

The Diagnosis from the Pathological Viewpoint of a Blood Disease

Masafumi Ito

Department of Pathology, Nagoya University Hospital, Japan

Abstract

The histopathological viewpoints of a blood diseases are namely following the cytological examinations except for the viewpoint of organ pathology and has been required only the usefulness in case with the difficulty of cytological examination. If it looks at a blood disease as hematopoietic organ disease, histopathological diagnostic study might make the new paradigm of blood diseases. In this time, I would like to present the hematological diagnosis from the pathological viewpoint by using histological bone marrow sections.

- *How to examine the bone marrow histology
- *Hematopoietic microenvironment disorder
- *Differential diagnosis of hypoplastic marrow lesions
- *Clinicopathological characteristics of Hypoplastic leukemia
- *Hemophagocytosis in bone marrow
- *How to diagnose MDS by histopathology

Bone marrow histology is the valuable diagnostic tool of many kinds of marrow disorders especial cases. By using immunohistochemistry and Giemsa staining, further information might obtain than smear film cytology. Bone marrow clot section aspirated from sternum is enough for histological examination except bone marrow biopsy. Precise cytomorphology might demonstrate by smear film than section histology. However surface phenotype would define by flow cytometric analysis, immunostaining of sections could demonstrate which cells show which markers. Structural and architectural disorder could only be represent by histology. The histological examination of bone marrow might introduce a new aspects of blood disease.

1. Introduction

Hematological disorders are unusually recognized as abnormality of peripheral blood, and are analyzed in blood cytology. Hematocytological diagnosis makes the mainstream of blood study. The histopathological viewpoints of a blood diseases are namely following the cytological examinations except for the viewpoint of organ pathology. Histopathological diagnosis of blood diseases has been required only the usefulness in case with the difficulty of hematocytological examination, for example myelofibrosis and metastatic tumors. If it looks at a blood disease as hematopoietic organ disease, histopathological diagnostic study might make the new paradigm of blood diseases.

2. How to Examine the Bone Marrow Histology

We have examined mainly the clot material of aspirated bone marrow juice from sternum. The marrow extracted from the sternum has a difference in cellularity, especially of elderly patients, compared with the bone marrow biopsy from iliac crest. Higher cellular marrow specimens demonstrate the true information of hypocellular marrow disorders. Usual paraffin embedded material is enough for histological evaluation. Giemsa staining or Giemsa and AS-D chloroacetate esterase double staining is certainly needed and HE staining is less usefulness of bone marrow examination. By using immunostaining of paraffin sections, surface phenotyping is possible to contrast with morphology and repeating im-

munostaining is also possible. Many antibodies are available for paraffin section immunostaining. The characteristic of histopathological examination is suitable for the diagnostic procedure of hematopoietic microenvironment disorders, hypoplastic marrow lesions and lymphoproliferative disorders.

3. Hematopoietic Microenvironment Disorder

Abnormalities of stromal element are not associated the cytological changes of bone marrow cells. Typical histological change is the myxofibromatous degeneration of the bone marrow stroma. In case with this stromal change, the bone marrow cells are proliferated with separated colony formation. This stromal change is introduced by hyponutritional situation, cachexic change with malignancy, renal failure and infectious disease; e.g. tuberculosis, HIV infection. Although the histological change is non-specific, aplastic anemia or MDS is less associated the myxofibromatous stromal change.

Case example:

73 y.o. Lady

CC: loss of appetite, body weight loss

Laboratory data showed mild pancytopenia, WBC 0.6 gig/L, Hb 7.5 g/dl, plt 151 gig/L, Fe, VB12, FA, and LDH were in WNL. Three months before, only mild anemia was detected (WBC 5.1 gig/L, Hb 9.5 g/dl, plt 310 gig/L).

Bone marrow examination was performed. Cytomorphological abnormality was not detected in smear film. Bone marrow clot section demonstrated normocellular marrow with marked myxofibromatous degeneration of the stroma and the proliferation of erythroid lineage was prominently separated. No metastatic tumor and granuloma was observed. Immunostaining of clot section demonstrated no HbF+ erythroblasts and p53+ cells. Histopathological diagnosis is secondary dyshematopoiesis with myxofibromatous degeneration of stroma caused by hyponutritional situation, bearing malignancy elsewhere suspected. No evidence of aplastic anemia and MDS was diagnosed.

