Light microscopic morphology of bone marrow cells of beagle dogs

A photographic atlas of cells from bone marrow smears

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1. Abstract

In toxicological studies, new chemicals and medications are always being tested on animals, for example beagle dogs, first. Further examination by pathologists like the evaluation of bone marrow smears is also recommended, as the test substance may has influence on the blood building system. This evaluation ist time-consuming and expensive. To sped up the process, an automatization is desired. As a first step towards automatization, bone marrow cells of beagle dogs were identified by light microscopy, photographed and then processed to record their normal morphology in an atlas. In a second step, for each cell type, defined sizes were measured.

For the atlas, some cells couldn't be found, which is compatible with the normal cell distribution in the bone marrow. Furthermore, some measurements are missing. Descriptiv statistics were not used, owing to the low number of data points. The collected data should be used in an image analysis system.

2. Introduction

To quantify changes in the blood building system triggered by a testing substance, bone marrow smears evaluation is recommended, although not required. For the bone marrow evaluation, at least 500 cells must be differentiated, including the description of abnormal cells and the calculation of various statistics. This is time-consuming and, due to the need of specialised staff, very costly. An automatization is expected speed up the process while simultaneously allowing more cells per smear to be differentiated. This would increase the accuracy of the result and lower the costs, as only cells which the automated system fails to identify must be differentiated by human operators [1].

Such automated systems may make use of artificial intelligence (AI) applications, which must be trained on a set of impeccable data points. To lay the groundwork towards such a set, morphologic features and data were collected in this thesis. In a first step, light microscopic pictures of bone marrow cells from beagle dogs were taken and processed, to create an atlas which is specific for beagle dogs. Second, the following sizes of cells were measured with the visual program Olympus Cellsense Dimension: area of the cell, area of the nuclei, horizontal and vertical diameter of the cell, diameters of the granulation, vesicle and nucleoli, if available. This data is expected to be useful for AI training sets aimed at beagle dogs and beyond.

3. Aims and leading questions

The aim of this thesis is establishing a specific photographic atlas of bone marrow cells from beagle dogs and the measuring of defined data:

- Can all bone marrow cells be found in the given bone marrow smears?
- Can at least three individual cells of each type be measured?

4. Material and Methods

The bone marrow smears were taken from different studies from beagle dogs (*canis lupus familiaris)* which originate from control animals of regulatory 28-days studies. The smears were prepared and stained by an May-Grünwald-Giemsa staining by the staff of the AnaPath services.

The pictures were taken using a BX45 light microscope with a connected Olympus UC30 camera attached to the Olympus Cellsense Dimension (version 1.17) software, using immersion oil and an 100x objective. The pictures were sharpened and formatted using the IrfanView software.

The measurement was made with Olympus Cellsense Dimension system, where the following distances were measured and entered in an Excel sheet: area of the cell and the nucleus, horizontal and vertical diameter and, if present, the diameter of granulation and nuclei. The average of each measurement and the nucleus-plasma-relation were calculated.

5. Results

Atlas: The figures are sorted by cell linage and different morphological features are noted beneath. The following cells couldn't be found: Basophilic band and segmented, osteoblasts, mitosis figures, reticulocytes and erythrocytes. Other cells were very numerous. Here are some figures as an example:

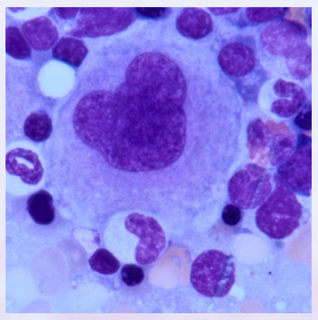

Figure 5.1: Promegakaryocyte

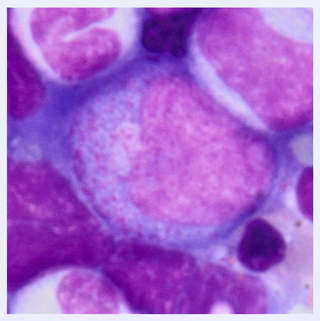

Figure 5.2: Promyelocyte

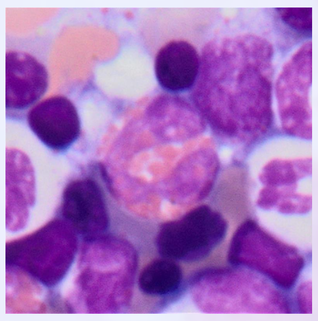

Figure 5.3: band eosinophil

Measurement: the data was entered into different tables, sorted by cell linage. As not all cell types could be found, not all could be measured three times or measured at all. The table below shows the measurement of the megakaryocyte. N-P-R means nucleus-plasma-relation.

Table 5.1: measured data of megakaryocytes

Cell	Cell size	Nuclei size	N-P-R	Horizontal	Vertical
Megakaryocyte	8'139.45	1'246.29	0.15	130.58	93.84
	11'434.98	3'265.72	0.29	143.35	114.54
	7'579.53	1'564.33	0.21	125.41	88.84

As megakaryocytes do not have granulation or nucleoli, they are not listed in this table.

6. Discussion

Atlas: Unfortunately, not all cell types could be found. This has a relation to the normal cell distribution in bone marrow. For example, basophilic granulocytes can normally not be found in bone marrow smears of beagle dogs, which explains this result. For Reticulocytes, different staining allowing the differentiation between erythrocytes and reticulocytes must done.

Measurement: The low number of samples and cell types therein does not allow for a quantitative discussion of the results. As this was never the goal for this work, this is an expected outcome.

Both leading questions must be answered in the negative. It is expected that this result is mainly due to the low frequency of appearance of some cell types and not enough time to screen more bone marrow smears. Next steps should include the methodical measurement of more samples as well as first steps at computer aided recognition of measurements.

References:

[1] Weber, K. (2020). Cell differentiation in bone marrow smears in laboratory animals. *Personal communication by Klaus Weber (10.03.2020)*

Figures:

Fig. 5.1: Maurer, N. (2020). *Promegakaryocyte.* Oberbuchsiten, AnaPath Services
Fig. 5.2: Maurer, N. (2020). *Promyelocyte.* Oberbuchsiten, AnaPath Services
Fig. 5.3: Maurer, N. (2020). *Band eosinophil.* Oberbuchsiten, AnaPath Services
Background: Maurer, N. (2020). *Bone marrow smear.* Oberbuchsiten, AnaPath Services

Tabel:

5.1: Maurer, N. (2020). *Measured data of megakaryocytes.*