Full title:

A METASTATIC SECRETORY GASTRIC PLASMACYTOMA WITH ABERRANT CD3 EXPRESSION IN A DOG

Running title:

CANINE CD3+ GASTRIC EXTRAMEDULLARY PLASMACYTOMA

Atherton MJ, Vazquez-Sanmartin S, Sharpe S, Waugh EM & Morris JS

Matthew J Atherton, McMaster Immunology Research Centre. Corresponding author. Email- athertmj@mcmaster.ca. Mailing- McMaster Immunology Research Centre, Department of Pathology and Molecular Medicine, McMaster University, 1280 Main St W, Hamilton, Ontario, L8S 4K1, Canada

Samantha Vazquez-Sanmartin, Oncology Unit, Animal Health Trust.

Sam Sharpe, Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary

Elspeth M Waugh, College of Medical, Veterinary and Life Sciences, University of Glasgow

Joanna S Morris, College of Medical, Veterinary and Life Sciences, University of Glasgow

ABSTRACT

A 10-year old crossbreed dog presented with a six-week history of hematemesis, melena, anorexia and lethargy. Clinical staging revealed a gastric mass with regional lymphadenomegaly as well as a monoclonal gammopathy manifesting as hyperglobulinemia. Cytologic and histopathologic analyses were consistent with a round cell neoplasm; neoplastic cells showed nuclear immunoreactivity for MUM1 and diffuse cytoplasmic reactivity for CD3. PCR performed on fixed and fresh tissue identified a clonal rearrangement with an IgH primer set. An extramedullary plasmacytoma (EMP) was confirmed by cellular morphology and molecular diagnostics. Following an objective response to chemotherapy the dog was euthanized 8 months after diagnosis and a post mortem examination confirmed the clinical findings. This is the first reported case of a monoclonal gammopathy secondary to a gastric EMP coupled with aberrant expression of CD3 in an aggressive plasmacytic tumor and highlights the utility of molecular diagnostics for classifying atypical hemolymphoid neoplasms.

Key words:

Atypical immunophenotype; plasmacytic tumor; PCR

Case Presentation

A 10yr old female neutered crossbred dog presented with a 6-week history of lethargy, anorexia, vomiting with progression to hematemesis and melena. Abdominal palpation was resented but physical examination was otherwise unremarkable. Hematology showed a mild non-regenerative anemia ($4.69x10^{12}$ erythrocytes/L; reference range 5.5-8.5x10^{12} erythrocytes/L). Serum biochemistry revealed hyperproteinemia (88 g/L; reference range 50-78 g/L) with hyperglobulinemia (66 g/L; reference range 28-42 g/L), hypoalbuminemia (22 g/L; reference range 29-36 g/L) and mildly elevated aspartate transaminase (73 U/L; reference range <40 U/L). A narrow spike in the gamma region of the densitometric trace of an agarose gel serum protein electrophoretic trace was present, consistent with a monoclonal gammopathy. An extensive, circumferential mass affecting the majority of the body of the stomach and progressing into the pylorus was present on abdominal ultrasound with mural thickening and loss of normal layering. Several hypoechoic masses of variable size (ranging in diameter from 13.9 mms to 53.5 mms), surrounded by reactive hyperechoic tissue, were observed in the mesentery caudal to the stomach, consistent with mesenteric lymphadenopathy. Two left-sided lymph nodes adjacent to the left kidney ~~were~~ appeared~~also~~ abnormally hypoechoic and a single well-defined hypoechoic hepatic nodule (6.5 mm in diameter) was present. A small amount of free fluid cranial to the bladder was noted. Thoracic radiographs revealed an enlarged sternal lymph node. Bone marrow aspiration as part of a complete staging process revealed normal myeloid and erythroid series without neoplastic infiltrate. A working diagnosis of metastatic gastric neoplasia was reached.

