Review of the Prognostic Evaluation of Canine Melanomas

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Objective:
Melanocytic neoplasms are one of the most common neoplasms in dogs and vary widely in their biological behavior. Numerous studies have evaluated various prognostic markers for canine melanocytic neoplasms either as primary or secondary goals, however, few true prognostic studies exist and there are no universally accepted criteria to prognosticate canine melanocytic neoplasms. The recently published “Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology” (Webster et al. Epub 2010) that were initiated by the ACVP oncology committee, reflect the current consensus opinion of veterinary pathologists and oncologists on how to evaluate prognostic classification systems of canine neoplasms. We conducted a detailed literature review of canine melanocytic neoplasms to identify which prognostic factors have been published and evaluated the different postulated prognostic classification schemes according to the above mentioned guidelines. Based on this evaluation, we determined which prognostic parameters have been shown to have statistical significance according to these standards, and provided recommendations for the prognostication of canine melanocytic neoplasms.

Terminology:
Throughout this paper, the term “melanocytoma” will be used to refer to a benign neoplasm of melanocytic origin and the term “malignant melanoma” will be used to refer to a malignant neoplasm of melanocytic origin per the current World Health Organization (WHO) classification (Goldschmidt et al. 1998), regardless of what term was used in the study being discussed. The term “melanocytic neoplasms” will be used to include both benign and malignant neoplasms. Junctional activity refers to proliferation of neoplastic cells at the dermo-epidermal junction (Goldschmidt et al. 1998, Goldschmidt and Hendrick 2002). Compound neoplasms have both an epidermal and dermal component whereas dermal neoplasms are confined to the dermis (Goldschmidt et al. 1998, Goldschmidt and Hendrick 2002).

Background:
Melanocytic neoplasms are one of the most common neoplasms diagnosed in dogs and malignant melanoma is reportedly the most common oral malignancy (Bergman 2007, Goldschmidt 1985, Goldschmidt et al. 1998, Goldschmidt and Hendrick 2002, Ramos-Vara et al. 2000, Smith et al. 2002, Todoroff and Brodey 1979) Despite the prevalence of these tumors, a fairly recent review of melanocytic neoplasms (Smith et al. 2002) states that “there is no single diagnostic technique capable of differentiating benign from malignant melanocytic neoplasms or of predicting survival time.” Another study (Roels et al. 1999) stated that the “behaviour of melanocytic tumors is a continuous spectrum ranging from strictly benign to highly malignant. Thus, the borderline between the two categories may be rather broad.” The primary source, or foundation literature, for melanoma morphology and basic behavior in dogs and cats begins, in the veterinary literature, in the late 1950’s and early 1960’s. In these early studies, some of which were written by MD or dental pathologists (Gorlin et al. 1960, 1969, 1971).
1959, Mulligan 1961), the morphology of neoplasms is often illustrated as malignant or benign without specifically enumerating the anatomic basis for that distinction. Tumor behavior was similarly determined anecdotally from necropsy reports, but often lacked correlation of specific histological criteria of malignancy to the metastatic lesions observed (Brodey 1960). Unfortunately, conclusions of both early and current studies regarding prognosis of melanocytic neoplasms have become widely used in routine tumor pathology, regardless of whether those conclusions are adequately supported by scientific studies with sufficient case numbers, a reference population, specific inclusion and classification criteria, uniform therapy, statistical analysis, survival data, etc. Also, it is often difficult to compare prognostic studies due to different classification systems, different groupings of tumors, e.g. by location or inclusion of both canine and feline tumors, and different methodologies for evaluating specific parameters, such as mitotic index (MI) or Ki67. Furthermore, most published reports are retrospective studies and few base their survival data on post-mortem findings. Without an accurate prognosis, an appropriate decision regarding primary and/or adjunct therapy for dogs with melanomas can not be made (Hahn et al. 1994).

