Use of Lymphoscintigraphy in the Staging of Canine Oral Neoplasia

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 1, 2015

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Abstract

Oral cancer is the fourth most common type of canine neoplasm. Metastatic potential is highly variable, and failure to accurately identify evidence of metastasis results in detrimental outcomes through inaccurate treatment recommendations and unrealistic prognostication. Lymphatic drainage within the canine head is highly complex with marked individual variability. Clinically evaluated mandibular lymph nodes may not accurately represent full lymphatic staging. The goals of this study are to evaluate lymphoscintigraphy and the effect of different injection techniques on sentinel lymph node identification. The hypotheses are that improved sentinel lymph node identification will be achieved by use of lymphoscintigraphy and that peri-tumoral injection will produce more consistent results. Lymphoscintigraphy was performed on dogs with oral neoplasia; each patient acted as their own control for injection site. Two cases were recruited by study completion. Variable lymphatic drainage was noted and differences relating to imaging time and identified sentinel lymph nodes were appreciated with different injection techniques.

Acknowledgements

I would like to thank the members of my graduate research committee: Drs. Harry Boothe, Annette Smith, Robert Cole, Peter Christopherson, Jey Koehler and Brad Matz. In addition, I would like to thank Dr. William Brawner for guidance in nuclear medicine and radiation protocols and Betty Files and Jennifer Spooner for technical assistance with clinical patients and performance of nuclear imaging procedures.

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Literature review

1. Anatomy

The lymphatic system of the canine head and neck is complex, and has been described in a number of manuscripts with variable anatomy reported. One detailed study evaluating cadaveric dogs following injection of blue dye described the primary cervical nodes[1]. The parotid lymph nodes were recorded as 0.5 to 1.0 cm in size and located superficial to the lateral internal maxillary vein. One node was typically present on each side, but rarely two nodes were identified. The lateral retropharyngeal node was 0.3 to 0.5 cm and was located in approximately 70% of specimens. When present, it was located adjacent to the caudal aspect of the parotid gland. Three mandibular lymph nodes ranging in size from 1 to 2 cm were located adjacent to the common facial vein; occasionally an additional smaller node medial to the termination of this vein was also identified. The medial retropharyngeal node, fusiform in shape and 3 to 4 cm long, was located lateral to the carotid artery, internal jugular vein and thyroid gland.

Lymphatic anatomy and drainage pathways of the head have also been described in the text Miller's Anatomy of the Dog[2]. The parotid lymphocentrum consists of one to three nodes located along the rostral edge of the parotid salivary gland and receives afferent drainage from the caudodorsal muzzle, eyelids and ocular glands, side of the cranium, external ear, temporomandibular joint, parotid salivary gland, lacrimal gland, the temporal, masseter and zygomatic muscles, the nasal, frontal, parietal and zygomatic bones and the mandible. The mandibular lymphocentrum consists of one to five nodes at the bifurcation of the jugular vein, receiving afferent drainage from all other parts of the head not drained by the parotid node, but also with overlapping afferents including the eyelids, ocular glands, skin of the dorsal cranium and temporomandibular joint. The buccal lymph nodes are single nodes that can be unilateral,

bilateral or absent and drain into the mandibular nodes. The retropharyngeal lymphocentrum consists of the medial and occasionally lateral retropharyngeal nodes. The medial retropharyngeal node is typically a solitary bilateral node, but 20% of dogs have two nodes, with afferent drainage from all deep parts of the head, including the tongue, walls of the oral, nasal and pharyngeal passages, salivary glands, deep external ear, larynx esophagus and non-cutaneous/non-mucocutaneous structures of the neck. The medial retropharyngeal node is also noted to receive efferent lymphatics from the parotid and mandibular nodes. This text reports less frequent lateral retropharyngeal lymph nodes, observed in only 30% of dogs, receiving afferent drainage from adjacent structures with efferent drainage to the medial retropharyngeal node.

Another study[3] evaluated 24 dogs and found the retropharyngeal lymphocentrum to contain lateral retropharyngeal nodes in only 8% of cases, with the medial center consisting of a single node in half of the cases, and the other half having additional small accessory nodes. This study also described the facial node, present in only 17% of cases, which, when present, was consistently bilateral, receiving afferent drainage from the nasal region and drained via efferent vessels to the mandibular node. The mandibular lymphocentrum contained two to three nodes; the most dorsal received lymph from the lateral surface of the head and the facial node, if present. Lymph then went to other nodes in the basin via interconnecting vessels. Efferent lymphatics draining the mandibular node followed two pathways; one to the ipsilateral medial retropharyngeal lymph node and the other joining the efferent lymphatics of the contralateral mandibular node by anastomotic connections and draining to the contralateral medial retropharyngeal node. The parotid node identified in this study, located at the rostral aspect of the base of the ear, also delivered lymph to the medial retropharyngeal lymph node. This study

concluded that the various traversing connections of lymphatic flow in the head did not support a concept of physiologic compartmentalization of lymphoid tissue.

Osshoff[4] investigated a propensity for contralateral drainage in the head. Lymphatic vessels were cannulated and injected with self-curing latex and neck dissection was performed. An efferent upper jugular communicating pathway was repeatedly identified that crossed midline and drained to a contralateral subdigastric group of nodes, bypassing the mandibular nodes. Two distinct systems of lymphatic vessels were identified draining the floor of the mouth, the superficial mucosal system and a deep collecting system. Drainage of the superficial mucosal capillary system was to the mandibular node. The deep collecting system drained to the mandibular node or the subdigastric nodes of the internal jugular chain. Superficial capillaries crossed randomly to drain to both the ipsilateral and contralateral mandibular nodes. This additional complexity of contralateral drainage has also been noted by Wright[5], when lymphoscintigraphy of a rostral mandibular injection site consistently revealed contralateral as well as ipsilateral drainage patterns to the mandibular nodes in healthy canine specimens.

2. Metastasis/Staging

2a. Lymphatics and tumor biology for metastasis

Metastasis can occur via lymphatic or hematogenous dissemination of neoplastic cells[6]. Lymphatic flow is more facilitating to metastasis as it is less turbulent than vascular flow and therefore exerts less fluid sheer stress to cells, and has a high hyaluronic content which nourishes the metastasizing cells[7]. The lymphatic system drains water, low molecular weight solutes, protein macromolecules, cell fragments and inflammatory cells from the interstitial space[8]. Lymphatic capillaries consist of an endothelial lining without a smooth muscle component and

with incomplete junctions resulting in a network of leaky valves that provide a mechanism of fluid uptake. Fenestrations are also present that provide an avenue for particle uptake[9]. Lymphatic capillary gap junctions vary in size from <10 nm to >500 nm, making them semi-permeable. The lymphatic capillaries join to form collecting vessels, which typically empty into lymph nodes via afferent channels in the node capsule[10]. Cellular events that occur with lymphatic metastasis include detachment of a malignant cell from a primary tumor, invasion to the lymphatic space and attachment, implantation and progressive growth in the regional lymph node[10]. For lymphatic metastasis to occur, cancer cells and lymph nodes require appropriate receptors, growth factors, and the ability for angiogenesis.

The previously discussed variability and highly complex normal lymphatic anatomy is further confounded by the presence of a neoplastic process, in which not only are native lymphatic vessels able to provide routes for metastasis, but lymphangiogenesis incited by the primary tumor can create additional possibilities of aberrant or unexpected lymphatic drainage for the tumor. Osshoff[4] reported that in oral carcinoma, contralateral metastasis occurred in 16% to 39% of cases, and suggested three possible mechanisms for this occurrence: via afferent lymphatic vessels, superficial lymphatic capillaries or by spread over the midline of efferent lymph vessels. Tumor lymphangiogenesis is grossly dysplastic with the following possible characteristics: prelymphatics that are not continuous with lymphatics, inconsistent and incongruent basal lamina, flattened endothelium, and abnormal interconnection of stroma with blood vessels and lymphatic structures[8]. Absent or inefficiently structured lymphatic drainage exists in most solid tumors, with the lymphatic fluid following the pathways of the normal tissues that surround the tumor[7, 8]. Additional confounding factors of neoplastic processes on lymphatic mechanics include obstruction of normal lymphatic vessels or nodes with metastatic

cells and physical disruption of normal lymphatic pathways by mass effect[11]. When this occurs, alternate lymphatic drainage pathways must be utilized. This can occur by collateral lymphatic channels, retrograde flow in normal lymphatics or via lymphaticovenous communication[12].

2b. Concept/origin of the sentinel lymph node

The sentinel lymph node concept was initially described by Gould in 1960[13] when describing tumors of the parotid gland. Nine patients were described spanning the dates 1950-1957. A lymph node was consistently identified at the junction of the anterior and posterior facial vein during dissection for a parotidectomy. Gould suggested that this node should always be removed for frozen section study, as evaluation may guide the decision for the necessity to perform a radical neck dissection. The initial use of the term "sentinel node" was simply based on its proximity to the tumor. However, within this study population, patients undergoing radical neck dissection had positive nodes only when the "sentinel node" was positive. This concept was further established in 1977 in relation to penile carcinoma[14]. A lymphangiogram of the penile lymphatics was performed to identify the sentinel lymph node as the initial site of penile carcinoma metastasis. Metastasis occurred as a chain of lymphatic spread with no second order node (e.g. inguinal) displaying metastasis without concurrent metastasis to the sentinel node. This study also highlighted the prognostic importance of metastasis on survival, noting a five year survival rate of 90% when there was no documented lymphatic involvement, 70% if only the sentinel node was involved, 50% with involvement of a second tier node (e.g. inguinal lymph node), 20% with iliac lymph node involvement and 12% when bilateral metastasis was appreciated. Twenty percent of these patients also possessed multiple first tier sentinel lymph nodes within the sentinel node basin.

The sentinel lymph node is defined as the first node to receive lymphatic drainage from a tumor. With lymphatic metastasis the sentinel lymph node should be first to be affected. Alternatively, if the sentinel node(s) is negative for metastasis, further lymphatic metastasis should not be expected[15]. A number of other concepts relate to the sentinel node theory, including transit nodes, interval nodes and skip metastasis. Transit nodes are those identified as sentinel but located between the primary tumor site and the nearest described lymphatic basin[16]. Interval nodes include any sentinel node that lies outside of a recognized lymphatic basin[17]. Skip metastasis is a concept that has been identified in conjunction with a number of neoplastic processes but is generally characterized by metastasis existing in a lower tier node while the "sentinel node" is negative for metastasis[18]. Rosen[19] described this phenomenon in 1.6% of cases in relation to breast carcinoma and axillary lymph nodes, confirming the rate noted in a previous investigation[20]. Level designation of lymph nodes relating to breast cancer is based on anatomic location; Level I: lateral to pectoralis minor, Level II: behind the clavicular head of the pectoralis minor, and Level III: medial to the head of the pectoralis minor muscle. It was hypothesized that lymph would flow in a sequential manner through these levels. Previously reported skip metastasis had been noted in 36% of cases, with 10% of metastatic disease found only in level III. It was found in the current study that the presence of skip metastasis was not affected by tumor size, location of the tumor in the breast, or histologic type of the primary tumor. The authors hypothesized that a possible explanation of skip metastases is the existence of micrometastasis that is not detected on evaluation of lower level nodes. Minamikawa[21] evaluated a similar phenomenon in squamous cell carcinoma of the oral cavity. Levels of cervical lymph basins were reported as; Level I: submandibular, Level II: upper jugular, Level III: mid jugular, Level IV: lower jugular and Level V: posterior. This study was performed with the underlying

theory that metastasis should not occur to lower levels (III-V) without first passing through levels I-II. A skip metastasis prevalence of 0.3% was identified when this criterion was applied.

The clinical use of the sentinel lymph node theory has been widely adopted in human medicine, especially for breast cancers and melanoma[8, 22, 23]. As well as providing comparable information regarding metastatic status as radical lymphatic dissection[24], the use of selective sentinel lymph node mapping and dissection reduces morbidity through reduced risk of lymphedema, post-operative pain, infection and iatrogenic loss of sensation, as well as improved mobility[14, 25]. A 20 to 30% complication rate has been associated with axillary lymph node dissection with a similar rate of chronic lymphedema. By comparison, sentinel lymph node biopsy has a relatively negligible risk of complication[26].

2c. Lymph node staging

All of the previously listed factors relating to variations in lymphatic anatomy and drainage give evidence that the chosen lymph node evaluated for staging of oral cancers is likely to be misguided unless specific diagnostics are performed to determine the sentinel node in each patient. In the absence of such diagnostics, to accurately perform full and accurate lymph node staging, full bilateral dissection of all lymph centers of the head would be required, leading to associated morbidity and increased surgical time. In a previous study by Herring[27], dissection of all major lymphatic centers of the head was performed and revealed that in 45% of cases, metastatic disease was detected in nodes other than the mandibular node in dogs and cats with oral and maxillofacial neoplasia. An additional study[28] evaluating a surgical approach for access to the main lymphocentrums of the head via a single lateral approach found that for patients

diagnosed with lymph node metastasis, the mandibular node was only involved in one third of cases.

Historically, the size, mobility, firmness and distribution of lymph nodes have been used to determine potential for presence of metastasis[29]; however, in the study by Herring[27], only 17% of palpably enlarged lymph nodes were found to be metastatic, while 8% of mandibular nodes that palpated normally were found to contain metastatic disease. Morton[24] determined a 15 to 20% false positive rate when melanoma patients were evaluated for nodal metastasis based on palpable enlargement of regional nodes. In another study[30] investigating dogs with oral melanoma, a 53% metastatic rate was identified, but only 70% of those were noted to have clinically enlarged lymph nodes and 40% of lymph nodes considered normal in size were found to have evidence of micrometastasis. This study found that evaluation of lymph nodes based on size alone had a sensitivity of 70%, a specificity of 51%, a positive predictive value of 62% and a negative predictive value of 60%, which was considered to be clinically insufficient. A more recent multi-institutional study investigated mandibular lymph node evaluation in dogs with oral melanoma[31]. Sensitivity, specificity, positive predictive value and negative predictive value of palpation vs. histologic evaluation for metastatic disease was 65%, 78%, 84% and 56%, respectively. Based on these findings, it was recommended that ipsilateral and contralateral mandibular and retropharyngeal lymph node biopsy be performed in all patients for accurate staging.

The correlation between cytologic evaluation and histologic evaluation of lymph nodes has also been reported with variable findings. The study performed by Williams[30] found agreement between the two diagnostic techniques in 5/7 lymph nodes. The sensitivity, specificity, positive predictive value and negative predictive value of cytologic vs. histopathologic results were 78%,

64%, 83%, and 56%, respectively, in a recent multi-institutional study involving 151 dogs[31]. Other studies with case numbers of 31 have reported better correlation, up to 90%[27]. The underlying causative factors between these discordant results is unclear; however, the low case numbers included in some of the studies is a potential contributing factor.

Lymph node metastasis has been postulated to act only as an indicator of aggressiveness for the possibility of vital organ metastasis, rather than having a direct outcome on survival, and that lymphadenectomy of micrometastases does not confer survival benefit[10]. However, other reports state that the presence of lymph node metastasis has been shown to have prognostic significance for survival in humans[10, 32, 33], and distant metastasis is reported twice as often in patients with lymphatic metastasis as those without[27]. The veterinary literature has only intermittently upheld this finding[31, 34]; however, it is also possible that this discrepancy is due to the decreased lymph node evaluation performed in veterinary oncology patients compared to human oncology counterparts and the true role of lymphadenectomy of sentinel nodes, whether diagnostic or prognostic, remains to be determined. The presence of metastatic disease also has an impact on clinical decisions regarding treatment recommendations. For these reasons, it is important to correctly and accurately determine those patients with lymph node metastasis, and correctly identify the sentinel lymph node.

2d. Lymph node histopathology

Neoplastic cells within lymph nodes can have many presentations. Occult metastases can be divided into micrometastases (indicating infiltration of < 2 mm diameter) and metastasis (> 2 mm diameter)[32], although other authors base categorization on percentage of lymph node involvement (e.g. micrometastasis defined as < 20% of node cross sectional area involvement)[26].

These categories also require differentiation from isolated tumor cells, which can be defined as a tumor embolus or the presence of tumor cell clusters in the intra-sinusoidal region without evidence of extravasation, extravascular stromal reaction or extravascular tumor cell proliferation[35]. The presence of neoplastic cells in these various categories may affect the TNM classification of a tumor, described by the World Health Organization to delineate the primary tumor, lymphatic and distant metastatic status of disease[36]. The presence of isolated tumor cells does not change the N status, but adds the qualifier "i+". Micrometastasis does change the N status but also adds the qualifier "mi", whereas the presence of occult metastases > 2 mm diameter changes the absolute N status to positive[35].

It has been suggested that the use of intra-operative techniques such as cytology and frozen biopsy section have poor sensitivity including false negative rates as high as 36% to 70% for frozen section, 52% for frozen section combined with cytology, and 14% for impression smear cytology[37]. In addition, these techniques can result in the loss of up to 50% of sentinel lymph node tissue that will therefore no longer be available for more accurate histopathologic processing[32].

Histopathology is the gold standard for diagnosis of metastatic disease. However, there have been a number of factors evaluated that have been suggested to influence the reliability of this diagnostic tool. By limiting the number of nodes that have to be evaluated by the pathologist, a more extensive study can be performed of each node, thus improving the false negative rates previously reported including up to 36% of frozen section evaluation in breast cancer and up to 71% in melanoma patients[26]. Herring[27] reported than when a single section of lymph node was evaluated, it was possible to miss metastatic disease that could be identified on serial sectioning. Treseler[26] also reported that the diagnostic yield of H&E stained slides increased with multiple

stained sections compared to single sections. When lymph nodes < 5 mm were embedded in paraffin whole and those > 5 mm were lamellated into 2 mm slices and subsequently sectioned into middle, deep and superficial layers, occult metastases were identified in 32% of sentinel nodes; 50% of those were micrometastases[35]. Mariani[8] recommends that nodes should be sectioned at 50-200 um intervals in order to identify small micrometastatic foci. Using this technique, starting from the node hilus, 77% of overall metastases are identified in the first section, 84% within the first 3 sections and 92% within the first 5 sections, whereas micrometastases within a sentinel node have only a 53% detection rate in the first 5 sections, and 91% in the first 10 sections. It was also determined that tumor cells causing metastases will initially be found in the most peripheral sinusoid spaces of the lymph node. Another study[38] found that disease was upstaged by 10% regarding nodal metastatic status when serial node sectioning was performed and an additional 10% with the use of immunohistochemistry.

Immunohistochemistry (IHC) and molecular techniques (e.g. PCR) are even more sensitive methods of detecting occult metastases, although false positives can be a problem, especially with molecular techniques[32]. Immunohistochemistry increased rates of node positive diagnosis from 7% to 31% with up to 50% conversion from node negative to node positive status with specific types of cancer (e.g. lobular vs. ductal carcinoma). Polymerase chain reaction is exquisitely sensitive, but there is no way to tell the origin of detected cells (i.e. metastatic carcinoma vs. benign epithelial inclusions vs. regular epithelium tracking in lymphatics after a biopsy can all appear as a positive result). Another study by Treseler[26] identified that the diagnostic yield can be increased by using immunoperoxidase stains specific for relevant tumor-related antigens to highlight metastatic deposits. For breast cancer, anti-keratin antibody or epithelial membrane antigen stains can be used. With melanoma, S-100 has been found to be

highly sensitive but less specific, HMB-45 is highly specific for cells of nevomelanocytic origin, and NK1/C3 and Melan-A have intermediate sensitivities and specificities. Use of these stains and evaluation of at least three levels of each lymph node detects 70 to 90% of sentinel lymph node metastases[26]. This study noted that while PCR is even more sensitive, it has a higher risk for false positive results. Some authors state that H& E performed on multiple sections is sufficient and immunohistochemistry is not recommended[39]; however, the general consensus appears to be that step-serial sectioning and the use of immunohistochemistry is the most accurate staging method[40].

3. Diagnosing the sentinel lymph node

3a. Discrepancy from expected anatomy

Initial determination of sentinel lymph nodes was often based on predicted lymphatic drainage patterns. This however, is likely an erroneous assumption as demonstrated previously by the large number of possible variables that could be responsible for altered individual lymphatic drainage patterns. A recent study by Sutton[33] found that when comparing pathologically positive nodes to predicted nodes, the clinical prediction was only correct in 73% of cases. This discrepancy was also noted to be a function of tumor thickness, as tumors classified as "thick" (up to 1cm) had unpredictable drainage patterns in up to 75% of cases. Between 32 to 62% of body tumors and 63 to 84% of head and neck tumors were found to have lymphatic drainage patterns different from those expected based on anatomic convention[23]. Lymphatics can be asymmetric bilaterally as well as expressing the possibility of contralateral drainage[41].

The identification of alternate pathway lymphatic drainage continues to be noted in animal studies as well. When drainage patterns of the canine ventral body wall were investigated, it was found that variable drainage patterns were identified, both crossing left and right as well as unexpected cranial or caudal drainage[42]. A study evaluating lymphatic drainage patterns from superficial sites on the heads of eight normal dogs found anomalous lymph nodes or unexpected drainage in approximately 10% of injections[43]. In another study investigating canine mammary lymphatics[44], there was evidence of individual variation of lymphatic drainage in areas previously considered to have well-described drainage patterns. Thoracic mammary glands drained to the cranial sternal and axillary lymphocentrums. The cranial thoracic glands also drained to the superficial cervical node in 40% of dogs. The cranial abdominal glands drained to the axillary lymph center while caudal abdominal glands drained to the superficial inguinal node in all cases, medial iliac lymph nodes in 80% and mediastinal and superficial cervical nodes in 20% of dogs. The inguinal mammary gland was drained by the superficial inguinal lymph node in all cases and the medial iliac lymph node in 20%. Forty-four percent of dogs were noted to have communication between lymph nodes.

