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Diagnostic immunohistochemistry for canine cutaneous round cell tumours — retrospective analysis of 60 cases

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Abstract

Introduction. Canine cutaneous round cell tumours (CCRCTs) include various benign and malignant neoplastic processes. Due to their similar morphology, the diagnosis of CCRCTs based on histopathological examination alone can be challenging, often necessitating ancillary immunohistochemical (IHC) analysis. This study presents a retrospective analysis of CCRCTs.

Materials and methods. This study includes 60 cases of CCRCTs, including 55 solitary and 5 multiple tumours, evaluated immunohistochemically using a basic antibody panel (MHCII, CD18, Iba1, CD3, CD79a, CD20 and mast cell tryptase) and, when appropriate, extended antibody panel (vimentin, desmin, α-SMA, S-100, melan-A and pan-keratin). Additionally, histochemical stainings (May-Grünwald-Giemsa and methyl green pyronine) were performed.

Results. IHC analysis using a basic antibody panel revealed 27 cases of histiocytoma, one case of histiocytic sarcoma, 18 cases of cutaneous lymphoma of either T-cell (CD3+) or B-cell (CD79a+) origin, 5 cases of plasmacytoma, and 4 cases of mast cell tumours. The extended antibody panel revealed 2 cases of alveolar rhabdomyosarcoma, 2 cases of amelanotic melanoma, and one case of glomus tumour.

Conclusions. Both canine cutaneous histiocytoma and cutaneous lymphoma should be considered at the beginning of differential diagnosis for CCRCTs. While most poorly differentiated CCRCTs can be diagnosed immunohistochemically using 1–4 basic antibodies, some require a broad antibody panel, including mesenchymal, epithelial, myogenic, and melanocytic markers. The expression of Iba1 is specific for canine cutaneous histiocytic tumours, and more sensitive than CD18. The utility of CD20 in the diagnosis of CCRCTs is limited. *(Folia Histochemica et Cytobiologica 2019, Vol. 57, No. 3, 146–154)*

Key words: canine cutaneous tumours; IHC diagnosis; histiocytoma; cutaneous lymphoma; plasmacytoma; mast cell tumour; alveolar rhabdomyosarcoma, amelanotic melanoma, glomus tumour

Introduction

Canine cutaneous round cell tumours (CCRCTs) are a heterogeneous group of neoplastic processes of similar morphology, but various histologic origins, with essentially different prognoses as well as treatments. CCRCTs generally include canine cutaneous histiocytoma, cutaneous lymphoma, plasmacytoma, and poorly differentiated mast cell tumours [1, 2], but some authors have also included amelanotic melanoma, neuroendocrine tumour, transmissible venereal tumour, and histiocytic sarcoma in this group [3–5]. Due to the similar morphology of tumour cells, routine histopathological examinations are not sufficient to obtain proper diagnoses in many cases of CCRCTs. Furthermore, veterinary oncologists require more specific diagnoses, which are essential for further therapy [6]. Some studies have shown that the histo-

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pathological diagnosis of a significant percentage of CCRCTs was modified after immunohistochemical (IHC) analysis [1, 7, 8].

The antibody panel for CCRCTs, described by Fernandez *et al.* (2005), includes MHCII, CD18, lymphocytic markers (CD3, CD79a), and mast cell tryptase [1]. While CD3, CD79a, and mast cell tryptase are highly specific for T-cell lymphoma, B-cell lymphoma/ /plasmacytoma, and mast cell tumours, respectively, MHCII and CD18 can be expressed by a wide range of cells [9, 10]. Although the immunoexpression of neither MHCII nor CD18 is specific for Langerhans cells of histiocytoma, the immunoexpression of both markers indicates histiocytoma, but only if tumour is negative for both CD3 and CD79a [1]. Recently, ionized calcium-binding adapter molecule 1 (Iba1), a pan-macrophage marker expressed by all subpopulations of cells of the monocyte/macrophage lineage, was shown to be specific for various cutaneous histiocytic disorders, including canine cutaneous histiocytoma and histiocytic sarcoma [11].

The diagnosis of cutaneous lymphoma is based on the immunoexpression of either CD3 or CD79a. While CD3 is a common marker of all T-cells [12], CD79a is an α-chain of the transmembrane heterodimer CD79, which is expressed exclusively by B-cells. In humans, the expression of CD79a continues throughout the phase of terminal plasma cell differentiation [13]. It was previously shown that 56–80% of canine plasmacytomas express CD79a [14, 15]. CD20, a phosphoprotein expressed from the pre-B cell stage to the activated B-cell stage, is also suitable for canine lymphoma immunophenotyping [16], but is rarely expressed in canine plasmacytomas [15].

