SURGICAL MARGIN EVALUATION IN VETERINARY MEDICINE: AN ASSESSMENT OF CANINE SKIN SHRINKAGE AND HISTOPATHOLOGIC REPORTING OF CANINE MAST CELL TUMORS

BY

JENNIFER K. REAGAN

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in VMS – Veterinary Clinical Medicine
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2017

Urbana, Illinois

Master's Committee:

Assistant Professor Laura Selmic, Chair
Clinical Associate Professor Laura Garrett
Assistant Professor Elizabeth Driskell

ABSTRACT:

When cutaneous mast cell tumors (MCT) are removed and evaluated for histopathology, the tissue undergoes many changes. Initially the mass is removed with planned surgical margins, which are a measure of grossly normal tissue around the tumor border. The cut margins are marked so that the pathologist can identify the margins in relation to the patient and the specimen is placed in formalin. Upon removal, however, the specimen undergoes noticeable shrinkage, which is further exacerbated by histologic processing. Upon being received by the laboratory, the gross appearance of the tissue has drastically changed from the time of surgery. This tissue is sectioned, stained and prepped for the pathologist to evaluate. Finally the pathologist prepares the histopathology report for the clinician. The histopathology reports, especially grade and margin reporting, are critically important for the clinician as they aid in the decision-making process regarding necessity and mode of adjunctive therapy recommended. However, the steps that occur from surgery until pathologic review can affect the pathologist's ability to report accurate histologic margins if appropriate measures are not taken. Likewise, it is important for the surgeon to understand the tissue shrinkage that occurs if histologic and surgical margins are ever to be correlated. Therefore, the second project focused on evaluating MCT histopathology reports, which is the major communication tool between the pathologist and the surgeon. The specific objectives of the first project were to evaluate the effects of anatomic location, histologic processing, and sample size on shrinkage of surgically excised canine skin samples. The purpose of the second project was to describe and evaluate information presented within canine MCT histopathology reports specifically focusing on how information that is important for determining future treatment recommendations and patient prognosis is reported.

In the first project, elliptical samples of the skin, underlying subcutaneous fat, and muscle fascia were collected from the head, hind limb, and lumbar region of 15 canine cadavers. Two samples (10 mm and 30 mm) were collected at each anatomic location of each cadaver (one from the left side and the other from the right side). Measurements of length, width, depth, and surface area were collected prior to excision (P1) and after fixation in neutral-buffered 10% formalin for 24 hours (P2). Length and width were also measured after histologic processing (P3).

The results of the first project found that length and width decreased significantly at all anatomic locations and for both sample sizes at each processing stage. Hind limb samples had the greatest decrease in length, compared with results for samples obtained from other locations, across all processing stages for both sample sizes. The 30-mm samples had a greater percentage change in length and width between P1 and P2 than the 10-mm samples. Histologic processing (P2 to P3) had a greater effect on the percentage shrinkage of 10-mm samples. For all locations and both sample sizes, the percentage change between P1 and P3 ranged from 24.0% to 37.7% for length and 18.0% to 22.8% for width. Based on the results of this project we concluded that histologic processing, anatomic location, and sample size affected the degree of shrinkage of a canine skin sample from excision to histologic assessment.

In the second project, MCT histopathology reports were collected from medical and surgical oncologists in 4 geographic regions of the United States: Midwest, Northeast, South and West. Up to 15 reports were obtained for cases presenting to these clinics between January 1st 2012 and May 31st, 2015. Inclusion criteria required that on the histopathology report the final diagnosis was MCT, a microscopic description was present, and the reports were from only cases where it was first attempt at surgical removal of the mass.

Three hundred and sixty-eight reports were collected from 26 contributors. While the majority of the reports contained a clinical history (85.9%), information to evaluate for prognostic indicators was lacking. Both Patnaik (Patnaik, Ehler, & MacEwen, 1984) and Kiupel (Kiupel et al., 2011) grading systems were described in 76.5% of reports with a single system being used in 7.1% and 15.2% of reports respectively. Subcutaneous MCT were assigned a grading scheme in 67.2% of reports with 33.3% stating appropriate limitations. Surgical margins were reported in 92% of the reports with 77.2% describing both deep and lateral margins. Tissue composing the deep margin was only described in 10.9% of the reports. Based on this project, it was concluded that reporting of MCT has variability across pathologists with inconsistencies present in the information provided for clinical history, the reporting of margin evaluation and grading of subcutaneous MCT.

ACKNOWLEDGEMENTS:

My sincerest thanks to my faculty and master's committee members, Dr. Laura Selmic, Dr. Heidi Phillips, Dr. Tisha Harper, Dr. Laura Garrett and Dr. Elizabeth Driskell for the support you have given me throughout my residency.

Thank you so much to my resident mates past and present. I would not have stayed sane without and you brought balance to my life.

I cannot show enough appreciation to my family, especially my dad. They have always supported me in everything that I do and without them I would not be here.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

When considering the treatment of cancer, there are 3 main pillars: surgical, medical, and radiation therapy. Surgery is the oldest of these pillars and surgical treatment of cancer in humans has been attempted for hundreds, potentially thousands of years. With the advent of antimicrobial and anesthetic drugs about 100 years ago, surgery became a viable treatment option and in the last 50 years incredible advances have been made in the control of various cancers through surgery and adjunctive therapies (Wyld, Audisio, & Poston, 2015).

At first glance the concept of surgical oncology is duplicitously simple: physically excise the cancer. In application though, questions quickly arise. One of the first questions to be asked is what tissue needs to be removed? The short, simplified and debatable answer: all cancer cells. Which quickly prompts the second question, how can one determine the microscopic delineation between neoplastic and normal cells in the macroscopic world of surgery? For some benign tumors, this line in the sand correlates well with the gross borders of the mass. However, for other malignant tumors, such as sarcomas, tumor cells may continue well beyond what is visible to the naked eye. Thus, the concept of "safety margins" was born which is the idea of taking a margin of normal tissue around a mass in hopes of complete excision (Kope, Bastos, Filho, & Gouvea, 2005). The recommended size of this safety margin is based on the suspected biologic characteristics of the tumor as well as information gleaned from research on surgical outcomes. In the youth of surgical oncology, it was thought that bigger was better and the trend was towards aggressive surgery to obtain wide margins (Kooby & Maithel, 2016). As surgical techniques, knowledge of tumor biology, adjunctive therapies as well as imaging capabilities have increased, a shift towards the recommendation for more conservative margins has occurred

(Kooby & Maithel, 2016). Decreasing the amount of normal tissue removed can greatly decrease surgical morbidity especially for large tumors or tumors in close association with vital structures (Kooby & Maithel, 2016; Kope et al., 2005).

In veterinary medicine, our understanding of margins in relation to specific tumor types is in its infancy. Mast cell tumors (MCT) are one of the most common cutaneous tumors and account for ~20% of all cutaneous tumors in canines (Garrett, 2014; London & Seguin, 2003). Its biologic behavior ranges from a benign lesion that is essentially cured with removal to an aggressive disease that can metastasize to the lymph nodes, spleen, liver, other abdominal organs and rarely lungs (London & Seguin, 2003). Histopathologic grading systems have been developed in an effort to help prognosticate the biologic behavior of the tumor based on histologic morphology. The two most common grading systems are the 2-tier system (high vs. low) by Kiupel et al. (2011) and the 3-tier system (1/low, 2/intermediate, 3/high) by Patnaik et al. (1984). Planned surgical and histologic margins have also been shown to be prognostic for MCT (Donnelly et al., 2015; Garrett, 2014; Mullins et al., 2006; Scarpa et al., 2012; Schultheiss, Gardiner, Rao, Olea-Popelka, & Tuohy, 2011; Seguin et al., 2006; Weisse, Shofer, & Sorenmo, 2002). The commonly held dogma has been that MCT should be excised with 3cm lateral margins and 1 fascial plane deep. However, recent studies suggest that for benign lesions, this level of resection may be excessive (Donnelly et al., 2015). These concerns mirror the shift that has occurred in human medicine.

Part of the difficulty in interpreting the current veterinary literature related to margins is the lack of standardization between study designs and variations in reporting of outcome measures. Some studies report outcome in relation to surgical margins, which are measured intra-operatively, while others describe outcome related to histologic margins. These

measurements are rarely equivalent especially for cutaneous tumors, such as MCT. The main cause of the discrepancy in measurements is related to shrinkage of the specimen that occurs after surgical excision (Becker, 2007). The phenomenon of shrinkage has been documented to a greater extent in humans and few studies have been performed in veterinary medicine. Understanding how shrinkage affects the relationship between surgical and histopathologic margins is important in order to be able to interpret the current literature on margins.

Anecdotally, the description of margins within MCT histopathology reports is variable. Histopathology reports are an important communication between the pathologist and the clinician and are often used to guide decisions related to the necessity or type of adjunctive therapy recommended for a patient (Kamstock et al., 2011). Histopathology reports are also a common source of information for retrospective studies. Within the medical record, MCT histopathology reports are a potential source of several prognostic factors including tumor size, location, histologic diagnosis, grade, histologic margins and, if performed, cellular markers of proliferation such as c-kit and PCNA. Standardization of MCT histopathology reporting, especially in relation to variably reported margins, is necessary to improve communication as well as improve their use for retrospective studies.

Related to histologic margins, there are several key points that should be included within the histopathology report (Kamstock et al., 2011). First, all histologic margins should be reported and the distance from the closest neoplastic cell to the margin should be given in metric units. Second, the tissue composing the margin should be described as some tissue types such as fascia are potentially more resistant to extension of tumor cells. Lastly, the terminology used to describe the histologic margins should not be subjective and terms such as close or narrow should be avoided. Ideally, all histopathology reports should list a mass as being incompletely or

completely excised. All of these recommendations greatly aid the clinician's ability to make recommendations related to adjunctive therapy and improve the communication between the pathologist and the clinician.

The overarching goal of this research was to add to the foundation of knowledge for understanding of the relationship between surgical and histopathologic margins in canine skin tumors, especially MCT. The objective of the first project was to evaluate effects of anatomic location, histologic processing, and sample size on shrinkage of excised canine skin samples. The objective of the second project was to describe and evaluate what information is presented within canine mast cell tumor (MCT) histopathology reports with a focus on the reporting of histologic margins.

CHAPTER 2

LITERATURE REVIEW

2.1 Concepts Affecting Margin Evaluation

When reviewing the human literature, numerous articles can be found discussing margins in relation to a specific tumor type. However, these articles are sparse within the veterinary literature. Therefore, both human and veterinary articles have been included together within this review. Generally, within both the human and veterinary literature, articles are focused on reporting or reviewing margin recommendations that have been made based on studies evaluating various outcomes such survival time, recurrence rate and metastatic rate in relation to the surgical or histopathologic margins. Recently the Journal of Surgical Oncology released an issue that was focused on the impact of surgical margins on patient outcomes for common solid organ tumors in humans such as breast cancer. The editorial briefly touched on the importance of surgical margins and the concept that margin status is also affected by histologic assessment which has not been standardized (Kooby & Maithel, 2016). Glimpses and minor references to the general concepts and complications associated with margin evaluation are common; however, there are few articles that thoroughly summarize the topic (Becker, 2007; Kope et al., 2005; Kott, 2008; Lurie, 2008).

2.1.1 Safety Margins:

Kope et al. attempted to review the literature on the concepts associated with "safety" margins. They discussed both macroscopic and microscopic safety margins with macroscopic safety margins being defined as the distance the surgeon plans to measure from the mass to the

cut edge (Kope et al., 2005). Microscopic safety margins are the distances measured from tumor cell to cut margin as determined by the pathologist (Kope et al., 2005).

Related to macroscopic margins, the main concept that complicates the clinician's ability to reliably use and define macroscopic margins is that the definition of the tumor margin is subjective and not reliable for infiltrative tumors (Kope et al., 2005). For some tumors, the visible/palpable margin correlates well with the distinction between normal and abnormal tissue while for other tumor types such as sarcoma, the neoplastic cells can extend beyond the visible margin of the tumor. In the case of cutaneous tumors, the properties of the skin can also complicate matters, as margin measurements will change depending on how much tension is placed on the skin. There are also other factors that could potentially affect the repeatability of macroscopic margin measurements and the execution of planned surgical margins in the patient. Measurements are sometimes made on surfaces that are irregular and may be difficult to measure accurately repeatedly. Another consideration is that the relative location of the tumor to other important anatomic structures (functional or aesthetic) may affect the clinician's decision to follow published macroscopic margin recommendations (Kope et al., 2005). These alterations in plan during surgery may not be well documented and could be a source of error, especially in retrospective studies.

The limitations of microscopic margins are related to changes that occur to the specimen after surgical excision, trimming methods, and the ability to distinguish normal cell from neoplastic cells (Becker, 2007). For example, in cutaneous tumors, translation of the tissue may make identifying the true cut margin difficult. In an effort to decrease error associated with this change, some clinicians mark the borders of the specimen with various inks or attempt to maintain the orientation the tissues with sutures or by pinning the sample to cardboard.

Inappropriate handling or rough transportation of a sample could result in loss of tissue from the periphery, resulting in a falsely positive margin or incomplete excision (Kope et al., 2005). The type of trimming method used by the laboratory can change the percentage of margins evaluated, which will be discussed in greater detail later in this chapter (Becker, 2007; Kamstock et al., 2011). Another, critical step is the decision of which portion of the specimens to trim (Becker, 2007; Kope et al., 2005). Related to certain tumors, such as MCT, the neoplastic cells can be difficult to distinguish from the normal populations of the cells within the tissue. Finally, how margins are reported on the histopathology report may affect treatment decisions or the ability to compare histologic margins to outcome.

2.1.2 Positive versus Negative Margins:

It is recommended that on histopathology reports margins be reported as incomplete versus complete and that the distance from the margin to the closest neoplastic cell be reported using metric measurements (Kamstock et al., 2011). Incomplete margins are defined as margins that have neoplastic cells touching the inked border (Kamstock et al., 2011). However, another paired terminology that commonly appears in the literature is positive and negative margins. For some, the terms positive and negative are synonymous with complete and incomplete, respectively. However, others use the terms positive and negative to include a certain width of normal tissue that has been defined within that respective paper or for a specific tumor type (Becker, 2007). Since the use of these terms is not standardized, one has to be careful when comparing outcomes across institutions in relation to positive and negative margins (Becker, 2007). Similarly for some tumors, margins will be reported as close/narrow and a definition may be associated. For example, for head and neck tumors in humans a close margin has been sited as

1-5mm while an involved margin was sited as <1mm (Sarode, Sarode, & Karmarkar, 2011). However, there is generally a lack of consensus on these definitions (Sarode et al., 2011).

The basic concept behind incomplete/complete and positive/negative margins is that what is truly important to the patient is whether or not tumor cells have been left behind. Related to this idea some advocate sampling the "true margin" which is the tissue around the area of the resection or the tumor bed (Becker, 2007; Sarode et al., 2011). Possible methods for evaluating the true margin include shaving, and optical diagnostics such as microendoscopy and optical coherence tomography (Sarode et al., 2011). Evaluating the true margin via shaving maintains many of the sources of error present within classical margin evaluation such as surgeon sampling or pathologists evaluating a non-representative portion of the tissue causing false negative results (Becker, 2007).

2.2 Specific Factors Affecting Margin Evaluation

2.2.1 Tissue Shrinkage

Numerous studies have been performed evaluating the phenomenon of post-operative surgical shrinkage in humans (Dauendorffer, Bastuji-Garin, Guero, Brousse, & Fraitag, 2009; Eid et al., 2007; El-Fol, Noman, Beheiri, Khalil, & Kamel, 2015; Gregory et al., 2003; Hudson-Peacock, Matthews, & Lawrence, 1995; Johnson, Sigman, Funk, Robinson, & Hoffman, 1997; Kerns et al., 2008; Sarode & Sarode, 2012; Sarode et al., 2011; Schned et al., 1996; Silverman et al., 1992; Wang et al., 2004) and in non-human species (Clarke, Banks, & Findji, 2014; George, Hyde, Wilson, & Smith, 2013; Jeyakumar et al., 2015; Johnson et al., 1997; Reimer, Seguin, DeCock, Walsh, & Kass, 2005; Risselada, Mathews, & Griffith, 2015; Upchurch, Malenfant, Wignall, Ogden, & Saile, 2014). Tissues evaluated in human medicine include skin, cervix,

intestine, rectum, prostate and oral mucosa. Studies on shrinkage have also been performed on various malignant cutaneous and mucosal tumors such as basal cell carcinoma, squamous cell carcinoma, and melanoma. In non-human species, shrinkage has been evaluated in the skin (dog and cat), oral mucosa (dog and pig) and intestine (dog). The only tumor type that has been specifically evaluated in relation to shrinkage is MCT (Risselada et al., 2015).

Focusing on shrinkage of skin, for cutaneous neoplasms in humans the mean percent shrinkage has been documented to range between 14-31% (Blasdale, Charlton, Weatherhead, Ormond, & Lawrence, 2010; Golomb et al., 1991; Gregory et al., 2003; Hudson-Peacock et al., 1995; Kerns et al., 2008; Silverman et al., 1992) with most studies reporting shrinkage to around 20%. Likewise in one study the surgical defect enlarged by 25% (Gregory et al., 2003). When evaluating depth after surgical excision and formalin fixation, one report found that depth increased, decrease, or stayed the same in 31%, 56% and 10% respectively (Gregory et al., 2003).

For normal canine skin and for canine cutaneous neoplasms, the range of skin shrinkage for lateral margins has ranged from 10.2%-18.45% (Upchurch et al., 2014), 17.6-32% (Reimer et al., 2005) and 35-42% (Risselada et al., 2015). The mean shrinkage of labiobuccal mucosa was 47.3% and the great portion of shrinkage was noted immediately after resection (Johnson et al., 1997). Only one study has evaluated skin shrinkage in cats and found that the length decreased by 32.40%, width decreased by 34.21%, and depth increased by 54.95% (Jeyakumar et al., 2015). In the canine, specimen thickness increased from 45.1-75.8% in one study (Reimer et al., 2005).

