

Progress in bone marrow pathology

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Myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) include a heterogeneous group of clonal hematopoietic disorders, characterized by irreversible derangement in the development of the hematopoietic cell lines which result from the progressive impairment of precursor cell maturation and ineffective hematopoiesis (1, 2). MDS usually present with peripheral blood cytopenia of one or several cell lines and with a hyper- or normocellular bone marrow.

MDS most often occur as idiopathic diseases (primary MDS) in elderly people but may also occur after radiotherapy or chemotherapy (secondary MDS). Primary MDS have occasionally been described in children (3).

The current concept of MDS is that they represent the early phase of an acute, most often nonlymphoid, leukemia (AL) or acute myelogenous leukemia (AML). An overt AL phase can supervene in one-third of the patients but this pathway of evolution is not obligatory and death is most commonly caused by infection or hemorrhage resulting from marrow failure.

Apoptosis plays a key role in MDS. It is related, in apparently opposite directions, to ineffective hematopoiesis and leukemic transformation; excessive apoptosis in the former and escape from apoptotic control in the latter. The cells undergoing apoptosis are restricted to CD34 negative cells (maturing compartment) whereas CD34-positive cells are almost never apoptotic (4-6). A possible unrestrained dual action of cytokines in the hematopoietic microenvironment is suspected. Candidates are tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β), interferon- γ (IFN- γ) and interleukin-1 β (IL-1 β) (5, 7). High proliferation activity of hematopoietic cells may be counteracted by the high level of medullary cell death, associated with lower bcl-2 expression (8).

The progression of MDS, which is related to accumulation of immature myeloid cells, is characterized by decreased apoptosis and increased bcl-2 expression (9).

Other biological parameters have been investigated in MDS. According to Orazi *et al.* (10), p53 + MDS and AML correspond to a distinct group of marrow disorders characterized by a high rate of intramedullary cell death. The role of MDR multidrug resistance gene encoding for a transmembrane protein, P-glycoprotein (PGP)-expression is at the preliminary stage of understanding (11). It seems that PGP-expression is common in the cases of MDS with other poor prognosis factors. RAS genes have potent effects on the differentiation and proliferation program of cells and appear to be involved in primary and secondary myeloid leukemias. The RAS gene family has been associated with disease progression from MDS to AML (12).

The diagnosis of MDS relies on a combination of data, including clinical status, morphologic evaluation of the peripheral blood smear, bone marrow aspirate and biopsy specimen as well as cytogenetic analysis.

The French-American-British (FAB) morphological classification (13) has allowed clearer delineation of MDS among workers as well as the publication of large series of patients. Five types of MDS are described according to the FAB proposals: refractory anemia, refractory anemia with ring sideroblasts, refractory anemia with excess of blasts, refractory anemia with excess of blasts in transformation and chronic myelomonocytic leukemia.

Scoring systems and multivariate analyses have generally demonstrated the prognostic value of age, the importance of cytopenias and the percentage of marrow blasts (14-17). An unfavorable cytogenetic group (patients with complex karyotypes) has been identified (18, 19).

Over the last 15 years, the introduction of bone marrow biopsies in MDS has led to consideration of histological prognostic parameters, such as cellularity, fibrosis, abnormal localization of immature precursors (ALIP) (20) and CD34 immunohistochemistry. The use of the FAB classification on sections allows a better correlation between the cytology and biopsy specimens (21).

The negative prognostic impact of histological parameters, such as quantity of blasts, marrow fibrosis and ALIP has been demonstrated (14, 16, 20, 22-26). According to Oriani *et al.* (25), the presence of ALIP identifies patients with a worse prognosis, irrespective of the FAB subtypes.

