SPLEEN PATHOLOGY

Chairpersons: A. Dogan (USA), M. Piris (Spain)

SPLENIC HISTOLOGY AND IMMUNOLOGY

Ahmet Dogan
Mayo Clinic, USA

Spleen is one of the most complex organs of the lymphoreticular system with regards to both function and structure. This complexity is the main reason for the difficulties in histopathological interpretation of both physiological and pathological changes.

The spleen develops from progenitor cells within the dorsal mesogastrium adjacent to stomach and pancreas. The process is controlled by a number of transcription factors expressed by early spleen mesenchymal cells. These include Nkx2.3, Nkx3.3, Pbx1, Sox11, Tcf21, Tlx1 and WT1. Mouse studies have shown that absence of any of these factors lead to either complete splenic agenesis or marked hypolasia indicating a very complex regulation of splenic development. In humans spleen is detectable by 5th week of gestation and blood vessel appear around the 9th week. Function of the spleen in the fetus varies from the postnatal spleen. The spleen is the main source of hematopoiesis in the fetus where is this function reduces dramatically after birth.

In the postnatal period, the spleen plays a major role in the production of antibodies against blood borne antigens and polysaccharide antigens. Next to this immunological function the spleen is also involved in removal of effete cells from the bloodstream. This dual role is reflected in the complex architecture of the spleen. The immunological section is formed by the white pulp that follows the branching central artery while the red pulp takes part in removal of defective erythrocytes and foreign elements from the bloodstream.

The red pulp is formed by a specialized venous system evolved to filter blood. The splenic artery enters the spleen at the hilum and then branches into smaller arteries called central arterioles. Branches of the central arterioles either terminate in the marginal zone sinus or directly into the cords of the red pulp. The cords are composed of fibroblasts, numerous macrophages and reticular fibrils and form an open blood system without endothelial lining. From the cords the blood flows into the venous sinuses which join together eventually to form the splenic vein. The structure of the cords and sinuses makes passage of aging erythrocytes difficult. These erythrocyte stick in the cords and are phagocytosed by the macrophages. Splenic macrophages, in addition to their role in phagocytosis, also endocytose free serum hemoglobin bound to haptoglobulin through haemoglobin receptor, CD163. They play a critical role in innate immunity, in particularly through Toll-like receptors (TLR). Numerous cells of lymphoid lineage are also present in the red pulp cords and sinuses. These include plasma cells, T-cells of both alpha-beta and gamma-delta lineage and small numbers of B-cells.

The white pulp consists of two separate compartments; the T-cell dependent periarteriolar lymphocyte sheath (PALS), B-cell compartment composed of the follicle and the surrounding marginal zone. The lymphocytes are recruited into the white pulp through a multistep process that is regulated by chemokines. As described above the central arterioles carrying the lymphocytes open into the marginal zone sinuses surrounding the white pulp areas. The marginal zone sinuses have a complex arrangement and contain sinus lining cells expressing adhesion molecule MADCAM-1, an inner and an outer ring of macrophages, dendritic cells and marginal zone B-cells. Chemokine CXCL13 which is secreted by follicular dendritic cells attracts the B-cells into the follicle centers by engaging receptor CXCR5 on B-cells. In contrast, the T-cells accumulate around periarteriolar T-zone by interacting chemokines CCL19 and CCL21 which are mainly secreted by the stromal cells in this area.

The B-cell component of the white pulp is distributed into three distinct zones: The follicle center, the mantle zone and the marginal zone. The follicle center may be absent in resting spleens and, in those instances, the white pulp is typically composed of a central mantle zone (primary follicle) and surrounding marginal zone. The mantle zone B-cells are mature but 'naïve' indicating that they have not been stimulated by antigen. They co-express IgM and IgD and immunoglobulin genes do not show any evidence of somatic hypermutation. In the presence of antigen, the mantle zone B-cells expressing immunoglobulin receptors with high affinity for that antigen are recruited into the follicle center and form secondary follicles. Phenotypically these cells express CD10, Bcl-6 and may show immunoglobulin class switch, expressing IgG. This is a complex process where the B-cell goes through somatic hypermutation of the immunoglobulin genes to create high affinity immunoglobulin receptors for the antigen. B cells successfully acquiring these receptors are then positively selected and clonally expand. B-cells unsuccessful in this process are deleted by apoptosis. At the end of the follicle center reaction, the B-cells exit the follicle center and become either plasma cells and locate to the red pulp cords or join the memory B-cell pool and occupy the marginal zone and circulate into the peripheral blood. Thus marginal zone B-cells show evidence of somatic hypermutation and express high levels of IgM and low or absent IgD. However it is believed that at least a subset of the marginal zone B-cells develop through other mechanisms outside the follicle.

The neoplasms arising in the spleen often mimic the anatomical distribution of their normal counter parts. For example follicular lymphoma, mantle cell lymphoma and marginal zone lymphoma preferentially occupy the compartments they are thought to rise from in the white pulp. Where as lymphoplasmacytic lymphoma and hepatosplenic T-cell lymphoma or large granular lymphocyte leukemia preferentially involve the white pulp where their normal cell counterparts mostly reside.

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SPLENIC MARGINAL ZONE B CELL LYMPHOMA

Miguel Piris, Spain

Presence of a discernible marginal zone was initially noticed in the spleen. It is usually a well developed functional and topographical area consisting of medium-sized lymphoid elements with regular nuclei and a variable amount of clear cytoplasm. Marginal zone cells in the spleen are CD20, CD79a+, IgM+, CD21+, alkaline phosphatase+, but CD23-, Bcl6-, and IgD negative or weakly positive. Recently, IRTA1 has been selectively associated with marginal zone cells [1]. The size and immunophenotype of the splenic marginal zone may vary depending of the age and specific pathologic conditions[2]. By analysis of the mutated IgVH genes, marginal zone have been demonstrated to be mostly memory B-cells, with a minor component of naive elements, these latter probably being responsible for thymus –independent type 2 antigen response, such as bacterial capsular polysaccharide [3]. Splenic marginal Zone B cells have been demonstrated to play an important role in transporting circulating immune complexes into the splenic follicles and depositing them on the surface of follicular dendritic cells[4]. This functional assignation seems to be consistent with the regular distribution of MZ B-cells around lymphoid follicles. The term splenic marginal zone lymphoma (SMZL) was introduced into the literature by Schmid et al. in 1992, in a study performed on a series of 4 cases[5]. Subsequently larger series were published, reediting some diagnostic criteria and growing up the knowledge about their molecular and clinical characteristics[6-8]. Tumors with the same features were reported previously and denominated as splenic lymphoma with villous lymphocytes[9]. Splenic Marginal zone lymphoma is a relatively rare type of lymphoma, which accounts for around 1-2% all non-Hodgkin´s lymphomas. Diagnostic criteria were initially based on the histopathological features of the spleen. However, in many cases, the conjunction of the clinical features, morphological and immunophenotypic characteristic of the peripheral blood and bone marrow involvement, as well as genetic studies, allows a reliable diagnosis even in the absence of the pathological study of the spleen.

Pathology

*Morpholo*gy: The tumour in the spleen is characterized by a micronodular lymphoid infiltrate located in white pulp, with variable red pulp infiltration, marginal zone differentiation and follicular replacement by neoplastic cells. The white pulp tumoral nodules are composed by inner central zone of small lymphocytes, located in the mantle zone and replacing the germinal center, and a peripheral zone of medium-sized cells with clear cytoplasm and scattered blasts, the marginal zone component. In the red pulp, both large and small cells could be observed. Rare cases show an exclusive diffuse infiltration of the red pulp with white pulp atrophy[10]. Lymphoma cells may show a variable degree of plasmacytic differentiation, including as a characteristic feature the presence of monoclonal plasma cells in the germinal centre. Is still a matter of controvery whether SMZL with plasmacytic differentiation should be classified as lymphoplasmacytic lymphoma. The tumor includes numerous admixed T cells.

The bone marrow infiltration is characterized by intertrabecular polymorphic nodules and intersticial and intrasinusoidal infiltration of small lymphocytes[11]. The intertrabecular nodules mimic the architecture and cell composition of tumoral follicles in the spleen, being centered in reactive germinal center (highlighted by CD21 immunostaining). Unlike the splenic nodules, the marginal zone differentiation is rare in this localization. The interstitial and intrasinusoidal involvement is better revealed by CD20 staining. This infiltration pattern (nodular and intrasinusoidal) is highly suggestive of the diagnosis.

The morphology of the tumoral cells in the peripheral blood includes villous cells, small cell, plasmacytic cells, centrocytoid cells and cells with monocytoid appearance. Although the presence of villous cells is a frequent finding in SMZL (as recognised by the term of splenic lymphoma with villous cells), villous cells can been observed in other lymphoproliferative diseases, such as CLL, FL or MCL. In fact, some of the cases denominated in the past as splenic lymphoma with villous cells and carrying t11; 14 are now proposed to be denominated as mantle cell lymphoma[12].

Splenic hilar lymph node involvement shows a micronodular pattern, centered in pre-existing replaced follicles. As in the bone marrow, marginal differentiation is generally absent. Peripheral lymph nodes are rarely involved by SMZL, but when are present they show findings similar to splenic hilar lymph nodes with greater architectural effacement[13]. These morphological variations suggest several points:
1.- SMZL cells, depending of the background, are showing morphological and immunophenotypical changes that reflect the tumoural growth dependence of the microenvironment. Thus, it seems that marginal zone differentiation is mainly restricted to the spleen, while in other localizations, the lack of the appropriate architecture or signalling mechanisms do not induce marginal zone differentiation.
2.- The cell composition of the tumoral aggregates somehow reflects the now recognised capacity of marginal zone B-cells of inducing germinal centre development through the transport of immune complexes to the follicular dendritic cells[14].

Immunophenotype: The tumoral cells express surface IgM and IgD and are CD20+, CD43-, IgD+, bcl2+, CD5-, CD23-, cyclin D1-, bcl6-, CD10-, DBA44-/+, cyclin D1-. Immunostaining with bcl2 highlighted follicular replacement of bcl2- cells germinal center by bcl2 + tumoral cells. Proliferative index is low, and MIB1 staining shows a distinctive annular pattern, outlining the presence of an increased growth fraction in the germinal center and marginal zone[7].

Absence of CD10 and bcl6 staining are useful in excluding follicular lymphoma, and staining of cyclin D1 is helpful for excluding mantle cell lymphoma. CD5 expression has been reported is documented in a few proportion of cases, especially when the more sensitive flow cytometry is used for the diagnosis, and can cause diagnostic difficulties with CLL. In theses cases, absence of CD23 and CD43 facilitates the distinction in most instances. To distinguish SMZL with DBA44 expression from Hairy Cell Leukemia (HCL) ,

the immunostaining for Anexin1 is useful to confirm a diagnosis of HCL.

Differential diagnosis with lymphoplasmacytic lymphoma (LPL) is more controversial, since SMZL may show plasmacytic differentiation and serum monoclonal paraproteinemia. Currently, LPL is a diagnosis of exclusion, being marginal zone lymphoma the most frequently overlapping entity. The presence of a mixed pattern of white and red pulp involvement by periarteriolar aggregates of plasmacytoid cells, small lymphocytes and immunoblasts, with absence of marginal zone differentiation are all of them findings pointing to the diagnosis of LPL. The combination of CD22 and CD25 has been reported to be useful to distinguish these entities by flow cytometry [15].

Cytogenetic and molecular findings

Cytogenetic and molecular studies have demonstrated that around 45% of SMZL cases have allelic loss at the chromosomal region 7q22-36. This alteration is found with higher frequency in SMZL than other small B cell lymphomas. The most frequently deleted microsatellite was D7S487 (45% of informative cases). Surrounding the D7S487 microsatellite, a smallest common deleted region of 5cM has been identified, defined between D7S685 and D7S514. Cases with 7q loss have been shown to show a more aggressive behaviour, with more frequent tumoral progression[16].

Other cytogenetic alterations include +3, +5, +9q, +12q, +18, +20q, t(10;14) (q24;q32), t(6;14)(p12;q32), and t(2;7)(p12;q22), with deregulation of cyclin D3 and CDK6 respectively[17-19]. Translocations involving CyclinD3 and CDK6, although biologically informative and challenging, have a low incidence [18,19].

The most frequent chromosomal numerical imbalances revealed by comparative genomic hybridization are gains of 3q, 5q, 12q, 20q, 9q and 4q, and loss of 7q, 6q, 14q and 17p[20,21]. Gene amplifications involving 3q26-q29, 5p11-p15 and 17q22-q25 regions are rare. A shorter overall survival has been shown for cases with higher losses of chromosomes, as identified by CGH[20].

Consistently with the findings in other small B-cell lymphomas, p53 abnormalities are infrequent in this disease (3-17% cases), but cases with p53 inactivation seem to run a more aggressive course. P16 alterations have not been found. Unlike MALT lymphomas, microsatellite instability is exceptional in this condition[22].

Somatic mutations of IgVH genes have been observed in about half of the cases and, in the cases treated with splenectomy, associated with longer overall survival[23]. This finding, similar to the observed in CLL, establish a striking parallel between these two conditions. Immunoglobulin genes studies have additionally revealed that roughly half of the SMZL cases use a selective use of the V(H)1-2 segment, suggesting that this tumour derives from a highly selected B cell population[22,23], this suggesting that either foreign or preserved autoantigens could play a role in the genesis of the disease. In addition to IgVH, a smaller proportion (13%) of SMZL cases display somatic mutations in the 5´non-coding region of the bcl6 gene[24].

Gene profiling studies, additionally to confirming the relative homogeneity of this entity, is indicating potential diagnostic markers and pathogenic pathways involved in the survival of the tumoral cell. Thus, the signature includes upregulated genes involved in apoptosis regulation, BCR and TNF signalling, and NF-κB activation, such as SYK, BTK, BIRC3, TRAF3, TRAF5, CD40 and LTB. Additionally, genes associated with the splenic microenvironment, like SELL and LPXN, were also overexpressed. Other genes of particular interest are lymphoma oncogenes such as ARHH and TCL1[25]. The increased expression of TCL1 is linked with the upregulation of genes associated with intracelular signalling via the AKT1 patway found in SMZL, as described by Thieblemont et al. [26]. Up-regulation of the AP-1 and Notch 2 transcription factors have been also described by Troen et al [27]. Consistently with the previous cytogenetic studies, genes located in the 7q31-7q32 region, such as CAV1, CAV2 and GNG11[25] are downregulated.