In humans, factors that have been implicated in affecting the degree of skin shrinkage include age (Golomb et al., 1991; Gregory et al., 2003; Silverman et al., 1992), location

(Dauendorffer et al., 2009; Gregory et al., 2003), tissue composition (Sarode & Sarode, 2012), tumor type (Blasdale et al., 2010; Hudson-Peacock et al., 1995) and method of resection (George et al., 2013; Smith, Hyde, & Wilson, 2008). However, the results of these studies have conflicting information and some state that age (Dauendorffer et al., 2009; Hudson-Peacock et al., 1995), location (Hudson-Peacock et al., 1995), and type of skin lesion (Dauendorffer et al., 2009) did not have an effect on shrinkage. In one study scar tissue did not correlate with shrinkage after excision and formalin fixation, but the scars were noted to be superficial (Kerns et al., 2008). While sex is often evaluated in relation to shrinkage, the majority of studies evaluating shrinkage have not found sex to be associated with shrinkage (Dauendorffer et al., 2009; Gregory et al., 2003; Hudson-Peacock et al., 1995).

In studies on the effects of shrinkage of normal canine skin and skin with MCT, factors that have been evaluated include location (Reimer et al., 2005), the presence of MCT tumor, lines of skin tension and presence of a muscle or fascial layer (Reimer et al., 2005) and size of the sample (Upchurch et al., 2014). Reimer et al found site/location to affect the degree of shrinkage, while Upchurch et al. did not find any correlation between site, sample size, or measurement orientation in relation to lines of tension (Reimer et al., 2005; Upchurch et al., 2014). The inclusion of a fascial layer did not have an effect on shrinkage, however, inclusion of muscle decreased the amount of shrinkage in length (17.6% vs. 26.6%) (Reimer et al., 2005). In contrast, in cats, inclusion of muscle on the thoracic samples had no effect on shrinkage (Jeyakumar et al., 2015).

Likely, the main cause of skin shrinkage after excision is due to skin's intrinsic tissue contractility (Kerns et al., 2008; Upchurch et al., 2014). In humans, skin looses tensile strength with age and the amount of shrinkage decreases by 0.3% for each year of age (Kerns et al.,

2008). Another study did not correlate age to skin shrinkage but they did note that younger patients had greater contraction of the skin edges (Hudson-Peacock et al., 1995). Specific factors of skin composition may be the underlying cause for different degrees of margin shrinkage within the same tissue specimen includes varying levels of elastin or tumor cells, degree of keratinization, inflammation, and desmoplasia (Kerns et al., 2008; Sarode & Sarode, 2012). In a study evaluating shrinkage of tumor in relation to tumor free margins, the tumor had a lesser degree of shrinkage (11%) compared to the tumor free margin (19%) (Blasdale et al., 2010). These results lend further support that tissue composition plays an important role in shrinkage. No studies in veterinary medicine have evaluated tumor shrinkage in relation to margin shrinkage.

2.2.1.1 Location:

In humans, truncal tumors had proportionally 5% more shrinkage than head and neck tumors (Kerns et al., 2008) and others have also documented a increased amount of shrinkage in truncal tissue (Hudson-Peacock et al., 1995). Dauendorffer found that a greater shrinkage in length was noted in specimens taken from the limb (Dauendorffer et al., 2009). Also, in their study the truncal site initially appeared to affect shrinkage, but was no longer significant when of specimen size was taken into account (Dauendorffer et al., 2009). In some these papers it was suspected that some locations (i.e. head) are more susceptible to photodamage with resultant loss of elasticity that may contribute to a loss in the degree of skin shrinkage after excision and formalin fixation compared to truncal sites (Gregory et al., 2003; Kerns et al., 2008). Another study did not find location was associated with shrinkage (Golomb et al., 1991).

In canines, one study showed site was significantly associated with shrinkage with the amount of shrinkage being largest in the hind limb (a region over the tibia), intermediate in the thoracic region, and least on the head (Reimer et al., 2005). These variations in shrinkage were suspected to be due the inherent properties of the subepithelial tissue and that the organization of the myoepithelial structures in the skin (Reimer et al., 2005). Another ideas was that areas of the body placed under greater tension with movement would be more elastic and therefore have greater contraction when excised (Reimer et al., 2005). As previously stated, Upchurch et al. did not find location to effect shrinkage which contradicts these results. However, when skin shrinkage was evaluated in cats, the tissue over the region of the tibia had the greatest amount of shrinkage (similar to the results of Reimer et al.) (Jeyakumar et al., 2015). While the Upchurch et al. and Reimer et al, both evaluated samples from the hind limb as well as thorax and head, possible reasons Upchruch et al. proposed for the discrepancy in findings included differences in sample excision technique, sample shape, breed, and live animals versus cadavers (Reimer et al., 2005; Upchurch et al., 2014).

2.2.1.2 Formalin Fixation:

The effects of formalin fixation may be tissue dependent. An MRI study was performed that evaluated formalin fixation on porcine limbs that were either whole or cross-sectioned into 2cm thick slices (Docquier et al., 2010). They found that the volume of fatty tissue shrunk by 4% (whole) and 9% (cross-sectioned) (Docquier et al., 2010). While muscle increased by 4-5% (whole) and 3% (cross-section) and formalin fixation had no effect on bone (Docquier et al., 2010). The study also showed that positioning effected the width/height of the whole samples, while the cross-sectioned samples which were placed flat had no overall change in width or

height(Docquier et al., 2010). Based on the MRI, gravity appeared to cause sagging of the muscle relative to the bone (Docquier et al., 2010).

Several studies have specifically evaluated the effect of formalin fixation on cutaneous tissue in humans (Dauendorffer et al., 2009; Golomb et al., 1991; Hudson-Peacock et al., 1995; Kerns et al., 2008). In a prospective study by Kerns et al. cutaneous lesions were excised and measured prior to excision, immediately after excision, and then 24 hours after formalin fixation. They found that significant shrinkage occurred after excision with the percent change in length being 23% and width being 14.6% (Kerns et al., 2008). Formalin fixation resulted in minor expansion of the samples (-3.6% length and -4.7% width). Other studies have also concluded that formalin is relatively non-contributory to cutaneous tissue shrinkage, with Dauendorffer et al. reporting that no difference in measurements after excision and formalin fixation being observed. (Dauendorffer et al., 2009; Golomb et al., 1991). However, there have been studies that found formalin did have a large effect on shrinkage. One study found that excision caused a 22% shrinkage while formalin fixation caused and additional 11% fixation (Hudson-Peacock et al., 1995).

For canines, only one study has specifically reported on the affects of formalin fixation on the degree of shrinkage in skin. Upchurch et al. reported that diameter measured immediately after excision and then formalin fixation did not change significantly with the overall percent changed being 1.69% (Upchurch et al., 2014). Reimer et al, performed the measurements necessary to assess the effects of formalin fixation but these measures were not specifically reported or discussed (Reimer et al., 2005). In a shrinkage study for feline skin (Jeyakumar et al., 2015), the effects of formalin fixation were not discussed, however, based on their results from

the time of excision until post-formalin fixation the median percent shrinkage for all locations/measurements was 3.9% (range -7.1-15.1%).

2.2.1.3 Method of Excision:

The type of instrument/method used to excise tissue may also have an effect on shrinkage. Shrinkage was evaluated in porcine lingual mucosa using cutting diathermy, coagulation diathermy, harmonic scalpel and a scalpel blade (Smith et al., 2008). They found that lesions excised with cutting or coagulation diathermy had less shrinkage compared to tissue excised with the harmonic scalpel or standard scalpel blade (George et al., 2013; Smith et al., 2008). It was theorized that the diathermy cause thermal damage and denaturation of the muscle which resulted in a decrease in contracture (George et al., 2013). Hudson-Peacock et al. noted that wound area decreased in size post-diathermy (Hudson-Peacock et al., 1995).

2.2.1.4 Relating Histologic Margins to Surgical Margins:

Many methods have been developed to evaluate shrinkage in an attempt to equate histologic margins to surgical margins. For basal cell carcinoma, a microscopic method was developed to measure shrinkage (Sarode & Sarode, 2012). The proposed advantages of this method were that it allowed the shrinkage of individual margins to be evaluated as well as was more accurate than macroscopic methods (Sarode & Sarode, 2012). Silverman et al. developed a formula to calculate surgical margins from histologic margins for melanoma. They found that 86.5% - 90.8% (depending on population used) of the specimens could have the surgical margins calculated to within +/-3.5mm from the histologic margins (Silverman et al., 1992). These types

of formulas are likely only useful for the specific populations they were derived for, in this case, human cutaneous melanoma for specific age groups.

Some advocate the margin guidelines should be shrinkage based, meaning the percent shrinkage should be calculated for each specimen (Sarode et al., 2011). As part of this recommendation, it was stated that histopathology reports should designate the distance of neoplastic cells from the deep and lateral cut edges (Sarode et al., 2011). In human medicine, accounting for shrinkage is not only useful when attempting to compare research studies but also has financial implications as it is a source of downcoding (Dauendorffer et al., 2009; Gregory et al., 2003). In this situation, downcoding is a term used to designate when costs related to surgical removal and evaluation of mass have been decreased inappropriately as cost is based on size. Since masses tend to decrease in size after excision and histologic processing, this is a potential source for financial loss for the providers.

2.2.2 Margin Marking

There are 3 benefits of marking margins with ink or sutures. 1) The marked margin defines the sample in relation to patient (Kamstock et al., 2011). 2) It visibly identifies all cut margins (Kamstock et al., 2011). 3) The marks can be used to denote an area of greatest concern (Kamstock et al., 2011). Other general guidelines that were listed by Kamstock et. al. for appropriately inking tissues included the following: 1) Recommend inking prior to formalin fixation as artifacts such as translation may alter the true margin. 2) Use official surgical ink or waterproof drawing ink to avoid loss of staining. 3) Blot tissue dry prior to inking. 4) Mark the margins with an object rather than dipping the specimen. 5) Allow ink to dry for 5-10 minutes prior to fixation. 6) Do not cut the specimen until all these steps have been performed. 7) The

best ink colors to use are black, yellow and green as they are easily seen against H&E stain. Also, if second margins are taken such as in the case of shaving, the orientation needs to be appropriately identified.

2.2.3 Tissue Trimming and Evaluation

Various trimming methods reported include cross-sectioning (i.e. radial method which uses perpendicular sectioning), breadloafing (i.e. parallel sections at regular intervals), modified technique that combines parallel and radial techniques, and tangential sections (shaved edges) (Kamstock et al., 2011). For cutaneous neoplasms, the margin can be evaluated either by perpendicular or parallel sectioning. Parallel sections (i.e. shave) of the margin allow a larger portion of the margin to be assessed for the presence of neoplastic cells, however they cannot determine the distance from the closest neoplastic cell to the margin (Lurie, 2008). Conversely, perpendicular sectioning allows the distance from the tumor to the margin to be measured but only represents a minor portion of the overall margin (Lurie, 2008). Perpendicular section is generally what's performed during radial sectioning and is commonly used for small and moderate sized masses. The disadvantages of radial sectioning are that this technique assumes symmetrical growth of the tumor when evaluating margins and only a very small portion of the tissue is being evaluated (<0.1%) (Kamstock et al., 2011). In the tangential technique multiple 2-3mm sections are shaved off the margin in a parallel fashion and any tumor cells noted within the shaved section make that margin incomplete (Kamstock et al., 2011) and has the disadvantages associated with parallel sections.

Often, a standard margin evaluation is generally performed unless otherwise requested by the surgeon. Without the surgeon specifically stating what they considered to be important areas

of evaluation, the most ideal positioning for transecting may not be performed (Kope et al., 2005). Likewise, the sectioning of the samples may be performed by non-pathologists and thus may not be as qualified to pick the best positioning for trimming (Kope et al., 2005). Both of these factors could potentially result in reporting of falsely complete margins.

2.2.4 Margin Reporting

Related to margin reporting, Kamstock et al. recommended that all histopathology reports include following: gross description/picture, diagnosis, microscopic description, comments section and references (Kamstock et al., 2011). Within each of these sections they made specific recommendations. For diagnosis, it was recommended that the grade should follow if applicable. Likewise if the clinician asked for a grade and one did not exist or wasn't created specifically for that tumor type, then a statement of limitation should be given. Also, the microscopic description should include all the findings that were used to derive that grade. For, MI it should be given as the number of mitotic figures per high powered field (400x) with ideally 10 fields with the highest activity being evaluated.

Related to margins, all the following should be included in the histopathology report (Kamstock et al., 2011): 1) method of specimen trimming, 2) description of cells closest to the margin, 3) an objective measurement of the margins and 4) a description of the tissue composing the margin. Kope et al. stated that they believe there may be a misunderstanding in the meaning of "free surgical margins" (Kope et al., 2005). This statement highlights 2 separate concepts related to margin reporting: 1) Factors previously discussed such as the biologic behavior of the tumor, post-operative tissue handling, tissue trimming, etc. can affect the pathologist ability to determine if tumor excision is complete. This is an idea that needs to be kept in mind when

reading the histopathology report. 2) The use of subjective description of margins can leave room interpretation and should be avoided (Kamstock et al., 2011). As previously discussed for positive vs. negative margins, the use of subjective terminology needs to be avoided (Kamstock et al., 2011).

The recommendations discussed in the section on margin marking should also be applied in this section as errors in inking can effect margin reporting. Possible reasons for falsely positive margins include separation of the tissue, lack of identified cut borders, translation and inability for the pathologist to definitively state complete or incomplete margins due lack of tissue or issues related to trimming (Kope et al., 2005). While the most likely reason for a falsely negative margin is related to the fact that in most cases only a small portion of the cut margin is evaluated and tumor growth is often asymmetrical (Kope et al., 2005).

2.3 Other Methods of Margin Evaluation

2.3.1. Mohs Micrographic Surgery:

In Mohs micrographic surgery (MMS), the surgeon, technician and pathologist may be (and ideally are) the same person (Kope et al., 2005). In this method of margin evaluation shaved sections are taken through the parallel and deep margins of the tumor (Lurie, 2008). The shave sections are meticulously labeled in relation to the to the tumor bed. The surgeon/pathologist immediately processes (inking and frozen section) and evaluates the sections while the patient waits. Further sections are then taken of any of the incomplete margins, essentially following the path of the tumor. The Mohs technique is most commonly used in the treatment of melanoma (Lurie, 2008)and basal cell carcinoma in humans. The advantages of this technique are that is allows for the minimal amount of healthy tissue to be removed while also helping to ensure

complete resection of the mass. Disadvantages include the process requires very specialized training, is time consuming and is only practical for relatively small tumors.

Mohs micrographic surgery has been reported for management of periocular mast cell tumor in a dog (case report) (Bernstein, Storey, & Bauer, 2013). The possible indications for MMS include: tumors with poorly defined local invasion, anatomic locations with greater subclinical invasion, locations that require tissue conservation and known perineural invasion (Bernstein et al., 2013).

2.3.2 Shave Technique:

The underlying concept of shaving in breast cancer is similar to the Mohs technique except modified for use with a larger tumor. Shaving is generally performed around the resection cavity and the number of shaved sections is variable. Because of the larger size of these masses, if intraoperative evaluation is performed only one frozen section perpendicular to the margin is evaluated (Lurie, 2008). The location of the section to be cut is generally chosen off of gross evaluation of the mass (Lurie, 2008) and has the same limitations as radial sectioning. Unsurprisingly, falsely negative complete margins are not infrequent (Lurie, 2008). Additional shaved margins are taken as deemed necessary by the surgeon. Like with the Mohs technique, inking and orienting the shaved sections in relation to the tumor bed is important. After standard formalin fixation and processing, the margins are evaluated, however the method of margin evaluation is not standardized (Lurie, 2008).

2.3.3 Intraoperative Surgical field Imaging:

Various modalities are being studied and used in human medicine to evaluate the wound bed or the "true margin" including: optical coherence tomography, radiofrequency spectroscopy,

Raman spectroscopy, positron emission tomography probes and near-infrared fluorescence optical imaging (Liptak, 2013). None of these modalities are currently standard of care in veterinary medicine. However, near-infrared fluorescence optical imaging has been used in research studies in veterinary medicine (Iida et al., 2013) and may gain more widespread use a promising technique for further evaluating margins in companion animals (Liptak, 2013).

2.4 Margin Evaluation and Reporting for MCT

2.4.1 General Information

Mast cell tumor (MCT) is one of the most common cutaneous tumors in the canine with reports ranging its frequency between 7-21% (Withrow & Vail, 2007). Its biologic behavior is variable and has been associated with multiple prognostic factors including: presence of clinical signs(Mullins et al., 2006), tumor location (Garrett, 2014; Gieger et al., 2003; Kiupel, Webster, Miller, & Kaneene, 2005), number of concurrent tumors (Kiupel et al., 2005), stage (Garrett, 2014) and tumor size (Mullins et al., 2006), mitotic index (MI) (Berlato et al., 2015; Elston, Sueiro, Cavalcanti, & Metze, 2009; Garrett, 2014; Romansik, Reilly, Kass, Moore, & London, 2007; van Lelyveld et al., 2015), histologic grade (Donnelly et al., 2015; Garrett, 2014; Kiupel et al., 2011; Murphy, Sparkes, Smith, Blunden, & Brearley, 2004; Patnaik et al., 1984; Sabattini, Scarpa, Berlato, & Bettini, 2015; Stefanello et al., 2015; Takeuchi et al., 2013), histologic margins (Donnelly et al., 2015; Garrett, 2014; Mullins et al., 2006; Scarpa et al., 2012; Schultheiss et al., 2011; Seguin et al., 2006; Weisse et al., 2002) and various cellular markers (Berlato et al., 2015; Costa Casagrande et al., 2015; Garrett, 2014; Kandefer-Gola et al., 2015; Maglennon et al., 2008; Scase et al., 2006; Seguin et al., 2006; Takeuchi et al., 2013; van

Lelyveld et al., 2015; Vascellari et al., 2013; Webster, Yuzbasiyan-Gurkan, Miller, Kaneene, & Kiupel, 2007).

