



Pesquisa cito-histológica de metástase nodal nos melanomas e mastocitomas

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DEFINIÇÕES

Lymphatic Territories in a Canine

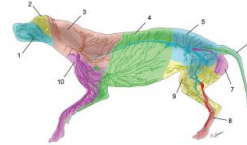
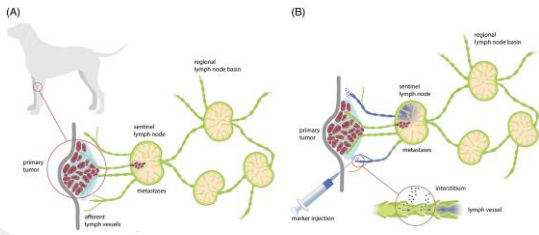


Figure 6. Color-coded diagram of the lymphatic territories (lymphosomes) with lymphatic vessels shown distally from their corresponding lymph nodes: 1, submandibular; 2, parotid; 3, dorsal superficial cervical; 4, axillary; 5, medial iliac; 6, lateral sacral; 7, hypogastric; 8, popliteal; 9, superficial inguinal; 10, ventral superficial cervical.  
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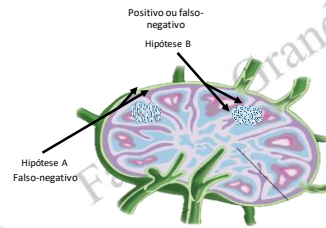
Linfocentros, linfonodo regional e sentinela



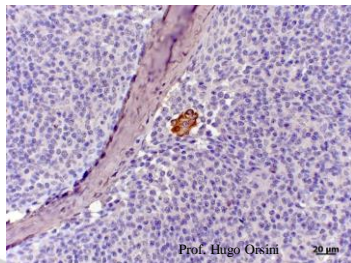
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<https://www.veterinarypracticenews.com/com-mon-forgotten-head-neck-lymphocenters/>

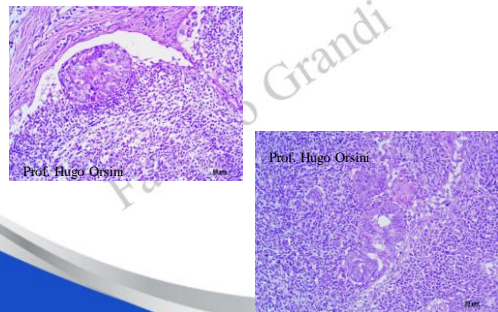
CONSIDERAÇÕES TÉCNICAS



Considerações técnicas



Considerações técnicas



- Dificuldades na avaliação das metástases nodais
  - Melanófago x melanócito
  - Mastócitos normal x mastócito neoplásico
  - % área comprometida do linfonodo
  - Amostragem de linfonodos de tamanho normal
  - Amostragem do linfonodo errado (não sentinela)
  - Metástase de células isoladas, micrometástases e macrometástases

#### Agreement Between Cytology and Histopathology for Regional Lymph Node Metastasis in Dogs With Melanocytic Neoplasms

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Janet A. Grimes<sup>1,2</sup>, Brad M. Matz<sup>1</sup>, Pete W. Christopherson<sup>3</sup>, Jey W. Koehler<sup>3</sup>, Kelsey K. Cappelle<sup>1</sup>, Katelyn C. Hlusko, DVM<sup>1</sup>, and Annette Smith<sup>1</sup>

- Neoplasias melanocíticas orais e cutâneas
  - Melanomas
  - Melanocitomas
  - Neoplasia melanocítica histologicamente bem diferenciada

- Citologia
  - Linfonodo “não metastático”
    - Células pigmentadas
      - Ausentes ou raras (0-5/esfregaço)
    - Células não pigmentadas com morfologia mesenquimal, coesas ou com indícios de malignidade
      - Ausentes

- Citologia
  - Linfonodo com metástase
    - Células pigmentadas com morfologia mesenquimal, coesas e/ou com indícios de malignidade
      - >5/esfregaço

- Citologia
  - Esfregaço equívoco
    - Células pigmentadas com morfologia redonda, individualizada, sem indícios de malignidade\*
      - >5/esfregaço

