



Cytology Over Size: Malignant Cytology in a Large Canine Mammary Mass without Metastasis

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Abstract | Mammary tumors are among the most frequently diagnosed neoplasms in intact female dogs, involves prolonged hormonal stimulation, genetic mutations, and environmental–metabolic influences that disrupt cell growth regulation, leading to uncontrolled proliferation, malignancy, and potential metastasis. Canine mammary tumor development involves activation of PI3K/AKT/mTOR, Wnt/ β -catenin, HER2/neu, and JAK/STAT pathways together with p53 inactivation and VEGF-mediated angiogenesis, promoting uncontrolled proliferation, dedifferentiation, and metastasis. This study aims to describe the diagnostic evaluation and clinical management of a suspected malignant mammary tumor in a geriatric female dog, emphasizing the diagnostic value of Fine Needle Aspiration (FNA). The novelty lies in the demonstration of FNA as a rapid, minimally invasive, and reliable diagnostic approach for assessing tumor malignancy in geriatric dogs, while also integrating the One Health framework to highlight shared hormonal and environmental risk factors between animals and humans. A 13-year-old intact female Golden Retriever dog presented with a firm, poorly defined, immobile mass measuring 8.2 cm in diameter in the right inguinal mammary gland. Cytological analysis revealed pleomorphic epithelial cells with prominent nucleoli, vacuolated cytoplasm, and anaplasia, features consistent with malignant neoplasia. Thoracic radiographs showed no evidence of pulmonary or regional metastases. Surgical excision (lumpectomy) was carried out as the primary treatment. Histopathological confirmation was not available in this case because surgical excision or biopsy of the mass was not performed due to the owner's financial constraints and concern about the animal's condition. The cytological findings, in conjunction with clinical and radiological assessments, supported the diagnosis of a malignant mammary tumor. This study highlights a key clinical gap in canine mammary tumor diagnosis, where tumor size alone cannot reliably indicate malignancy. Cytological evaluation provides a rapid, minimally invasive, and cost-effective tool to assess tumor behavior before surgical intervention. The findings emphasize the importance of integrating cytology into routine diagnostic protocols to avoid misclassification and delayed treatment. Early detection and timely intervention remain essential for improving prognosis in canine mammary tumors.

Keywords | Canine, Disease, Fine needle aspiration, Tumor, Anaplasia, Non-invasive

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The mammary gland in female dogs is a modified apocrine gland composed of alveoli and ducts embedded within connective tissue, blood vessels, and nerves. Dogs possess five pairs of mammary glands two thoracic, two abdominal, and one inguinal with the inguinal glands being the most frequently affected by neoplastic growths. Mammary tumors can arise from epithelial, myoepithelial, or stromal components of the gland. These neoplasms are the most commonly diagnosed tumors in intact female dogs, with approximately 50% reported as malignant (Hazirolu *et al.*, 2010).

Benign mammary tumors typically present as small, well-circumscribed, slow-growing, and non-painful masses. In contrast, malignant tumors are often characterized by rapid growth, irregular margins, adhesion to underlying tissues, ulceration, and possible hemorrhage (Shabrina *et al.*, 2023). In this case, the tumor was firm in consistency, poorly defined in shape, and immobile, suggesting adherence to the underlying tissue and a higher likelihood of malignancy. Metastasis may occur, primarily targeting regional lymph nodes and lungs, but may also spread to the liver, bones, and brain in advanced cases (Misdorp, 2002; Sorenmo *et al.*, 2011).

Cancer, including mammary tumors, remains a major cause of mortality in aging dogs, particularly those older than 10 years (Rickyawan *et al.*, 2022; Vazquez *et al.*, 2023a). Older dogs experience cumulative hormonal stimulation and genomic instability, predisposing mammary epithelial cells to malignant transformation (Vazquez *et al.*, 2023b). Interestingly, the incidence of mammary tumors in dogs is approximately three times higher than in humans (Pastor *et al.*, 2018), rendering canine mammary tumors an excellent natural model for studying human breast cancer pathogenesis and therapy (Ushio *et al.*, 2023). This similarity extends to hormonal dependency, molecular subtype classification, metastatic behavior, and response to treatment, where canine tumors closely mimic human breast cancer, thereby offering valuable translational insights for comparative oncology (Bhutta and Choi, 2025). Beyond hormonal factors, environmental and lifestyle-related influences are increasingly recognized in tumor pathogenesis (Bollati *et al.*, 2023). Carcinogenic exposures from environmental pollution, endocrine-disrupting compounds, and high-fat diets contribute to the development of cancer (Olaifa *et al.*, 2025). Obesity, particularly in early life, further elevates the risk and is also linked to more aggressive tumor phenotypes (Xu *et al.*, 2025).

