

Pyometra and mammary gland tumor in a Beagle dog at the Veterinary Teaching Hospital of Brawijaya University, Malang, Indonesia

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ABSTRACT

A 12-year-old Beagle dog weighing 7.7 kg was brought to the Brawijaya University Veterinary Teaching Hospital in a state of dehydration, lethargy, and jaundice. On the 2nd day of hospitalization, the dog had mucopurulent discharge from the vagina. From the results of a complete blood count and ultrasonography (USG), the patient was diagnosed with pyometra and was treated with an ovariohysterectomy. After surgery the dogs was given cefotaxime 10 mg/kg bw, meloxicam 0.2 mg/kg bw, 0.9 ml vitamin K, and supportive therapy with Biodin® 0.5 mL and Modivitasan® 0.5 mL. Pyometra infection causes progesterone levels to rise, thereby triggering mammary gland tumor. On the 23rd day of hospitalization, a lump was found in the mammary gland. Fine needle aspiration (FNA) results showed the presence of Mott cells. The mammary gland tumor was treated with a single mastectomy. Mastectomy was performed when the dog was in stable condition, about a month after the ovariohysterectomy. Ovariohysterectomy and mastectomy were not performed simultaneously due to consideration of the patient's clinical condition, which made it impossible to get prolonged exposure to anesthesia. Post-mastectomy surgery the dogs were given cefixime 10 mg/kg bw, the analgesic meloxicam 0.2 mg/kg BW, and Biodin® 0.5 ml as supportive therapy. The postoperative wound healed well without complications. The patient recovered after one month of treatment.

Keywords: blood count test, mastectomy, mucopurulent discharge, ovariohysterectomy, ultrasonography

INTRODUCTION

Pyometra is an accumulation of pus in the lumen of the uterus. The etiology of pyometra can be caused by factors such as age, breed, bacterial infection, or hormonal imbalance (Rickyawan *et al.*, 2022). Pyometra is classified

into open and closed pyometra. Dogs with open pyometra will show clinical symptoms of purulent discharge from the vagina; these symptoms are not found in closed pyometra cases (Baithalu *et al.*, 2010). Closed pyometra is more dangerous because bacterial infections in the uterus produces toxins that can cause sepsis

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and even death. The incidence of pyometra is closely related to reproductive hormone imbalance, which often triggers other diseases such as mammary gland tumors. The hormones involved are estrogen and progesterone (Patrick, 2016).

The hormone progesterone, both endogenous and synthetic, can trigger an increase in Growth Hormone (GH) production, which increases the concentration of Insulin-Like Growth Factor 1 (IGF1). The IGF1 stimulates cell proliferation in the mammary glands and acts as a local growth factor (Benavente *et al.*, 2016). Age factor is one of the predisposing factors for mammary gland tumors. The tumor incidence rate increases rapidly in animals from approximately six years of age. The average age of dogs with mammary gland tumors is 10 to 11 years (Fesseha, 2020; Valdivia *et al.*, 2021). Treatment of mammary gland tumors is through the surgical approach of mastectomy namely removal of mammary gland tumors. Mastectomy in this case, is a single mastectomy (Fossum, 2019).

MATERIALS AND METHODS

The procedures consist of anamnesis and physical examination, hematological and blood chemistry examination, ultrasound examination, cytology examination, operative management, and post-operative histopathological confirmation.

Patient history was obtained by interviewing the owner or patient handler. The medical history aims to obtain specific information about the reason for visit, past medical history and living condition. Physical examination of the patient begins with inspection, palpation, percussion and auscultation. The result of the physical examination can help establish a diagnosis based on clinical findings. Based on these clinical findings, it was decided to carry out hematological and blood chemistry examinations as well as ultrasonography (USG).

Hematological examination was carried out using a Rayto 7600® Veterinary Hematology Hemo-analyzer machine, while for blood

chemistry examinations ICHEM I-YBIO 535® was used. A 3 ml blood sample was taken from the saphenous vein and stored in an EDTA tube for hematological examination and a heparin tube for blood chemistry examination.

An Ultrasonography (USG) procedure was performed to confirm findings of abnormalities from the vagina in the form of purulent discharge leading to reproductive disorders. The dog was shaved in the abdominal area before applying the ultrasound gel. The dog was positioned dorsally lying (dorsal recumbency). A probe was applied along the linea alba to evaluate the uterine.