\*anisocitose, anisocariose, variação e/ou aumento na razão N:C, multinucleação, e aumento na atividade mitótica

Grimes et al

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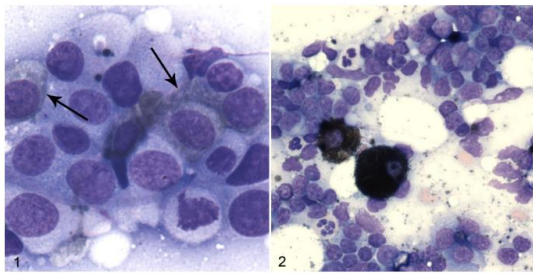


Figure 1. Metastatic melanoma, lymph node, canine, fine needle aspirate. Neoplastic cells are generally round to spindle in shape with large round nuclei, 0 to 2 prominent nucleoli, and a small to moderate amount of pale basophilic cytoplasm that contains low numbers of discrete melanin granules (arrows). Modified Wrights stain. Figure 2. Equivocal for metastatic melanoma, lymph node, canine, fine needle aspirate. Two heavily pigmented melanin-containing cells are seen. The histopathologic diagnosis for this node was metastatic melanoma. Modified Wrights stain.

### Histopatologia

#### Micrometástase

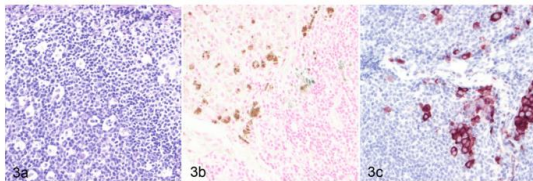
- 5-15 melanócitos agrupados ou individualizados em seios ou parênquima perisinusoidal com morfologia de melanócitos, negativos para Azul da Prússia e positivos para Melan A.

#### Macrometástase

- Mantos de melanócitos pigmentados neoplásicos obliterando a arquitetura normal

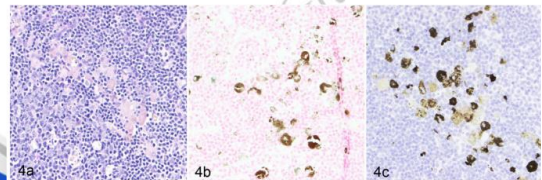
### Histopatologia (positivo para metástase)

- A) *Bleached*
- B) Azul da Prússia
- C) Melan-A (NovaRED)



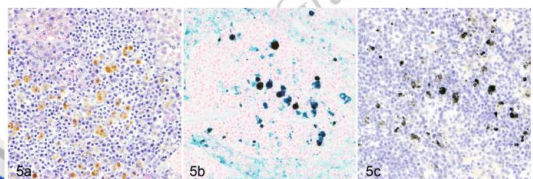
### Histopatologia (melanófagos)

- A) *Bleached*
- B) Azul da Prússia
- C) Melan-A (NovaRED)



### Histopatologia (hemossiderófagos)

- A) *Bleached*
- B) Azul da Prússia
- C) Melan-A (NovaRED)



### Conclusões do estudo

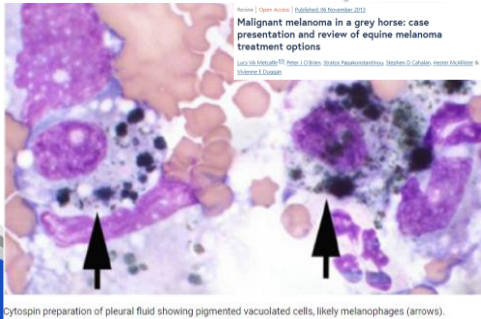
- Como tratar o resultado citológico de rotina?

Table 1. Relationship Between Palpation of Lymph Nodes (Normal or Enlarged) and Review of Cytology and Histopathology Slides for 29 Lymph Nodes From 27 Dogs With Melanocytic Neoplasms.

