NOTE *Internal Medicine*

Predicting Metastatic Potential of Gastrointestinal Stromal Tumors in Dog by Ultrasonography

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ABSTRACT. Gastrointestinal stromal tumor (GIST), a mesenchymal neoplasm affecting the gastrointestinal tract, shows a variety of clinical behaviors from inactive benign to aggressive malignant in dogs. In this study, the feasibility of using clinically significant ultrasonographic features to predict the metastatic potential of canine GIST was investigated through comparison with actual metastatic incidence and findings of malignancy obtained by postoperative pathological examination. Ultrasonographic features, including large tumor size, irregular margin and heterogeneous internal echogenicity with large hypoechoic areas, related closely with the presence of metastasis as well as a high-risk ranking by the human classification system according to pathological findings. Based on these ultrasonographic features, the potential of metastasis in canine GIST could be preoperatively predicted.

KEY WORDS: canine, gastrointestinal stromal tumor, metastatic potential, ultrasonography.

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Gastrointestinal stromal tumor (GIST) is a mesenchymal neoplasm originating primarily from the stomach and intestine. The tumor typically expresses KIT protein, a transmembrane tyrosine kinase receptor for stem cell factor, encoded by the c-kit gene [9]. Although GIST has been confused with smooth muscle tumors, this tumor recently has been recognized, through the demonstration of KIT protein expression, as a unique neoplasm, likely originating from the interstitial cells of Cajal [20]. In human medicine the first-line diagnostic examination for this tumor is ultrasonography, and some criteria for using ultrasonographic findings to predict the potential for malignancy of GIST preoperatively have been proposed based on comparative studies between pathological findings and the ultrasonographic appearance of the tumors [16, 17, 19, 22]. It is clinically important in treating GIST to know the prognosis preoperatively because GIST includes many heterogeneous types of tumor with a variety of biological behaviors, from inactive benign to aggressive malignant. However in veterinary medicine there is no published report, to our knowledge, that has identified how to evaluate malignant risk by ultrasonography. In this manuscript, we report significant findings from ultrasonography for predicting metastatic potential in canine GIST through a retrospective comparative study based on pathological and clinical findings.

GIST, a rare canine tumor, was diagnosed in 12 dogs based on postoperative pathological examination at 3 central hospitals including the Veterinary Teaching Hospital of Rakuno Gakuen University, Japan Animal Referral Medical Center and Medical Center of Animal Clinical Research Foundation during a period from 2004 to 2010. Of these patients, 11 dogs were enrolled in this study because detailed information on pathology, clinical features and preoperative ultrasonography was available. For these dogs, physical examinations, blood tests (CBC and serum biochemical analysis), chest and abdominal radiography and ultrasonography (together with computed tomography in 2 cases) were performed at initial presentation. As a result of these examinations, the dogs were delivered to surgery based on a provisional diagnosis of neoplasm in the gastrointestinal tract. After surgical resection, detailed histopathological examination was performed for the resected tumor masses. A definitive diagnosis of GIST was made on the basis of histological features and expression of KIT protein in tumor tissues. Upon pathological examination, macroscopic findings, including regions of origin, tumor sites and sizes, along with microscopic findings, including mitotic count and presence or absence of internal tissue necrosis, were recorded. Mitotic count was determined by counting the mitotic figures in 50 consecutive high-power fields on sectioned tissues stained with hematoxylin and eosin. Based on tumor size and mitotic count, the cases were classified into 4 classes, very low-risk, low-risk, intermediate-risk or high-risk, according to human criteria for estimating meta-

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static risk proposed by the US National Institutes of Health, as shown in Table 1 [1]. Analysis of c-kit gene mutation in exon 11 was conducted for 7 of 11 patients by commercial laboratories to investigate whether it has clinical importance in canine GIST as it does in canine mast cell tumor. Metastasis was postoperatively confirmed pathologically using tissue specimens biopsied from livers (3 patients) or omentum (1 patient) during surgery. Transabdominal ultrasonography was performed preoperatively for all patients with convex (6 to 8 MHz) or linear probes (10 to 13 MHz) using various scanners (EUB-6500, HITACHI Medical Corporation, Tokyo, Japan; PROSOUND α7, ALOKA, Tokyo, Japan) by 3 experienced veterinarians, without any prior diagnostic information, under commonly recognized ultrasound criteria. The ranges of transducer frequencies used, within which optimal imaging was possible, were determined based on previous reports [16, 22]. Ultrasonographic findings on tumor size, tumor margin (regular or irregular) and internal echogenicity (homogeneous or heterogeneous) were recorded. The internal echogenicity patterns were further classified into the following 4 types for detailed analysis, referring to Chounan *et al.* [2]: (1) Type A is uniformly hypoechoic, as compared with surrounding tissue; (2) Type B is a heterogeneous mass containing hyperechoic areas within Type A; (3) Type C is a heterogeneous mass containing hyperechoic and hypoechoic areas within Type A; and (4) Type D is a heterogeneous mass containing hypoechoic areas within the mass, which is hyperechoic or isoechoic as compared to the surrounding tissue (Fig. 1).