RESULTS

A 12 year old Beagle dog weighing 7.7 kg was admitted to Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Brawijaya University, with a lethargic and dehydrated condition. The dog has received treatment at other clinic before, but has not shown any progress. On July 29, 2022 the owner brought this patient to the Brawijaya University Veterinary Teaching Hospital.

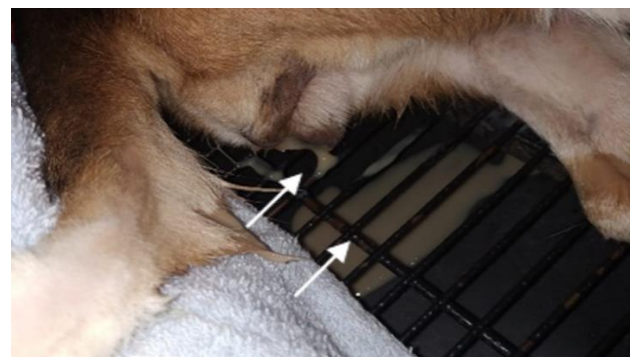


Figure 1 Mucopurulent discharge; out of Alexa's dog vagina (white arrow), mucopurulent discharge came out on the second day of hospitalization

Physical examination showed a degree of dehydration of 6%, icteric mucous membrane, and cardiac auscultation detected S3 and S4 murmur (abnormalities of ventricular filling and ejection of blood through the aorta) and arrhythmic heart beats. It was decided that the dog was admitted to the Veterinary Teaching

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Hospital for further observation. On the second day of hospitalization, the Beagle dog was depressed, vomiting and purulent discharge came out of the vagina (Figure 1a).

Hematology and blood chemistry examination

Hematology and blood chemistry test was performed on June 29th 2022. The results showed that the dog had leukocytosis, lymphocytosis, granulocytosis, monocytosis, hypochromic normocytic anemia, thrombocytosis, azotemia, and increased SGPT and ALP enzymes (Table 1).

Table 1 Results of hematology and blood chemistry tests

Examination	results	unit	normal value
Hematology			
white blood cell (WBC)	53	103/ μ L	6.0 - 17.0
lymphocyte	5	103/ μ L	1.0 - 4.8
monocyte	3	103/ μ L	0.15 - 1.35
granulocyte	45	103/ μ L	3.5 - 14.0
lymphocyte (%)	10.9	%	12.0 - 30.0
monocyte (%)	6.7	%	3.0 - 10.0
granulocyte (%)	82.4	%	60.0 - 80.0
red blood cells (RBC)	2.04	106/ μ L	5.5 - 8.5
hemoglobin (Hb)	0	g/dL	12.0 - 18.0
hematocrit (HCT)	14	%	37.0 - 55.0
MCHC	0	g/dL	32.0 - 36.0
MCH	0	Pg	19.5 - 24.5
MCV	67.4	fL	60.0 - 24.5
RDW-CV	16.7	%	12.0 - 16.0
RDW-SD	47.6	fL	35 - 56
thrombocyte	563	103/ μ L	200 - 500
MPV	7.5	fL	6.7 - 11.0
PDW	5.6	fL	0.0 - 50.0
Blood chemicals			
SGPT	78.8	U/L	28 - 76
BUN	36	mg/dL	15 - 34
CREA	4.9	mg/dL	0.8 - 2.3
ALP	118.5	IU/L	0 - 62
CK-MB	5.23	IU/L	4.9 - 6.3

Red notation indicates an increase, and blue notation indicates a decrease from the normal range value (Rickyawan *et al.*, 2022).

Diagnostic imaging ultrasonography

Ultrasonography examination was performed on June 30th 2022 after two days hospitalization. The ultrasound results of the uterus (Figure 2) were obtained using a transverse plane that divides the uterine organs into two halves in a manner opposite to the body

axis. The ultrasound image shows an enlargement of the uterine horns, inflammation of the endometrial mucosa and an irregular appearance, and accumulation of hypoechoic fluid in the uterine lumen.

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Diagnosis and prognosis

The diagnosis was made based on clinical sign and laboratory examination (complete blood count, biochemistry test), and ultrasonography performed at Veterinary Teaching Hospital of Brawijaya University. The dog has diagnosed with open pyometra, because the Beagle's dog is included in the geriatric age and decreased organ

function due to pyometra infection the prognosis of this case is dubious. The treatment for pyometra is ovariohysterectomy, and in high infection before surgery bitch should be stabilized with fluid therapy, broad spectrum antibiotics and supplement to improve the function of organs affected by systemic infection.