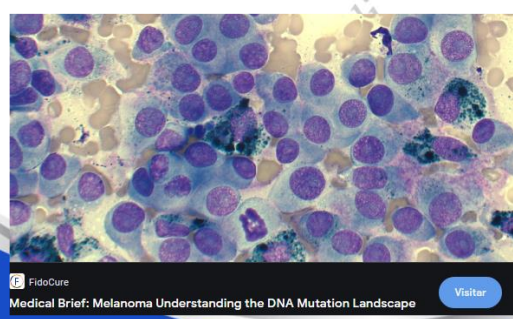
Lymph Node	Cytology Result	Histopathology Result
Normal size on palpation (13)	Equivocal (9)	Negative (9)
	Negative (4)	Negative (2) Metastatic (2)
Enlarged on palpation (15)	Equivocal (6)	Negative (5) Metastatic (1)
	Negative (5)	Negative (4) Metastatic (1)
	Metastatic (4)	Negative (2) Metastatic (2)
	Normal/enlarged* (1)	Equivocal (1) Negative (1)

\*Normal size on palpation at time of cytology and enlarged at time of histopathology.

- Conclusões do estudo
  - Melanófago x melanócito

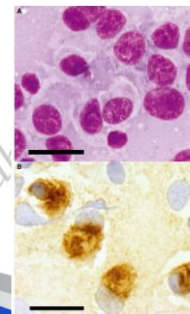


- Conclusões do estudo
  - Melanófago x melanócito



- Conclusões do estudo
  - Melanófago x melanócito
    - Imunocitoquímica
    - “cell block”

ORIGINAL RESEARCH  
**Accuracy of routine cytology and immunocytochemistry in preoperative diagnosis of oral amelanotic melanomas in dogs**  
 Rafał Przędziecki<sup>1</sup>, Michał Czopowicz<sup>2</sup>, Rafał Sapierzyński<sup>1</sup>  
<sup>1</sup>Department of Pathology and Veterinary Diagnostics, and <sup>2</sup>Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Warsaw, Poland



- Conclusões do estudo
  - Melanófago x melanócito

- Conclusões do estudo
  - Painéis imunohistoquímicos
    - Melan A, IBA-1, S-100, PNL-2, HAM-56
  - Amostragem citológica de linfonodos de tamanho normal

Association between lymph node size and metastasis in dogs with oral malignant melanoma: 100 cases (1987–2001)  
 Leonard E. Williams<sup>1</sup>, Rebecca A. Foster  
**Results**—Forty-seven (47%) dogs, of which 23 (49%) had enlarged mandibular lymph nodes, had no cytologic or histologic evidence of metastasis. Of 53 (53%) dogs with cytologic or histologic evidence of mandibular lymph node metastasis, 37 (70%) had enlarged mandibular lymph nodes, and 16 (30%) had mandibular lymph nodes of normal size. Overall, 16 of the 40 (40%) dogs with normal-sized lymph nodes had microscopic evidence of metastatic disease. Sensitivity and specificity of lymph node size as a predictor of metastasis were 70 and 51%, respectively, and the positive and negative predictive values were 62 and 60%, respectively.

Original Article

DOI: 10.1111/j.1476-5829.2009.00185.x

### Cytological lymph node evaluation in dogs with mast cell tumours: association with grade and survival\*

E. L. Krick<sup>1</sup>, A. P. Billings<sup>2</sup>, F. S. Shofer<sup>1</sup>, S. Watanabe<sup>1†</sup>, and K. U. Sorenmo<sup>1</sup>

Evaluation of regional lymph node metastasis in canine cutaneous mast cell tumours  
 Erika L. Krick and Donald J. Meuten



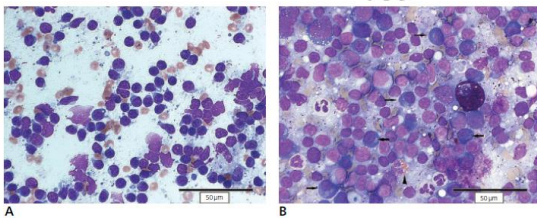
- Justificativa do estudo
  - Baixa reprodutibilidade dos critérios citológicos que definem metástase

