FACT SHEET 1

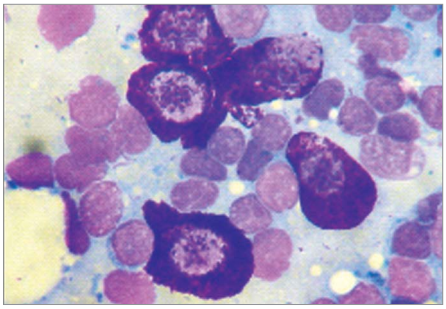
FINNPathologists

The Veterinary Pathologists

Canine Cutaneous Mast Cell Tumours and Supplemental Testing

There are a plethora of additional tests available to aid further prognostication of canine cutaneous mast cell tumours (MCTs).

These are offered either alone or in various combinations and all measure different things, which can be somewhat bewildering…



Mast cells, from an FNA, stained with Giemsa

This hand-out aims to summarise the tests available via Finn Pathologists including AgNOR, Ki67, c-KIT mutations and KIT staining patterns, describing what they measure and correlating them with their usefulness in terms of prognosis. There is currently no single marker which will reliably differentiate the benign Patnaik grade II tumours from the malignant and potentially fatal minority, therefore these prognostic markers must always be considered in conjunction with histological grading, other markers, clinical staging and other clinical information such as tumour location and number.

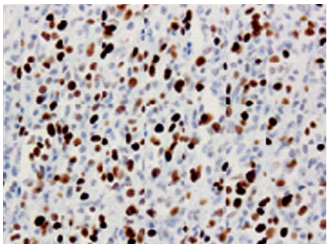
Consultation with a clinical oncologist is always advisable if considering further tests and/or treatment options for these tumours.

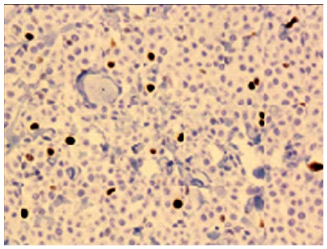
Ki67

Ki67 is a nuclear protein expressed in all active phases of the cell cycle but which is absent in non-cycling cells. It is detected by immunohistochemical (IHC) staining. The relative number of Ki67-positive cells indicates the growth fraction, or the relative number of cells actively involved in cell cycle growth at that point in time. It is possible to divide intermediate grade (grade II) tumours into two groups based on the Ki67 score; those with higher Ki67 scores have a tendency for shorter survival times compared to those with lower scores. Ki67 has been shown to be a prognostic indicator independent of histological grade, meaning it provides further prognostic information in addition to that given by the Patnaik grade. This test is available on its own, in conjunction with AgNOR or as part of the MCT Prognostic Profile.

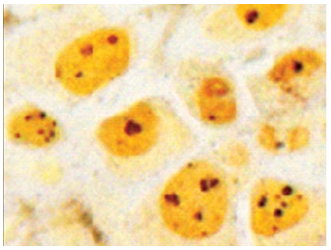
AgNOR

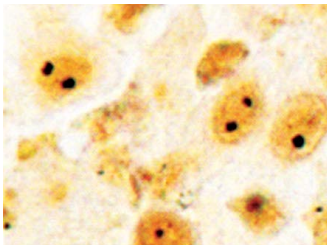
AgNOR (argyrophilic nucleolar organiser regions) are areas within the nucleus associated with proteins involved in ribosomal RNA transcription. AgNORs are detected by a histochemical (silver) stain, and the number of AgNORs present within each nucleus is proportional to the rate of cell proliferation in vivo. Therefore the AgNOR count gives an indication of generation time, a component of the rate of cellular proliferation. Higher AgNOR counts in MCTs are associated with increased mortality, local recurrence and metastasis. Lower AgNOR counts correlate with longer survival times but are not predictive of clinical behaviour independent of histological grade; this reduces the usefulness of the test since it does not provide prognostic information additional to that already provided by the Patnaik grade. Hence this test is available in conjunction with Ki67 or as part of the MCT Prognostic Profile.





Mast cell tumours with high and low Ki67 scores respectively (courtesy of GD Laboratories)





Mast cell tumours with high and low AgNOR scores respectively (courtesy of GD Laboratories)

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Combining Ki67 and AgNOR

Cellular proliferation is a product of both growth fraction and generation time, and so Ki67 and AgNOR scores are sometimes combined (also referred to as the Ag67 index) to give an indication of overall cellular proliferation within a tumour. The growth fraction and generation times are independent of one another, and thus are providing complementary biological information about the tumour cells, which may be more useful when the two indices are used in concert rather than individually.

c-KIT mutation

c-KIT is a proto-oncogene; these are normal genes involved in regulating cell growth and differentiation. *c-KIT* encodes a tyrosine kinase receptor (KIT), which is normally expressed on the cell surface and acts as a receptor for a growth factor. Mutations in the *c-KIT* gene convert it to an oncogene; these are genes that are abnormally activated and promote autonomous cell growth in cancer cells. Such mutations in the *c-KIT* gene can result in a KIT receptor which is permanently activated even in the absence of its ligand. *c-KIT* mutations can be an important contributory factor in MCT development and growth, for example by increasing cellular proliferation. *c-KIT* gene mutations are detectable by PCR, and some are associated with a worse prognosis. Knowledge of their presence may also influence the choice of chemotherapeutic agents, particularly the tyrosine kinase inhibitors. This test is available on its own as well as part of the MCT prognostic profile. A test has also recently been developed which utilises DNA obtained from the cells in an air-dried smear made from fine needle biopsy samples (using non-formalin fixed samples only).

KIT staining patterns

KIT is the tyrosine kinase receptor encoded by the *c-KIT* gene, as discussed above. Normally, the KIT receptor is present on the cell surface (i.e. membrane-associated), and several studies have looked for changes in KIT staining patterns in tumour cells using IHC, and whether these are associated with outcome. Staining pattern I is membrane-associated staining with little or no cytoplasmic staining (i.e. normal), while staining pattern II is intense focal or stippled cytoplasmic staining, and pattern III is diffuse cytoplasmic staining of neoplastic mast cells. Staining patterns II and III, i.e. increased cytoplasmic staining of KIT, are thought to be associated with shorter overall survival times and increased risk of local recurrence. The histological grade, *c-KIT* mutations and KIT staining patterns are all independent factors, thus all can theoretically provide useful prognostic information in their own right. This test is available as part of the MCT prognostic profile.

If you require any further assistance in understanding the further prognostic tests available for mast cell tumours please do not hesitate to contact us.

Recommended reading:

"European consensus document on mast cell tumours in dogs and cats" published in 2012 by Blackwood, Murphy et al. (issue 10, vol 3, pages e1 - e29) in Veterinary and Comparative Oncology – a very comprehensive review, including useful diagnostic algorithms and extensive further references.

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