Results and Recommendations:

When attempting to predict the biological behavior of a particular melanocytic neoplasm, accurate determination of melanocytic origin of the neoplasm is crucial prior to prognostication. Since amelanotic melanomas are difficult to distinguish from soft tissue sarcomas, which commonly exhibit an aggressive biological behavior, inaccurate differentiation will impact the validity of any prognostic study. According to the current literature, immunohistochemical labeling with a combination of Melan-A, PNL2, TRP-1 and TRP-2 as single antibodies, or as a cocktail, has been shown to have the highest sensitivity for detecting amelanotic melanomas while maintaining 100% specificity (Cangul et al. 2001, Choi and Kusewitt 2003, Giudice et al. 2010, Koenig et al. 2001, Ramos-Vara et al. 2000, Sandusky et al. 1985, Smith et al. 2002, Smedley et al. in press, Sulaimon et al. 2002). Other commonly used antibodies, such as S-100 or MiTF are highly sensitive for detecting amelanotic melanomas, but lack the specificity necessary to provide an accurate diagnosis (Choi and Kusewitt 2003, Granter et al. 2001, Ohsie et al. 2008, Sandusky et al. 1985, Smith et al. 2002, Smedley et al. in press).

This review focused on prognostic parameters for canine cutaneous and oral melanocytic neoplasms. Ocular neoplasms are not discussed. According to the literature, parameters in each of the following categories have been evaluated for their prognostic significance: Signalment, Clinical Presentation, Location, Gross Morphologic Features, Microscopic Features, and Molecular Biology (Table 1). Only the most important parameters are discussed in more detail below.

Location:

In general, oral melanocytic neoplasms have been shown to have a worse prognosis than cutaneous neoplasms and those on the lip or digit have been shown to have a worse prognosis than cutaneous melanocytic neoplasms at other sites. However, as several studies (Bergin et al. in press, Esplin 2008, Laprie et al. 2001, Schultheiss 2006, Spangler and Kass 2006) have demonstrated, location alone can not be used to predict prognosis, as there are definitely exceptions to the above generalizations. Some factors have been shown to have prognostic significance for neoplasms of a particular site, but not for neoplasms at other locations. See Table 1.
**Gross Morphologic Features:**

Neoplasms less than 0.5 cm are expected to have a favorable prognosis and neoplasms greater than 1.0 cm are expected to have a poor prognosis (Spangler and Kass 2006). Hahn *et al.* 1994 also found that among dogs with oral melanomas, those with neoplasms less than 8 cm³ had significantly longer remission lengths and survival times. Distant metastasis is a negative prognostic factor for all neoplasms regardless of location.

**Microscopic Features:**

Histological classification of canine melanocytic neoplasms as benign or malignant has generally been associated with clinical outcome and survival. However, “morphologic classification” as a prognostic parameter by itself is difficult to evaluate in the currently published literature, as each study used different classification criteria.

Nuclear atypia and mitotic index (MI) have been shown to be the histologic features that have the highest positive predictive values in terms of predicting prognosis for neoplasms of various sites (Bergin *et al.* in press, Spangler and Kass 2006), but no single parameter should be used alone.

If the neoplasm is composed of epithelioid cells, we recommend assessing the degree of nuclear atypia, which should be based on the strict criteria described by Spangler and Kass 2006 and ideally evaluated on an incremental scale from 1 to 10, signifying the subjectively estimated percentage of nuclei involved (Spangler and Kass 2006). In reality, such a rigorous grading scale is unlikely to be applied in a routine diagnostic setting; however, the following detailed histologic features should be used to characterize the nuclei as well-differentiated or undifferentiated so that subjective scores of mild, moderate, or severe can be used to score the degree of atypia.

**Well-differentiated or typical melanocytic neoplastic cells** (Spangler and Kass 2006)

- small nucleus
- single, centrally oriented nucleolus
- minimal clumping of chromatin
- may have condensed strands of nuclear chromatin extending from the nucleolus to the nuclear membrane
- condensation of chromatin along the inner surface of the membrane
- cells that lack a nucleolus have fine and evenly dispersed chromatin at the periphery of the nucleus.

**More undifferentiated neoplastic cells (atypical)** (Spangler and Kass 2006)

- larger nucleoli of less-regular shape that are eccentrically located in the nucleus
- often multiple nucleoli
- in some cases, multiple nucleoli are haphazardly connected to the inner surface of the nuclear membrane by thin strands of chromatin and give the appearance of a coarsely vacuolated nucleus