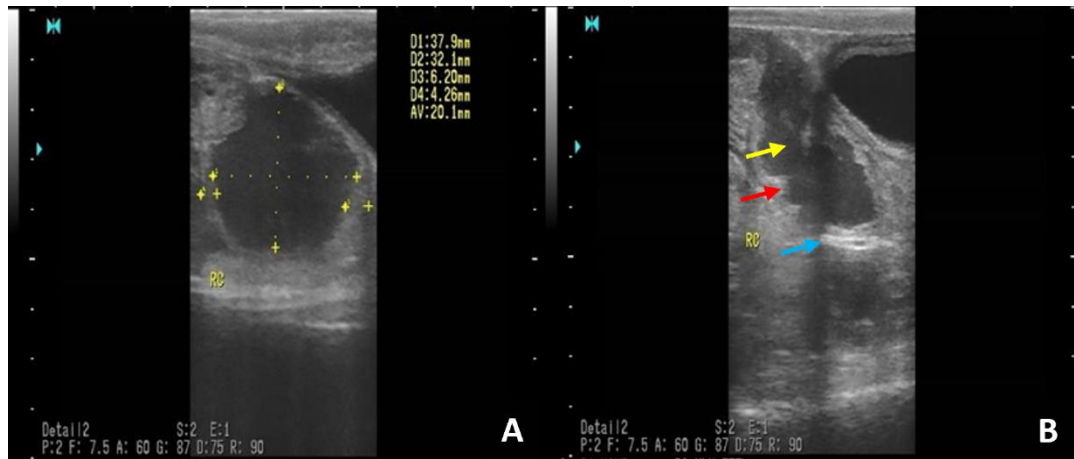


Figure 2 Uterine horn of a beagle with pyometra; A: uterine horn enlargement with a diameter of 1-4 cm; B: lumen of uterine horn; accumulation of hypoechoic masses suspected of fluid in the lumen of the uterus (yellow arrows), endometrial mucosal lining is irregular (red arrows), thickened, and appears hyperechoic (blue arrows).

Treatment

Septic pyometra caused disfunction of liver and kidney. Treatment of septic pyometra is not enough with OHE, but it is necessary to deal with clinical symptoms and improving the function organ that affected by bacterial pyometra infection. The Beagle dog has clinical sign of jaundice as suggestive of liver disease. Causative therapy for liver disfunction was given ursodeoxycholic acid (Urdahex®) that useful as adjunctive therapy for the medical management in patient with chronic liver disease. Kidney injury are present marked by increase BUN and creatine in biochemistry test. The bitch was given kidney supplement Renal N® to decrease BUN level.

Surgical treatment (ovariohysterectomy)

The ovariohysterectomy surgery was carried out on 30 July 2022 after one month hospitalization and the condition of patient was

stable. The preparation of the patient before the operation was that the animals were fasted for 8 hours. Infusion of fluids using 0.9% NaCl solution intravenously through the cephalic antebrachial vein using a 22G IV catheter and administration of diazepam premedication 0.4 mg/kg bw intramuscularly. Anesthetic induction propofol 4mg/kg bw was given after 15 minutes intravenously, followed by administration of general anesthesia, a combination of 10 mg/kg bw ketamine and 2 mg/kg bw xylazine. The patient is transferred to the operating table, and an endotracheal tube is placed to deliver isoflurane inhalation anesthetic as 1-2% maintenance. The patient's position was lying dorsal, and the surgical area that had been shaved was cleaned using 70% alcohol and 10% povidone-iodine and then covered using a sterile towel.

The incision in the midline below the umbilicus was carefully done. The layers that are

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incise start from the skin, subcutaneous and muscular. Exploration of the abdominal cavity for the uterus. The uterus with pyometra (Figure 4) was removed from the abdominal cavity to facilitate ovary ligation. The dexter and sinister ovaries were ligated using a 3/0 absorbable monofilament suture and then cut. The body of uterus was ligated using the same thread and then cut. The abdominal cavity is cleaned using 0.9% NaCl solution to prevent organ adhesion. The muscle was sutured using an interrupted simple suture pattern, and the subcutaneous suture was sutured using an intradermal suture pattern with a 3/0 absorbable monofilament suture. The skin was sutured using 3/0 nonabsorbable silk thread with a simple interrupted pattern. The suture marks were cleaned using 10% povidone-iodine and covered with sterile gauze. Postoperative drug administration consisted of Cefotaxime® 10 mg/kg bw, meloxicam 0.2 mg/kg bw, vitamin K 0.9 mL, and supportive therapy Biodin® 0.5 mL and Modivitasan® 0.5 mL.



