in the spleen is miliary and/or diffuse. Histology may be typical of the same lymphoma occurring in lymph nodes elsewhere (e.g., small lymphocytic lymphoma and follicular lymphoma) or may have distinctive features in spleen-predominant lymphomas such as splenic marginal zone B-cell lymphoma.

Optimizing splenic histology for lymphoma diagnosis

Spleens, especially if substantially enlarged, tend to fix poorly due to poor formalin penetration of the blood-rich tissue. Ideally, splenectomy specimens should be delivered promptly, unfixed, to the pathology laboratory from the operating theatre so that they can be measured and described then sliced to aid fixation. In

the fresh state, depending on congestion and the extent of lymphomatous infiltration, splenic tissue can readily be sliced at centimetre or sub-centimetre intervals for further fixation; all or selected slices from the organ can then be fixed successfully. Slices should be rinsed free of blood gently but thoroughly (conveniently done in a large container with several changes of neutral-buffered formalin; NBF) before being immersed in clean 10% NBF for fixation. Re-slicing after 8–12-hours immersion ("Melba toast" technique) to a thickness suitable for embedding is necessary in most cases to optimize fixation while minimizing distortion from curling of the tissue.

When spleens are received in formalin, the same approach remains extremely valuable although it is almost impossible to avoid a 5–10 mm rim effect from initial fixation of subcapsular tissue. Without slicing, even in normally sized spleens, formation of a fixed rind of tissue in this way inhibits further penetration of formalin. Deeper tissue becomes autolyzed rather than fixed. To minimize the adverse effects of this, slicing should be undertaken as soon as possible after receipt of any spleen in the laboratory. Over-fixation (more than 72-hours exposure to formalin before processing) should also be avoided, since formalin pigment accumulates rapidly in splenic tissue. More detailed information can be found in the Royal College of Pathologists guidance on pathways for tissue handling (www.rcpath.org/resourceLibrary/tissue-pathways–lymph-spleen-bone-version-aug-10.html).

Laparoscopic splenectomy specimens require separate consideration. Highly morcellated fragments are of no histological value. Prior discussion with the surgeon is helpful, to ensure that at least a proportion of the tissue is removed in larger ($\geq$5 cm) pieces handled with surgical care to minimize distortion during removal. In the laboratory, it is advisable to separate these as soon as possible from the highly fragmented tissue and fix in fresh NBF. This will minimize inhibition of fixation by the abundant blood that is typically released into the original formalin.

Lymphomas primarily or predominantly involving the spleen

Hairy cell leukaemia

For many years the most cytologically and histologically distinctive splenic lymphoma, hairy cell leukaemia (HCL) was first described in 1972. It is rare, representing fewer than 2% of non-Hodgkin lymphoma and shows no trend of increasing frequency. It is still poorly understood; despite more than 25 years of research, its cell of origin remains a mystery. Expression of CD11c by the neoplastic cells, resulting from upregulation of *RAS* proto-oncogenes and expression of the transcription factor activator protein-1 (AP-1) is thought to underlie its unusual adhesion and migration properties.[2] Related to these mechanisms, underexpression of RhoH, a Ras inhibitor expressed exclusively by haemopoietic cells, has been demonstrated and has been investigated further as a potential therapeutic target.[3] There is currently great excitement following the consistent identification of a specific *BRAF* mutation (V600E; as found in malignant melanoma) in almost all cases of HCL, offering diagnostic and therapeutic opportunities. Moreover, the resultant overexpressed BRAF protein can conveniently be demonstrated by immunohistochemistry.[4]

The descriptive name of HCL reflects its characteristic cytological features in peripheral blood films, with the cells having multiple hair-like processes extending radially from their surface membranes. Splenic involvement is usually massive and patients present with symptoms related to the spleen's bulk. However, lymphoma growth is indolent and most patients probably have a long prodromal period before seeking medical attention. Rupture, even of a spleen only modestly enlarged by the disease, may occur following minor or unnoticed trauma, leading to emergency admission with acute abdominal symptoms and shock.

Splenectomy has been good treatment for HCL even though most patients have disseminated disease at diagnosis; the reduction in tumour burden can provide lengthy, if partial, remission. However, chemotherapeutic approaches are favoured currently and fewer splenectomies are performed, in line with general trends to avoid major surgery and preserve splenic function whenever possible.

Hairy cell leukaemia is unique in the extent of destruction that it causes to the underlying splenic architecture. A few, widely scattered white pulp nodules may remain (Figure 1), but typically the spleen appears homogeneously enlarged, both macroscopically and microscopically, completely lacking a military pattern. Multifocal haemorrhage reflects peliosis-like destruction of sinusoidal and capillary endothelium; the endothelium becomes replaced by infiltrating neoplastic lymphoid cells lining the resultant ectatic vascular spaces. Reticulin staining reveals the extensive destruction of cordal architecture.

Individual neoplastic cells have the same characteristics as are recognized in the bone marrow. They are of medium size with abundant pale cytoplasm, so that nuclei appear widely spaced. The nuclei are characteristically oval to bean-shaped (Figure 2). Infiltration is accompanied by relatively few reactive, inflammatory cells but among these mast cells are often prominent. The typical immunophenotype of neoplastic cells in HCL, demonstrable in fixed spleen sections by immunohistochemistry, is summarized in Table 1. Development of monoclonal antibodies to detect tartrate-resistant acid phosphatase has allowed previous enzyme histochemical methods to be abandoned. Features of the immunophenotype that may be unfamiliar outside larger haemato-oncology centres are:

- Expression of CD25 and CD123 in most cases
- Nuclear expression of cyclin D1 in more than 50% of cases (although generally weak/heterogeneous)
- Expression of CD10 in up to 20%

Hairy cell leukaemia variant

The existence of hairy cell leukaemia variant (HCL-v) as an entity within the WHO system is regarded as provisional, since reclassification is highly likely in the foreseeable future as molecular studies of such entities proceed.[5] Despite its name, this is not related to true HCL and probably encompasses at least two separate entities. One group of patients has a TRAP-negative lymphoma with morphology overlapping that of HCL and B-cell prolymphocytic leukaemia. Bone marrow is aspirable and the diagnosis rests on a combination of compatible cytological and immunophenotypic features, splenomegaly and, sometimes, cytopenias. Comparison of the major immunohistochemical features of this form of HCL-v in comparison with typical HCL