This case report aims to provide a comprehensive clinical, cytological, and radiographic assessment of a mammary

tumor in a geriatric intact female dog, emphasizing the diagnostic value of Fine Needle Aspiration (FNA) as a minimally invasive approach.

MATERIALS AND METHODS

A 13-years old intact multiparous female Golden Retriever was presented to Maha Pet Care Animal Clinic Bandung, Indonesia with a history of a progressively enlarging mammary mass on the right inguinal gland, initially noted one year prior, with accelerated growth over the past six months. The dog had a body condition score of 6/9. The dog was alert and responsive, with a stable appetite and no overt signs of systemic illness. The case was subsequently examined and managed at Maha Pet Care Animal Clinic Bandung, where diagnostic, cytological of mammary mass, and radiographic evaluations were performed.

CLINICAL EXAMINATION

A general physical examination was performed to assess the patient's overall health status. Vital parameters temperature, heart rate, respiratory rate, mucous membrane color, capillary refill time, and hydration status were within normal limits. No significant abnormalities were observed in behavior or general condition, and no lymphadenopathy was detected on palpation.

MAMMARY GLAND ASSESSMENT

Systematic palpation of all mammary glands (from cranial thoracic to caudal inguinal) revealed a solitary, firm, and poorly mobile mass measuring 8.2 cm in the right inguinal gland. Clinically, the mass had irregular borders and was firmly attached to the underlying tissues, but no ulceration or pain was noted upon palpation. Although these features may not be typical of highly aggressive malignancies, such presentation can occur in slowly developing mammary carcinomas.

CYTOLOGICAL EVALUATION (FINE NEEDLE ASPIRATION BIOPSY)

After aseptic preparation, FNA was performed using a 22-gauge needle attached to a 5 mL syringe. The aspirated material was transferred onto glass slides, air-dried, and stained with Giemsa for microscopic examination (Ciliberti *et al.*, 2024). The cytological findings, characterized by epithelial cells with multiple prominent nucleoli, marked pleomorphism, and vacuolated cytoplasm, are consistent with a malignant epithelial neoplasm. Additional cytomorphological features such as coarse chromatin, anisocytosis, anisokaryosis, and loss of cellular cohesion are observed in the smear. Although cytology provides strong evidence of malignancy, histopathological evaluation remains the gold standard for definitive diagnosis.

RADIOGRAPHIC ASSESSMENT

Thoracic radiographs were taken in right lateral and ventrodorsal projections to evaluate for pulmonary metastasis (Kusumawardani *et al.*, 2021). The lungs showed no radiographic signs of metastatic nodules, and the mediastinum and cardiac silhouette appeared unremarkable. No evidence of lymphadenopathy or thoracic involvement was found.

SURGICAL INTERVENTION

Standard general anesthesia was applied for the surgical procedure. The regional inguinal lymph node was palpated and found to be normal; however, fine-needle aspiration was not performed as the owner declined additional diagnostic procedures. A lumpectomy was selected rather than a radical mastectomy due to the patient's advanced age, the absence of detectable metastasis, and financial limitations of the owner. The dog was placed in dorsal recumbency, and the surgical site was shaved and disinfected. A skin incision was made over the mass, followed by careful blunt and sharp dissection to remove the mass along with margins of surrounding tissue. Hemostasis was achieved using electrocautery, and the wound was closed in multiple layers with absorbable sutures for the subcutis and non-absorbable sutures for the skin (Yilmaz *et al.*, 2011).

RESULTS

A 13-years old intact female Golden Retriever weighing 24.6 kg was presented with a history of a mammary mass that had been present for approximately one year and had shown rapid enlargement over the past six months, accompanied by a decreased appetite. On physical examination, the mass was firm in consistency, measuring approximately 8.2 cm in diameter, and located in the mammary region (Figure 1A). The overlying skin was intact, and no ulceration or pain was observed upon palpation. The regional lymph nodes were not enlarged, and other vital parameters were within normal limits. Thoracic radiographs were obtained in both left lateral (Figure 1C) and ventrodorsal (Figure 1D) projections to evaluate the potential for metastatic spread. Radiographic assessment revealed no abnormalities in the lungs, heart, or other thoracic structures. No evidence of pulmonary nodules, mediastinal masses, or pleural effusion was observed. These findings suggest that, at the time of examination, the mammary tumor was confined to the primary site without detectable metastasis to the thoracic organs. The absence of radiographic signs of distant spread is consistent with a localized disease stage and supports the consideration of surgical excision as a primary management approach.