Figure 3 Uterus with pyometra

Postoperative observation

On the 22nd day of hospitalization, a lump was found in the left mammary gland number 2.

The mass of the lump had a stiff consistency, was movable, and had a diameter of ± 1.5 cm (Figure 4). Based on owner's information, the mass in mammary gland has appeared in the first the bitch came to Veterinary Teaching Hospital, Brawijaya University, but it's getting bigger over time.



Figure 4 Mammary gland lump mass detected on the 23rd day of hospitalization; the lump had a firm consistency and was movable.

Cytology examination

A cytological sampling of the mammary gland mass on the second left nipple used the fine needle aspiration (FNA) technique and stained it using the Diff-quick stain. Collection of sample was performed on August 9th 2022. Cytological results showed Foam cells, Mott cells, and erythrocytes (Figure 5).

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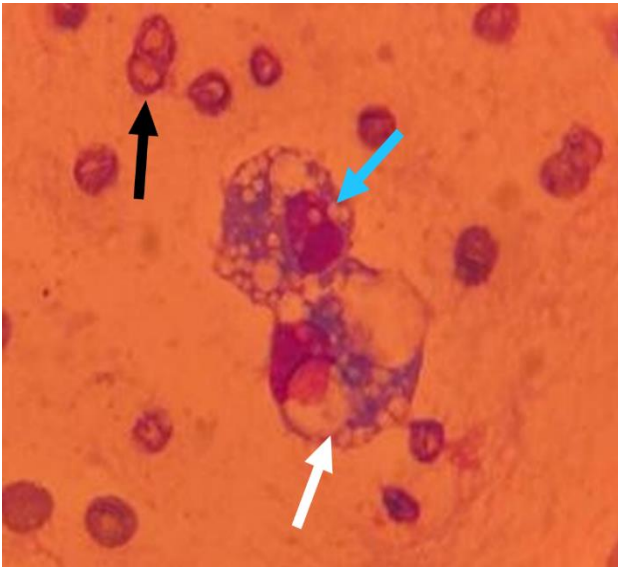


Figure 5 Cytological examination of the lump in the mammary gland of the dog shows erythrocytes (black arrows), Foam cells (white arrows), and Mott cells (blue arrows); diff quick staining; 1000x objective lens

Diagnosis and treatment

Diagnosis for the formation of mass in mammary gland was temporally suspected to be caused by chronic inflammation, because tumor cell was not found. However the mass must be removed and confirmed with histopathological examination. Removal of the mass in mammary gland through the surgical approach of single mastectomy.

The mastectomy procedure for the treatment of mammary gland tumors was carried out at a different time (22 August 2022) because the patient was old, with anemia and decreased function of the liver and kidneys. Therefore, it was decided that the mastectomy should be carried out after the OH closure stitches healed. The incision was made circularly about 2 cm from the mass of the lump. The skin was dissected subcutaneously using blunt scissors. Superficial cranial et caudal epigastric veins were ligated using absorbable thread 3/0, then rinsed using 0.9% NaCl solution. The skin was sutured using 3/0 nonabsorbable silk thread with a simple interrupted pattern. Treatment for postoperative mastectomy is Cefixime® 10 mg/kgBB, analgesic meloxicam 0.2 mg/kgBW, and Biodin® 0.5 ml as supportive therapy.

Post-operative histopathological confirmation

Histopathological preparations were made at the Laboratory of Anatomical Pathology, Faculty of Veterinary Medicine, Brawijaya University; the aim was to confirm microscopic changes in the mammary glands (Figure 5a, 5b) and uterus with pyometra (Figure 5c, 5d). Histopathological preparations were stained using Hematoxylin and Eosin.