Pathogenesis

The cell origin for SMZL is still a controversial issue. Although the term seems to indicate a close relationship with marginal zone B cells, the absence of IgVH somatic mutations in half of the cases, the absence of marginal differentiation when the tumor is located outside the spleen and the expression of IgD by the tumoral cells puts into question the marginal zone cell origin for this tumor, and suggests the possibility that SMZL tumoral cells could represent the tumoral expansion of hitherto uncharacterised marginal zone precursor splenic B-cell subpopulation. This is supported also by the findings of Troen et al, where Notch 2, a transcription factor that induces marginal zone B cell differentiation is overexpressed in SMZL[27].

Molecular studies of SMZL reveal selection for precise VH1 regions, thereby suggesting a hypothetical role for antigens in the promotion of the growth of the tumoral cells. A relationship between HCV infection and SLVL has recently been established[28], witch lends support to the hypothesis that the stimulation of marginal zone B-cells in the spleen by persistent HCV antigens, particularly the E2 viral antigen, might be involved in the pathogenesis of SMZL. Thus, positivity for hepatitis C infection was reported positive in a proportion of SMZL patients oscillating between 1 to 16% to [29]. Significantly, a higher proportion of HCV positivity (up to 50%) has been found in splenic DLBCL[29]

The role of infectious agents in the promotion of SMZL pathogenesis is also supported by the findings establishing a link between SMZL and hyperreactive malarial splenomegaly[30,31]

Clinical findings

SMZL is a symptomatic disease, usually diagnosed in patients older than 60 years. The most relevant feature at clinical presentation is a splenomegaly of variable size, and the majority of the symptoms and signs of the disease are derived from the splenomegaly, such as anaemia and thrombocytopenia. In cases presenting sympthoms, abdominal discomfort or pain due to the enlarged spleen are the most frequent complaints[32,33].

The most of the patients are diagnosed in clinical stage IV, with bone marrow involvement being the rule. Hemolytic autoimmune anemia in found in 10-15% of patients, and can be the first clinical manifestation of the disease. Rarely patients may develop immune thrombocytopenia and other autoimmune phenomena such as auto-antibodies against coagulation factor, lupus anticoagulant or anticardiolipin antibodies. Peripheral blood involvement was detected in different series in a frequency of 64%- 84%, depending of the criteria for selection of patients. The term Splenic Lymphoma with villous lymphocytes (SLVL) has been applied in the past to describe those cases in which peripheral blood involvement by the tumour cells was a striking finding. A monoclonal component is detected in a small number of patients Peripheral lymphadenopathy is infrequent (15-25 % of cases), but splenic hilar lymph nodes are more frequently involved (35%-65% of cases).

Splenic MZL is a low grade lymphoma with an indolent clinical course, and a overall median survival between 8-10 years. A comparative analysis of the survival of the series of cases so far described reveal some differences between those denominated as SLVL and those called SMZL, probably related with the fact that the diagnosis of SMZL is usually preceded by splenectomy, which implies a more aggressive behaviour of the tumor, requiring earlier terapy[32]. After therapy, the most of the patient have persistent disease with bone marrow or peripheral blood involvement, in spite of splenectomy or even chemotherapy. A small proportion of cases, most of them carrying p53 alterations, develop since the debut of the disease an aggressive behaviour.

Similarly to other indolent B cell neoplasm, transformation into high-grade lymphoma may occur (13%), with a frequency that is lower than in follicular lymphoma(25-60%) and mantle cell lymphoma (11-38%), and similar to that observed in CLL (10%). SMZL progression seems to be mainly independent of p53 or p16 inactivation, and to be characterized by a higher growth fraction and more frequent 7q deletion[34].

Treatment

SMZL is an indolent lymphoma, and the therapeutic approach “watch and wait” is recommended in asymptomatic patients with favourable prognostic factors. Data available from reported series have demonstrated that patients not requiring treatment at diagnosis have a favourable clinical course[32,33,35]. In the presence of cytopenias and /or huge symptomatic splenomegaly, splenectomy is the treatment of choice, and usually leads to a favorable response, correcting cytopenias. Radiation therapy is an optional approach in the treatment if surgery is contraindicated.

Chemotherapy is choice for patients with clinical progression after splenectomy, or when surgery is contraindicated for patients requiring therapy. Alkylating agents (chlorambucil or cyclosphosphamide) or purine analogues (fludarabine) have been reported as effective treatment.

As other B-cell lymphomas, Rituximab may be employed, in association with conventional therapy [36,37].

A potentially interesting data is the successful therapy of HCV-positive SMZL cases treated with α interferon and rivabirin, in analogy with Helicobacter pylori eradication in gastric lymphoma of MALT type[28,38].

Prognostic factors

The few studies performed on relatively large series show that adverse clinical prognostic factors are related to high tumoral mass or poor general status. The presence of an immunological event or of an M component was associated with a significantly higher risk of disease progression. Shorter survival was associated with high concentration of β2 microglobulin, high counts of blood leukocytes, and lymphocytes[35]. The lack of involvement of non-haematopoietic sites and complete response to treatment were associated with longer survival and failure-free survival[32].

A number of biological variables have been associated with poor outcome. These include p53 alterations, 7q deletion and the absence of somatic mutation in IgVH genes. Recently, survival analysis using microarray data highlighted new variables associated with survival. Shorter survival was associated with CD38 expression, naïve IgV_H genes and the expression of a set of NF-κB pathway genes, including TRAF5, REL and PKC-alpha[25].

Perspectives

The diagnosis of SMZL is often hindered by the lack of specific molecular markers. Unfortunately, the considered hallmarks of SMZL, i.e.: micronodular pattern and marginal zone differentiation in the spleen, villous cells in the peripheral blood and intrasinusoidal infiltration in the bone marrow may be present in others small B cell lymphomas such as follicular lymphoma, mantle cell, or LLC-B. The occasional presence of plasmacytic differentiation makes the distinction with LPL especially difficult. In order to define better the borders of the entity and to facilitate the routine diagnosis, the identification of routinely available diagnosis markers is still in need of further investigation and validation. This applies singularly to the newly described markers such as CD40, ILF1, Senataxin, Selectin L, Leupaxin, ARHH and TCL1 [25].

Clinical variability of SMZL poses an additional challenge. Thus, like CLL or FL, patients diagnosed of SMZL often exhibit a striking variability. There is a group of indolent patients not requiring splenectomy during prolonged follow-up, and in contrast the disease in a subset of patients is pursuing an aggressive course. Additionally to the already described prognostic markers, new clinically applicable predictors markers need to be validated into the context of clinical trials that could identify risk-stratified therapeutic protocols.

Fortunately, the molecular studies of SMZL are starting to reveal aspects of its pathogenesis that could eventually lead to the proposal and identification of new drugs targeted for SMZL key genes and pathways, such as spleen microenvironment, BCR signalling and NF-kB activation. This study would be enormously facilitated by the development of appropriate experimental models or the immortalization of tumoral cells.

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SPLENIC B CELL LYMPHOMAS – OTHER THAN SPLENIC MARGINAL ZONE LYMPHOMA

Manuela Mollejo, Spain

Splenic Small B Cell Lymphoma

The spleen is frequently involved in lymphoproliferative disorders, especially by small B cell lymphoma; however, the initial diagnosis of lymphoma is rarely performed in splenectomy specimens. Splenic marginal zone lymphoma is the most common diagnosis stablished in the splenectomy specimens. The morphological appearance of SMZL can be closely mimicked by a variety of primary nodal low-grade B cell lymphomas when they involve the spleen. The differential diagnosis of SMZL with other small B-cell lymphomas require the integration of clinical, morphological, immunophenotypic and genetic data. Immunohistochemical studies are extremely useful in the differential diagnosis of small B cell lymphomas in the spleen.

Follicular lymphoma (FL): Macroscopically, unlike SMZL, tumoral nodules are usually of variable size. The tumoral follicles accumulate principally in the white pulp and the cytological features of theses nodules show that they are composed entirely of a mixture of centrocytes and centroblasts. Frequently, follicular lymphoma in the spleen shows a peripheral zone without an intervening mantle, in which the tumoral cells contain more abundant cytoplasm, resembling marginal zone cells. In some cases, there is true marginal zone differentiation; usually with a preserved mantle. Expression of CD10 and bcl6 are useful for follicular lymphoma diagnosis. Interestingly, there are some splenic follicular lymphomas with absence of bcl2 expression, the differential diagnosis being especially difficult with SMZL. Morphological features of tumoral cells, MIB1 staining pattern, residual mantle cell, IgD staining of tumoral cells, in addition to bone marrow and/or lymph nodes histological findings help to establish the diagnosis.

Mantle cell lymphoma (MCL): Splenic involvement by MCL shows white pulp nodules and a variable intensity of diffuse red pulp infiltration. The distribution of the tumoral cells around reactive germinal centres and marginal zone differentiation may also be found in MCL. However, the morphology is monomorphous, and nucleolated large cells are only rarely seen in MCL, while are commonly seen in SMZL. The differential diagnosis between mantle cell lymphoma and SMZL is easily achieved by cyclin D1 expression by tumoral cells in MCL.

Chronic lymphocytic leukaemia (CLL): Splenic involvement in CLL is characterised by an extensive and diffuse red-pulp infiltration with effacement of follicles. Cytological composition of these cases shows a predominance of small lymphocytes, with scattered prolymphocytes and paraimmunoblasts. However, in some cases, splenic involvement shows micronodular infiltrate with marginal zone differentiation. The immunophenotype CD23+, CD43+, CD5+, IgD+ and low proliferation fraction (without annular pattern of staining) are in favour of CLL diagnosis.

Lymphoplasmacytic lymphoma (LPL): Differential diagnosis with lymphoplasmacytic lymphoma can be difficult since SMZL may show plasmacytic differentiation with serum monoclonal paraproteinemia. Currently, LPL is a diagnosis of exclusion, marginal zone lymphoma being the most frequently overlapping entity. The presence of a mixed pattern of white and red pulp involvement by periarteriolar aggregates of plasmacytoid cells, small lymphocytes, plasma cells and a variable number of immunoblasts, with absence of marginal zone differentiation are all findings pointing towards a diagnosis of LPL. Although the presence of t(9;14)(p13;q32) and other PAX5 translocations has been reported in LPL, these molecular findings are uncommon and unspecific events in low-grade B-cell lymphomas with plasmacytic differentiation. Isolated del-6q is in favor of LPL/Waldenstrom.

MALT lymphoma: Occasionally, the differential diagnosis with MALT-type marginal zone lymphoma is necessary due to the existence of cases of MALT lymphomas infiltrating the spleen with a micronodular pattern. In theses cases, the spleen shows widening of marginal zone external to a preserved mantle, with small cluster of similar cells in the red pulp. Two useful features are the absence of t(11;18) in SMZL cases , and the frequent IgD expression in SMZL that is only rarely observed in MALT lymphomas.

Large B Cell Lymphoma

Three morphologic patterns of splenic involvement by large B cell lymphoma have been identified: macronodular, micronodular and diffuse.

Macronodular: The splenic parenchyma is partially replaced by macroscopically discernable large, solitary or multiple large nodules. Tumoral nodules are composed of homogeneous compact masses of large lymphocytes, totally replacing splenic architecture in the involved areas. The residual splenic tissue usually shows a preserved architecture unaffected by the tumor. Most cases are bcl6 positive. Clinically are characterized by predominantly stage I disease and favorable clinical outcome.

Micronodular: In some cases the spleen shows a relatively uniform miliary pattern, with focal coalescence of splenic white pulp micronodules. The tumoral nodules are centered in the white pulp with variable infiltration of the red pulp. Nodules are composed predominantly of large B-cells, mixed with a few small T lymphocytes. The tumoral cells are bcl6 positive.

In other cases, the nodules contained scattered large B-cells in a background of numerous T-cells and histiocytes, with a pattern overlapping that described in the lymph nodes involved by TCRBCL. In this type, the tumoral cells are usually EMA positive.

Most of the patients in this group are diagnosed at advanced clinical stages and died of the disease.

Diffuse red pulp infiltration. In this pattern, the spleen shows a firm, homogeneous beefy red appearance at the cut surface, discrete tumoral lesions being absent. The spleen display diffuse distribution of the tumoral infiltrate, with infiltration of the red pulp

cords and permeation of the sinusoids, and it was possible to identify scattered residual white pulp islands. Tumoral cells have centroblast, polylobated centroblast or pleomorphic cytology. Frequently theses cases are IgD+.

This group is characterized by advanced clinical stages and with an aggressive behavior.

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SPLENIC INVOLVEMENT BY T-CELL LYMPHOPROLIFERATIVE DISORDERS

[1]Philippe Gaulard, [2]William G. Morice
[1]Département de Pathologie & Inserm U841, Hôpital Henri Mondor, Créteil, France
[2]Department of Pathology & Laboratory Medicine, Mayo Clinic, Rochester, Minnesota, USA

Introduction

The spleen can be involved by a number of T-cell lymphoproliferative disorders (T-LPDs). In some T-LPDs splenic involvement is a characteristic feature and its recognition can aid disease diagnosis; in others splenic involvement can be a secondary finding which varies from case to case. For these reasons, a basic understanding of the T-LPDs which can exhibit splenic involvement can aid greatly in interpretation of the significance of observed pathologic changes.

Hepatosplenic T-cell lymphoma

Hepatosplenic T-cell lymphoma (HSTL) is rare, with approximately 100 cases reported(1, 2, 3). Its incidence, however, might be underestimated as the clinical features are not typical for lymphoma. HSTCL occurs mainly in young adults with a male predominance (median age around 35 years)(1, 2, 3). A number of cases have been reported in solid organ transplant patients and in this context, HSTL is regarded as a late-onset post-transplant lymphoproliferative disorder of host origin(3, 4). HSTCL has also been reported in children and cases with an □□ TCR phenotype may be more often encountered in older adults. Recently, several cases of HSTL have been reported in adolescents and young adults receving infliximab along with purine analogs for Crohn's disease (Rosh JR et al... review in Inflamm Bowel Dis) Patients typically present with systemic symptoms associated with marked hepatosplenomegaly without lymphadenopathy(1-3, 5). Thrombocytopenia is common, it is associated with anemia and/or leukopenia in about half of the patients. Peripheral blood involvement at presentation is usually absent or minimal, however this may become more prominent with disease progression(3, 6). HSTCL is highly aggressive with a median survival of 16 months despite high-dose chemotherapy with or without bone marrow stem cell transplantation. Rare cases with prolonged disease remission have been described(3). The neoplastic cells of HSTCL are usually monomorphic small to medium-sized cells with a round/oval or slightly irregular nucleus with inconspicuous nucleoli. They frequently have abundant, pale-staining, variably granulated cytoplasm(7). At splenectomy, the *spleen* is typically massively enlarged - commonly weighting 1000 to 3500g – due to diffuse red pulp expansion without identifiable gross lesions, and without hilar lymph node enlargement. Histopathology shows diffuse red pulp invasion by neoplastic cells with small to intermediate sized nuclei, round or slightly irregular nuclear contours, and small nucleoli. The massive red pulp infiltration by these cells leads to a commensurate marked attenuation of the white pulp. The neoplastic cells are present within the red pulp cords and, to variable degrees, within the sinuses which may be dilated. An associated increase in red pulp histiocytes may also be present and in some cases hemophagocytosis may be seen. The splenic hilar lymph nodes, although not significantly enlarged, often show involvement confined to sinuses or perisinusal areas(8). *Cytological variants of HSTCL*, (ie large cells or cells with a blastic appearance), have been occasionally observed at diagnosis but frequently occurs with progression during the course of the disease. Sinusoidal involvement of the *liver* by HSTCL is also uniformly present. In some instances tumoral involvement can cause the formation of pseudopeliotic lesions (9). Additional mild portal and periportal lymphomatous infiltrates may be observed, but are not predominant.

