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# Microscopic architecture of murine bone marrow: a histological and cytological perspective#

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**Abstract**

*Bone marrow, the primary lymphoid organ in mice, plays a pivotal role in haematopoiesis and immune function. In this study, we performed a comprehensive structural and cellular characterisation of murine bone marrow using histological sections and cytological smears from the tibia. Haematoxylin and eosin (H&E) staining and field staining techniques were employed to elucidate the architecture and cellular composition of the marrow. The bone marrow was found to be highly cellular, with three distinct anatomical zones-endosteal, intermediate and central, though not sharply demarcated. The endosteal zone, adjacent to bony trabeculae, predominantly contained stromal and supporting cells, including fibroblasts and osteoblasts. Hematopoietic precursor cells were primarily located in the endosteal and intermediate zones, whereas mature blood cells were concentrated in the central zone. All major hematopoietic lineages were identified based on cell morphology, nuclear characteristics and staining patterns. The erythroid lineage displayed progressive maturation stages from proerythroblast to reticulocyte, characterised by changing cytoplasmic colour and nuclear morphology. The myeloid lineage included identifiable stages such as myeloblast, myelocyte, metamyelocyte and band forms, with mature granulocytes like neutrophils, eosinophils and basophils present. Monocytic lineage cells, including monocytes, were morphologically distinguishable by their kidney-shaped nuclei. Lymphoid cells were identified as small cells with dense nuclei and a peripheral crescent of cytoplasm. Megakaryocytes were prominent in histological sections, exhibiting multilobed nuclei and granular cytoplasm. Stromal elements such as adipocytes, fibroblasts, reticular and endothelial cells were observed in varying proportions.The myeloid to erythroid (M:E) ratio was calculated to be 2.62 ± 0.11, indicating a predominance of myeloid elements in the marrow. This study provides a detailed anatomical and cytological map of murine bone marrow, serving as a reference for future haematological and immunological research.*

**Keywords:** *Megakaryocytes, erythroid lineage, myeloid lineage, fibroblasts, murine bone marrow*

Bone marrow is a vital primary lymphoid organ in mice, serving as the central site for haematopoiesis, the generation of all blood cell lineages, including erythrocytes, leucocytes and thrombocytes. In mice, bone marrow resides primarily within the medullary cavities of long bones and also in other bones of the skeleton. It contributes significantly to both haematopoietic and immune functions (Lucas, 2021). Understanding the structural and cellular architecture of murine bone marrow is essential, as mice serve as a primary model in preclinical and translational research (Weinreb *et al*., 2020).

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Detailed knowledge of murine bone marrow histology and cytology is essential for interpreting haematopoietic responses in experimental models of cancer, infection, anaemia and immune dysfunction. The general anatomy of murine bone marrow niches, including endosteal, perivascular and stromal compartments, has been well described. Advanced single-cell and spatial studies have recently mapped hematopoietic stem and progenitor localisation within these niches (Baccin *et al*., 2020; Lucas, 2021). However, important gaps remain. Most recent work emphasises molecular and omics-based profiling, while systematic morphological and cytological descriptions of murine bone marrow cell types in smears and histological sections are comparatively limited (Meza-León *et al*., 2021). Finally, despite progress in high-resolution imaging and spatial transcriptomics, the integration of classical cytology with molecular identity is lacking, leaving a gap in linking microscopic morphology with single-cell resolution datasets (Weinreb *et al*., 2020).

This work contributes to bridging these gaps by providing a detailed account of bone marrow gross anatomy, histological features and systematic cytological identification of haematopoietic lineages in murine smears, thereby complementing a morphological reference to the existing molecular studies (Baccin *et al*., 2020), while addressing the scarcity of the standard murine bone marrow cytology and histology datasets (Meza-León *et al*., 2021) necessary for translation to human maps. Although murine and human bone marrow shares fundamental features, notable differences exist. For instance, mice exhibit a higher bone marrow cellularity and possess different proportions of lineage-committed progenitors compared to humans. The regenerative capacity and hematopoietic kinetics also vary significantly between species (Mestas and Hughes, 2004).