The most common method of diagnosis for MCTs is through fine needle aspiration; but only recently a current method has been documented to predict grade based off of cytological samples. The 2 studies that have been published attempting to predict grade based on cytology have shown promising results, but are not commonly used in the clinical setting (Camus et al., 2016; Scarpa, Sabattini, & Bettini, 2014). Established grading methods currently applied to MCT are based on histopathology samples. Currently there are 2 common methods for grading MCT: the 2-tier method (Kiupel et al., 2011) and the 3-tier method (Patnaik et al., 1984) which are described in greater detail later within this chapter. Low grade tumors that are completely excised generally do not require further treatment, while adjunctive therapies are often recommended if there are incomplete margins or metastatic disease (Donnelly et al., 2015). However, the lack of standardization in the research studies related to the use of MCT grading systems and the cut off values for complete margins make comparisons across studies difficult.

Mast cell tumors have been reported to metastasize to local lymph nodes, lungs, spleen, liver and bone marrow (Withrow & Vail, 2007). Therefore, possible staging modalities that may be considered include local lymph node fine needle aspirates, thoracic radiographs or CT, and abdominal US or CT. Depending on the results of the imaging and clinician preference, fine needle aspirates of the spleen or liver may also be considered. However, it's important to note that the ultrasonographic appearance of the liver/spleen can appear normal in the face of metastatic disease or abnormal in appearance without the presence of disease. However, detection of metastatic disease within a draining lymph node is strongly correlated with prognosis; one study documented that distant metastatic disease was only noted in dogs that had

local metastasis to the region lymph node (Warland, Amores-Fuster, Newbury, Brearley, & Dobson, 2014). The same study also found that none of the dogs had visible metastatic disease on thoracic radiographs calling into question the utility of this diagnostic test. Instead they suggested that staging initially rely on assessment of the regional lymph nodes for the presence of disease (Warland et al., 2014). Rates of distant metastatic disease have been reported as 6.8% (Warland et al., 2014), while rates of local metastatic disease have been reported as 30.9% (Warland et al., 2014).

In addition to grade and margins, overall survival time is effected by presence of metastatic disease and various markers of proliferation such as c-KIT, AgNOR and PCNA (Berlato et al., 2015; Costa Casagrande et al., 2015; Garrett, 2014; Kandefer-Gola et al., 2015; Maglennon et al., 2008; Scase et al., 2006; Seguin et al., 2006; Takeuchi et al., 2013; van Lelyveld et al., 2015; Vascellari et al., 2013; Webster et al., 2007). The location of metastatic disease also effects survival as dogs that have metastatic MCT within the bone marrow have a shorter median survival time of 43 days with a range of 14-57 days (Marconato et al., 2008).

2.4.2 Histopathology and Grading

One of the first papers that attempted to correlate histologic findings (grade) to biologic behavior was written by Bostock in 1973 (Bostock, 1973). This grading system published in this paper was modified into the Patnaik system published in 1984 (Patnaik et al., 1984). The Patnaik system is a 3-tier system grading MCT as grade 1, grade 2, and grade 3. Generally grade 1 tumors are considered well differentiated and confined to the interfollicular dermis. Grade 2 tumors are intermediately differentiated and extend into the deep dermis/subcutaneous tissue while grade 3 tumors are poorly differentiated with infiltration (Patnaik et al., 1984). Specific

histologic features that are used to determine the grades are the following: location, cell morphology, nuclear morphology, architecture, cellularity, stromal reaction, mitotic figures, edema and necrosis (Northrup et al., 2005; Patnaik et al., 1984). The 3-tiered system has been shown to prognostic when evaluating grade 3 tumors versus grade 1 & 2 tumors. In a study of 340 cutaneous MCT, grade 3 tumors had a median survival time of 278 days, versus over 1300 days for grade 1 & 2 tumors (Murphy et al., 2004). In the same study recurrence was found in 19% of grade 3 tumors and 6% of grade 2 tumors. Other studies have reported recurrence rates for grade 2 MCT to by between 4-21% (Scarpa et al., 2012; Schultheiss et al., 2011; Weisse et al., 2002) while for grade 3 and high grade tumors the recurrence rate was 36% (Donnelly et al., 2015). For grade 3 tumors, lymph node status was consistently associated with outcome and metastatic disease generally was found to be the cause treatment failure (Donnelly et al., 2015; Hume et al., 2011). This study also reported the overall survival time to 257 days which similar to Murphy et al.

Multiple studies have shown that grade 2 tumors can have a variable behavior and there appears to a subpopulation that behaves like high-grade tumors (Murphy et al., 2004; Stefanello et al., 2015). Furthermore, one of the main criticisms of the Patnaik grading system is that majority of MCT are classified as grade 2. In a study of 100 dogs, only 22% and 4.3% had grade and 1 and 3 MCT respectively while 74% had grade 2 MCT (Schultheiss et al., 2011). The guidelines used to grade under the Patniak system are subjective. In one study, grade 3 tumors only had a 75% concordance and grade 1 & 2 tumors had a 63% concordance (Kiupel et al., 2011). Because of this subjectivity and potentially the pathologist's reluctance to pick a side, many the majority are classified as grade 2 diminishing the prognostic value of this grading system (Northrup et al., 2005; Sabattini et al., 2015).

In an attempt to overcome the deficiencies of the Patnaik system, the 2-tiered system was developed (Kiupel et al., 2011). In this system, tumors are either classified as low grade or high grade, which eliminates the option for indecision. This system is also less subjective as a MCT is considered high grade if it has any one of the following characteristics: 1) ≥7 mitotic figures/hpf, 2) ≥3 multinucleated giant cells, 3) ≥3 bizarre nuclei or 4) karyomegaly in 10% of cells(Kiupel et al., 2011). However, karyomegaly and bizarre nuclei can still be subjective measures. The 2-tiered system has been shown to be prognostic with high grade tumors having a 4 month survival time. It has also been shown to have 97% consistency between observers (Kiupel et al., 2011). Low grade tumors also have a low risk (4%) of local recurrence (Donnelly et al., 2015). It has also been shown that the 2-tiered system can potentially identify the biologically more aggressive tumors that are classified as grade 2 on the 3-tiered system (Donnelly et al., 2015).

When it comes to histologic reporting of MCT, it has previously been recommended to use both systems ("VCS Oncology-Pathology Working Group MCT Subgroup Consensus on Grading Canine Cutaneous MCT," 2013) as each has potential strengths/weaknesses and each have been associated with prognosis (Donnelly et al., 2015; Kiupel et al., 2011; Murphy et al., 2004; Patnaik et al., 1984; Schultheiss et al., 2011; Stefanello et al., 2015; Takeuchi et al., 2013). However, recently studies have been published comparing both grading systems (Sabattini et al., 2015; Stefanello et al., 2015; Takeuchi et al., 2013). Both Sabattini et al. and Takeuchi et al. evaluated the grading systems in relation to survival and both concluded the Kiupel system had superior prognostic value. Stefanello et al. evaluated the grading systems for prognosticating metastatic disease and concluded prognostication should not rely solely on grade but factor in the results of staging as both grade 1/2 and low grade tumors had metastatic disease (5.8%/16.5% and 14.9% respectively).

Mitotic index is used as one of the criteria for the 2-tiered grading system; however, MI has been shown to be prognostic in its own right. Mitotic index has been shown to correlate with grade and survival (Romansik et al., 2007). A MI ≤5 had a median survival time of 70 months, compared to 2 months (all grades) or 5 months (grade 2) (Romansik et al., 2007). It was also shown that dogs with grade 3 tumors and MI ≤5 did not reach median survival time (Romansik et al., 2007) suggesting that is one of the key factors in the 2-tiered Kiupel system's ability to distinguish biologically aggressive tumors. Other studies have evaluated MI and Ki67 in relation to survival (Berlato et al., 2015; van Lelyveld et al., 2015). Mitotic index >5 was had poor sensitivity (32.4%) but good specificity (96%) as a predictor of death, while generally the inverse was true for Ki-67 (van Lelyveld et al., 2015). Another study used a different stratification for MI with MI>7, MI 1-7 and MI <1 and found that median survival time was 3 months, 15 months and not reached respectively (Elston et al., 2009).

2.4.3 Margins

Multiple studies have been performed attempting to correlate histologic and planned surgical margins of MCT to prognosis (Donnelly et al., 2015; Garrett, 2014; Mullins et al., 2006; Scarpa et al., 2012; Schultheiss et al., 2011; Seguin et al., 2006; Weisse et al., 2002). While it can be agreed that patients with complete margins have a better prognosis than patients with incomplete margins, the safety margin (histologic or surgical) necessary to obtain complete margins is still being debated. Classically it has been recommend that MCT be excised with 3cm lateral margins and 1 fascial plane deep. However, the evidence to support this recommendation is lacking especially for low grade tumors. Simpson et al. prospectively evaluated 1cm, 2cm, and 3cm planned surgical margins and found that all grade 1 MCT and 75% of grade 2 MCT were

complete excised with 1cm, while all grade 2 MCT were complete excised with 2cm surgical margins (Simpson et al., 2004). Based on this study it was suggested at 2cm margins and 1 fascial plane deep should be sufficient for grade 1 & 2 MCT (Simpson et al., 2004). Fulcher et al. excised 23 MCT using these recommendations and had complete margins in all but tumors. No local recurrence was noted and the survival time was never reached (> than 538 days). Another study found that low grade tumors with histologically tumor free margins less than 3mm did not recur and that high grade tumors had significant risk of local recurrence regardless of histologic tumor free margin width (Donnelly et al., 2015).

These studies highlight the difficulty in comparing results and recommendations related to margins and outcome. For example, while the Donnelly study suggests that 3mm may be sufficient margin, they are referring to histologic margin, which can't be correlated back to surgical margins. The Simpson et al. study reported outcome in planned surgical margins related to histologic margins, however this study had relatively few dogs and there are potential factors that could have skewed their results such as tissue loss and measurement errors secondary to histologic processing. Also all dogs in the Fulcher and Simpson et al. studies were grade 1 and 2 MCT. Also, error associated with defining the border of the MCT could affect the results as the intraoperative determination of margins can be difficult if excessive edema formation from spontaneous cytokine release occurs (Matz, 2015) or if the borders of the tumor are irregular.

Currently, there is no standardization in histologic reporting for what is considered a complete or "clean" margin. Many studies report the use of 1-2mm (Pratschke, Atherton, Sillito, & Lamm, 2013; Thompson et al., 2011; Weisse et al., 2002) away from the margin as considered clean, however some require at least 5 or 9 mm of normal tissue between the margin and neoplastic cells (Kry & Boston, 2014; Murphy et al., 2004).

A method of surgical margin determination for MCT has been proposed that is based on dimension of the tumor and proportional margins (Pratschke et al., 2013). In this system, the measurement of the lateral surgical margins was based on the largest diameter of the mass (maximum of 4cm) while keeping the deep margin as one fascial plane deep. Using this approach 85% of the tumors had a histologically complete resection (Pratschke et al., 2013). One of the major limitations of this study however were that the majority of the tumors were low grade (90% based on the Kiupel system), therefore while only 1 dog was suspected to have progression of disease, this may not reflex a normal population of MCT.

2.4.4 MCT and recurrence

Despite the presence of incomplete margins, grade 2 MCT often do not recur with a 23.3% recurrence rate being reported (Seguin et al., 2006). Lack of recurrence with positive margins may be related to a falsely positive margin due to the presence of non-neoplastic mast cells (Becker, 2007; Kope et al., 2005). However, in a study evaluating outcome of dogs with either incomplete excision or "close" margins that underwent additional adjunctive therapy (re-excision or radiation), it was found that survival time was improved compared to dogs that did not receive additional treatment (Kry & Boston, 2014). In that study, median survival times for dogs with re-excision or radiation was 2930 days and 2194 days respectively while in the untreated group survival time was 710 days (Kry & Boston, 2014). This data may suggest that while some dogs may be false positives, some could be truly incomplete resections.

Possible reasons for tumor recurrence in the face of supposedly complete margins include the previously mentioned possibility of erroneous sampling or margin evaluation. However, a more interesting possibility is the concept of field cancerization, which is the idea that the cancer

changes the microenvironment around the tumor, predisposing the cells in close association to the tumor to become cancerous (Becker, 2007). Another possibility relatively unique to MCT is that since MCT produce chemotactic factors, it can be difficult to tell if the mast cells near the margins are neoplastic cells, if they are secondary to local inflammation or if they are local mast cells that are naturally present (Fulcher et al., 2006; Gieger et al., 2003; Kry & Boston, 2014).

2.4.5 Subcutaneous and other Non-Cutaneous MCT

It has been recommended that the standard grading systems designed for cutaneous MCT should not be applied to subcutaneous/non-cutaneous MCT as they demonstrate different biologic behaviors (Elliott et al., 2016; Newman, Mrkonjich, Walker, & Rohrbach, 2007; Thompson et al., 2011). Generally subcutaneous MCT are considered to a more benign biologic behavior than their cutaneous counterparts. Thompson et al. reported that the median survival time was unable to be reached and the probability of survival was 95%, 93%, 92% and 86% at 6 months, 1 year, 2 years, and 5 years respectively (Thompson et al., 2011). Also, only a 4% metastatic and 8% local recurrence rate was present in spite of the fact that 56% had incomplete resections (Thompson et al., 2011).

The primary grading systems used for grading MCT were specifically developed for cutaneous MCT and have not been validated for non-cutaneous MCT (Elliott et al., 2016; Thompson et al., 2011). In subcutaneous tumors it has been shown that grade is not indicative of behavior. However other factors such as an infiltrative growth pattern have been associated with MCT mortality with mortality being approximately 3x more likely with an infiltrative growth than in well circumscribed tumors (Thompson et al., 2011). Mitotic index has also been associated with prognosis in subcutaneous MCT with a MI >4 having a median survival time of

140 days and being 54x more likely to have metastatic disease than a dog with a MI of 0 (Thompson et al., 2011).

Other types of non-cutaneous MCT in canines include mucosal MCT and conjunctival MCT (Elliott et al., 2016; Fife, Blocker, Fife, Dubielzig, & Dunn, 2011). Similar to subcutaneous tumors, conjunctival tumors have a low risk of local recurrence, are unlikely to have metastatic disease and overall have a good prognosis (Fife et al., 2011). In contrast to subcutaneous MCT, mucosal MCT are associated with a worse prognosis. A recent study evaluating mucosal MCT found that there was a 55% lymph node metastatic rate at the time of diagnosis and that both MI (MI >3 and MI >5 were prognostic cutoff points) and lymph node metastasis had a poor prognosis (Elliott et al., 2016). Dogs with lymph node metastasis had an overall survival time of 276 days and dogs without adequate local control had a median overall survival time of 150 days (Elliott et al., 2016). Where adequate local control was defined as a complete excision on histopathology or a microscopically (not grossly) incomplete excision followed by radiation therapy.

CHAPTER 3:

EVALUTION OF THE EFFECTS OF ANATOMIC LOCATION, HISTOLOGIC PROCESSING AND MARGIN SIZE ON THE DEGREE OF SHRINKAGE OF SKIN SAMPLES AFTER SURGICAL EXCISION

3.1 INTRODUCTION

Safety margins are considered to be the required distance from a given tumor that will theoretically result in complete excision of the tumor; safety margins are used in surgical planning (Kope et al., 2005). These margins usually are derived from studies conducted to evaluate outcome related to histologic margins or outcome compared to the macroscopic safety margins used during surgery. The concept of a safety margin has many confounding factors; however, understanding the relationship of these planned surgical margins to histologically determined margins is necessary to better interpret outcomes related to these measures (Becker, 2007; Kope et al., 2005; Reimer et al., 2005).

Histopathologic margins are often the standard used to measure success of surgical resection and have been associated with the prognosis for a patient (Kamstock et al., 2011; Reimer et al., 2005). The working definition of a histopathologic margin is the distance from the cut edge of the surgical sample to the closest identifiable tumor cell (Auw-Haedrich, Frick, Boehringer, & Mittelviefhaus, 2009; Donnelly et al., 2015). Although histopathologic margins are often considered a defined and objective measure, there are many sources of error and subjectivity that can add uncertainty to interpretation of the results. For cutaneous tumors, separation of deeper anatomic layers from the skin, type of tumor, surgical method, postoperative handling, method of histologic sectioning, and various patient factors can all influence measurements of histopathologic margins (Becker, 2007; Donnelly et al., 2015; Giudice et al., 2010; Hudson-Peacock et al., 1995; Kope et al., 2005). Also, the phenomenon of shrinkage (i.e.,

change in sample size between presurgical measurements and post-histologic processing) has been described (Dauendorffer et al., 2009; Gregory et al., 2003; Hudson-Peacock et al., 1995; Kerns et al., 2008), and this phenomenon further complicates interpretation of histopathologic margins in relation to planned surgical margins.

Most of the studies conducted to evaluate skin shrinkage after surgical excision have been performed on samples obtained from humans. Factors reported to influence the amount of skin shrinkage include patient age, location of the tumor, inherent elasticity of the skin in that patient, and presence of scar tissue (Kerns et al., 2008). However, there have been conflicting results that indicate age, sex, and skin lesion type (benign vs. malignant) do not affect shrinkage (Dauendorffer et al., 2009). It was once commonly believed that histologic processing, specifically formalin fixation, may affect skin shrinkage. However, studies in human (Dauendorffer et al., 2009; Kerns et al., 2008) and veterinary (Upchurch et al., 2014) medicine have found that formalin fixation has little or no effect on change in sample size.

