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# Standardization of Bone Marrow Biopsy Reporting in Ph$^-$ CMPD

## Introduction

In Ph$^-$ CMPD, an ideal classification system based on the molecular pathogenesis of each entity is actually not yet available. In accordance with the WHO classification, the diagnosis of an individual patient‘s disease should be based on the correlation of clinical, laboratory, molecular and cytogenetic data with morphologic findings (1-4). The morphologic evaluation includes peripheral blood smears, bone marrow aspirate smears and bone marrow trephine biopsies. Cytological smears do not allow to asses exactly the cellularity, the histotopography of haematopoietic cells and the fiber content in polycythemia vera, chronic idiopathic myelofibrosis or essential thrombocythaemia and are non-representative in the fibrotic stage. Therefore, bone marrow biopsies are mandatory at initial diagnosis and during follow-up. Their evaluation helps to find the diagnosis, to predict prognosis and to document therapy-related changes. Moreover, trephine biopsies can be used for molecular studies (5). Studies performed on trephines provide informations on the spatial distribution of hematopoietic, fibrogenic and angiogenic growth factors as well as their modulation and their shift in the site of expression with disease progression. The complex network of interactions between hematopoietic, stromal cells and matrix can be analysed under in in situ conditions.

## Specimen processing

By standardized protocols for sample handling and processing processing, reproducible results will be obtained in different institutions (6). Bone marrow trephines performed from the posterior iliac crest, should be fixed in 4% buffered formaldehyde for 12 to 48 hours maximum, decalcified in 10% buffered ethylene-diamine tetra-acetic acid (EDTA) pH 7.2 for 2 days and paraffin-wax embedded. Sectons cut at 2-4µm are stained with H&E or Giemsa to evaluate histologic and cytologic features. Naphthol-AS-D-chloroacetate esterase should be used to assess granulopoiesis, Gomori‘s silver impregnation for fiber content and Perl`s reaction for iron. Valuable diagnostic and prognostic informations can be added by CD34 immunohistochemistry (IHS) for hematopoietic progenitors and CD61 IHS for

megakaryocytes. A broader IHS panel which is optional includes appropriate antibodies to detect hemoglobin, myeloperoxidase, elastase, lysozyme, CD117 (c-kit), mast cell-tryptase, CD68, Ki-67, CD20, CD3, CD4, CD8, CD56, CD57, CD138, κ, λ (6).
Using optimized protocols, total RNA can be isolated from total sections or laser-assisted microdissected single cells of formalin-fixed paraffin-embedded bone marrow trephines (5, 7).

**Standardized reporting of bone marrow trephines**

A standardized evaluation and reporting of bone marrow trephines is mandatory to correlate systematically clinical and morphological data, to define biological markers and to investigate pathophysiological concepts of $Ph^-$CMPD. Increased, decreased or unchanged marrow cellularity should be documented. Reports should include informations on quantity and distribution of hematopoietic cell lineages, i. e. erythropoiesis, myelopoiesis (neutrophils, eosinophils and monocytes), and megakaryopoiesis. The degrees of maturation defects and dysplasia observed in each lineage, and the percentage of $CD34^+$ progenitors have to be taken into account. A major point is the reproducible grading of marrow fibrosis and of bone formation. A semiquantitative score has been proposed: grade 0: no fibrosis; grade 1: minimal to mild increase in reticulin; grade 2: marked increase in reticulin throughout the section plus some bundles in collagen; grade 3: coarse collagen and reticulin fibrosis, often associated with different degrees of osteosclerosis (8, 9). In addition, the iron content of macrophages, the presence of Pseudo-Gaucher cells, plasma cells and lymphoid infiltrates in the stroma, and the marrow vasculature should be considered. The whole spectrum of parameters is listed in the so-called „Cologne Bone Marrow evaluation sheet“ designed by J. Thiele and H.M. Kwasnicka (see below) which is recommended in these guidelines. A synoptical approach considering morphological and clinical parameters will contribute to the final diagnosis of the different Ph- CMPD entities by positive criteria and not by exlcusion of other subtypes of CMPD or reactive cases.

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# COLOGNE BONE MARROW EVALUATION FORM





| HISTOPATHOLOGICAL FINDINGS AT TIME OF_____ BIOPSY | |
|---|---|
| biopsy-nr.: ..... | date of biopsy: ___ / ___ / ___ |

**SEMIQUANTITATIVE SCORING SYSTEM**

| Biopsy | size: length ..... (mm) width ..... | representative [ ] yes [ ] no<br>artifacts [ ] yes [ ] no |
|---|---|---|

| ❑ Cellularity | |
|---|---|
| age-related | |

| ❑ Megakaryopoiesis | |
|---|---|
| quantity | |
| loose clusters | |
| dense clusters | |
| size (small) | |
| size (large to giant) | |
| maturation defects | |
| nuclear lobulation | |
| bulky nuclei (quantity) | |
| naked nuclei | |

| ❑ Granulopoiesis | |
|---|---|
| quantity | |
| left shift | |
| maturation defects | |
| blasts | |
| eosinophils | |

| ❑ Erythropoiesis | |
|---|---|
| quantity | |
| left shift | |
| maturation defects | |

| SEMIQUANTITATIVE GRADING SYSTEM | | |
|---|---|---|
| *Reduction* | *Normal = 0 (not present)* | *Increase* |
| -1<br>-2<br>-3 | minimal<br>moderate<br>overt | +1<br>+2<br>+3 |

| ❑ Fibers | |
|---|---|
| score | |
| osteosclerosis | |

| ❑ Macrophages | |
|---|---|
| iron deposits | |
| pseudo-Gaucher cells | |

| ❑ Stroma | |
|---|---|
| perivascular plasma cells | |
| cell debris | |
| lymphoid nodules | |

| ❑ Sinusoids | |
|---|---|
| proliferation | |
| dilatation | |
| intrasinusoidal hematopoiesis | |

| FINAL DIAGNOSIS |
|---|
| ❑ IMF |
| ❑ ET |
| ❑ PV |
| ❑ CML |
| ❑ MPD-unclass. |
| ❑ no MPD ❑ MDS |

*USE NEW FORM FOR EACH BONE MARROW BIOPSY !*