- Critérios

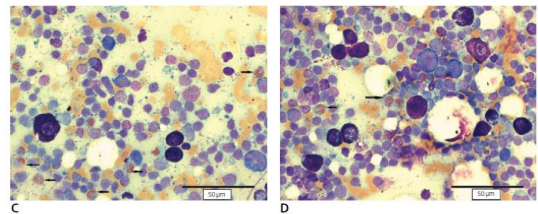
**Table 1.** Cytological criteria utilized by the Clinical Pathology Service at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania for determination of metastatic mast cell disease in regional lymph nodes

Interpretation	Description
Normal	No mast cells seen
Reactive lymphoid hyperplasia	Greater than 50% small lymphocytes with a mixed population of polymorphocytes, lymphoblasts, plasma cells, and/or few to moderate numbers of macrophages, neutrophils, and eosinophils, and/or rare individual mast cells
Possible metastasis	On at least one slide, two to three incidences of mast cells in aggregates of two to three cells
Probable metastasis	On at least one slide, greater than three foci of mast cells in aggregates of two to three cells and/or two to five aggregates of more than three mast cells
Certain metastasis	On at least one slide, effacement of lymphoid tissue by mast cells, and/or the presence of aggregated, poorly differentiated mast cells with pleomorphism, anisocytosis, anisokaryosis, and/or decreased or variable granulation, and/or greater than five aggregates of more than three mast cells

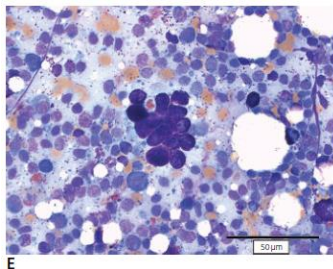
- A) Normal, B) Reativo



- C) Possível, D) Provável



- E) Metástase certa



- Principais resultados
  - Estágio 2 (metástase certa)
    - Maior associação aos MCT's grau III
  - Provável metástase x metástase certa: tempo de sobrevida médio similar

- Vantagens
  - Critérios objetivos (reprodutibilidade)
- Problemas
  - Padrão-ouro ausente (histopatologia dos linfonodos)
  - Coeficiente de correlação inter e intraobservador ausente

**Table 1.** Cytological criteria utilized by the Clinical Pathology Service at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania for determination of metastatic mast cell disease in regional lymph nodes

Interpretation	Description
Normal	No mast cells seen
Reactive lymphoid hyperplasia	Greater than 50% small lymphocytes with a mixed population of prolymphocytes, lymphoblasts, plasma cells, and/or few to moderate numbers of macrophages, neutrophils, and eosinophils, and/or rare individual mast cells
Possible metastasis	On at least one slide, two to three foci/areas of mast cells in aggregates of two to three cells
Probable metastasis	On at least one slide, greater than three foci of mast cells in aggregates of two to three cells and/or two to five aggregates of more than three mast cells
Certain metastasis	On at least one slide, effacement of lymphoid tissue by mast cells, and/or the presence of aggregated, poorly differentiated mast cells with pleomorphism, anisocytosis, anisokaryosis, and/or decreased or variable granulation, and/or greater than five aggregates of more than three mast cells

## Correlation of Nodal Mast Cells with Clinical Outcome in Dogs with Mast Cell Tumour and a Proposed Classification System for the Evaluation of Node Metastasis

K. M. Weishaar, D. H. Thamm, D. R. Worley and D. A. Kamstock

First Animal Cancer Center, Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

### Nodal Metastasis of Canine Mast Cell Tumour

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Table 1

Novel classification system for microscopic evaluation of node metastasis in dogs with mast cell tumour

Classification	Histopathological criteria	Proposed interpretation
HN0	None to rare (0–3), scattered, individualized (isolated) mast cells in sinuses (subcapsular, paracortical or medullary) and/or parenchyma per ×400 field (0–3 mast cells per ×400 field), or does not meet criteria for any other classification below.	Non-metastatic
HN1	Greater than three individualized (isolated) mast cells in sinuses (subcapsular, paracortical or medullary) and/or parenchyma in a minimum of four ×400 fields (unless otherwise stated, at least four ×400 fields each, which contain more than three mast cells)	Pre-metastatic
HN2	Aggregates (clusters) of mast cells (>3 associated cells) in sinuses (subcapsular, paracortical or medullary) and/or parenchymal, or sinusoidal sheets of mast cells	Early metastasis
HN3	Disruption or effacement of normal nodal architecture by discrete foci, nodules, sheets, or overt masses composed of mast cells	Overt metastasis