Mast cell tryptase belongs to the group of mast cell-specific proteases that are expressed exclusively by mast cells [17] and are widely used as markers of these cells [18, 19]. Tryptase immunostaining shows high sensitivity for the detection of both normal and atypical mast cells, as shown in studies of human mastocytosis and other diseases associated with an increase in mast cells [19]. A previous report revealed that immunoperoxidase staining with monoclonal antibody AA1 (anti-tryptase) is both highly specific and sensitive for the detection of mast cells in routinely processed tissues [18].

The first aim of this study was to evaluate the utility of histochemical and immunohistochemical assessments in the diagnosis of CCRCTs. The second aim of this study was to identify tumours that should be considered at the beginning of differential diagnosis in cases of CCRCTs and tumours that are rare and unexpected but still possible. A properly structured list of conditions to be considered in differential diagnosis will allow the development of a cost-effective stepwise approach for the complementary immunohistochemical analysis of CCRCTs.

Materials and methods

The canine cutaneous tumours analysed in this study were archival diagnostic specimens (2013–2017) from the Department of Pathological Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland. Cutaneous tumours (solitary n = 55 and multiple n = 5) were collected from 59 dogs by surgical excisional or incisional biopsies. The tissue samples were fixed in 10% buffered formalin, embedded in paraffin, cut into 3-μm sections, and mounted onto silanized glass slides. The sections were processed routinely and stained with Mayer's haematoxylin and eosin (HE). All tumours were diagnosed originally by histopathologic examination, as undifferentiated round cell tumours, without specifying the tumour type. Due to the lack of features indicating the specific tumour type, a more definitive diagnosis was not obtained. Immunohistochemical examination of each tumour was performed manually using a basic antibody panel (MCHII, CD18, Iba1, CD3, CD79a, CD20 and mast cell tryptase) and a visualization system based on the immunoperoxidase method with 3,3-diaminobenzidine (DAB) as a substrate (Table 1). In cases where the final diagnosis could not be determined on the basis of the results of the basic antibody panel, an extended antibody panel was applied (vimentin, desmin, α-SMA, S-100, melan-A and pan-keratin; Table 1). The specimens were counterstained with Mayer's haematoxylin. For the positive control, normal canine tissues (tonsil for MHCII, CD18, Iba1, CD3, CD79a and CD20; colon for vimentin, desmin, α-SMA, and S-100; and skin for mast cell tryptase, melan-A and pan-keratin) were processed together with the evaluated sections. For the negative control, the primary antibody was replaced by the isotype-matched mouse IgG (Dako, Glostrup, Denmark) at the appropriate dilution (for monoclonal primary antibodies) or omitted (for polyclonal primary antibodies). Additionally, selected slides were stained using May-Grünwald-Giemsa (MGG staining kit; Bio-Optica, Milan, Italy), methyl green pyronine (MGP staining kit; Bio-Optica), or periodic acid-Schiff (Sigma-Aldrich, Steinheim, Germany).

The slides were evaluated using an Olympus BX51 light microscope (Olympus, Hamburg, Germany), and the microphotographs were prepared using the U-TVO.5XC-3 camera and cellˆB imaging software (both Olympus).

Results

All evaluated cutaneous tumours comprised a dense infiltration of round to polygonal cells with variable (low to fairly high) anisocytosis, anisokaryosis and chromatin distribution. The mitotic activity of the

Table 1. Primary antibodies and antigen retrieval and visualization systems

Primary antibody	Clone	Dilution	Antigen retrieval	Visualization system
HLA-DR α chain (MHCII)[a]	Monoclonal mouse anti-human TAL.1B5	1:20	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
CD18[c]	Monoclonal mouse anti-canine CA16.3C10	1:10	5 min. proteinase K[a]	EnVision+ System-HRP, Mouse (DAB)[a]
Iba1[d]	Polyclonal rabbit	1:500	Tris-EDTA buffer pH = 9[b]	ImmPRESS HRP Universal Antibody (Anti-Mouse/Rabbit IgG)[e]
CD3[a]	Polyclonal rabbit anti-human	1:50	Tris-EDTA buffer pH = 9[b]	ImmPRESS HRP Universal Antibody (Anti-Mouse/Rabbit IgG)[e]
CD79a[f]	Monoclonal mouse anti-human HM57	1:100	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
CD20[g]	Monoclonal rabbit anti-human SP32	1:100	Tris-EDTA buffer pH = 9[b]	ImmPRESS HRP Universal Antibody (Anti-Mouse/Rabbit IgG)[e]
Mast cell tryptase[a]	Monoclonal mouse anti-human AA1	1:200	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
Vimentin[a]	Monoclonal mouse anti-bovine VIM 3B4	1:100	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
Desmin[a]	Monoclonal mouse anti-human D33	1:50	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
α-SMA[a]	Monoclonal mouse anti-human 1A4	1:50	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
S-100[a]	Polyclonal rabbit anti-bovine	1:50	Citrate buffer pH = 6[b]	ImmPRESS HRP Universal Antibody (Anti-Mouse/Rabbit IgG)[e]
Melan-A[f]	Mouse anti-human A103	1:50	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]
Pan keratin[h]	Monoclonal mouse anti-human AE1/AE3/PCK26	ready to use	Tris-EDTA buffer pH = 9[b]	EnVision+ System-HRP, Mouse (DAB)[a]

