Clinical and pathological evaluation of the injection site masses in 12 dogs

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SUMMARY

In this study, histopathological characterization after surgical removal of the injection site masses in dogs was performed. Twelve dogs (11 males and 1 female), mainly Labrador retrievers (n = 8), 5 to 40 month old, stemming from the same dog farm have exhibited injection site masses 75 days after miscellaneous subcutaneous injections for cough treatment. All masses were removed with radical surgical resection under general anaesthesia and no radiotherapy or chemotherapy was performed. By conventional histopathological analysis with haematoxylin-eosin, Masson’s trichrome and Alcian blue staining, and immunohistochemistry using anti-vimentine or anti-α-smooth muscle actin as primary antibodies, chronic inflammatory granulomas were identified in the majority of cases (6/12) but fibrosarcoma (characterized by spindle shaped well-differentiated vimentine positive neoplastic fibrocytes associated with giant cells) was diagnosed in 4 cases and fibromyxosarcoma spindle shaped well-differentiated vimentine positive neoplastic fibrocytes identified in the majority of cases (6/12) but fibrosarcoma (characterized by fibrocytes fusiformes néoplasiques bien différenciés exprimant la vimentine mais pas l’actine α) were also identified in the 2 other cases. No local recurrence or metastasis was observed in any case. It was concluded that among soft tissue sarcomas which occurred at the injection site, fibrosarcoma was the most common in dogs but this tumour type was less aggressive in dogs than in cats.

Keywords: Dog, subcutaneous injection site, tumour, chronic granuloma, soft tissue sarcoma, immunohistochemistry.

RÉSUMÉ

Évaluation clinique et pathologique de masses tumorales formées sur le site d’injection chez 12 chiens

Dans cette étude, la caractérisation histopathologique de masses tumorales formées sur un site d’injection a été réalisée chez le chien. Pour cela, 12 chiens (11 mâles et 1 femelle) principalement de race Labrador, âgés de 5 à 40 mois, issus d’un même chenil et présentant des masses tumorales sur les sites d’injections réalisées 75 jours auparavant dans le cadre d’un traitement d’une toux de chien ont subi une exérèse chirurgicale complète des tumeurs sous anesthésie générale. Aucune radiothérapie ou chimiothérapie n’a été entreprise en parallèle. Après analyse des tumeurs par histopathologie conventionnelle et colorations à l’hémalun-éosine, au trichrome de Masson et au Bleu Alcian et par immunohistochimie utilisant comme anticorps primaires des monoclonaux dirigés contre la vimentine ou contre l’actine α des fibres musculaires lisses, des granulomes inflammatoires chroniques ont été identifiés dans la majorité des cas (6/12), mais, de plus, le diagnostic de fibrosarcome (caractérisé par des fibrocytes fusiformes néoplasiques bien différenciés exprimant la vimentine mais pas l’actine α) a été porté dans 4 cas et les 2 derniers chiens présentaient respectivement un fibromyxosarcome et un hémangiopericytome (caractérisé par des fibrocytes néoplasiques exprimant les 2 marqueurs). Aucune récidive locale et aucune métastase n’ont été ultérieurement mises en évidence. En conclusion, parmi les sarcomes des tissus mous qui surviennent sur le site d’injection, le fibrosarcome en est le type le plus fréquent et serait moins agressif chez le chien que chez le chat.

Mots clés : Chien, site d’injection sous-cutanée, tumeur, granulome chronique, sarcome des tissus mous, immunohistochimie.

Introduction

Tumours of the skin and subcutaneous tissues are the most common tumours affecting dogs, accounting for approximately one third of all tumours encountered in the specie [3, 11]. Of all skin tumours, mast cell tumour (18.8%), perianal adenoma (10.1%), lipoma (7.1%), sebaceous adenoma (7.1%), histiocytoma (6.7%), squamous cell carcinoma (6.2%), melanoma (6.2%), fibrosarcoma (6.1%), basal cell tumour (4.6%), and hemangiopericytoma (4.4%) are the most common [31].