4. Differential Diagnosis of Hypoplastic Marrow Lesions

Hypoplastic marrow lesion was consisted with small number of marrow cells. Differential diagnosis between aplastic anemia (AA), hypoplastic MDS (hypo MDS) and microenvironment disorder was sometimes difficult. Typical morphology of AA is reduced cellularity, low number of megakaryocytes, and a predominance of lymphocytes, plasma cells and mast cells. However, bone marrow of hypo MDS shows also similar morphological changes as aplastic anemia. Especially in case of hypo MDS with monosomy 7, cytomorphological atypia is often seldom, number of megakaryocytes is decreased and mast cells is prominent as AA. Recently we have demonstrated to be able to distinguish both lesions by using HbF and p53 immunostaining. To investigate the

significance of HbF-containing erythroblasts (F-blasts) and p53 expression in making the distinction between hypoplastic MDS and acquired AA, we immunohistochemically assessed F-blasts and p53 in bone marrow specimens from 16 patients with hypo MDS, 31 patients with acquired AA, and 15 hematological normal individuals. F-blast production was elevated in 87.5% (14/16) of patients with hypo MDS, but in only 3.2% (1/31) of patients with AA ($P < 0.01$). p53 was overexpressed in 75.0% (12/16) of hypo MDS patients and in 6.4% (2/31) of AA patients ($P < 0.01$). The mean contents of F-blasts and p53-positive cells in patients with hypo MDS were 6.31/3.27% and 7.54/4.36% of all bone marrow cells, which were significantly higher than for patients with AA (0.35/0.46% and 0.58/1.29%, $P < 0.01$). We conclude that a high prevalence of elevated F-blast production is noted in hypo MDS, suggesting that the assessment of F-blasts in bone marrow can be used as an additional tool for differentiating hypo MDS from acquired AA.

Case example:

75 y.o. gentleman,

CC: fever of unknown origin

PH: chronic renal failure, operation of colon cancer

Patient was admitted by fever of unknown origin. Laboratory findings showed pancytopenia (WBC 1.8 gig/L, Hb 6.8 g/dl, and plt 30 gig/L).

Bone marrow clot section was demonstrated marked hypocellular marrow (<5%) with low number of megakaryocytes without prominent mast cells. Erythroblasts with mild dysplasia and micromegakaryocytes were observed. Immunohistochemical examination was revealed increased number of HbF+ erythroblasts and p53+ cells. Our histopathological diagnosis was hypoplastic MDS without excess blasts. Cytogenetic results demonstrated trisomy 8.

5. Hypoplastic Leukemia

Hypoplastic acute leukemia (HAL) is a rare atypical leukemia characterized by a hypocellular or normocellular marrow (less than 50% cellularity on core biopsy), more than 30% marrow blasts, and absent to low levels of circulating blasts. The incidence of HAL is 5-10% of all acute leukemia. It is sometimes difficult to diagnose accurately, because determination of the blast percentage may be challenging by its hypocellularity. Also the optimal treatment remains unclear. Since HAL presents with pancytopenia and hypocellular bone marrow, it is important to distinguish HAL from hypo MDS or AA. All blasts of HAL were CD34 expression, and less of CD13, MPO, TdT occasionally expression. These blasts are categorized in AML M0. Blasts proliferate to replace of normal structure without expansion. Low power view showed almost normal proliferation pattern. Decreased number of megakaryocytes and an often-increased number of mast cells are associated. Dyserythropoiesis is not detected. Cytogenetics shows no chromosomal abnormality. Immunostaining shows no expression of

HbF on erythroblasts. P53+ cells are seldom observed. Long time follow up cases show no increasing blasts counts and rather favorable prognosis without cytoreduction therapy. Early phase of normal AML must be distinguished. Histopathological examination could distinguish each other. Diagnosis of definite HAL is clinically important.

Case example:

86 y.o. Gentleman,
CC: general fatigue

He was suffered from general fatigue without causes. No evidence of lymphadenopathy and hepatosplenomegaly was observed. Laboratory data showed pancytopenia (WBC 1.8 gig/L, Hb 9.9 g/dl, and plt 32 gig/L) and no blast was identified. Bone marrow examination was performed. Bone marrow clot section was showed hypocellular marrow (cellularity <20%) and 40% blasts, which had scarce basophilic cytoplasm, round nuclei with prominent small nucleoli. Increased number of mast cells and decreased number of megakaryocytes with micromegakaryocytes, no dyserythropoiesis was observed. Immunostaining demonstrated that the blast showed CD34+, MPO+/-, and TdT-, CD13-/, p53-, Ki67 index 50%. Hematopathologically he was diagnosed hypoplastic leukemia. He was treated only supportive therapy with blood transfusion except for any cytoreduction therapy. Frequent bone marrow examinations were showed almost same morphology, hypocellular marrow, excess blasts and no dysplastic change. Cytogenetics showed normal karyotype. Seven years later, he was died with pneumonia. Autopsied bone marrow demonstrated same morphology as initial bone marrow examination.

6. Hemophagocytosis in Bone Marrow

Hemophagocytic syndrome (HSP) presents with fever, splenomegaly, pancytopenia, liver dysfunction and coagulation changes. Histiocytic hyperplasia and hemophagocytosis occur in bone marrow. Reactive HSP is commonly caused by bacterial or viral infection, occurring either in previously healthy subjects or as terminal complication in patients with immunocompromised host. EBV infection and T cell malignancy is sometimes associated hemophagocytosis. Recently, in Asian country specific type of B cell lymphoma is associated hemophagocytosis. B-cell lymphomas associated with HPS are rare in Western countries, while the association is being reported increasingly in Asian countries. Such cases showing IVL characteristics have been reported exclusively from Asian countries, so this disease was proposed to be a new variant of IVL. BM involvement, HPS, and a rapidly aggressive clinical course characterized this variant. We immunohistochemically investigated 146 bone marrow (BM) clots from the patients with fever of unknown origin (FUO; n = 124) or hemophagocytic syndrome (HPS; n = 22). Among the 146 patients, 12 cases (8.2%) of intravascular lymphoma (IVL) primarily involving BM were detected. Diagnosis was made based on the presence of CD20+ and CD79a+ tumor cells confined