As with the liver, involvement of the *bone marrow* is a universal feature of HSTCL although histologic clues to the diagnosis can lacking. This is due both to the characteristic sinusoidal pattern of marrow infiltration by HSTCL and an associated panmyeloid hyperplasia which is frequently present and results in marrow hypercellularity. In fact, the combination of marrow hypercellularity and splenic enlargement can lead to initial misinterpretation of the bone marrow findings as indicative of a myeloproliferative disease. Sinusoidal marrow infiltration by the HSTCL cells which typically have small to medium-sized, variably irregular nuclei is strongly highlighted by immunoperoxidase staining using antibodies to CD3 and TIA-1(5,6). With disease progression marrow involvement tends to become more pronounced with both interstitial and sinusoidal infiltrates present.

An unusual feature of HSTCL is the proclivity of the cells to express $\gamma\delta$ T cell receptor, leading the disorder to be called hepatosplenic $\gamma\delta$ T cell lymphoma when it was first described. Since these initial descriptions bone fide HSTCL cases expressing $\alpha\beta$ T cell receptor have been recognized, and as a result HSTCL is now the preferred moniker for this disease in the WHO classification scheme. Regardless of TCR type the phenotype of HSTCL is relatively uniform; these cells typically express CD3, CD2 and often CD7, but usually lack CD5. They may be either CD4-/CD8- or, less often, CD4-/CD8+. Most cases are CD56+, but CD57 is negative. They may express CD16. All cases have a non activated cytotoxic phenotype, ie TIA-1+, without expression of granzyme B and perforin (5), with the exception of rare cases with blastic cytologic features. HSTL is negative for CD25, CD30, and other activation-associated antigens. Expression of killer immunoglobulin-like receptors (KIRs) and of CD94/NKG2A has been recently reported in several cases(10, 11). On frozen sections or flow cytometry, the majority of cases express the $\gamma\delta$ T cell receptor, as discussed above. It is noteworthy that determination of the $\alpha\beta$ or $\gamma\delta$ T-cell origin may be difficult to establish in a number of cases due to the absence of reliable marker for □□ T cells in routinely-fixed specimen. Irrespective of their $\gamma\delta$ or $\alpha\beta$ phenotype, HSTL show a clonal rearrangement of the TCR γ gene, as demonstrated by polymerase chain reaction (PCR) studies used in routine practice.

Isochromosome 7q [i(7)(q10)] is the most commonly described cytogenetic abnormality in HSTCL, occurring in the majority of cases(12). i7q may appeared as the sole karyotypic abnormality, however it is frequently associated with other abnormalities such as trisomy 8 and/or loss of chromosome Y. i(7)(q10) has also been

found in hepatosplenic cases with αβ-phenotype(13), thus further supporting that both γδ and αβ cases may represent variants of the same entity. The biological significance of i(7)(q10) is not established. Despite a strict correlation between i(7)(q10) and HSTL, this aberration is not associated exclusively with this type of lymphoma, since isochromosome 7q can be seen in other malignant disorders, such as acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome and Wilms tumor. To date, association with viral pathogens (HTLV-1 and -2, HIV, HHV-8, HCV) has been reported in HSTCL. One case has been reported in a patient positive for HHV-6. The vast majority of cases do not show EBV association as well, with the exception of rare cases with cytological features of transformation, suggesting that EBV might be regarded as a secondary event.

T-Cell Large Granular Lymphocytic Leukemia (T-LGL)

T-cell large granular lymphocytic leukemia (T-LGL) is a relatively rare entity, although, like HSTCL, this disease has protean clinical manifestations which make it difficult to recognize. The hallmark features of T-LGL are the presence of a distinct, clonal population of cytotoxic T-cells in the peripheral blood accompanied by unexplained anemia and/or neutropenia. This disorder typically occurs in adults (median age 55 years) and an association with rheumatoid arthritis and other autoimmune phenomenon has long been recognized (14). The lymphocytosis in T-LGL is usually modest (median lymphocyte count 7800 cells/uL). Many T-LGL patients are asymptomatic and and the diagnosis is often first considered due to unexplained, persistent neutropenia and/or anemia. Clinical signs and symptoms which may lead to the diagnosis include recurrent bacterial infections and/or fatigability which are secondary to the disease-associated neutropenia and anemia, respectively (15).

The cytologic features of T-LGL are those of cytoxic lymphocytes and are typified by the presence of cells with small, bland nuclei and usually abundant cytoplasm containing variable numbers of azurophilic granules. Pronounced cytologic atypia in T-LGL is rare, and when encountered should lead one to consider the possibility of a different, more aggressive lymphoproliferative disorder of cytotoxic lymphocytes such as aggressive NK-cell lymphoma/leukemia and HSTCL. It is also noteworthy that the prominence of the cytoplasmic granularity varies between cases and the cytoplasm of these cells is not always voluminous. For these reasons, performing absolute granular lymphocyte counts is problematic and this no longer emphasized as an important element of the diagnostic criteria. Given the lack of distinguishing cytologic features in T-LGL, the identification of an immunophenotypically distinct, clonal cytotoxic T-cell population is a fundamental element in establishing the diagnosis. Splenic involvement by T-LGL is frequent, occurring in more than 50% of cases (16, 17). The splenomegaly resulting from T-LGL involvement is usually modest, especially when compared to other T-LPDs such as HSTCL. T-LGL infiltrates the splenic red pulp, similar to HSTCL, and T-LGL may show a slight predilection for involving the splenic cords. Despite the infiltration of the red pulp by T-LGL cells the splenic architecture is typically intact with sparing of the white pulp and capsule. In fact, the white pulp may also be expanded due to an associated follicular hyperplasia. Cytologically the T-LGL cells are quite bland with small, minimally irregular nuclear contours and variable amounts of pale staining cytoplasm. There have been isolated case reports in the literature of transformation of T-LGL to a more aggressive disease. However the precise relationship of such cases to the universally recognized T-LGL disorder is unclear and if there is pronounced cytologic atypia or effacement of the splenic architecture in a red pulp cytotoxic lymphocyte infiltrate other disease entities such as HSTCL should be strongly considered.

T-LGL is a disorder of memory cytotoxic T-cells which are typically CD8-positive. As such these cells will be positive for cytotoxic granule proteins such as TIA-1, perforin and granzyme B. The frequent positivity of T-LGL for CD8 and granzyme B can help distinguish this entity from HSTCL, which is usually negative for these antigens (17). T-LGL frequently shows abnormalities of T-cell antigen expression with diminished or lost CD5 expression detected in approximately 90% of by flow cytometry (18, 19). Diminished staining for T-LGL cells in the splenic red pulp may also be seen by immunohistochemistry, however one must exercise caution in interpreting the significance of this finding as diminished CD5 expression may be seen in other cytotoxic T-cell disorders including HSTCL.

Bone marrow involvement by T-LGL is common, likely occurring in over 80% of cases (20, 21). When T-LGL involves the marrow it typically infiltrates in a sinusoidal and/or subtle interstitial pattern. These patterns of infiltration, in conjunction with the bland cytologic features can make this disease extraordinarily difficult to detect by routine histologic examination. Indeed, the non-neoplastic interstitial lymphoid aggregates containing admixed T- & B-cells are frequently present in T-LGL may be the most obvious histologic clue to the diagnosis. Immunohistochemistry using antibodies to CD8, TIA-1, and granzyme B can be extraordinarily helpful in revealing the intrasinusoidal T-LGL infiltrates and thereby establishing the diagnosis (20, 21). Intrasinusoidal T-LGL infiltrates may also be seen in the liver. Involvement of the lymph nodes and other tissues by T-LGL appears to be exceedingly uncommon. Aberrant expression of NK-cell associated antigens is a pathognomic feature of T-LGL which is best demonstrated by flow cytometry. Early reports emphasized the importance of CD57 co-expression in the diagnosis of T-LGL. While frequent in T-LGL, this finding lacks disease specificity as CD57 is also expressed by normal memory cytotoxic T-cells (18). Furthermore, immunohistochemistry appears to have much less sensitivity for CD57 detection as compared to flow cytometry further limiting the utility of CD57 immunohistochemical assessment in T-LGL diagnosis (17,20). CD16, an antigen normally found on NK-cells, is expressed in over 80% of T-LGL. Unlike CD57, CD16 is rarely expressed by normal T-cells, and the detection of a CD16-positive T-cell population comprising over 30% of the total T-cells present by flow cytometry should lead one to consider T-LGL (18). Unfortunately, this antigen is difficult to detect in paraffin embedded tissues. CD56 is expressed in only a minority of T-LGL cases (18).

A group of receptors expressed by NK-cells and a subset of cytoxic T-cells which recognize MHC I and related antigens on potential target cells have recently been described (22). These receptors mentioned above in the discussion of HSTCL, are termed the NK-cell associated receptors or NKRs and are broadly divided into two types, the lectin-family receptors which include the CD94/NKG2 heterodimeric complexes, and the killer cell immunoglobulin-like receptors (KIRs). Of particular interest in T-LGL are the KIR antigens. There are multiple different KIRs, each of which recognize specific MHC I ligands. Whereas normal cytotoxic lymphocytes show a polymorphic pattern of KIR expression, in T-LGL restricted expression of a single KIR antigen is seen in approximately 2/3^{rds} of cases (18,19). This restricted pattern of KIR expression in T-LGL both aids in characterization of the abnormal cell population and provides indirect evidence of clonality. CD94 expression is also seen in T-LGL, although the frequency of CD94 positivity varies between reports. In comparison to the KIRs, the expression of CD94 is of somewhat limited utility in diagnosing T-LGL as this antigen is more commonly expressed by normal T-cells and it does not provide a surrogate marker of clonality. Unfortunately, antibody reagents to these antigens which allow for the evaluation of these antigens in paraffin embedded tissues are not currently available.

Cytotogenic abnormalities in bona fide T-LGL cases appear to be extraordinary rare. As discussed above however, detection of T-cell clonality by molecular genetic analysis of T-cell antigen receptor gene rearrangements is a uniform disease feature. Evidence from a number of sources strongly suggest that T-LGL leukemia represents an antigen-driven proliferation of cytotoxic cells with dysregulated apoptosis leading to both the accumulation of these abnormal clonal T-cells and the associated auto-immune diseases. Indeed, although the leukemic LGLs constitutively express FAS (CD95) and its ligand which is found at high levels in the sera of T-LGL patients, they commonly show FAS mutations which results in a defective Fas-mediated apoptotic pathway.

T-LGL is difficult to diagnose because of both the great degree of phenotypic overlap between T-LGL and reactive CD8-positive T-cells and the fact that many causes of reactive CD8-positive T-cell expansions, such as viral infections and myelodysplastic syndromes, can be associated with cytopenias. Furthermore, T-cell clonality may not serve as a “gold standard” for making this distinction, as these reactive processes which can lead to splenic enlargement (such as viral infection) may also yield apparently clonal TCR gene rearrangement results due to the limited clonal diversity in the reactive cell population. For these reason correlation of the splenic pathology with flow cytometric and molecular genetic results and other clinical and laboratory findings is critical in distinguishing T-LGL from reactive cytotoxic T-cell expansions. Furthermore, although T-LGL and HSTCL are clinically readily distinguished due the indolent nature of the former and the aggressive behavior of the latter these disease can exhibit considerably overlapping pathologic features. For these reasons if clinicopathologic correlations are not assiduously performed when evaluating splenic red pulp cytotoxic lymphocyte infiltrates, one is at risk of rendering an incorrect diagnosis. (Recently reviewed by Loughran in The Oncologist 2006;11:263-73)

Aggressive NK cell lymphoma/leukemia

Although the focus of this discussion is on T-cell lineage disorders aggressive NK cell lymphoma/leukemia is a major consideration in the differential diagnosis of splenic malignancies of cytotoxic lymphocytes such as HSTCL and T-LGL and therefore is briefly addressed. Aggressive NK cell lymphoma/leukemia is an extraordinarily rare malignancy most often encountered in Asian populations, like HSTCL it commonly present as hepatosplenic disease with B symptoms and is aggressive with poor clinical outcome. Despite these attributes common to the two entities there are clinicopathologic features which help distinguish aggressive NK-cell leukemia from HSTCL including the presence of a mild leukemic picture at presentation, occasional involvement of extranodal sites such as skin and digestive tract, and a frequently associated hemophagocytic syndrome (including biological manifestations with cytopenia, increased ferritinemia, and/or hypertriglyceridemia). Histologically aggressive NK-cell leukemia is characterized by relatively minimal peripheral blood and bone marrow involvement by monotonous, cytologically atypical large cells which frequently containing azurophilic granules. The neoplastic cells have attributes consistent with NK-cell lineage, they lack T-cell receptor gene rearrangement TCR expression or a fully assembled CD3 complex. They do however express the CD3 epsilon subunit as well as CD56; they lack CD57and have an activated cytotoxic (Granzyme B+/perforin+) profile. Finally, EBV association is nearly constant and the disease has been proposed as the leukemic variant of nasal-type NK/T cell lymphoma. Aggressive NK-cell lymphoma/leukemia can show overlapping morphologic features with HSTCL with medium to large, atypical tumor cells infiltrating the splenic red pulp and the sinusoids of the liver. In the spleen, however, tumor cell density can be low and blood vessels walls are commonly infiltrated. Another distinguishing morphological feature of aggressive NK-cell leukemia is the pattern of bone marrow infiltration which is diffuse and interstitial - often minimal - without a dectable sinusoidal predilection. This pattern of marrow infiltration, as well as the frankly neoplastic cytology and acute clinical presentation also distinguish this entity from LGL disorders of either T- or NK-cell lineage. Bone marrow examination is often the initial diagnostic procedure performed and, in the context of severe illness with an associated hemophagocytic syndrome, immunohistochemistry using antibodies to CD3e, CD5, granzyme B and/or perforin as well as immunohistochemical or in situ hybridization studies for EBV are mandatory to reveal the neoplastic infiltrates. The clinical course is unfortunately highly aggressive.