These unpredictable drainage patterns are the likely explanation for the previously discussed phenomenon of skip metastasis. Rather than neoplastic cells actually passing through a primary node without establishing metastasis, it is more likely that the drainage pattern in that specific individual follows an unexpected path and the "second tier" node with "skip metastasis" is actually the first node encountered by neoplastic cells undergoing lymphatic invasion[37]. This is supported by the finding that the incidence of skip metastasis is noted to decrease with surgeon experience of performing sentinel node imaging techniques[15] as well as the absence of skip metastases noted when sentinel lymph node mapping is performed prior to biopsy[45]. As an

additional confounding factor to diagnosis, the number of identified sentinel nodes is often > 1, with a mean reported as 2.6[46, 47] to 3.7 per patient[48], and in one study[49] all patients were noted to have at least two sentinel nodes. What continues to remain unknown, is if there is an incubation period within a single node where metastatic cells grow before spreading to other systemic sites, or if this is a simultaneous process[7]. Regardless, the frequency of unexpected drainage patterns found throughout the literature highlights the need for individual investigation for accurate sentinel node detection.

3b. Blue dye and lymphoscintigraphy

Early studies of sentinel lymph node mapping involved the injection of vital blue dye for visual tracing of lymphatic channels and identification of draining lymph nodes. The injected dye is taken up by lymphatics and the channels are physically followed by dissection to discolored sentinel nodes. When evaluated by Thompson[17], blue dye mapping revealed enormous individual variation in lymphatic drainage patterns. Multiple sentinel lymph nodes were frequently detected and noted in up to as many as five different "node fields". This study also showed contralateral drainage in up to 15% of cases and concluded there was overall an unpredictability of lymphatic head and neck drainage. Another early study[24], reported successful identification of sentinel nodes using blue dye technique in 194/237 nodes in human patients with melanoma. A false negative rate of < 1% was found when evaluating only sentinel nodes for metastasis. This report noted that extreme caution must be used when elevating skin flaps to follow lymphatic channels as it is possible to transect these channels. Careful dissection of lymphatic channels is also necessary to ensure a sentinel lymph node is not accidently bypassed for a second order node. There was a very steep learning curve with substantial experience required to have a high rate of

success with this technique[24]. Additional drawbacks to the use of the blue dye technique include unnecessary dissection owing to the fact that the location of nodes is difficult to determine prior to skin incision, difficulty verifying successful removal of all first echelon draining lymph nodes, the fact that nodes retain dye for only approximately 15 minutes and dye stains local tissues as well as lymphatics obscuring tissues and having negative cosmetic outcomes[49]. Blue dye has also been reported to have the possibility of inciting allergic reactions in people, although this has not been previously noted in veterinary patients.

Another study[37] used isosulfan vital blue dye injected into the primary tumor site with the lymphatics being followed to the sentinel node. Nodes were successfully identified in 65% of cases with accurate prediction of nodal status in 95%. The second half of the study cases had increased predictive accuracy for metastasis. There was a 4% false negative rate, 88% sensitivity and 100% specificity. The reported probability of excising a positive node with random sampling was 17% compared to 62% with lymphatic mapping. A 24% rate of "skip metastasis" was seen, defined as second level sentinel node turning blue without a level 1 node taking up dye[37]. Although results of blue dye mapping show better results than random lymph node sampling, the results are still low enough to be clinically significant and concerning when used as a sole diagnostic technique.

Lymphoscintigraphy involves the use of local injection of a radioisotope that enters the lymphatics and passes to the draining lymph node. Photon emissions given off by the isotope are detected by a gamma camera and converted to an image. An intra-operative gamma probe can also be used to detect these emissions through the skin, enabling a small targeted incision to be made directly over the node; this results in much less dissection than what is required for tracing lymphatics using the blue dye technique[50]. Lymphoscintigraphy has been recommended to

identify the drainage basin, determine the number of sentinel nodes, differentiate first tier and lower tier nodes, locate sentinel nodes outside expected node basins, and potentially to mark the location of a sentinel node on the skin[51]. Lymphoscintigraphy has shown that individual lymphatic drainage patterns can be discrepant from "expected" anatomical pathways in up to 60% of patients[49]. The benefits of performing lymphoscintigraphy are that it allows identification of the node by indirect means rather than having to perform complete dissection of node basins, which is previously reported to be associated with a number of complications and morbidities. Short-term complications associated with full elective neck dissection include seroma, infection, dehiscence, lymphedema and pain. Long-term complications include lymphedema, pain and hernia formation[23]. Gamma probe guidance has been used to identify and remove first draining lymph nodes in the inguinal basin of cats. This study showed comparable results to a more invasive method with blue dye, but much less dissection was required and resulted in less overall morbidity. Additional benefits of the gamma probe include confirmation of removal of the correct node, evaluation of the remaining lymph basin for any additional nodes of interest and being more easily and rapidly performed than blue dye dissection. The gamma probe was able to identify 100% of the nodes localized by intra-operative dissection. Maximal counts were obtained 3 to 6 hours after injection and began to drop to one-half maximum by 8 to 9 hours after injection. There was no apparent correlation between size of lymph node and gamma emission[52].

Intra-nodal retention of a radionuclide is the result of phagocytosis by macrophages lining the sinusoid spaces of lymph nodes[8]. Clearance from the interstitial space and trapping in the lymph node of colloidal particles is dependent on particle size and on the functional state of the reticuloendothelial system[53]. Particle size has been noted to play a key role in the speed of lymphatic uptake as well as retention within the node. Smaller particle radiocolloids migrate

faster than larger particle radionuclides and tend to pass more quickly into the second and third-echelon nodes. Larger particle colloids may be less likely to penetrate the sentinel lymph node either due to diseased or blocked lymphatics[38]. Although local administration of technetium results in uptake into lymphatic drainage, up to 95% of the dose will remain at the injection site[54, 55]. There is a recommendation for a large volume to be used when intra-tumoral injection is performed to increase intra-tumoral pressure and therefore encourage lymphatic drainage. Minimal radiocolloid leaves the injection site via this method, and so higher doses are also required; intra-tumoral doses are recommended to be approximately 10 times larger than intradermal, and intra-parenchymal dose is advised to be 5 times larger[11]. Use of these larger doses and volumes can cause difficulty in evaluating nodes close to the injection site due to increased scatter artifact in the image.

It was initially thought that the appearance of the lymphoscintigraphy image represented the metastatic status of the node. It was theorized that the process of uptake by the node is influenced by its phagocytic activity; it could possibly be increased by induced immunologic response to tumor antigen in the early stages of metastasis, leading to an initially higher uptake. This would be followed by a progressive fall in uptake as tumor replaced functional nodal tissue. Finally there would be complete mechanical obstruction due to gross metastasis causing absence of tracer uptake with permeation of the radionuclide to the adjacent tissues resulting in a radioactive "blush"[41]. Hyperplastic lymph nodes or those undergoing fatty degeneration may also demonstrate a slow and reduced uptake[11]. These changes were thought to be prognostic for local recurrence. These theories, however, have mostly been replaced with the idea that lymphoscintigraphy acts simply as a map to guide dissection of the relevant lymph nodes, and it is only by histologic analysis that the metastatic status of those nodes can be determined[56].

3c. Equipment used in lymphoscintigraphy

The main components of lymphoscintigraphy equipment include the gamma camera and the gamma probe. A gamma camera detects photons from the radioactive tracer, and the counts and locations are recorded. The lower and upper level discriminator is called a pulse height analyzer and is set dependent on the energy emission of the radiotracer being used. The lens of the camera is made of septated lead (parallel hole collimator) that permits only perpendicularly-directed photons to react with the crystal in the detector. Pinhole collimation can be used to help identify sentinel nodes by focusing on an area and obscuring other higher intensity sources of activity to enhance the definition of areas of less activity. It is possible to use an external radiation sheet behind the patient to obtain a body outline (^{99m}Tc or cobalt). Alternatively, it is possible to use the residual dose in the syringe as a pointer or to trace the body outline, give an intravenous injection of $^{99m}TcO_4$ to obtain a body contour, or inject ^{99m}Tc-methylenediphosphonate intravenously to obtain bone phase images[44]. A persistence scope permits continuous monitoring of the radioactivity and is useful for defining external point source of ^{99m}Tc in relation to body locations[15]. The ability of the gamma camera to distinguish between two adjacent areas of activity is determined by the resolution of the camera, the amount of signal from each of the points, the distance from the camera to the subject, when shorter acquisition time is used or when the patient moves during acquisition. Recommendations to improve accuracy during lymphoscintigraphy include: performing dynamic imaging at the time of injection, use of high resolution collimators, positioning the camera as close as possible to the patient, and creating images in two projection planes[57]. The pulse height analyzer settings for a gamma camera when

using technetium should be centered on 140keV emission peak with a +/- 10% window. A high-resolution collimator and an acquisition matrix of 256x256 pixels is preferable[8].

The gamma probe consists of a detector, collimator, digital or analog display and an audio signal generator[6]. The detector is made of a material that will interact with a gamma photon when it strikes the detector surface. Detectors can be made of crystals such as cadmium zinc telluride or sodium iodide or they can be a cadmium telluride semiconductor detector. Semiconductor based probes have a lower sensitivity than scintillation based probes, especially at energies higher than 140keV[6]. The crystal is made of materials that have electron densities that provide stopping power to interact with the incoming gamma photons[15]. For example, a 0.5 inch crystal of sodium iodide will interact with approximately 98% of the gamma photons that strike the surface; 80% for the cadmium crystal. Technetium gives off energy of 140keV as it decays. When a photon with this energy strikes the crystal, it is considered a primary event. A scattered photon will have less energy and probes have a discriminator to eliminate scatter radiation from the counts detected. When a primary event occurs, an electronic pulse is initiated, amplified and if of the correct energy, recorded as a count. When a count occurs, the probe converts this information to an audible signal and the count rate is proportional to the amount of activity striking the crystal. Shielding is needed to isolate the radiation source of interest from other sources (e.g. node vs. injection site). Shine through effect is noted when the radiation given off by one site "masks" the signal from another site; shielding is needed to counteract this effect. Shielding is present on the sides and back of the detector. The opening of the detector is collimated to add to its precision. When the shielding is insufficient, it is sometimes necessary to surgically remove the primary tumor before proceeding with sentinel node dissection to reduce background activity levels; this has been reported in up to 14% of patients[54]. Inverse square law

applies to the gamma probe in that as the distance between the probe and radiation site doubles, the counts recorded by the probe will decrease by a factor of four. Characteristics of an ideal gamma probe should include: counting efficacy of ^{99m}Tc, small diameter to fit into small incisions, ease of handling, collimation capability, side shielding, and energy discrimination[15]. Sensitivity is one of the main parameters of performance of a gamma probe; this is the ability to detect a count rate per unit activity, or counts per second. This reflects the efficiency of the probe in converting radiation into an electrical signal. Contrast (target to background ratio) is actually more important than definitive count number. Energy resolution is another key performance parameter; a higher resolution will eliminate more counts corresponding to scatter radiation and discard fewer counts corresponding to the primary radiation of the target. Spatial resolution is the third performance parameter; it is important for identifying the location of the radiation source. It is determined by the ability of the probe to detect counts as a function of the lateral distance from the central axis of the detector. The major determinant for this feature is lateral shielding; heavier shielding improves spatial resolution but reduces sensitivity and increases probe weight[8]. Subjectively, a variable frequency audio signal has also been reported to be easier to use intra-operatively[6]. Specificity of the gamma probe over the camera has also been appreciated[52]; in one cat two nodes were separated from each other by 1.2 cm (nodes 7 mm and 4 mm in diameter) and the probe was able to identify them as 2 distinct nodes while they appeared as a single spot on the camera image. With a gamma camera, the mean nodal counts were typically 2.5 to 15 times background counts at 3 to 4 hours whereas nodes localized by the probe at this time had a ratio of 100 to 400 times background.

Criteria have been established to define the identification of a sentinel lymph node with a gamma probe intra-operatively. Initial definitions used an absolute count of at least 20 counts

over 10 seconds[46, 52]. This use of absolute values has been questioned, and more commonly, a ratio is used to determine radioactivity. Typically a ratio is compared to background values, which can be determined by four equidistant points from the point of injection or the sentinel node[57]. When 0.45mCi of ^{99m}Tc was injected, the average hot spot to background ratio was noted to be 3.24 and average *in vivo* sentinel lymph node to background ratio was 8.22. Based on these findings, a definition of "hot" node is made based on criteria of > 2:1 activity over background *in vivo* or more than 10 times activity over *ex vivo* non-sentinel lymph nodes[22]. In 85% of cases, the "hot spot" returned to 150% of background measurement after excision of all sentinel lymph nodes. A "hot" node was any node with > 3:1 activity over background *in vivo*, or > 10:1 ratio over a non-sentinel lymph node *ex-vivo.* Recommendations for the definition of sentinel node intra-operatively are: any blue stained node with blue-staining afferent lymphatics (when blue dye is used), *in vivo* activity > 3:1 vs. background, and it is recommended to attempt to identify additional nodes if area activity remains > 150% background[16]. Alex[49] also defined an active node as having a ratio of three times greater than background to achieve an emission measurement precision of 98%. A study evaluated these definitions of radioactivity and determined that the "miss rate" could be reduced to acceptable levels by modification of the suggested activity levels[48]. Lymph nodes with radioactivity at least 10% that of the highest activity node were resected. The "miss rate" was successfully reduced to 2.5% by removing all nodes with activity counts 10% or more of the most active node, compared to a 16% miss rate when only the hottest node was removed. The additional use of blue dye did not significantly reduce the miss rate compared to the use of radionuclide alone when the 10% rule was used. Nodes of interest were also determined to be at least 3 times more active than the adjacent normal tissue background levels[48]. Although it has been postulated that metastatic sentinel nodes

will have the highest activity, it has been shown that in up to 5% of positive nodes, they were not the hottest nodes in the basin[47] and that in 16% of cases, the positive lymph node was found to have a lower radioactive count than that of the "hottest" lymph node. Further, in approximately 10%, the positive node had less than 50% the activity level[48]. When evaluating for nodes that are at least 10% the activity of the hottest node in the area, it was found that 96% had activity more than four times greater than the node basin[47].

3d. Agents used for lymphatic mapping and lymphoscintigraphy

Studies have been performed comparing different types of vital blue dyes to determine the best dye for lymphatic mapping. Morton[24] compared methylene blue, isosulfan blue, patent blue-V, Cyalume, and fluorescein dye by performing an intradermal injection of 0.5 to 1.0 mL five minutes prior to surgery. Injections were repeated every 20 minutes intra-operatively. An incision was made over the lymph basin of an expected drainage site and dissection was continued to evaluate for blue nodes. Patent blue-V and isosulfan blue produced the best results with regards to accuracy; these dyes entered the lymphatics rapidly after injection, were associated with minimal diffusion and were readily visible. Other dyes were mostly unsatisfactory because of a tendency to diffuse out of the lymphatics. Methylene blue had poor lymphatic retention causing the nodes to stain only faintly. Patent blue-V enabled the best viewing of lymphatics and brightest staining of sentinel nodes. It was also concluded that in addition to the notable technical difficulty of this procedure and high learning curve, additional morbidity is also associated with the increased tissue dissection required. Postoperative complications included wound edge necrosis (4%), seroma (5.5%) and infection (4.8%)[24].

Characteristics of an ideal imaging agent for lymphoscintigraphy include rapid clearance from the interstitial space, ability to produce high quality images, good retention in the lymph nodes, and delivery of a low radiation dose[23]. Another study determined that optimal imaging results are obtained once doses reach values above 65 MBq (1 MBq = 0.027 mCi)[58]. Any agent to be used for lymphoscintigraphy must also be evaluated for molecular size, physical and biological distribution in the interstitial space and potential for problems relating to radionuclide labeling[53]. Particle size and stability over time are the key tracer characteristics that allow for effective lymphoscintigraphy. Larger particle size limits the number of sentinel lymph nodes identified and possibly enhances identification of true sentinel nodes[59]; however, particles > 500 nm tend not to leave the injection site. Human serum albumin (^{99m}TC-HSA) nanocolloid has particle sizes ranging from 4 to 100 nm and ^{99m}Tc-antimony trisulfide has a particle size ranging from 3 to 30 nm. ^{99m}Tc-rhenium sulfide is reported to be 100nm in size, but actually has trimodal size distribution of 40 nm, 440 nm, and 650 to 2,200 nm. ^{99m}Tc-sulfur colloid has a particle size of 15 to 5,000 nm (unfiltered). The most commonly used radionuclide, filtered ^{99m}Tc-sulfur colloid, tends to yield particles 10 to 100 nm; typically has rapid lymphatic visualization (within 30 minutes) and good lymph node retention[23]. The recommended dose of ^{99m}Tc-sulfur colloid varies between 0.45 to 1.0 mCi[59]. Commercially available technetium sulfur is made by incubating with thiosulfate resulting in a colloidal compound which is then filtered through a 0.22 micron filter to remove the larger particles. ^{99m}TC antimony sulfide (not available in the US) contains smaller particles of 10 to 40 nm, and has a recommended dose of 500 μCi in up to 0.3 mL volume[41]. Albumin colloid (also not in the US) has a particle size < 80 nm. A previous study showed that HSA, which is not a colloid and instead acts like blue dye, is not retained in the node, although otherwise has similar kinetics to sulfur or albumin colloids[15].

Previous studies comparing ^{198}Au-colloid, ^{99m}Tc-antimony sulfide colloid, ^{99m}Tc-tin colloid, ^{99m}Tc-phytate and ^{99m}Tc-sulfur colloid revealed that ^{198}Au-colloid provided the best image quality but only at doses that caused excessive patient radiation exposure and therefore was not considered suitable for clinical use[42]. ^{99m}Tc-antimony sulfide colloid had the best image quality at lower radiation doses. Technetium is transported in lymph when in a colloid form and imaging may take up to 2 hours to be performed. This is compared to dextran, which is soluble in lymph, and scans can be performed 15 minutes after injection. However, despite faster images, the sulfide colloid provided better imaging quality (both visual and quantitative) than dextran as there was a higher background activity observed with dextran[42].

^{99m}Tc-albumin colloid, ^{99m}Tc-HSA and ^{99m}Tc-sulfur colloid were evaluated by injecting 500 to 800 μCi and performing dynamic and static imaging at the time of injection, 30 min (early) and 2 to 4 h (late) post-injection[60]. All 3 agents showed better imaging at 30 min than at 2 to 4 hours. Human serum albumin had faster washout times and better definition of lymphatic channels. All agents showed better lymphatic channels in early than late images. Individual patient variation had a greater impact on imaging than type of agent. Delayed images were unreliable in differentiating sentinel lymph nodes from non-sentinel lymph nodes. Half times for washout from the injection site were 7.5, 4.3 and 13.9 h for albumin, HSA and sulfur, respectively. HSA had more uniform washout time between patients. Particulate agents demonstrated more nodal retention. Lymphatic flow of all agents was also noted to be affected by local hyperthermia and massage[60].

Dextran as a technetium carrier agent has been assessed numerous times. Henze[53] performed interdigital injection in dogs, and the radiotracer was noted to reach the knee or elbow lymph nodes in 12.4 seconds and inguinal or axillary lymph nodes in 98 seconds. It was cleared from

the injection site with a half time of 31 minutes. When lymphedema was present, tracer migration was markedly delayed. Dextran, with a molecular weight less than 40,000, is able to penetrate capillary membranes. There was no evidence of phagocytosis of dextran, so it is not retained in the lymph node, but ultimately is trapped by the hepatic reticuloendothelial system [53]. ^{99m}Tc-dextran has also been compared to ^{99m}Tc-sulphide colloid for suitability as a lymphoscintigraphy agent in rabbits and dogs[61]. Absorption from an interstitial injection site was evaluated including popliteal lymph node sequestration and total body uptake and distribution. Dextran uptake was faster and greater than sulfide, but total lymph node sequestration was significantly lower likely due to its non-colloidal, non-particulate nature. In general, lymph node uptake was higher in dogs than in rabbits, highlighting species-based differences. It was determined that reduced uptake, as well as instability, make dextran an unsuitable transport molecule of technetium for the purposes of pathologic lymph node imaging. In addition, the labeling efficacy of sulfide was always greater than 95%, whereas for dextran, it varied from only 12% to 32%[61].

3e. Reliability, reproducibility, and factors affecting quality of lymphoscintigraphy

The ability to reproduce results of lymphoscintigraphy and lymphatic mapping has been evaluated, and the factors affecting the reliability and repeatability of this diagnostic tool have been investigated. The first description of intra-operative use of radiolymphoscintigraphy reported technical difficulties resulting in unsuccessful explorations following vital blue dye technique in up to 20% of procedures[16]. Sixty-nine percent of nodes were identified by blue dye staining, 84% by radioactivity detected by the gamma probe. Use of both techniques allowed for 96% identification. When sentinel lymph nodes were found to be negative on histology, there

was a < 2% chance of those patients developing lymphatic metastasis in the same basin in the future. A high learning curve was associated with use of blue dye, relating to both injection technique and dissection, and success was directly proportional to surgeon experience. Improved localization with less tissue dissection was found when using a gamma probe. Two of sixteen metastatic nodes were found to have radioactivity without any blue coloration. Three to four hours was identified as the ideal interval between injection and sentinel lymph node biopsy with a gamma probe to allow for optimal distinction between activity from nodes vs. background[16]. With additional use, it was determined that lymphoscintigraphy reliably localizes sentinel lymph nodes, allows the lymphatic bed to be checked intra-operatively to verify complete sentinel node biopsy and is relatively simple. In humans, this procedure can be performed under local anesthesia. Lymphoscintigraphy showed nodal uptake as early as 10 minutes after injection and remains clearly localizable by gamma probe for at least 5 hours[62].