Mild nuclear atypia would support a favorable prognosis, marked atypia would support a poor prognosis, and moderate atypia would be non-determinant.
The mitotic index should be determined by counting the number of mitotic figures in 10 consecutive 400x fields with counting starting in the area of highest mitotic activity for oral/lip neoplasms (Bergin et al. in press, Spangler and Kass 2006) and in random fields for cutaneous neoplasms (Laprie et al. 2001). Areas under ulceration should be avoided. The MI should be reported as the number of mitoses per 10 hpf (Bergin et al. in press, Goldschmidt et al. 1998, Laprie et al. 2001, Spangler and Kass 2006). Dogs with cutaneous neoplasms having a MI greater than or equal to 3 per 10 hpf (Bostock, Laprie et al. 2001) and dogs with oral/lip neoplasms having a MI greater than or equal to 4 per 10 hpf (Bergin et al. in press) are expected to have shorter survival times, whereas dogs with melanocytic neoplasms with a MI less than those values, for each respective site, are expected to have longer survival times.

The presence of inflammation and/or necrosis supports a poor prognosis for melanocytic neoplasms at any site (Spangler and Kass 2006). Some studies found an association between the degree of pigmentation and clinical outcome for oral and cutaneous neoplasms, respectively (Bergin et al. in press, Esplin 2008, Laprie et al. 2001). Especially when paired with assessment of nuclear atypia, the degree of pigmentation had prognostic significance (Bergin et al. in press). Dogs with highly pigmented, well-differentiated oral/lip neoplasms were found to have longer post-diagnosis survival times that approached two years (Esplin 2008). This parameter was less predictive in neoplasms with medium or low pigmentation (Bergin et al. in press).

One study (Millanta et al. 2002) demonstrated lymphatic invasion as a negative prognostic factor for oral and cutaneous melanocytic neoplasms. Even though lymphatic invasion was not specifically evaluated as a prognostic factor for melanocytic neoplasms of the lip or digit in that study (Millanta et al. 2002), vascular invasion is considered by some as the best indicator of malignancy (Goldschmidt et al. 1998).

For cutaneous melanocytic neoplasms, the presence of ulceration has also been associated with a poor prognosis (Laprie et al. 2001). This study included 12 digital melanocytic tumors. The level of infiltration/invasion has been shown to be a negative prognostic factor for cutaneous melanocytic neoplasms (also including 12 digital neoplasms) (Laprie et al. 2001), and possibly for oral neoplasms (Roels et al. 1999). Junctional activity has been associated with shorter survival times for feet and lip neoplasms (Spangler and Kass 2006) but its significance for cutaneous melanocytic neoplasms at other sites is uncertain due to conflicting reports (Laprie et al. 2001, Spangler and Kass 2006).

Histologic features should be combined to classify a melanocytic neoplasm as benign or malignant. If these features conflict with one another, the neoplasm should be classified as a melanocytic neoplasm and both the positive and the negative prognostic factors should be discussed.

Molecular Parameters:

No criteria have been established to allow for completely objective assessment of histologic parameters for prognostic purposes in a routine laboratory setting. In contrast, the Ki67 index, as a measure of the growth fraction of neoplastic cells, is a highly objective test that has a higher predictive value than histologic criteria (Bergin et al. in press, Laprie et al. 2001) and is especially useful for cases with conflicting histologic parameters, so called “gray zone” cases. For cutaneous melanocytic neoplasms, the Ki67 index should be reported as a percentage of positive staining nuclei per 500 cells (Laprie et al. 2001). Neoplasms with a Ki67 index greater than or equal to 15% are expected to have a poor prognosis and neoplasms with a Ki67 index less than 15% are expected to have a favorable prognosis (Laprie et al. 2001). For oral/lip
melanocytic neoplasms, the Ki67 index should be reported as the average number of positively
labeled nuclei per 1 mm² grid reticle at 400x by counting the number of positive nuclei in 5 grid
areas (Bergin et al. in press). Neoplasms with a Ki67 index greater than or equal to 19.5 are
expected to have a poor prognosis and neoplasms with an index less than 19.5 are expected to
have a good prognosis (Bergin et al. in press). For both methods, counting should commence in
an area with a high labeling index, but areas of inflammation and areas underlying ulceration
should be avoided (Bergin et al. in press, Laprie et al. 2001).

