Secondary myelodysplastic syndrome

Secondary myelodysplastic syndrome is seen as a complication of radiotherapy and/or chemotherapy. Several papers have reported the development of MDS post-therapy in children. Juvenile chronic myeloid leukemia and infantile monosomy-7 do not occur as secondary myelodysplastic disorders.

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Bone marrow involvement in non-Hodgkin's lymphoma according to the REAL classification

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Introduction

The Revised American European Lymphoma (REAL) classification, a proposal by the International Lymphoma Study Group, was published in 1994. This classification was an attempt to simplify the existing plethora of lymphoma classifications, to update them with recent phenotypical and genotypical data and, most importantly, to offer a reproducible classification acceptable to both Europeans and Americans. While the Working Formulation published in 1982 was indeed meant to serve as a translation system between the various classifications, it became very popular in the United States as "a classification on its own"; in contrast, European pathologists continued to use the Kiel classification. Moreover, it should be noted that both classification systems were based on pure morphology as cases were studied on hematoxylin and eosin or Giemsa stained sections only. To complicate the situation even more, lymphomas presenting with blood and/or bone marrow involvement were identified as lymphoid leukemias but were separately subtyped according another classification, which was proposed by the French American British (FAB) group. As immunohistochemistry and flow cytometry for phenotyping lymphomas became widely performed and as genotyping on these neoplasms by karyotyping and molecular techniques became available, these lymphoid malignancies were better understood and "new" lymphoma-entities were identified. As the lymphoma classifications used until then needed to be revised, a new proposal was necessary. Bone marrow examination is a routine procedure in the staging post-therapy follow-up of patients with non-Hodgkin's lymphoma. In addition, this investigation may serve as "the" diagnostic tool in certain subtypes of lymphoma and in clinical settings in which enlarged peripheral nodes are absent or splenomegaly is the sole presenting finding. Bone marrow investigation should include trephine biopsy sections (and trephine imprints) as well as aspirate smears and may be completed by the examination of blood smears. All these specimens contribute to the final diagnosis and frequently complement one another.

Traditionally, the morphological evaluation of a bilateral trephine biopsy is the method of choice in assessing bone marrow involvement in lymphoma. In view of the REAL classification, the use of previously defined criteria, such as the number and growth pattern of the lymphoid infiltrates and/or the cytological features of the lymphoid cells in the trephine, is not always helpful in identifying the biological meaning of these infiltrates or in subtyping the lymphoma.

Identification of the biological meaning of lymphoid infiltrates in the trephine

The usefulness of phenotyping on paraffin-embedded trephine sections is limited; frozen sections theoretically allow for a more extended panel of antibodies to be applied but this technique is more demanding and difficult to incorporate in a routine practice. Moreover, incorrect interpretation of the staining results may occur. Alternatively, immunophenotyping may be performed on bone marrow aspirate samples but may give discordant results due to sample variation between the trephine biopsy and the bone marrow aspirate.

With the development of molecular biological techniques such as the polymerase chain reaction (POR), it has become possible to recognize the clonal character of lymphoid infiltrates by analyzing the presence of lymphoma-specific genetic translocations or clone-specific antigen receptor gene rearrangements. In addition, due to its increased sensitivity, the PCR technique offers the potential to detect small numbers of malignant cells, allowing the monitoring of minimal residual disease.

Detection of minimal residual disease is mostly performed in peripheral blood and bone marrow aspirate samples because these samples can be freshly processed. Bone marrow trephine biopsies, which have been fixed and decalcified, are more problematic but they may be analyzed with some PCR techniques. However, in our experience as well as in that of others, the analysis on bone marrow sections of IgH gene rearrangements with the PCR technique works well and this technique may be more sensitive than morphological and immunohistochemical analysis.

Subtyping non-Hodgkin's lymphomas

Whether a correct subtyping of the lymphoma is possible using the previously described growth pattern and/or the cytological features of the trephine infiltrate is debatable. A reevaluation of these morphological parameters, based on a detailed analysis of fully documented cases and taking the REAL classification into account, seems to be mandatory. At present we have such a study underway, including more than 300 cases documented by phenotypical and genotypical data. Our first results indicate that in comparison with blood smears and bone marrow smears, the study of bone marrow trephines offers the most sensitive technique for detecting lymphomatous infiltrates. Nevertheless, preliminary results indicate that the various growth patterns as defined in the past (nodular, interstitial, massive and paratrabecular) are not specific to all lymphoma subtypes and that cytological features of the lymphomatous infiltrate may be misleading. While this phenomenon has been described for nodal large cell lymphomas, as they may present with a small cell component in the trephine, it is less well known that the cytological features of the lymphomatous infiltrate in the trephine does not always allow the various subtypes of small cell lymphomas to be cor-

rectly differentiated. Finally, our preliminary results also point towards trephine as the most informative and occasionally unique material for identifying certain particular lymphoma entities.

