Diagnostic Problems in Follow-Up Bone Marrow Biopsies of Patients Treated for Acute and Chronic Leukaemias and MDS

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Abstract
Bone marrow biopsy evaluation after therapy for hematolymphoid disorders is complex. The difficulties encountered by the pathologist are due to the fact that a variety of heterogenous diseases for which different treatments can be applied have to be considered. Moreover, usually different clinical questions have to be addressed for which different analyses are performed, typically in different laboratories by different investigators. In the present paper, we suggest a systematic, integrative and interdisciplinary approach that includes knowledge of the original diagnosis and of the type of prior therapy, considers treatment effects and a use of standardized response criteria for AML and MDS. Finally, we discuss how to deal with discrepancies between morphological findings in a bone marrow biopsy and the corresponding aspirate.

Introduction
Among the many indications to perform a bone marrow biopsy, the so-called follow-up biopsies of patients treated for hematolymphoid neoplasms deserve special interest. The interpretation and reporting of follow-up bone marrow biopsies or post-therapy bone marrow biopsies is difficult [1]. The most common problems are related to several factors (fig. 1):

(1) A variety of heterogenous diseases regarding clinical presentation, genetics, morphology and immunophenotypical profile are included. These consist of acute myeloid leukemias, acute lymphoblastic leukemias, chronic myeloproliferative disorders, lymphomas with a predisposition to manifest in the bone marrow and myelodysplastic changes (MDS) [2].

(2) Not only for these different diseases but usually also for a single disease – depending on the clinical presentation – different types of treatments are applied such as watch and wait, supportive or high-dose chemotherapy, radiotherapy, hematopoietic stem cell transplantation (autologous, allogenic with normal or reduced intensity conditioning) and so-called specific targeted therapies (e.g. imatinib).

(3) Different clinical questions have to be addressed in the final report. These questions can principally be grouped in two categories: (i) addressing the underlying disease and (ii) addressing the hematopoiesis, and include evaluation of these issues specifically regarding the administered therapy. Examples are: remission status (complete remission, partial remission, minimal residual disease), resistance to therapy, relapse after a disease-free interval, rejection of the transplant, regeneration of hematopoiesis, opportunistic infections and disease-specific changes after therapy.
To answer these complex questions, different analyses are performed such as blood count, bone marrow aspiration, bone marrow biopsy, flow cytometry (of bone marrow and/or peripheral blood), cytogenetics, molecular genetics, etc. The results need to be integrated into the history of the patient and the clinical findings. To complicate the final report, these analyses are often performed in different laboratories by different investigators.

Taking into consideration this complex situation (fig. 1), there is no doubt that a state-of-the-art follow-up bone marrow evaluation in an individual patient requires knowledge of the original disease, the prior therapy, and the exact time of the marrow evaluation with respect to the treatment. In other words, the pathologist needs the possibility to review the original and previous biopsies, and integrate these findings to all clinical and biological results to draw firm conclusions.

**Treatment Effect**

Chemotherapy, with or without radiation, is the treatment of choice for various hematolymphoid neoplasms including acute leukemias. Systemic chemotherapeutic agents have an effect on both normal hematopoietic cells and neoplastic cells. The common changes after myeloablative therapy are marrow aplasia or hypoplasia, reduction of marrow fat, interstitial edema, fibrinoid necrosis, sinus dilatation and a relative increase in stromal cells, lymphocytes, plasma cells and histiocytes.

Signs of marrow recovery include reappearance of fat, foci of erythroid and granulocyte islands on days 7–10, especially in children, or after T-cell-depleted stem cell transplantation and at times of dramatic increase in precursor B cells, so-called hematogones [3]. Later, there is resolution of reticulin fibrosis and appearance of small megakaryocytes in clusters. Finally, in a standard case, the bone marrow shows an age-adjusted normalcellularity about 4–5 weeks after the last cycle [4].