Fine-needle aspirates of the mesenteric lymph nodes were obtained and submitted for cytology. Modified Wright-Giemsa staining of slides revealed a pleomorphic population of round cells. Cellular

margins varied along a spectrum from indistinct to well defined. Nuclei number was highly variable ranging from mono to multinucleated with moderate to large amounts of basophilic and occasionally vacuolated cytoplasm present**.** Some cells exhibited perinuclear halos. Chromatin was described as being clumped or coarsely reticulated. Morphology was consistent with a round cell neoplasm with plasmacytoid features (Figure 1a). As an atypical hemolymphoid malignancy was suspected immunocytochemistry (ICC) was performed using monoclonal antibodies raised against CD3 (1:100; Dako, Glostrup, Denmark) and CD79a (1:100; Dako, Glostrup, Denmark) antigens. Neoplastic cells exhibited dual positivity for both antigens, thus a definitive cytologic diagnosis could not be reached.

Histologic evaluation of a needle-core biopsy of one of the affected mesenteric lymph nodes confirmed the presence of a malignant round cell population, obliterating normal nodal architecture. The morphology of the cells reflected the cytologic findings; mitotic figures were abnormal but mitotic rate was low (4 per 10 HPF). Very large cells were frequently observed with nuclear diameters in excess of 40 µms recorded. Marked anisocytosis and anisokaryosis were noted with numerous bi- and multinucleated cells present (Figure 1b). An immunohistochemical panel (IHC) revealed moderate neoplastic reactivity against CD79a (1:100; Dako, Glostrup, Denmark) in 20% of neoplastic cells and marked nuclear reactivity against multiple myeloma oncogene 1 (MUM1) (1:50; Dako, Glostrup, Denmark) in virtually all cells (Figure 1c). Cells also exhibited diffuse cytoplasmic reactivity against CD3 (1:100; Dako, Glostrup, Denmark) (Figure 1d) in 50% of the population and this finding was consistent when staining was repeated. Neoplastic cells were negative for CD18 (1:20; UC Davis Leukocyte Antigen Biology Lab, Davis, CA) and Pax5 (1:100; Dako, Glostrup, Denmark). Histology was consistent with a neoplastic population of lymphoid lineage, the main differentials being a functional B cell tumor, T cell or double positive neoplasm with an associated monoclonal gammopathy.

PCR was performed on fresh and formalin fixed paraffin embedded (FFPE) tumor tissue using TCRγ and IgH primer sets. ~~with a clonal rearrangement detected with one set of the IgH primers and polyclonal products detected with the remaining IgH and TCRγ primers (Figure 2).~~ DNA was extracted using QIAamp DNA Mini kits or DNA FFPE Tissue kits (Qiagen Ltd, Manchester, UK). PCR was carried out using primers described by Gentilini et al [1] and Chaubert et al [2], with modifications to reaction conditions. Briefly, reactions were performed in a total volume of 25 µl, and contained 100 ng DNA, each primer at 250 nM (IDT, Leuven, Belgium) and 1 x HotStarTaq Plus Master Mix (Qiagen Ltd, Manchester, UK). Thermal cycling was carried out on a GeneAmp PCR System 9700 (Applied Biosystems, Life Technologies, Waltham, MA) using the following conditions: 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, with a final extension of 72 °C for 30 minutes. Products were visualised using GeneScan methodology on an ABI 3130xl Genetic Analyser (Applied Biosystems, Life Technologies, Waltham, MA) with a 36 cm capillary length loaded with POP-4 polymer. Appropriate electrophoretic readouts were obtained from the positive (Figure 2a.) and negative (Figure 2b.) control reactions. A clonal product of approximately 100 bases was amplified with one set of the IgH primers used (Figure 2c). With the remaining IgH and TCRγ primer sets either no product or polyclonal/ skewed polyclonal products were detected (Figures 2d, e, f, g and h). These PCR results confirmed a tumor of B cell lineage and combined with the morphologic features and the gammopathy a secretory plasmacytoma with aberrant CD3 expression was diagnosed.