The reliability of lymphoscintigraphy and intra-operative gamma probe usage has been further evaluated with Ege[41] determining the most important requirements for an effective study to be selection of an appropriate injection site to enable visualization of lymphatics and use of an agent with optimum properties. Jansen[63] injected 1.6 mCi of ^{99m}Tc nanocolloid and obtained 2 hour static imaging. The next day, gamma probe and blue dye techniques were used to retrieve sentinel nodes. Sentinel nodes were defined if an afferent lymphatic was identified originating from the injection site, or if it was the first spot to appear in a basin if no afferents could be seen. All other spots were defined as non-sentinel nodes. Different numbers of nodes were detected on pre-operative scintigraphy than intra-operatively in 23% of patients. More nodes were found intra-operatively because a vessel was not seen on pre-operative lymphoscintigraphy, multiple nodes appeared to be one node, or rarely, because a node was blue but not radioactive. Fewer

sentinel nodes were identified at the time of surgery because of a lymphangioma being mistaken for a node on lymphoscintigraphy, or a single elongated node being misidentified as two separate nodes. Only 1% of nodes were noted to be blue but not radioactive. Active nodes were not removed in this study if the authors felt the nodes were in a location of non-sentinel nodes based on pre-operative lymphoscintigraphy, which is in disagreement with recommendations of many other studies[22, 48, 49] and may partially be responsible for the relatively low reported accuracy of 81%. The authors also suggest caution when interpreting gamma probe results as deeper nodes will appear to emit less signal than superficial ones; they advise to always recheck the lymphatic bed after removal of nodes to evaluate for the continued presence of additional sentinel nodes[63].

Another study noted that radical neck dissection for lymph node biopsy without the use of pre-operative identification of lymph nodes was only accurate in classifying metastasis in 90% of cases, vs. 94% accuracy when performing sentinel node biopsy using additional blue dye mapping technique[21]. Experimental studies in animals have also shown reasonable accuracy of lymphoscintigraphy and blue dye mapping. When used to determine the lymphatic drainage of the anterior commissure of the canine vulva using a gamma probe and blue dye, 88% of sentinel lymph nodes were successfully identified[57].

In general, it has been determined that reproducibility of lymphoscintigraphy is good; however, discordant findings may occur resulting in the identification of different nodes or even completely different nodal basins[15]. Imaging over time after a single injection has shown to have an effect on reproducibility. Images obtained at the initial injection period were compared to those obtained 18 to 24 hours later with results revealing that 71% had similar scans on day 1 and 2, 10% offered clarification of questionable nodes with the second scan and 19% showed new nodes on day 2[47]. It was also noted that approximately 25% of ^{99m}Tc-sulfur colloid is

detectable at 24 hours post-injection, with a washout time of 13.9 ± 12.7 h[47]. Contradictory findings were noted by Rubio[59] when time to evaluation did not appear to impact reproducibility of results. A single injection was given and evaluation was performed within 4 hours of injection and compared to results the following day. There was no difference noted in node numbers, or increased labeling of identified nodes[59]. In another study, repeated intra-tumoral injection over 2 days resulted in the same lymphatic drainage patterns in all patients[64]. A study evaluated scintigraphic studies performed 2 to 4 weeks apart by a similar imaging method involving peri-tumoral quadrant injections[65]. Primary tumor removal had been performed prior to either of the imaging studies. Sentinel nodes were always identified within 20 minutes throughout this study, but 12% of the cases showed differences in study results between the first and second injection, involving different numbers of nodes being identified. It was concluded that although lymphoscintigraphy is highly reliable, it is not 100% reproducible and possible explanations for the limited reproducibility included: small variations in injection technique, impact of delayed injection of sulfur colloid (particle size increases after 5 hours and a delay in drawing it up may impact lymphatic uptake), individual physiologic variations including hydration status or previous exertion, and the potential impact of adjacent wound healing (effect of granulation and fibrous tissue development on lymphatic drainage)[65].

It has been suggested that intra-tumoral injection of radionuclide more accurately represents the true tumor lymphatic draining pathways than injection at a distant site due to specific tumor lymphangiogenesis and intra-tumoral lymphatics[64]. This theory was supported by a single human case report[66]; however, it has not been evaluated on a larger scale. In the case report, a 1.5 cm non-palpable breast tumor was evaluated. Tracer was inadvertently injected initially approximately 3cm distant from the tumor and lymphoscintigraphy revealed two sentinel nodes

in the axilla. A second injection was given the following day using an intra-tumoral technique and a second lymphoscintigraphy revealed additional sentinel nodes in 2 regions outside the axilla[66]. Valdes Olmos[58] also agreed with the finding that visualization of the lymphatic vessels is less frequent with peri-tumoral injection. Intra-tumoral injection has been described for tumors of the gastrointestinal tract, thyroid and head and neck. Peri-tumoral injection may enhance sentinel lymph node detection as lymphatic drainage from the skin and sub-areolar plexus is richer than drainage directly from the tumor, however injection away from the tumor risks crossing a lymphatic watershed and the identified lymph node may not actually be the one that drains the tumor[58]. While intra-tumoral injection theoretically introduces the risk of needle tract metastasis when the tumor is located deeper in the parenchyma, this has not been noted clinically; however, peri-tumoral injection spreads tracer over a wider area, potentially impacting visualization of adjacent lymph nodes[58]. An additional consideration relating to intra-tumoral injection is that there remains an unanswered question relating to the possibility of tissue disruption and resultant decreased ability to appropriately analyze the tissue with histopathology when an intra-tumoral injection is used[54].

Extra-tumoral injection locations have also been compared. An evaluation between dermal, subdermal, peri-areolar or sub-areolar injection for breast cancer showed results that sentinel node identification was improved with dermal (98%) as compared to peri-tumoral injection technique (90%). The false negative rate was also improved with the use of dermal (6.5%) compared to peri-tumoral (9.5%) technique[67]. Morton[24] found an apparent difference in drainage patterns between intradermal and subcutaneous injection techniques when using blue dye for lymphatic identification. Injection technique was also investigated in an experimental study involving canine prostate glands[56]. Transrectal ultrasound guided technetium injections were

performed comparing central vs. peripheral injection techniques as well as the effect of large or small injection volumes (10% prostate volume and 1% prostate volume). The number of sentinel nodes visualized ranged from 4 to 7 and it was noted that there was marked inter-individual variation in drainage pattern as well as disparate drainage within an individual based on specific area of the prostate injected. It was found that a central injection technique had better reproducibility at 84% with either volume of technetium. The largest amount of scatter was noted after central, volume-reduced injection. The authors postulate that the best results would be obtained by combining central and peripheral injections. Theoretically, lymphatic flow is initialized by an increase in the interstitial pressure triggered by a volume of fluid causing distention of endothelial cells mediated by anchoring filaments. The volume required to achieve this is dependent on the density of the involved tissue. However, this does not appear to actually correspond to *in situ* physiology, and smaller volumes can provide good imaging results[56].

Comparison of intradermal vs. peri-tumoral injection technique has been performed. Sentinel lymph node identification was improved by using a dermal injection technique (98% identification) compared to subdermal (95%) or peri-tumoral (90%) techniques, but there were similar false negative rates among the three techniques[68]. Blue dye identified significantly fewer sentinel nodes (87%). The relative degree of radioactivity of the nodes was five to seven times higher with the dermal injection technique. Transit time was also much faster with the intradermal technique and a biopsy can be performed within 30 to 60 minutes of dermal injection compared to the 2 to 3 hour delay recommended for peri-tumoral injection technique. Much smaller volumes are used for dermal injection (0.5 mL) vs. peri-tumoral (6 mL) but with similar doses of radionuclide (0.5 mCi). A discussion point referencing unpublished data mentioned that different tracers have been used for injection in the skin and in the tumor and have found that

different nodes were identified, with each potentially disputing the argument that the surrounding lymphatics of normal tissue represent the draining pathways of the tumor. It was also noted in this study that there were occasional nodes that are completely infiltrated with tumor and would not take up blue dye or radiocolloid[68]. The overreaching goals of sentinel node biopsy are to identify the nodes correctly in at least 90% of patients and have a false negative rate < 5%.

The effect of previous surgery on the tumor site and associated lymphatics has been investigated by a number of authors. Failure rate of sentinel node detection was significantly higher in patients with previous excisional biopsy (36%) vs. those with the primary tumor in place (4%)[11]. Similar findings were noted by Estourgie[51] with a discrepancy in drainage pattern observed in 68% of patients and a reproducibility of only 32% when lymphoscintigraphy was performed prior to and 2 weeks to 6 months after surgical resection of the primary tumor. This study involved intra-tumoral or intra-cavitary injection of technetium. Reproducibility of the drainage pattern was also different based on the lymphatic basin involved; reproducibility of axillary drainage patterns was 56% vs. that of mammary chain drainage of 23%. It was also noted that after intra-cavitary injection following excisional biopsy, the tracer tended not to disperse into the lymphatics[51]. Krag[46] also found that previous excisional biopsy reduced the likelihood of successfully identifying a sentinel node. Another study, however, found conflicting results[69] that sentinel lymph node dissection has a high success rate in breast cancer patients regardless of previous tumor biopsy method (stereotactic core biopsy, fine needle aspirate or excisional biopsy). In addition, the interval from biopsy to node dissection, tumor size, tissue excision volume, and tumor location were noted not to have an effect on the success rate of sentinel node biopsy. It was postulated that the difference in results may be related to injection technique (into biopsy bed compared to peri-tumoral injection), and the authors suggest that peri-

tumoral injection may continue to provide adequate identification even after excisional biopsy. Wong[67] and Martin[39] supported these results and found no difference in success and accuracy of sentinel node biopsy with relation to identification and false negative rates when core needle or excisional biopsy had been performed, or when the excisional biopsy involved lumpectomy or mastectomy. They concluded that the concern that excisional biopsy of a breast tumor causes disruption of tumor lymphatics and incorrect lymphoscintigraphy results was unjustified. In addition, although there was previous thought that when clinically positive nodes are present, the tracer will be redirected to "false" sentinel nodes due to tumor obstruction of lymphatic drainage, there is no evidence to substantiate this theory[59].

Primary tumor and lymph node characteristics as well as surgeon experience have also been suggested to impact the accuracy of lymphoscintigraphy. Tumor type, preparation of the radiopharmaceutical, injection technique and imaging technique can influence the efficacy of lymphoscintigraphy[23]. When the volume of injection is increased, the number of identified nodes may increase by up to 15%[15]. Older people have fat replacement of lymph nodes, which decreases the node's ability to retain the radioactive colloid, but increasing the amount of tracer and diluent can offset this limitation[46]. It is unknown if a similar phenomenon may be seen in veterinary patients. It has also been suggested that there is an increased risk of false negative results associated with primary tumor size < 2.5 cm, tumor location, removal of a single sentinel lymph node, minimal surgeon experience, and use of immunohistochemistry for analysis. Of those factors, surgeon experience had the largest impact on the false negative rate; experience with < 4 cases was associated with a 12% rate, while > 4 cases had a rate of 7%[39]. Chagpar[25] reported a similar surgeon learning curve of 10 cases, while other authors have found a much higher learning curve associated with the use of radioguided biopsy. Mariani[8] identified a

learning curve to reach 97% successful identification of 40 to 60 procedures and Rubio[59] identified a 60 to 80 procedure learning curve while Giuliano[37] commented that the first 87 cases were associated with a 58% successful identification rate while the second 87 cases were associated with a 72% success rate.

Mariani[8] also recommend not to perform sentinel node imaging if nodes are palpably enlarged, if the primary tumor is > 4 cm in diameter or multicentric, or if there has been previous regional dissection. Morita[15] agreed with this last statement, and noted that injection into a biopsy site (i.e. scar tissue) results in failure of imaging as the tracer does not move from the injection site. It has also been postulated that blue dye or radionuclide lymphoscintigraphy may be impeded by adjuvant chemotherapy or radiation therapy as these may interrupt or alter lymphatic drainage[38]. Other authors have found that sentinel node biopsy remains feasible and reliable even in a previously-treated lymphatic basin[40]. Chagpar[25] also found palpable tumors and the use of a dermal injection technique to be associated with identification of more than 4 sentinel lymph nodes using a combination of lymphoscintigraphy and blue dye. The drawback to this finding relates to the fact that removal of more nodes does not confer survival benefit, but is associated with increased patient morbidity including increased pain, numbness, decreased range of motion of the shoulder (with axillary dissection) and arm swelling as well as increased operative time. When metastatic disease is present, in at least 97% of patients, it is identified within the first 3 lymph nodes removed, even when more than 3 nodes are resected[25].

Patients with elective neck dissection have better regional control and survival outcome than patients with clinically negative necks that were not treated until clinically evident disease was present; however, there is also substantial morbidity associated with elective neck dissections. To perform evaluation of nodes while minimizing associated morbidity, sentinel node biopsy is

recommended, and this has become standard of care practice for many human neoplasms with successful identification rates up to 94 to 97%[22, 39, 40, 70]. Sentinel node biopsy has proven useful for malignant melanoma, breast cancer, penile cancer, vulvar cancer, Merkel cell carcinoma and thyroid cancer. It is easy and quick to perform lymphadenectomy once the target has been identified with a gamma probe[50]. Some studies report complete success in the ability to localize and remove sentinel lymph nodes when combining pre-operative lymphoscintigraphy and intra-operative gamma probe usage[35].

Sentinel lymph node biopsy with the use of blue dye alone has been suggested to be an inaccurate reflection of pathology of the neck[38]; however, up to a 97% success rate of identification has been reported when radioactivity and blue dye techniques are combined[22]. Overall, sentinel node biopsy has been associated with a negative predictive value of 89% to 96% in node negative cases and 74% in node positive cases[40]. Although radiotracers and blue dye have been used together, and it has been noted that it is possible for the only metastatic node to stain blue but have minimal radioactivity[68]. Many studies have found that the sensitivity of lymphoscintigraphy is much higher than that of dye, and that the incidence of identifying a sentinel node with blue dye that would not have otherwise been located is low enough to be clinically insignificant. Tanis[54] reported that sentinel nodes could be visualized pre-operatively in 93% of patients and identified intra-operatively in 97%; 5% of those nodes were identified based only on blue discoloration, whereas 37% of the nodes had no dye uptake and were only identified by radioactivity. Glass[22] found that 20% of nodes that had metastasis were radioactive but did not stain blue. Dequanter[71] found that no sentinel nodes were only blue, 5/17 were radioactive but not blue and 12 were both; while all metastatic nodes were radioactive and blue. Another study[47] noted that 79% of radionuclide identified sentinel nodes were also seen to stain

blue. At the same time, only 0.5% of blue nodes were not radioactive, whereas 5% of radioactive nodes did not contain blue dye. All positive nodes had radioactivity as well as blue dye. This report again referred to the concept that the blue dye technique is technically challenging and often requires extensive dissection[47].

Successful resection of radiolabeled lymph nodes occurred in 96% of patients when a gamma probe was used for sentinel lymph node identification in a clinically negative neck[70]. Success rate of identification with blue dye was 75% and with lymphoscintigraphy alone was 91%. Drawbacks to the blue dye technique include that it is difficult to determine the location of blue nodes prior to making a skin incision, resulting in the need for additional dissection, that there is a high learning curve to achieve good success rates, that it is difficult to verify removal of all identified nodes, and that it causes staining of local tissues that can impede further dissection[70].

Stoeckli[72] evaluated sentinel node diagnostic techniques for squamous cell carcinoma of the head. Localization of the sentinel lymph node by lymphoscintigraphy was possible in 18/19 patients; the additional use of a gamma probe made it possible in all 19. There was no evidence of skip metastasis but occult metastasis was noted in 6/19 cases. The ability to identify sentinel lymph nodes with lymphoscintigraphy took an average of 9 minutes in this study. Methylene blue was injected around the tumor in the first 7 patients in this study; it was only possible to detect a blue node in 2 of these and there was notably increased difficulty associated with primary tumor resection caused by the presence of the blue dye. Use of dye was abandoned after these 7 cases. Conclusions relate to poor experience with use of blue dye in agreement with other studies as compared to easy and efficient use of the gamma probe[72].

Pre-operative lymphoscintigraphy and intra-operative gamma probe use after injection with ^{99m}Tc–colloidal albumin showed that sentinel lymph node identification is possible in 89% of

patients[11]. The negative predictive value of the biopsied sentinel nodes was 98% in this study and there was a 10% failure rate in identifying any nodal targeting. The number of sentinel nodes identified was not affected by the size or location of the tumor. Gamma probe guided search was concluded to be very efficient and easy to learn compared to the more technically demanding and tedious wide dissection required for blue dye tracing[11].

In squamous cell carcinoma of the head and neck, use of peri-tumoral quadrant injection with pre-operative lymphoscintigraphy and intra-operative gamma probe has shown identification of sentinel lymph nodes in all cases[71]. 30% of nodes were identified as having occult metastasis that would not have been detected without lymph node dissection. A false negative was only detected in 1 case[71]. The technique has also been described for breast cancer patients[46]. A 93% identification of sentinel nodes was obtained with the use of a gamma probe only. When followed with full axillary dissection, it was determined that there was 97% predictive accuracy for metastatic status. There was also a significant correlation between the number of pathologically positive sentinel lymph nodes and the number of positive non-sentinel nodes. With the blue dye technique, the only way to evaluate for additional sentinel nodes is via additional random dissection and the end point is unclear, as compared to use of a gamma probe where the wound bed can be evaluated for residual radioactivity[46].

3f. Safety concerns

The blue dye often used in conjunction with lymphoscintigraphy has been reported to have potential side effects in the human population, however these have not been reported in veterinary patients[7]. A reported 1 to 3% risk of allergic reactions exists related to isosulfan blue

dyes in people with a smaller potential risk of skin reactions including dermal necrosis with intradermal methylene blue.

As lymphoscintigraphy involves the injection of a radioactive material, and subsequent exposure to multiple personnel, the inherent safety associated with the procedure is of concern. Although the amount of radioisotope injected is of minimal concern when considered as a single exposure to the patient, the cumulative effect of multiple exposures to hospital personnel may be more problematic. A number of studies have been performed to investigate potential exposure.

Brenner[55] performed calculations using dosimetry readings based on standard injection dose of 30 MBq (approximately 0.8 mCi) in 1 mL of ^{99m}Tc-nanocolloid. Measurements taken 3 to 5 hours post-injection gave the following results: 84 μGy/h at 2.5 cm, 3.6 μGy/h at 30 cm, 0.9 μGy/h at 1 m, and 0.4 μGy/h at 150 cm in the operating room and 44 μGy/h at 2.5 cm and 1.66 μGy/h at 30 cm in the pathology labs. Calculations based on an estimate of 250 operations per year with a mean exposure time of 30 minutes for the surgical team and 10 minutes for the pathology team gave resultant total exposure doses as follows: finger dose: 10.5 mGy for the surgeon and 5.5 mGy for the pathologist; whole body doses: 0.45 mSv, 0.11 mSv, 0.05 mSv and 0.21 mSv for the surgeon, operating room nurse, anesthetist and pathologist, respectively. Pathologist exposure would be expected to be even lower in veterinary patients, as intra-operative frozen section samples are not routinely evaluated, and as such, additional decay time occurs during sample fixation before processing. Radiation exposure to all personnel is of low enough levels that classification as radiation-exposed workers is not considered necessary. Five hundred milliSieverts is the accepted exposure dose in the US for occupational exposure; 50 mSv finger dose and 5 mSv whole body dose are the accepted exposure doses for non-occupational

exposure in Germany, where this study was performed and safety levels were confirmed even with these more restrictive guidelines.

Additional calculations and investigation[15] have found similar safety results. Anyone potentially exposed to more than 500 mrem per year would be classified as a radiation worker whose whole body dose limit is 5000 mrem and 50,000 mrem to the hands. With a 0.135-0.27 mCi injection dose, surgeon hand exposure over 100 operations was 450 mrem. Materials (instruments and disposables) used during a procedure have been found to have levels near background activity after a sentinel node dissection procedure. Monitoring levels for staff in recovery, operating room and pathology (other than the surgeon and pathologist) have been found to be insignificant. Rubio[59] determined that a surgeon's torso receives 1.33 mrem/h, the pathologist's torso receives 0.34 mrem/h and the surgical nurse receives 0.15 mrem/h. Surgeon's hands would be within limits until 2,190 hours of procedure time per year. The reported safety limits for this study were 5000 mrem total body dose and 75,000 mrem finger dose.

Even when a higher dose of radiation is given to patients, Treseler[26] found that radiation safety monitoring of pathology workstations have not shown levels to be above background. They recommend tissues be held for 5 half-lives (approx. 30 hours) before further processing, however at other institutions, samples undergo same day processing and after extensive monitoring this protocol has been deemed safe. Expected radiation exposure to a surgeon over a 3 hour procedure on a patient given 20 mCi ^{99m}Tc-sestamibi is approximately 1 mrem.

Based on the dose of radioisotope used in a recent clinical veterinary study (0.125 mCi), a non-pregnant surgeon would have to perform 500 to 600 intra-operative lymphoscintigraphy procedures to reach 10% of the annual radiation exposure limit based on US guidelines[7]. Similar safety was found with tissues to be evaluated with histopathology, although for added safety it is

recommended that tissues were isolated overnight and were typically equivalent to background activity levels by the next day.

4. Alternative methods of sentinel lymph node identification

Although the most commonly reported, and current standard of care for many human cancers is the use of lymphoscintigraphy with or without the use of a vital blue dye, other diagnostics have also been evaluated for detection of sentinel nodes. Some techniques, including radioguided surgery using IV injection of tumor-seeking agents (e.g. somatostatin analogs) have revealed limited sensitivity that prevented their general acceptance[54]. Other diagnostic approaches, however, have shown more promise.

The use of contrast CT for sentinel lymph node identification has been evaluated. Iopamidol was injected submucosally in dogs to evaluate esophageal and gastric lymphatic drainage patterns. CT was performed prior to and over a 10 minute period after injection[73]. Contrast was noted to extend to sentinel nodes within 5 minutes of injection. Lymph node enhancement was considered positive if the attenuation on post contrast imaging was increased by more than 30 Hu. Leakage of contrast into the lumen of the esophagus or stomach was noted occasionally, however, it was easily distinguished from the lymphatic pathways. Marked individual variation in drainage pattern was noted including both cranial and caudal lymphatic flow. No noticeable enhancement of structures other than lymphatic pathways were observed. This diagnostic tool was noted to have 100% sensitivity and accuracy and a 0% false negative rate. The only limitation reported was a difficulty detecting nodes located close to the injection site because of shine through effect[73].