Soft tissue sarcomas are a heterogeneous population of mesenchymal tumours that constitute 15% of all skin and subcutaneous tumours in the dog [26]. These are locally aggressive tumours with a high tendency of surrounding tissue invasion despite the low metastasis rate. Most soft tissue sarcomas are solitary tumours in middle to older aged dogs and cats. The incidence of soft tissue sarcomas seem to arise in young cats with a higher recurrence rate [14]. Clinical course and histopathological aspects of these tumours are more aggressive in cats compared to dogs [7, 27, 28]. In dogs, sarcomas have been associated with radiation, foreign bodies, and orthopaedic implants [13, 17, 22, 25]. Recently it was reported that a myofibroblastic fibrosarcoma was occurred at injection site after equine influenza vaccination [18].

Dogs and cats sometimes can develop subcutaneous inflammatory reactions at sites of injections. There is some evidence to further suggest that, although other drugs may be involved, those reactions are mainly associated with the use of inactivated virus vaccines containing aluminium-based adjuvants [1, 14]. Hendrick and Dunagan reported necrotizing panniculitis at sites of rabies vaccine administration in both dogs and cats [15]. These lesions were characterized by a central area of necrosis rimmed by an inflammatory reaction, often with lymphatic follicles formation [28]. Furthermore, in cats an unusual tumour which developed at sites of rabies and FeLV vaccine administration was reported by Hendrick and Goldschmidt [16].
Postinjection fibrosarcoma is a well-known pathological entity, first described in cats and recently observed in dogs and ferrets [23, 28, 30]. It is accepted that substances other than aluminium can be involved in the pathogenesis of these fibrosarcoma. Approximately for 100 years, investigators have observed that irritation, inflammation, and/or wounds are potential tumour promoters [21]. Virtually anything that causes a local inflammatory reaction may potentially be responsible for neoplastic initiation [31]. Sarcomas developing at sites of subcutaneous administration of long-acting drugs are clinical examples that support these findings [10].

The role of chemotherapy and radiotherapy alone in the management of dogs with soft tissue sarcoma is not clear. In the treatment protocol aggressive surgical resection is absolute indication and may be combined with both chemotherapy and radiotherapy. However, surgical removal of a malignant suspicious mass without adequate margins will result in incomplete resection and a high risk of local tumour recurrence [19]. The minimum recommended margins for surgical resection are 3 cm lateral to the tumour and one fascial layer deep to the tumour [1, 6]. Diameter below 5 cm, superficial localisation, and clear surgical resection found to be advantageous for the prognosis [31].

The purpose of the present study was to report the pathological evaluation and identification of postinjection subcutaneous masses of 12 dogs of various breed, age, and sex following aggressive surgical resection.

**Materials and Methods**

A total of 12 dogs (11 males and 1 female) exhibiting subcutaneous persistent masses in the chest or back regions 75 days after injections for cough treatment were included in the present study. They were 5 to 40 months old and 5 breeds were represented: 8 Labrador retrievers, 1 German pointer, 1 German shepherd, 1 Gordon setter and 1 Anatolian shepherd. The following information was recorded for each case: the dog signalment and history, a description of the mass, localisation and pathological diagnosis (Table I).

Complete blood counts and serum biochemistry profiles were preoperatively determined in each case. All animals received perioperative cefazolin sodium (Cefozin 500mg, Bilim, Istanbul, Turkey, 20 mg/kg, IV) at the time of the anaesthetic induction and every 2 hours throughout the surgical procedure. All cases were premedicated with diazepam (Diazem, Deva, Istanbul, Turkey, 0.1 mg/kg, IV) induced with propofol (Propofol 1%, Fresenius Kabi, Upsala, Sweden, 6 mg/kg, IV), and maintained with isoflurane (Isoflurane-Usp, Adeka, Samsun, Turkey). In all cases meloxicam (Maxicam 5 mg/ml, Sanovel, Istanbul, Turkey, 0.2 mg/kg/day, SC) injection was administered immediately before and for 3 days following surgery. No any other treatment protocol such as chemotherapy or radiotherapy was performed except surgical intervention. Masses were removed with radical resection technique (with 3 cm of margins around the masses) under general anaesthesia. Operation site was closed routinely and covered with a soft padded bandage for a 10-day-period until suture removal.