Other T-cell Lymphoproliferative Disorders

Both HSTCL and T-LGL have been extensively discussed as the spleen represents a major, or primary organ of involvement by these diseases. Other T-LPDs can secondarily involve the spleen and in some instances cause pronounced splenomegaly. These entities will be briefly discussed.

T-cell Prolymphocytic Leukemia

T-cell prolymphocytic leukemia (T-PLL) is an uncommon, distinct lymphoproliferative disorder of post-thymic mature CD4-positive T-cells (23). T-PLL affects the middle aged to elderly and typically presents with a striking lymphocytosis, anemia, and thrombocytopenia. Splenomegaly is common and is less frequently accompanied by hepatomegaly, generalized adenopathy, and cutaneous eruptions. (24). T-PLL is an aggressive lymphoproliferative disorder with a median survival of less than one year when treated with conventional chemotherapy. A subset of patients show a more indolent course in which the disease may respond to anti-CD52 monoclonal antibodies (25).

The neoplastic T-cells of T-PLL show a wide morphologic spectrum ranging from cases with numerous prolymphocytes having large nuclei, reticular chromatin, and single prominent central nucleoli (the majority of cases) to cases with increased numbers of small, cytologically bland lymphocytes (the minority of cases). Some cells may have irregular nuclei and in rare cases the degree of nuclear convolution may approximate that of Sezary cells. Some controversy persists regarding the nomenclature of this disorder as cases with small, bland nuclei have been termed the 'small cell variant' of T-PLL by some authors and T-cell chronic lymphocytic leukemia (T-CLL) by others [15-16]. Although splenomegaly may be less prominent in cases with small lymphocyte cytology, the clinical behavior and prognosis are otherwise indistinguishable and as a result, many use the combined moniker T-PLL/CLL to encompass all of the cytologic variants of this entity.

As noted above, splenic involvement by T-PLL is common and may lead to marked splenomegaly (up to 3 Kg). Histologically splenic involvement by T-PLL is characterized by diffuse red pulp infiltration by cytologically atypical lymphoid cells with irregulary shaped nuclei which may vary in size from small to large (17). Unlike T-LGL, this infiltrate typically effaces the splenic architecture with attenuation of the red pulp, angioinvasion, and perforation of the capsule. Involvement of splenic hilar lymph nodes with effacement of the lymph node architecture may also be seen. The neoplastic T-cells of T-PLL have an immunophenotype typical of mature post-thymic T-cells with expression of CD2, CD3, CD5, and CD7. Over 60% of cases are CD4+ and CD8-, the remaining are usually dual CD4+ and CD8+ although rare CD8+ and CD4- T-PLL have been described. The CD8+ T-PLL lack the properties of cytotoxic T-cells such as the expression of cytotoxic granule proteins TIA-1 & granzyme B, allowing for ready distinction of these cases from T-LGL and HSTCL. In over 80% of T-PLL/CLL cases either an inv(14)(q11;q32) or a t(14;14)(q11;q32) can be identified. Both of these cytogenetic abnormalities lead to fusion of the *TCL1* gene on chromosome 14q32.1 with the *TCR* □□□ gene(s) on chromosome 14q11 which causes over-expression of TCL-1 oncoprotein (26, 27). When a diagnosis of T-PLL/CLL is suspected one should test for either *TCL1/TCR* □□□ gene fusion or TCL-1 protein expression, as these are disease specific findings in this setting. T-PLL/CLL cases with a t(X;14)(q28;q11) which results in dysregulation of MTCP1 gene have also been described.

Adult T-cell Leukemia/Lymphoma

Adult T-cell Leukemia/Lymphoma (ATLL) is a mature TLPD associated with the human T-cell lymphotropic virus-1 (HTLV-1) and therefore it is most commonly encountered in geographic areas where HTLV-1 sero-positivity is endemic, such as southern Japan, the Caribbean, sub-Saharan Africa, and Central and South America (28, 29). The clinical features of ATLL vary. Most cases present as a systemic illness characterized by constitutional symptoms, prominent peripheral blood involvement, hepato-splenomegaly, cutaneous eruption, adenopathy, and hypercalcemia (29). This is often referred to as the "acute" form of ATLL, and is associated with a particularly dismal prognosis. Infrequently, ATLL may have a less fulminant clinical presentation; included in this group are "chronic" ATLL which typically has persistent lymphocytosis and little adenopathy and smoldering ATLL which typically manifests with transient skin lesions and little peripheral blood involvement. The prognosis of these more indolent ATLL-variants is still relatively poor with median 2 year survivals of 78% and 52% for the chronic and smoldering forms, respectively (as compared to 20% for the acute ATLL). Although splenic involvement by acute ATLL is common this diagnosis is often made based on the peripheral blood findings and therefore there are few descriptions of the splenic histopathology in this disease. A prototypic peripheral blood finding in acute ATLL is the presence of increased numbers of lymphocytes with medium to large-sized polylobate nuclei (leading to the eponym "flower cells") and deeply basophilic cytoplasm. Nucleoli may be prominent. The cytologic atypia is less pronounced in the chronic and smoldering variants. The phenotype of all forms of ATLL is typically that of mature CD4-positive T-cells. Diminished expression of CD7 is a frequently described abnormality in ATLL, however this "aberrancy" has little disease specificity as it may be encountered in leukemic phase cutaneous T-cell lymphomas and in reactive T-cell populations. The expression of the T-associated antigens CD2, CD3, and CD5 in ATLL usually differs little from normal cells, ATLL cells may be distinguished by high levels of CD25 expression and FoxP3.

Other T-LPDs

In addition to the entities described above a number of other T-LPDs such as angioimmunoblastic T-cell lymphoma, can potentially involve the spleen. Splenic involvement is not prototypical, or even frequent, however for any of these diseases which are in and of themselves uncommon. For this reason, detailed discussion of these other T-LPDs is beyond the scope of this discussion.

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VASCULAR DISORDERS OF THE SPLEEN

Aysegul Uner
Department of Pathology, Hacettepe University, Ankara, Turkey

Splenic vascular tumors are uncommon neoplasms generally identified incidentally. Vascular changes associated with systemic disorders are more common than tumors. Vascular tumors of the spleen include several entities some of which are unique to this organ including splenic hamartoma, sclerosing angiomatoid nodular transformation (SANT), and littoral cell angioma. Other vascular tumors, such as hemangioma, lymphangioma, hemangioendothelioma and angiosarcoma can be encountered at other sites in the body.

Hemangioma and hemangiomatosis

Hemangioma is the most common primary benign neoplasm of the spleen. Usually it represents as a small localized tumor, however, rarely diffuse hemagiomatosis could also be encountered. The incidentally identified lesions are generally less than 4 cm. Typically they are nonencapsulated and may be difficult to discriminate from the surrounding splenic parenchyma. Morphologically variably sized dilated spaces which contain red blood cells is seen. The majority are cavernous in nature or may contain varying proportions of cavernous and capillary components. Pure capillary hemangiomas are very rare. Hemangiomatosis is generally aymptomatic but may also present with symptoms of hypersplenism, splenic rupture and portal hypertension. Typically splenic parenchyma is replaced by variably sized blood vessels.

Lymphangioma and lymphangiomatosis

Splenic lymphangiomas are characterized by endothelim-lined spaces filled with eosinophilic proteinaceous material which are situated under the capsule and/or along the trabeculae. On gross examination they may be difficult to differentiate from hemangiomas and mesothelial cysts. Lining cells are positive for CD31, *Ulex europeaus*, vWF Ag and D2-40 and negative for CD8. There might be faint expression of CD68 and/or lysozyme.

Littoral Cell Angioma

Littoral cell angioma (LCA) is an unusual and rare splenic vascular tumor first described in 1991 by Falk *et al* as “a novel splenic vascular lesion demonstrating hystiocytic differentiation”. It is believed to be derived from the normal splenic lining of the red pulp (littoral cell), cells with mixed vascular and histiocytic features. Although LCA cells show minimal immunophenotypic differences from the normal littoral cell, the tumor cells manage to mimic the dual differentiation potential of the reticuloendothelial system – endothelial and histiocytic, with features intermediate between those of endothelial cells and macrophages, similar to littoral cells. There are about 100 reported cases in the literature with no autopsy series to determine its true incidence and clinical characteristics. These tumors are known to present at any age, with no known gender predilection. In the literature, a significant proportion of the reported cases were noted to be associated with immune deregulations related to various malignancies (adenocarcinoma of the colon, gastric leiomyosarcoma, pancreatic and ovarian cystadenoma, renal cell carcinoma, meningioma, neuroendocrine tumor of the pancreas, nonsmall cell carcinoma of the lung, seminoma, papillary thyroid carcinoma, hepatocellular carcinoma, urothelial carcinoma, cervix carcinoma), drugs (immune supression due to renal transplantation, inflammatory bowel disease, Ankylozing spondylitis) or diseases (hepatitis, Wiskott Aldrich syndrome, Evans syndrome, Gaucher’s disease, psoriasis, lymphocytic colitis). This association suggests that, chronic inflammation and systemic immune suppression may have a role in its etiology.

Patients usually present with findings of hypersplenism (such as splenomegaly, anemia and/or thrombocytopenia) or incidentally (most often during staging evaluations for malignancies). Ultrasonography reveals multiple echogenic nodules. On MRI the lesions display heterogeneous signal intensity during contrast enhancement, due to its dual differentiation, with uptake of contrast media in macrophages.

Macroscopically, splenic LCA presents as multiple nodules varying in size from minute lesions barely noticeable to the unaided eye, to giant lesions involving the whole spleen.

Microscopically, they are characterized by anastomozing vascular channels, with pseudopapillary and cystic areas, resembling an exaggerated red pulp. These vascular channels are lined by plump cells with light eosinophilic or clear cytoplasm and bland cytology. Some cells may have vesicular, indented or grooved nuclei and may display scattered nucleoli. Pleomorphism and atypia is normally absent. Mitotic figures are lacking. Some of the lining cells are seen to display hemophagocytosis and to slough off into the lumen of the vascular channels. Immunohistochemically these cells are positive for factor VIII, CD31, *Ulex europeaus* (endothelial markers), KP1,CD68, lyzozyme, CD163, cathepsin D, alpha-1-antitrypsin and S100 (histiocytic markers). They are negative for CD34, CK, EMA and CD8. This immunoprofile is unique to LCA. It differs minimally from that of the normal littoral cell, the immunoprofile of which is CD8+, CD31+, CD68+ (focal) and CD34-.

There have been rare cases of tumors in this spectrum with the immunoprofile of LCA displaying areas of solid growth pattern, minute foci of necrosis and mild nuclear atypia but lacking mitosis and clearly malignant cytology. In one study these lesions have been shown to have diploid DNA, low S-phase fraction and Ki-67 proliferation index. These cases are referred to as ‘littoral cell hemangioendothelioma” and believed to represent a low grade variant of malignant LCA. Although the clinical behaviour of LCA’s is considered to be benign, cases in this spectrum have been shown to present with metastasis years later, even though they too normally lead a protracted clinical course. These cases emphasize the importance of careful search for solid foci and necrosis and longer follow-up periods for LCA patients, especially if the atypical features are identified.

Splenic angiosarcoma is the most important differential diagnosis in terms of growth pattern. However its rapidly progressive and fatal clinical course and overt malignant cytology and CD34 positivity help differentiate it from LCA. Whether or not there is a malignant end of this spectrum of these tumors similar to an angiosarcoma remains to be seen.

Sclerosing Angiomatoid Nodular Transformation (SANT)

SANT is a recently recognized vascular lesion of the spleen first described by Martel *et al* in 2004. Approximately 33 cases are presented in the English literature by this name. The entity however is thought to have previously been reported under various names such as "capillary cord hemangioma", splenic hamartoma, splenic hemangioendothelioma and "multinodular hemangioma".

The lesion usually presents in the 5-6th decades (age range 22-74) and is approxiamately twice as common in women. The splenic mass is usually an incidental finding during evaluation for unrelated conditions. Occasionally patients have been reported to have presented with splenomegaly. İmaging studies reveal a hypodense multinodular mass. Based on the reported cases to date the lesion has a benign clinical course and splenectomy is curative.

Macroscopically, the spleen is either normal in size or slightly enlarged, the lesion being characterized by a solitary unencapsulated white sclerotic mass within which small red-brown nodules can be discerned.

Microscopically, the lesion is composed of multiple vascular/angiomatoid nodules surrounded by an inflammatory fibrocollageneous stroma resembling inflammatory pseudotumors (IPT). Some of the nodules are demarcated by fibrin, some show coalescence, some have irregular outlines and some may be highly sclerotic as to resemble a granuloma. The center of the nodules consist of a mixture of vascular structures lined by plump endothelial cells immunophenotypically resembling splenic sinusoids (CD34-, CD31+, CD8+), capillaries (CD34+, CD31+, CD8-) and veins (CD34-, CD31+, CD8-). CD68 can also be present focally. Minimal atypia and prominence of nucleoli can be seen, but necrosis and prominent mitotic activity is lacking. A recent study has demonstrated CD30 positivity in the endothelial component of their series of 6 cases and EBV RNA expression in one case and have suggested that a subset of SANTs may be related to IPTs as postulated by Martel *et al*.

Hamartoma is an important differential diagnostic lesion, since both represent proliferatoins of red pulp tissue, in fact, it is thought that SANT may represents a peculiar sclerosed hamartoma. However hamartomas are usually not considered to be composed of a mixture of vascular structures or show angiomatoid nodules. Martel *et al.* have also presumed that SANT represents an exaggerated stromal response or the final common pathway of various lesions including inflammatory pseudotumor, hamartoma and hematoma.

Splenic Hamartoma

Splenic hamartomas are benign lesions first described by Rokitansky in 1861. Its synonyms include splenoma, spleen within spleen, nodular hyperplasia, and fibrosplenoma. There are different opinions on its pathogenesis; some consider it to be a congenital malformation, some think that they are neoplastic and yet others believe they represent a posttraumatic lesion.

Hamartomas may occur at any age with equal gender predilection. Most patients are asymtomatic, and it discovered incidentally. Pancytopenia, anemia and thrombocytopenia are the most common findings in symptomatic patients. Uncommonly palpable mass, splenomegaly, rupture, fever, malaise and weight loss may be the initial manifestation.