Although Stoeckli[40] commented that positron emission tomography/computed tomography (PET/CT) has poor sensitivity for micrometastases, a study evaluating its use in canine prostatic drainage was more favorable[74]. Transrectal intra-prostatic injection of approximately 10 MBq per lobe of Ga-68 labeled tilmanocept was performed and pelvic lymph nodes were imaged every 20 minutes with PET/CT. Injections were divided into a superficial and deep injection for each lobe. Imaging was followed by prostatectomy and extended lymphadenectomy. *Ex vivo* radioactivity was recorded with a gamma probe and percent injected dose was calculated. Sentinel nodes were defined as containing more than 10% of the maximum percent of injected dose. PET/CT identified a mean of 4.25 lymph nodes per dog, with a mean number of dissected nodes being 4. Of the excised nodes, only 29% were located in the expected external iliac and obturator distribution. Sensitivity of PET/CT was 93% with a high signal to background activity attained within 70 minutes of injection; 85% of nodes were identified by 20 minutes. Prevalence of lymphatic metastasis in patients with negative cross sectional imaging ranged from 1.1 to 26%. Thirty-one percent of positive nodes were reported to block uptake of technetium by neoplastic obstruction of the lymphatic vessels[74].

Near infrared emitting polymer nanogels (NIR-PNG) have also been evaluated[75]. Intradermal injection of nanoprobes into the thigh of a pig permitted real time imaging of the lymphatic flow towards the sentinel lymph node. Position of the node was identified within 1 minute with aid of the near-infra red fluorescence images. NIR-PNG has enhanced photostability and retention time. Agents with hydrodynamic diameters of 10 to 50 nm (optimal size) are taken up rapidly into the lymphatic system, those smaller than 5 nm will partition into the bloodstream, 5 to 10 nm will pass through the sentinel node to second tier nodes, whereas particles larger than 300 nm rarely leave the site of injection. This study utilized a polymer with a 30 nm hydrodynamic

diameter. NIR-PNG does not have cytotoxic effects or affect cytokine levels. Lymph node retention time is at least 48 hours. On reaching the lymph node, particles were primarily phagocytosed by macrophages and dendritic cells. The near infrared (NIR) spectrum is 700 to 1000 nm and is useful for medical imaging because of low autofluorescence background, low optical scatter and the possibility of significant imaging depths. Other NIR fluorescent dyes investigated include ondocyanine green and heptamethine cyanine, but these were found to pass readily through the sentinel lymph node rather than being retained. Attempts to polymerize with biodegradable polymers increases the particle size too much and these do not leave the primary injection site.

The most frequently investigated alternative diagnostic is the use of contrast assisted ultrasound. In one study, contrast medium was injected subcutaneously in the distal extremities of dogs with comparison between submicron, near micron and conventional sized ultrasound contrast microbubble suspensions. Popliteal and superficial cervical lymph nodes were evaluated with power Doppler intermittently over 120 minutes. Contrast enhancement occurred in 85% of sentinel nodes overall and in 94% of nodes when submicron or near-micron diameter bubble formulations were used[9]. A further study investigated the effects of various contrast injection techniques by comparing subcutaneous, submucosal or parenchymal injections of sonographic contrast agent for detection of sentinel lymph nodes. Gray scale pulse inversion harmonic imaging was used to evaluate lymphatic channels and nodes. Nodes were identified regardless of injection technique; however, it was noted that a change in injection site by as little as 1 cm could result in drainage to a different sentinel node. It was determined that the contrast was retained within the sentinel nodes, and did not proceed to second echelon nodes. It was possible to trace the contrast from the injection site to the lymph node and nodes as small as 3 mm were

identifiable. Complete enhancement was typically noted within 15 minutes. Massaging the injection site increased the rate of contrast movement, and also increased the reflectivity of the affected nodes. Injection in a single location was seen to occasionally drain to multiple nodes. After ultrasound analysis, blue dye injection and dissection of lymph nodes were performed and it was determined that there was good agreement between the lymphosonography and surgical dissection. Some limitations of ultrasound evaluation were identified. Specifically, that if the lymphatics drained deeper to the pelvis or into the thorax, air filled lung or bowel could obscure the imaging. Scanning electron microscopy was performed on a few cases and confirmed that the microbubbles were contained within macrophages, explaining the retention within the sentinel lymph nodes[76]. Sentinel lymph nodes of canine mammary glands were also identified with contrast enhanced ultrasound using octafluoropropane-filled lipid microspheres[77]. Ultrasound was used to follow the contrast through the lymphatics to the sentinel node. It was found that the contrast agent was easily visualized; eight nodes from 3 dogs were identified and successfully biopsied percutaneously. Nodes were visualized 4 to 5 minutes after injection. 0.2 mL was reported from a pilot study to provide the best contrast enhancement with the smallest volume. Advantages reported for contrast enhanced ultrasound include direct visualization of the node, increased specificity for first order lymph nodes and lack of ionizing radiation[77].

Contrast enhanced ultrasound has also been investigated in clinical veterinary patients involving canine head and neck tumors[78]. Peri-tumoral injection of microbubbles was performed and regional lymph nodes were imaged up to 20 minutes after injection. Comparative lymphoscintigraphy was then performed using a subcutaneous or submucosal peri-tumoral injection technique. Nodes were considered positive with ultrasound if they revealed a previously described color flare within the node parenchyma and adjacent lymphatic vessels.

Eighty percent of dogs had sentinel nodes identified with ultrasound and these corresponded to nodes identified with lymphoscintigraphy. Multiple sentinel nodes were identified in 20% of dogs[78].

5. Use of lymphoscintigraphy in veterinary medicine

Although most previous studies evaluating this technology in animals were experimental research performed in healthy animals, some studies have been performed evaluating this approach to clinically affected animals. Norris[79] evaluated the use of lymphoscintigraphy for cases of canine mammary neoplasia. Interstitial injection of $^{99m}TcSb_2S_3$ colloid was used in 4 clinically normal dogs and 13 dogs with mammary neoplasia and it was determined to be possible to effectively evaluate individual variants of the lymphatic system. This technique correctly identified 100% of metastatic lymph nodes and 82% of non-metastatic nodes with an overall accuracy of 87%. In line with earlier theories depicting lymph node status, this study reported the ability to reflect physiologic processes such as lymphatic transport, filtration and reticuloendothelial function and described decreased radioactivity in a node, failure to visualize a node, or deviation of normal lymphatic flow as being indicative of metastasis. Considerable individual variability was found in the number of sentinel nodes identified (ranging from 1 to 3) and also noted occasional bilateral involvement. A sex difference in lymphatic drainage was also reported in this study, noting that inguinal nodes drain the caudal mammae in female dogs, but only the penis, scrotum and preputial skin in males. Aberrant nodes in the region of the caudal mammary glands, but not in line with the inguinal nodes were also identified in this report, consistent with previously described transit or interval nodes[16, 17.]

More recently, lymphoscintigraphy was used in clinical patients with mast cell disease, and the utility of this diagnostic evaluation for most appropriately staging disease was emphasized[7]. Spontaneously occurring or incompletely excised mast cell tumors were investigated using regional peri-tumoral injection lymphoscintigraphy, gamma probe and blue dye mapping. Forty-two percent of dogs were found to have a sentinel node that was not the closest proximity node. Pre-operative lymphoscintigraphy identified sentinel lymph nodes in 18/19 dogs; use of an intra-operative gamma probe resulted in identification of sentinel nodes in all 19 dogs. It was noted that intra-operative lymphoscintigraphy was particularly helpful for dogs with non-palpable or small lymph nodes. Because of this staging diagnostic, treatment recommendations were altered in 42% of dogs. This emphasizes the significantly improved patient care that is possible with adoption of technologies to more reliably stage neoplastic disease, however additional studies are still required to prove the utility of this tool in a wider variety of veterinary patients.

Introduction

Oral cancer is the fourth most common cancer in dogs[80]. The most common types of neoplasia noted in the canine oral cavity are melanoma, squamous cell carcinoma and fibrosarcoma[27]. Each of these displays varying biologic behavior regarding invasiveness, tendency to metastasize and response to various treatment modalities. Oral and maxillofacial neoplasms previously associated with the highest propensity for lymphatic metastasis include squamous cell carcinoma, melanoma, fibrosarcoma and salivary carcinoma[27]. The metastatic rate of malignant melanoma is reported to be up to 80%, non-tonsillar squamous cell carcinoma is approximately 20% and fibrosarcoma has a likewise lower rate of approximately 30%[80]. Like any cancer, the presence of metastatic disease typically confers a worse prognosis, and usually results in the recommendation for adjunctive systemic therapy in addition to methods of local control. Metastasis occurs either by lymphatic drainage to lymph nodes, or hematogenously to other organs, such as lungs and liver. The World Health Organization TNM classification scheme highlights the importance of three main factors when considering the aggressiveness of the neoplastic disease: the primary tumor itself (T) with regards to size and local invasiveness, the status of regional lymph nodes (N) with regards to mobility and presence of metastasis, and distant metastasis (M) with regards to presence or absence thereof[36]. To evaluate these factors, various diagnostic techniques are undertaken. For evaluation of the lymph node, due to accessibility, the mandibular node is often the only node examined on initial diagnostics, including palpation and fine needle aspirate for cytology.

The first line of therapy for oral neoplasia is typically local treatment with surgical resection with appropriate margins, with or without secondary radiation therapy. The use of adjuvant systemic therapies, such as chemotherapy, or the melanoma vaccine, is dictated by the presence

or absence, or likelihood thereof, of metastatic disease. Often, nodal metastasis can result in palpable differences to the nodes themselves with features such as enlargement, decreased mobility or change in texture. However, these findings can also signify reactive nodes, giving false impression of metastasis on palpation alone, or can be palpably normal while containing micrometastases[30]. Because of this, it is generally advisable to evaluate regional lymph nodes in all cases of neoplasia with some form of invasive diagnostic, such as aspirate or biopsy. Central to this concept, however, is to ensure that the diagnostics are performed on the appropriate lymph node.

The canine head contains three primary lymphocentrums; the mandibular, parotid and medial retropharyngeal. The drainage patterns of these lymphatic centers, as well as descriptions of nodes contained within each basin and the description of additional nodes outside of these main centers, have been noted to have wide individual variation in the healthy population. In addition to this variation, a neoplastic process adds additional complexity in that normal lymphatic routes may become obstructed and new lymphatic pathways may develop through oncogenic lymphangiogenesis. With this complexity, it is evident why determination of the correct draining lymph node for accurate staging of neoplastic disease is crucial.

Purposes/hypotheses

1. To improve the accuracy of identifying the clinically relevant sentinel lymph node in cases of canine oral neoplasms.

1a.The hypothesis of this study is that by investigating all potential draining nodes, the correct sentinel lymph node will be detected more frequently using lymphoscintigraphy than typical staging tools (i.e. mandibular lymph node aspirate) alone.

2. To investigate the tumoral drainage patterns when intra-tumoral radionuclide injection is used vs. quadrant peri-tumoral injection.

2a. The hypothesis of this study is that for cases of successful lymphatic drainage detection the flow pattern will be similar regardless of the pattern of radionuclide injection, however failure to successfully identify lymphatic drainage will occur more commonly with intra-tumoral injection compared to peri-tumoral quadrant injection.

3. To investigate the correlation between pre-operative cytologic mandibular lymph node diagnosis with histopathologic results of identified sentinel lymph nodes with regards to metastatic disease.

3a. The hypothesis of this study is that lymph node metastasis will be identified more frequently based on histopathology of the identified sentinel lymph node compared to pre-operative cytology of mandibular lymph node aspirates.

4. To investigate the radiation safety associated with both the animal and dissected tissues following lymphoscintigraphy and surgical resection.

4a. The hypothesis of this study is that given the extremely low levels of radioisotope used in the injections, the animal as a whole will be within limits of institutional safety release criteria immediately after the lymphoscintigraphy procedure, and that tissue samples obtained during surgery, if not immediately within safety limits, will be within those limits by the morning after surgery.

Materials and methods

Inclusion criteria for this study were client owned dogs presenting to Auburn University Veterinary Teaching Hospital (AU VTH) for a naturally-occurring primary malignant neoplasm of the oral cavity, with a plan for surgical excision. Exclusion criteria included patients with any previous oral surgery that removed gross disease or involved significant maxillofacial/mandibular reconstruction (e.g. fracture repair) but not including dental prophylaxis, uncomplicated tooth extraction, or incisional tumor biopsy. Additional exclusion criteria include dogs that are pregnant, lactating, or unable to undergo general anesthesia. The number of desired patients for a 95% confidence level with a minimum detectability of 25% difference for identification of sentinel node based on lymphoscintigraphy vs. current standard of care of evaluation of mandibular nodes resulted in a calculated minimum sample size of 16. An additional 4 dogs were included to recruitment numbers to offset potential non-normal distribution or other statistical effects.

This study was approved by the Auburn University Institutional Animal Care and Use Committee. Patients were admitted to hospital following informed owner consent to enroll in the study. Standard staging procedures (blood work, urinalysis, thoracic radiographs, fine needle aspirate/cytology of the mass, +/- abdominal ultrasound, +/- head CT for surgical planning) were performed as indicated on an individual case basis and based on previous diagnostics performed prior to referral.

On admission, the patient was assigned by random number generator to group A or B (A= intra-tumoral radionuclide injection on day 1 and peri-tumoral quadrant injection on day 2; B= peri-tumoral quadrant injection on day 1 and intra-tumoral injection on day 2). On day 1, the dog was placed under heavy sedation according to routine protocols. 0.5 mCi technetium (^{99m}Tc)-

sulfur colloid in < 1 mL volume was injected in the predetermined location. A single injection was made for the intra-tumoral injection site. For peri-tumoral injection, the total dose was divided and injected into 3 to 4 sites 1 to 2 mm around the periphery of the tumor. A scintillation camera [General Electric 400 Maxi Cam with scintillation crystal dimensions of 37.5 cm X 51 cm with Mirage [PCI] acquisition application software (version 5.715f7f) developed by Segami] was used to image lymphatic drainage and sentinel node(s) at time 0 and every 5 minutes until 30 minutes of imaging were achieved. If nodes were not successfully identified at 30 minutes, the scan was repeated at 60 minutes then every hour until nodes were identified, to a maximum time of 3 hours. At 3 hours time, if nodes were still not identified, the study was discontinued and considered unsuccessful. Location and number of sentinel node(s) were recorded as well as duration required for identification. At the completion of scanning, radiation emissions from the patient were measured by a Geiger counter at 1 meter distance from the injection site (measured by meter stick) and repeated every 15 minutes for the first hour, then hourly for 6 hours then every12 hours until patient release criteria (< 0.5 mR/h at 1 m) was achieved. Time to safe release levels was recorded. On recovery from sedation, the patient was hospitalized overnight and a radiation shield was placed in front of the cage if indicated.

Within 72 hours (Day 2), the patient was again placed under routine heavy sedation for repeated lymphoscintigraphy using the alternate injection method from that performed on the first day of imaging. Apart from the injection site, the procedure was similar as for day 1, except that a single image was taken with the gamma camera prior to injection to assess the patient for residual radiation emissions. Following the second lymphoscintigraphy procedure, and confirmation of safe working radiation levels, the patient was placed under general anesthesia using routine protocols. The specific anesthetic protocol was at the judgement of the

anesthesiologist on duty. Fine needle aspiration for cytology of the mandibular lymph node was performed a single time following both lymphoscintigraphy procedures to minimize potential effect on the image acquisition. Aspirates were evaluated by a single clinical pathologist (PC) for consistency. Following aspiration of the mandibular lymph node, the patient was prepared for surgery.

Lymph node(s) identified as sentinel on pre-operative lymphoscintigraphy were dissected. Surgical technique varied depending on the node(s) identified with the main goal of creating as little surgical trauma as possible; either a single approach over a single identified node/node basin, or using the previously described approach to access all 3 main lymphocenters of the head[29]. Once the lymph basin was accessed, lymphadenectomy was performed and the area evaluated digitally for any palpable evidence of additional lymph nodes. All palpable lymph nodes in the identified basin were removed. The location and number of node(s) removed was recorded. Following lymphadenectomy, the primary tumor was resected according to appropriate oncologic surgical techniques. A Geiger counter was used to monitor for residual radiation emission from the patient, and a radiation shield was placed in front of the patient cage during recovery, if indicated. The patient was recovered from anesthesia and was monitored in hospital until deemed suitable for discharge to the owner. Postoperative care was dictated on an individual patient need basis.

The primary tumor margins were appropriately inked, and all removed tissues were placed in 10% buffered formalin. The tissues were assessed for radioactive emissions at 1 meter distance to determine radioactive safety. If they measured > 0.2 mR/h, tissues were stored in isolation overnight and reassessed the following morning and every 12 hours until sufficient decay time had occurred to result in safe samples, at which time the samples were submitted for

histopathology. Time to safety of tissue samples was recorded. For consistency, all tissues were evaluated by a single pathologist (JK). Tumors were assessed according to standard histopathologic technique for definitive diagnosis, tumor grade and surgical margins. Lymph nodes were evaluated for evidence of metastasis and compared to initial cytology from pre-operative fine needle aspirates. For histopathologic analysis of lymph nodes, they were sectioned according to the following methods: for lymph nodes < 1 cm, a single longitudinal section through the node was made; for nodes larger than 1 cm in any dimension, multiple parallel cuts perpendicular to the long plane of the node were made at 5 mm intervals. An assessment of the degree of artifact produced by previous aspiration (in the mandibular nodes) and ^{99m}Tc-sulfur colloid injections (in the primary tumor) were made based on the following criteria: 0 = no impact on diagnostic integrity of the tissue, 1 = > 1 and < 50% of the tissue is disrupted in examined sections, 2 = 50 to 75% of the tissue is disrupted in examined sections, 3 = tissue is disrupted to the point of being non-diagnostic.

Descriptive statistics were performed regarding frequency of which specific node basin was sentinel for different locations, sizes and types of tumors as well as number of sentinel nodes per animal for different locations, sizes and types of tumors. Comparison of histopathology with pre-operative cytology (evidence of metastasis vs. not) was evaluated using chi square analysis. For comparison of sentinel node lymphoscintigraphy findings following intra-tumoral vs. peri-tumoral injection (similar or different drainage pathways; speed of scan results; if different pathways are present, does one technique correspond more consistently with histopathologic evidence of malignancy), Chi square analysis was used for any appropriate data gathered between the two treatment groups. Descriptive analysis of radiation safety regarding time to

meeting state safety guidelines for both patient and tissue samples was performed. For any statistical analysis other than descriptive, significance was set at $p < 0.05$.

Results

Due to poor case accrual, only 2 cases were obtained over the 15-month data collection period. See table 1 for summary of results from cases.

CASE 1

A 12-year-old male neutered medium size mix breed dog presented with an approximately 3 cm diameter non pigmented mass located on the right maxilla extending from the canine to the 3rd premolar tooth. The mass was initially noted on physical examination by the primary care veterinarian, and the owners report that the mass had grown rapidly over the past month, although it did not appear to otherwise affect the dog. The remainder of the physical exam was unremarkable except for a palpably enlarged right mandibular lymph node. Initial staging revealed a radiographically normal thorax, mild liver and renal changes on abdominal ultrasound, and head CT revealing bony destruction and local invasion of the nasal cavity. Ultrasound guided aspirates of the liver revealed regenerative changes and glycogen degeneration; aspirates of the kidney revealed normal renal tubular epithelial cells. Lymphoscintigraphy was performed as outlined in the materials and methods section. Peri-tumoral injection of a total of 0.5 mCi of technetium in 0.35 mL was performed on day 1. The injection was divided into 3 quadrants (rostral, caudal and dorsal) due to extent of the mass to the ventral level of the maxillary mucosa. Radioactive markers were placed adjacent to the dog's head to identify the location of the mandibular and medial retropharyngeal lymph nodes, and

residual isotope in the syringe was used to trace the outline of the head and neck. Lymphatic flow of the radioisotope was noted immediately on gamma camera images and was followed to the first order lymph node, identified as the mandibular node (Figures 1 and 2). After scanning, a Geiger counter was used to assess the patient for continued radiation emission. Emission levels were well below standard release criteria (0.2 mR/h) on initial evaluation, and radiation isolation housing was deemed unnecessary. The second lymphoscintigraphy procedure (intra-tumoral injection) was not performed until 2 days after the first due to scheduling conflicts. Prior to injection, a scan was performed to evaluate for residual radioisotope signal. A minor amount of signal was detected, however the counts were sufficiently low that they would not interfere with the planned procedure (approximately 100 counts vs. > 20,000 counts with fresh injection; Figure 3). Intra-tumoral technetium (0.5 mCi in 0.35 mL) injection was performed and images were obtained at previously described intervals. Images obtained immediately after injection revealed no evidence of lymphatic drainage (Figures 4 and 5). Initial lymphatic movement was not detected until hour 1 post-injection, at which time initial lymphatic drainage was appreciated (Figures 6 and 7). Complete drainage pathway to the sentinel lymph node was not identified until the hour 3 post-injection images, at which time it was confirmed that the mandibular node was the first order draining lymph node (Figures 8 and 9). Of note, although the mandibular basin was confirmed to be the drainage path from the tumor, on retrospective evaluation of the images, the lymph node identified within the basin was different than that identified on the first day, being located slightly medial to the first identified node. A Geiger counter assessment determined that the patient was immediately safe to proceed to surgery (0.15 mR/h). A fine needle aspirate of the mandibular lymph node was performed: cytologic evaluation was consistent with a reactive lymph node with no evidence of neoplastic cells**.** A partial rostral right

maxillectomy and right mandibular lymphadenectomy were performed. On dissection and palpation, only a single mandibular node was identified and removed. The patient recovered from anesthesia and the procedure without incident. The excised tissues were assessed with the Geiger counter, and radiation emission levels were below required limits. Tissues were deemed safe to submit for processing and analysis. Histopathologic diagnosis revealed a malignant giant cell tumor of bone with no evidence of neoplastic cells within the examined lymph node sections. Neoplastic cells were noted to extend to the surgical margins. Lymph nodes were graded as histologic grade 1 for impact from fine needle aspirate with approximately 30% of tissue involvement in the most affected section. The tumor was evaluated as histologic grade 1 effect of radionuclide injection with approximately 50% tissue involvement in the most affected section. In both the lymph node and tumor, tissue disruption by aspiration or injection was not noted to interfere with the overall ability to make a histologic diagnosis. Due to the incomplete surgical excision, local treatment was continued with radiation therapy (definitive protocol; 18 fractions of 3 Gy) as well as systemic chemotherapy (doxorubicin 30 mg/m^2 every 3 weeks for 5 doses) starting approximately 3 weeks postoperatively. These treatments were performed to completion with only mild discomfort and mucositis noted secondary to the radiation therapy and chemotherapy-associated gastrointestinal upset requiring hospitalized supportive care. The patient continues to do well at home and stage negative for evidence of local recurrence or metastasis at the most recent follow up, 10 months postoperatively.