Given the extensive use of genetically modified mouse strains and syngeneic tumour models, a thorough understanding of murine bone marrow microenvironment is crucial to extrapolate findings such as cancer progression and the changes in bone marrow which were accurately defined to human conditions. Thus, elucidating murine bone marrow architecture not only enhances basic understanding but also strengthens its application in translational medicine. The objectives of the study was to investigate the advanced gross anatomical, histological and cytological features of the murine bone marrow for interpreting complex translational studies such as in cancer.

## Materials and methods

*Animals*

Seven adult female BALB/c mice (6–8 weeks old, 18–22 g) were housed under standard laboratory conditions (12 h light/dark cycle, 22 ± 2°C, 50–60% humidity) with *ad libitum* access to food and water. All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and approval was obtained (IAEC approval No. CVAS/MTY/IAEC/24/72) before initiation of the study.

*Cytological and histological studies*

Mice were euthanised using 150mg/kg thiopentone sodium. The hind limbs were cleaned with 70 per cent ethanol and dissected aseptically.The femur was carefully separated from surrounding tissue and excised proximally at the hip joint and distally at the knee joint using sterile scissors.The remaining muscle tissue was gently stripped off using sterile gauze and forceps without damaging the bone.Both the ends of the femur (epiphysis) were cut using a sterile scalpel or scissors to expose the medullary cavity. A 26G needle attached to a one mL syringe filled with 1 mL of ice-cold PBS was inserted into the open end. The bone marrow was flushed into a sterile 1.5 mL microcentrifuge tube by gently pushing the phosphate-buffered saline (PBS, pH 7.4) through the femoral marrow cavity. The process was repeated until the bone appeared pale. The flushed marrow was centrifuged to obtain the cellular suspension and kept on ice until further use.A small aliquot of the suspension was smeared and stained with fields staining and counted using oil immersion under light microscope. Cells were counted in 10 fields (100 cells) each in seven animals. The mean and standard error is calculated with the respective number of cells.

The tibiae were harvested during the euthanised post-mortem period as described above, with care taken to remove all soft tissues. Intact tibiae were fixed in 10 per cent neutral buffered formalin (NBF) for 24–48 hours at room temperature with gentle agitation. Bones were decalcified in five per cent nitric acid for two to three weeks, with solution changed every two to three days, until bones became pliable. After decalcification, bones were washed in PBS and dehydrated through a graded ethanol series (70% to 100%), followed by xylene. Bones were embedded in molten paraffin wax and oriented longitudinally in embedding cassettes. Paraffin blocks were sectioned at 5 µm thickness using a rotary microtome. Sections were floated on a warm water bath and mounted on poly-L-lysine–coated glass slides. Slides were deparaffinised in xylene, rehydrated through decreasing concentrations of ethanol, and rinsed in distilled water. Sections were stained with haematoxylin for 5 minutes, rinsed and counterstained with eosin for 1–2 minutes. After dehydration, slides were mounted using DPX and allowed to dry (Luna, 1968; Singh and Sulochana, 1997). Stained sections were observed under a light microscope to assess cellular architecture, haematopoietic components and marrow cellularity.

## Results and discussion

Mice are widely regarded as the best animal models for translational cancer studies due to their

genetic similarity to humans, ease of manipulation and well-characterised immune system (Jayalakshmi *et al*., 2024). In mammals, including humans and mice, blood and immune cells are replenished from hematopoietic stem cells (HSCs) within the bone marrow, maintained in specialised niches whose exact composition is still under investigation (Omatsu, 2023). Examination of bone marrow in both humans and experimental animals is central to clinical haematology and pathology. Histological sections reveal the organisation of hematopoietic tissue, vascular sinusoids and stromal components, while cytological smears allow identification of cell lineages and assessment of maturation. These analyses establish a baseline for evaluating pathological conditions, including cancer and treatment-induced marrow suppression (Lucas, 2021).