Macroscopically, hamartomas are usually well-circumscribed, bulging, nodular lesions that tend to compress and deform the adjacent parenchyma. Generally they are solitary but may also manifest as multiple nodules. The cut-surface is typically dark red to grayish-white. Histologically the lesions are more difficult to discern from the surrounding splenic parenchyma. Reticulin stain may help to demonstrate the compressed red pulp of the surrounding spleen. Histopathologically, they are composed of irregularly arranged sinusoids lined by endothelial cells and surrounded by fibrotic cords of splenic red pulp. The lining cells show typical phenotype for splenic-type endothelium. White pulp may or may not be present. Plasmacytosis; extramedullary hematopoiesis; and increased numbers of macrophages, eosinophils, and mast cells may be noted.

Hemangioendothelioma

These vascular lesions have morphologic and clinical features that are intermediate between those of hemangioma and angiosarcoma. Their malignant potential is considered borderline. Morphologically they exhibit mild cellular atypia, low mitotic rate and lack of necrosis. Cells may be spindle and/or epithelioid in appearance with a range of architectural features.

Angiosarcoma

Primary angiosarcomas are rare, aggressive tumors with dismal prognosis. It is the most common primary, malignant, non-lymphoid tumor of the spleen with less than 100 cases reported in the literature. Most patients are older than 40 years of age. Although thorium oxide (Thorotrast), vinyl chloride and arsenic exposure have been implicated in the etiology, a clear association has not been documented for splenic angiosarcomas.

In contrast to other vascular lesions of the spleen, most patients are symptomatic at presentation. Left upper quadrant pain, fatigue, weight loss and fever are among frequent complaints. Anemia, elevated erythrocyte sedimentation rate and coagulopathy may be identified in laboratory evaluation.

Macroscopically the lesions are usually multinodular and hemorrhagic with foci of necrosis. Histologically the appearance may be extremely variable. However, in almost all cases at least focally vasoformative

architecture is present. Irregularly anastomosing spaces lined by cytologically atypical cells is seen. In cases in which vascular nature of the tumor is not apperant, immunohistochemical studies are necessary to confirm the diagnosis. Most cases are positive for one or more of the markers of vascular differentiation such as CD31, CD34, *Ulex europeaus*, VEGFR3, and vWF. CD31 is the most specific and sensitive among these markers and tends to be expressed in even poorly differentiated tumors. A significant proportion of the primary splenic angiosarcomas may also express CD68 and/or CD8 which is an evidence for their derivation from sinus endothelial cells.

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BONE MARROW PATHOLOGY

Chairpersons: J. van der Walt (UK), S. Alkan (USA)

PLASMA CELL AND PLASMACYTOID CELL DISORDERS INVOLVING BONE MARROW

S. Alkan (USA)

Plasma cell (PC) disorders are monoclonal neoplasms that develop from common progenitors in the B-lymphocyte lineage. The most characteristic feature of these disorders is production of immunoglobulin molecules or fragments from abnormal PCs.
A number of different disorders with monoclonal proliferation of PCs are encountered in routine bone marrow evaluations (Table 1). Some of these disorders primarily arise from the bone marrow ;however, PCs or plasmacytoid lymphocyte proliferation may be seen as secondary involvement of the bone marrow and it may be quite challenging to make a definitive diagnosis. Therefore, a thorough clinical history is essential for rendering final diagnosis.

Multiple Myeloma

Multiple myeloma (MM) is characterized by the proliferation of PCs in the bone marrow. During the past few years very exciting development took place particularly inunderstanding the biology of malignant plasma cells and development of novel therapies. Various signaling pathways activating malignant PC proliferation and its relationship to apoptosis, migration, and invasion. Novel targets including the inhibition of proteasomes, tyrosine kinases, histone deacetylases, farnesyltransferases, molecular chaperones are currently under clinical investigation. However, the basic role of hematopathologists in the diagnosis of myeloma remains the same. Diagnosis of myeloma requires integration of clinical information such as M-protein level and type, and the presence of lytic lesions.

Flow cytometric evaluation may be performed in clinical evaluation. Typically plasma cells show expression of CD38, CD138, CD56, light chain restriction and lack expression of CD19, CD20, CD22 and CD45. However, CD20 and CD45 could be detected in the minority of patients. Although CD56 expression is more commonly encountered in MM, 30-40% of the cases may fail to express this marker. Plasma cell quantification is essential part of MM diagnosis and analysis of therapy response. Traditionally, bone marrow aspirate smear is used for quantitative analysis of plasma cells ;however, aspirate smear evaluation may not be accurate. I recommend that CD138 immunohistochemical staining be included in routine evaluation along with aspirate smear differential counting to accurately quantify plasma cell involvement of the bone marrow in MM patients.[1]Although the diagnosis of myeloma is not challenging in the great majority of cases, some rare forms such as spindle cell, small cell or anaplastic variants may cause misdiagnosis.

Recent advances particularly in the field of cytogenetics and molecular biology is adding useful information for the determination of prognosis. One of the most significant developments in this area is related to adaptation of FISH using probes against 13q. Deletion of 13q as a negative prognostic factor had been known for the past several years and assays for 13q using FISH probe had been already integrated in many laboratories. [2] Interestingly, patients treated with bortezomib appear to respond better compared to patients treated with dexamethazone. Based on a recent publication, it appears that the integration of two other targets [(t4;14)(p16;q32) and 17p13 deletions] are going to be also useful in prognostic evaluation of myeloma patients.[3] Patients with t(4;14) have a poor response to high-dose therapy and need development of alternative therapy while the 17p13 deletion involving p53 locus usually have a shorter survival and likely develop extramedullary disease, plasmacytomas and hypercalcemia. Due to ease of these assays using FISH probes, screening these additional cytogenetic markers will soon find a wider utilization in primary clinical investigations of myeloma patients.

Several recent studies using molecular profiling also showed that specific molecular signatures distinguish different prognostic groups. [4] However, these studies had not been integrated in daily routine clinical evaluations by hematologists or pathologists.

Plasmacytoma

This category constitutes 1% of monoclonal gammopathies and plasmacytomas are seen in 5% of patients with plasma cell myeloma. It is characterized by a solitary clonal mass of plasma cells presenting either in the bony tissue or soft tissue without systemic bone marrow plasmacytosis. The presence of M-spike is related to the size of the tumor and/or the secretion levels of the immunoglobulin chain. In some cases the M–spike may be noticeable on the serum and urine protein electrophoresis analysis ; however, the level of protein seen is significantly lower than multiple myeloma patients. While extraosseous lesions rarely spread and can be surgically excised, the bone lesions usually progress to multiple myeloma. In microscopic analysis of tissue based plasma cell infiltrates, one should keep in mind that marginal zone lymphomas (nodal and extranodal) sites may have marked plasmacytic differentiation.[5] [6] [7] Therefore, if plasmacytoma is considered as a differential diagnosis at an unusual site i.e. MALT organs, ocular adnexa, skin etc, the diagnosis of marginal cell lymphoma should be excluded.

Monoclonal gammopathies of uncertain significance (MGUS)

MGUS refers patients with the presence of a serum monoclonal protein in the absence of clinical other known cause of monoclonal gammopathy (eg, multiple myeloma, Waldenström macroglobulinemia, primary amyloidosis, or other related disorders). MGUS itself is harmless but over many years some individuals with MGUS will progress to myeloma. The level of M-protein in MGUS should be less than 3 g/dl. A bone marrow biopsy is needed if the M-protein level is 1.5

g/dl or above. The main differential diagnosis of MGUS from myeloma is based on the presence or absence of end-organ damage. The incidence of MGUS increases with age (1% at age 50 years; 5% at age 70 years; 10% at age 80 years).

Kyle et al reported an actuarial probability of malignant evolution of 17% at 10 years, 34% at 20 years, and 39% at 25 years, with an annual rate of 1.5%.[8] The factors significantly associated with malignant transformation in MGUS are the serum M protein size (> 1.5 g/dL); the proportion of plasma cells (higher than 5%) ; immunoglobulin isotype (IgA>IgG); and the type of MGUS (evolving vs nonevolving). [9] The evolving type was defined only as a progressive increase in the M protein size on serum protein electrophoresis in each of the annual consecutive measurements during a period of 3 years (ie, any annual increase in the serial M protein measurements through baseline plus years 1, 2, and 3 needed to be higher compared with each previous one) while M protein size does not significantly change in the non-evolving type of MGUS.

Rajkumar et al shown that patients with non-IgG MGUS, an M protein size larger than 15 g/L, and an abnormal kappa/lambda light chain ratio had an actuarial probability of malignant evolution of 58% at 20 years of follow-up, whereas for patients with IgG type, an M protein size less than 15 g/L, and a normal [kappa]/[lambda] light chain ratio, the probability of malignant evolution at 20 years was only 6%. [10]
Various cytogenetic abnormalities seen in MM are also encountered in MGUS patients. The poor cytogenetic finding of del of chromosome 13 is also seen upto 50% of MGUS patients and could not be used in differential diagnosis of MM versus MGUS.

Amyloidosis
Amyloidosis is a rare monoclonal plasma cell proliferative lesion that presents with a systemic disorder of protein metabolism with progressive extracellular deposition of insoluble fibrillary protein with subsequent organ dysfunction. The structural subunit of AL amyloid usually consist of monoclonal light chain or fragment and occurs secondary to deposition of fibrils derived from the N-terminal amino acid residues of LC immunoglobulin variable regions. AL amyloidosis is structurally unrelated to other forms of amyloidosis, including Alzheimer's disease.

The histology of bone marrow infiltration could be very subtle and may be missed if insufficient clinical information provided. [11] Usual histomorphologic findings include plasma cell dyscrasia with light chain restriction (lambda> kappa). Plasma cells are typically mature and show no atypia or large cluster formation. The great majority of cases show plasma cell counts less than 15% in the bone marrow evaluation. Diagnostic work should always include Congo red staining of a BM biopsy. However, this staining demonstrates amyloid deposits only in ~60% of patients. Usually amyloid deposition is noticeable around the vessels but some cases particularly kappa light chain related AL may have interstitial deposition. There are no distinctive features in peripheral blood. Thrombocytosis secondary to functional hyposplenism from amyloid replacement of the spleen may be encountered and presence of Howell-Jolly bodies may support this physiologic change.Rarely, AL amyloidosis may be encountered in the context of B-cell lymphoproliferative disorder, usually CLL or lymphoplasmacytoid lymphoma.[12]

It appears that AL amyloidosis shares numerical chromosomal changes with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM), such as 13q14 deletions and t(11;14).

Patients are usually referred to hematologist with the observation of M-protein. The bone marrow examination typically reveals plasma cell count less than 10%. Systemic clinical evaluation reveals various symptoms, such as nephropathy secondary to renal amyloidosis, cardiac symptoms, peripheral neuropathy. High degree of suspicion of amyloidosis in patients with MGUS may be very valuable for arriving correct diagnosis. Screening with serum protein electrophoresis to make a diagnosis of AL amyloidosis is not recommended since 50% of patients fail to reveal M-protein while serum and urine immunofixation and free light chain analysis shows abnormality in the majority of patients. Therefore, serum/urine immunofixation and free light chain analysis is the best non-invasive tests for patients being investigated for AL amyloidosis. Patients with positive test results then could be investigated by bone marrow evaluation. Bone marrow or subcutaneous fat aspirate biopsy shows Congo-Red positivity in 75% of the patients. BM evaluation is necessary to exclude the diagnosis of myeloma.

POEMS syndrome
POEMS syndrome is characterized by a chronic progressive polyneuropathy with a predominant motor disability.The acronym for the POEMS includes polyneuropathy, organomegaly,endocrinopathy, M protein, and skin changes. There are other features not included in the main category such as sclerotic bone lesions, Castleman disease, papilledema peripheral edema, ascites, effusions, thrombocytosis, polycythemia, clubbing and fatigue. To make diagnosis of POEMS syndrome, the peripheral neuropathy; osteosclerotic myeloma (i.e., a clonal plasma cell dyscrasia and at least one sclerotic bone lesion) or Castleman disease; and at least one of the other features should be seen (table 2). All of the patients show a monoclonal plasma cell population and the light chain is restricted to lambda light chain in almost all of the cases. Thrombocytosis is seen in 50% of the patients and may potentially cause misdiagnosis of essential thrombocytothemia. Bone marrow findings are usually non-specific. In approximately 20% of the cases plasma cells are slightly increased ; however, rarely, a plasma cell count greater than 20% may be noticed. [13]

Several cytokines are documented to be increased in this disorder including vascular endothelial growth factor (VGEF), tumor necrosis factor-alpha, IL-1beta, and IL-6 . A novel approach in therapy targeting VEGF by bevacizumab in POEMS patients appears to be promising.[14]

Waldenström macroglobulinemia (Lymphoplasmacytoid Lymphoma)

Waldenström macroglobulinemia (WM) is a clinicopathologic syndrome characterized by a proliferation of lymphocytes, plasmacytoid lymphocytes and plasma cells associated with the presence of a monoclonal gammopathy (IgM) in the serum.

The most common heavy chain expressed in MM are IgG and IgA ; however there are some cases of MM with IgM expression that may be difficult to distinguish from LPL (WM). Diagnosis of MM in this instance is usually not difficult since the most cases of MM has multiple bony lytic lesions and other findings of myeloma. Markedly elevated IgM monoclonal protein and monoclonal lymphoplasmacytoid infiltration of the bone marrow, hepatosplenomegaly, anemia, hyperviscosity and lymphadenopathy is consistent with diagnosis of WM. In some cases of IgM myeloma bony lytic lesions may not be present and making diagnostic evaluation difficult. In these instances, predominance of lymphocytic and lymphoplasmacytic infiltrate observed by microscopic evaluation, demonstration of CD20 and bright CD45 expression and lack of CD138 favors diagnosis of LPL. Cytogenetic evaluation may also be very valuable as it may show karyotypic findings specific for each entity such as 13q del, t(4;14), t(11;14) favoring myeloma.