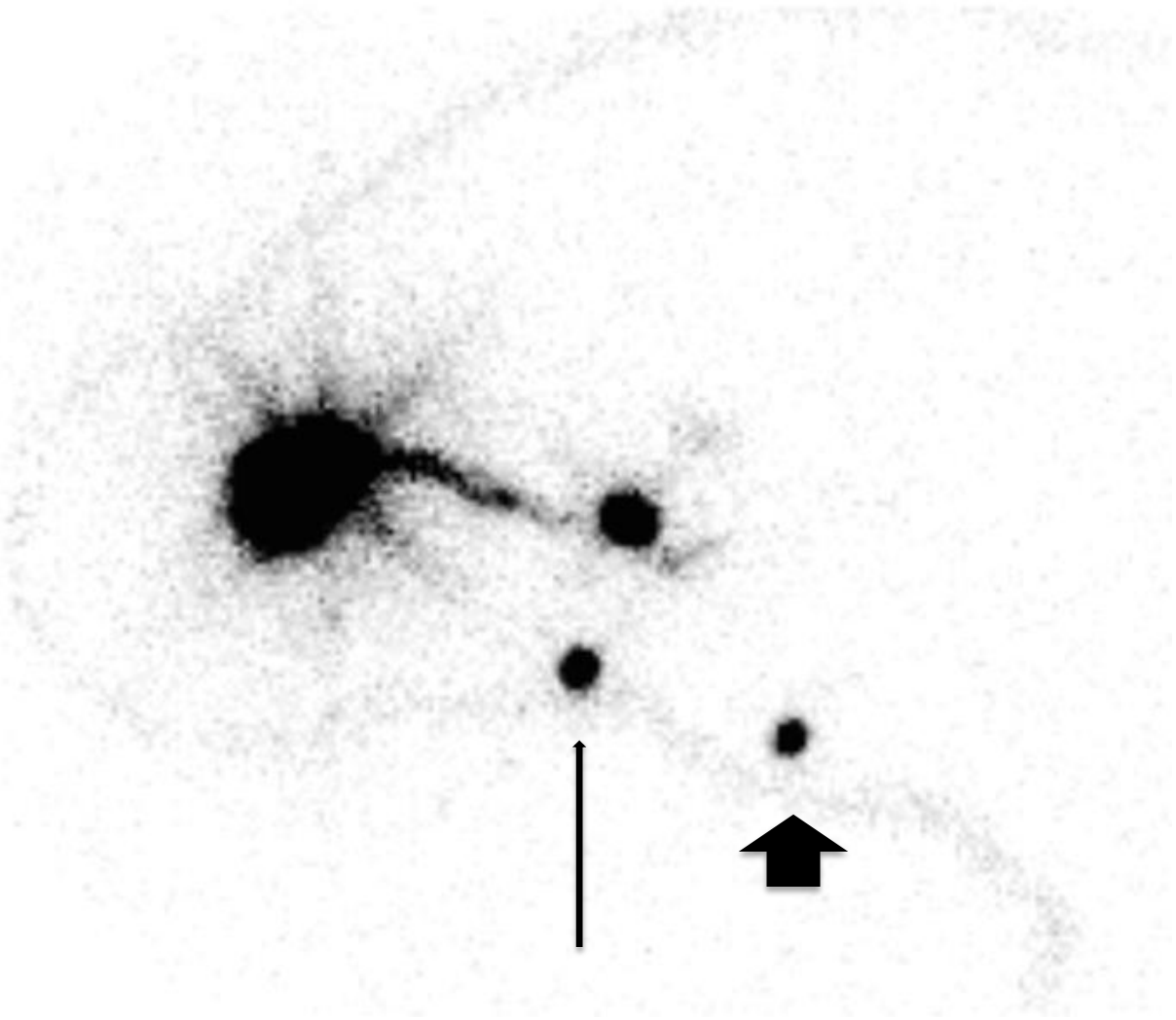

Figure 1: Case 1 lateral view image immediately following peri-tumoral injection. Markers identify location of mandibular (⬆) and retropharyngeal (⬆) lymph nodes. Lymphatic drainage to mandibular node is evident.

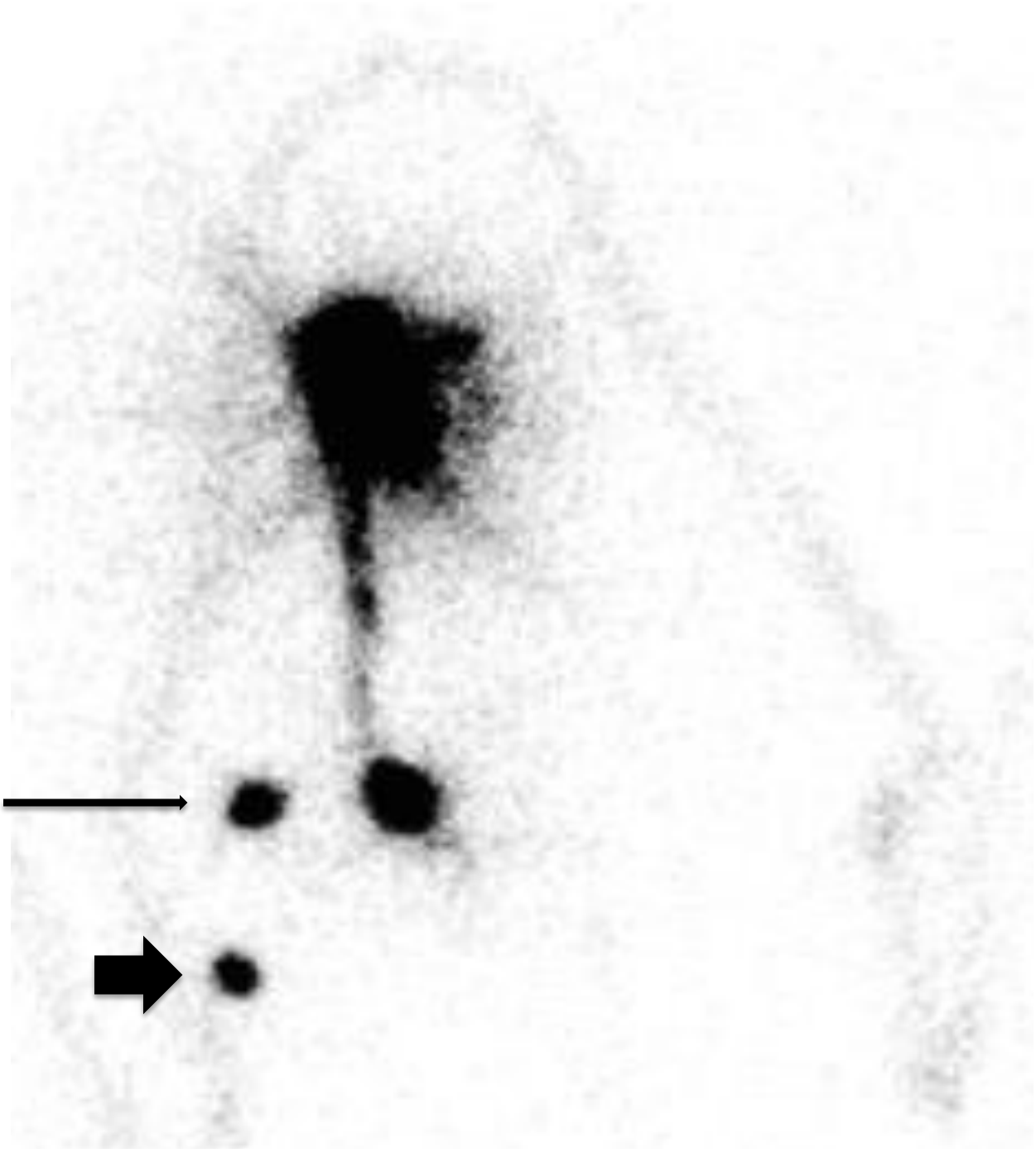

Figure 2: Case 1 ventral view image immediately following peri-tumoral injection. Markers identify location of mandibular (➞) and retropharyngeal (➧) lymph nodes. Lymphatic drainage to mandibular node is evident.

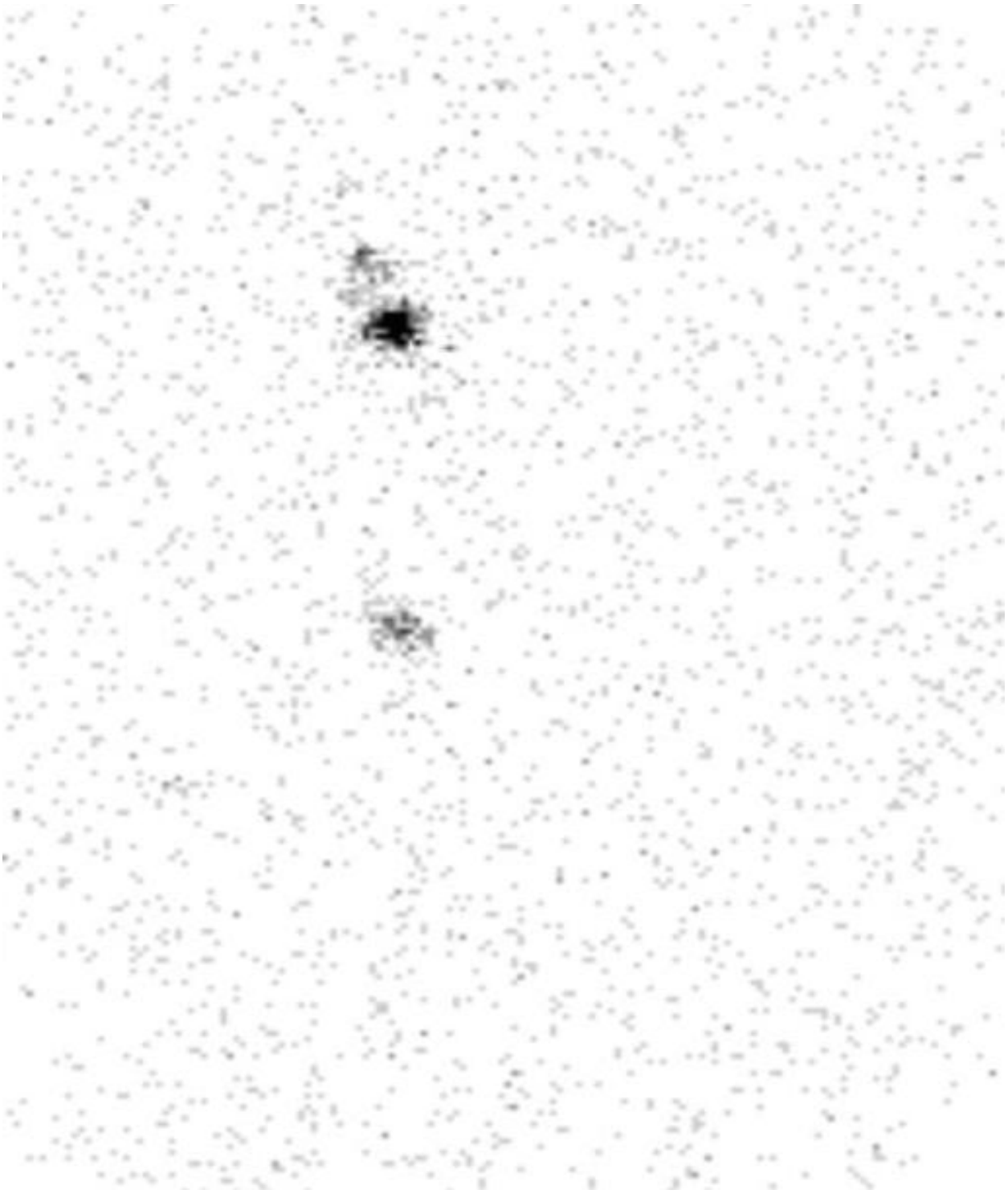

Figure 3: Case 1 ventral view image prior to second injection. Mild residual radioactive activity present.

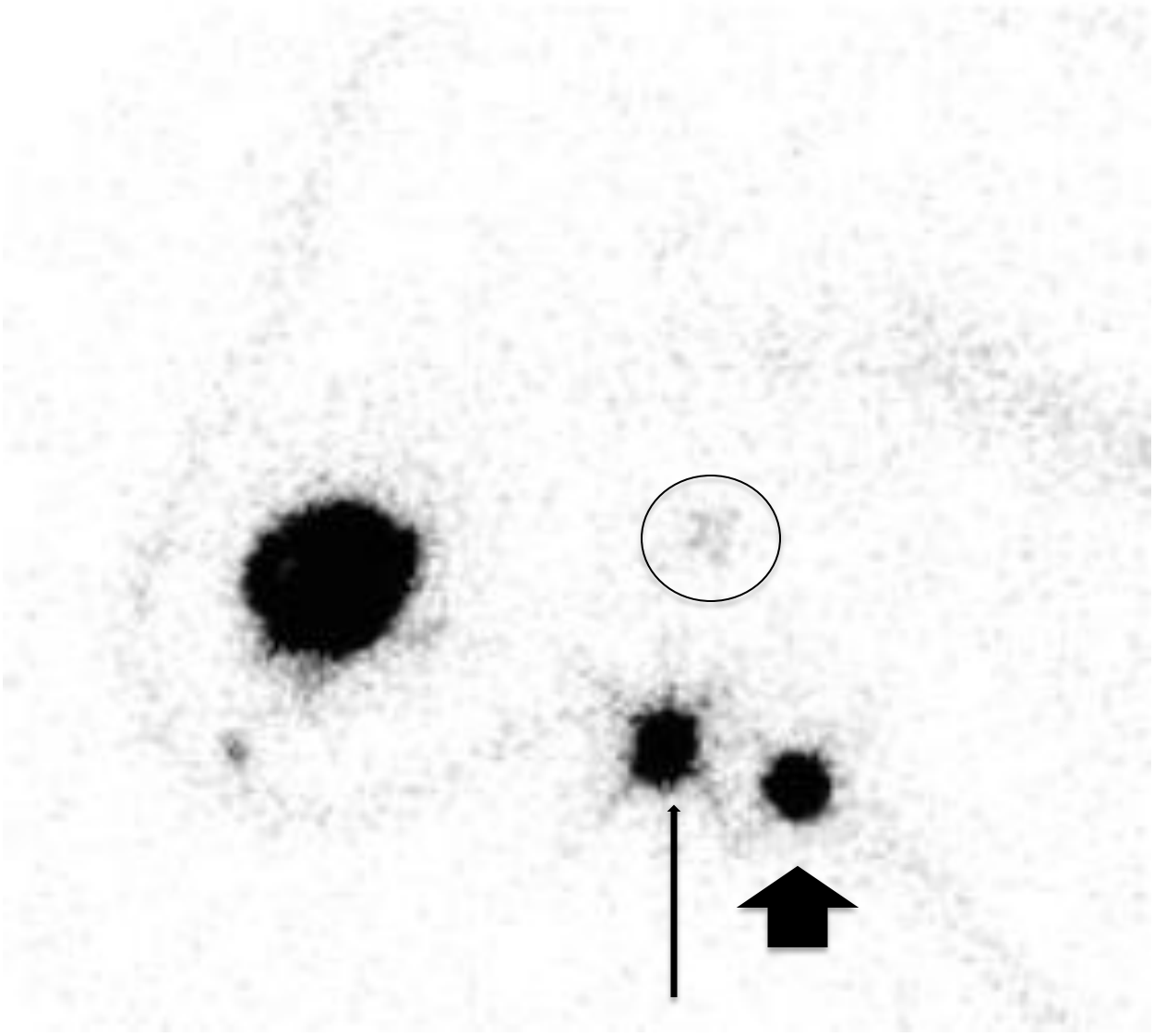

Figure 4: Case 1 lateral view image immediately after intra-tumoral injection. Markers identify location of mandibular (⬆) and retropharyngeal (⬆) lymph nodes. No lymphatic drainage evident. Previous injection isotope faintly visible at mandibular lymph node (◯).

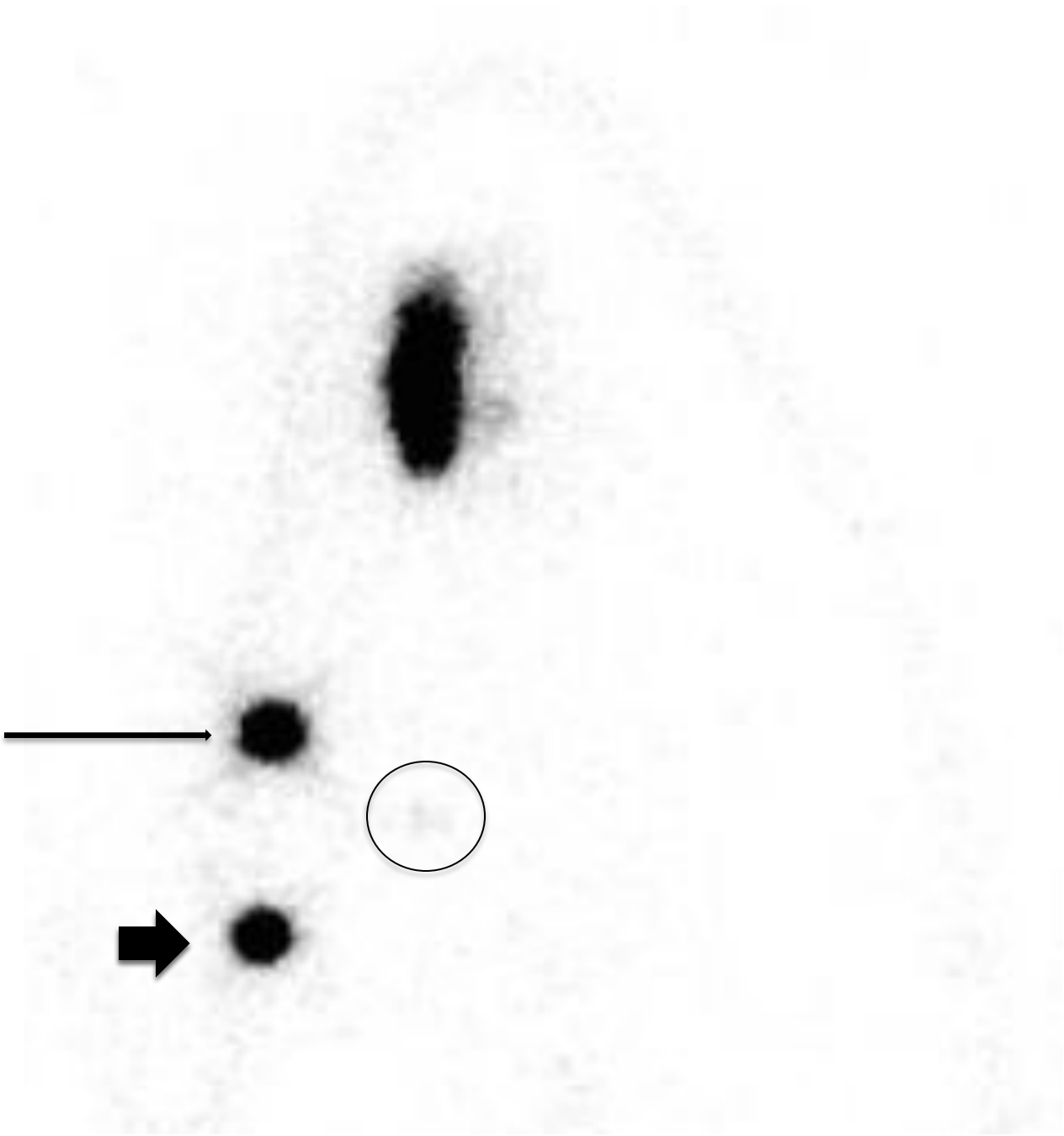

Figure 5: Case 1 ventral view image immediately after intra-tumoral injection. Markers identify mandibular (⟶) and retropharyngeal (➡) lymph nodes. No evidence of current lymphatic drainage. Isotope from previous injection faintly visible at mandibular lymph node (◯).

