FACT SHEET 1

FinnPathologists *The* Veterinary Pathologists

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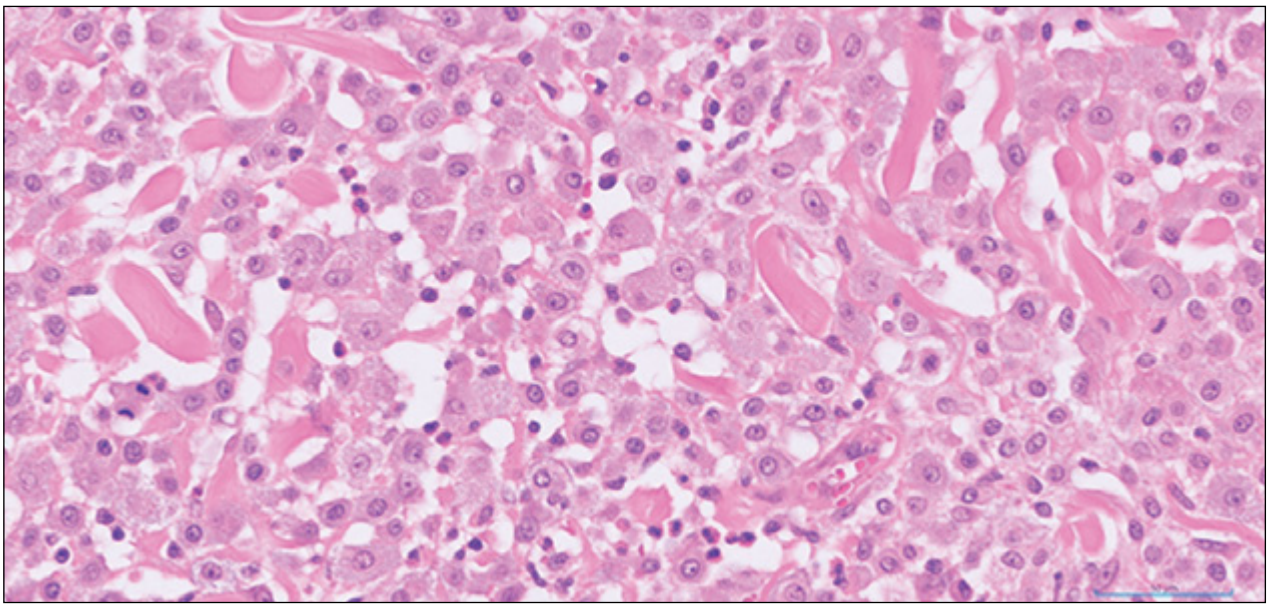
Canine cutaneous and subcutaneous mast cell tumours

– supplemental testing on histopathological samples

There is a plethora of additional tests available to aid the further prognostication of canine cutaneous and subcutaneous mast cell tumours (MCTs). These tests all measure slightly different things, and this can be a little bewildering.

This fact sheet aims to summarise the tests available, describing what they measure and how useful they are in terms of prognosis. There is currently no single individual marker which will reliably differentiate between the relatively benign majority of MCTs and the more malignant and potentially fatal minority. This means it is very important that these prognostic markers are always considered in conjunction with the histological grading, other markers, clinical staging and any additional clinical information such as tumour location and clinical behaviour. Please note this fact sheet only applies to canine cutaneous and subcutaneous MCTs and is not for MCTs arising at other sites such as the oral cavity, or MCTs arising in other species.

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



Mast cell tumour, from the skin of a dog, haematoxylin and eosin stain, high power (400x)

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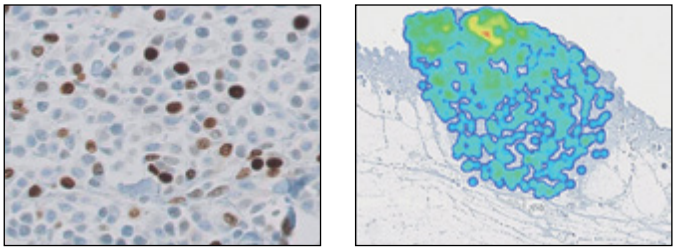
Ki-67

This is the most routinely requested test here at Finn Pathologists, and is a test often recommended by oncologists. Ki-67 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 Ki-67 positive cells indicates the growth fraction, or the relative number of cells actively involved in cell cycle growth at that particular point in time. It is possible to divide mast cell tumours into two groups based on the Ki-67 score; those with higher Ki-67 scores have a tendency for shorter survival times compared to those with lower scores.

Here at Finn Pathologists, in association with Aiforia Technologies, we have developed and validated an artificial intelligence (AI) model to help our pathologists more accurately assess Ki-67 expression in canine MCTs. This AI-assisted method results in a faster, less subjective and more reliable Ki-67 score than traditional manual methods of counting, which can be subjective and prone to variation.