Conclusions:

Ki67 index has been shown to be a better predictor of the behavior of canine melanocytic
neoplasms than histologic features and is currently the most objective predictor that can easily be
used in a routine diagnostic setting (Bergin et al. 2011 in press, Laprie et al. 2001). We
recommend that Ki67 index be used as the determining factor for prediction of prognosis.
However, it is important to recognize that if the histologic and clinical features are in conflict
with the Ki67 index for a particular neoplasm, or if the Ki67 index is very close to the “cut-off”
or “threshold” value, it may not be possible to accurately predict the prognosis of that neoplasm.
Although it is impractical to accurately predict, on an individual basis, the biological behavior of
melanocytic neoplasms by applying elaborate numeric criteria to specific histologic features
(Spangler and Kass 2006), evaluation of nuclear atypia and MI in combination with Ki67 index
and clinical features will maximize the percentage of correctly classified neoplasms. An accurate
prognosis is becoming more and more important in veterinary oncology as various treatment
options are now available for specific types of neoplasms and many more clients are pursuing
these options. These treatments are often costly, may involve radical surgeries, and may have
significant or yet unknown side effects. Thus, it is important to identify which melanocytic
neoplasms require additional therapies and which will likely be cured by excision alone.

None of the previously published studies met all the standards outlined by the
“Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary
Oncology”(Webster et al. Epub 2010). We based our recommendations regarding prognostic
factors for canine melanocytic neoplasms on the studies that came the closest. Very few studies
had adequate sample numbers, strict selection criteria, and detailed statistical analysis with
survival data. A major difficulty in veterinary prognostic studies is accurately evaluating survival
data, as many pets are euthanized, rather than die, due to neoplastic disease. Often, it is even
difficult to associate the cause of euthanasia with the progression of neoplastic disease. Some
dogs with melanocytic neoplasms may be euthanized due to deterioration of health secondary to
neoplastic disease, others may be euthanized due to management issues that the owner can not
handle, and others may be euthanized for completely unrelated causes. It is also often difficult to
determine a cause of death, even in dogs that die a natural death, as necropsy is often not
performed. Additionally, none of the published studies specifically stated the expected
magnitude of each factor’s contribution or the expected prognosis of the population. Also, few
studies specifically defined a reference population. In some cases, these could be inferred by the
reader. Another major pitfall is that many of the studies that have examined various treatments
for dogs with melanocytic neoplasms have failed to use specific selection criteria. Thus, few
conclusions can be drawn from those studies. All of the studies examined were retrospective
studies, thus, prospective studies are greatly needed to confirm the validity of the described
prognostic parameters. Future canine melanocytic neoplasm prognostic studies should utilize the
“Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary
Oncology” (Webster et al. Epub 2010) to help with the study design. An effort should be made to design more prospective studies, especially to evaluate specific treatment protocols that would allow a more targeted therapy approach.
Table 1. Significance of Prognostic Factors for Canine Melanocytic Neoplasms Based on Location

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Oral Neoplasms</th>
<th>Lip Neoplasms</th>
<th>Digit Neoplasms</th>
<th>Other Cutaneous Neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signalment</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Weight of dog</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Stage of disease</td>
<td>Yes</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>No***</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Size of neoplasm</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Symmetry of neoplasm</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
</tr>
<tr>
<td>Morphologic Classification (benign vs. malignant)</td>
<td>Variable**</td>
<td>Variable**</td>
<td>Variable**</td>
<td>Variable**</td>
</tr>
<tr>
<td>Nuclear atypia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mitotic index</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>Cell type</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cellular pleomorphism</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Degree of pigmentation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>Junctional activity</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Uncertain</td>
</tr>
<tr>
<td>Invasiveness of epithelium</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ulceration</td>
<td>No</td>
<td>NE</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>Level of infiltration/invasion</td>
<td>Possible</td>
<td>NE</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>Yes</td>
<td>NE</td>
<td>NE</td>
<td>Yes</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ki67 index</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>Proliferation index</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
</tr>
<tr>
<td>Expression of Melan-A, S-100, vimentin, NSE</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DNA Ploidy</td>
<td>Possible</td>
<td>NE</td>
<td>NE</td>
<td>Possible</td>
</tr>
<tr>
<td>MCC and MVD</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
</tr>
<tr>
<td>Plasma VEGF</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
</tr>
</tbody>
</table>

* Only 12 digit neoplasms were included in the Laprie et al. study and they were grouped together with the other cutaneous neoplasms
** Dependent on which classification system used
*** Only limited data available
NE = site not specifically examined
Possible = Limited data support that this factor has prognostic significance for this location
IN = Insufficient data available

(Additional references for table provided upon request)
References