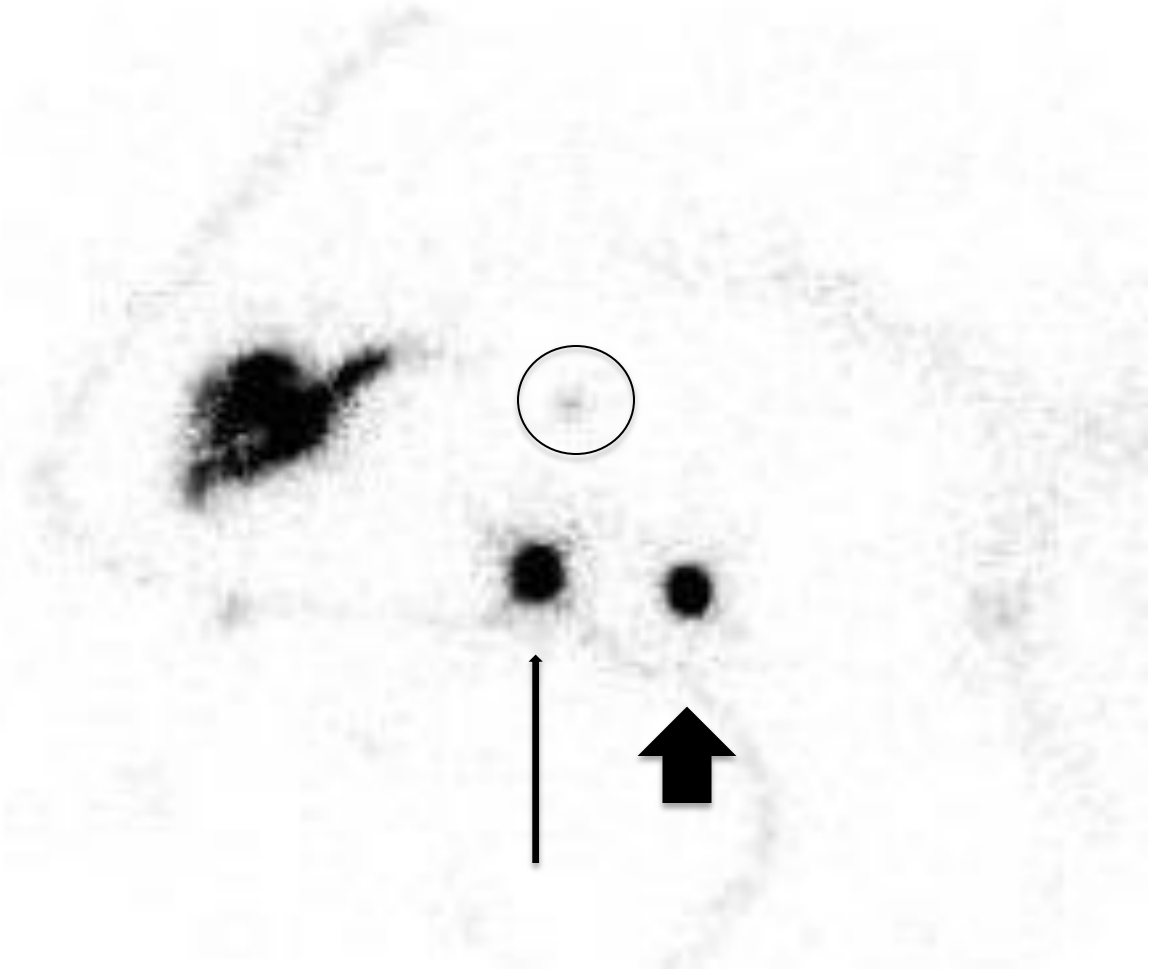

Figure 6: Case 1 lateral view image 2 hours after intra-tumoral injection. Markers identify mandibular (⬆) and retropharyngeal (⬟) lymph nodes. Initial lymphatic drainage identified. Isotope from previous injection faintly visible at mandibular lymph node (◯).

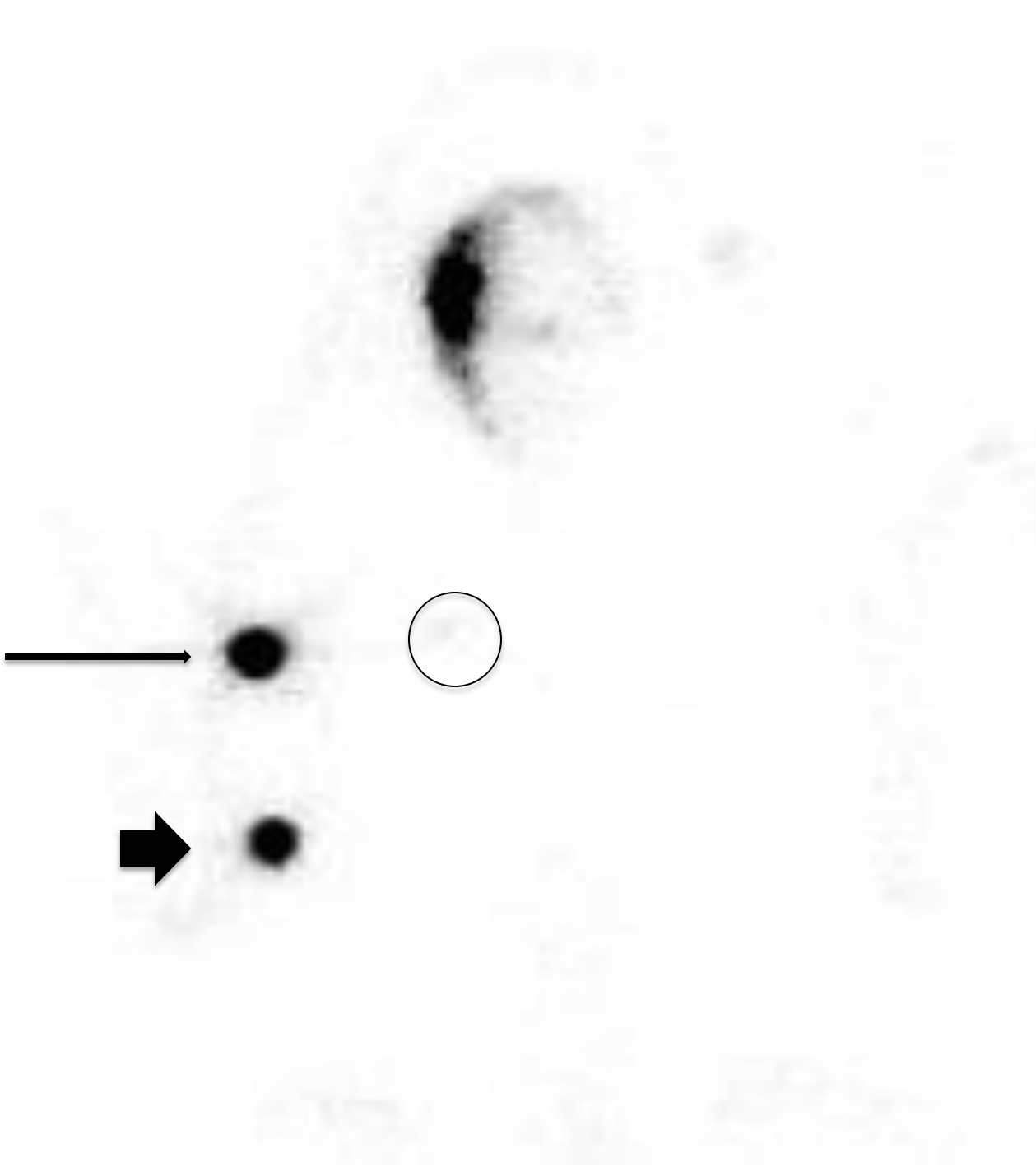

Figure 7: Case 1 ventral view image 2 hours after intra-tumoral injection. Markers identify mandibular (➞) and retropharyngeal (➧) lymph nodes. Initial lymphatic drainage identified. Isotope from previous injection faintly visible at mandibular lymph node (◯).

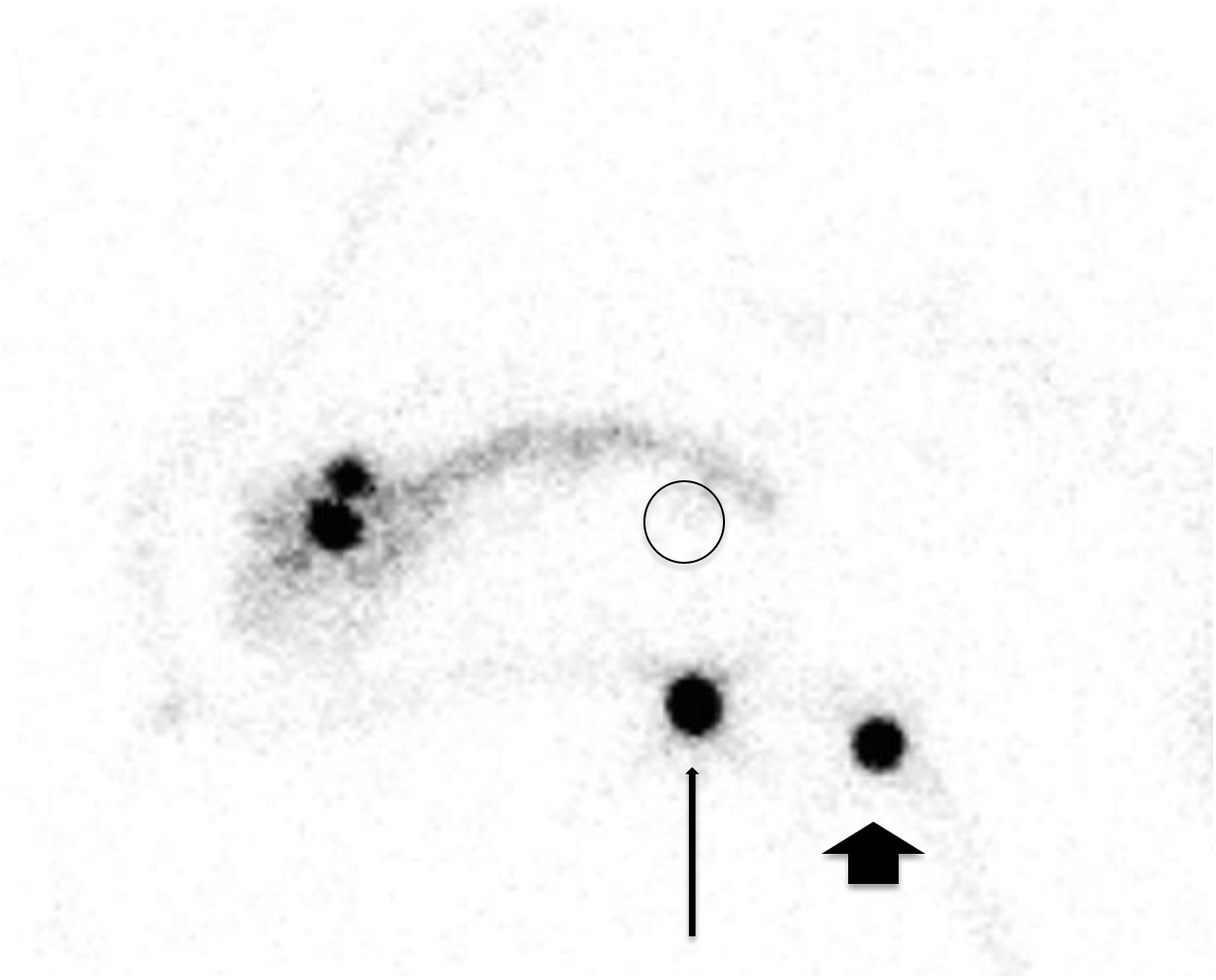

Figure 8: Case 1 lateral view image 3 hours after intra-tumoral injection. Markers identify mandibular (⬆) and retropharyngeal (⬟) lymph nodes. Lymphatic drainage to mandibular lymph node identified. Isotope from previous injection faintly visible at mandibular lymph node but slightly offset from current drainage (◯).

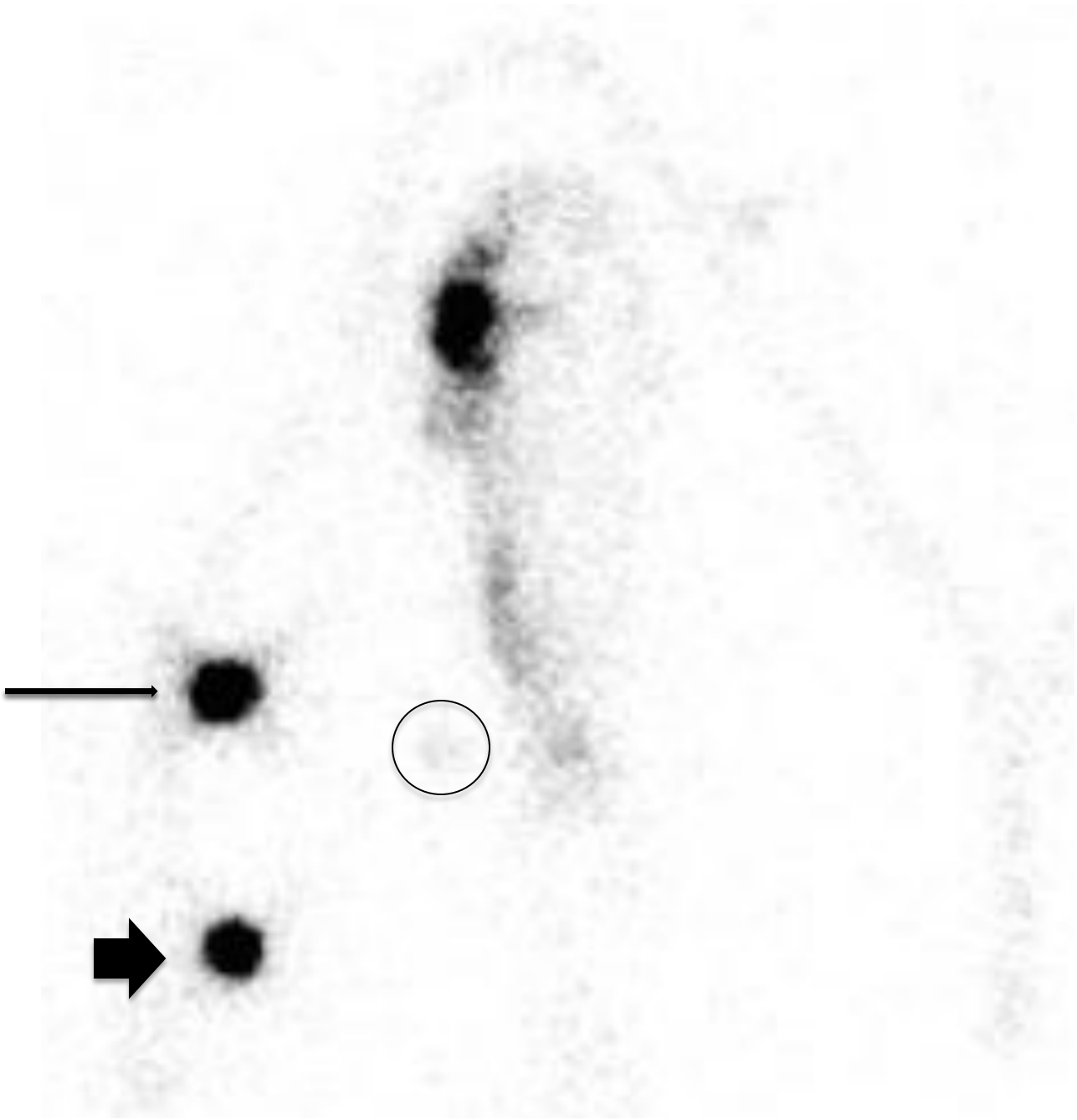

Figure 9: Case 1 ventral view image 3 hours after intra-tumoral injection. Markers identify mandibular (➞) and retropharyngeal (➡) lymph nodes. Lymphatic drainage to mandibular lymph node identified. Isotope from previous injection faintly visible at mandibular lymph node but slightly offset from current drainage (◯).

CASE 2

A 4-year-old male castrated Labrador Retriever/hound mix presented for a mass on the left rostral mandible initially noted by the owners 2 weeks prior to presentation. An incisional biopsy of the mass was performed by the primary care veterinarian with histopathologic results consistent with a sarcoma, however amelanotic melanoma could not be ruled out. Thoracic radiographs at that time were unremarkable. On presentation, the mass was located caudal to the mandibular incisors and measured approximately 2 cm in diameter. A head CT was performed and revealed mild lysis of the rostral mandible, mass extension from the incisors to the caudal aspect of the left mandibular canine tooth and mild enlargement of the left retropharyngeal lymph node relative to the right. Lymphoscintigraphy was performed as outlined in the materials and methods section. Day 1 involved intra-tumoral injection of a total of 0.4 mCi of technetium in 0.4 mL, and images were obtained at previously described intervals. Radioactive markers were used to locate the position of the mandibular and medial retropharyngeal lymph nodes and residual radionuclide in the syringe was used to trace the outline of the head and neck. Images obtained immediately after injection showed focal radionuclide activity at the site of injection with no evidence of lymphatic drainage (Figures 10 and 11). Movement of the radioisotope was not identified at any time point, including 3 hours post-injection (Figures 12 and 13); the study was terminated. Immediately after study completion, a Geiger counter assessed radiation emission levels to be well below standard release criteria (0.09 mR/h), and radiation isolation housing was deemed unnecessary. The second lymphoscintigraphic procedure (peri-tumoral injection) was not performed until 2 days after the first procedure. Prior to injection, a scan was performed to evaluate for residual radioisotope signal. A minor amount of signal was detected, however the counts were sufficiently low and continued to be contained to the injection site

(Figures 14 and 15). Technetium (0.4 mCi in 0.4 mL) was injected into three quadrants surrounding the tumor (ventral, rostral and caudal). The fourth quadrant was not included due to the tumor extending to the dorsal extent of the mandibular mucosa. Lymphatic flow of the radioisotope was noted immediately; however, it did not extend to the first order lymph node (Figures 16 and 17). By the 15-minute images, the lymphatic path was followed the full distance to the first order lymph node, which was identified as the left mandibular node. In addition, a second lymphatic path was identified coursing medially on the left and ending rostrally at an undescribed second smaller node (Figures 18 and 19). A Geiger counter determined that the patient was immediately safe to proceed to surgery (radiation emissions 0.1 mR/h). Fine needle aspirate of the mandibular lymph node was performed: cytologic evaluation was consistent with a reactive lymph node with no evidence of neoplastic cells. A partial rostral mandibulectomy and left mandibular lymphadenectomy were performed. The smaller rostral node noted with lymphoscintigraphy was not identified intra-operatively: however, it may have been excised with the mandibulectomy. Two lymph nodes were identified at the mandibular location and both were removed. The patient recovered from anesthesia and surgery without incident. On evaluation of the dissected tissues with the Geiger counter, radiation emission levels were below required limits, and it was determined safe to submit for processing and analysis. Histopathology of the mass revealed a high-grade fibrosarcoma with complete surgical margins and no evidence of vascular or lymphatic invasion. Lymph nodes were confirmed reactive on histopathology. Lymph nodes were graded as histologic grade 1 for impact from fine needle aspirate with approximately 20% of tissue involvement in the most affected section. The tumor was evaluated as histologic grade 1 effect of radionuclide injection with approximately 5% tissue involvement in the most affected section. In both the lymph node and tumor, tissue disruption by aspiration or

injection was not noted to interfere with the overall ability to make an effective diagnosis**.** The owners declined further treatment, including chemotherapy. There was delayed incisional healing, requiring the sutures to be left in place for one month, at which time full healing was complete. On follow-up contact with the owners 9 months postoperatively, the patient had recovered fully, and was doing well at home. There was no evidence of recurrence, however no follow up staging diagnostics had been performed since the time of surgery.

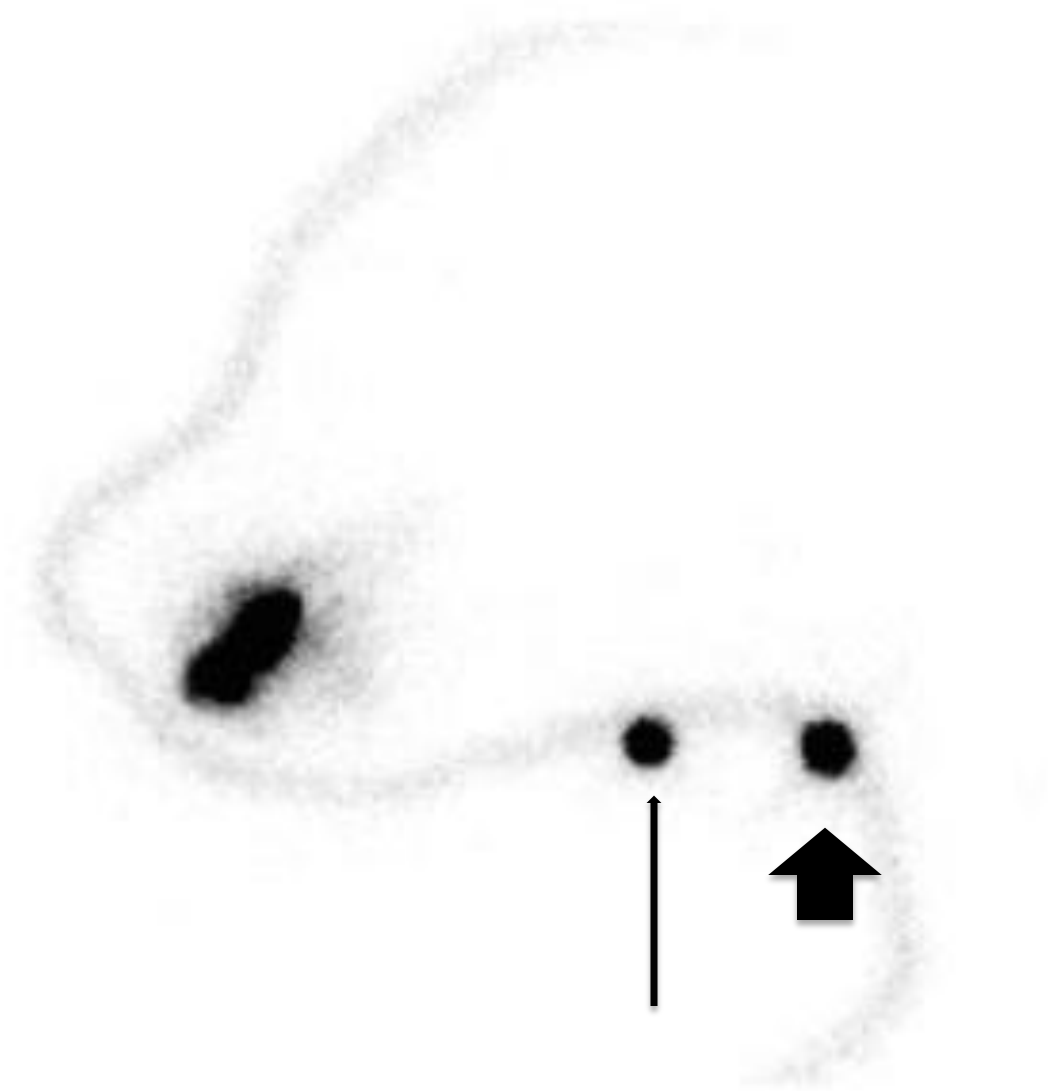

Figure 10: Case 2 lateral view image immediately after intra-tumoral injection. Markers identify mandibular (⬆) and retropharyngeal (⬆) lymph nodes. No evidence of lymphatic drainage.