Shrinkage of samples of clinically normal skin of dogs has been evaluated in 2 studies (Reimer et al., 2005; Upchurch et al., 2014). Investigators in the earlier study (Reimer et al., 2005) found that the degree of shrinkage is dependent on location and reported that inclusion of a muscle layer but not a fascial plane may decrease the amount of shrinkage. Investigators in the latter study (Upchurch et al., 2014) evaluated sample size, location, and tension lines; however, in contrast to the earlier study, they did not find that location affected skin shrinkage. Although the earlier study did include histologic processing, effects of processing were not assessed independently from effects of formalin fixation. The effect of histologic processing on size of skin samples was not assessed in either study.

The purpose of the study reported here was to examine the relationship between planned surgical and histopathologic margins by evaluating factors (e.g., size of skin sample, anatomic location, and histologic processing) that may affect shrinkage. We hypothesized that the degree of shrinkage of histologically processed clinically normal skin samples differs on the basis of sample size and location and that shrinkage occurs following histologic processing but to a lesser degree than the shrinkage that occurs between excision and formalin fixation.

3.2 MATERIALS AND METHODS

3.2.1 Sample

Skin samples were obtained from 15 canine cadavers consisting of 13 pit bull–type terriers and 2 mixed-breed dogs (6 sexually intact males, 6 sexually intact females, and 3 neutered males). Dogs of breeds with thin skin (e.g., greyhounds) or excessively loose skin were excluded. Cadavers were restricted to dogs with a body weight between 15 and 35 kg and with a body condition score of 4 to 6 on a scale of 1 to 9 (Laflamme, 1997). The skin was required to have a grossly normal appearance with no evidence of neoplasia, scars, current lesions, or previous dermatologic disease. The cadavers were obtained from a local animal shelter, and samples were collected within 2 to 8 hours after dogs were euthanized. All dogs used in this study were euthanized for reasons unrelated to the study.

3.2.2 Sample Collection and Processing

Templates were created to serve as a guide for excision of skin samples. Templates consisted of durable water-resistant foam and represented 10-mm and 30-mm margins from a central marker. For the 10-mm samples, length and width of the ellipse was 40 mm and 20 mm, respectively. For the 30-mm samples, the length and width of the ellipse was 120 mm and 60 mm, respectively. These dimensions were chosen because they replicated lateral margins created from a central point (e.g., tumor), with the length being 2 times the width. Because the tumor in this study was a pinpoint, the sample size was considered equal to half the diameter of the width. This elliptical shape is commonly used clinically to increase the ease of skin closure.

A 10-mm sample and a 30-mm sample were collected from each of 3 anatomic locations (head, hind limb, and lumbar region) on each cadaver **(Figure 1)**. Sample size (10 mm or 30

mm) was randomly allocated via coin toss to a side for each cadaver; all 10-mm samples were collected from one side of a cadaver, and all 30-mm samples were collected from the other side of that cadaver. Cadavers were placed in lateral recumbency for collection of skin samples from the hind limbs and lumbar region and in sternal recumbency for collection of skin samples from the head. Hair was clipped from each location prior to collection of samples.

Samples from the head were centered over the temporal region, with the long axis of the ellipse placed in a cranial-to-caudal direction. For samples from the lumbar region, the center was placed at the midpoint between the last rib and the crest of the wing of the ilium at a point approximately 4 to 5 cm lateral to the vertebral spinous processes; the long axis of the ellipse was placed in a cranial-to-caudal direction. For skin samples collected from the hind limbs, the patella and greater trochanter were detected by use of manual palpation, and the sample was centered between these 2 points. Samples were obtained cranial to the long axis of the femur, and the long axis of the ellipse was placed parallel to the line of tension (Irwin, 1966). Direction of the ellipse was chosen on the basis of a consistent fascial layer with minimal muscle attachment, to facilitate bilateral positioning for collection of the samples, or both.

The template was lightly held in place so that the skin was pulled or stretched as little as possible. By use of the template, an ellipse was drawn on the skin with a fine-point marker, and a needle coated in surgical ink (Davidson marking system, Bradley Products Inc, Bloomington, Minn.) was used to mark the center **(Figure 2)**. The template was removed, and the margins were measured for accuracy by use of a soft ruler accurate to 1 mm.

A partial-thickness skin incision through the epidermis was made along the marked ellipse (Figure 2). A photograph of the ellipse and ruler was obtained. Photographs were subsequently used to calculate SA. Length (long axis of the ellipse) and width (widest point of

the ellipse perpendicular to the long axis) were measured prior to excision of the samples (i.e., first processing stage; P1). Initially, 3 measurements were obtained and the mean value calculated for use; however, this practice was discontinued because of the highly repeatable nature of length and width measurements. After the length and width were measured, the incision was extended deeper into the skin at the points used to measure the length and width until the fascial layer could be identified. A depth gauge typically used for cortical bone measurements was used to measure the P1 depth (distance from the surface of the skin to the fascial layer). The most cranial, caudal, lateral (or medial) and dorsal (or ventral) extents of the incision were marked with different colors of surgical ink (Davidson marking system, Bradley Products Inc., Bloomington, Minn.); the ink was allowed to air dry for 10 minutes. A full- thickness skin incision was made on half of the sample, and the remainder of the deep fascial layer was undermined. The skin was loosely sutured to the fascia at 3 of the ink-marked points to prevent movement of fascia prior to completion of the skin incision and sample removal. Once the sample was excised, a fourth suture was placed at the remaining ink-marked point. Samples were placed flat on the bottom of a sample container and immersed in neutral-buffered 10% formalin (approx. 1 part tissue to 10 parts formalin) for 24 to 48 hours. Measurements as described for P1 were again obtained at 24 to 48 hours after the start of formalin fixation (i.e., P2). The same investigator (JKR) performed all measurements at P1 and P2. After the length and width measurements were obtained at P2, photographs were obtained and subsequently used to calculate SA.

Samples were trimmed via radial cuts. The first cut was made on the short axis between the ink-marked points. Samples were then cut on the long axis between the ink-marked points. The trimmed samples were subjected to standard embedding in paraffin. Slides were stained with

H&E stain and evaluated by a board-certified veterinary pathologist (KS). Measurements of the long and short axis for each of the trimmed sections for each slide were added to determine the length and width, respectively, at this processing stage (i.e., P3). Because of tissue sectioning and creation of artifacts during histologic processing, depth was not measured at P3.

Photographs obtained at P1 and P2 were used to calculate SA. Publically available software (Rasband WS. ImageJ, version 1.48, 1997–2014. Bethesda, Md: US National Institutes of Health. Available at: http://imagej.nih.gov/ij/. Accessed April, 30th, 2014.) was used for SA calculations.

3.2.3 Statistical Analysis

Descriptive statistics were calculated for body condition score and body weight. Continuous variables were assessed for normality by analyzing histograms for skewness and kurtosis and by use of the Shapiro-Wilk test. Mean and SD were reported for normally distributed variables, and median and range were reported for nonnormally distributed variables. Percentage change for length, width, depth, and SA between P1 and P2 was calculated by use of the following equation: ([P1 measurement – P2 measurement]/P1 measurement) X 100. Percentage change for length and width between P2 and P3 was calculated by use of the following equation: ([P2 measurement – P3 measurement]/P2 measurement) X 100. Percentage change for length and width between P1 and P3 was calculated by use of the following equation: ([P1 measurement – P3 measurement]/P1 measurement) X 100. All calculations were performed with commercially available software (Microsoft Excel for Mac 2011, version 14.4.4, Microsoft Corp, Redmond, Wash.). Linear mixed models of commercially available statistical software (SAS software, version 9.3 of the SAS system for PC, SAS Institute Inc, Cary, NC.) were used to

evaluate the effect of time, anatomic location, and sample size on changes in measurements of length, width, and SA. Length, width and SA represented fixed effects, and dog was a random effect. This statistical method was used because of the loss of 1 data point for SA. Significance was set at values of $P < 0.05$.

3.3 RESULTS

3.3.1 Samples

A total of 90 samples were obtained from the 15 cadavers (6 samples/cadaver). Median body condition score was 5 (range, 4 to 6), and mean ± SD body weight was 23.6 ± 3.9 kg. All measurement data were available for all samples, except for 1 missing data point for the SA calculation of 1 dog because of a lost photograph.

3.3.2 Change in Absolute Length and Width of Samples

Time, anatomic location, and sample size all influenced length, width, and SA. Mean and SD of the length and width for each anatomic location and sample size were summarized **(Tables 1 and 2)**.

Absolute length and width decreased significantly ($P < 0.001$) from P1 through P3 for both sample sizes and all anatomic locations. There were significant ($P < 0.001$) decreases in length and width between P1 and P2, P2 and P3, and P1 and P3. Generally the decrease in length and width between P2 and P3 was less than that between P1 and P2 for the 3 anatomic locations; these changes were more marked in the 30-mm samples.

Length and width of all samples at the 3 anatomic locations decreased significantly ($P < 0.001$) over time (P1 through P3) for both sample sizes. Hind limb samples had a significantly ($P < 0.001$) greater decrease in length, compared with results for samples obtained from the lumbar region and head, between P1 and P2, P2 and P3, and P1 and P3 for both sample sizes. Samples from the lumbar region had an intermediate decrease, and samples from the head had the smallest decrease in length; results for each of these anatomic locations differed significantly ($P < 0.001$) from results for all other locations for all processing stages and both sample sizes. The

absolute change in width was similar for both sample sizes for all locations among all 3 processing stages.

Sample length decreased significantly ($P < 0.001$) for both 10-mm and 30-mm samples between PI and P2, P2 and P3, and P1 and P3 for all anatomic locations. Decrease in length was significantly ($P < 0.001$) greater for 30-mm samples than for 10-mm samples for all anatomic locations and processing stages. The decrease in sample width between P2 and P3 was less that between P1 and P2 for all anatomic locations and both sample sizes.

3.3.3 SA

Mean and SD of the SA for each anatomic location and sample size were summarized **(Table 3)**. The SA decreased by 10.3% to 59.3% between P1 and P2 for all samples. Mean ± SD percentage change for the SA for the 10-mm samples from the head, lumbar region, and hind limb was 33.5 ± 12.6%, 37.5 ± 9.8%, and 40.0 ± 10%, respectively. Mean percentage change for the SA for the 30-mm samples from the head, lumbar region, and hind limb was 34.1 ± 9.9%, 39.4 ± 0.1%, and 48.8 ± 6.3%, respectively.

3.3.4 Percentage Change in Length and Width

Percentage change in length and width between P1 and P2, P2 and P3, and P1 and P3 was evaluated for each sample size and anatomic location **(Tables 4 and 5)**. Percentages were calculated because they were believed to be a more clinically relevant and intuitive measure than was the absolute change.

For all locations and sample sizes, the percentage change between P1 and P3 ranged from 24.0% to 37.7% for length and from 18.0% to 22.8% for width **(Tables 4 and 5)**. Mean

percentage change in width between P2 and P3 was less than the mean percentage change in width between P1 and P2. In general, the mean percentage change in length was greater than the mean percent change in width between P2 and P3, relative to their corresponding changes between P1 and P2. Mean percentage change in length or width of all samples was greater between P1 and P3, compared with the percentage change between P1 and P2.

Hind limb samples had a greater difference in the mean percentage change in length between P1 and P2, compared with results for lumbar region samples, which was greater than results for head samples **(Tables 4 and 5)**. Mean percentage change in width between P1 and P2 was similar for all anatomic locations and sample sizes, except for the 10-mm hind limb sample. Mean percentage change in length between P1 and P3 was greater for hind limb samples than for the other locations.

Mean percentage change for 10-mm samples between P2 and P3 generally was greater than that for the corresponding 30-mm samples **(Tables 4 and 5)**. Mean percentage change for the 30-mm samples between P1 and P2 generally was greater than that the 10-mm samples. Mean percentage change in length and width between P1 and P3 was similar for both sample sizes, although there was greater variation in length for the 30-mm samples.

3.3.5 Percentage Change in Depth

Mean ± SD percentage change in depth between P1 and P2 ranged from –30.9 ± 22.7% to 6.0 ± 13.6%. In general, depth increased between P1 and P2.

3.4 DISCUSSION

Tumor type and histopathologic margins are two of the most important factors considered when making treatment recommendations for cutaneous tumors. Although the diagnosis of tumor type may be straightforward, interpretation of histopathologic margins has many complicating factors. Efforts have been made in human medicine to further define the relationship between histopathologic and surgical margins for various tumor types and tissues, including tongue or labiobuccal tissue (Johnson et al., 1997), rectal cancer (Eid et al., 2007), and nonmelanoma skin cancer (Lane & Kent, 2005). However, this relationship is poorly understood in veterinary medicine, with only a handful of studies (Fulcher et al., 2006; Reimer et al., 2005; Risselada et al., 2015; Simpson et al., 2004; Upchurch et al., 2014) conducted to correlate planned surgical margins to histopathologic margins. Before attempting to define this relationship for various tumor types in clinical settings, it is important to gain a better understanding of the manner in which surgical handling, histologic processing, and patient factors affect shrinkage in anatomically normal canine skin.

In the study reported here, decreases in absolute length and width following excision and histologic assessment (P1 to P3) and shrinkage in relation to sample size and anatomic location corroborated results of other veterinary studies conducted to evaluate shrinkage. The overall percentage shrinkage from the time of excision planning through histologic processing ranged from 24.0% to 37.7% for length and 18.0% to 22.8 % for width. This is similar to previously reported findings of a total surface change of 21.2% to 32% for canine skin samples (Reimer et al., 2005) and 21% for human skin samples (Gregory et al., 2003). Sample shrinkage is an important consideration when attempting to plan surgical margins based on histopathologic margins. Although these results cannot be directly translated to clinical cases, if these findings

were similar for a given tumor type, a 10-mm histologic margin may equate to approximately a 13- to 16-mm planned surgical margin (safety margin).

Recommendations for size of planned surgical margins differ greatly depending on tumor type. Marginal resections are recommended for some benign cutaneous tumors, whereas at the other extreme, planned surgical margins of up to 5 cm are recommended for vaccine-associated feline sarcomas (Phelps, Kuntz, Milner, Powers, & Bacon, 2011). Except for a recent study, (Upchurch et al., 2014) investigators in other studies have not evaluated whether size of the sample has an effect on shrinkage. In the present study, we found that histologic processing, anatomic location, and sample size all had an effect on the amount of change in length, width, and SA between the processing stages. To our knowledge, this was the first study of canine samples that revealed sample size affected the amount of skin shrinkage. In comparing the commonly planned margins of 10 and 30 mm, length had a greater absolute decrease for 30-mm samples than for 10-mm samples at all anatomic locations and processing stages. When the percentage change was evaluated, percentage change in length between P1 and P2 for the 30-mm samples was greater than the change for the equivalent 10-mm sample (13.7% vs 15.1%, 23.8% vs. 30.6%, and 17.2% vs. 19.7% for head, hind limb, and lumbar region, respectively). However, when the percentage change between P1 and P3 was evaluated, percentage changes were relatively similar when comparing 10-mm and 30-mm samples for the same anatomic location.

Each anatomic location had differing degrees of skin shrinkage. The hind limb sample had the greatest decrease in length and width for all processing stages for both sample sizes, whereas the lumbar region samples had an intermediate decrease, and the head samples had the smallest decrease. Investigators in another study (Reimer et al., 2005) also found that samples obtained from a hind limb distal to the stifle joint (as opposed to proximal to the stifle joint in the

present study) had an increase in shrinkage, compared with shrinkage for sites on the head and thorax, providing further support that the hind limb may be predisposed to more shrinkage than other regions of the body. This behavior may be related to the inherent increase in elasticity or mobility of the skin in that hind limb region necessary for movement. However, results for the study reported here are in contrast to those of a recent study (Upchurch et al., 2014) in which investigators found differences in margins and location had no effect on shrinkage. In that study, (Upchurch et al., 2014) investigators specifically aimed to evaluate shrinkage related to tension lines; however, the study design differed from that of the present study because they collected circular samples (2, 4, and 6 cm). Possible reasons proposed for the differences in findings related to location include differences in sample excision technique, sample shape, breed, and live animals versus cadavers. Given that similar animals were used in both the aforementioned study (Upchurch et al., 2014) and the present study, shape, location, and excision technique were the most probable contributors. Skin in the pelvic limb, particularly in the cranial portion of the thigh, is highly mobile, and even mild tension can change the amount of skin removed. Also, evaluation in the other study included only up to the point at 24 hours of formalin fixation (equivalent to P2 in the present study) and did not include histologic evaluation. Because the present study found that histologic processing contributed substantially to the final amount of shrinkage, the lack of histologic evaluation in the previous study (Upchurch et al., 2014) may also have accounted for the differences in results related to the use of various sample sizes. In the aforementioned study, (Upchurch et al., 2014) results of total percentage shrinkage (equivalent to between P1 and P2 for the present study) ranged from 10.25% to 18.25% and were similar to our results for the same processing stage when hind limb samples were excluded (13.7% to 19.7%).

In general, the mean percentage changes in length between P1 and P2 were greater for the 30-mm samples at a given location than for the 10-mm samples; however, this pattern was diminished when comparing changes between P1 and P3 for the 30-mm and 10-mm samples. Because the 10-mm samples proportionally had a greater mean percentage change between P2 and P3, in comparison to the percentage change for the 30-mm samples, this variation was likely attributable to effects of histologic processing. Histologic processing had a major effect on shrinkage. The mean ± SD percentage change between P2 and P3 ranged from 5.1 ± 7.3% to 19.5 ± 5.8% for the 10-mm samples and 4.2 ± 8.8% to 11.9 ± 3.7% for the 30-mm samples. Cutting of samples for histologic examination results in the loss of 1 to 2 mm of tissue. It follows that this loss would have a greater effect on a small sample than on a large sample. Another possible factor that may have contributed to the difference between the results was interobserver measurement error.