Many treatment regimens for hematolymphoid neoplasms are supported by application of cytokines and hematopoietic growth factors aiming at reduction of side effects of the chemotherapy, acceleration of bone marrow recovery after chemotherapy or stem cell transplantation (e.g. erythropoietin, G-CSF, GM-CSF) or given for their known anti-neoplastic properties (e.g. interferon-α). All these factors induce specific morphological bone marrow changes that should be taken into account in the assessment of the follow-up biopsies in such patients. The most commonly applied cytokines and hematopoietic growth factors and their morphologic effects are as follows: GM-CSF can induce overall increase of cellularity and a particular left shift in myelopoiesis, increase of blasts and monocytosis, which can be difficult to distinguish from AML/MDS on histomorphological criteria alone without knowledge of the prior hematopoietic growth factor application. Morphologic comparison with the blast population before therapy is very helpful in this situation. Additionally, the topographical distribution should be assessed carefully because a physiological pattern is maintained in growth factor administration, whereas in residual disease, the blast population is distributed irregularly. Persistence of an aberrant immunophenotype and molecular genetic analysis is helpful in this situation because a blast population of >5% does not necessarily equal residual disease.

Erythropoietin and thrombopoietin are able to induce transient morphologic changes that may resemble chronic myeloproliferative disorders.

Effects of growth factors should disappear after 2–3 weeks after administration.

Distinguishing reactive – ‘regenerative’ – atypia from persistent myelodysplasia is one of the most difficult problems. Similarly, during induction chemotherapy, it is difficult to distinguish residual leukemia from early re-

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**Fig. 1.** Overlapping issues to be considered in the follow-up bone marrow biopsies.
Morphologic Features of Bone Marrow Transplantation

Bone marrow transplantation or hematopoietic stem cell transplantation is a therapeutic option for patients with a variety of diseases, including neoplastic and non-neoplastic disorders. Depending on the clinical setting, either an autologous or an allogeneic hematopoietic stem cell transplantation can be performed. The morphological features in the early transplantation period (days 1–28) include hypocellularity/aplasia with extensive necrosis, proteinaceous debris, fat necrosis, stromal edema and increased phagocytic macrophages. Approximately 7–10 days after hematopoietic stem cell transplantation, bone marrow regeneration is apparent with small, non-paratrabecular colonies of uniform immature cells. In general, erythroid and myeloid regeneration precede megakaryocytic regeneration.

By day 21, bone marrow cellularity reaches about 50% of normal levels and by day 28 all cell lines should have engrafted resulting in a more or less normocellular appearance. However, transient dyshematopoietic changes including ringed sideroblasts may persist for a longer period of time. If no regeneration is evident after 4 weeks, the hematopoietic stem cells have failed to engraft.

Engraftment of the hematopoiesis is demonstrated by chimerism analysis, allowing to determine the proportion of donor and recipient cells in peripheral blood or marrow. Actually the most current laboratory approach is a PCR-based analysis amplifying DNA sequences which are highly polymorphic between donor and recipient, such as for instance the determination of short tandem repeats.

Disorders that can occur in the late (> day 28) transplantation period include rejection of graft, opportunistic infections, relapse of the primary disease, florid hematogone proliferations, post-transplant myelodysplasia, post-transplant lymphoproliferative disorders and some others. The morphological features of early, middle and late bone marrow transplantation periods are described in detail in textbooks of bone marrow pathology [5].

Definition of Response

Acute Myeloid Leukemia (AML)

Generally accepted and clinically relevant definitions of outcomes and reporting standards are a prerequisite to compare clinical studies and to determine the individual therapy strategy. Recently, revised recommendations for standardized response criteria in AML have been reported [6]. These new criteria of response in AML define a ‘morphologic leukemia-free state’ as <5% bone marrow blasts, no extramedullary disease and the absence of any aberrant leukemia immunophenotype by flow cytometry during early assessment with persistent neutropenia (<1.0 g/l) and thrombocytopenia (<100 g/l). Accurate histopathological blast quantification requires immunohistochemistry for CD34, CD117 and potential other markers. Even in high-quality trephine sections, a reliable distinction between true blasts and immature cells is only rarely possible.