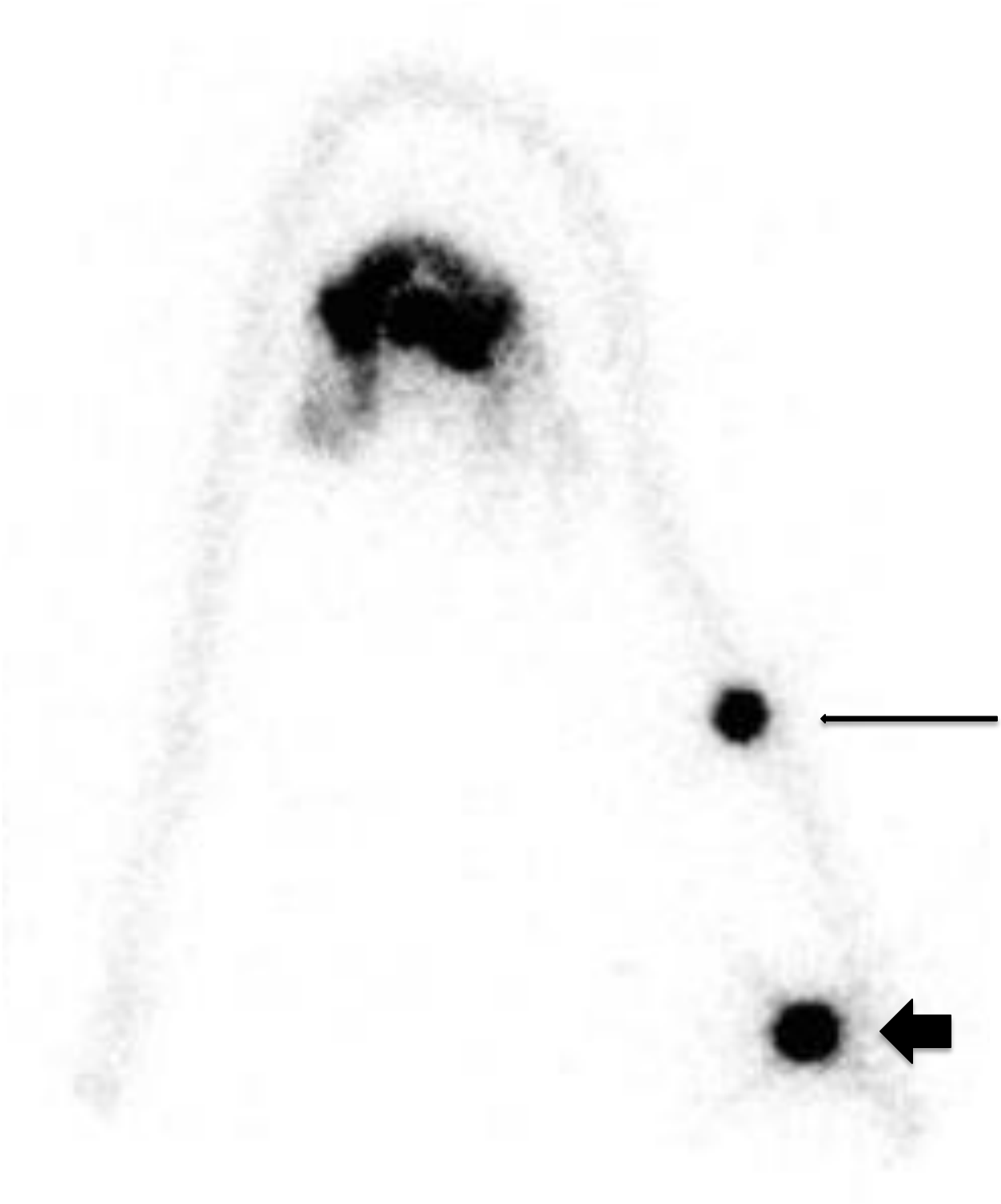

Figure 11: Case 2 ventral view image immediately after intra-tumoral injection. Markers identify mandibular (⟵) and retropharyngeal (⬅) lymph nodes. No evidence of lymphatic drainage.

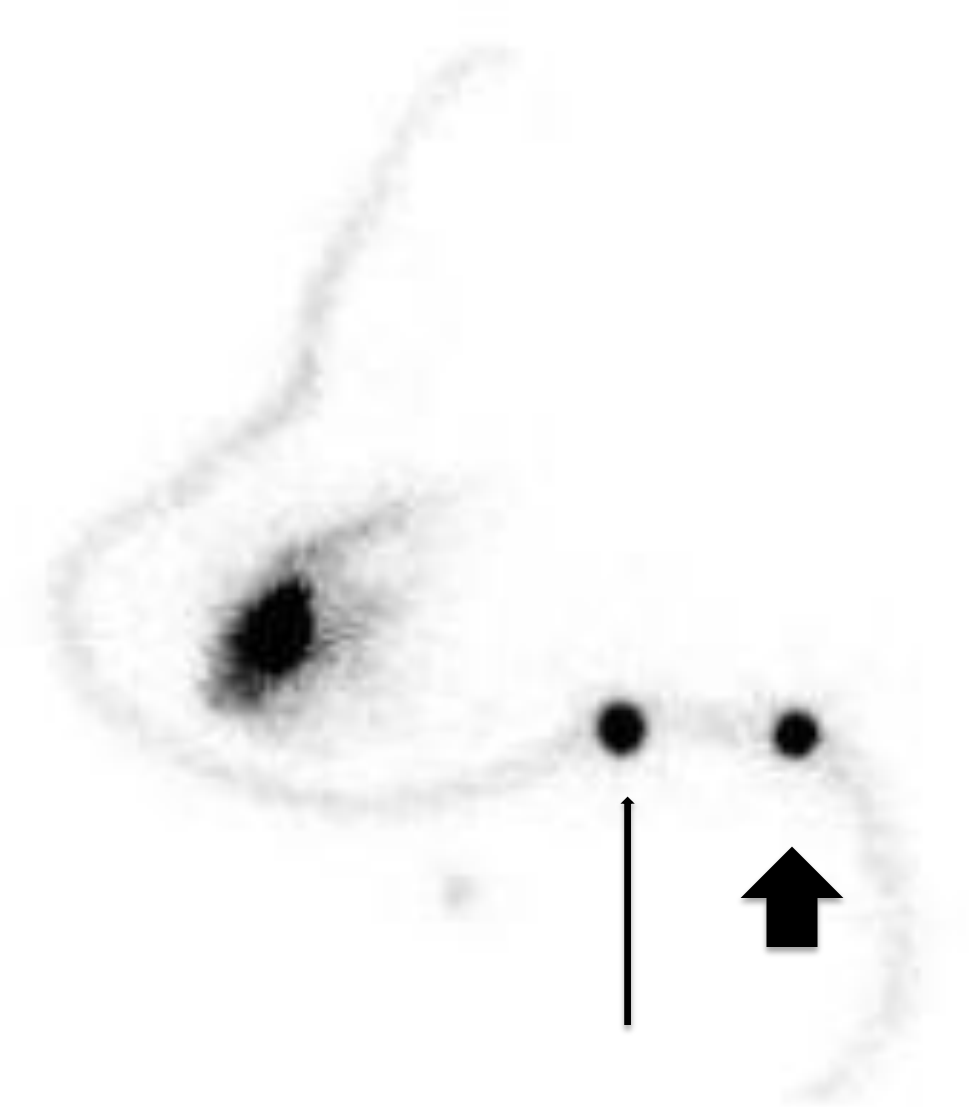

Figure 12: Case 2 lateral view image 3 hours after intra-tumoral injection. Markers identify mandibular (⬆) and retropharyngeal (⬆) lymph nodes. No evidence of lymphatic drainage.

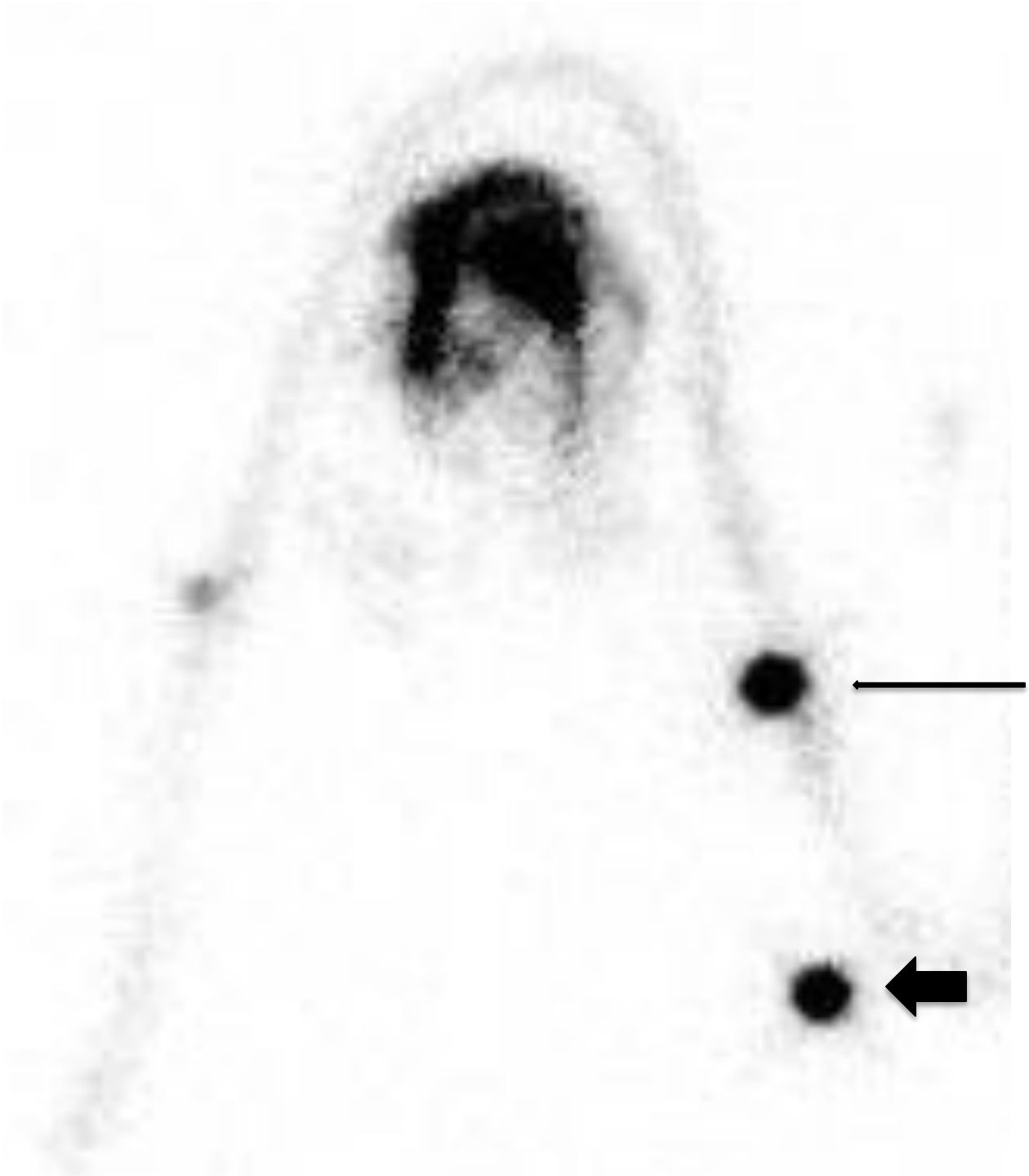

Figure 13: Case 2 ventral view image 3 hours after intra-tumoral injection. Markers identify mandibular (⟵) and retropharyngeal (⬅) lymph nodes. No evidence of lymphatic drainage.

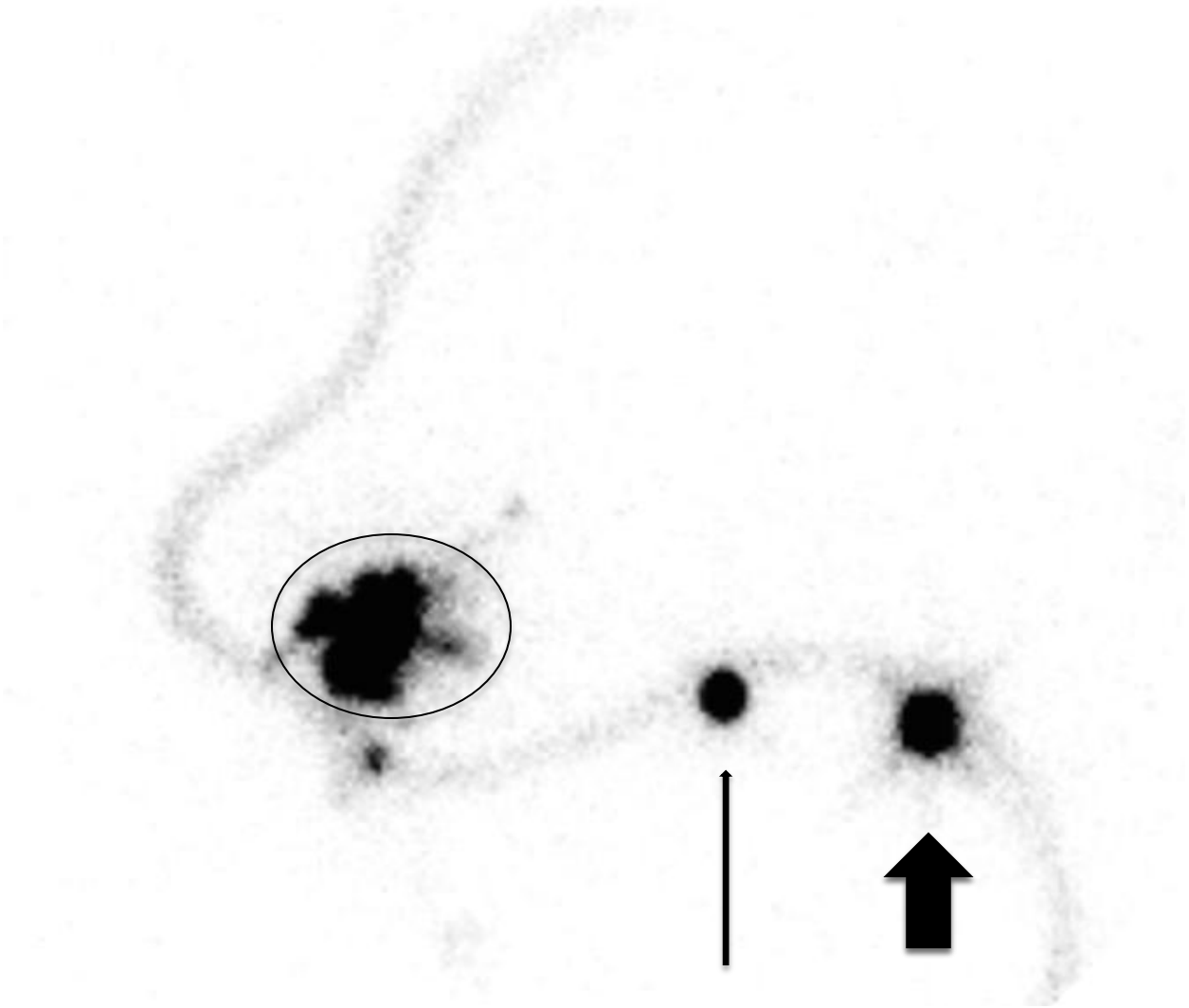

Figure 14: Case 2 lateral view image immediately prior to peri-tumoral injection. Markers identify mandibular (⬆) and retropharyngeal (⬆) lymph nodes. No evidence of lymphatic drainage from previous injection. Isotope at injection site still evident (◯).

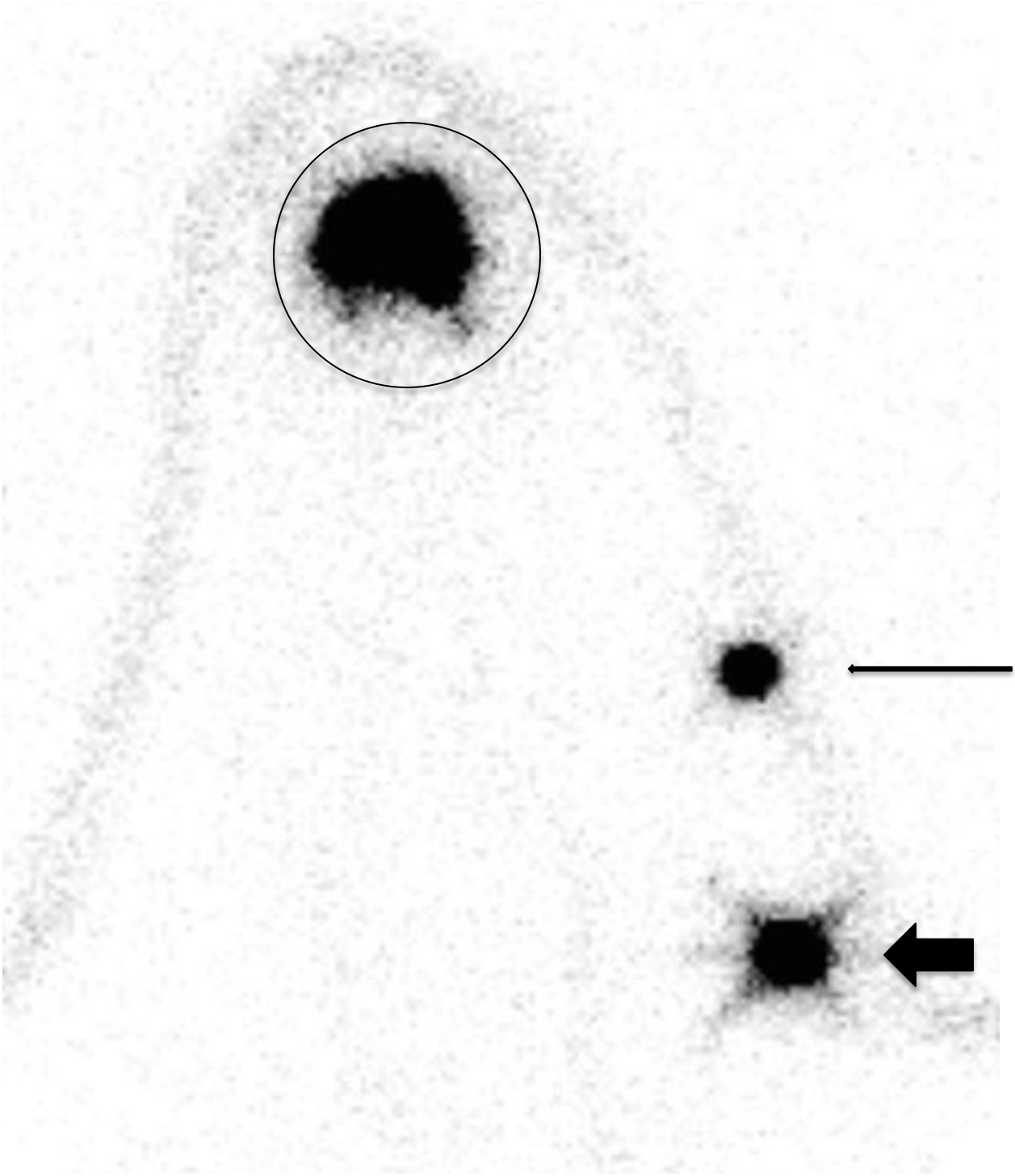

Figure 15: Case 2 ventral view image immediately prior to peri-tumoral injection. Markers identify mandibular (⟵) and retropharyngeal (⬅) lymph nodes. No evidence of lymphatic drainage from previous injection. Isotope at injection site still evident (○).

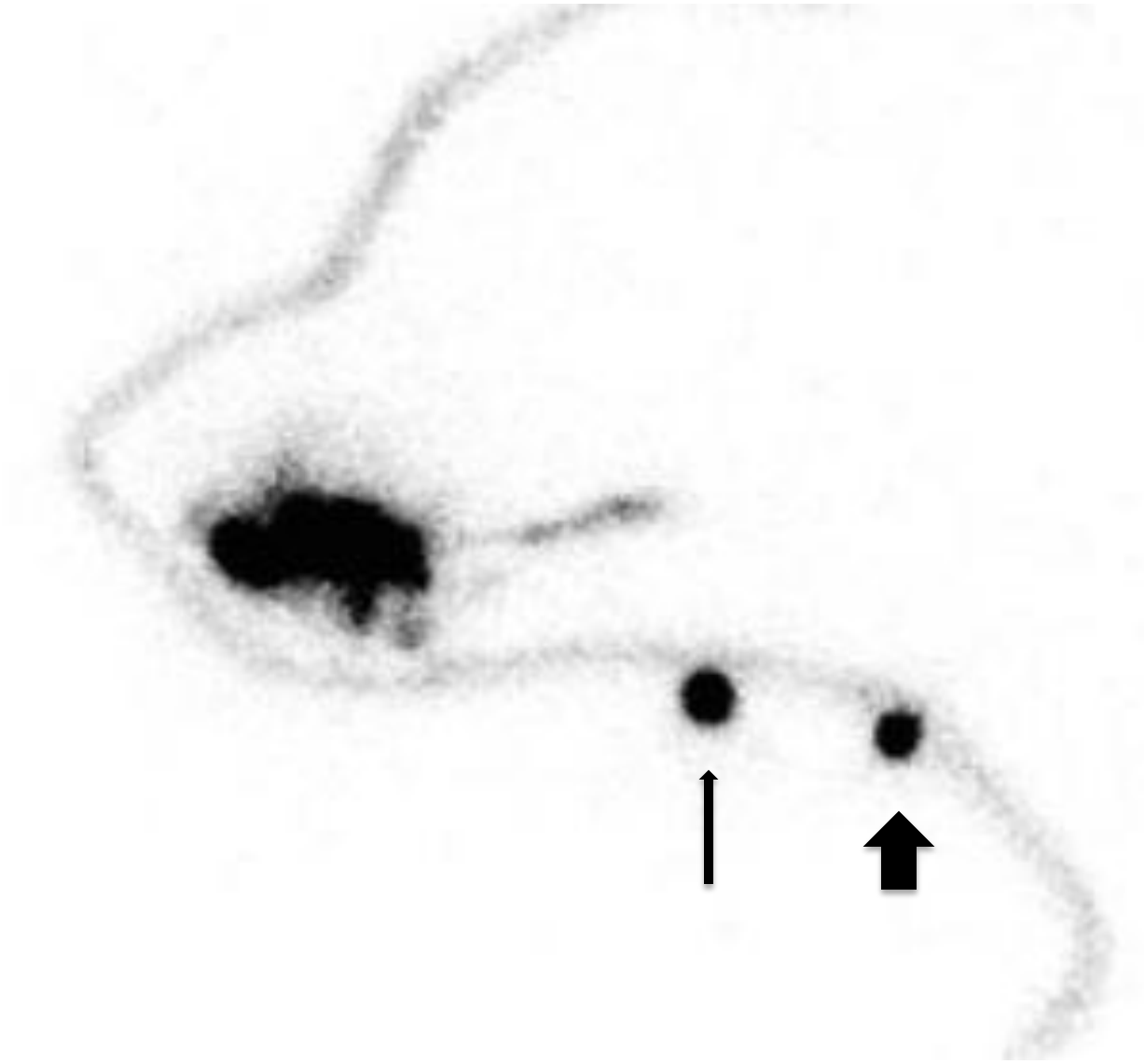

Figure 16: Case 2 lateral view image immediately after peri-tumoral injection. Markers identify mandibular (⬆) and retropharyngeal (⬆) lymph nodes. Evidence of initial lymphatic drainage is present.