Histologic processing consists of many steps, including fixation in formalin, embedding in paraffin, trimming of tissue, tissue staining, and microscopic evaluation of the tissue (Kamstock et al., 2011). Skin shrinkage during formalin fixation has been evaluated in both human and veterinary medicine, and most reports (Dauendorffer et al., 2009; Kerns et al., 2008; Reimer et al., 2005; Upchurch et al., 2014) indicate that shrinkage as a result of histologic processing is minimal. Because this change reportedly is minimal, it was not directly evaluated in the present study, but it may have contributed to some of the differences between P1 and P3. In addition to the tissue that is lost during cutting, there may be some changes to the conformation of the tissue during histologic preparation of the tissue prior to cutting. These losses are difficult to quantify and will likely differ according to each sample being processed.

As part of the methods use in the present study, locations that were measured were marked with surgical ink in an attempt to decrease the error associated with obtaining measurements at different locations. It was evident during examination of samples after formalin fixation that considerable conformational changes occurred in the samples that made it challenging to obtain accurate measurements despite precise marking of the locations to be measured. Some of these changes included rolling of the sample edges; movement, separation, and rotation between the subcutaneous, fascial, and dermal layers; and general distortion of the sample as a result of contraction. Specific protocols were implemented in the present study to attempt to limit these changes associated with tissue handling and formalin fixation. Only half of each sample was dissected at a time to ensure the layers remained appropriately aligned. A loose, simple-interrupted suture was placed at the measurement locations before removal of a sample from a cadaver to limit shifting of dermal layers. Samples were placed flat on the bottom of formalin containers, and formalin was gently added to ensure the samples remained as flat as possible. Anecdotally, we found that these practices greatly increased the ability to more consistently measure differences between processing stages. Without use of these practices, it would have been difficult or impossible to obtain consistent measurements. Difficulties encountered in this study and the benefits of specific practices in sample acquisition and preparation highlight the importance of meticulous sample preparation in clinical cases to ensure that accurate margin interpretation by a veterinary pathologist is possible. Practices regarding perioperative and postoperative handling of tissue samples can impact the interpretation of margins, and further studies to evaluate these practices are warranted. Currently, these practices are not standardized. For example, in some studies (Fulcher et al., 2006; Simpson et al., 2004) conducted to evaluate mast cell tumors of dogs, the specimens were pinned to cardboard in their

approximate original shape prior to formalin fixation. The manner in which changes in post-excisional handling of tissues (e.g., pinning) affect shrinkage and margin interpretation is unknown.

In an attempt to further quantify overall shrinkage, SA was compared between P1 and P2. The SA decreased between 10.3% and 59.3 % for all samples between P1 and P2; however, the mean ± SD percentage change between samples ranged from 34.1 ± 9.9% to 48.8 ± 6.3%. The major source of this variability likely was related to the method of SA evaluation. The SA was calculated from 2-D images; however, the samples curved with the lines of the cadaver and were not in a flat plane in P1 SA images. Images were centered over the samples to account for this as much as possible in P1 images; however, distortion caused by normal body curvature, compared to flat skin, likely caused considerable variability in SA calculations.

In general, samples appeared to increase in thickness (i.e., depth) between P1 and P2 for the 4 measurement points. However, there was a wide range in the mean ± SD percentage change among the measurement points (range, –30.9 ± 22.7% to 6.0 ± 13.6%). Most of the means were negative, and a negative number represented an increase in thickness. To the authors' knowledge, this was the first study in veterinary medicine in which investigators attempted to measure depth at P1. A previous study (Reimer et al., 2005) revealed an increase in thickness between stages equivalent to P2 and P3. Because of the distortion (i.e., movement or separation between the fascial layers) that occurs during histologic processing, depth was not measured at P3 in the study reported here because it was thought likely to be inaccurate.

Limitations of the present study were related to the nature of basic research and use of cadavers. It was possible that postmortem changes within canine skin could have affected the innate properties of skin and therefore skin shrinkage. Skin samples in this study were obtained

within 2 to 8 hours after dogs were euthanized to mitigate postmortem effects. Results of this study were consistent with those of another study (Reimer et al., 2005) that involved the use of live dogs, which suggested that the use of cadaveric tissue had a small or no effect on outcomes. Also, use of cadavers allowed collection of a greater number of samples, which decreased inherent statistical error. Another limitation of this study was that it was performed with anatomically normal canine skin and did not account for effects that a tumor may have on skin shrinkage. A recent retrospective study (Risselada et al., 2015) conducted to evaluate histologic shrinkage specifically for mast cell tumors found that the mean shrinkage was 35% to 42% and that there was a greater percentage shrinkage in appendicular than in truncal samples in certain directions. These percentages are similar to but overall greater than those for the present study. This difference may have been a reflection of the effect of the tumor, use of live vs. cadaveric tissue, size of the tissue excised or limitations inherent within a retrospective study design such as variations in record keeping, individual measurement techniques, and tumor morphology.

3.5 CONCLUSIONS

On the basis of the results for the present study, the primary hypotheses were accepted that anatomic location and sample size affected the degree of shrinkage when comparing surgical margins to histopathologic margins. Also, the secondary hypothesis that shrinkage following histologic processing (P2 to P3) was less than between excision and formalin fixation (P1 to P2) was also accepted. Given that investigators of previous studies (Dauendorffer et al., 2009; Kerns et al., 2008; Upchurch et al., 2014) have found that formalin fixation has minimal effects on shrinkage, this suggests that most of the skin shrinkage likely occurred directly after excision. The next step in this line of research would be to further define factors that affect margin evaluation, such as evaluating shrinkage in relation to margins for various cutaneous tumor types to determine the role of the tumor in shrinkage. Other factors to be evaluated that may affect shrinkage include other potential tumor locations and various methods of periexcisional and post-excisional handling of samples. The information presented within this chapter has been previously published (Reagan et al., 2016).

CHAPTER 4:

EVALUATION OF INFORMATION PRESENT WITHIN MAST CELL TUMOR HISTOPATHOLOGY REPORTS: 2012-2015

4.1 INTRODUCTION

The histopathology report is the primary form of communication between the clinician and pathologist (Kamstock et al., 2011; Newman, 2003). The pathologist's ability to provide useful information to the clinician is dependent on the quality of the clinician's input such as providing an accurate history with detailed physical exam findings as well as performing appropriate sample submission and margin marking (Brannick, Zhang, Zhang, & Stromberg, 2012; Kamstock et al., 2011). Likewise, the pathologist is expected to provide a diagnosis when possible and any histologic parameters that may predict biologic behavior (e.g. recurrence or metastasis) for that specific tumor type such as reporting of margins (Kamstock et al., 2011; Newman, 2003).

Kamstock et al. published a consensus providing guidelines and recommendations on veterinary surgical pathology reporting (Kamstock et al., 2011). Generally they recommended that a report should include the following components: diagnosis, grade when applicable, microscopic description, comments/remarks and references (Kamstock et al., 2011). Related to grade, it was recommended that grade be listed after the diagnosis, the features used to arrive at that grade should be described, and a reference should be reported for the grading scheme (Kamstock et al., 2011). Other specific features of the report that were discussed included mitotic index (MI) and histologic margins. They stated that MI should be listed as the number of mitotic figures per number of high power field (ideally minimum of 10 high power fields) (Kamstock et al., 2011). When reporting margins, the method of trimming should be reported. Also, margin evaluation should include a description of the closest neoplastic cells, an objective measure from

the closest neoplastic cell to the margin, and the tissue types/quality composing the margin (Kamstock et al., 2011).

Recently the VCS Oncology-Pathology Working Group MCT Subgroup published a consensus on cutaneous mast cell tumor (MCT)("VCS Oncology-Pathology Working Group MCT Subgroup Consensus on Grading Canine Cutaneous MCT," 2013) grading which recommended that both Kiupel (Kiupel et al., 2011) and Patnaik (Patnaik et al., 1984) grading systems be reported and that MI should be standardized in a similar fashion as that recommended by Kamstock et al. ("VCS Oncology-Pathology Working Group MCT Subgroup Consensus on Grading Canine Cutaneous MCT," 2013). It was also stated that grade should be used in conjunction with the overall clinical picture and other prognostic indicators to predict biologic behavior and recommend treatment options ("VCS Oncology-Pathology Working Group MCT Subgroup Consensus on Grading Canine Cutaneous MCT," 2013). For MCT, many prognostic factors have been evaluated that may be present in the histopathology report. Prognostic information that may be included within the history section includes presence of clinical signs (Mullins et al., 2006), tumor location (Garrett, 2014; Gieger et al., 2003; Kiupel et al., 2005), number of concurrent tumors (Kiupel et al., 2005), stage (Garrett, 2014) and tumor size (Mullins et al., 2006). While information supplied by the pathologist associated with MCT prognosis includes MI (Berlato et al., 2015; Elston et al., 2009; Garrett, 2014; Romansik et al., 2007; van Lelyveld et al., 2015), histologic grade (Donnelly et al., 2015; Garrett, 2014; Kiupel et al., 2011; Murphy et al., 2004; Patnaik et al., 1984; Sabattini et al., 2015; Stefanello et al., 2015; Takeuchi et al., 2013), histologic margins (Donnelly et al., 2015; Garrett, 2014; Mullins et al., 2006; Scarpa et al., 2012; Schultheiss et al., 2011; Seguin et al., 2006; Weisse et al., 2002) and various cellular markers (Berlato et al., 2015; Costa Casagrande et al., 2015; Garrett, 2014; Kandefer-

Gola et al., 2015; Maglennon et al., 2008; Scase et al., 2006; Seguin et al., 2006; Takeuchi et al., 2013; van Lelyveld et al., 2015; Vascellari et al., 2013; Webster et al., 2007).

Histopathology reports are critically important in the clinician's decision-making process regarding necessity and mode of adjunctive therapy recommended after tumor removal (Kamstock et al., 2011; Newman, 2003). However, anecdotally considerable variation exists in the types of information in histopathological reports for MCT especially relating to histologic margins and the grading system used. The purpose of this study was to describe and evaluate the information present within histopathology reports for surgically resected canine cutaneous MCT. We hypothesized that both Kiupel and Patnaik grading systems would be used for cutaneous MCT but not all reports would contain both grades. Also, margin reporting would be variable and not all reports would contain complete information on lateral and deep surgical margins.

4.2 MATERIALS AND METHODS

Histopathology reports for cases diagnosed as MCT from January 1st 2012 to May 31st 2015 were collected from various clinicians across the United States. The United States were divided into 4 regions (**table 6**) based on the regions described in by the United States Census Bureau (website).

All clinicians contributing histopathology reports were board certified specialists (medical oncologist or surgeon), however the reports submitted could have been requested by a referring veterinarian or the contributing veterinarian or their colleagues. The institutions associated with the contributing clinicians were comprised of veterinary teaching hospitals and private specialty practices. It was requested that reports from their most recent MCT cases that were eligible were submitted for evaluation. A maximum of 15 reports were collected from each contributor.

All clinicians participating in the study were asked to complete a basic questionnaire related to their associated institution and to submit a copy of each final histopathology report. The questionnaire gained information related to the practice including: type of practice (specialty referral practice or veterinary teaching hospital), the practice's name, contact information and the name/credentials of the clinician collating the reports. Inclusion criteria for the study required the following: submission of a completed questionnaire and final canine histopathology report with diagnosis of MCT, a microscopic description must be included in the histopathology report, and this must have been the first attempt at MCT excision. Incisional biopsies were excluded. The reports were evaluated based on recommended general histopathologic reporting guidelines (Kamstock et al., 2011)and types of data evaluated are listed in **table 7**. The reports were evaluated by 2 individuals (JKR and CF). Interobserver error was not assessed, however, JKR

reviewed all columns that contained subjective interpretation (see table 2) and any disagreement was adjudicated by LES.

Descriptive statistics were calculated for each category. Continuous variables were assessed for normality by analyzing histograms for skewness and kurtosis, and by Shapiro-Wilk test. If variables were normally distributed mean and standard deviation was presented or if they were not normally distributed the median and range were presented. All statistics were performed using commercially available software (Microsoft Excel for Mac 2011, version 14.4.4, Microsoft Corp, Redmond, Wash. and SAS software, version 9.3 of the SAS system for PC, SAS Institute Inc, Cary, NC.).

.

4.3 RESULTS

A total of 395 MCT histopathology reports were received from 26 contributors across the United States. Twenty-seven reports did not meet the inclusion criteria and were excluded from statistical analysis. Of the remaining 368 reports, 96 (26.1%) were from the Midwest, 82 (22.3%) were from the Northeast, 99 (26.9%) were from the South and 91 (24.7%) were from the West. Across all regions, laboratories associated with veterinary universities produced 121 of the reports (32.9%), private laboratories created 227 of the reports (61.7%) and in 20 reports (5.4%) the laboratory could not be determined.

4.3.1 Signalment and Clinical History:

Information on the patient signalment was present in 316 (85.9%) of the reports with 65 breeds represented. The highest frequencies of reports were from the following breeds: Labrador retriever (13.1%, 48 reports), mixed breed (12.3%, 45 reports), boxer (7.1%, 26 reports) and golden retriever (6.5%, 24 reports). For sex, 38.3% (141 reports) were male, 46.2% (170 reports) were female and 15.5% (57 reports) were missing this data. A clinical history or description of the lesion was given in 315 reports (86%). The median number of words in the history was 17 (range 0-317). The description of the clinical history was considered adequate in 175 reports (47.6%) (see Table 7 for the definition of adequate). The most common historical information provided was the location of the lesion (292 reports, 79.3%), suspected tumor type (219 reports, 59.5%) and method of diagnosis of the suspected tumor type (122 reports, 33.2%). Other information provided included duration of disease (96 reports, 26.1%), number of masses present (81, 22%), size (70 reports, 19.0%), growth rate (48 reports, 13.0%), age at diagnosis (41 reports, 11.1%) and medications the patient has received (31, 8.4%).

4.3.2 Gross and Microscopic Description:

A gross description was reported in 97 reports (26.4%) with 1 report containing images of the sample. Of the reports that contained a gross description, 43 reports measured the sample size in three dimensions, 23 in two dimensions and 21 reports in one dimension. Mitotic index was given in 126 reports (34.2%) and mitotic figures per high power per field were listed in 342 reports (92.9%). Seven reports (1.9%) did not list a MI or mitotic figures per high power field. The tissue of origin was recorded as cutaneous in 280 reports (76.1%), subcutaneous in 52 reports (14.1%), muscular in 4 reports (1.1%), unknown in 4 reports (1.1%), submucosal/mucosal in 2 reports (0.5%) and the description of the tissue of origin was missing in 26 reports (7.1%).

4.3.3 Histologic Margins:

Some description of histologic margins was present in 356 reports (96.7%), however description of both lateral and deep margins was present in only 284 reports (77.2%). The histologic margins were quantified in centimeters or millimeters in 287 reports (78.0%). Subjective descriptors (e.g. clean, close, etc.) were used in 88 reports (23.9%) while 188 reports (51.1%) specifically stated complete vs. incomplete margins. The direction (e.g. cranial, caudal, etc.) of the closest lateral margin was noted in 60 reports (16.3%). The tissue type composing the margin, the quality of the tissue composing the margin and a description of the neoplastic cells closest to the margin were rarely recorded (40 reports/10.9%, 4 reports/1.1% and 2 reports/0.5% respectively). The method of sample trimming was noted in 62 reports (16.8%), and 123 (33.4%) of the reports had an indication that the margins were marked with ink or suture. Only 16 reports (4.3%) contained the surgeon's planned margin at the time of surgery.

4.3.4 Grading System:

For cutaneous tumors, a grade was given in 308 reports (98.7%). For non-cutaneous tumors, a grade was stated on 39 reports (67.2%), while 26 of these reports (44.8%) did not state the limitations of the application of grading systems to these tumor types. The number of reports that were graded with each grading system is listed in **Table 8**.

4.3.5 Comments Section:

Comments were provided in 363 reports (98.6%) with 275 reports (74.7%) giving general comments on the biologic behavior of MCT and 8 reports (2.2%) giving treatment recommendations. Additional immunohistochemistry (IHC) stains and diagnostics were performed or offered in 131 reports (35.6%) with the most common being c-KIT PCR (124 reports, 33.7%), c-KIT IHC (106 reports, 28.8%), Ki-67 (95 reports, 25.8%), AgNOR (72 reports, 19.6%) and PCNA (30 reports, 8.2%). The percent of reports that offered or performed additional stains and/or diagnostics for cutaneous MCT were 13.3% (6 reports) for grade 1, 38.6% (78 reports) for grade 2, 40.5% (15 reports) for grade 3, 34.3% (71 reports) for low grade and 52.9% (27 reports) for high grade. For non-cutaneous tumors, additional IHC stains and diagnostics were performed or recommend in 20 reports (34.5%). A medical oncology consultation was recommended or offered in 71 reports (19.3%). References for the comments were provided in 296 reports (80.4%).

4.4 DISCUSSION

The main purpose of a histopathology report is as a conveyor of information. Therefore, in order to evaluate a report, the important information that needs to be conveyed needs to be determined. Related to MCT, the clinician needs to know factors that influence prognosis and thus further treatment recommendations.. These factors include confirmation of tumor type, histopathologic grade of the MCT, information on the histologic margins and potentially recommendations for further diagnostic tests (Kamstock et al., 2011; Newman, 2003). This study found that for MCT, while the majority of these factors were presented within the histopathology reports, variation existed in the reporting especially related to histologic margins and grading of MCT.