The clinical and experimental importance of bone marrow is further underscored by its role in transplantation, a cornerstone therapy for haematological malignancies and marrow failure. Murine transplantation studies have provided crucial insights into HSC engraftment, immune reconstitution and graft-versus-host disease, directly informing clinical practice (Scheurer *et al*., 2022). In cancer immunology, bone marrow functions both as a source of immune effector cells and as a site of tumor-induced immunosuppression, where malignant cells remodel niches, alter hematopoietic output and create a permissive environment for metastasis (Kusmartsev, 2025). Despite its importance, detailed morphological comparisons between murine and human marrow remain limited, though such studies are essential to bridge experimental findings with clinical relevance (Chen *et al*., 2021; Meza-León *et al*., 2021). A comprehensive evaluation of bone marrow structure, cytology and niche organisation in animal models therefore provides a strong foundation for advancing cancer biology and immunology.

Bone marrow is the largest primary lymphoid organ in mice, primarily located within the medullary cavities of long bones and also in other bones of the skeleton. The bone marrow is a highly cellular primary lymphatic tissue that serves as the primary site for haematopoiesis. In the present study, there were three zones observed in the bone marrow of mice, which were not clearly demarcated, representing the functional compartments in haematopoiesis. They were endosteal zone, intermediate zone and central zone (Fig. 1). The endosteal zone was

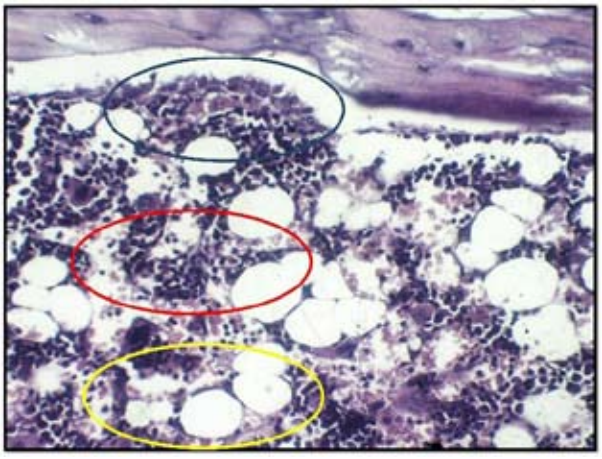

**Fig. 1.** Three zones in the bone marrow. Black circle- endosteal zone, Red circle- intermediate zone, Yellow circle- central zone. H&E. x 200