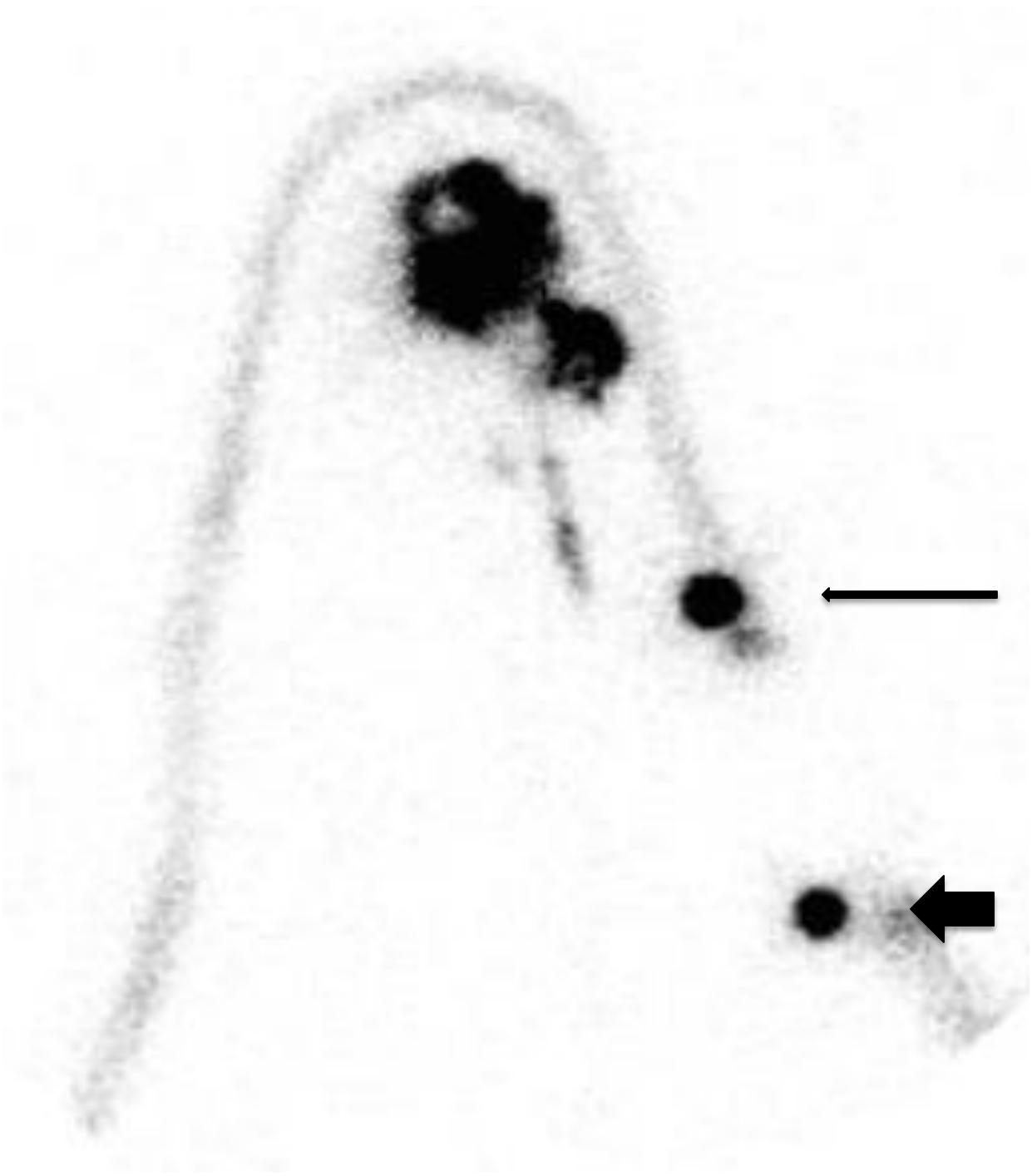

Figure 17: Case 2 ventral view image immediately after peri-tumoral injection. Markers identify mandibular (⟵) and retropharyngeal (⬅) lymph nodes. Evidence of initial lymphatic drainage is present.

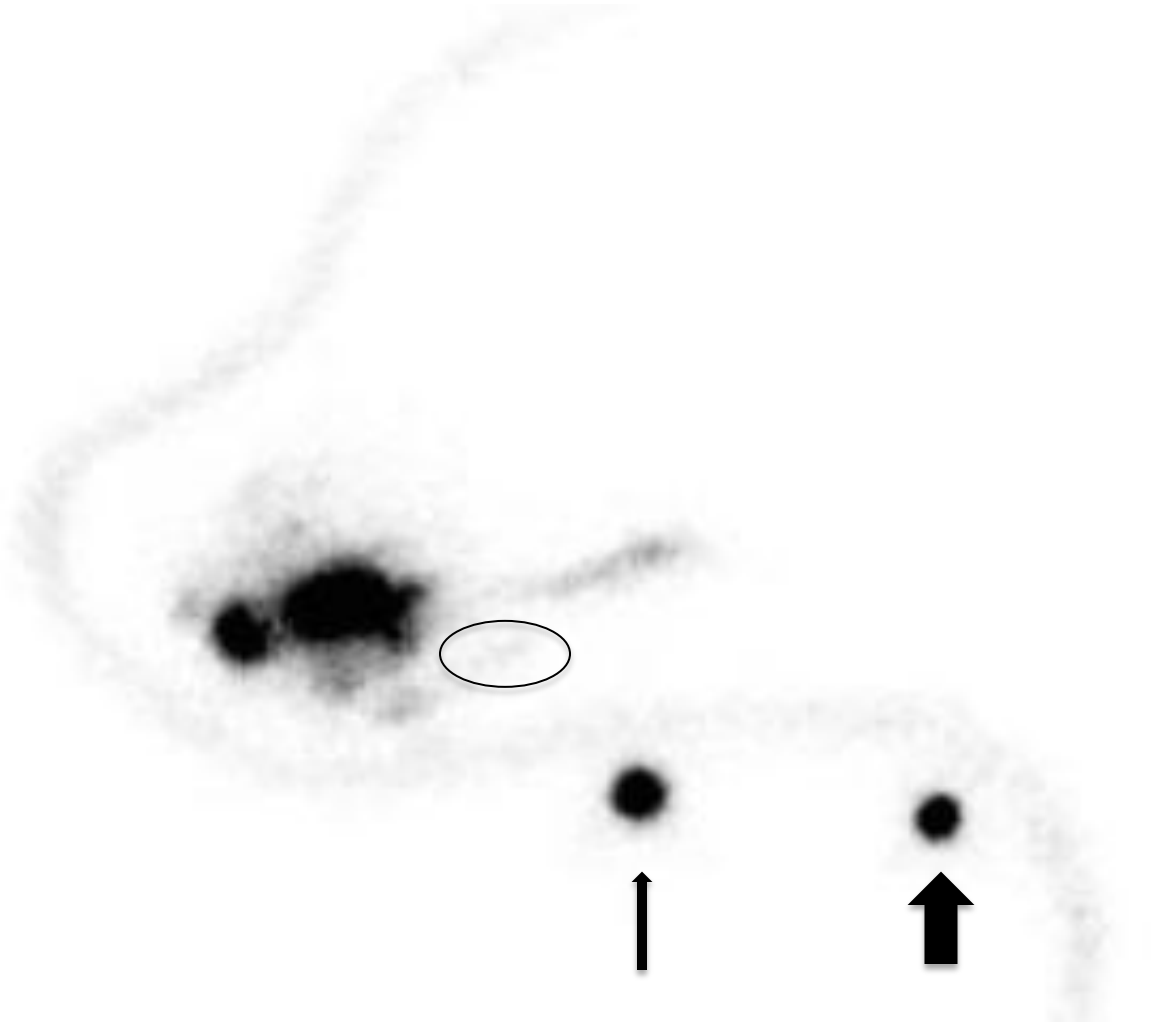

Figure 18: Case 2 lateral view image 15 minutes after peri-tumoral injection. Markers identify mandibular ( ) and retropharyngeal ( ) lymph nodes. Lymphatic drainage to the mandibular lymph node is evident. Secondary lymphatic drainage noted ventral to injection site to an additional, undescribed lymph node ( ).

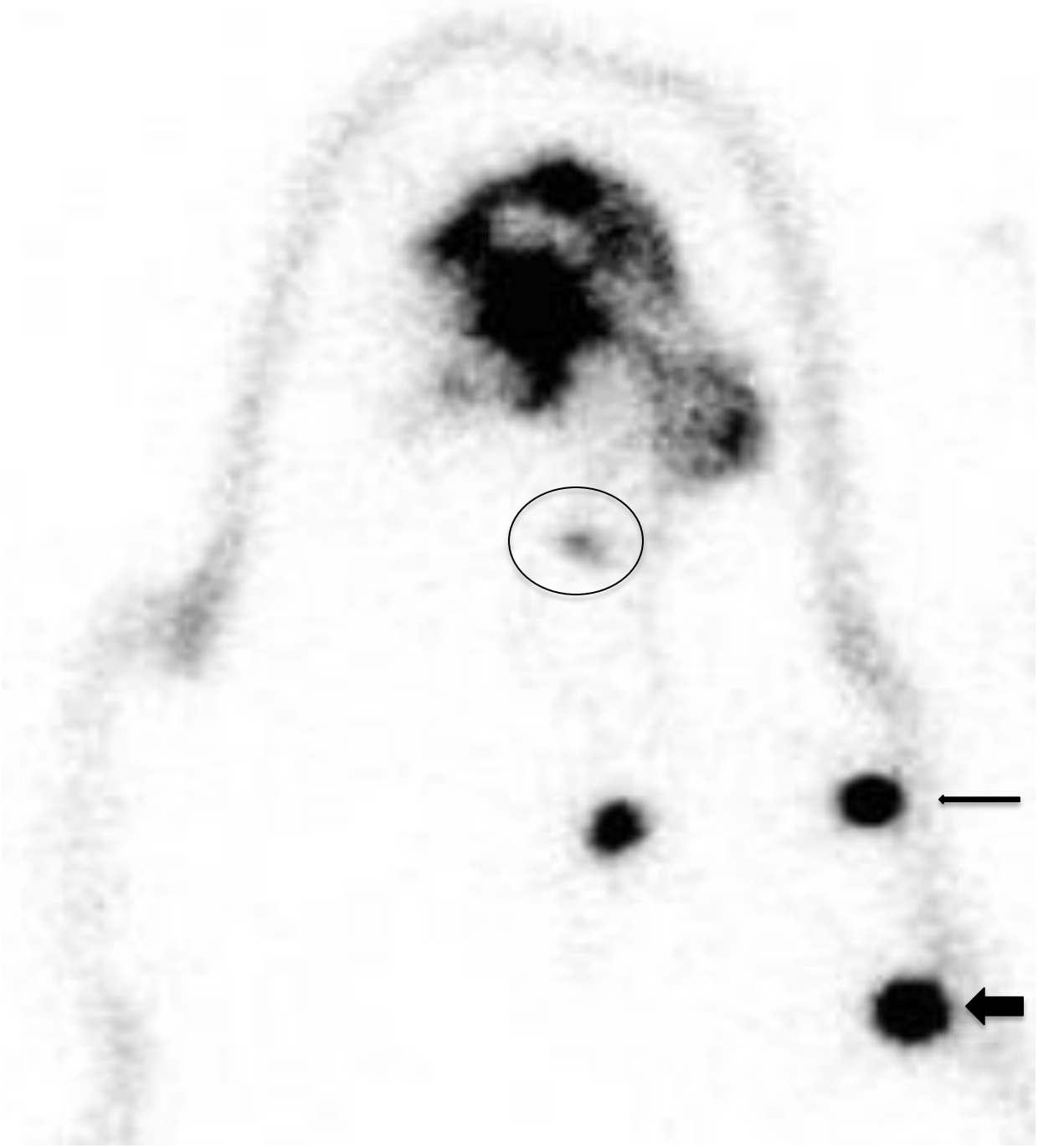

Figure 19: Case 2 ventral view image 15 minutes after peri-tumoral injection. Markers identify mandibular (⟵) and retropharyngeal (⬅) lymph nodes. Lymphatic drainage to the mandibular lymph node is evident. Secondary lymphatic drainage noted rostral and medial to an additional, undescribed lymph node (◯).

Table 1: Case summary

Injection technique	Tumor location	Tumor diagnosis	Sentinel lymph node	Time to identify sentinel node	Cytology of mandibular node	Sentinel lymph node histology	Time to radiation safety
Case 1: intra-tumoral	Right maxilla	Giant cell tumor of bone	Ipsilateral mandibular	3 hours	Reactive	Reactive	Immediate
Case 1: peri-tumoral	Right maxilla	Giant cell tumor of bone	Ipsilateral mandibular	Immediate	Reactive	Reactive	Immediate
Case 2: intra-tumoral	Left mandible	Fibrosarcoma	N/A	N/A	Reactive	Reactive	Immediate
Case 2: peri-tumoral	Left mandible	Fibrosarcoma	Ipsilateral mandibular; second undescribed node	15 minutes	Reactive	Reactive	Immediate

Discussion

Although it is difficult to derive definitive conclusions from this study based on the small case numbers, preliminary findings of potential interest were identified. Preliminary results suggest that injection technique is likely to play a role in the results obtained from lymphoscintigraphy studies. Intra-tumoral injections were less reliable in identifying sentinel lymph node. Peri-tumoral injections resulted in rapid identification of the sentinel lymph node; whereas intra-tumoral injection resulted in identification in only 1 of the 2 subjects, and only after 3 hours. Providing additional complexity to this matter, although a similar lymphatic course was identified when there was successful drainage identified with the intra-tumoral technique, different nodes within the lymphatic basin were identified. Unfortunately, this discrepancy was not identified until retrospective evaluation of the images, rather than being noted prior to surgery. In addition, based on intra-operative palpation, only a single node was removed from that patient, making it impossible to know if the excised lymph node represents the node identified by the intra-tumoral or peri-tumoral injection technique. Definite recommendations regarding the optimal injection technique to perform lymphoscintigraphy in the patient with oral neoplasia await further investigation. The clinical significance of the different lymph nodes identified in the same basin is unknown and additional studies would be required to further evaluate this finding.

Regarding the first purpose of this study, it is difficult to conclude that lymphoscintigraphy enabled better staging for oncology patients with oral tumors. Although unique drainage patterns were identified, without additional diagnostics (blue dye or gamma probe), the information was of limited value. Both patients in this study were noted to have sentinel nodes located in the mandibular basin. It is also difficult to draw conclusions based on this low population number;

however, based on historic literature[27, 28], it is likely that this is not representative of the majority of patients with oral tumors. The fact that successful imaging was performed with lymphoscintigraphy suggests that staging will be improved with the use of this technology for the population of dogs whose sentinel node is not the mandibular node.

Peri-tumoral injection resulted in rapid image acquisition; however, there is the possibility that the lymph node identified by this technique does not represent the true sentinel node draining the tumor, as suggested by Estourgie[66]. It has been previously reported[11] that an increased injection volume is required when performing intra-tumoral injection to increase the pressure and cause lymphatic uptake of the tracer. Although other studies have found that this is not necessarily the case[56], it is a possible explanation for the lack of drainage, or slow lymphatic uptake seen with the intra-tumoral injection technique in this study. In addition, procedures such as massage or application of heat have been suggested to increase the speed of lymphatic uptake and may be beneficial to enhancing images when this technique is used[60]. Such techniques were not performed in this study in an attempt to maintain consistency and limit extraneous variables.

The second purpose of this study also remains to be fully answered and will require additional case accrual. Although there was improved speed of image acquisition with peri-tumoral injection technique, assessment of clinical significance requires additional cases. The question relating to "true" tumor lymphatic drainage based on radionuclide injection technique will also require substantially more investigation.

Another feature emphasized by both of these cases highlights the potential importance of the combination use of an intra-operative gamma probe as well as pre-operative lymphoscintigraphy. In the first case, two separate nodes were identified pre-operatively, one by each scan; however, only a single node was identified intra-operatively. It is suggested when using a gamma probe

that after a radioactive node is removed, the basin should be evaluated for radiation counts > 150% of background and if noted, additional investigation should be performed[16]. This allows for detection of smaller nodes not visible or palpable within the draining basin and would aid not only in recognition of the previously undiagnosed second node, but also assist in identification of the location of the node when it was determined to be non-palpable.

The second case was noted to have a second sentinel node on pre-operative lymphoscintigraphy. This was noted to be in a location that is not a previously described lymphatic basin in dogs. It may be an example of the previously defined transit or interval node[16, 17]. Again, at the time of surgery, it was not possible to visualize or palpate this node, and it is unknown if the node was excised. To perform full sentinel lymph node staging of this patient, the second node would also require histopathologic analysis. The ability to trace this node with a gamma probe intra-operatively could have improved the ability to perform this complete staging. Although the use of pre-operative lymphoscintigraphy in this study agreed with previous studies in that sentinel node identification was possible, complete sentinel node biopsy seems to require additional use of an intra-operative gamma probe. The results of true lymph node staging would be much more accurate with the use of this technology. It has been previously documented by Albertini[16] that pre-operative lymphoscintigraphy and intra-operative gamma probe usage are complimentary diagnostics, rather than one being able to replace the other. A gamma probe can identify the true radioactive node intra-operatively within the basin, and pre-operative lymphoscintigraphy may identify interval or transit nodes that are not in typically recognized node basin locations that would be otherwise missed if only an intra-operative gamma probe were used[63]. Alternatively, the use of a blue dye could have been used in this study; however, given the previously described technical difficulties of this procedure and

associated patient morbidity, it is difficult to recommend this technique for routine use with oral tumors[24, 49]. The second tumor in the study was also noted to cross rostral midline, and there was pre-emptive expectation that the resultant lymphogram would show bilateral drainage; however, it was seen that the drainage continued to be unilateral to the side of the origin of the tumor. This may indicate that as tumors grow, even if they enlarge to the point of crossing midline, neoplastic lymphangiogenesis may continue to proceed in a direction that follows the initial lymphatic flow.

The third purpose of this study also remains to be elucidated. Both cases of this report were found to have corresponding cytologic and histopathologic results of lymph node analysis; however, both cases were also reported to have only reactive nodes with no evidence of metastasis, and both sentinel nodes were recorded within the mandibular lymphocentrum. Verification or contradiction of the diagnosis of metastasis was not possible. When evaluating the effect of aspiration or radionuclide injection on tissue disruption and interference with histopathologic diagnostic ability, no negative impact on diagnostic accuracy was noted. Even in the sections that were moderately affected by needle tracts or disruption from local injection, additional sections of tissue were noted to be completely clear of any such artifact and diagnosis was easily made.

Although too few cases were included to be able to statistically analyze, radiation emission immediately after the scan was completed for each patient or dissected tissue, was noted to be below release criteria and no radiation isolation was required for any patient or tissue. This suggests that this diagnostic tool may be readily adopted in case management without significant disruption to the typical treatment schedule, required hospital stay, or in obtaining microscopic diagnosis and that it conforms to radiation safety guidelines.

Future directions

In addition to completing case accrual to successfully determine the advantage of intra- vs. peri-tumoral injection within the oral cavity as well as to validate the theory of correct sentinel lymph node identification with this technique, there are many additional avenues of research that warrant further exploration:

1. Evaluation of lymphoscintigraphy for use of sentinel node detection in other anatomic locations (e.g. perineal/inguinal region, or on the thoracic or abdominal wall).
2. Evaluation of the use of an intra-operative gamma probe combined with pre-operative lymphoscintigraphy for detection of small or non-palpable nodes within or outside of the lymphatic basin.
3. Comparison of sentinel lymph node detection between various types of tumors, and more accurately determining risk of metastatic potential of these tumor types.
4. Evaluating the effect of distance of peri-tumoral injection from the actual tumor margins on sentinel node detection.
5. Effect of size of tumor on injection technique/distance from tumor edge.
6. Cases included in this study resulted in having their second scan performed 2 days after the first, rather than the following day due to scheduling conflicts not related to this study. When the second procedures were performed, continued radiation was detected from the previous injection, however the levels were only slightly higher than background and did not interfere with obtaining quality results with the second injection. It is unknown if this would have been the case if the second procedure were to be

performed only 24 hours after the first. The clinical implications of this are likely minimal, as a typical clinical case would only receive a single injection; however, it may be important to know how quickly a second injection can be given without risk of non-diagnostic results due to interference from the initial radionuclide in case an initial failed or poor quality imaging study required a second injection.

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