The majority of reports (356 reports, 96.7%) had a description of histologic margins; however, the way the histologic margins were reported varied. Almost a quarter of the reports did not describe both the lateral and deep histologic margins and only 60 reports (16.3%) described the direction of the closest histologic margin. While omission of this data may be due to lack of thorough evaluation, more likely the failure is related to incomplete reporting, vague wording of the report or an inability of the pathologist to completely evaluate these margins secondary to how the samples were submitted for evaluation. Many reports had presumptive wording such as the closest lateral margin was X mm. This statement fails to describe the deep margin as well as assumes that all other lateral histologic margins were measured but not reported specifically. Likewise, if a mass was incompletely resected, it was not consistent as to whether all histologic margins were described and to what level of detail. It is important to note that if the clinician did not identify the border of the cut margins of the sample in relation to the

patient, it would not be possible for a pathologist to accurately report all histologic margins in a way that could be related back to the patient.

Information on all histologic margins, especially the distance of the closest neoplastic cells from the margin, which margins contain the closest tumor cells and the composition of the tissue composing the margin may effect the decision of whether or not to recommend a scar revision. For example, having tumor cells within 1mm of the margin may not be as concerning if the tissue composing the margin is fascia versus adipose tissue, which is generally thought to act as a poor barrier for tumor cell invasion. In this study, the tissue type composing the margin, the quality of the tissue composing the margin and a description of the neoplastic cells closest to the margin were all rarely recorded (40 reports/10.9%, 4 reports/1.1% and 2 reports/0.5% respectively). For MCT, identifying the tumor cells closest to the margin can pose a particular challenge given that clusters of mast cells within tissue or mast cells related to inflammation can be indistinguishable from neoplastic mast cells (Michels, Knapp, DeNicola, Glickman, & Bonney, 2002; Scarpa et al., 2012).

Kamstock et al. recommended reporting histologic margins in objective measures and to avoid using subjective descriptors such as close or narrow (Kamstock et al., 2011). In this study, the histologic margins were quantified in centimeters or millimeters in 287 reports (78.0%) while subjective descriptors were used in 88 reports (23.9%). The use of subjective descriptors leaves room for interpretation, as the definition of "close" will vary between individuals. It has also been recommended to specifically state that a histologic margin is complete or incomplete (Kamstock et al., 2011), which was stated in 188 reports (51.1%). By stating complete versus incomplete histologic margins there is no room for misinterpretation. For MCT, it can be debated that the distance of the closest tumor cell to the histologic margins is unimportant as the width of

the histologic tumor free margin has not been associated with recurrence (Donnelly et al., 2015). Therefore, complete versus incomplete excision may be one of the main considerations when advising adjunctive therapy.

The ability of the pathologist to interpret the histologic margins can be affected by the method of post-operative handing of the sample and the histologic processing. The most common method used for specimen trimming and histologic margin evaluation is the radial method for small or moderately sized masses (Kamstock et al., 2011). Using this method, an incredibly small portion of the margin (generally <0.1%) (Becker, 2007; Rapini, 1990) is actually evaluated while using other methods such as tangential sectioning or parallel slicing, a larger percentage of the margin can be evaluated (Kamstock et al., 2011). The radial method also assumes that tumors are symmetric/evenly distributed (Kamstock et al., 2011). Understanding which method was used for trimming and therefore the associated limitations is valuable information for the clinician when interpreting histologic margin results in relation to the true cut margins both for their patient as well as in research studies. In this study the method of sample trimming was only reported in 62 reports (16.8%). Another important aspect of histologic margin evaluation lies in the handling of the sample and post-operative marking of the margins. In this study, 123 (33.4%) of the reports had an indication that the margins were marked with ink or suture. While this may underestimate the number of specimens that were marked if this information was omitted from the report, it is likely that many specimens were unmarked. Without marking the borders of the sample, the orientation of the sample in relation to the animal cannot be determined and in some cases it can be difficult for the pathologist to determine the true cut borders or margins. The presence of a gross description within the reports would also be able to provide information related to how specimens were marked.

It was relatively uncommon for a gross description to be present, with only 97 reports (26.4%) containing this section and only 1 report contained images of the sample. The reports were also not standardized in how the dimensions of the sample were reported, with 43 reports measuring the sample in three dimensions, 23 in two dimensions and 21 reports in one dimension. The importance of the gross description is that it can help orient the clinician to the pathologist's perspective. Images or a gross description can be especially useful when the case has been referred and the clinician using the report is not the clinician who initially removed the tumor. In relation to histologic margins, a gross description or image can also help report post-surgical changes that occur in a specimen such as the degree of translation that occurred between the skin, subcutaneous tissue and fascial layers which may effect the pathologists ability to reliably access the histologic margins.

For MCT, the biologic behavior of mucosal, subcutaneous and cutaneous tumors is different (Elliott et al., 2016; Newman et al., 2007; Thompson et al., 2011), which highlights the importance of the pathologist clearly indicating the tumors' suspected origin on the histopathology report within the diagnosis. Overall, the vast majority of reports in this study clearly indicated the origin of the tumor in either the microscopic description and/or diagnosis with description of the tissue of origin only missing in 26 reports (7.1%). However, the grading of non-cutaneous versus cutaneous MCT was far more variable. For cutaneous MCT, a grade was given in 306 reports (98.7%) while for non-cutaneous MCT a grade was stated on 39 reports (67.2%). The primary grading systems used for grading MCT are the Patnaik and Kiupel systems (Kiupel et al., 2011; Patnaik et al., 1984). These systems were specifically developed for cutaneous MCT and have not been validated for non-cutaneous MCT (Elliott et al., 2016; Thompson et al., 2011). In subcutaneous MCT it has been shown that grade is not indicative of

behavior (Thompson et al., 2011). Therefore, while 13 of the 39 reports gave this as a limitation, 26 of these reports (44.8% of the total reports for non-cutaneous MCT) used these grading systems and did not state the limitations of these grading systems related to non-cutaneous MCT. For clinicians that are unaware of this literature, failure to acknowledge these limitations could result in inappropriate monitoring or treatment recommendations for the patient.

It has been recommended that both Patnaik and Kiupel grading systems be reported for cutaneous MCT ("VCS Oncology-Pathology Working Group MCT Subgroup Consensus on Grading Canine Cutaneous MCT," 2013). In this study, 23.6% of the reports did not comply with this recommendation. Using both of these systems has been recommended as each system has its strengths/weaknesses and both of these systems have been associated with prognosis in previous papers (Donnelly et al., 2015; Kiupel et al., 2011; Murphy et al., 2004; Patnaik et al., 1984; Schultheiss et al., 2011; Stefanello et al., 2015; Takeuchi et al., 2013). For the Patnaik system, the main concerns are 1) that the majority of tumors are intermediate in grade thereby diminishing its prognostic utility and 2) there is a significant amount of interobserver variation due to the subjective nature of the grading system (Northrup et al., 2005; Sabattini et al., 2015). The Kiupel system is more objective than the Patnaik system but it has not been evaluated as thoroughly. Recently, studies have been published comparing both grading systems (Sabattini et al., 2015; Stefanello et al., 2015; Takeuchi et al., 2013). Both Sabattini et al. and Takeuchi et al. evaluated the grading systems in relation to survival and both concluded the Kiupel system had superior prognostic value. Stefanello et al. evaluated the grading systems for prognosticating metastatic disease and concluded prognostication should not rely solely on grade but factor in the results of staging as even grade 1, grade 2, and low grade tumors had metastatic disease (5.8%, 16.5% and 14.9% respectively). However, it was also noted that using both grading schemes

showed a difference in metastatic potential as grade 3/high grade tumors metastasized more frequently than grade 2/high grade tumors (49% vs. 15%) (Stefanello et al., 2015). Based on the results of this study, continued reporting of both grading schemes is supported.

The history section contains important information for the pathologist as well as clinicians that may subsequently treat the case. The most common historical information provided was the location of the lesion (292 reports, 79.3%), suspected tumor type (219 reports, 59.5%) and method of diagnosis of the suspected tumor type (122 reports, 33.2%). However, the size of the mass was only present in 75 reports (19.8%). Size has been shown to be an important prognostic indicator for MCT and is useful information for clinicians (Mullins et al., 2006). If a gross description from the pathologist is present, size may be indicated within that section of the report; however, the size of the mass may be decreased secondary to surgical removal and histopathologic processing (Risselada et al., 2015). Generally, it was noted that the history section was truncated, with the median number of words in the history being 17 (range 0-317). The brief histories supplied on the reports may be due to the history not being reported to the pathologist or the history being shortened or omitted by the pathologist. Historical information that is pertinent to diagnosis or prognosis is important both for the pathologist as well as for future clinicians reviewing the case and should ideally be included within the report. While adding this information may be beneficial, it needs to be weighed against time constraints and possible legal implications (e.g. accidental incorporation of incorrect information).

Additional immunohistochemistry stains and/or PCR testing were recommended or offered in 131 reports (35.6%) with the most common being c-KIT PCR (124 reports, 33.7%), c-KIT IHC (106 reports, 28.8%), Ki-67 (95 reports, 25.8%), AgNOR (72 reports, 19.6%) and PCNA (30 reports, 8.2%). Subjectively, certain diagnostic laboratories routinely offered or

recommended these tests suggesting that this is part of their protocol. Some immunohistochemistry stains and PCR testing have been associated with prognosis for MCT (Berlato et al., 2015; Costa Casagrande et al., 2015; Elston et al., 2009; Garrett, 2014; Kandefer-Gola et al., 2015; Maglennon et al., 2008; Romansik et al., 2007; Scase et al., 2006; Seguin et al., 2006; Takeuchi et al., 2013; van Lelyveld et al., 2015; Vascellari et al., 2013; Webster et al., 2007), and the use of these tests in conjunction with histopathologic grade may help determine prognosis (Scase et al., 2006). The relatively low proportion of reports offering these additional immunohistochemistry stains and diagnostics shows a lack of general consensus on whether this should be included within the report. The discussion of which tests should be recommended or offered and by whom is beyond of the scope of this paper, however, this highlights an area of inconsistency that may be worthy of discussion.

The main limitations of this paper are due to the subjective nature of evaluation of histopathology reports as well as the difficulty in obtaining a representative population of histopathology reports for the USA. The authors attempted to make the evaluation of the reports as objective as possible by making most categories a question of present versus absent. We also attempted to obtain reports from both academic and private practices from various geographic regions across the USA. However, those chosen to contribute reports were based on knowledge of individuals in the geographic regions and potential willingness to contribute reports, which may have introduced bias. Another source of bias could have been introduced in the assessment of subjective elements of the reports evaluated by two individuals (JKR and CF) potentially introducing interobserver variation in the interpretation of the reports. Since columns that were considered more subjective were evaluated by both individuals, the effects of interobserver are likely small, however interobserver error was not specifically assessed. Also, if the presence of

information was questionable, favor was generally given towards the information being present, rather than absent. Therefore, any bias would make the results presented within this paper more favorable towards the clinician and pathologist.

4.5 CONCLUSIONS

The study findings suggested that while histologic margins are generally reported, details about the margins (e.g. direction of the closest margin and tissues composing the margin) and consistency of how histologic margins are reported are generally lacking. While the majority of reports included both the Kiupel and Patnaik grading systems, about a quarter used only one grading system. Also, grade is often reported for non-cutaneous MCT without stating the appropriate limitations of using grading systems developed for cutaneous MCT. The histopathology report represents a vital communication between the pathologist and clinician. It is important for clinicians to improve communication with pathologists in the form of improved clinical history reporting and specimen marking. Likewise, this paper highlights the need for discussions on standardization of certain elements of the histopathology report especially in relation to grading and margin reporting.

CHAPTER 5

FUTURE RESEARCH

For cutaneous tumors the best method of margin evaluation has yet to be determined. The topic of margin evaluation is deceptively complex as we are currently attempting to reconcile microscopic and macroscopic parameters. To complicate matters, the information being used has been derived with no standard protocols of methods or measurements.

What may have the greatest impact (especially on veterinary medicine) on advancing the understanding of margins would be to develop standard protocols for reporting of margins (both surgical and histologic) as well as other factors suspected to be directly related to prognosis such as MCT grading. This would better allow information to be shared across institutions and make developing large multi-institutional prospective and retrospective studies more feasible. Several points related to histopathology reports were discussed in Chapter 4 and it seems important to define what an incomplete versus complete margin is specific to MCT.

For MCT there is a large amount of data comparing margins and grade to outcome. Several studies have suggested that the planned surgical margins necessary to completely excise low grade tumors are less than the previously reported 3cm margins. One potential approach that could be used to help answer this question would be to perform a systematic review and plan prospective studies based on its results. Another possible direction would be to further evaluate the use of FNA as a proxy for grading and then plan surgical margins based off of FNA using small margins for suspected low grade tumors. A third possibility would be to plan a prospective study using shaved margins as this could be a better bridge to correlate surgical to histologic margins.

The most direct way to avoid the conundrum of histologic versus planned surgical margins is the concept of directly imaging of the tumor bed. Currently this is in the very early phases of use in both human and veterinary medicine, however, further studies on optical coherence imaging near-infrared fluorescence optical imaging are warranted as this is likely the future of margin evaluation.

CHAPTER 6

FIGURES AND TABLES

Figure 1—Photographs of the locations for the collection of skin samples from the head (A), hind limb (B), and lumbar region (C) of canine cadavers. In panel A, notice the placement for the 30-mm template on the left side of the head and the 10-mm template on the right side of the head.

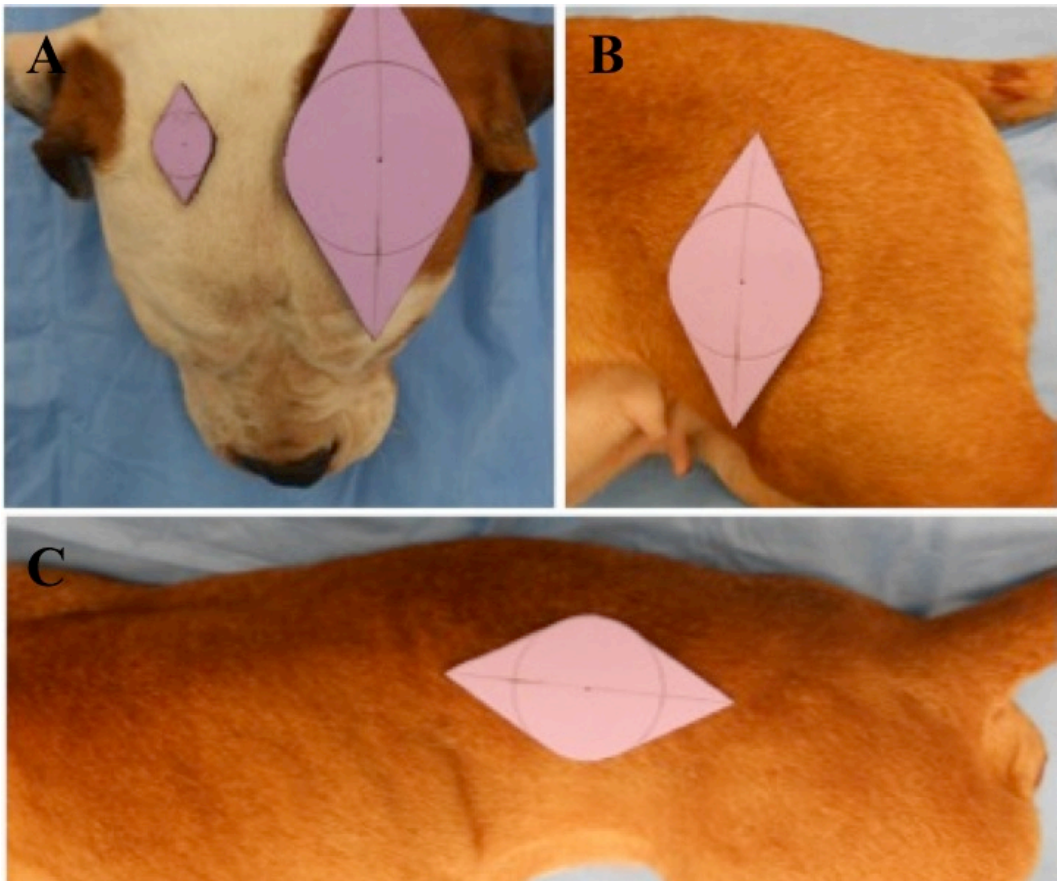

Figure 2—Photographs illustrating steps involved in processing of skin samples obtained from canine cadavers. A—An elliptical area was drawn on the skin with an indelible marker; the black circle in the middle represented a tumor. A partial-thickness skin incision was made; length and width were measured at this stage (prior to excision [P1]). B—The incision was extended deeper into the skin until the fascial layer could be identified; depth of the sample was measured (P1) Ink dots of different colors were placed on the sample for orientation. C—A full-thickness incision was made on one side of the sample, and the sample was undermined beneath the fascial layer. Sutures were placed in the sample, and the sample was then excised. D—Excised skin samples were placed in neutral-buffered 10% formalin, and measurements were obtained after fixation for 24 hours (P2). E—Skin samples were embedded in paraffin. F—Slides of tissue samples were used for measurement of length and width (after histologic processing [P3]).