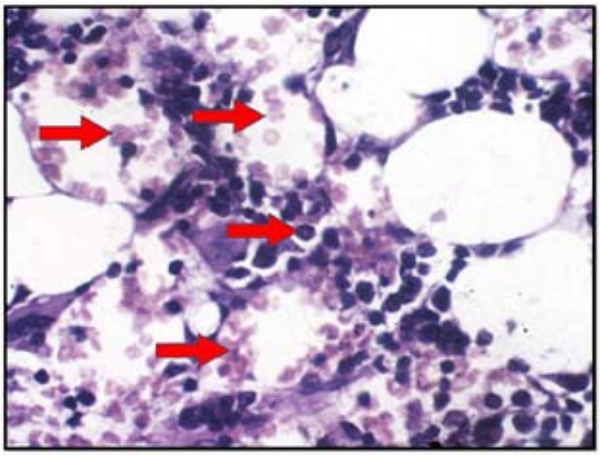

**Fig. 3.** Central zone in the bone marrow showing the mature RBC (arrows). H&E. x 400

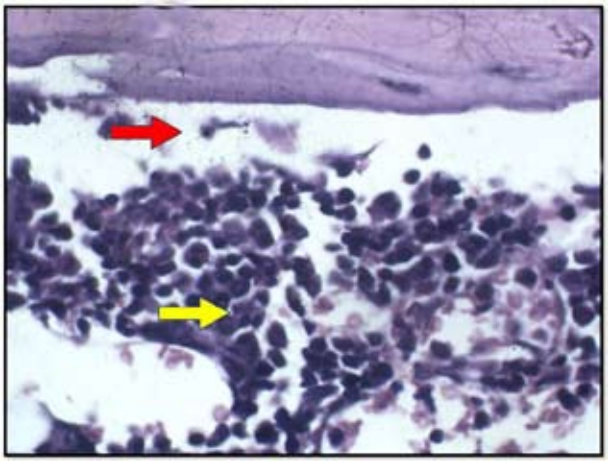

**Fig. 2.** Decalcified endosteal zone (red arrow) and intermediate zone (yellow arrow) in the bone marrow. H&E. x 400

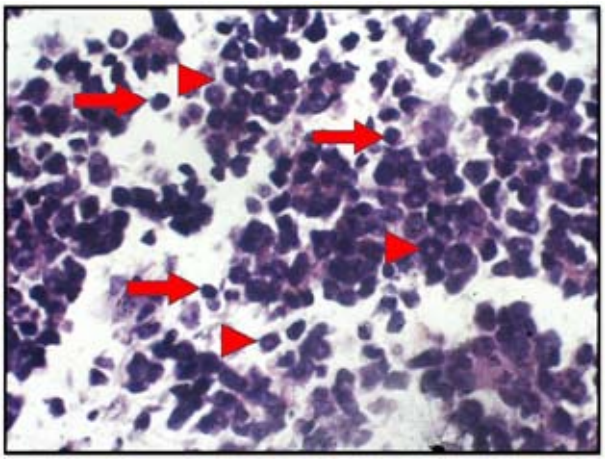

**Fig. 4.** Central zone in the erythroid (arrows) and myeloid (arrowheads) precursors in the bone marrow. H&E. x 400

close to the bony trabeculae and the compact bone (Fig. 1). It contained the stromal and supporting cells such as fibroblasts in majority of the fields. The osteoblasts were also observed to be closely associated to the bony tissue in few areas (Fig.1). It is represented consistent with its role in maintaining the HSC niche (Morrison and Scadden, 2014). There were decalcified in few areas showing a vacant space (Fig. 2). Osteoblasts and fibroblasts in this region have been shown to regulate HSC quiescence and activation through cell-to-cell interaction and cytokine signalling (Zhang *et al*., 2003).

Majority of the precursor cells were observed in the endosteal and intermediate zones. The intermediate zone showed several progenitor and immature cells (Fig. 2). Mature blood cells primarily constituted the central zone (Fig. 3). The predominance of erythroid and myeloid precursors in the endosteal and intermediate zones supports the model of progressive maturation from periphery to centre. Similar zonation and differentiation gradients have been reported in murine models by Kiel *et al*. (2005), who identified lineage commitment and proliferation patterns based on niche localisation.

Everds (2007) reported similar findings that different types of cells could be identified based on their size, shape, staining properties and nuclear characteristics in the bone marrow of mice. The major cell types were haematopoietic cells (blood cell precursors) which were responsible for producing all the blood cells (red cells, white cells and platelets). These haematopoietic cells were further classified into erythroid lineage/red blood cell precursors (Fig. 4), myeloid lineage/white blood cell precursors (Fig. 4), monocyte lineage (Fig.5), lymphoid cells/lymphocyte precursors (Fig. 5) and megakaryocyte lineage/platelet precursors (Fig. 5).

The erythroid lineage consisted of various developmental stages such as proerythroblast, basophilic

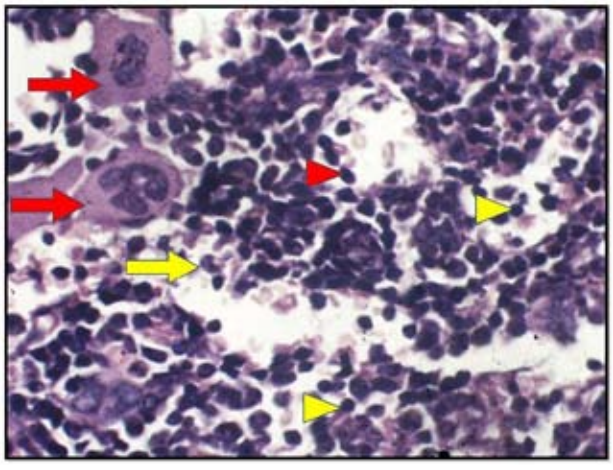

**Fig. 5.** Mature megakaryocytes (red arrows) in the bone marrow. Yellow arrows- monocyte lineage, red arrow heads-erythroid cells, yellow arrow heads-lymphoid cells. H&E. x 400