Sometimes, lymphoplasmacytoid appearance on the bone marrow morphologic evaluation may be difficult to appreciate. Therefore, it is necessary to have a high degree of suspicion of lymphoplasmacytoid lymphoma diagnosis in small B-cell lymphoproliferative disorder lacking CD5 co-expresion. Presence of Dutcher bodies and mast cells in the bone marrow aspirate are good clues for the diagnosis of LPL. The lymphocytes, plasmacytoid lymphocytes, and plasma cells diffusely infiltrate the bone marrow, lymph nodes, spleen, and liver. Differential diagnosis includes chronic lymphocytic leukemia with plasmacytoid differentiation, splenic marginal zone lymphoma, small cell variant of myeloma and recently described lymphoplasmacytoid variant of mantle cell lymphoma.[15] There are rare cases of extranodal marginal zone lymphomas arising from the MALT sites that may have clinical features of WM. [16] In these cases, biopsy of the involved MALT site shows typical histology of MALT lymphoma. Interestingly, a recent study using gene expression profiling demonstrated that the gene expression pattern of WM shows more similarity to chronic lymphocytic leukemia than myeloma. [17]

Castleman's disease

Castleman's disease (CD) is histologically divided into the hyaline vascular form and a plasma cell variant. Hyaline vascular (HV) form of CD is more common form seen as a localized disease while plasma cell variant is more common in multicentric Castleman's disease (MCD). The great majority of patient's with MCD is associated with human herpesvirus 8 (HHV8) and encountered predominantly in HIV patients This virus encodes a homologue of interleukin 6 (vIL 6), which may mediate some systemic features of MCD.

MCD may involve the bone marrow and histologic findings are usually non-specific. Most patients show mild degree of plasmacytosis (varying 5-20%). Occasionally small lymphoid follicles with depleted/hyalinized germinal centers similar to those seen in the CD involved lymph nodes. [18], [19] Within the mantle zone area of these follicles, plasmablasts are present and infected with HHV-8. Immunohistochemical staining is very helpful for diagnosis as it usually shows positivity with antibody against HHV-8 latent nuclear antigen (LNA). LNA positivity is seen only within the lymphoid follicle whereas interstitial plasma cells are negative. This diagnosis should be considered especially in HIV positive patients with Kaposi's sarcoma and presence of regressed lymphoid follicles as it most commonly occurs in this setting. However, the hallmark histology is not seen in the majority of patients. Therefore, biopsy of lymph node is usually necessary for the precise diagnosis.

Heavy chain disease

This disorder is characterized by presence of only a monoclonal heavy chain detected by serum protein electrophoresis without evidence of light chain production. The type of the heavy chain produce results in a different clinical presentation.[20]

Alpha chain disease, most common of the HCD, is also referred as Mediterranean lymphoma or Immunoproliferative small intestinal disease (IPSID) Typically the patients are young presenting with severe malabsorption. IPSID has been described mainly in the Mediterranean, Middle East, and African countries. It occurs rarely in western countries. The usual presentation of these patients is related to gastrointestinal symptoms as the plasmacytic infiltrate typically involves the lamina propria of the small intestinal mucosa.The lamina propria of the intestinal mucosa results in villous atrophy, malabsorption, diarrhea, steatorrhea, and hypocalcemia. Abdominal lymph nodes may be infiltrated by lymphocytes, plasmacytoid lymphocytes, and plasma cells. Rarely, the initial material submitted to pathologists may be the bone marrow smear and biopsy. Since advanced cases of Alpha chain disease show marked monotypic lymphoplasmacytic/plasmacytic infiltrate, the diagnosis of this disease should be considered in young patients presenting with malabsorption, diarrhea, and steatorrhea. SPEP evaluation may be very critical for diagnosis as it reveals the presence of Alpha heavy chain disease. This disease may be caused by Campylobacter jejuni and treatment of this early phase with antibiotics may cause remission in some patients.[21] Some cases of HCD may progress to a large cell B- immunoblastic lymphoma carrying a poor response to therapy.

Gamma chain disease patients most commonly presents as lymphadenopathy, hepatosplenomegaly, anemia and fever. There is no consistent histopathology associated with this disease ;however, the most common finding is pleomorphic lymphoplasmacytic infiltrate. In some cases, it may present with plasmacytoma or marked plasmacytic infiltrate in the bone marrow. Monoclonal gamma heavy chain is detected by SPEP in the majority of patients and useful for consideration of this rare disorder. [22]

Mu chain disease is an extremely rare form of HCD . This disorder manifests with a variable histopathologic findings including chronic lymphocytic leukemia, lymphoma, Waldensrom macroglobulinemia or myeloma like features. The plasma cells noted in the bone marrow aspirates are characteristically vacuolated. Hepatosplenomegaly is common as a presenting symptom, but without accompaning lymphadenopathy. Diagnosis again depends on the careful analysis of the SPEP and demonstration of mu heavy chain.

Table 1. Differential diagnosis of hematopoietic disorders that may present with an increased number of plasma cells in the bone marrow

- Multiple myeloma
- Plasmacytoma
- Monoclonal gammopathy of uncertain significance (MGUS)
- Amyloidosis
- POEMS syndrome (osteosclerotic myeloma)
- Heavy-chain diseases

Gamma heavy-chain disease
Alpha heavy-chain disease
Mu heavy-chain disease

- Castleman's disease
  HV, plasma cell, multicentric
- Lymphoma

Lymphoplasmacytoid (Waldenström's macroglobulinemia)
Marginal zone (splenic, MALT)
Diffuse large cell lymphoma (immunoblastic plasmacytoid)

Table 2. Criteria for the diagnosis of POEMS syndrome.

(Diagnosis is made with two major criteria and at least 1 minor criterion.)
Major criteria: Polyneuropathy, Monoclonal plasma cell-proliferative disorder
Minor criteria:Sclerotic bone lesions,Castleman disease, Organomegaly, Edema, Endocrinopathy, Skin changes, Papilledema

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CHRONIC MYELOPROLIFERATIVE DISEASES AFTER JAK2. DO WE NEED A NEW CLASSIFICATION?

Hans Kreipe, Kais Hussein, Oliver Bock
Institute of Pathology, Medizinische Hochschule Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. e-Mail: Kreipe.Hans@MH-Hannover.de

Chronic myeloproliferative disorders (CMPD) represent clonal proliferations of a neoplastic haematopoietic stem cell (HSC) and affect all or a subset of the haematopoietic lineages, resulting in a mono-, bi- or trilinear proliferation of megakaryocytic, erythroid and granulocytic precursor cells. CMPDs encompass chronic myeloid leukaemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF, synonymously also chronic megakaryocytic granulocytic myelosis, CMGM or myelofibrosis with myeloid metaplasia, MMM or agnogenic myeloid metaplasia, AMM). The latter three types of CMPD overlap in clinical presentation as well as in histomorphology of the bone marrow although the majority of cases can be subtyped according to the presentation in the bone marrow. Unlike CML which is characterized by the bcr-abl junction no clonal marker was available thus far. A breakthrough was achieved with the most recently discovered 1849G>T mutation in exon 12 of the Janus tyrosine kinase 2 (JAK2) gene. This hot spot point mutation is located on chromosome 9p and leads to a gain of function due to a defect Valine to Phenylalanine exchange at the amino acid position 617 (V617F) of the autoinhibitory JH2 pseudokinase domain of JAK2. Thus the mutated $JAK2^{V617F}$ tyrosine kinase is constitutively activated and conveys erythropoietin hypersensitivity and growth-factor independence to transfected cell lines. After antecedent irradiation transplantation of $JAK2^{V617F}$ transfected cells was sufficient to mimic human PV and its evolution towards myelofibrosisin murine *in vivo* models.

Since its discovery Janus kinase 2 gain of function V617F mutation has emerged rapidly as a novel marker for chronic myeloproliferative diseases and has provided new insights into the pathogenesis of Philadelphia chromosome negative chronic myeloproliferative disorders (Ph^- CMPD). Only exceptionally the mutation can be observed in chronic myeloid leukaemia (CML), AML or MDS. The mutation appears to be almost specific for Ph^- CMPD but the different entities comprising polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF) are not discriminated by the mutation. It is still unclear how the diversity with heterogeneous clinical and pathoanatomical presentation comes about. It has been suggested that differences in $JAK2^{V617F}$ gene dosage or different degrees to which the haematologic lineages are affected by the mutation leads to the differences in clinical presentation, histomorphology and tendency to transform into myelofibrosis or blast crisis. With regard to myelofibrosis $JAK2^{V617F}$ appears not to be a causative factor. Only about 50-60% of ET and CIMF reveal the $JAK2^{V617F}$ mutation. In PV not only the highest incidence of $JAK2^{V617F}$ mutation can be observed exceeding 95% but also the allele frequencies are higher in PV than in ET and CIMF with the majority of cases being homozygous for the mutation. During the course of CMPD there is usually no novel acquisition of the mutation, but CMPD either start as $JAK2^{V617F}$ - positive or - negative and will remain in this state. This is surprising because in humans there is evidence that the mutation might be secondary to pre-existing clonal defects. In familial cases of CMPD $JAK2^{V617F}$ does not affect the germ line but represents an acquired somatic mutation, which can be found in several generations. In ET the allele frequency is usually below 50% and an increase does not take place during the course of the disease. In CIMF a higher allele frequency occurs and transitions from hetero- to homozygouity can be observed. The latter, however can not be linked to fibrotic progression. The differences in allele frequencies of $JAK2^{V617F}$ which can be observed between PV, ET and CIMF are not sufficient to explain the heterogeneity of CMPD. Although CMPD are considered to be stem cell disorders not every cell lineage is affected equally by the mutation. Granulocytes despite being clonal may be spared from the mutation. This has been interpreted as hint that the mutation represents a second event in an already neoplastic cell. Furthermore, blast crisis developing in $JAK2^{V617F}$ positive CMPD are frequently negative for the mutation. The detection of JAK2 mutation is usually technically undemanding and reliable. Costs range from relatively cheap to expensive devices and methods. For diagnostic and research purposes DNA and RNA/cDNA can be obtained from peripheral blood (mononuclear cell fraction, granulocytes, platelets and flow cytometrical fractioned cells), formalin-fixed and paraffin-embedded bone marrow trephines or bone marrow derived laser microdissected cells, such as megakaryocytes. The JAK2 status can be expressed in different ways, either simply as mutated/unmutated, or wild-type/heterozygous/homozygous or as the gene dosage/allele frequency of the percentage of mutated G>T allele. Different methods can be used to detect the 1849G>T transition in DNA and/or cDNA. A well known and simple to use detection method for single nucleotide polymorphisms (SNP) are restriction enzymes. BasX I restriction enzyme is suitable for digestion of the wild-type JAK2 PCR product, but fails to recognize the mutant G>T hot spot, leaving the PCR product unfragmented [11,17]. Amplification refractory mutation system (ARMS) assay is based on a tetra-primer JAK2-PCR, consisting of two primer pairs for the simultanious ampification of the wild-type and mutated SNP alleles. Both assays have the advantage of implying only standard PCR devices and some specific reagents. Pyrosequencing® is based on a luciferase dependent light signal, which is proportional to pyrophosphate release for every incorporated nucleotide at the SNP hot spot. Apart from Pyrosequencing®, also real-time polymerase chain reaction based assays and capillary electrophoresis alllow a quantification of the ratio of wild-type G to mutant T allele. Of note, primer sequences and products length may vary among different publication without affecting the overall result. Actually, a systematic comparison of all available methods has not been done so far, but for validation reasons the generated JAK2-PCR products are often used for more than one method. For example, if requested ARMS assay and BsaX I restriction analysis can be combined.

Currently, JAK2^{V617F} provides a valuable adjunct to the diagnosis of Ph$^-$ CMPD, in particular with regard to discrimination from reactive proliferations. It is not sufficient to discriminate the different types of CMPD and will on its own not form the basis for a new classification. In the diagnosis of PV JAK2 mutation besides high haemoglobin levels will provide the major criteria in the future, supported by minor criteria such as bone marrow histology and low erythropoietin level. In case of the other CMPD, ET and CIMF, diagnosis will still predominantly rest on bone marrow histology.

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MASTOCYTOSIS AND MYELOMASTOCYTIC OVERLAP SYNDROMES*

Hans-Peter Horny, M.D.
Institute of Pathology, Ansbach, Germany

*THIS LECTURE IS MAINLY BASED ON AN ARTICLE RECENTLY PUBLISHED IN PATHOBIOLOGY: "MASTOCYTOSIS: STATE OF THE ART" BY H-P HORNY, K SOTLAR AND P VALENT

SUMMARY

Mastocytosis is a neoplastic disease involving mast cells and their CD34+ progenitors. Basically, mastocytosis should be separated from reactive states with mast cell (MC) hyperplasia. Symptoms in mastocytosis and mast cell hyperplasia are caused by inappropriate release of biological mediators from MC. A WHO consensus classification for mastocytosis exists, which is widely accepted and includes 3 major subvariants. 1. Cutaneous mastocytosis (CM), a benign disease in which MC infiltration is confined to the skin, is preferentially seen in young children and exhibits a marked tendency to regress spontaneously. 2. Systemic mastocytosis (SM) which is commonly diagnosed in adults and includes four major categories: i. indolent SM/ISM (the most common form involving mainly skin and bone marrow); ii. a unique subcategory termed SM with an associated non-mast cell clonal hematological disease/SM-AHNMD: iii. aggressive SM/ASM usually presenting without skin lesions; and iv. MC leukemia/MCL, probably representing the rarest variant of human leukemias, and 3. The extremely rare localized extracutaneous MC neoplasms, either presenting as malignancy (MC sarcoma) or as benign tumor termed extracutaneous mastocytoma. Diagnostic criteria for mastocytosis include one major criterion (multifocal compact tissue infiltration by MC) and 4 minor criteria: 1. Prominent spindling of MC, 2. Atypical immunophenotype of MC with coexpression of CD2 and/or CD25 (antigens which have not been found to be expressed on normal/reactive MC), 3. Activating (usually somatic) point mutations of the c-kit proto-oncogene commonly involving exon 17, with the imatinib-resistent type D816V being most frequent, and 4. Persistently elevated serum tryptase level (>20 ng/ml). To establish the diagnosis of SM, at least one major and one minor criteria, or at least three minor criteria have to be fulfilled. The natural clinical course of mastocytosis is variable. Most patients, in particular those with CM and ISM, remain in an indolent stage over many years or even decades, while others, in particular those with ASM, SM-AHNMD, or MCL show a progressive course, usually with a fatal outcome.

DEFINITION

Mastocytosis is a very heterogeneous disease of bone marrow origin and characterized by abnormal growth and/or accumulation of clonal MC in one or more organs. In SM, at least one extracutaneous organ is involved. It cannot be overemphasized that mastocytosis basically must be diagnosed morphologically, by investigating biopsy specimens of skin (cutaneous mastocytosis) and/or bone marrow (to reveal or exclude SM). Cytomorphological diagnosis of SM in bone marrow smears is also possible in a minority of cases but is inevitable in all cases of MC leukemia.