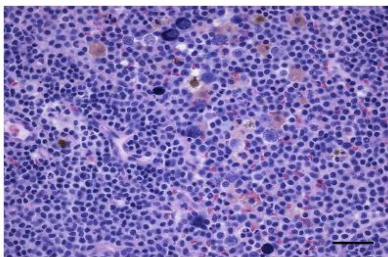


Fig. 2. Canine lymph node with 18, variably granulated, individual mast cells within the nodal parenchyma, consistent with classification HN1. HE. Bar, 50 µm.

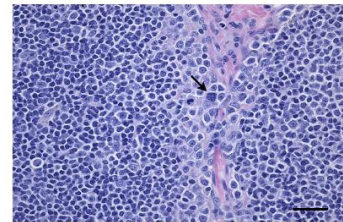


Fig. 1. Canine lymph node demonstrating a single individual mast cell in the paracortical sinus (arrow), consistent with classification HN0. HE. Bar, 50 µm.

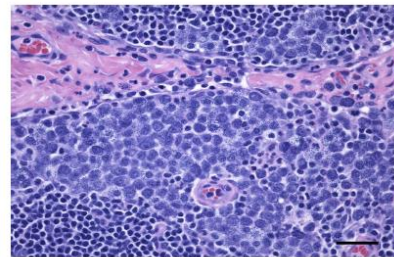


Fig. 4. Canine lymph node with sheets of mast cells in the paracortical sinuses, consistent with classification HN2. HE. Bar, 50 µm.

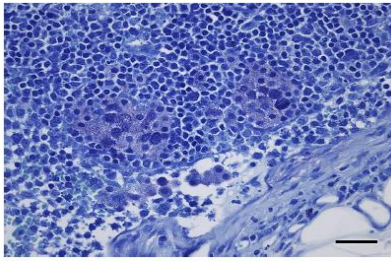


Fig. 3. Canine lymph node with aggregates of mast cells within both the subcapsular sinus and nodal parenchyma, consistent with classification HN2. Toluidine blue. Bar, 50  $\mu$ m.

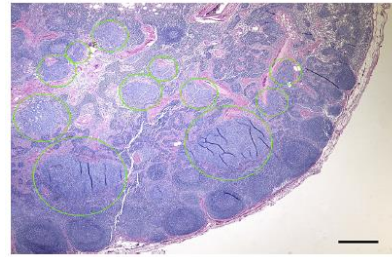


Fig. 5. Canine lymph node with multiple, variably sized, discrete nodules of mast cells (encircled in green) disrupting normal nodal architecture, consistent with classification HN3. Bar, 500  $\mu$ m.

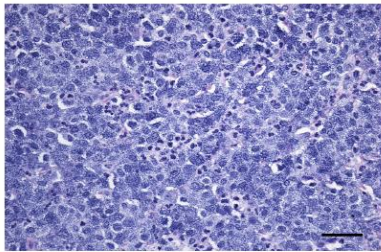
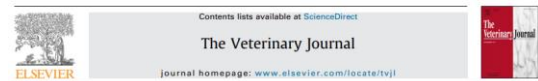


Fig. 6. Canine lymph node. High-power magnification of a single nodule shown in Fig. 5 demonstrating dense and compact sheets of mast cells admixed with eosinophils. HE. Bar, 50  $\mu$ m.



Fig. 7. Canine lymph node demonstrating extensive effacement of normal nodal architecture with replacement by dense sheets of mast cells admixed with eosinophils, consistent with classification HN3. Peripheral residual lymphoid tissue is present. HE. Bar, 500  $\mu$ m.