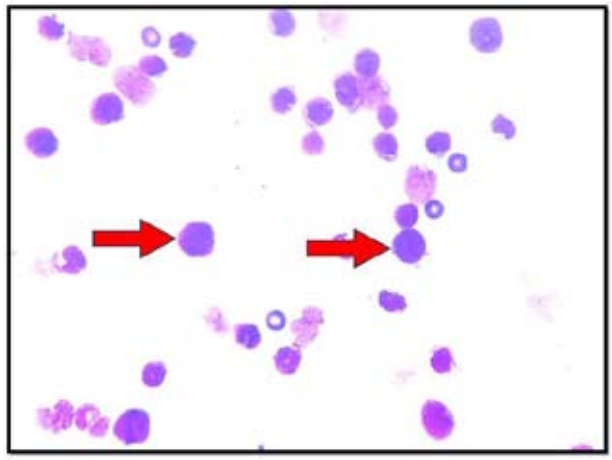

**Fig. 6.** Erythroid cells (red arrows) in the bone marrow. Fields stain x 1000

erythroblast, polychromatic erythroblast, orthochromatic erythroblast and reticulocyte. The erythroblasts were clear rounded cells with round nucleus (Fig. 6) and the nucleus extruded at normoblast (orthochromatic erythroblast) (Fig. 7) stage and formed mature red blood cells without nucleus. The cytoplasm was bluish in the early stages, reddish blue in the intermediate stages and reddish in the late mature stages (Figs. 6 and 7). The mature red blood cells were found evenly in all the three zones. In histological sections, the erythroid cells were clear round cells with typical round nucleus. Similar findings were reported by Cha *et al*. (2024) in mice.

The myeloid lineage had different developmental stages such as myeloblast, promyelocyte, myelocyte, metamyelocyte, band cells and mature granulocytes. The myeloblasts were very rare in the cytological smear. It had a moderate amount of cytoplasm with one or two granules. The promyelocyte had many prominent granules and was similar to the myeloblast. The myelocytes were large cells with round or slightly indented or kidney shaped nucleus with more specific granules (Fig. 8). The metamyelocyte had more nuclear indentation (Fig. 8). The band or stab form had more prominent indentation with C-shaped or horse shoe-shaped nucleus (Fig. 9). Mature granulocytes such as neutrophils, basophils and eosinophils were similar to those found in the blood smear. The basophils and eosinophils were rare and occasionally found in the cytological smears. These findings were in accordance with O'Connell *et al*. (2015) in mice.

The monocyte lineage was similar to the myeloid lineage with less or absence of specific granules (Fig. 10). This lineage had different developmental stages such as monoblast, promonocyte and monocyte. However, all these stages were not clearly identified in the cytological smears. The monocytes were similar to the neutrophil precursor, metamyelocyte. It was larger than the metamyelocyte with kidney shaped nucleus (Fig. 10). The lymphoid cells were differentiated from lymphoblasts to lymphocytes with