Pending the PCR result, the dog was initially treated for a presumptively diagnosed anaplastic lymphoid neoplasm with one dose of L-Asparaginase (4000 IU SC) and received prednisolone at 2mg/kg PO q24h. After definitive classification of the tumor as a plasmacytoma the dog was prescribed melphalan (2mg PO q48h) and the prednisolone was gradually tapered over the following month to 0.5mg/kg q48h. One month after presentation t~~T~~he hyperglobulinemia normalized to 32

g/L following initiation of melphalan chemotherapy and repeat abdominal ultrasound ~~one month after presentation~~ revealed a marked reduction in size of both the gastric mass and associated nodes with resolution of the hepatic nodule. The gastric mass had resolved and the nodes were smaller again when ultrasound was performed at three months, however at six months progressive disease was noted ultrasonographically with tumor recurrence in the stomach and associated lymphoid tissue. Although at this time point the dog was clinically well, with normal hematology and biochemistry parameters (globulin of 36 g/L), the owners declined rescue therapy and elected for euthanasia shortly after when clinical signs recurred (over-all survival time from first chemotherapy was 219 days).

Post-mortem examination was performed. Gross pathology and histopathology were consistent with a metastatic plasma cell tumor involving stomach, pancreas, gastric nodes, liver, mesentery, esophagus, diaphragm and spleen. The histologic appearance of the necropsy samples was consistent with the biopsy specimen, but cellular pleomorphism and mitotic rate were increased. Post-mortem findings confirmed clinically advanced neoplasia, which explained the clinical relapse and confirmed ante-mortem diagnosis.

DISCUSSION

The case reported here is a very uncommon presentation of canine stomach cancer; gGastric neoplasms are diagnosed infrequently in dogs, accounting for less than 1% of all tumors and gastric extramedullary plasmacytomas (EMPs) are rarely reported [3]. Typically EMPs are benign small solitary masses found in the skin and the oral cavity[3]. Gastrointestinal EMPs tend to be more aggressive with associated lymphatic metastasis [4]. Monoclonal gammopathy is frequently part of the multiple myeloma (MM) clinical syndrome but is less frequently reported secondary to non-cutaneous EMPs [4]. Only two other cases of secretory gastrointestinal EMP have been reported in dogs, both of which were intestinal [5,6]. This is the first reported case of a secretory gastric EMP.

Gastrointestinal EMPs, such as the case reported here, represent a rarely encountered form of canine plasma cell pathology. Plasma cells are a terminally differentiated population of B cells that produce immunoglobulin and their malignant transformation can manifest as a spectrum of different neoplastic entities. Both multiple myeloma and IgM (Waldenstom's) macroglobulinemia are reported in dogs as systemic disease syndromes with bone marrow infiltration critical for diagnosis [7]. Alternatively solitary plasmacytomas can occur either intraosseously or in an extremedullary form (EMP) [4]. Gastrointestinal EMPs are infrequently reported in dogs but generally behave in aggressive manner with frequent nodal metastasis [4,7]. Only one previous report of a gastric plasmacytoma with nodal metastasis exists in a dog, however it was non-secretory[8]. The case reported here is a unique presentation of a gastric EMP with distant metastasis and an associated monoclonal gammopathy.

Diagnosis of this gastric neoplasm was partly facilitated by the application of panels of ~~Various~~ immuno-cytologic and ~~/~~ -histologic cellular markers ~~are available~~ with the aim of ~~to~~ identifying ~~aid with~~ hematopoietic cell lineage ~~identification. of hematopoietic malignancies.~~ For canine plasmacytic tumors, multiple myeloma oncogene 1/ interferon regulatory factor 4 (MUM1/IRF4) is a transcription factor with a key role in plasma cell production, which is a specific and sensitive immunohistochemical marker [9]. A second transcription factor, Pax5, is involved in B cell development and acts as a useful immunohistochemical marker for identifying canine B cell lymphoma but is absent in differentiated plasma cells [10]. The expression of the cell surface markers CD79a (a B cell receptor signaling component) and CD18 (a leukocyte adhesion molecule subunit) are detected variably using antibodies for immunohistochemistry in canine plasma cell neoplasia [9]. Cell membrane staining for CD3, a signaling protein associated with the T cell receptor, has been used to identify canine T cells for over 20 years [11] and has been documented in aggressive human plasma cell tumors as an exceedingly rare occurrence [12]. Interestingly a 67-year-old male patient previously diagnosed with multiple myeloma subsequently developed gastrointestinal bleeding with clinical investigation revealing the presence of CD3 positive neoplastic plasma cells within the stomach, however any possible relationship between CD3 positive plasma cells and gastrointestinal localization would be highly speculative given the rarity of such pathology in both species [12]. In a case series classifying lymphoid malignancies in the dog and cat by the WHO system, two indolent cases of canine plasmacytoma were reported as positive for both CD3 and CD79a, however no additional information was given and diagnosis was based solely on histopathologic morphology [13]. Understanding the physiologic roles of marker molecules and appreciating the limitations of immunophenotypic markers assisted with the definitive diagnosis of EMP in this case.