After surgical removal, masses were fixed in 10% formalin solution and sent for pathological examination. Tissue samples were embedded in paraffin after routine tissue processing and section (4-6 μm in thickness) were stained with haematoxylin-eosin, Masson’s trichrome, and Alcian blue and examined under light microscope.

For immunohistochimical examinations, Streptavidin-Biotin Complex Peroxidase (SBC-P) method was used. As primary antibodies, mouse monoclonal anti-α-smooth muscle actin (Actin, Smooth muscle, Lab Vision, USA) and mouse monoclonal anti-vimentin (Vimentin Ab2-Clone V9, Lab Vision, Turkey). In all cases meloxicam (Maxicam 5 mg/ml, Sanovel, Istanbul, Turkey, 0.2 mg/kg/day, SC) injection was administered immediately before and for 3 days following surgery. No any other treatment protocol such as chemotherapy or radiotherapy was performed except surgical intervention. Masses were removed with radical resection technique (with 3 cm of margins around the masses) under general anaesthesia. Operation site was closed routinely and covered with a soft padded bandage for a 10-day-period until suture removal.

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<table>
<thead>
<tr>
<th>Case</th>
<th>Breed</th>
<th>Age</th>
<th>Sex</th>
<th>Size</th>
<th>Localisation</th>
<th>Histological type</th>
</tr>
</thead>
<tbody>
<tr>
<td>n°</td>
<td>Dog signalment</td>
<td></td>
<td></td>
<td>Macroscopic mass characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Labrador retriever</td>
<td>18</td>
<td>M</td>
<td>15 cm$^3$ (2.5 x 2.7 x 2.2)</td>
<td>Right thorax</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td>2</td>
<td>Labrador retriever</td>
<td>18</td>
<td>M</td>
<td>48 cm$^3$ (4 x 3 x 4)</td>
<td>Right thoraco-abdominal borderline</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td>3</td>
<td>Labrador retriever</td>
<td>12</td>
<td>M</td>
<td>21 cm$^3$ (4 x 3.5 x 1.5)</td>
<td>Interscapular region</td>
<td>Chronic granulation tissue</td>
</tr>
<tr>
<td>4</td>
<td>Labrador retriever</td>
<td>12</td>
<td>M</td>
<td>25 cm$^3$ (5 x 2.5 x 2)</td>
<td>Left thoraco-abdominal borderline</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
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<td>M</td>
<td>22 cm$^3$ (4 x 2.8 x 2)</td>
<td>Interscapular region</td>
<td>Chronic granulation tissue</td>
</tr>
<tr>
<td>6</td>
<td>Labrador retriever</td>
<td>24</td>
<td>M</td>
<td>13 cm$^3$ (3 x 2.5 x 1.7)</td>
<td>Right thorax</td>
<td>Chronic granulation tissue</td>
</tr>
<tr>
<td>7</td>
<td>Labrador retriever</td>
<td>24</td>
<td>M</td>
<td>672 cm$^3$ (12 x 8 x 7)</td>
<td>Left thoraco-abdominal borderline</td>
<td>Hemangiopericytoma</td>
</tr>
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<td>8</td>
<td>Labrador retriever</td>
<td>24</td>
<td>F</td>
<td>27 cm$^3$ (6 x 3 x 1.5)</td>
<td>Left thorax</td>
<td>Fibromyxosarcoma</td>
</tr>
<tr>
<td>9</td>
<td>German pointer</td>
<td>12</td>
<td>M</td>
<td>21 cm$^3$ (7 x 2 x 1.5)</td>
<td>Interscapular region</td>
<td>Chronic granulation tissue</td>
</tr>
<tr>
<td>10</td>
<td>German shepherd</td>
<td>24</td>
<td>M</td>
<td>107 cm$^3$ (6.5 x 5.5 x 3)</td>
<td>Left thorax</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td>11</td>
<td>Gordon setter</td>
<td>40</td>
<td>M</td>
<td>11 cm$^3$ (3 x 2.5 x 1.5)</td>
<td>Left thorax</td>
<td>Chronic granulation tissue</td>
</tr>
<tr>
<td>12</td>
<td>Anatolian shepherd</td>
<td>5</td>
<td>M</td>
<td>63 cm$^3$ (4.5 x 4 x 3.5)</td>
<td>Left thorax</td>
<td>Chronic granulation tissue</td>
</tr>
</tbody>
</table>

M: male, F: female.