- Principais resultados
  - Comparação entre as categorias HN agrupadas
    - Intervalo livre da doença e tempo de sobrevida em 2 anos
      - HN2/3: *outcome* pior
      - HN0/1: *outcome* melhor
  - Comparação entre as categorias HN não agrupadas
    - Não houve diferença estatística (número de casos baixo em cada categoria)



Utility of flow cytometry in canine primary cutaneous and matched nodal mast cell tumor

M. Sulce<sup>a</sup>, L. Marconato<sup>b</sup>, M. Martano<sup>c</sup>, S. Iussich<sup>d</sup>, A. Dentini<sup>e</sup>, M. Melega<sup>a</sup>, B. Miniscalco<sup>a</sup>, F. Rondato<sup>a,\*</sup>



**Table 3**  
Percentage of mast cells in lymph nodes detected by cytology and flow cytometry. Histologic classification was done in six cases according to Vitshauer et al. (2014) and cytologic evaluation was done as reported by Krick et al. (2009).

Sample	Cytology (%)	Flow cytometry (%)	Histologic classification	Cytologic classification
1	0	0.2	HN3 <sup>a</sup>	Reactive lymphoid hyperplasia
2	0	5	HN2 <sup>b</sup>	Reactive lymphoid hyperplasia
3	0.2	0.1	HN2	Reactive lymphoid hyperplasia
4	1.6	0.6	-	Probable metastasis
5	2	0.4	-	Probable metastasis
6	2	1.8	HN1 <sup>c</sup>	Probable metastasis
7	5.5	8.9	HN2	Certain metastasis
8	6	7.6	-	Probable metastasis
9	7.7	2.3	HN2	Probable metastasis
10	35.2	42.4	-	Certain metastasis
11	51.5	88.1	-	Certain metastasis
12	88.8	83.1	-	Certain metastasis

<sup>a</sup> HN1 (non-metastatic),  
<sup>b</sup> HN2 (early metastasis),  
<sup>c</sup> HN3 (overt metastasis).

## CITOMORFOLOGIA LINFONODO NORMAL

- LINFÓCITOS PEQUENOS T E B (>80%)
- CENTRÓCITOS (5-10%)
- CENTROBLASTOS (1-5%)
- IMUNOBLASTOS (1-5%)
- MMC (<1% EM CÃES, AUSENTE EM GATOS)
- PLASMÓCITOS (<2%)
- *FLAME CELLS* (<1%)
- *MOTT CELLS* (<1%)
- CÉLULAS DENDRÍTICAS (<1%)
- MACRÓFAGOS (<2%)
- NEÚTRÓFILOS, EOSINÓFILOS E **MASTÓCITOS** (<3% CADA)
- CORPÚSCULOS LINFOGLANDULARES

FORTE BARGER A. SMALL ANIMAL CYTOLOGICAL DIAGNOSIS

### Cytologic comparison of the percentage of mast cells in lymph node aspirate samples from clinically normal dogs versus dogs with allergic dermatologic disease and dogs with cutaneous mast cell tumors

Mast cells play an important role in immunologic, inflammatory, and allergic reactions. It is intuitive that they would be present in LNs of dogs with various disease conditions, and it is widely accepted that mast cells can be present in regional LNs because of nonneoplastic, reactive conditions. Despite this, literature<sup>37-39</sup> exists in which metastasis is described as the presence of any mast cells in an organ. Further complicating the issue, no standardized cytologic or histologic criteria exist for determination of MCT metastasis to the LNs. Smears of LN aspirate samples from clinically normal dogs in 1 study<sup>37</sup> contained between 1 and 16 well-differentiated mast cells/slide, for a mean of 6.4 cells/slide. In that study,<sup>37</sup> only 1 slide/dog was evaluated and no description was given as to which LNs were chosen for evaluation. It was later suggested that MCT metastasis to the LNs can be diagnosed by cytologic examination if mast cells represent > 3% of the cell population in LN aspirate samples.<sup>40</sup> If that criterion were used, metastatic MCT would be diagnosed in up to 25% of clinically normal dogs,<sup>41</sup> emphasizing again that more standardized criteria are needed for determination of MCT metastasis to the LNs. In the study reported here, clinically normal dogs and dogs with allergic skin disease (primarily atopy) had a median of 0 to 1 mast cell/2,000 cells counted in LN aspirate samples. Dogs with MCTs had a median of 8 mast cells/2,000 cells counted, which would represent 0.4% of the cell population evaluated for these dogs.