The final diagnosis of EMP with aberrant immunophenotypic features was reached using a combination of clinicopathologic and molecular tests, namely histopathologic and cytologic

morphology alongside a monoclonal gammopathy and notably a clonal IgH rearrangement. Co-expression of T cell (e.g. CD3) and B cell lineage (e.g. CD79a) markers can be an aberrant finding and at times confound the diagnosis of lymphoid neoplasms in dogs [7]. As documented in this case, monoclonal gammopathies are usually associated with B-cell neoplasms although rare, however although in human oncology monoclonal gammopathy monoclonal gammopathies secondary to T cell neoplasms have has also been reported; in human oncology but this has not been observed documented in dogs, however [14,15]. In cases of hemolymphoid malignancy which have ambiguous results, PCR for clonality of antigen receptor rearrangements is usually considered the diagnostic method of choice [16], as proved to be the case for the EMP reported here. Additional diagnostic confirmation was demonstrated by shrinkage of the mass in response to the appropriate treatment for an aggressive plasma cell neoplasm, achieving an objective, albeit short-lived clinical response with excellent quality of life. The case reported here not only documents for the first time, aberrant CD3 expression in an aggressive canine gastric EMP with monoclonal gammopathy but also emphasizes the role of molecular diagnostics in ensuring appropriate therapeutics for veterinary patients.

REFERENCES

1. Gentilini F, Calzolari C, Turba ME, Bettini G, Famigli-Bergamini P. GeneScanning analysis of Ig/TCR gene rearrangements to detect clonality in canine lymphomas. *Vet Immunol Immunopathol*. 2009;127(1-2):47-56.

2. Chaubert P, Chaubert ASB, Sattler U, et al. Improved Polymerase Chain Reaction–Based Method to Detect Early-Stage Epitheliotropic T-Cell Lymphoma (Mycosis Fungoides) in Formalin-Fixed, Paraffin-Embedded Skin Biopsy Specimens of the Dog. *J Vet Diagn Invest*. 2010;22(1):20-29.

3. Withrow SJ. Cancer of the Gastrointestinal Tract, Gastric Cancer. In: Withrow SJ, Vail DM, Page RL. *Withrow and MacEwen's Small Animal Clinical Oncology (Fifth Edition)*. Saint Louis, MO: W.B. Saunders; 2013: 402-404.

4. Jacobs RM, Messick JB, Valli VE. Tumors of the Hemolymphatic System. In: Meuten DJ, ed. *Tumors in Domestic Animals*. Ames, IA: Iowa State Press; 2002:119-198.

5. Jackson MW, Helfand SC, Smedes SL, Bradley GA, Schultz RD. Primary IgG secreting plasma cell tumor in the gastrointestinal tract of a dog. *J Am Vet Med Assoc*. 1994;204(3):404-406.

6. Trevor PB, Saunders GK, Waldron DR, Leib MS. Metastatic extramedullary plasmacytoma of the colon and rectum in a dog. *J Am Vet Med Assoc*. 1993;203(3):406-409.

7. Vail DM. Hematopoietic Tumors, Myeloma-related disorders In: Withrow SJ, Vail DM, Page RL. *Withrow and MacEwen's Small Animal Clinical Oncology (Fifth Edition)*. Saint Louis, MO: W.B. Saunders; 2013: 665-678.