Table I: Dog signalment, clinical and pathological characteristics of the injection site masses in the 12 dogs.
Results

According to the anamnesis, it was reported that masses were formed following subcutaneous injections of ceftiofur (2.2 mg/kg SID, 7 days), carprofen (4mg/kg SID, 4 days), vitamin C (10 mg/kg SID, 5 days) and 4 mL vitamin B complex (B1 5 mg/mL, B2 2 mg/mL, B6 2 mg/mL, B12 4 mg/mL, Nicotinamide 20 mg/mL, Panthenol 10 mg/mL, SID, 5 days) for the treatment of a kennel cough outbreak in a dog farm. The treatment process was reported to be carried out in 54 dogs in quarantine. Because the injections during the treatment process were performed by 5 different veterinarians, there was no uniformity for the injection site in response to a specific drug. Following the treatment process, between 25 to 50 days, various sized masses were formed on the injection suspected areas in the 12 dogs of the present study. None of the masses were existed before the treatment process and no subcutaneous drug administration was performed for an 8 months long period before the disease outbreak.

The various mass localisations were the left thorax in 4 cases, the interscapular region in 3 cases, the right thorax in 2 cases, the left thoraco-abdominal borderline in 2 cases and the right thoraco-abdominal borderline in 1 case (Table I). Volumes of the removed masses were ranged from 11 cm3 to 672 cm3 (mean 87.2 cm3); 3 masses exhibited a reduced volume (below 15 cm3), and 3 a large volume (above 60 cm3) while the volume sizes of the others 6 were comprised between 16 and 50 cm3 (Table I). The mass consistencies were various between elastic to firm. Cut surfaces revealed a greyish-white colour with jelly and a reddish-brown central necrosis area was also observed in all cases.

In 6 cases (dogs n°3, 5, 6, 9, 11 and 12) a chronic granulation tissue composed of connective tissue cells, collagen fibres and mononuclear cells was diagnosed. Fibrosarcoma was diagnosed in 4 cases (dogs n°1, 2, 4 and 10). The neoplastic cells were associated with giant cells (figure 1) and numerous mitotic figures were found. Ovoid or spindle shaped well-differentiated neoplastic fibroblasts and fibrocytes blue stained with the Masson’s Trichrome were observed (figure 2). In these cases, positive vimentine immunolabelling was evidenced (figure 3) whereas the α-smooth muscle actin immunoreactivity was negative in this tumour type. Fibromyxosarcoma was diagnosed in one case (dog n°8) and was characterized by ovoid and spindle shaped well-differentiated neoplastic fibroblasts and fibrocytes arranged interwoven or with herringbone and the presence of atypical embryonic connective tissue cells (figure 4). Hemangiopericytoma was diagnosed in the last case (dog n°7). Histologically perivascular whorls of fusiform shaped well-differentiated neoplastic cells were seen. These cells were ranged from thick to thin, and spindle shaped to almost pyriform, also they were separated by variable amounts of collagenous stroma and mucinous matrix (figures 5 and 6). In some areas mitotic figures were also observed. In the case of hemangiopericytoma, neoplastic cells were both immunopositive for vimentin and α-smooth muscle actin (figures 7 and 8).

During a following-up period of 8-12 months, no local recurrence or other tumour (metastases) development was recorded in any case.

Discussion

In the present study, among masses spontaneously formed after subcutaneous injections, the majority of cases (6/12) consisted in chronic granulation tissues but fibrosarcoma was identified in 4 cases and hemangiopericytoma and fibromyxosarcoma in the 2 resting cases (dogs n°7 and 8, respectively). In agreement, it was previously reported that the most common injection site tumour was fibrosarcoma [30].