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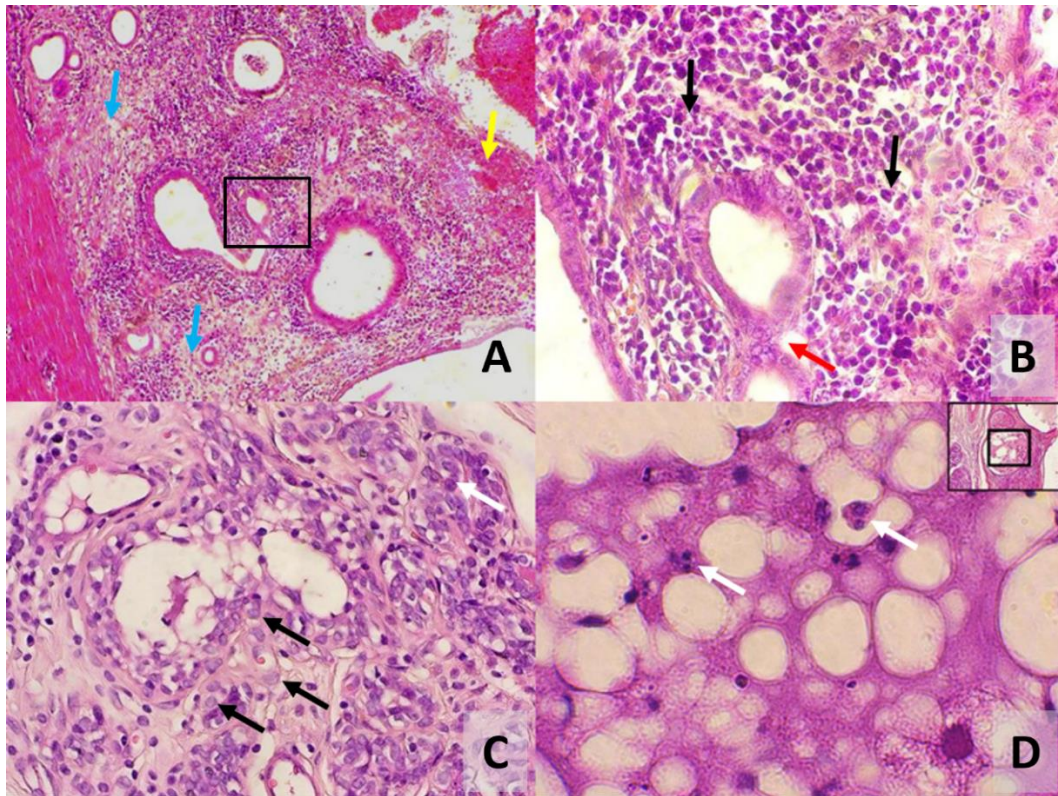


Figure 6 Postoperative histopathological confirmation of the uterus; A: layers of the uterine endometrium, exudate accumulation in the endometrium (blue arrow) and hemorrhage (yellow arrow), (100x magnification); B: endometrium. inflammatory cell infiltration (black arrows) and necrosis of endometrial gland cells characterized by karyolytic cell nuclei (enlargement of image A, 400x magnification); C: mammary gland tubules, tubular cell necrosis (black arrow), nuclear pleomorphism, and nuclear mitotic index (white arrow), 400x magnification; D: lymphatic vessels of the mammary glands; the mitotic index is found in the lumen of lymphatic vessels (white arrows), 1000x magnification; HE staining.

DISCUSSION

The results of the anamnesis, physical examination, and supporting examinations in the form of hematological tests, blood chemistry, and ultrasonography, as well as histopathology, as confirmation of microscopic tissue changes, informed that the dog had pyometra and mammary gland tumors. Female dogs not neutered have a greater risk of developing pyometra and mammary gland tumors (Rickyawan *et al.*, 2022). Pyometra can initiate the emergence of tumor cells in the mammary glands due to a hormonal imbalance between estrogen and progesterone. High progesterone impacts decreasing myometrial contractility and increasing endometrial gland stimulation,

thereby increasing mucus production and reducing leukocyte response. Mucus accumulation in the uterus during diestrus is used as a medium for normal vaginal flora bacteria growth. Ascending bacteria move from the vagina to the uterus. Mucus mixed with bacteria fails to be cleaned by the uterus due to decreased myometrial contractility (Patrick, 2016; Nelson and Guillermo-Couto, 2019).

Pyometra is classified into two based on clinical findings in the form of purulent discharge from the vagina, namely open and closed pyometra (Patrick, 2016). Open pyometra is a condition where the cervix is open so that purulent discharge can come out continuously or intermittently. This condition is not found in closed pyometra cases (Hagman, 2018). Closed

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pyometra is more dangerous because of the potential for uterine and fallopian tube rupture due to the accumulation of purulent discharge resulting in septic peritonitis (Nelson and Guillermo-Couto, 2019).