Conclusion

Histological examination of the bone marrow based on an evaluation of trephine biopsies is the gold standard for the diagnosis of bone marrow involvement in non-Hodgkin's lymphomas while immunohistochemistry and molecular analysis may increase the accuracy and the sensitivity of lymphoma detection. Subtyping lymphomas on the trephine, according to the REAL classification, needs further investigation.

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Philadelphia chromosome-negative (Ph1-) chronic myeloproliferative disorders. A synoptic approach

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Progress in the understanding and definition of the three main subtypes of Ph1- chronic myeloproliferative disorders (CMPDs), idiopathic (primary) myelofibrosis (IMF), polycythemia vera (PV) and essential thrombocythemia (ET) seems to be most promising when pursuing a synoptic approach to this problem. This implies not only close cooperation With the clinicians and reference to hematological data (1) but also a clear-cut distinction between reactive lesions *(i.e.,* spurious polycythemias *vs.* PV or thrombocytosis *vs.* ET) in the initial stages of the disease process (2, 3). Considerable difficulties may arise in distinguishing thrombocythemias (4) and (osteo)-myelofibrosis (5), which eventually occur in all three subtypes of Ph1- CMPDs (4, 6). In addition to semiquantitative staging systems (Hannover Classification), which are based on histological features in pretreatment (6) and sequential bone marrow biopsies (5, 7), more elaborate teChniques such as histochemistry and morphometry are warranted (Table 1). In this context controversy still persists regarding morphological criteria (8), in particular megakaryopoiesis as one of the most distinctive hallmarks of histopathology (4-7). Moreover, a difference of opinion has been expressed concerning the so-called prefibrotic initial stages of IMF. This implicates predominantly their stage-like evolution into myelofibrosis (4, 5, 7), presenting clinical features and diagnostic criteria (9-11) and, when accompanied by an elevated platelet count, also their differentiation from ET (Table 2). Altogether, laboratory data and morphological variables should be considered equally to construct corresponding risk groups which are essential for the design of clinical trials aiming to improve treatment strategies (10,12). The determination of features indicating prognosis have to include parameters Characterizing dynamics of hematopoietic cell kinetics (13), such as proliferating cell nuclear antigen (PCNA), Ki-S 1 (topoisomerase-II alpha) and apoptosis. The rationale of this procedure is to obtain a deeper insight into leukemic cell turnover (14) and to significantly

Table 1. Morphometric characteristics (mean ±**SD) at diagnosis of patients regarding several bone marrow features** in **Ph 1- chronic myeloproiferative disorders compared** with a control group.

	Normal bone marrow	Polycythemia vera	Idiopathic myelofibrosis	Essential thrombocythemia
Number of patients	20	115	150	40
Cellularity (%)	50.5±13.9	88.1±7.9	81.9±12.4	70.8±6.6
Erythropoiesis x10^2/mm^2 (Ret 40 f)	14.6±2.7	36.9±6.7	7.2±3.8	16.1±3.0
Granulopoiesis x10^2/mm^2 (naphthol-AS-D-chloroacetate esterase reaction)	39.0±6.3	58.7±8.8	40.1±25.0	37.4±6.3
Megakaryopoiesis/mm^2 (0061)	24.4±5.3 (14.0-30.1)	102.6±24.2 (52.1-145.2)	79.9±29.9 (36.8-184.0)	126.6±35.6 (66.5-197.2)
Macrophages x10^2/mm^2 (0068)	3.0±1.2	3.9±1.2	7.4±1.3	3.7±0.7
Fibers x10^2/mm^2 (Gomori's stain)	16.1±5.1 (4.1-19.5)	21.6±10.3 (5.4-38.6)	93.5±32.8 (16.1-160.3)	14.9±6.1 (6.6-30.5)
Fraction with myelofibrosis (%)	0	17	84	0

Because of the wide ranges, corresponding values (in brackets) are given for megakaryocytes and fibers. The incidence of (reticulir - collagen) myelofibrosis for all entities (cut-off point, doubling of normal reticulin stain, measured fiber density) la also indicated.