The cytological examination using Fine Needle Aspiration (FNA) demonstrated epithelial cells with multiple

prominent nucleoli, marked pleomorphism, anisocytosis, coarse chromatin, and vacuolated cytoplasm (Figure 1B). The presence of conspicuous nucleoli reflects increased nucleic acid and protein synthesis, a hallmark of active malignant proliferation. Cellular pleomorphism indicates a loss of uniformity and structural regulation, which is commonly observed in high-grade mammary tumors (Ismail *et al.*, 2021). Meanwhile, cytoplasmic vacuolation may represent degenerative changes or enhanced secretory activity associated with neoplastic transformation. Collectively, these cytomorphological features support the diagnosis of a malignant mammary neoplasm, cytologically consistent with carcinoma (Queiroga *et al.*, 2005; Hazirolu *et al.*, 2010). These cytological features align with established diagnostic criteria for malignancy in canine mammary tumors, emphasizing the critical role of FNAB in differentiating benign from malignant lesions prior to surgical excision (Sewoyo *et al.*, 2023). While cytology alone cannot establish a definitive diagnosis, it offers significant diagnostic value, especially when histopathology is not feasible.

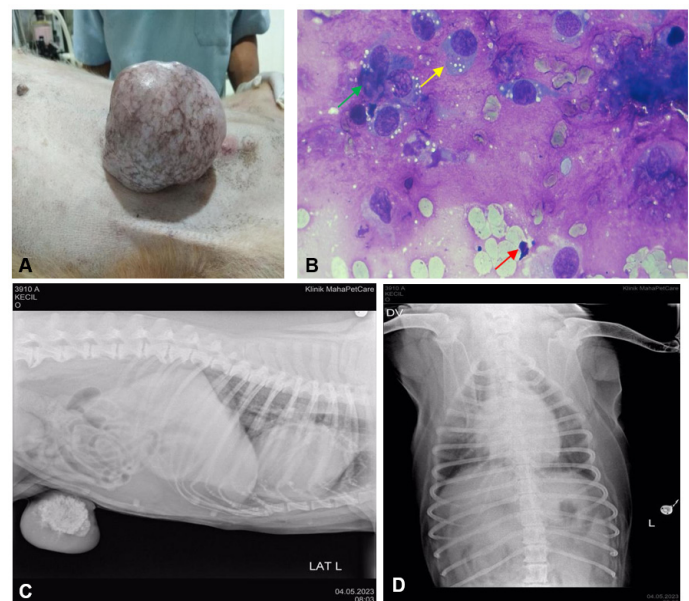


Figure 1: (A) Macroscopic image of a firm, poorly circumscribed mass measuring approximately 8.2 cm in diameter located in the right inguinal mammary gland of a female Golden Retriever dog. The mass appeared non-ulcerative, immobile, and poorly demarcated from surrounding tissues. (B) Cytological smear from fine needle aspiration biopsy (FNAB) showing malignant features, including cellular pleomorphism (green arrow), prominent nucleoli (red arrow), and vacuolated cytoplasm (yellow arrow), consistent with epithelial mammary carcinoma (Giemsa stain, 1000x). (C–D) Thoracic radiographs in left lateral (C) and ventrodorsal (D) projections. Radiographic evaluation showed no evidence of metastatic spread to the lungs or thoracic organs, indicating the tumor was likely confined to the primary site at the time of examination.

Canine mammary neoplasms arise under the influence of hormonal, metabolic, and environmental factors, and this patient exhibited multiple high-risk characteristics, including advanced age, intact reproductive status, and delayed spaying (Rickyawan *et al.*, 2023). Estrogen and progesterone stimulate mammary epithelial proliferation and may promote neoplastic transformation by enhancing growth hormone (GH) and insulin-like growth factor I (IGF-I) activity, reducing apoptosis, and inducing genotoxic metabolites (Russo and Russo, 2004).