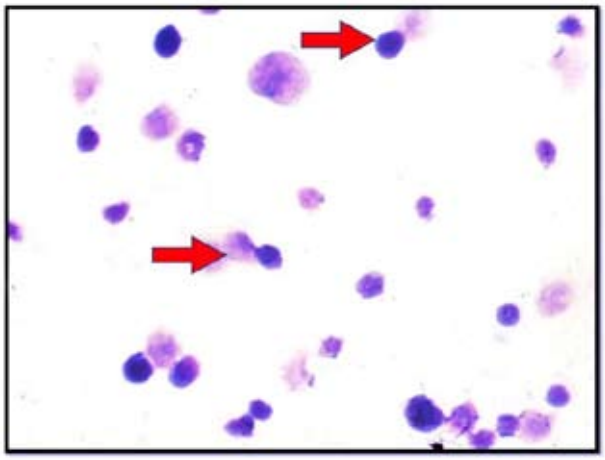

**Fig. 7.** Normoblast cells (red arrows) in the bone marrow smear. Fields stain x 400

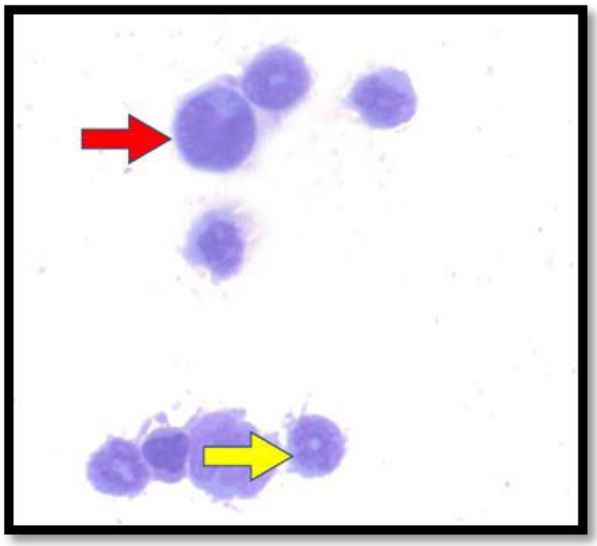

**Fig. 8.** Central zone in the meta myelocyte (red arrow) and myelocyte (yellow arrow) in the bone marrow smear. Fields stain x 1000

the round dense nucleus with scant pale blue cytoplasm (Fig. 10). These cells were similar to the erythroblasts. However, there was crescentic cytoplasm at one corner in the lymphoid cells (Fig. 10) against the centrally placed nucleus with evenly distributed cytoplasm surrounding the nucleus in the erythroblast cells. Similar findings were observed by O'Connell *et al*. (2015) in mice

The megakaryocyte lineage had different developmental stages such as megakaryoblast, promegakaryocyte and megakaryocyte. These cells were large with vesiculated nucleus with prominent lobation forming multilobed nucleus and pale clear pink cytoplasm with few granules. The lobulation was different in different stages of development (Fig. 11). These were found to be less in the cytological smear (Fig. 12) and were clearly identified in the histological section. These cells are essential for platelet production and reside near marrow sinusoids, consistent with findings of Takaku *et al*. (2010) in mice, who highlighted their interaction with vascular niches.

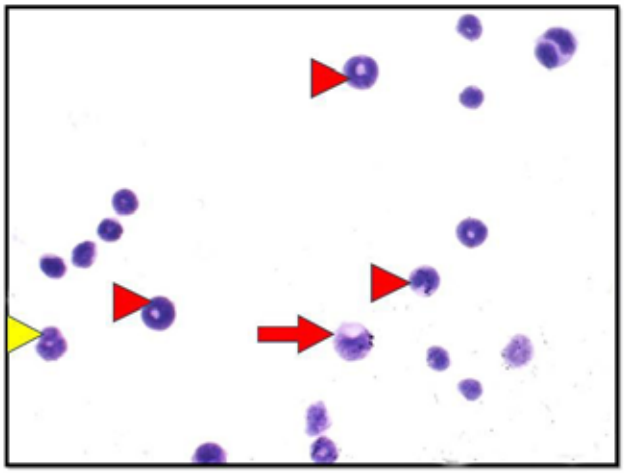

**Fig. 9.** Band form or stab cells (red arrow heads) in the bone marrow smear. red arrow- metamyelocyte, yellow arrowhead- mature neutrophil. Fields stain x 400