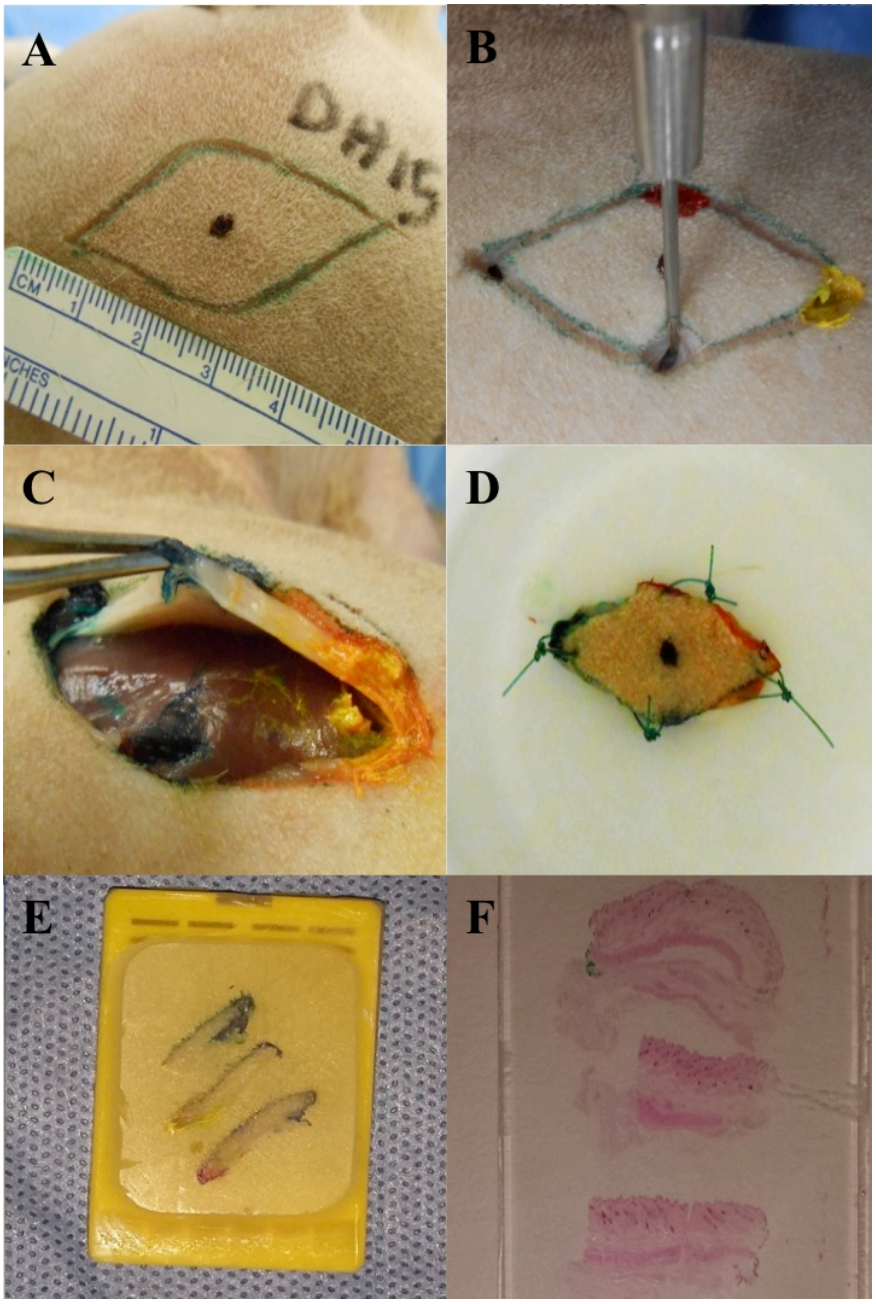

Table 1—Mean ± SD absolute length at various stages of processing* for 2 sizes of skin samples obtained from 3 anatomic locations of 15 canine cadavers.

Sample size (mm)	Anatomic location	P1 length (mm)	P2 length (mm)	P3 length (mm)
10	Hind limb	40.9 ± 1.4[a]	31.1 ± 2.3[d]	26.3 ± 2.4[g]
10	Lumbar	40.0 ± 1.1[b]	33.1 ± 2.9[e]	26.7 ± 2.9[h]
10	Head	40.9 ± 1.4[c]	35.3 ± 2.3[f]	27.9 ± 4.7[i]
30	Hind limb	121.7 ± 3.2[a]	84.4 ± 4.5[d]	75.9 ± 2.2[g]
30	Lumbar	120.5 ± 3.5[b]	96.7 ± 6.5[e]	85.3 ± 7.0[h]
30	Head	121.1 ± 2.9[c]	102.9 ± 8.0[f]	92.1 ± 11.2[i]

*Measurements were obtained prior to excision (P1), 24 to 48 hours after samples had been fixed in neutral-buffered 10% formalin for 24 hours (P2), and on slides prepared for histologic evaluation (P3).

** Within a row, all values differ significantly (P<0.001).

*** Within a column, values with different superscript letters differ significantly (P<0.001).

Table 2—Mean ± SD absolute width at various stages of processing* for 2 sizes of skin samples obtained from 3 anatomic locations of 15 canine cadavers.

Sample size (mm)	Anatomic location	P1 width (mm)	P2 width (mm)	P3 width (mm)
10	Hind limb	18.9 ± 0.9[a]	17.9 ± 1.6[d]	15.5 ± 1.5[g]
10	Lumbar	20.9 ± 1.1[b]	17.7 ± 0.9[e]	16.1 ± 1.6[h]
10	Head	20.9 ± 1.0[c]	17.1 ± 0.9[f]	16.9 ± 1.8[i]
30	Hind limb	59.5 ± 1.7[a]	49.3 ± 2.9[d]	47.2 ± 4.2[g]
30	Lumbar	61.3 ± 1.8[b]	51.1 ± 2.1[e]	47.3 ± 3.5[h]
30	Head	61.3 ± 1.6[c]	50.9 ± 3.4[f]	47.9 ± 4.1[i]

See Table 1 for key.

Table 3—Mean ± SD SA at various stages of processing* for 2 sizes of skin samples obtained from 3 anatomic locations of 15 canine cadavers.

Sample size (mm)	Anatomic location	P1 SA (mm^2)	P2 SA (mm^2)
10	Hind limb	492.2 ± 47.6	293.8 ± 46.4
10	Lumbar	545.5 ± 67.4	337.2 ± 46.8
10	Head	530.1 ± 54.9	349.5 ± 57.0
30	Hind limb	4,145.1 ± 335.9	2,107.7 ± 160.3
30	Lumbar	4,229.4 ± 314.0†	2,504.5 ± 360.4
30	Head	4,197.3 ± 482.2	2,751.4 ± 437.8

†Represents results for only 14 samples because the photograph for 1 sample was lost. ***See*** Table 1 for remainder of key.

Table 4—Mean ± SD percentage change in length between various stages of processing* for 2 sizes of skin samples obtained from 3 anatomic locations of 15 canine cadavers.

Sample size (mm)	Anatomic location	Change between P1 and P2 (%)	Change between P2 and P3 (%)	Change between P1 and P3 (%)
10	Hind limb	23.8 ± 4.3	15.3 ± 6.8	35.6 ± 4.8
10	Lumbar	17.2 ± 6.8	19.5 ± 5.8	33.3 ± 7.0
10	Head	13.7 ± 4.8	18.7 ± 8.6	35.6 ± 4.8
30	Hind limb	30.6 ± 3.9	10.1 ± 4.8	37.7 ± 2.0
30	Lumbar	19.7 ± 5.5	11.9 ± 3.7	29.3 ± 5.3
30	Head	15.1 ± 6.0	10.7 ± 5.9	24.0 ± 8.4

Percentage change was calculated by use of the following equations: change between P1 and P2 = ([P1 measurement – P2 measurement]/P1 measurement) X 100, change between P2 and P3 = ([P2 measurement – P3 measurement]/P2 measurement) X 100, and change between P1 and P3 = ([P1 measurement – P3 measurement]/P1 measurement) X 100. ***See*** Table 1 for remainder of key.

Table 5—Mean ± SD percentage change in width between various stages of processing* for 2 sizes of skin samples obtained from 3 anatomic locations of 15 canine cadavers.

Sample size (mm)	Anatomic location	Change between P1 and P2 (%)	Change between P2 and P3 (%)	Change between P1 and P3 (%)
10	Hind limb	9.5 ± 3.9	9.4 ± 6.7	18.0 ± 6.9
10	Lumbar	15.1 ± 6.0	8.1 ± 7.5	22.6 ± 7.9
10	Head	14.3 ± 7.6	5.1 ± 7.3	18.7 ± 9.4
30	Hind limb	17.1 ± 4.4	4.2 ± 8.8	20.6 ± 8.4
30	Lumbar	16.6 ± 3.7	7.5 ± 4.9	22.8 ± 5.9
30	Head	17.1 ± 5.0	5.8 ± 5.2	21.8 ± 6.7

See Tables 1 and 4 for key.

Table 6: Distribution of the states by region of the United States of America based on the United States Census Bureau.

Region of the United States	States within Region
Northeast	ME, NH, VT, NY, MA, CT, RI, NJ, PA, DE, MD, WV, VA
South	KY, NC, TN, SC, GA, AL, MS, FL, TX, OK, AR, LA
Midwest	ND, MN, WS, MI, SD, IA, IL, IN, OH, NE, KS, MO
West	WA, OR, ID, MT, WY, AK, CA, NV, UT, CO, AZ, NM, HI

Table 7: Information evaluated within each histopathology report.

Report section	Information evaluated within each section	Answer format
Signalment	Age at the time of the report Breed Sex	Year or missing Breed or missing Sex or missing
Clinical history	Clinical history present Number of words† Adequate history‡, § Age at diagnosis Location Adequate description Number of masses, Mass size Mass growth rate Suspected tumor type Method diagnosis Expected surgical margin Marked margins (sutured or inked)*, § Current medications	Yes or no Number or missing Remaining information evaluated as yes, no or missing if a clinical history was not present
Gross description	Gross description present Tumor size Location	Yes or no 3D, 2D, 1D or missing Yes or no
Microscopic description	Mitotic index Number of mitotic figures per HPF Tissue of origin§	Yes or no Yes or no Subcutaneous, cutaneous, or non-cutaneous
Diagnosis/MCT grading system	Grading system used If non-cutaneous/subcutaneous were limitations stated§ Grade given	Both, Kuipel, Patnaik or none Yes, no High, low or 1,2,3

Table 7 (cont.)

Margin evaluation	Margins reported Description of neoplastic cells closest to the margin § All margins described§ Metric measurements used Direction of closest lateral margin § Tissue composing the margin§ Margin tissue quality§ Trimming method§ Subjective descriptors used§ Margins stated complete or incomplete	Yes or no Remaining information evaluated as yes, no or missing if margins were not reported
Comments	Comments section present Additional diagnostics recommend or performed If applicable the diagnostic recommended was recorded Comments on biologic behavior Oncologist consultation recommended References	Yes, no Yes, no, missing if no comment section AgNOR, PCNA, c-kit IHC, c-kit PCR, ki-67, other Yes, no, missing if no comment section Yes, no, missing if no comment section Yes, no, NA

* If margins were inked or marked within the gross description this was included within this category.

† MCT and FNA considered 1 word for count purposes.

‡ Subjectively adequate history was defined as yes if 3 or more of the specific pieces of information listed within this section below adequate history was present.

§ Columns evaluated by both JKR and CF. For information that was considered more subjective in nature reports were evaluated by both evaluators and if a concern arose LES was consulted.

Table 8: Grading systems used for reporting the grade of MCT that were cutaneous and non-cutaneous in origin. The cutaneous category contains all tumors that were reported as cutaneous in origin or that were not specified and presumed to be cutaneous based on the report.

MCT tissue of origin	Both	Kiupel	Patnaik	No grade
Cutaneous	237 (76.5%)	22 (7.1%)	47 (15.2%)	4 (1.3%)
Non-cutaneous All reports	28 (48.3%)	3 (5.2%)	8 (13.8%)	19 (32.8%)
Non-cutaneous Stating grading limitations*	9 (32.1%)	2 (66.7%)	2 (25%)	10 (52.6%)

* For non-cutaneous MCT no grade should be given, as these grading systems do not apply to non-cutaneous MCT. These numbers reflect the number of reports that stated this limitation with the percentage being of the total number of reports within the grading category for non-cutaneous tumors.

CHAPTER 7

REFERENCES

Auw-Haedrich, C., Frick, S., Boehringer, D., & Mittelviefhaus, H. (2009). Histologic safety margin in basal cell carcinoma of the eyelid: correlation with recurrence rate. *Ophthalmology, 116*(4), 802-806. doi:10.1016/j.ophtha.2008.11.012

Becker, G. D. (2007). The many faces of surgical margins. *Am J Clin Oncol, 30*(5), 556-559. doi:10.1097/01.coc.0000264178.06004.46

Berlato, D., Murphy, S., Monti, P., Stewart, J., Newton, J. R., Flindall, A., & Maglennon, G. A. (2015). Comparison of mitotic index and Ki67 index in the prognostication of canine cutaneous mast cell tumours. *Vet Comp Oncol, 13*(2), 143-150. doi:10.1111/vco.12029

Bernstein, J. A., Storey, E. S., & Bauer, R. W. (2013). Moh's micrographic surgery for the management of a periocular mast cell tumor in a dog. *Vet Ophthalmol, 16*(3), 234-239. doi:10.1111/j.1463-5224.2012.01059.x

Blasdale, C., Charlton, F. G., Weatherhead, S. C., Ormond, P., & Lawrence, C. M. (2010). Effect of tissue shrinkage on histological tumour-free margin after excision of basal cell carcinoma. *Br J Dermatol, 162*(3), 607-610. doi:10.1111/j.1365-2133.2009.09577.x

Bostock, D. E. (1973). The prognosis following surgical removal of mastocytomas in dogs. *J Small Anim Pract, 14*(1), 27-41.

Brannick, E. M., Zhang, J., Zhang, X., & Stromberg, P. C. (2012). Influence of submission form characteristics on clinical information received in biopsy accession. *J Vet Diagn Invest, 24*(6), 1073-1082. doi:10.1177/1040638712458783

Camus, M. S., Priest, H. L., Koehler, J. W., Driskell, E. A., Rakich, P. M., Ilha, M. R., & Krimer, P. M. (2016). Cytologic Criteria for Mast Cell Tumor Grading in Dogs With Evaluation of Clinical Outcome. *Vet Pathol*. doi:10.1177/0300985816638721

Clarke, B. S., Banks, T. A., & Findji, L. (2014). Quantification of tissue shrinkage in canine small intestinal specimens after resection and fixation. *Can J Vet Res, 78*(1), 46-49.

Costa Casagrande, T. A., de Oliveira Barros, L. M., Fukumasu, H., Cogliati, B., Chaible, L. M., Dagli, M. L., & Matera, J. M. (2015). The value of molecular expression of KIT and KIT ligand analysed using real-time polymerase chain reaction and immunohistochemistry as a prognostic indicator for canine cutaneous mast cell tumours. *Vet Comp Oncol, 13*(1), 1-10. doi:10.1111/vco.12010

Dauendorffer, J. N., Bastuji-Garin, S., Guero, S., Brousse, N., & Fraitag, S. (2009). Shrinkage of skin excision specimens: formalin fixation is not the culprit. *Br J Dermatol, 160*(4), 810-814. doi:10.1111/j.1365-2133.2008.08994.x

Docquier, P. L., Paul, L., Cartiaux, O., Lecouvet, F., Dufrane, D., Delloye, C., & Galant, C. (2010). Formalin fixation could interfere with the clinical assessment of the tumor-free margin in tumor surgery: magnetic resonance imaging-based study. *Oncology, 78*(2), 115-124. doi:10.1159/000306140

Donnelly, L., Mullin, C., Balko, J., Goldschmidt, M., Krick, E., Hume, C., . . . Sorenmo, K. (2015). Evaluation of histological grade and histologically tumour-free margins as predictors of local recurrence in completely excised canine mast cell tumours. *Vet Comp Oncol, 13*(1), 70-76. doi:10.1111/vco.12021

Eid, I., El-Muhtaseb, M. S., Mukherjee, R., Renwick, R., Gardiner, D. S., & Macdonald, A. (2007). Histological processing variability in the determination of lateral resection margins in rectal cancer. *J Clin Pathol, 60*(6), 593-595. doi:10.1136/jcp.2006.040873

El-Fol, H. A., Noman, S. A., Beheiri, M. G., Khalil, A. M., & Kamel, M. M. (2015). Significance of post-resection tissue shrinkage on surgical margins of oral squamous cell carcinoma. *J Craniomaxillofac Surg, 43*(4), 475-482. doi:10.1016/j.jcms.2015.01.009

Elliott, J. W., Cripps, P., Blackwood, L., Berlato, D., Murphy, S., & Grant, I. A. (2016). Canine oral mucosal mast cell tumours. *Vet Comp Oncol, 14*(1), 101-111. doi:10.1111/vco.12071

Elston, L. B., Sueiro, F. A., Cavalcanti, J. N., & Metze, K. (2009). The importance of the mitotic index as a prognostic factor for survival of canine cutaneous mast cell tumors: a validation study. *Vet Pathol, 46*(2), 362-364, author reply 364-365. doi:10.1354/vp.46-2-362

Fife, M., Blocker, T., Fife, T., Dubielzig, R. R., & Dunn, K. (2011). Canine conjunctival mast cell tumors: a retrospective study. *Vet Ophthalmol, 14*(3), 153-160. doi:10.1111/j.1463-5224.2010.00857.x

Fulcher, R. P., Ludwig, L. L., Bergman, P. J., Newman, S. J., Simpson, A. M., & Patnaik, A. K. (2006). Evaluation of a two-centimeter lateral surgical margin for excision of grade I and grade II cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc, 228*(2), 210-215. doi:10.2460/javma.228.2.210

Garrett, L. (2014). Canine mast cell tumors: diagnosis, treatment, and prognosis. *Veterinary Medicine: Research and Reports*, 49. doi:10.2147/vmrr.s41005

George, K. S., Hyde, N. C., Wilson, P., & Smith, G. I. (2013). Does the method of resection affect the margins of tumours in the oral cavity? Prospective controlled study in pigs. *Br J Oral Maxillofac Surg, 51*(7), 600-603. doi:10.1016/j.bjoms.2013.03.016

Gieger, T. L., Theon, A. P., Werner, J. A., McEntee, M. C., Rassnick, K. M., & DeCock, H. E. (2003). Biologic behavior and prognostic factors for mast cell tumors of the canine muzzle: 24 cases (1990-2001). *J Vet Intern Med, 17*(5), 687-692.