CD34 immunostaining is a simple and reproducible investigation, which can be easily performed on routine bone marrow biopsy due to the availability of monoclonal antibodies reactive on aldehyde-fixed, paraffin-specimens (25-27). CD34 is a transmembrane glycoprotein selectively expressed on human hematopoietic bone marrow stem cells and on lymphoid and myeloid progenitors. Its expression is normally confined to 0.1-0.5% of nucleated cells in the peripheral blood and to 0.8-5% of mononuclear cells in adult bone marrow (25). An increased percentage of CD34-positive cells has been observed in bone marrow biopsy from MDS patients (25, 28). According to Oriani *et al.* (25), the presence of "large" and CD34-positive aggregates of immature cells represents a negative prognostic factor. Moreover, CD34 immunostaining could be a reliable method of distinguishing ALIP from "pseudo-ALIP". In our experience, CD34/QBEND10 appears to be an excellent marker for hemopoietic progenitor cells in decalcified bone marrow biopsies and is of great help in the assessment and follow-up of MDS on bone marrow biopsy (27).

Although not included in the FAB system, two variants of MDS correspond to clinicopathologic entities: MDS with bone marrow fibrosis and hypoplastic form of MDS.

In both situations, bone marrow biopsy is required for the diagnosis. Several studies have reported an unfavorable prognosis in MDS with fibrosis (14, 23, 24). In a study by Maschek *et al.* (24),

17.3% of 352 MDS patients presented with marrow fibrosis. Fibrotic cases were characterized by higher frequency of cytogenetic aberrations, lower value of hemoglobin and lower platelet counts, marrow hyperplasia, dysplasia in megakaryopoiesis and poor prognosis. A higher incidence of marrow fibrosis was observed in chronic myelomonocytic leukemia.

The differential diagnosis between fibrotic MDS and chronic myeloproliferative disease (idiopathic myelofibrosis, chronic myeloid leukemia) may be extremely difficult and borderline cases probably exist (29, 30). In this situation, clinical history, precise hematological data and chromosomal analysis are mandatory.

Although bone marrow is usually described as normo- or hypercellular in MDS, it appears to be hypoplastic in 7.7-19% of the cases, according to the literature (31, 32). Bone marrow cellularity does not appear to be an important factor in prognosis (33).

Differentiating hypoplastic MDS from aplastic anemia can at times be extremely difficult. Careful attention should be paid to the presence of clusters of blasts in the bone marrow biopsy. Significant morphological dysplasia (dysmegakaryopoiesis, presence of micromegakaryocytes) and reticulin fibrosis are essential in the diagnosis of MDS. The finding of a cytogenetic abnormality common to MDS and AML would support a diagnosis of MDS. Orazi et al. (32) have recently reported that immunohistochemistry could enable these conditions to be distinguished. They demonstrated that aplastic anemia is characterized by low expression of proliferating cell nuclear antigen in bone marrow and reduced CD34 frequency compared with MDS, supporting the concept of an early deficiency of stem cells in aplastic anemia.

Overlapping cases probably exist. Recently, increasing evidence has been collected supporting the hypothesis that acquired aplastic anemia, paracytic nocturnal hemoglobinuria and MDS may be linked by a common hematopoietic stem cell defect (34). Long-term observation of patients with acquired aplastic anemia demonstrates that the incidence of late clonal evolution after treatment with antilymphocyte globulin is much higher than previously suspected (35).

When considering the diagnosis of MDS on biopsy, the following entities must be considered in the list of differential diagnosis: i) marrow disturbances resulting from nutritional disorders (vitamin $_{12}$ or folate deficiencies), infectious diseases (tuberculosis, HIV and parvovirus infection) and toxic effects of drugs; ii) chronic myeloproliferative syndromes; iii) aplastic anaemia; iv) paroxysmic nocturnal hemoglobinuria; v) pure red cell aplasia; vi) hypoplastic leukemia; and vii) large granular lymphocyte disorders with cytopenia.

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Pediatric preleukemic disorders

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For many pathologists, diagnosing preleukemic disorders is often a difficult diagnostic problem, not least because the incidence of these diseases is very low. Diagnosing preleukemic disorders in children is even more difficult. Bone marrow biopsies in these situations are usually seen only by pathologists in specialized centers. Nevertheless, every pathologist should know about preleukemic situations in childhood. The situation is not only complicated by the fact that most pathologists have little experience with pediatric bone marrow but also because there is little information about the normal histology of bone marrow in newborn and young children. Another complicating factor is the large spectrum of diseases that can develop into leukemia, many of which are very rare. A final complicating factor is that the diagnosis of these diseases is often based on very subtle and difficult to diagnose bone marrow changes.