[a]Dako, Glostrup, Denmark; [b]Antigen retrieval was heat-induced, conducted in a microwave oven at 650 W. Samples were microwaved twice to the boiling point, and incubated in a hot buffer for 20 min after boiling each time; [c]PF. Moore, Davis, CA, USA; [d]Wako Pure Chemical Industries, Ltd., Osaka, Japan; [e]Vector Laboratories Inc., Burlingame, CA, USA; [f]Bio-Rad Laboratories Inc., Hercules, CA, USA; [g]Abcam, Cambridge, UK; [h]Ventana, Tucson, AZ; USA.

tumour cells also varied from low to high. The majority of the evaluated tumours (55/60, 91.7%) were differentiated using the basic antibody panel. The detailed basic results of IHC analyses are presented in Table 2.

Canine cutaneous histiocytoma was diagnosed in 27/60 tumours (45%), collected from dogs aged 8 months to 12 years (mean age: 6.4). The tumours were solitary except for one case (a 1.5-year old dog presented with multiple tumours localized within the pinna). The tumour cells expressed MHCII, Iba1, and — in 23 cases — CD18, but were negative for CD3, CD79a, CD20 and mast cell tryptase (Fig. 1A). The tumour cells showed moderate epitheliotropism, which was difficult to observe in massively ulcerated tumours. In one dog, the cutaneous tumour of the paw pad showed a morphology similar to that of the simultaneously excised testicular tumour, which was diagnosed morphologically as seminoma, and therefore cutaneous metastasis of seminoma was suspected. Tumour cells massively infiltrated skin and subcutis, without any epitheliotropism. However, the IHC assessment revealed, that the neoplastic cells of the cutaneous tumour showed the membranous expression of MHCII and Iba1 (while the testicular tumour was negative to Iba1, but approximately 10% of tumour cells expressed MHCII), and were negative to CD18, CD3, CD79a, CD20 and mast cell tryptase. On the basis of these findings, histiocytic sarcoma was diagnosed (Fig. 1B).

Cutaneous lymphomas of either T-cell or B-cell origin were diagnosed in 18/60 cases (30%). These tumours were collected from dogs aged 1.5–13 years (mean age: 8.8 years). In 14 cases, cutaneous lymphoma was solitary, while in 4 — multiple (epitheliotropic T-cell lymphoma). In epitheliotropic cutaneous lymphomas (10/60 cases, 16.7%), the tumour cells showed prominent epitheliotropism. The tumour cells expressed either CD3 (9/60 cases, 15%, Fig. 1C) or CD79a (one case). In nonepitheliotropic cutaneous

Table 2. Basic antibody panel results in the evaluated canine cutaneous round cell tumours

