**Lymph node histology for the assessment of residual neoplastic disease in canine mast cell tumours: does the presence of metachromatic granules always identify mast cells?**

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Evaluation of loco-regional disease is a crucial step in the staging work-up of canine mast cell tumours (MCT).1 This includes cytology and/or histology of the primary lesion and the draining lymph node/s.1 Identification of mast cells in groups or large numbers is currently accepted as morphological sign of lymph node metastasis in both cytology and histology.2-3 If necessary, Toluidine blue stain can be used in order to better identify mast cells infiltrating the node.

Particularly in patients with clinical stage II disease, which may be candidate for a long survival, lymph node monitoring acquires a crucial role in the assessing of treatment response and the evaluation of residual disease prior to treatment discontinuation.1,4

In our institution, lymph node histology is recommended if cytology suggests residual disease despite presence of a normal lymph node (microscopic disease). Cytological and histological evaluation is performed on tissues exposed to the effect of chemotherapy and/or radiotherapy, and effects on tissue morphology are not well documented either in human or veterinary literature; however, the mechanisms of action of antineoplastic compounds and ionising radiations share the final outcome of causing cell apoptosis.5

This particular type of cell death physiologically triggers phagocytosis by the reticulo-endothelial system and clearance of cell debris in the draining lymph node.

In this scenario we came across some cases where identification of mast cells in haematoxilin and eosin was unrewarding, with a predominant histological picture characterised by macrophages (Fig.1A). After toluidine blue stain, groups of cells bearing purple metachromatic granules were detected (Fig.1B), however such cells were characterised by peripheral nucleus and occasionally exhibited intracytoplasmic red blood cells (Fig.1B-inset). Histochemical staining for Perl’s Prussian blue confirmed that the majority of such cells were bearing haemosiderin (Fig.1C). Immunohistochemistry performed on the lymph nodes failed to identify groups of KIT positive cells (Fig.1D) comparable with previously detected toluidine blue positive cells (Fig.1B), and only isolated well differentiated KIT positive mat cells scattered throughout the nodes (Fig. 1D–-inset) were detected. Conversely, immunohistochemistry for Iba1 (Fig. 1E) confirmed the histiocytic nature of the cell aggregates.6 Finally, electron microscopy identified clustered groups of electrondense granules within the cytoplasm of cells consistent with macrophages (Fig. 1F) and lacking typical ultrastructural features of mast cells.7 In summary our findings suggest that macrophages may be particularly activated in lymph nodes of dogs receiving chemotherapy; moreover they may phagocytose mast cell granules and therefore they may mimic mast cells.

In the light of this hypothesis, we do believe that chemo-radiotherapy treated tissues may reveal a specific biological niche, which may present diagnostic challenges that could make interpretation of MCT residual disease misleading.

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**Caption to figure:**

**Figure 1**. Histomorphology (A), histochemistry (B,C), immunohistochemistry (D,E) and transmission electron microscopy of cells infiltrating lymph node in sequential sections of the same lymph node area. A: lack of evidence of mast cells on HE stain. B: multifocally scattered cells with intracytoplasmic tolouidine blue metachromatic granules. In some cells a red blood cell is detected in association to intracytoplasmic metachromatic granules (inset). C: large numbers of Perls’ Prussian blue positive cells indicating intracytoplasmic haemosiderin, suggesting a histiocytic origin. D: absence of KIT positive stain indicative of mast cell origin. Scattered rare KIT positive cells (normal mast cells) are recognised in the lymph node. E: large numbers of cells are positive for Iba1 immunohistochemistry, consistent with histiocytic origin. F. ultrastructure of a representative cell (black dashed line) with peripheral nucleus (asterisk) and clustered intracytoplasmic round homogeneous round to oval electrondense granules (white arrow and inset) often evident within small vacuoles (arrowhead). Larger aggregates of granular electrondense material is also recognised (black arrow) consistent with haemosiderin. A to E: bar = 50 microns. F: bar= 5 microns; inset: bar=200 nanometers.