MORPHOLOGICAL DIAGNOSIS

The diagnosis of mastocytosis can be established when multifocal compact MC infiltrates consisting of spindle-shaped MC, are detected in a given tissue. MC often show cytomorphological atypia with a reduced content of metachromatic granules. Usually, the compact MC infiltrates also contain varying amounts of intermingled eosinophils and lymphocytes. Compact lymphocytic infiltrates in the immediate vicinity of MC aggregates are commonly found in ISM and have been shown to be reactive in nature in almost all cases. When compact infiltrates consist exclusively of round mature-appearing MC, other minor SM criteria must be fulfilled to achieve the definitive diagnosis of mastocytosis. In very rare instances there is a focal and/or diffuse collagen fibrosis of the bone marrow containing an abundance of loosely scattered spindle-shaped MC which lack both expression of CD25 and an activating point mutation of c-kit. This condition therefore must be regarded as reactive thus being an important mimicker of mastocytosis and might be tentatively be termed "fibromastocytic lesion".

In all cases of suspected mastocytosis a limited panel of antibodies against at least three antigens should be applied: i. anti-tryptase which is highly specific and sensitive (with the exception of both neoplastic tryptase+ myeloblasts and basophils), and therefore allows screening for both the number of loosely scattered MC and immediate detection of even small compact MC infiltrates. In extramedullary tissues (e.g. mucosa of the gastrointestinal tract), non-specific background-staining of anti-tryptase may easily lead to overestimation of MC numbers and misinterpretation as mastocytosis; ii. antibodies against KIT (CD117) should therefore also be applied to reconfirm the presence of MC in such cases. Although anti-KIT antibodies are highly non-specific because KIT is also expressed on hemopoietic stem cell, melanocytes, germ cells, and CAJAL cells, they have been found to be of superior sensitivity allowing verification of tryptase+ cells as MC without significant background-staining. iii. antibodies against CD25 which is also a very non-specific antigen which is expressed on activated T cells but also on certain B-cell malignancies like hairy cell leukemia, should also be applied in all cases of suspected SM because neoplastic MC coexpress CD25 whereas normal and reactive MC are CD25-negative. CD25 immunohistochemistry is of particular diagnostic value in the bone marrow where CD25+ lymphatic cells are found only in very small number or virtually are absent and the CD25-reactivity of megakaryocytes can be easily used as internal control. The diagnostic value of anti-CD2 antibodies is limited because of a lower sensitivity for detection of atypical MC and the presence of CD2+ T cells in almost all tissue infiltrates of mastocytosis.

WHO CLASSIFICATION OF MASTOCYTOSIS

Based on significant advances in mastocytosis research, an updated consensus classification for mastocytosis has been proposed in 2001. This classification system was fully adopted by the WHO and has now become widely accepted because it

provides both criteria to discriminate between SM and MC hyperplasia and between SM and CM. Moreover, it even allows separation of SM from related myeloid disorders with signs of MC differentiation. Three major subgroups of the disease were defined: i. Cutaneous mastocytosis (CM); ii. Systemic mastocytosis (SM) including ISM, ASM, SM-AHNMD, and MCL; and iii. extracutaneous mastocytoma.

CM is an indolent disease and, by definition, can only be diagnosed when SM is excluded by appropriate investigations. Most patients are children, whereas only a minority of adult patients have pure cutaneous mastocytosis. In adults, a trephine biopsy specimen including immunohistochemical and molecular analyses must always be investigated to assess or exclude SM. The most common subvariant of CM presents as disseminated macular or maculopapular rash, and has been descriptively termed urticaria pimentosa. Diffuse cutaneous mastocytosis is much less frequent and usually seen only in very young children. The solitary or localized mastocytoma (of the skin) is also rare, and has a totally benign clinical course. Most MC tumors of the skin show a benign course or even resolve spontaneously at puberty. However, more recently, a unique case of a primary cutaneous mast cell sarcoma (with secondary infiltration of the bone marrow) has been detected (own unpublished observation).

ISM is the most common variant of SM comprising about two thirds of all cases. Usually, ISM involves both skin and bone marrow. The bone marrow infiltration may be difficult to detect in some cases and then can only be diagnosed when appropriate immunohistochemical stains including an anti-CD25 antibody and molecular (D816V) studies, are performed. ISM shows a prolonged clinical course in almost all patients with survival times of two decades and more. However, in a small group of patients, transformation into another disease category, such as aggressive SM, or SM with an associated hematological malignancy (SM-AHNMD) occur.

ASM is by far much less common than ISM comprising only about 5% of all SM patients. Clinically, ASM may present with hepatosplenomegaly and/or generalized lymphadenopathy, but usually without skin lesions. ASM is often revealed only by histological examination, but not suspected by the clinician. ASM is characterized by progressive MC infiltration of various organs with clinically significant impairment of their function including severe cytopenia, malabsorption, bone fractures, and signs of hepatopathy with loss of liver function. Such findings are termed C-findings. MC infiltration leading to marked organomegaly should not be regarded as a C-finding unless accompanied by signs of impaired organ function. Siginificant organomegaly is also found in patients with an indolent or a smouldering course, and then represent B-findings. A rare subvariant of ASM with prominent eosinophilia of blood and tissues and generalized lymphadenopathy (clinically mimicking malignant lymphoma) has been described as lymphadenopathic mastocytosis with eosinophilia.

In about one fourth to one third of the patients with SM, an AHNMD is diagnosed thus making SM-AHNMD the second most frequent subtype of SM. In these patients, WHO criteria for both SM and the AHNMD must be fulfilled. SM-AHNMD is a unique disorder amongst hematological neoplasms in that it combines two completely different histologies and disease-categories into one defined entity of SM. The vast majority (about 80 to 90%) of "AHNMDs" are myeloid disorders including almost all defined disease entities: myelodysplastic syndromes (MDS), myelodysplastic/myeloproliferative syndromes (MDS/MPS), myeloproliferative syndromes (MPS), acute myeloid leukemia (AML), chronic eosinophilic leukemia (CEL), and chronic myeloid leukemia (CML). Most common are disorders of the MDS/MPS group, usually termed chronic myelomonocytic leukemia (CMML). Of special importance is the clear-cut separation of chronic eosinophilic leukemia (CEL) containing loosely scattered CD25+ abnormal MC but not fulfilling the criteria of SM, and SM-CEL with multifocal compact diagnostic mast cell infiltrates and criteria for both SM and CEL. Associated lymphatic malignancies comprise only about 10 to 20% of all "AHNMDs" with plasma cell myelomas being most frequent within this group. Acute and chronic lymphatic leukemia and hairy cell leukemia have also been reported. Clinical picture and prognosis of SM-AHNMD patients are mainly determined by the "AHNMD". Rarely, infiltrates of SM have been detected after successful chemotherapy of an AML. This condition is tentatively termed "occult mastocytosis". SM-AHNMD may present with three histomorphological pictures: i. extremely hypercellular marrow with multifocal infiltrates of SM and the diffuse-compact infiltrates of "AHNMD"; ii. normo- or even hypocellular marrow usually seen in patients with plasma cell myeloma or chronic lymphatic leukemia both exhibiting a multifocal but minor infiltration with widely intact hemopoiesis; and iii. "occult" mastocytosis which is revealed only after chemotherapy because diffuse-compact blast cell infiltrates obscured SM.

MCL is extraordinarily rare and characterized by leukemic infiltration of various organs by atypical MC. MCL is the only subvariant of mastocytosis diagnosed cytologically in smear preparations: MC numbers in bone marrow smears must exceed 20% of all nucleated cells. The cut-off level of 20% for bone marrow MC only refers to the cytological assessment in smears, but not to the percentage of MC in the histological analysis. In most cases with MCL, circulating MC are found. In typical MCL, MC make up more than 10% of blood cells, while aleukemic variants of MCL are rare. The prognosis of patients with MCL is grave. The most important differential diagnosis to be considered is myelomastocytic leukemia.

Localized MC proliferations are also extremely rare and include both the extracutaneous mastocytoma (of the lung) and the "true" MC sarcoma of which less than five published cases have been published. Since cytomorphological atypia of MC sarcoma is usually very high (according to a grade 3 sarcoma), it is impossible to achieve the correct diagnosis without appropriate immunohistochemical stainings. It is noteworthy that most reported MC sarcomas occured in tissues not commonly involved by SM (larynx, colon, meningal site). All cases showed rapid progression

and generalization with a terminal phase resembling ("secondary") MC leukemia.

RARE SUBVARIANTS OF MASTOCYTOSIS AND DIFFERENTIAL DIAGNOSES

The following subcategories are either rare subvariants of mastocytosis not yet included in the WHO classification system (1.-4.) or are disorders closely related and therefore often confused with "true" mastocytosis (5.-7.):

1. *SMOULDERING MASTOCYTOSIS (SSM)*: SSM is a defined subcategory of ISM that clinically assumes an intermediate position between ISM and ASM, with a high degree of tissue infiltration including the bone marrow, a high serum tryptase level (>200 ng/ml), and organomegaly, e.g. lymphadenopathy or splenomegaly (B-Findings). By definition, C-findings are not detected in SSM.

2. WELL-DIFFERENTIATED SYSTEMIC MASTOCYTOSIS (WDSM): WDSM is another subcategory of ISM. Compact tissue infiltrates of mastocytosis consisting exclusively of round mature-appearing hypergranulated MC belong to the spectrum of the so-called tryptase-positive round cell infiltrate (of the bone marrow), preliminary termed TROCI-bm. TROCI-bm may present with both localized or diffuse infiltration patterns, and WDSM is included within the differential diagnostic spectrum of localized TROCI-bm. Morphologically, WDSM can be separated from "common" SM by the absence of both CD25 expression on MC and the typical exon 17 point mutations. The only published case of WDSM reported on a unique point mutation of c-kit within the transmembranous domain (F522P) which did not lead to imatinib-resistence seen in patients carrying the typical D816V mutation. In addition, a unique case of WDSM was recognized within the spectrum of SM-AHNMD presenting with an associated monoblastic leukemia (own unpublished observation).

3. MONOCLONAL MAST CELL ACTIVATION SYNDROME: This disorder comprises a group of patients clinically presenting with recurrent episodes of anaphylaxis, no skin lesions, and only one or two minor diagnostic criteria for SM but lacking the major criterion of a compact infiltrate. In this subdiagnostic condition, MC may display cytomorphological atypia, aberrant expression of CD25 or the presence of D816V but all three features (which would be sufficient for the diagnosis of SM) are not detectable. A follow-up of such patients may reveal SM, whereas progression into high gade SM seems unlikely and has not described so far.

4. *OCCULT MASTOCYTOSIS*: Occult mastocytosis presents in two different variants. On the one hand, it can be a rare occurence within the spectrum of SM-AHNMD and is detected after eradication of the "AHNMD" by adequate chemotherapy and then, retrospectively, may be also found in the initial biopsy specimens where it was obscured by the widespread neoplastic non-MC clone. This can only be achieved after appropriate immunohistochemical and molecular analysis. Despite complete hematological remission infiltrates of SM usually persist or even progress signaling that still one part of the neoplastic process is still present. On the other hand, it was possible to analyse tissues which had been removed years before the diagnosis of SM was established. Although there was no morphological evidence of a tissue infiltrate of SM, molecular analysis yielded the presence of an activating c-kit mutation up to 10 years before morphological manifestation of SM.

5. *MYELOMASTOCYTIC LEUKEMIA (MML)*: MML represents a rare advanced myeloid neoplasm (usually a myelodysplastic syndrome of RAEB type or even AML by WHO criteria) exhibiting more than 10% metachromatic immature cells (often metachromatic blasts) in a bone marrow or blood smear but not fulfilling criteria for diagnosis of SM. In particular, there are no compact MC infiltrates, there is no aberrant phenotype of MC with coexpression of CD25 and there is no activating point mutation of c-kit. Usually, a significant increase in CD34+ progenitor/blast cells is detected. Tentatively, MML is best categorized as a subgroup within the MDS/MPS overlap syndromes.

6. *TRYPTASE+ ACUTE MYELOID LEUKEMIA*: Tryptase+ AML is also a rare finding and characterized by strong expression of tryptase and less frequently also of KIT (CD117) by myeloblasts in an otherwise morphologically unremarkable AML (often subtypes FAB M1, M2, or M4-eo). Tryptase+ AML lacks criteria for diagnosis of SM. The separation of tryptase+ AML from MML is possible by counting metachromatic cells in blood and/or bone marrow smears: presence of more than 10% metachromatic cells with signs of MC differentiation argues for the diagnosis of MML.

7. *BASOPHILIC LEUKEMIA (BAL)*: BAL is an extremely rare subvariant of myeloid leukemias and until now could not be diagnosed histologically in bone marrow trephine biopsy specimens. BAL also belongs to the spectrum of TROCI (diffuse type) since it could be shown that neoplastic basophils do express immunohistochemically detectable amounts of tryptase. Definitive diagnosis of BAL is only possible when basophil-related antibodies like 2D7 and/or BB1 are used for immunohistochemical analysis of a trephine biopsy specimen. In contrast to MC granules, metachromatic granules of basophils are water-soluble and therefore cannot be detected in routinely processed formalin-fixed tissues. In most published cases of BAL, the underlying disease was classified as Ph+ CML. Recently, a unique case of secondary basophilic leukemia in a patient with Ph+ CML with associated SM was diagnosed retrospectively in an analysis of almost 200 cases of CML (own unpublished observation).

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Table 1
WHO criteria for Diagnos of Systemic Mastocytosis

Major:* Multifocal compact infiltrates of MCs in bone marrow or other extracutaneous organ(s) (>15 MCs)

Minor:* a. MCs in bone marrow or other extracutaneous organ(s) show an abnormal spindle-shape morphology (>25%)
b. c-kit mutation D816V in extracutaneous organ(s)**
c. MCs in the bone marrow express CD2 or/and CD25
d. Serum tryptase >20 ng/ml (does not count in patients who have an associated hematopoietic clonal non MC lineage disease (= AHNMD)

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* if at least one major and one minor criterion or three minor criteria are fulfilled, the diagnosis SM can be established
** other activating mutations at codon 816 of c-kit also count as a minor criterion MCs, mast cells

Table 2
WHO - Classification of Mastocytosis

Variant - Subvariants	**Abbreviation**
Cutaneous Mastocytosis	CM
- Maculopapular CM*	MPCM
- Diffuse CM	DCM
- Mastocytoma of skin	
- (Mast cell sarcoma of skin)	
Indolent Systemic Mastocytosis	ISM
- Smouldering SM	SSM
- Isolated bone marrow mastocytosis	BMM
Systemic Mastocytosis with an associated clonal hematologic non mast cell lineage disease	SM-AHNMD**
Aggressive Systemic Mastocytosis	ASM
- lymphadenopathic SM with eosinophilia***	
Mast Cell Leukemia	MCL
- Typical MCL	
- Aleukemic MCL****	
(Extracutaneous) Mast Cell Sarcoma	MCS
Extracutaneous Mastocytoma	

* also termed urticaria pigmentosa; ** the subtype of the "AHNMD" has to be defined by WHO criteria as well; *** in a subgroup of these patients, the FIPL1-PDGFRA fusion gene is detectable; **** circulating mast cells are <10%.