within the lumen of sinuses and surrounded by CD34+ endothelial cells. Among the 12 cases, 6 were CD5+ and of these, 5 were positive for vimentin. A considerably high prevalence of IVL in BM from the patients with FUO or HPS suggests that immunohistochemical examination of BM may be helpful in the diagnosis of IVL in these patients. Vimentin co-expression in CD5+ IVL might be evidence of origin from a subset of prefollicular B cells.

Case example:

69 y.o. Gentleman
CC: pneumonia

He was suffered from pneumonia and admitted. In spite of multiple antibiotics and steroid therapy, fever of unknown causes was continued and pancytopenia was progressed. Lymphadenopathy and hepatosplenomegaly was not detected. Bone marrow examination was performed. Bone marrow clot section presented normocellular marrow with marked hemophagocytosis and cluster of small numbered large atypical cells. These large atypical cells were CD20+ B-cells and located intrasinusoidal space. The number of lymphoma cells was less than 1% of total bone marrow cells. Histopathologically, he was diagnosed intravascular lymphoma of large B-cell type with hemophagocytosis. He was treated with intensive chemotherapy, two months later he was died.

7. How to Diagnose MDS by Histopathology

The MDS are diseases consequent on a clonal hemopoietic disorder characterized by dysplastic, ineffective hemopoiesis. There is thus often a discrepancy between a hypercellular marrow and peripheral cytopenia. Bone marrow smear film could demonstrate the cytomorphological atypia with dysplastic features. The count of blasts could subdivide the MDS. Histopathological finding of the MDS is characterized not only the cytological atypia but destruction of well-ordered marrow cells proliferation such as destroyed erythroid islets. By immunostaining, CD34 staining could demonstrate the blast population. Recently we have published the immunohistochemical study of MDS cases concerning the phenomenon of ineffective erythropoiesis with HbF. To investigate the relationship between the HbF-containing erythroblasts (F-blasts) and apoptosis in MDS, we immunohistochemically assessed F-blasts, F-cells, and apoptosis in 137 patients with MDS. A marked increase in the number of F-blasts in the bone marrow was identified in 116 of 137 patients (84.7%), and the number of F-cells was elevated in 54 patients (39.4%). Among the erythroblasts stained by anti-glycophorin C antibody, the mean percentage of F-blasts was 14.63/9.17% in MDS, which was significantly higher than that in non-MDS patients with stress erythropoiesis (4.82/3.35%, $P < 0.01$), although there were no significant differences in the number of F-cells between these groups. In particular, 62 of the 137 MDS patients (45.3%) had an apparent increase in F-blasts but no elevation of F-cells. The apoptotic rate was significantly higher in the

patients with a F-blast/F-cell (Fb/Fc) ratio >5.0 than in those with a Fb/Fc ratio <1.0 ($P < 0.01$). The results indicate that F-cell precursors are incapable of maturing into functioning end-stage F-cells, presumably owing to apoptotic cell death. The measurement of F- blasts in the bone marrow is needed for the precise evaluation of fetal-type erythropoiesis in MDS. We diagnose and subcategorize MDS by using histopathological sections with HbF, p53 and CD34 immunostaining.

Case example:

54 y.o. Gentleman,

CC: angina, coronary angio-bypass graft operation

He was suffered angina with coronary artery stenosis and treated with coronary angiobypass operation. Pancytopenia was detected after operation. WBC 1.6 gig/L, Hb 6.0 g/dl, plt 15 gig/L, 1% of blast was detected. Bone marrow examination was performed. Bone marrow clot section revealed hyper cellular marrow (cellularity 60-80%), M/E=4-5, megakaryocyte count 8-10/HPF with micromegakaryocytes, prominent dyserythropoiesis and excess blast (20%). Erythroid islet formation was destroyed. Immunohistochemical findings were increased number of p53+ cells, gathering and clustered strong positive HbF of erythroblasts with dysplastic features and increased numbered CD34+ blasts. These blasts were small clustered, that was suspected blastic transformation.

Conclusions

Bone marrow histology is the valuable diagnostic tool

of many kinds of marrow disorders especial cases. By using immunohistochemistry and Giemsa staining, further information might obtain than smear film cytology. Bone marrow clot section aspirated from sternum is enough for histological examination except bone marrow biopsy. Precise cytomorphology might demonstrate by smear film than section histology. However surface phenotype would define by flow cytometric analysis, immunostaining of sections could demonstrate which cells show which markers. Structural and architectural disorder could only be represent by histology. Although the histological examination of bone marrow is quite old fashion method, new aspects of blood disease might elicit insofar as blood disease depends on bone marrow as hematopoietic organ.

References

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