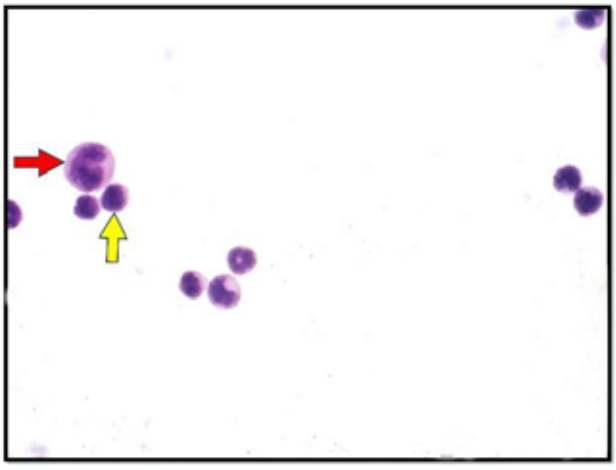

**Fig. 10.** Monocyte (red arrow) and lymphoid (yellow arrow) precursors in the bone marrow smear. Fields stain x 1000

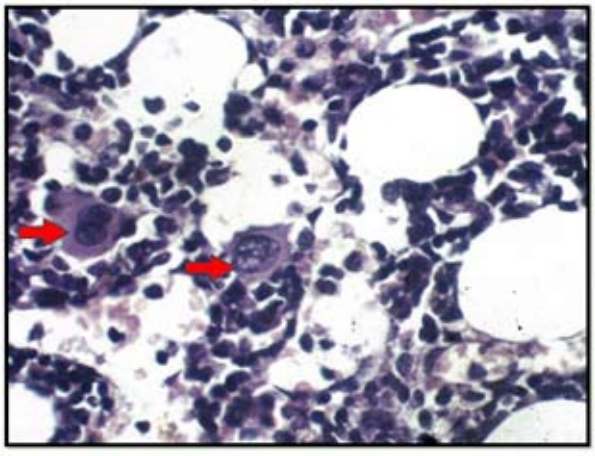

**Fig. 11.** Megakaryocytes (red arrow) in the bone marrow. H&E. x 400

There were few stromal and supporting cells such as adipocytes, fibroblasts, reticular cells and endothelial cells (Fig. 13). The stromal and supporting

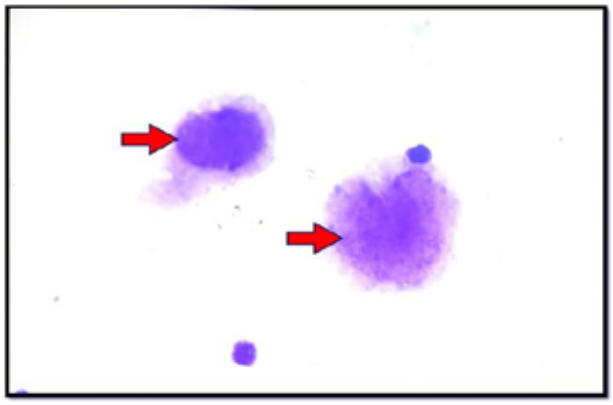

**Fig. 12.** Megakaryocytes (red arrow) in the bone marrow smear. Fields stain x 1000

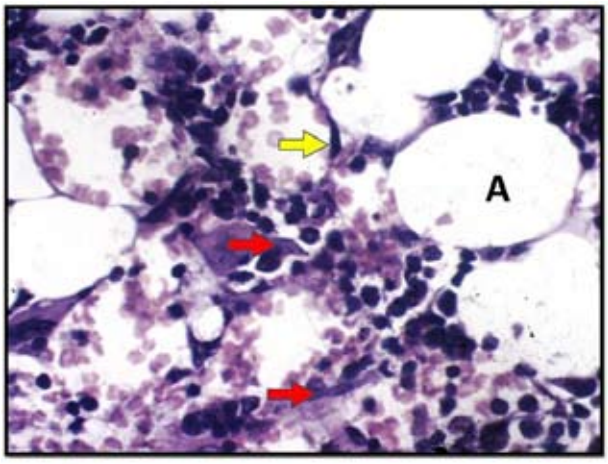


**Fig. 13.** Reticular cells (red arrow) and endothelial cells in the sinusoids (yellow arrow) in the bone marrow. A-Adipocyte. H&E. x 400

