Deep Learning Method for Quantification of Mast Cells in Toluidine Blue Stained Tissue Sections of Mouse Skin

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Introduction

Mast cells are present in the connective tissues throughout the body which are of myeloid origin. Various aspects of pathological conditions are significantly modulated by the activation and degranulation of mast cells. Mast cells *(Fig. 1)* play a significant role in skin immunity and the pathogenesis of multiple skin diseases, including atopic dermatitis, scleroderma, contact dermatitis, blistering cutaneous disorders, and chronic graft versus host disease. It is also responsible for physiological functions such as innate and adaptive responses, angiogenesis, regulation of vasodilation, vascular homeostasis, and detoxification of venom.

A common characteristic across skin diseases is an increase in mast cells, which undergo degranulation in the affected skin. Accurate quantification of mast cells is an important step in assessing the efficacy of study models for skin diseases. We propose a deep learning (DL)-based method for quantification of mast cells in skin sections of mice stained with toluidine blue.

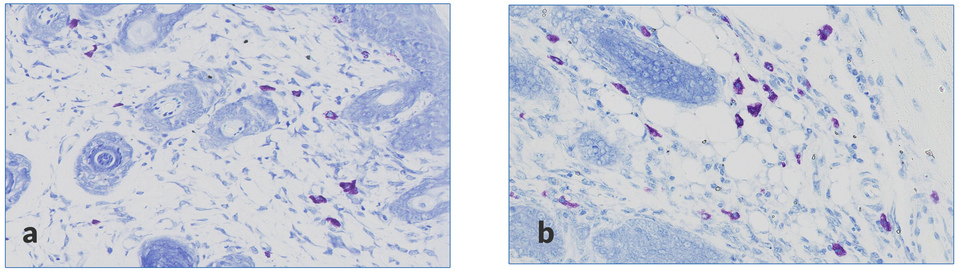

Fig. 1. Toluidine blue-stained skin images of mice at 20x magnification. (a & b) Mast cells are highlighted in purple color.

Materials and Methods

Training data generation

- 170 whole slide images (WSI) scanned at 40x magnification on mice skin tissue sections from three different drug-induced efficacy studies stained with toluidine (C57BL/6 mice, 10-12 weeks of age) were included in this study.
- 933 patches of size 512×512 under 20x magnification were extracted from 10 WSI.
- Ground- truth data was created *(Fig. 2)* under the supervision of pathologists.

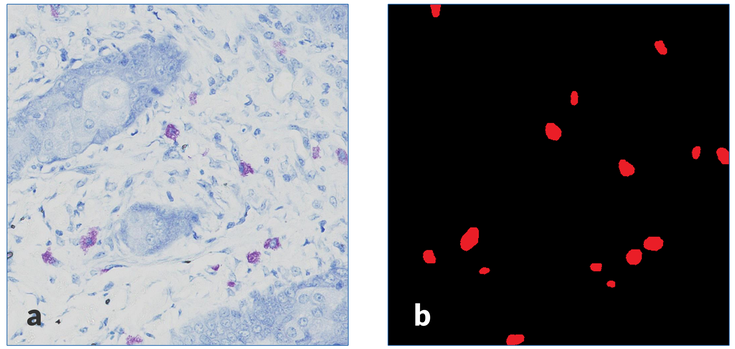

Fig. 2. Ground truth on tiles extracted at 20x magnification. (a) Input image; (b) Ground truth.

Model development and validation

- A U-Net model with an EfficientNet backbone was trained to identify mast cells.
- The foreground dice for training and validation were 0.95 and 0.91, respectively *(Table 1)*.
- A new set of 160 WSI from three different studies was used for evaluating the model.
- Pathologists validated all the model outputs for 160 WSI.
- Mast cell count was determined for each of the WSI from control and three different studies *(Fig. 3)*.

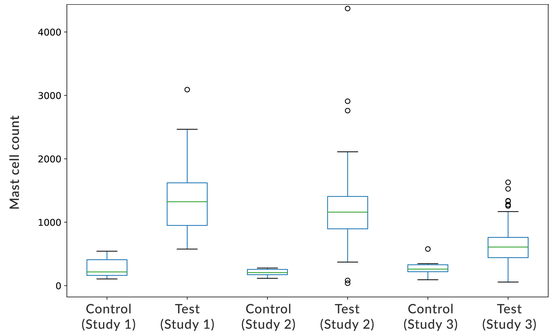

Fig. 3. Boxplot showing the Mast cell count distribution in three different studies

Results

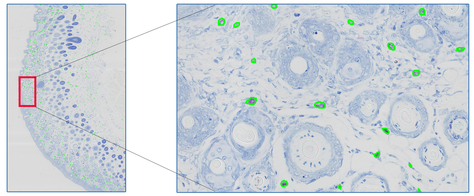

Fig. 4. Mast cell detected output using the proposed algorithm.

Table 1. Model training and validation performances for Mast cells

	Training performances (Foreground Dice)			WSI Validation performances on blind dataset		
Parameter	Training WSI	Training	Validation	Number of WSI	Precision(%)	Recall(%)
Mast Cell	10	0.95	0.91	160	98.8±0.8	99.1±0.7

- Pathologists' validation was used to identify true positives, false positives, and false negatives, and model performance was computed *(Figs. 4 and 5)*.
- The model achieved an average of 98.8% precision and 99.1% recall for 160 validation images *(Table 1)*.

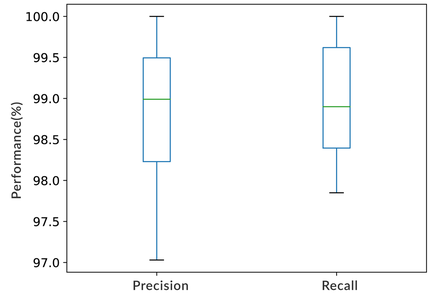

Fig. 5. Precision and recall measures for Mast cell detection using the proposed methodology.

Conclusions

This model was able to accurately detect and quantify the mast cells in toluidine blue-stained skin sections, which is faster and more accurate than manual counting by pathologists.

Impact Statement

This study suggests that this deep learning-based model was able to detect and quantify mast cells on mice skin stained with toluidine blue. The model could be a useful and supportive tool for histopathological evaluations, especially in efficacy studies of dermal products. Quantification of mast cells in toluidine blue-stained sections would help grade the severity of lesions. In this study, methods used to quantify mast cells in mice skin sections can be extended for quantifying mast cells in other organs and tissues.

References

Raja N, Naikodi S, Govindarajan A, and Palanisamy K. Toluidine blue staining of murine mast cells and quantitation by a novel, automated image analysis method using whole slide skin images. J Histotechnol. 2021 Dec;44(4):190-195. doi: 10.1080/01478885.2021.1915934. Epub 2021 May 17. PMID: 33998401.