Table 3
Diagnostic Work up in Patients with Suspected Mastocytosis

Initial Sign/Symptom	Recommended Diagnostic Procedures
UP-like skin lesions in pediatric cases	1. Skin biopsy (with analysis of c-kit D816V) & serum tryptase (monitoring)* Bone marrow investigation in cases with suspected hematologic disease / SM
UP-like skin lesions in adult patients	1. Bone marrow examinations, skin biopsy & serum tryptase (>20 ng/ml in most cases) 2. In case of SM → complete staging: GI tract, osteodensitometry, y-ray of bones, ultrasound of abdomen, complete blood count, serum chemistry, coagulation parameters, c-kit mutations
Reported mediator symptoms but no skin lesions (UP)**	1. Serum tryptase, if >20 ng/ml → 2. 2. Bone marrow examination, if SM → 3. 3. SM – Staging**
Severe unexplained allergic reaction / anaphylaxis at presentation	1. Serum tryptase, if >20 ng/ml → 2. 2. Repeat serum tryptase a few weeks later: if then, serum tryptase is >20 ng/ml → 3. 3. Bone marrow examination, if SM → 4. 4. SM – Staging**

* In young infants, a serum tryptase level slightly exceeding 20 ng/ml is not regarded as safe indicator for systemic mastocytosis. Therefore, it is recommended to wait and to monitor the serum tryptase level over time in these patients (but do not perform a bone marrow puncture) unless other signs for a systemic hematologic disease are found (organomegaly, osteolyses, severe cytopenias, others).
** Especially in patients with aggressive mast cell disorders, skin lesions are absent. Therefore it is of pivotal importance to know the subtype of SM in these patients as soon as possible. In aggressive SM the serum tryptase level is usually higher than in patients with isolated bone marrow mastocytosis (often <20 ng/ml), a benign mast cells disease in which skin lesions are also absent.

IATROGENIC CHANGES IN THE BONE MARROW

Isinsu Kuzu
Ankara University School of Medicine, Department of Pathology, Morphology Building Level 01, Sihhiye, Ankara Turkiye
isinsukuzu@yahoo.com,ikuzu@medicine.ankara.edu.tr

Bone marrow examination is essential between or at the end of the courses of chemotherapy protocols applied for the therapy of hematological malignancies. The chemotherapeutics, radiotherapy, recombinant growth factors and cytokines create morphological and phenotypic changes to the cellular composition of the bone marrow. The main reason for the bone marrow examination during or after the therapy is monitoring the residual disease or to examine the cause of unexpected prolonged cytopenia during the therapy. The pathologists should be aware of the effects of chemotherapeutics on bone marrow morphology in order to true interpretation of the bone marrow biopsies and the aspirates. The clinicians are also responsible for the true interpretation by giving the full therapy history. The pathologists should address the status of the residual disease, the status of the normal hematopoetic cells regarding the administered therapy. In order to answer several questions, different analysis should be asked and the results are usually combined together for the final decision. The pathologists usually need to review the diagnostic specimen and the previous follow up biopsies and integrate all clinical and biological findings to give a final conclusion report. In this session our aim is to focus on the morphological changes seen in the bone marrow samples following several different therapy regimens used for the treatment of hematological malignancies. While discussing the morphology we also would like to give the goals of therapies for the several different hematological malignancies.

Acute Myeloid Leukemia (AML):
Except Acute promyelocytic leukemia the standard therapy for AML contains remission induction and consolidation regimens. Remission induction therapy contains cytarabine and daunorubicine or other antracyclins. These drugs are used for reduction of the number of blasts less than 5% and to induce the regeneration of the normal bone marrow precursors. The cytarabine is given for 7 days and followed by 3 days antracyclin. Usually bone marrow biopsy is performed for examination of chemotherapy related hypoplasia on the 14^{th} day of therapy.

If cellularity is > %15 and the cellular composition is mostly composed of blasts this means ineffectivity of the first remission induction. If the blasts are <15% this means to repeat the therapy. The patients are received if the remission is achived by the first therapy. The consolidation therapy includes 6 courses of high dose cytarabine. The patients are usually examined by peripheral blood smears.

There are three main morphological steps examined in the bone marrow samples following the therapy. In the first week the cellular death features are dominant with most commonly individual cell death followed by fibrinoid necrotic features. The prominent cellular death causes hypocellularity, stromal edema, sinusoidal dilatation, relative increase in plazma cells and lymphocytes, increase in macrophages and slight increase in reticular fibers. The hypocellularity is striking within the first 2 weeks following the chemotherapy. Bone marrow regeneration usually starts within 2 weeks. The biopsy is usually performed at the regeneration period. Following the acute tissue injury period the adipocytes increase. This process is thought to play a critical role on hematopoetic regeneration ( ). The multilocular lipocytes mature into unilocular forms and hematopoetic regeneration follows this process. In the early phases the hematopoetic regeneration starts at the paratrabecular region. Generally erythroid and granulocytic series appear before megakaryocytic regeneration. At the early stages of erythroid regeneration dysplastic changes can be seen which disappears shortly. With ongoing regeneration of the hematopoetic cells with adipoctytes the fibrnoid necrosis and reticular fibers disappear. The complete remission for the AML patients is characteristic with absence of blastic cells and achievement of normal hematopoesis in the bone marrow. The patients without remission the bone marrow is consistent with ongoing cellular damage and blastic cells.

The other scenarios of the post chemotherapy effects on bone marrow can be increase in hematogones, hematopoetic regeneration failure, increased groups of megakaryocytes and promyeloctes with fibrosis. Increased groups of promyeloctes could be misinterpreted as the relapse of acute leukemia or acute promyelocytic leukemia. Prominent increase in megakaryocytes could be seen in patients having induction therapy. But spontaneous regression of this feature is expected.

Fibrosis after chemotherapy usually regresses in AML patients. But patients who have secondary AML transformed from chronic myeloproliferative diseases may have prominent fibrosis.

Acute Promyelocytic Leukemia (AML M3)
This type of acute leukemia is characteristic with the genetic abnormality t(15:17) and with clinical signs of pancytopenia and quagulation defects. The characteristic genetic abnormality causes maturation defect in myeloid precursors. For this reason the pathogenesis and also the therapy for this type of leukemia differs from the other types. Antracylins and all-transretinoic acid (ATRA) are combined on remission induction for these patients. Usually bone marrow examination is performed on the 28^{th} day of the therapy. The cellularity of the bone marrow should be examined. The bone marrow features are different than the other AML forms in AML M3 patients. Frequently the patients do not have bone marrow hypoplasia and do show granulocytic maturation due to ATRA effect. ATRA induces leukocytosis at the beginning of the therapy and by its maturation effect it causes maturation of the promyleocytes with translocation to granulocytes. The maturation of the leukemic clone can be examined by FISH. There is usually prominent leukocytosis present but very rarely necrosis or fibrosis may be also be seen following ATRA therapy.

Arsenic trioxde can be performed for its apoptosis inducing effect on neoplastic promyelocytes. This therapy can cause leukopenia at the beginning but within 15-20 days the increase in mature granulocytes with degreased blast are examined in the bone marrow. Combination therapy of arsenic trioxide and ATRA may induce leukocytosis and necrosis in the bone marrow.

Precursor Acute Lymphoblastic Leukemia (ALL)

The therapy stages of ALL are remission induction, central nervous system prophylaxis, and consolidation. The remission induction therapy contains prednisolon, vincristine and antracyclins. Cyclophospamide and L-asparaginase may be added. Remission with 60-90% can be achieved by 4-6 weeks of therapy courses. The bone marrow should contain less than 5% blasts with normal cellularity, regression of clinical symptoms with normal blood count are the hallmarks of remission. There should be long consolidation therapy periods in order to keep the patients in remission. The bone marrow examination is performed on the 14^{th} and the 28^{th} day of treatment for evaluation of the leukemia. The true interpretation of the bone marrow biopsies can be done if the pathologists are well informed about the therapy status of the patient at the time of the biopsy was taken. The bone marrow changes are the same as described in the AML cases at the early stages of the biopsy consistent with cellular necrosis. But the biopsies are usually performed after the hypocellular stage. The main reason for the biopsy is examination of the residual disease. The biopsy should be examined with the aspiration smears and /or touch prints. The amount of blasts and the cellular composition of the bone marrow should be evaluated. Flow cytometry and cell smear results should be compared to the biopsy for the good representation status. Immunohistochemistry and cytogenetics for minimal residual disease evaluation can be performed.

Myelodysplastic Syndrome (MDS)

MDS is a heterogeneous disease; therefore the biopsies at the diagnosis are very important for making true interpretation of the follow up biopsies after the treatment. The goal of therapy in MDS includes hematological response, cytogenetic response and maintaining the quality of life. The effective therapy of MDS is bone marrow transplantation. Because of the mortality and morbidity of transplantation due to the age of the patient and the risk category of the disease is different, several different therapy alternatives could be performed. Growth factors such as erythropoetin (EPO) and granulocytic colony stimulating factor (G-CSF); immunosuppressive agents such as Anti thymocyte globuline (ATG), cyclosporine; antiangiogenic agents such as thalidomide, revlimide; hypomethylating agents such as azacitidine and decitabine; and chemotherapeutics used in AML could be performed.

Supportive therapy with (EPO) increases the number of erythroid precursors and dicreases the myeloid/ erythroid ratio in the bone marrow samples. When G-CSF is added to the therapy the granulocytic precursors increase. The promyelocytes are usually uniform in morphology in treated patients which could be helpful for the differentiation of therapy related and disease related changes. There could be reactive plasma cell and histiocyte proliferation seen.

The interactions with dysplastic hematopoetic precursors and the microenvironment may be important in the biology of the disease. Vascular endothelial growth factor (VEGF) and increased amount of inflammatory cytokines such as Tumor Necrosis Factor (TNF) may cause the down regulation of the receptors for the cellular maturation. Thalidomide, and revlimide are used for regulating the effects of cytokines and may be useful for the MDS patients who are dependent to transfusion.

Epigenetic changes such as hypermethylation may play an important role on inactivation of some genes which play role in impairment of normal cellular differentiation and may cause leukemic transformation. Hypomethylating agent azacitidine or decitabine are preferentially used for alternative therapeutics in MDS. Their major toxicity is severe myelosupression which may increase the infection rates.

One important problem for the pathologists and the hematologists is the differentiation of the reactive changes and the residual disease following aggressive therapy in MDS patients. Megaloblastic changes and slight displastic changes in erythroid calls are non specific. The reactive changes are expected to disappear within 2-3 months. If they are still examined more than 3 months following therapy, depending on the therapy regimen this finding could be interpreted as residual disease.

Chronıc Myeloid Leukemia (CML)

The thyrosine kinase inhibitors, most frequently Imatinib is used in achievement of the hematological, morphological and molecular remission of the chronic phase CML patients. These agents cause reduction in overall marrow cellularity, and building up the normal ratios and morphology of myeloid, erythroid and megakaryocytic elements of the bone marrow. This treatment can also be effective on decreasing the bone marrow fibrosis. The most important parameter in the fallow-up of the CML patients is the molecular remission.

Hodgkin's and Non Hodgkin's Lymphomas:

The bone marrow biopsy should be performed at the time of diagnosis in lymphomas. Combination therapies are performed by using different chemotherapeutics in Non Hodgkin's and Hodgkin's Lymphomas. The bone marrow changes include early effects of drugs seen during and at the end of therapy or late effects seen several years following the therapy. The drugs are toxic either to the neoplastic or to the normal hematopoetic cells. Control bone marrow biopsy at the end of treatment is performed for the patients who have bone marrow involvement at the diagnosis. If neutropenia is observed early after treatment, G-CSF could be applied for 7-10 days. Additional anti CD20 immunotherapy is performed for the B cell lymphomas. Anti CD52 is another immunotherapy alternative for the lymphomas which express this antigen. Several aggressive combination regimens can be applied for aggressive recurrent cases. Radiotherapy could be performed for the patients who have localized disease. Recovery of

hematopoesis could be delayed due to the drugs used for therapy. In these circumstances bone marrow biopsy could be performed earlier and effects of therapy can be examined. These are decreased amount of hematopoetic elements due to the delayed hematopoesis or increased myeloid precursors due to the growth factors. Massive necrosis can be seen if massive involvement is present. Prominent decrease in hematopoetic and stromal elements can be seen in patients who had radiotherapy. Dysmophic hematopoetic elements can be seen. Erythroid elements can be affected more and the myeloid / erythroid ratio can be increased. The erythroid elements are more sensitive to the drugs comparing to the myeloid elements and megacaryocytes.

Bone marrow biopsy is most commonly performed to examine the residual disease for the patients who have involvement at the diagnosis. In anti CD20 treatment for B cell lymphomas, the antibody combines with the antigen on the membrane of the neoplastic and non-neoplastic B lymphocytes. The biology of immunotherapy with anti CD20 depends on the increased reactive T cell response. The anti CD20 immunohistochemistry for highlighting the neoplastic cells will be unsuccessful as the antigens are opsonized by the drug. For this reason another specific B cell marker such as CD79a should be used for imunohistochemistry. On hematoxylin and eosine (HE) stained sections, increased reactive T lymphocytes due to the therapy could be miss interpreted as residual disease if immunophenotyping is not performed. Immunophenotyping is also important on examination of the residual disease in T cell lymphomas involving the Bone marrow. Residual lymphomas can be monitored by using clonality analysis or quantitative molecular methods and FISH analysis for the lymphomas which have specific chromosomal abnormalities.

Late Effects of Chemotherapy:

About 5-10% of patients who receive multiagent chemotherapy and /or radiotherapy develop chromosomal damage and clonal hematopoetic diseases. Secondary MDS and secondary leukemia are the most common types of neoplasia. Alkylating agents and radiotherapy are the most frequent therapies responsible for this. Myelodysplasia with blastic elements are examined in the bone marrow biopsies. Topoisomerase II inhibitor is another agent that causes secondary leukemia. These leukemias are most commonly monoblastic and do not show myelodysplasia.

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