8. Brunnert SR, Dee LA, Herron AJ, Altman NH. Gastric extramedullary plasmacytoma in a dog. *J Am Vet Med Assoc*. 1992;200(10):1501-1502.

9. Ramos-Vara JA, Miller MA, Valli VEO. Immunohistochemical detection of multiple myeloma 1/interferon regulatory factor 4 (MUM1/IRF-4) in canine plasmacytoma: comparison with CD79a and CD20. *Vet Pathol*. 2007;44(6):875-884.

10. Willmann M, Müllauer L, Guija de Arespacochaga A, Reifinger M, Mosberger I, Thalhammer JG. Pax5 immunostaining in paraffin-embedded sections of canine non-Hodgkin lymphoma: a novel canine pan pre-B- and B-cell marker. *Vet Immunol Immunopathol*. 2009;128(4):359-365.

11. Ferrer L, Fondevila D, Rabanal R, Ramis A. Detection of T lymphocytes in canine tissue embedded in paraffin wax by means of antibody to CD3 antigen. *J Comp Pathol*. 1992;106(3):311-314.

12. Tang YL, Chau CY-P, Yap WM, Chuah KL. CD3 expression in plasma cell neoplasm (multiple myeloma): a diagnostic pitfall. *Pathology (Phila)*. 2012;44(7):668-670.

13. Vezzali E, Parodi AL, Marcato PS, Bettini G. Histopathologic classification of 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO. *Vet Comp Oncol*. 2010;8(1):38-49.

14. Viny AD, Lichtin A, Pohlman B, Loughran T, Maciejewski J. Chronic B-cell dyscrasias are an important clinical feature of T-LGL leukemia. *Leuk Lymphoma*. 2008;49(5):932-938.

15. Lin P, Hao S, Handy BC, Bueso-Ramos CE, Medeiros LJ. Lymphoid neoplasms associated with IgM paraprotein: a study of 382 patients. *Am J Clin Pathol*. 2005;123(2):200-205.

16. Thalheim L, Williams LE, Borst LB, Fogle JE, Suter SE. Lymphoma immunophenotype of dogs determined by immunohistochemistry, flow cytometry, and polymerase chain reaction for antigen receptor rearrangements. *J Vet Intern Med*. 2013;27(6):1509-1516.

FIGURE LEGENDS

Figure 1: Cytologic and histologic images from a mesenteric lymph node.

1a: Cytologic smear of fine needle aspirate taken from a mesenteric lymph node and stained with modified Wright-Giemsa revealing a pleomorphic population of malignant round cells with erythrocytes and neutrophils noted in the background (x40 objective lens).

1b: Needle-core biopsy taken from a mesenteric lymph node and stained with haematoxylin and eosin showing the same population of malignant round cells obliterating normal nodal architecture (x10 objective lens).

1c: Immunohistochemical staining of a needle-core biopsy taken from a mesenteric lymph node revealing marked nuclear reactivity against MUM1 (x20 objective lens).

1d: Immunohistochemical staining of a needle-core biopsy taken from a mesenteric lymph node revealing diffuse cytoplasmic reactivity against CD3 (x20 objective lens).

Figure 2: PCR electrophoretograms from fresh mediastinal lymph node aspirate sample. Approximate product size in bases (b) is indicated at the top of each plot. Amplification products are in blue or green; size markers are in red.

2a: Positive DNA amplification controls in green (Cμ; approx. 128 b) and blue (γ-actin; approx. 272 b).

2b: No template control. No amplification within the expected size range is seen.

2c: Clonal product of approx. 100 b amplified using an immunoglobulin heavy chain (IgH) primer set. A clonal product of the same length was amplified from FFPE material from this site using the same primer set, and also from post-mortem material from mediastinal lymph node and stomach mass.

2d,e,f: Polyclonal or skewed polyclonal products are amplified using T-cell receptor gamma primer sets.

2g,h: Polyclonal or poor amplification is noted with a further four IgH primer sets.