A ‘morphologic complete remission’ requires a morphologic leukemia-free state as well as an absolute neutrophil count of >1.0 g/l and platelets of >100 g/l. There is no requirement for bone marrow cellularity.

To verify the impact on treatment outcome of these criteria for complete remission, each criteria was separately evaluated after induction chemotherapy in a large analysis encompassing >1,200 patients with newly diagnosed AML [7]. It appeared that a bone marrow blast count of <5%, recovery of neutrophils and platelets and the absence of extramedullary disease are indeed the cornerstones for the definition of a hematological complete remission in AML patients. Again, assessment of cellularity of the bone marrow and also the presence of peripheral blasts can be omitted from the definition of complete remission.

The meaning of dysplasia after treatment is not clear. Mild megaloblastic changes and karyorrhexis of erythropoiesis can be considered in the early phase after treatment as reactive. However, if the dysplasia was present...
already at diagnosis (AML with multilineage dysplasia), persistence or reappearance suggests residual leukemia.

More sensitive techniques to determine the remission status include molecular and cytogenetic analysis, particularly for AML (and acute lymphoblastic leukemia) with recurrent genetic aberrations. Therefore, in such patients, the special categories of cytogenetic complete remission and molecular (RT-PCR, multidimensional flow cytometry) complete remission should be considered in a final, integrative report. Patients with a morphologic complete remission but with residual (genetic) disease have a poorer prognosis.

**Acute Lymphoblastic Leukemia (ALL)**

Although it has not been defined in a recent publication, the remission criteria for ALL should be similar to those used for AML. Blast count <5% in patients with normal recovery of neutrophil and platelet counts (see above) and without any evidence of extramedullary blast infiltration are considered as complete morphological remission. The difficulty in assessment of remission in ALL during the early post-treatment phase is the distinction between hematogones and leukemic blast cells. This problem should be addressed in an integrative way considering microarchitectural and immunophenotypic features (e.g. CD10, CD19, CD20, CD34, CD79a, CD99 and TdT). Hematogone populations exhibit a continuous and complete maturation spectrum of antigen expression typical of the normal evolution of B-lineage precursors, lack aberrant or asynchronous antigen expression and rarely form large sheets. Thus, detection of single cells or small clusters of immature lymphoid cells with various but not aberrant or asynchronous phenotypic characteristics, reflecting the sequential marker expression of normal B-cell maturation, should be considered consistent with hematogones rather than with persistent leukemic blasts [8]. Again – as for AML – molecular and multidimensional flow cytometric techniques are clearly helpful in detecting minimal residual disease if specific genetic markers and/or aberrant immunophenotypes are present.

**Chronic Myeloid Leukemia (CML)**

Currently, the vast majority of patients with CML in chronic phase are treated initially with imatinib, a tyrosine kinase inhibitor, that directly inhibits the BCR/ABL fusion protein. Most patients achieve a clinical, hematological, morphologic and cytogenetic remission [9]. Morphologically, in a typical case, there is a reduction in overall marrow cellularity, normalization of the myeloid:erythro (M:E) ratio and a normalization of megakaryocyte number and also morphology. Imatinib therapy also gradually eliminates bone marrow fibrosis. In a recent study we defined morphologic criteria such as absence of dry tap, absence of abnormal megakaryocytes, normalization of cellularity, reduction of fibrosis, normalization of M:E index, blast and basophil count that showed an early and late correlation with cytogenetic response. Based on these findings, we proposed a morphologic response score for follow-up bone marrow biopsies of imatinib-treated CML patients [10].

Clearly, however, the most important parameter in the follow-up of a CML patient is assessment of cytogenetic and molecular remission of bcr/abl. All these results have to be summarized in the final report.