Giudice, C., Stefanello, D., Sala, M., Cantatore, M., Russo, F., Romussi, S., . . . Grieco, V. (2010). Feline injection-site sarcoma: recurrence, tumour grading and surgical margin status evaluated using the three-dimensional histological technique. *Vet J, 186*(1), 84-88. doi:10.1016/j.tvjl.2009.07.019

Golomb, F. M., Doyle, J. P., Grin, C. M., Kopf, A. W., Silverman, M. K., & Levenstein, M. J. (1991). Determination of preexcision surgical margins of melanomas from fixed-tissue specimens. *Plast Reconstr Surg, 88*(5), 804-809.

Gregory, N., Mulvaney, M., Pattison, T., Hill, J., Carlson, J. A., & Goncharuk, V. (2003). Shrinkage of skin excision specimens and downcoding. *Arch Dermatol, 139*(4), 542-543. doi:10.1001/archderm.139.4.542

Hudson-Peacock, M. J., Matthews, J. N., & Lawrence, C. M. (1995). Relation between size of skin excision, wound, and specimen. *J Am Acad Dermatol, 32*(6), 1010-1015.

Hume, C. T., Kiupel, M., Rigatti, L., Shofer, F. S., Skorupski, K. A., & Sorenmo, K. U. (2011). Outcomes of dogs with grade 3 mast cell tumors: 43 cases (1997-2007). *J Am Anim Hosp Assoc, 47*(1), 37-44. doi:10.5326/JAAHA-MS-5557

Iida, G., Asano, K., Seki, M., Ishigaki, K., Teshima, K., Yoshida, O., . . . Kagawa, Y. (2013). Intraoperative identification of canine hepatocellular carcinoma with indocyanine

green fluorescent imaging. *J Small Anim Pract, 54*(11), 594-600. doi:10.1111/jsap.12148
Irwin, D. H. (1966). Tension lines in the skin of the dog. *J Small Anim Pract, 7*(9), 593-598.
Jeyakumar, S., Smith, A. N., Schleis, S. E., Cattley, R. C., Tillson, D. M., & Henderson, R. A. (2015). Effect of histologic processing on dimensions of skin samples obtained from cat cadavers. *Am J Vet Res, 76*(11), 939-945. doi:10.2460/ajvr.76.11.939
Johnson, R. E., Sigman, J. D., Funk, G. F., Robinson, R. A., & Hoffman, H. T. (1997). Quantification of surgical margin shrinkage in the oral cavity. *Head Neck, 19*(4), 281-286.
Kamstock, D. A., Ehrhart, E. J., Getzy, D. M., Bacon, N. J., Rassnick, K. M., Moroff, S. D., . . . American College of Veterinary Pathologists' Oncology, C. (2011). Recommended guidelines for submission, trimming, margin evaluation, and reporting of tumor biopsy specimens in veterinary surgical pathology. *Vet Pathol, 48*(1), 19-31. doi:10.1177/0300985810389316
Kandefer-Gola, M., Madej, J. A., Dzimira, S., Nowak, M., Janus, I., & Ciaputa, R. (2015). Comparative analysis of markers of cell proliferation in canine mast cell tumours according to current classifications. *Pol J Vet Sci, 18*(2), 241-247. doi:10.1515/pjvs-2015-0031
Kerns, M. J., Darst, M. A., Olsen, T. G., Fenster, M., Hall, P., & Grevey, S. (2008). Shrinkage of cutaneous specimens: formalin or other factors involved? *J Cutan Pathol, 35*(12), 1093-1096. doi:10.1111/j.1600-0560.2007.00943.x
Kiupel, M., Webster, J. D., Bailey, K. L., Best, S., DeLay, J., Detrisac, C. J., . . . Miller, R. (2011). Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol, 48*(1), 147-155. doi:10.1177/0300985810386469
Kiupel, M., Webster, J. D., Miller, R. A., & Kaneene, J. B. (2005). Impact of tumour depth, tumour location and multiple synchronous masses on the prognosis of canine cutaneous mast cell tumours. *J Vet Med A Physiol Pathol Clin Med, 52*(6), 280-286. doi:10.1111/j.1439-0442.2005.00726.x
Kooby, D. A., & Maithel, S. K. (2016). Negative surgical margins: Main course or just icing on the cake? *J Surg Oncol, 113*(3), 247. doi:10.1002/jso.24131
Kope, L. F. F., Bastos, J. C. F., Filho, J. S. A., & Gouvea, P. S. (2005). Safety margin: an old and relative concept. *An Bras Dermatol., 80*(3), 279-286.
Kott, I. (2008). The importance of margins in surgery. *J Surg Oncol, 98*(8), 570-571. doi:10.1002/jso.21039
Kry, K. L., & Boston, S. E. (2014). Additional local therapy with primary re-excision or radiation therapy improves survival and local control after incomplete or close surgical excision of mast cell tumors in dogs. *Vet Surg, 43*(2), 182-189. doi:10.1111/j.1532-950X.2014.12099.x
Laflamme, D. P. (1997). Development and validation of a body condition score system for dogs. *Canine Pract, 22*, 10-15.
Lane, J. E., & Kent, D. E. (2005). Surgical margins in the treatment of nonmelanoma skin cancer and mohs micrographic surgery. *Curr Surg, 62*(5), 518-526. doi:10.1016/j.cursur.2005.01.003
Liptak, J. (2013). The future for surgical margins. *J Small Anim Pract, 54*(11), 563. doi:10.1111/jsap.12145

London, C. A., & Seguin, B. (2003). Mast cell tumors in the dog. *Vet Clin North Am Small Anim Pract, 33*(3), 473-489, v.
Lurie, M. (2008). Evaluation of surgical margins: viewpoint of the pathologist. *J Surg Oncol, 98*(8), 616-617. doi:10.1002/jso.21037
Maglennon, G. A., Murphy, S., Adams, V., Miller, J., Smith, K., Blunden, A., & Scase, T. J. (2008). Association of Ki67 index with prognosis for intermediate-grade canine cutaneous mast cell tumours. *Vet Comp Oncol, 6*(4), 268-274. doi:10.1111/j.1476-5829.2008.00168.x
Marconato, L., Bettini, G., Giacoboni, C., Romanelli, G., Cesari, A., Zatelli, A., & Zini, E. (2008). Clinicopathological features and outcome for dogs with mast cell tumors and bone marrow involvement. *J Vet Intern Med, 22*(4), 1001-1007. doi:10.1111/j.1939-1676.2008.0128.x
Matz, B. M. (2015). Current concepts in oncologic surgery in small animals. *Vet Clin North Am Small Anim Pract, 45*(3), 437-449. doi:10.1016/j.cvsm.2015.01.003
Michels, G. M., Knapp, D. W., DeNicola, D. B., Glickman, N., & Bonney, P. (2002). Prognosis following surgical excision of canine cutaneous mast cell tumors with histopathologically tumor-free versus nontumor-free margins: a retrospective study of 31 cases. *J Am Anim Hosp Assoc, 38*(5), 458-466. doi:10.5326/0380458
Mullins, M. N., Dernell, W. S., Withrow, S. J., Ehrhart, E. J., Thamm, D. H., & Lana, S. E. (2006). Evaluation of prognostic factors associated with outcome in dogs with multiple cutaneous mast cell tumors treated with surgery with and without adjuvant treatment: 54 cases (1998-2004). *J Am Vet Med Assoc, 228*(1), 91-95. doi:10.2460/javma.228.1.91
Murphy, S., Sparkes, A. H., Smith, K. C., Blunden, A. S., & Brearley, M. J. (2004). Relationships between the histological grade of cutaneous mast cell tumours in dogs, their survival and the efficacy of surgical resection. *Vet Rec, 154*(24), 743-746.
Newman, S. J. (2003). Diagnostic pathology for the cancer patient. *Clin Tech Small Anim Pract, 18*(2), 139-144. doi:10.1053/svms.2003.36633
Newman, S. J., Mrkonjich, L., Walker, K. K., & Rohrbach, B. W. (2007). Canine subcutaneous mast cell tumour: diagnosis and prognosis. *J Comp Pathol, 136*(4), 231-239. doi:10.1016/j.jcpa.2007.02.003
Northrup, N. C., Howerth, E. W., Harmon, B. G., Brown, C. A., Carmicheal, K. P., Garcia, A. P., . . . Gieger, T. L. (2005). Variation among pathologists in the histologic grading of canine cutaneous mast cell tumors with uniform use of a single grading reference. *J Vet Diagn Invest, 17*(6), 561-564.
Patnaik, A. K., Ehler, W. J., & MacEwen, E. G. (1984). Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol, 21*(5), 469-474.
Phelps, H. A., Kuntz, C. A., Milner, R. J., Powers, B. E., & Bacon, N. J. (2011). Radical excision with five-centimeter margins for treatment of feline injection-site sarcomas: 91 cases (1998-2002). *J Am Vet Med Assoc, 239*(1), 97-106. doi:10.2460/javma.239.1.97
Pratschke, K. M., Atherton, M. J., Sillito, J. A., & Lamm, C. G. (2013). Evaluation of a modified proportional margins approach for surgical resection of mast cell tumors in dogs: 40 cases (2008-2012). *J Am Vet Med Assoc, 243*(10), 1436-1441. doi:10.2460/javma.243.10.1436
Rapini, R. P. (1990). Comparison of methods for checking surgical margins. *J Am Acad Dermatol, 23*(2 Pt 1), 288-294.

Reagan, J.R., Selmic, L.E., Garrett, L.D., & Singh, K. (2016). Evaluation of the effects of anatomic location, histologic processing, and sample size on shrinkage of skin samples obtained from canine cadavers. *Am J Vet Res*, 77(9), 1036-1044.

Reimer, S. B., Seguin, B., DeCock, H. E., Walsh, P. J., & Kass, P. H. (2005). Evaluation of the effect of routine histologic processing on the size of skin samples obtained from dogs. *Am J Vet Res, 66*(3), 500-505.

Risselada, M., Mathews, K. G., & Griffith, E. (2015). Surgically planned versus histologically measured lateral tumor margins for resection of cutaneous and subcutaneous mast cell tumors in dogs: 46 cases (2010-2013). *J Am Vet Med Assoc, 247*(2), 184-189. doi:10.2460/javma.247.2.184

Romansik, E. M., Reilly, C. M., Kass, P. H., Moore, P. F., & London, C. A. (2007). Mitotic index is predictive for survival for canine cutaneous mast cell tumors. *Vet Pathol, 44*(3), 335-341. doi:10.1354/vp.44-3-335

Sabattini, S., Scarpa, F., Berlato, D., & Bettini, G. (2015). Histologic grading of canine mast cell tumor: is 2 better than 3? *Vet Pathol, 52*(1), 70-73. doi:10.1177/0300985814521638

Sarode, S. C., & Sarode, G. S. (2012). A novel 'microscopic method' of shrinkage calculation in the pursuance of shrinkage based histopathological guidelines for interpretation of surgical margins. *Oral Oncol, 48*(6), e15-16. doi:10.1016/j.oraloncology.2012.01.011

Sarode, S. C., Sarode, G. S., & Karmarkar, S. (2011). Shrinkage based assessment of surgical margins: a need of the hour in oral cancer. *Int J Oral Maxillofac Surg, 40*(11), 1336-1337. doi:10.1016/j.ijom.2011.05.005

Scarpa, F., Sabattini, S., & Bettini, G. (2014). Cytological grading of canine cutaneous mast cell tumours. *Vet Comp Oncol*. doi:10.1111/vco.12090

Scarpa, F., Sabattini, S., Marconato, L., Capitani, O., Morini, M., & Bettini, G. (2012). Use of histologic margin evaluation to predict recurrence of cutaneous malignant tumors in dogs and cats after surgical excision. *J Am Vet Med Assoc, 240*(10), 1181-1187. doi:10.2460/javma.240.10.1181

Scase, T. J., Edwards, D., Miller, J., Henley, W., Smith, K., Blunden, A., & Murphy, S. (2006). Canine mast cell tumors: correlation of apoptosis and proliferation markers with prognosis. *J Vet Intern Med, 20*(1), 151-158.

Schned, A. R., Wheeler, K. J., Hodorowski, C. A., Heaney, J. A., Ernstoff, M. S., Amdur, R. J., & Harris, R. D. (1996). Tissue-shrinkage correction factor in the calculation of prostate cancer volume. *Am J Surg Pathol, 20*(12), 1501-1506.

Schultheiss, P. C., Gardiner, D. W., Rao, S., Olea-Popelka, F., & Tuohy, J. L. (2011). Association of histologic tumor characteristics and size of surgical margins with clinical outcome after surgical removal of cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc, 238*(11), 1464-1469. doi:10.2460/javma.238.11.1464

Seguin, B., Besancon, M. F., McCallan, J. L., Dewe, L. L., Tenwolde, M. C., Wong, E. K., & Kent, M. S. (2006). Recurrence rate, clinical outcome, and cellular proliferation indices as prognostic indicators after incomplete surgical excision of cutaneous grade II mast cell tumors: 28 dogs (1994-2002). *J Vet Intern Med, 20*(4), 933-940.

Silverman, M. K., Golomb, F. M., Kopf, A. W., Grin-Jorgensen, C. M., Vossaert, K. A., Doyle, J. P., & Levenstein, M. J. (1992). Verification of a formula for determination of preexcision

surgical margins from fixed-tissue melanoma specimens. *J Am Acad Dermatol, 27*(2 Pt 1), 214-219.
Simpson, A. M., Ludwig, L. L., Newman, S. J., Bergman, P. J., Hottinger, H. A., & Patnaik, A. K. (2004). Evaluation of surgical margins required for complete excision of cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc, 224*(2), 236-240.
Smith, G. I., Hyde, N. C., & Wilson, P. (2008). Does the method of resection affect the surgical margin in oral cavity tumour excision? *British Journal of Oral and Maxillofacial Surgery, 46*(7), e42. doi:10.1016/j.bjoms.2008.07.030
Stefanello, D., Buracco, P., Sabattini, S., Finotello, R., Giudice, C., Grieco, V., . . . Marconato, L. (2015). Comparison of 2- and 3-category histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009-2014). *J Am Vet Med Assoc, 246*(7), 765-769. doi:10.2460/javma.246.7.765
Takeuchi, Y., Fujino, Y., Watanabe, M., Takahashi, M., Nakagawa, T., Takeuchi, A., . . . Tsujimoto, H. (2013). Validation of the prognostic value of histopathological grading or c-kit mutation in canine cutaneous mast cell tumours: a retrospective cohort study. *Vet J, 196*(3), 492-498. doi:10.1016/j.tvjl.2012.11.018
Thompson, J. J., Pearl, D. L., Yager, J. A., Best, S. J., Coomber, B. L., & Foster, R. A. (2011). Canine subcutaneous mast cell tumor: characterization and prognostic indices. *Vet Pathol, 48*(1), 156-168. doi:10.1177/0300985810387446
Upchurch, D. A., Malenfant, R. C., Wignall, J. R., Ogden, D. M., & Saile, K. (2014). Effects of sample site and size, skin tension lines, surgeon, and formalin fixation on shrinkage of skin samples excised from canine cadavers. *Am J Vet Res, 75*(11), 1004-1009. doi:10.2460/ajvr.75.11.1004
van Lelyveld, S., Warland, J., Miller, R., Maw, H., Foale, R., Goodfellow, M., & Dobson, J. (2015). Comparison between Ki-67 index and mitotic index for predicting outcome in canine mast cell tumours. *J Small Anim Pract, 56*(5), 312-319. doi:10.1111/jsap.12320
Vascellari, M., Giantin, M., Capello, K., Carminato, A., Morello, E. M., Vercelli, A., . . . Mutinelli, F. (2013). Expression of Ki67, BCL-2, and COX-2 in canine cutaneous mast cell tumors: association with grading and prognosis. *Vet Pathol, 50*(1), 110-121. doi:10.1177/0300985812447829
VCS Oncology-Pathology Working Group MCT Subgroup Consensus on Grading Canine Cutaneous MCT. (2013). [Press release]. Retrieved from http://www.vetcancersociety.org/members/files/2014/05/Consensus_CCMCT-Grading.pdf
Wang, L., Shen, J., Song, X., Chen, W., Pan, T., Zhang, W., . . . Wu, J. (2004). A study of the lengthening and contractility of the surgical margins in digestive tract cancer. *Am J Surg, 187*(3), 452-455. doi:10.1016/j.amjsurg.2003.12.006
Warland, J., Amores-Fuster, I., Newbury, W., Brearley, M., & Dobson, J. (2014). The utility of staging in canine mast cell tumours. *Vet Comp Oncol, 12*(4), 287-298. doi:10.1111/vco.12012
website, U. S. C. B. Regions and Divisions. http://www.census.gov/econ/census/help/geography/regions_and_divisions.html

Webster, J. D., Yuzbasiyan-Gurkan, V., Miller, R. A., Kaneene, J. B., & Kiupel, M. (2007). Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Vet Pathol, 44*(3), 298-308. doi:10.1354/vp.44-3-298

Weisse, C., Shofer, F. S., & Sorenmo, K. (2002). Recurrence rates and sites for grade II canine cutaneous mast cell tumors following complete surgical excision. *J Am Anim Hosp Assoc, 38*(1), 71-73. doi:10.5326/0380071

Withrow, S. J., & Vail, D. M. (2007). *Withrow & MacEwen's small animal clinical oncology* (4th ed.). St. Louis, Mo.: Saunders Elsevier.

Wyld, L., Audisio, R. A., & Poston, G. J. (2015). The evolution of cancer surgery and future perspectives. *Nat Rev Clin Oncol, 12*(2), 115-124. doi:10.1038/nrclinonc.2014.191