cells were observed with various shapes, sizes and nuclear characteristics. The adipocytes (fat cells) were large clear cells with nucleus at the periphery giving a signet ring appearance. The adipocytes were commonly observed in the control group animals with no tumour induction. The fibroblasts and reticular cells which provided supporting framework for haematopoietic cells were spindle to elliptical-shaped with elongated oval nucleus (Fig. 13). The sinusoids were lined by endothelial cells (Fig. 13). Adipocytes and fibroblasts were particularly noted in control animals, aligning with studies suggesting that bone marrow adiposity increases with age and in non-proliferative marrow states (Naveiras *et al*., 2009). The presence of endothelial cells lining sinusoids also confirms the functional vascular niche crucial for cell trafficking and oxygen/nutrient exchange (Acar *et al*., 2015).

The myeloid to erythroid ratio (M:E ratio) is the ratio of the myeloid cells to erythroid cells in the cytological smear. Acoording to Elmore (2006), the M:E ratio compared the granulocytic (myeloid) to erythrocytic cells, excluding lumphocytes in the cytologic preparations. However, lymphoid and monocytic cells were not easily distinguished from other nucleated cells in the bone marrow sections. So, the lymphoid and monocytic lineages were considered as myeloid and the erythroid lineage was considered as such. The ratio was 2.62 ±0.11 (Table. 1), similar findings were reported in mice by Elmore (2006). The M:E ratio observed in our study indicated a myeloid-dominant marrow, which was typical in adult mice under homeostatic conditions (Ogawa, 1993). The scarcity of basophils and eosinophils is expected, as these granulocytes are rare in normal marrow but increase under allergic or parasitic stimuli.

**Table.1** The count of different cells in murine bone marrow

| Parameters | Count (Mean±SE) |
|---|---|
| Myeloid cells (%) | 55.36± 5.36 |
| Erythroid cells (%) | 30.00 ±0.58 |
| Megakaryocytes (%) | 2.00 ±0.00 |
| Lymphocytes (%) | 10.00 ±0.58 |
| Monocytes (%) | 3.33 ±0.88 |
| Other precursors (%) | 17.00 ±0.58 |
| M:E ratio | 2.62 ±0.11 |
| Myelocytes | 8.00 ±0.58 |
| Metamyelocytes | 16.67 ±1.45 |
| Band cells | 13.33 ±1.67 |
| Neutrophils | 16.33 ±2.03 |
| Eosinophils | 0.67 ±0.33 |
| Basophils | 0.33 ±0.33 |
| Total myeloid % | 55.33± 5.36 |

The current study offers a detailed structural and cytological characterisation of murine bone marrow, with a focus on cellular localisation, lineage development and marrow organisation. Taken together, our study confirms and expands upon existing literature by providing visual and morphological clarity on murine marrow architecture. These findings offer critical reference points for evaluating disease-induced marrow alterations in cancer, infection and immunological studies using mouse models.

## Conclusion

The present study provides a comprehensive morphological and cytological atlas of murine bone marrow, highlighting its intricate architecture and diverse cellular composition. By delineating the three anatomical zones-endosteal, intermediate, and central—we established a clear spatial organisation of hematopoietic activity within the marrow. The identification of various hematopoietic lineages, including erythroid, myeloid, monocytic, lymphoid and megakaryocytic cells, along with supporting stromal elements, underscores the functional complexity of the marrow microenvironment. The predominance of myeloid elements, as reflected by the myeloid-to-erythroid (M:E) ratio of 2.62 ± 0.11, emphasises the marrow's dynamic response to hematopoietic demands. Our detailed cytological analysis reveals not only the maturation stages of each lineage but also the unique morphological markers that distinguish them.These findings offer valuable

baseline data for researchers working with murine models of disease. Understanding the normal architecture and cellularity of mouse bone marrow is essential for recognising pathological alterations in conditions such as cancer, anaemia, immunodeficiency and bone marrow failure. This foundational insight bridges the gap between experimental data and clinical relevance, enhancing the translational value of murine studies. Ultimately, this work contributes to the refinement of mouse models as reliable tools in biomedical and therapeutic research.

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**Conflict of interest**

The authors declare no conflict of interest

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