Interestingly, both benign and malignant mammary tumors can present as large, palpable masses, making clinical assessment based on size alone insufficient for accurate differentiation (Shi *et al.*, 2023). While physical examination may provide initial information regarding the consistency, mobility, and surface characteristics of the mass, it cannot reliably distinguish between benign hyperplasia, adenoma, or malignant neoplasms (Viëtor *et al.*, 2021). Cytological analysis, such as Fine Needle Aspiration (FNA), is therefore indispensable, as it allows detailed evaluation of cellular architecture, including nuclear atypia, mitotic activity, chromatin patterns, nucleolar prominence, and cytoplasmic characteristics, all of which are key indicators of malignant transformation (Pakdeesaneha *et al.*, 2024). Furthermore, cytology can reveal subtle features of aggressiveness, such as loss of cellular cohesion and vacuolation, that are not apparent through palpation or gross examination (El-Chamieh *et al.*, 2022). Consequently, relying solely on tumor size as a prognostic parameter is potentially misleading, emphasizing the need for a combined approach integrating clinical, cytological, and, when necessary, histopathological assessments to guide diagnosis and management of canine mammary neoplasia (Quanyang *et al.*, 2024). Obesity and synthetic hormone exposure (e.g., medroxyprogesterone acetate) have also been implicated in mammary carcinogenesis. Environmental endocrine disruptors and high-fat diets contribute to cumulative risk, further highlighting the One Health implications of this disease. Companion animals such as dogs serve as sentinels of shared environmental exposure to carcinogens that also pose risks to human health (Maharjan *et al.*, 2021; Crafts *et al.*, 2022).

In this case, Fine Needle Aspiration (FNA) provided rapid, minimally invasive, and practical diagnostic guidance, allowing clinicians to make timely decisions regarding surgical intervention, specifically lumpectomy, without waiting for formal histopathological analysis. FNA enabled early evaluation of cellular morphology, including nuclear atypia, pleomorphism, prominent nucleoli, and cytoplasmic characteristics, collectively suggesting malignancy (Behzatoğlu and Schmitt, 2022). Although FNA cannot

replace histopathology in providing definitive tumor grading, margin assessment, or precise identification of histologic subtypes, it serves as a valuable initial diagnostic tool, particularly in general veterinary practice or resource-limited settings where prompt surgical planning is necessary (Shen *et al.*, 2025). Early cytological assessment also helps prioritize cases for intervention, reduces patient morbidity, and guides selection of additional imaging or staging procedures (Pedro and Wood, 2025). It should be noted that histopathological confirmation and grading of the excised mass were not performed in this case, limiting the ability to determine tumor invasiveness, exact subtype, and prognostic classification. Moreover, as a single case report, these findings cannot be generalized to the wider canine population. Future studies are recommended to combine cytological, histopathological, and molecular analyses in larger cohorts to validate diagnostic reliability and to explore the molecular mechanisms underlying canine mammary tumorigenesis. This case underscores the importance of integrating clinical examination, cytology, and imaging in the assessment of canine mammary tumors. While surgical excision remains the definitive treatment, early diagnosis through FNA and awareness of multifactorial risk factors are essential to guide prognosis, management, and client education.

CONCLUSION

Fine Needle Aspiration proves to be a valuable, rapid, and minimally invasive tool for diagnosing mammary tumors in female dogs. Despite the large tumor size, cytological evaluation accurately confirmed malignancy without evidence of metastasis. This case emphasizes that tumor size alone does not determine malignancy and highlights the importance of combining cytology with clinical and radiographic assessments for optimal management of canine mammary neoplasia.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this case report are fully available within the article. Any additional clinical, cytological, or imaging information can be obtained from the corresponding author upon reasonable request.

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We sincerely thank the dog owner for their cooperation and for granting permission to publish the clinical data presented in this case report.

NOVELTY STATEMENT

This case provides novel insight into the diagnostic value of Fine Needle Aspiration Biopsy (FNAB) for evaluating

large, non-ulcerative mammary tumors in geriatric intact female dogs, particularly when histopathology is unavailable. Unlike most reports that rely solely on surgical biopsy, this study demonstrates that cytological analysis combined with clinical and radiographic findings can yield reliable diagnostic information for malignancy assessment.

AUTHOR'S CONTRIBUTION

Fatimah Alaydrus diagnosed the case and performed the surgical procedure. Wong Aihuwa Diah Lestari and Agus Widodo conducted the FNAB and interpreted the cytological results. Clarissa Setyorini was responsible for thoracic radiographic imaging. Bakti Sri Utami performed the physical examination. Amalia Rizqi Octerrya and Nur Ashiddiqi Mundiri contributed to data collection. Annastasya Yuliani Rahayu and Abdullahi Baso conducted the cytological observations. Lita Rakhma Yustinasari reviewed and revised the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

The study did not involve any experimental procedures and therefore did not require Institutional Animal Care and Use Committee (IACUC) approval. Owner consent for diagnosis and treatment was obtained prior to case management.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

Generative AI tools were used solely for language editing and grammar improvement. The authors reviewed, verified, and approved all content. No AI tools were used for data analysis, interpretation, or creation of scientific content.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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