**Chronic Myeloproliferative Disorders (MPD) Other than CML**

In most patients with chronic MPD other than CML, a non-curative treatment is given. Still, patients may improve or even their peripheral blood counts normalize and they are then in partial or complete hematological remission, irrespective of the marrow findings. With conventional treatment, cellularity of the bone marrow can usually be decreased; however, the marrow retains its proliferative aspect. After allogeneic stem cell transplantation, similar criteria are used for remission definition as for other malignant hematological neoplasms.

**Myelodysplastic Syndromes (MDS)**

As for other hematopoietic stem cell disorders, standardized criteria for assessing response are crucial to ensure comparability among clinical trials also for patients with MDS. However, the MDS are a heterogenous group of diseases that differ from many other hematologic malignancies in their chronicity, morbidity and mortality caused by cytopenias, without progression to AML. As a result, the therapeutic goals in MDS patients encompass a broad spectrum from altering the natural history of the disease (partial remission, complete remission) to alleviation of disease-related complications (time to progression, quality of live, etc.). To address these problems, standardized response criteria for clinical trials involving patients with MDS have been established [11]. These include hematologic response, altering the natural history of the disease, cytogenetic response, and quality of life.

For the hematopathologists, the most challenging task is to distinguish between residual MDS and reactive changes, especially after treatment with aggressive ther-
apy and/or hematopoietic stem cell transplantation. As mentioned above, megaloblastic changes of erythropoiesis as well as slight dyserythropoiesis are non-specific. However, severe dysplasia, in particular the presence of micromegakaryocytes, speaks in favor of residual MDS.

As a general rule, reversible therapy-related atypia should disappear within 2–3 months, otherwise it should be interpreted as therapy-related (depending on the type of administered chemotherapy) or residual MDS.

**Discrepancies**

A discrepancy between the morphological findings and/or interpretation in the bone marrow biopsy and the corresponding aspirate is not unusual but should be elucidated in an integrative report. An explanation for this discrepancy has to be given. How to deal with this situation? In our institution, any case with a discordant report is re-evaluated separately by the hematologist (aspirate) and the pathologist (bone marrow biopsy). In case of a misinterpretation of morphological findings by one of the investigators, a corrected report is generated by the person (hematologist or pathologist) who is concerned (an example would be the interpretation of atypia as reactive instead of residual MDS in a bone marrow biopsy).

However, in many cases the discrepancy between bone marrow biopsy and cytology is explained by differences in samples analyzed or in the diagnostic strength of each technique. Thus, in case that each report is correct, the ‘worse’, i.e. treatment- and prognosis-determining, result determines further patient management. Additionally, the discrepancy is noted and commented in a final, integrative report (an example would be focal lymphomatous infiltration, e.g. by follicular lymphoma or Hodgkin lymphoma, that is present in a bone marrow biopsy but not in the aspirate). The most common reason for discrepant results is a heterogenous distribution of cells (peritrabecular, focal groups of blasts, etc.) or findings that are related to accompanying bone marrow fibrosis (systemic mastocytosis, metastatic tumors, etc.).

**Summary**

State-of-the-art bone marrow biopsy evaluation after therapy in individual patients requires knowledge of the original diagnosis and of the clinical history, including the type of prior therapy. The original diagnosis has to fulfill the criteria of the WHO classification of hematolymphoid tumors. Accordingly, the hematopathologist has to be familiar with the respective diagnostic criteria.

In many cases, in particular in cases with a diagnosis of myelodysplasia, re-evaluation of the original diagnostic biopsy and comparison to the follow-up findings is crucial. Effects and toxicities of therapy have to be carefully considered. For certain diseases such as AML and MDS, standardized response criteria and terminology have been suggested. The clinical value of these response criteria has been confirmed in clinical studies. Pathologists should adhere to these response criteria. In discrepant cases (aspirate vs. biopsy), re-evaluation of the findings is mandatory. In case of a true discrepancy, the clinically relevant result determines further patient management.

**Conclusion**

The reporting of follow-up bone marrow biopsies of patients treated for hematopoietic stem cell disorders requires a multidisciplinary, multivalued, integrative approach.
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