The results of hematological and blood chemistry examinations showed that the patient had leukocytosis, lymphocytosis, granulocytosis, monocytosis, hypochromic normocytic anemia, thrombocytosis, azotemia, increased SGPT and ALP enzymes. Most female dogs with pyometra experience change in the results of their hematological examination, which correlates with severe infection or chronic inflammation. Anemia is found in 70% of pyometra patients due to disruption of the process of erythropoiesis caused by a bacterial toxin. Hypochromic normocytic anemia is seen in septic patients. In cases of sepsis, the red blood cell profile has decreased, caused by impaired red blood cell production and red blood cell death. Production of red blood cells can be due to iron deficiency, decreased synthesis of the hormone erythropoietin, infection, and inflammation (Ettinger and Feldman, 2010; Scott and Stockham, 2013).

Leukocytosis is common in pyometra patients, accompanied by increased neutrophils and monocytes (Ettinger and Feldman, 2010). Azotemia conditions indicate kidney disorders due to endotoxins produced by bacteria. The toxin causes nephron cell death, so the kidneys experience a decreased function in urine filtering (Nelson and Guillermo-Couto, 2019). Elevated liver enzymes such as ALP and SGPT are also common in female dogs with pyometra. An increase in ALP and SGPT indicates a decrease in liver function which may cause intrahepatic cholelithiasis (Ettinger and Feldman, 2010). Ultrasound results in dogs with pyometra show thickening of the endometrial mucosa accompanied by a distended uterine lumen due to fluid accumulation with a hetero-echogenic appearance. In contrast, in cases of mucometra, the ultrasound results will appear hypoechoic (Ettinger and Feldman, 2010).

The incidence of pyometra is related to the finding of mammary gland tumors in dogs. The

increase in progesterone levels in pyometra cases stimulates growth hormone (GH). The GH hormone causes an increase in the concentration of Insuline-like Growth Factor I (IGFI), which works to stimulate cell proliferation in the mammary glands and acts as a local growth factor (Fesseha, 2020; Gobello and Corrada, 2001). FNA results indicate the presence of foam cells, a type of macrophage localized to fat deposits, which absorb low-density lipoproteins and become loaded with lipids. These cells secrete various substances that are involved in plaque growth, and their death causes inflammation (Rickyawan *et al.*, 2022). The presence of Mott cells indicates the presence of lymphocyte neoplasia or lymphoma. Mott cell (Figure 3) is a defect resulting from the secretion of immunoglobulin derived from plasma cells. Multiplication of inclusion bodies in the cytoplasm of Mott cells contains immunoglobulins that accumulate in the reticulum-endoplasmic region. Mott cell differentiation is associated with pathological conditions in several diseases, including chronic inflammation, autoimmunity, and tumors. Several case studies reported that Mott cells were found in cases of lymphosarcoma (Kim *et al.*, 2022).

The histopathology of the lump mass in the mammary gland was confirmed to be a tumor. Judging from the type of cell with the characteristics of a pleomorphic cell nucleus, there is a mitotic index, and many cells were necrotic. Tumor cells are thought to metastasize via lymph vessels, where lymph vessels are characterized by a single layer of endothelial cells (Sartini *et al.*, 2015). Active mitotic tumor cells were found in the lymphatic vessels' lumen. Histopathological feature of mammary gland tumors displays areas of necrosis accompanied by inflammatory cell infiltration and multifocal bleeding. Tumor malignancy criteria can be seen from a) tumor type; b) cellular pleomorphism; c) mitotic index; d) distribution of cells experiencing necrosis; e) lymphatic gland invasion; and f) regional lymphoglandular metastases (Goldschmidt *et al.*, 2011). Based on the metastatic node tumor classification, dog

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Alexa's mammary gland tumor belongs to the stage I because the tumor mass has a size of <3cm, there is no evidence of metastasis to regional glandular lymph node, and there was no evidence of systemic tumor cell metastasis (Maiti, 2017).

CONCLUSION

The clinical examination results, hematological tests, blood chemistry, and ultrasound indicated an open pyometra with surgical ovariohysterectomy. Mammary gland tumor disease was detected on the 23rd day of hospitalization and was diagnosed based on the results of a physical examination and cytological investigation of FNA. The results of the cytological examination indicated a mammary gland tumor. Treatment was done by removing mammary gland tumors (single mastectomy). Mammary glands that have tumors were incised, and histopathological preparations were made as confirmation of microscopic pathological changes. The postoperative wound healed well without complications, and the patient was cured after one month of treatment.

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AUTHOR'S CONTRIBUTIONS

Nofan Rickyawan (NR), Karlina (K), Dian Vidiastuti (DV), Shelly Kusumarini (SK). Study concept and design: NR. Data collection and interpretation: NR, K. Writing draft articles: NR, K. Critize and review: NR, DV, SK.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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