Pediatric preleukemic disorders can be divided into three main groups: i) hereditary preleukemic disorders, clinically manifested by single-cell cytopenias as well as by pancytopenias; ii) primary myelodysplastic syndromes, including juvenile chronic myeloid leukemia and infantile monosomy-7; and iii) secondary myelodysplasia as a late complication of radiotherapy and/or chemotherapy.

Hereditary preleukemic disorders

Most syndromes involving bone marrow failure (single-cell cytopenias and pancytopenias of both the inherited and the acquired type) are, in at least a few patients, associated with the subsequent appearance of leukemia. These disorders involve the red cell series, the granulocytic series as well as lymphopoiesis abnormalities. In a number of cases all cell lines are involved. The most important single-cell cytopenias with an increased risk of development of leukemia are pure red cell aplasia, Kostmann's syndrome and Shwachman's syndrome. Pancytopenias with an increased risk of developing leukemia are Fanconi's anemia, Bloom's syndrome, aplastic anemia, and familial bone marrow failure. Other disorders with increased risk are trisomy-21 and ataxia telangiectasia.

Single cell cytopenias

Pure red cell aplasia

Pure red cell aplasia (Diamond-Blackfan anemia) is a severe macrocytic or normocytic anemia characterized by an isolated depletion of erythroid precursors. The disease is usually seen in the first year of life.

The pathogenesis of the disease is probably an intrinsic defect of the erythroid precursor cells. Histologically, most bone marrow

shows erythroid hypoplasia or aplasia. Many congenital abnormalities are seen in children with pure red cell aplasia among which abnormalities of the head and upper limbs are prevalent. There is a slightly increased risk of acute myelogenous leukemia (AML) and myelodysplastic syndromes (MDS).

Kostmann's syndrome

Kostmann's syndrome is a rare disease, also known as infantile genetic agranulocytosis. It is also referred to as severe chronic neutropenia. Usually, children with this disease develop severe pyogenic infections and extreme neutropenia in the first half-year of life. Histologically, the bone marrow shows an absence or markedly decreased number of myeloid precursors. Most patients die of infections and some develop AML.

Shwachman's syndrome

Schwachman's syndrome is a rare multiorgan disorder characterized by a variable neutropenia in patients with a large number of other abnormalities. Disease symptoms may resemble those of cystic fibrosis. Approximately 25% of the patients develop aplastic anemia. The bone marrow is histologically usually hypocellular. Occasionally MDS or AML is seen.

Pancytopenias

Fanconi's anemia

Fanconi's anemia is the most common inherited form of congenital pancytopenia. It may take years before the hematological abnormalities reveal themselves. Bone marrow examination shows hypocellular or aplastic bone marrows, often with dyserythropoiesis.

Bloom's syndrome and ataxia telangiectasia are characterized by pancytopenic marrow failure.

Primary myelodysplastic syndromes

Primary myelodysplastic syndromes are characterized by maturational disturbances resulting in ineffective hematopoiesis, morphological abnormalities in one or more cell lines and an increased chance of developing acute leukemia. The different MDS subgroups of the French-American-British (FAB) classification apply also to children.

In addition, juvenile chronic myeloid leukemia and the infantile monosomy-7 syndrome are incorporated in the pediatric MDS group.

Juvenile chronic myeloid leukemia

Juvenile chronic myeloid leukemia is a chronic myeloid leukemia without the Philadelphia chromosome. It is characterized by a marked monocytosis and high fetal hemoglobin levels. The peripheral blood and the bone marrow cells show dysplastic features and the prognosis of this disease is poor.

Infantile monosomy-7

The prominent feature of this disease is monosomy or partial deletion of chromosome-7. The histology of this disease is the same as that of the other myelodysplastic diseases. Since monosomy-7 is also seen in other myelodysplastic and leukemic disorders, infantile monosomy-7-related MDS is only diagnosed in children under 3 years of age. The prognosis is relatively good with 40% of the patients surviving 5 years.