Using our new AI-assisted method, a representative section of the mast cell tumour is first stained for Ki-67 using IHC, and AI-based image analysis is then performed on the entire slide to detect the positive-staining nuclei of mast cells within the tumour. This results in a "heatmap", highlighting areas of the tumour containing the most positive-staining nuclei. This heat map is then utilised by the pathologist to place five grids (0.25mm^2) in those areas of the tumour with the highest density of positive-staining nuclei, and the average number of positive-staining nuclei per grid is calculated.



The image on the left is a mast cell tumour with a high Ki-67 score; note the large number of positive staining (brown) nuclei. The image on the right is an example of the AI model results: a heatmap showing the density of Ki-67 positive staining nuclei of mast cells, within a typical low-grade canine MCT, immunohistochemically stained for Ki-67. Red represents an area of highest density of positive-staining nuclei and blue the lowest density.

c-KIT mutation PCR

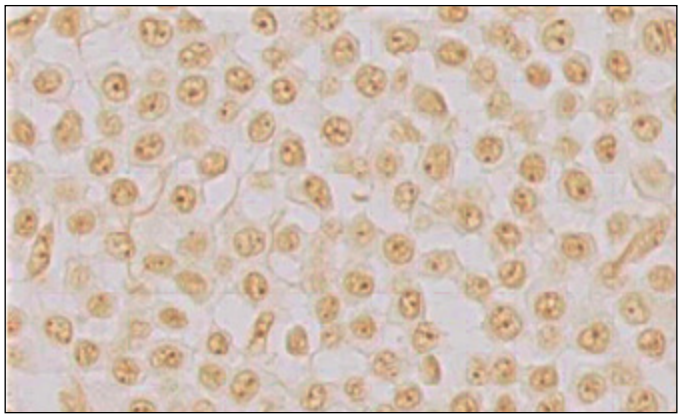
c-KIT is a proto-oncogene; these are normal genes involved in regulating cell growth and differentiation. *c-KIT* encodes a tyrosine kinase receptor (KIT), which is normally expressed on the cell surface and acts as a receptor for a growth factor. Mutations in the c-KIT gene convert it to an oncogene; 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. Canine mast cell tumours positive for the activating duplication mutation in exon 11 of *c-Kit* have been shown to have a shorter disease-free interval and survival time compared to mast cell tumours without a *c-Kit* mutation. However, tumours with such mutations have been shown to respond more favourably to therapy with tyrosine kinase inhibitors. Canine mast cell tumours that are positive for the activating duplication mutation in exon 8 of *c-Kit* **do not** have a more aggressive biological behaviour, but these tumours are also more likely to respond favourably to therapy with TKIs.

The Ki-67 scoring and *c-KIT* mutation PCR are the two tests we routinely offer in our canine cutaneous and subcutaneous MCT reports. The additional tests described below are also available on request.

AgNOR

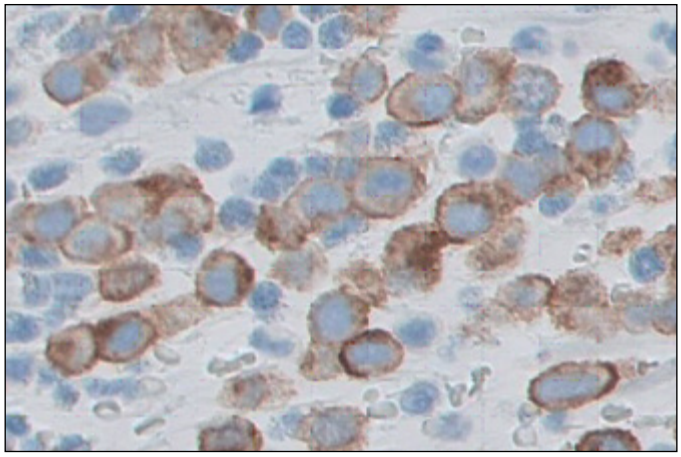
AgNOR (argyrophilic nucleolar organiser regions) are areas within the nucleus associated with proteins involved in ribosomal RNA transcription. AgNORs are detected by a histochemical (silver) stain, and the number of AgNORs present within each nucleus is proportional to the rate of cell proliferation 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.



A mast cell tumour with an AgNOR stain. The AgNORs are small dark brown dots within the yellow nuclei.

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, although this does depend on the study. Please note that due to the methodology involved in assessing KIT staining patterns, these results can be somewhat subjective in nature.



A mast cell tumour stained for KIT using immunohistochemistry; these mast cells demonstrate positive (brown) staining consistent with a pattern I (membrane-associated).

Please note that these additional tests for mast cell tumours rely on there being an adequate number of identifiable mast cells present in the sample for accurate assessment. Also for incisional biopsies, it is assuming that the sample submitted is fully representative of the mass as a whole.

Hence, these tests become less accurate in the following circumstances:

- Small samples, with low numbers of mast cells (small incisional biopsies)
- Samples with artefact, such as crush or cauterization, where the mast cells cannot be accurately identified
- Mast cell tumours which are overrun by eosinophils
- Mast cell tumours with necrosis and/or significant inflammation

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

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