# Light microscopic morphology of bone marrow cells of rats - preparation of an atlas

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## 1. Abstract

Bone marrow examination is an important part of the evaluation of the hematopoietic system. In pharmaceutical and toxicological research. Bone marrow evaluation can help determine the potential hematotoxicity or effects of new compounds on hematopoietic cells. BMD is nevertheless carried out for various reasons (determination of the threshold of toxic effects, mechanistic studies, studies on pharmacodynamics, etc.). There is currently no automatic procedure for bone marrow assessment. There are different programs for automated cell differentiation available; however, nobody has been able to deal with the bone marrow cells. The aim of this work is to provide clinical pathologists and researchers with an updated overview of bone marrow assessment in rats as well as practical guidance for methods and microscopic evaluation. Methods for obtaining and preparing for smear as well as morphological characteristics of rat bone marrow cells are discussed. The cell and nuclear sizes the nucleus-plasma ratio and the size of the inclusions were measured using the Olympus CellSense Dimension system (Version 1.17). The descriptions, picture samples, and measurements, should be used for another computer system. QuPath v0.2.0., a system working with (artificial intelligence) in order to learn and automatize BMD.

## 2. Introduction

Bone marrow smears should be collected, stained and archived in each toxicological study. Bone marrow differentiation (BMD) is not a standard in regulatory studies. However, if considered necessary, BMD should never be performed as a stand-alone evaluation, but always in conjunction with values from peripheral blood and results from histopathology evaluation. In practice, bone marrow examination is recommended when there is a primary or secondary hematologic disorder that cannot be explained by peripheral blood examination alone [1].

Bone marrow can be taken from the femur (preferably rodents), the sternum or from the ribs (preferably non rodents). However, the smears should be made not later than 30 minutes after death [2].

Each cell population and lineage should be determined exactly because a change in every single cell lineage may cause a shift within the bone marrow that need to be interpreted correctly. However, there are cell lineages bearing diagnostic difficulties, e.g. it may be hard to differentiate between prorubricytes and rubriblasts, basophilic rubricytes or lymphoblasts or others.

## 3. Aims/ Leading Questions

The general aim of this thesis is to describe the cell morphology in detail as much as possible so that it can be entered into an artificial intelligence computer program (QuPath). The program must be able to be recognize all cells types with the given details and differentiate it automatically and save the measured data.

The following leading questions were:

- Is it possible to classify each cell type/cell lineage by cellular details in order to describe the cell lineage correctly and in a constant manner?
- Does the morphology vary within a given cell lineage?
- Is it possible to create an atlas?

## 4. Material and Methods

The bone marrow smears used for this work are from a pre-existing collection of control rats. Therefore the thesis starts from the point "Bone marrow differentation“.




## 5. Results

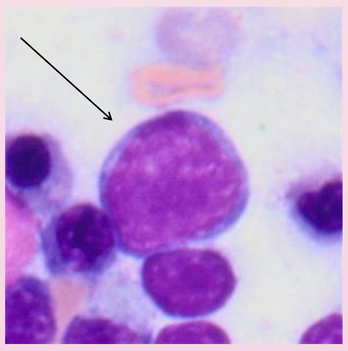

Fig. 5.1: Erythropoietic cells: Rubriblast

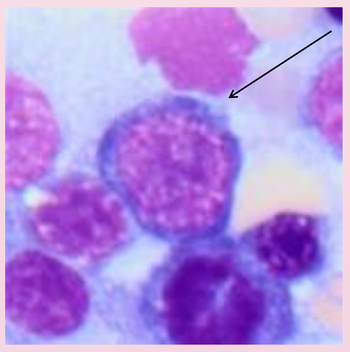

Fig. 5.1: Erythropoietic cells: Prorubriblast

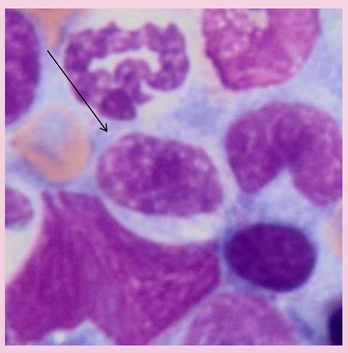

Fig. 5.2: Granulopoietic precursors and neutrophils: Neutrophilic myelocyte

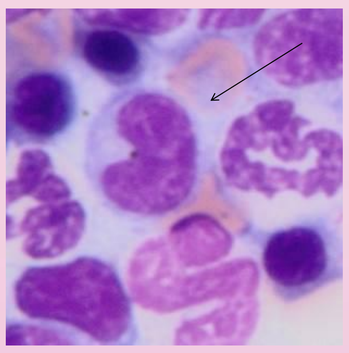

Fig. 5.2: Granulopoietic precursors and neutrophils: Neutrophilic metamyelocyte

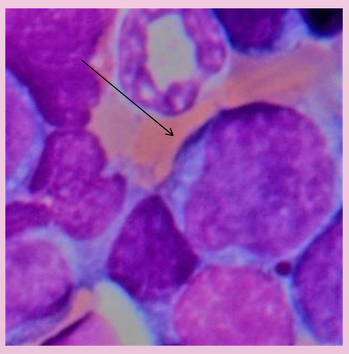

Fig. 5.8: Monocytic lineages: Monoblast

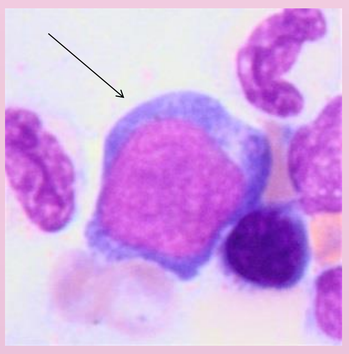

Fig. 5.6: Lymphopoiesis: Lymphoblast

## 6. Discussion

In some cases it was difficult to classify the cell type even though the staining with May-Grünwald was useful. This are some examples of cells which were difficult to classify:

### ❖ Prorubricyte vs. rubriblast:

In both cell lineages, the cytoplasm is dense and stain in May-Grünwald intense basophilic, and the karyoplasm of the round, centrally located nuclei is clumpy and dark. A reliable morphological parameter are 1-2 (rarely more) nucleoli in the karyoplasm of rubriblasts.

### ❖ Neutrophilic myelocyte vs neutrophilic metamyelocyte:

The nuclei of neutrophilic metamyelocytes are cleaved or donut-shaped, less round than in myeloytes, and more centrally located. Metamyelocytes are much smaller than myelocyte.

### ❖ Monoblast vs lymphoblast:

Similar to lymphoblasts, the monoblasts present pale zones adjacent to the nuclei. However, the mononblasts show indentations causing a kidney-shaped nucleus. Furthermore, there are pale round nucleoli in the karyoplasm.

**References**

[1] Provencher-Bolliger, A. (2008). Cytologic evaluation of bone marrow in rats: indications, methods, and normal morphology *33* (2): 58-67
[2] Weber, K. (2020). Personal communication, Oberbuchsiten

**Figures**
Fig. 5.1: Erythropoietic cells: Rubriblast, own figure
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Fig. 5.2: Granulopoietic precursors and neutrophils: Neutrophilic myelocyte, own figure
Fig. 5.2: Granulopoietic precursors and neutrophils: Neutrophilic metamyelocyte, own figure
Fig. 5.8: Monocytic lineages: Monoblast, own figure
Fig. 5.6: Lymphopoiesis: Lymphoblast, own figure