Table 1. Metastatic risk classification proposed in human GIST

Risk	Tumor size	Mitotic count (per high-power field)
Very low-risk	< 2 cm	< 5/50 HPF
Low-risk	2–5 cm	< 5/50 HPF
Intermediate-risk	< 5 cm	6–10/50 HPF
	5–10 cm	< 5/50 HPF
High-risk	> 5 cm	> 5/50 HPF
	> 10 cm	Any mitotic rate
	Any size	> 10/50 HPF

Information based on the US National Institutes of Health GIST Workshop convened in April 2001.

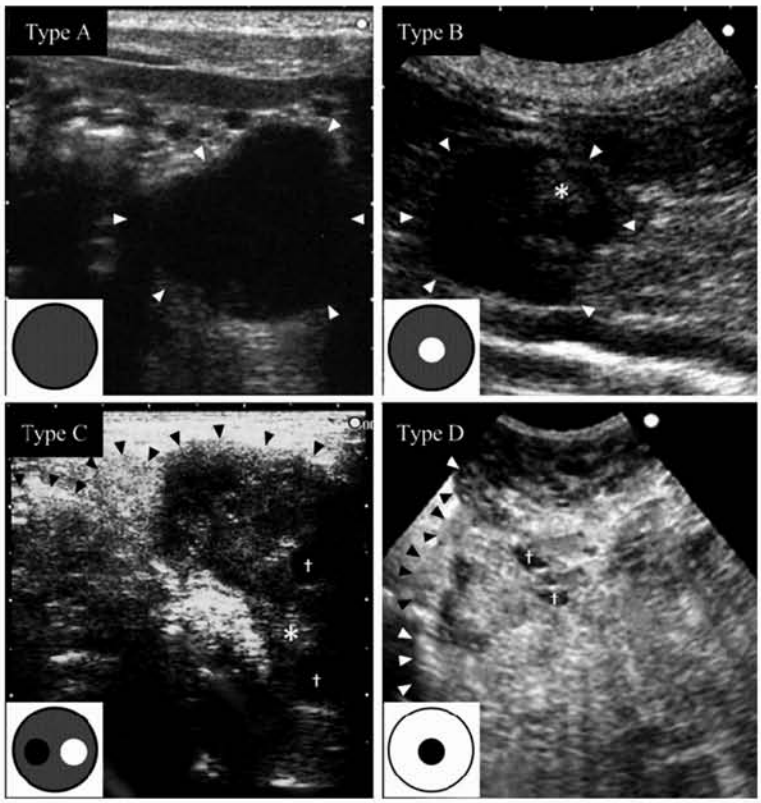

Fig. 1. Classification based on 4 different internal echogenicity patterns Representative images with associated schema (lower left) are shown (Types A, B, C, and D). Type A is a uniformly hypoechoic mass, as compared with surrounding tissue. Type B is a hypoechoic mass containing hyperechoic areas (asterisk). Type C is a hypoechoic mass containing hyperechoic (asterisk) and hypoechoic areas (cross). Type D is a hyperechoic mass containing hypoechoic areas (cross). Arrowheads show outlines of GIST masses.