Postinjection fibrosarcoma is a well-known pathological entity, first described in cats and recently observed in dogs, ferrets, and horse [18, 23, 28]. Histologically, feline postinjection fibrosarcomas are characterized by inflammatory peritumoral infiltration, multinucleated giant cells and myofibroblastic cells [9]. Data suggest that local inflammation caused by aluminium or other potentially irritant inoculated substances may predispose tissues to tumour development. The most common reported cause of vaccine associated feline sarcomas is
FIGURE 3: Fibrosarcoma located in the right thorax in a male 18 month old Labrador retriever (dog n°1). Vimentine immunopositive neoplastic cells (arrows), Immunohistochemistry (Streptavidin-Biotin Complex Peroxidase method), X100.

FIGURE 4: Fibromyxosarcoma located in the left thorax in a female 24 month old Labrador retriever (dog n°8). Note the spindle shaped well-differentiated neoplastic fibroblasts and fibrocytes (red arrows) and myxomatous tissue (black arrow), Masson’s trichrome, X40.

FIGURE 5: Hemangiopericytoma located in the left thoraco-abdominal borderline in a male 24 month old Labrador retriever (dog n°7). Note the perivascular whorls of fusiform shaped neoplastic cells (arrow), Haematoxylin-eosin, X100.

FIGURE 6: Hemangiopericytoma located in the left thoraco-abdominal borderline in a male 24 month old Labrador retriever (dog n°7). Note the mucinous matrix (arrow), Alcian blue, X100.

FIGURE 7: Hemangiopericytoma located in the left thoraco-abdominal borderline in a male 24 month old Labrador retriever (dog n°7). Vimentine immunopositive neoplastic cells (arrow), Immunohistochemistry (Streptavidin-Biotin Complex Peroxidase method), X100.

FIGURE 8: Hemangiopericytoma located in the left thoraco-abdominal borderline in a male 24 month old Labrador retriever (dog n°7). Alpha-smooth muscle actin immunopositive neoplastic cells (arrows), Immunohistochemistry (Streptavidin-Biotin Complex Peroxidase method), X100.
aluminium which is used in inactivated virus vaccines containing aluminium-based adjuvants [14]. Substances other than aluminium can also be involved in the pathogenesis of fibrosarcomas such as irritation, inflammation and wounds [12, 20, 21]. Anything that causes a local inflammatory reaction may potentially be responsible for neoplastic development [31]. Sarcomas developing at sites of subcutaneous administration of long-acting drugs [10], vaccines [18], at sites with deep non-absorbable sutures [4], ocular post-traumatic sarcomas [8], as well as fibrosarcoma [30] and liposarcoma [29] development after microchip implantation, are clinical examples that support these findings. Additionally, some authors report that the vaccine reaction was exacerbated by multiple vaccines that support these findings. Furthermore, some authors report that repeated subcutaneous drug injections resulted in inflammation or tumour development.

Canine haemangiopericytoma is a cutaneous tumour that is more often found in the hind limbs and appears as a round nodule [24]. By contrast, this tumour type was rarely located in the head and in oral cavity and involvement of the orbit is highly uncommon [2, 5]. However, one haemangiopericytoma is identified in the dog no.7 in the present study on the left thoraco-abdominal borderline which is also a very rare development site for this tumour.

Whereas a high recurrence rate of soft tissue sarcomas was found in cats, and particularly in young cats [14] and these tumour types exhibit more aggressive histopathological characteristics (invasiveness (extending away from the mass along deep tissue), necrosis, local inflammation) and induce a rapid and fatal clinical outcome in cats compared to dogs [7, 27, 21], most of the reported cases were determined in dogs. Whereas a high frequency of soft tissue sarcomas was found in cats, and particularly in young cats [14] and these tumour types exhibit more aggressive histopathological characteristics (invasiveness (extending away from the mass along deep tissue), necrosis, local inflammation) and induce a rapid and fatal clinical outcome in cats compared to dogs [7, 27, 28], no recurrence or metastasis were observed in the present study in any dog.

As a conclusion, mass formation may be triggered by subcutaneous injections of long acting and irritant injectable drugs for 4 to 7 days which initiated the inflammation process and caused neoplastic changes in some cases. However, the injection site tumours do not proceed in dogs with high local recurrence and mortality rates as in cats if early radical surgical resection is performed.

References