Clinical features of the dogs, such as breed, sex, age, body weight and clinical symptoms at initial presentation are listed in Table 2. Chief clinical symptoms, which probably resulted from the tumors, included vomiting, anorexia, diarrhea and weight loss. Based on CBC and biochemical analysis, 3 cases with normocytic normochromic moderate anemia (PCV, 32–34%; reference range 37–55%), 1 case with mild leukocytopenia (23,900/μl; reference range 6,000–17,000 μl), 3 cases with moderate hypoalbuminemia (1.8–2.4 g/d*l*; reference range 2.5–3.5 g/d*l*), 2 cases with increased alkaline phosphatase activity (336–2,067 U/*l*; reference range 20–156 U/*l*), 1 case with increased alanine transaminase activity (166 U/*l*; reference range 21–102 U/*l*) and 1 case with increased aspartate transaminase activity (352 U/*l*; reference range 23–66 U/*l*) were noted. Through abdominal radiographic examination, soft tissue masses, which were located in upper (1 case) or middle (7 cases) abdominal regions, having discernible relationships with surrounding parenchymal organs such as liver, spleen and kidney, were noted in 8 cases, whereas no abnormality by thoracic radiography was observed in any patients. Through comprehensive ultrasonographic scanning, round to oval well-defined masses in apparent or probable connection with the gastrointestinal tract were demonstrated in the abdominal cavities. Detailed ultrasonographic characteristics for each patient are summarized in Table 3. The tumor masses had an irregular margin in 5 (45%) or regular (smooth) margin in 6 cases (55%). The internal echogenicity was heterogeneous in 10 cases (91%) and homogeneous (Type A) in 1 case (9%). The heterogeneous tumors with Type B, Type C and Type D were 4 (36%), 4 (36%) and 2 cases (18%), respectively. Nodular hyperechoic areas suggestive of metastasis were noted in the liver in 3 cases (27%). Other abdominal organs showed no ultrasonographic abnormality suggestive of metastasis. Surgical examination revealed that tumors appeared to originate in both the small (3 cases: 36%) and large intestines (7 cases: 64%). The most common site of occurrence was the cecum (6 cases: 55%), while a minority of tumors originated in the jejunum, ileum and colon (Table 3). This observation is in accordance with a previous report that found the most common region of origin to be the large intestine (48%), with 70% of those tumors occurring in the cecum [6]. Abnormal liver regions, which were observed as nodular hyperechoic areas in 3 cases, were biopsied during surgery. Pathological examination of the tissue samples obtained demonstrated that the hyperechoic areas in the liver were metastatic lesions. Omental metastasis was also confirmed in 1 patient through biopsy during surgery and pathology in spite of no observed abnormality in preoperative ultrasound examination. Including the omentum metastasis case, 4 of 11 cases (36%) in total had metastatic lesions in abdominal organs (Table 3). Another 7 dogs showed no macroscopic abnormality suggestive of metastasis, as observed during the surgical procedure. The finding that liver was the most probable organ for GIST metastasis coincides with similar observations in humans [4]. On the basis of the risk classification system, which has been used to guide prognosis in human GIST [13], canine patients classified as very low-risk, low-risk, intermediate-risk or high-risk were 1 (10%), 2 (20%), 3 (27%) or 5 (45%), respectively (Table 3). To assess the validity of the human

Table 2. Clinical features of dogs enrolled in this study

No	Breed	Sex	Age	Body weight (kg)	Symptom
1	Maltese	M	14	3.4	Anorexia Weight loss
2	Golden Retriever	MC	10	34.0	Vomit
3	Shiba-Inu	FS	9	10.5	Vomit
4	Miniature Dachshund	M	8	4.4	Anorexia Vomit Weight loss
5	Mix	MC	12	24.9	Anorexia
6	Puli	MC	11	11.8	Diarrhea Weight loss
7	Miniature Dachshund	M	5	6.2	Diarrhea
8	Labrador Retriever	FS	9	24.8	Vomit
9	Mix	FS	10	8.0	Anorexia Vomit
10	KCC Spaniel	M	9	8.0	Anorexia Vomit
11	Labrador Retriever	M	11	36.2	-

M: Male. MC: Castrated male. F: Female. FS: Spayed female.
KCC Spaniel: King Charles Cavalier Spaniel.

Table 3. Pathological, ultrasonographic and genetic examination

Patient	Pathological examination					Risk classification	Ultranonographic examination		Ultrasonographic classification	Genetic examination
No	Occurrence Site	Tumor size (cm)	Mitotic count (/50HPF)	Necrosis	Metastasis (location)	Risk group	Margin	Internal echogenecity	Type	c-kit mutation (Exon)
1	Large intestine (cecum)	6.0	21	+	+ (liver)	High	Irregular	Heterogeneous	C	-
2	Large intestine (cecum)	12.6	11	+	+ (liver)	High	Irregular	Heterogeneous	C	-
3	Large intestine (cecum)	5.0	6	+	+ (omentum)	High	Irregular	Heterogeneous	C	-
4	Small intestine (jejunum)	5.8	7	+	+ (liver)	High	Regular	Heterogeneous	D	NE
5	Large intestine (colon)	15.0	7	+	-	High	Irregular	Heterogeneous	C	NE
6	Large intestine (cecum)	9.8	3	+	-	Intermediate	Irregular	Heterogeneous	D	+ (Exon 11)
7	Small intestine (jejunum)	2.0	8	-	-	Intermediate	Regular	Homogeneous	A	+ (Exon 11)
8	Small intestine (ileum)	5.0	4	+	-	Intermediate	Regular	Heterogeneous	B	NE
9	Large intestine (cecum)	2.2	1	-	-	Low	Regular	Heterogeneous	B	NE
10	Large intestine (cecum)	4.7	3	+	-	Low	Regular	Heterogeneous	B	-
11	Small intestine (jejunum)	1.8	2	+	-	Very low	Regular	Heterogeneous	B	-

NE: Not examined.

risk classification system in canine GIST, we compared the actual incidence of metastasis at the time of surgery among the risk groups. Interestingly, all metastatic dogs were included in the high-risk group, and the metastatic incidence in the high-risk group was very high (4 cases: 80%), suggesting that the human GIST classification scheme can be used to rank metastatic risk in canine GIST. Focusing on ulrasonographic characteristics in the high-risk group, 4 (80%) of the 5 high-risk patients had tumors with an irregular margin while no tumors with irregular margin were noted among the low-risk group or the very low-risk group. The ultrasonographic irregular margin pattern would be a marker for predicting metastasis in dog, concurring with findings in human patients that border irregularity significantly correlates with higher risk for metastasis and unfavorable prognosis [16, 18]. In human medicine it has been reported that irregular ultrasonographic margin patterns correspond to macroscopic nodular formation on the tumor surface [17]. These patterns are seen most often in histologically malignant GIST masses [8], although no detailed pathological mechanism of nodule formation in malignant GIST has been elucidated. As in human GIST, canine tumors with irregular margins exhibited many variously sized macroscopic surface nodules (Fig. 2). Additionally, the internal echogenicity of Type C could be a marker for metastatic potential because all (100%) 4 tumors categorized as Type C were ranked in the high-risk group. In tumors with heterogeneous ultrasonographic patterns, Types C and D, necrosis areas were pathologically confirmed.

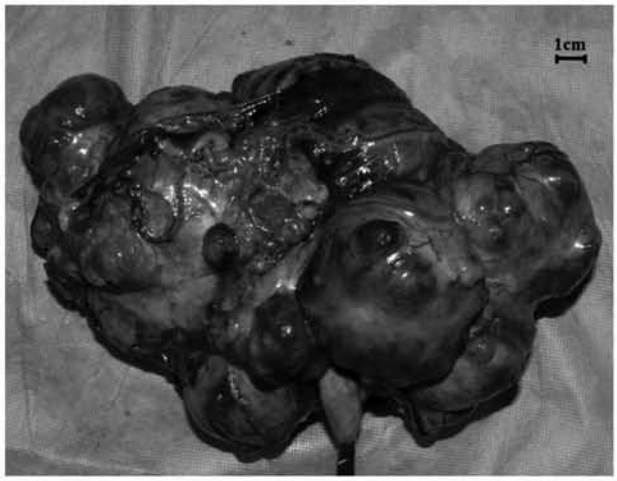

Fig. 2. A GIST mass removed by surgery
There were multiple nodules in a variety of sizes on the surface of the tumor mass. The tumor margin was observed to be irregular by ultrasound examination because of the nodules.

Considering a previous report on human patients in which necrosis areas were observed as hypoechogenic areas within heterogeneous masses [10], it is not unreasonable to consider that the prominent and large hypoechogenic areas of Type C and Type D found within the heterogeneous masses could be necrosis regions. Tumors of Type C or Type D with large necrosis areas, which were categorized as high-risk, tended to be larger than other types of tumors. These results agree with earlier studies of canine GIST reporting that necrosis areas positively correlate with tumor size [7] and that larger tumor diameter is a negative prognostic indicator in canine GIST [12]. The negative prognosis may be a result of the aggressive nature of malignant tumors, which grow rapidly and contain large, expanding internal necrosis regions. [14]. The c-kit gene mutation in exon 11 was found in 2 cases (28%) of 7 samples examined (Table 3). Many studies of human GIST have shown that c-kit gene mutation in exon 11 occurs preferentially in the malignant type [5, 9, 11, 15, 21]. Although these observations suggest that this mutation might be a useful molecular marker for malignant GIST, the contribution of c-kit gene mutation to malignant potential in canine GIST has not yet been confirmed. Based on our observation that no c-kit gene mutation was found in the high-risk group, it would be difficult to accept the mutation as a molecular marker for malignancy in dog. However, one study points out that the detection of c-kit gene mutations in human GIST may be of limited prognostic importance because activating mutations in c-kit gene are acquired in the very early stages of GIST development [3]. Thus, in our study, the 2 tumors of intermediate-risk in which c-kit gene mutation was detected might have progressed to high-risk tumors with metastasis potential.

Our studies of metastasis were conducted only at the time of surgery and did not follow the clinical course or postoperative outcomes in our canine patients. Therefore our study does not permit us to reach a clear conclusion as to whether the presence of metastasis actually leads to poor prognosis in canine GIST. However in human GIST, it has been demonstrated that median survival time is shortened in patients, who exhibit metastasis along with primary tumors at initial diagnosis [4]. In conclusion, although this study was retrospective and targeted at a limited population, our results show that the metastatic potential, which may be related to poor prognosis in canine GIST, could be clinically and preoperatively predicted based on ultrasonographic features such as large tumor size, irregular margin and internal echogenicity of Type C or Type D.

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