No	MHCII	CD18	Iba1	CD3	CD79a	CD20	Tryptase	Diagnosis
1	+	+	+	–	–	–	–	Histiocytoma
2	+	+	+	–	–	–	–	Histiocytoma
3	+	+	+	–	–	–	–	Histiocytoma
4	+	+	+	–	–	–	–	Histiocytoma
5	+	+	ND	–	–	ND	–	Histiocytoma
6	+	+	ND	–	–	ND	–	Histiocytoma
7	+	+	+	–	–	–	–	Histiocytoma
8	+	+	+	–	–	–	–	Histiocytoma
9	+	+	+	–	–	–	–	Histiocytoma
10	+	+	+	–	–	–	–	Histiocytoma
11	+	+	+	–	–	–	–	Histiocytoma
12	+	+	+	–	–	–	–	Histiocytoma
13	+	+	ND	–	–	ND	–	Histiocytoma
14	+	+	+	–	–	–	–	Histiocytoma
15	+	+	+	–	–	–	–	Histiocytoma
16	+	+	+	–	–	–	–	Histiocytoma
17	+	+	ND	–	–	ND	–	Histiocytoma
18	+	+	+	–	–	–	–	Histiocytoma
19	+	+	+	–	–	–	–	Histiocytoma
20	+	+	+	–	–	–	–	Histiocytoma
21	+	+	ND	–	–	ND	–	Histiocytoma
22	+	+	+	–	–	–	–	Histiocytoma
23	+	+	+	–	–	–	–	Histiocytoma
24*	+	+	+	–	–	–	–	Histiocytoma
25	+	–	+	–	–	–	–	Histiocytoma
26	+	–	+	–	–	–	–	Histiocytoma
27	+	–	+	–	–	–	–	Histiocytoma
28	+	–	+	–	–	–	–	Histiocytic sarcoma
29	+	+	–	+	–	–	–	Epitheliotropic T-cell lymphoma
30	+	–	–	+	–	–	–	Epitheliotropic T-cell lymphoma
31*	+	–	–	+	–	–	–	Epitheliotropic T-cell lymphoma
32*	+	+	–	+	–	–	–	Epitheliotropic T-cell lymphoma
33	+	–	–	+	–	–	–	Epitheliotropic T-cell lymphoma
34*	+	+	–	+	–	–	–	Epitheliotropic T-cell lymphoma
35	–	–	–	+	–	–	–	Epitheliotropic T-cell lymphoma
36	–	–	ND	+	–	ND	ND	Epitheliotropic T-cell lymphoma
37*	+	+	ND	+	–	ND	ND	Epitheliotropic T-cell lymphoma
38	+	–	ND	–	+	–	–	Epitheliotropic B-cell lymphoma
39	+	–	ND	+	–	ND	–	Nonepitheliotropic T-cell lymphoma
40	–	–	–	+	–	–	–	Nonepitheliotropic T-cell lymphoma
41	–	–	–	+	–	–	–	Nonepitheliotropic T-cell lymphoma
42	+	–	–	+	–	–	–	Nonepitheliotropic T-cell lymphoma
43	–	–	–	–	+	–	–	Nonepitheliotropic B-cell lymphoma
44	–	–	ND	–	+	–	–	Nonepitheliotropic B-cell lymphoma

→

Table 2 (cont.). Basic antibody panel results in the evaluated canine cutaneous round cell tumours

No	MHCII	CD18	Iba1	CD3	CD79a	CD20	Tryptase	Diagnosis
45	–	ND	ND	–	+	+	–	Nonepitheliotropic B-cell lymphoma
46	+	–	–	–	+	+	–	Nonepitheliotropic B-cell lymphoma
47	+	+	–	–	+	+	–	Plasmacytoma
48	+	–	ND	–	+	–	–	Plasmacytoma
49	–	+	ND	–	+	–	–	Plasmacytoma
50	–	+	–	–	+	–	–	Plasmacytoma
51	–	–	ND	–	+	–	–	Plasmacytoma
52	–	–	ND	–	–	ND	+	Mast cell tumour
53	–	–	–	–	–	–	+	Mast cell tumour
54	–	ND	ND	–	–	ND	+	Mast cell tumour
55	–	–	ND	–	–	ND	+	Mast cell tumour
56	–	–	ND	–	–	ND	–	Alveolar rhabdomyosarcoma
57	–	–	ND	–	–	ND	–	Alveolar rhabdomyosarcoma
58	–	–	–	–	–	–	–	Amelanotic melanoma
59	–	–	–	–	–	–	–	Amelanotic melanoma
60	–	–	–	–	–	ND	–	Glomus tumour

Symbols: +, positive; –, negative; ND — not determined; * multiple tumours

lymphomas (8/60 cases, 13.3%), the tumour cells expressed either CD3 (4 cases, 6.7%) or CD79a (4 cases, 6.7%; Fig. 1D). The expression of CD20 was seen in two cases of nonepitheliotropic B-cell lymphoma. The expression of MHCII was observed in 8 cases of epitheliotropic lymphoma and 3 cases of nonepitheliotropic lymphoma, while the expression of CD18 — in 4 cases of epitheliotropic lymphoma. All evaluated lymphomas were negative to Iba1 and mast cell tryptase.

In cutaneous plasmacytomas (5/60 cases, 8.3%) collected from dogs aged 1.5–14 years (mean age: 8.3 years), the tumour cells expressed CD79a, and, in one of these cases — also CD20, but the nuclear:-cytoplasmic ratio was substantially lower than that in tumours diagnosed as cutaneous B-cell lymphomas. In four of these cases, the cytoplasm stained magenta with methyl green pyronine. The expression of MHCII was observed in 2 cases, while CD18 — in 3 cases. All evaluated plasmacytomas were negative to Iba1 and mast cell tryptase.

In four other cases (4/60, 6.7%), tumour cells expressed mast cell tryptase and were negative to other markers; therefore, their final diagnosis was mast cell tumour. The metachromatic granules, which were indiscernible in routine HE staining, were visualized by May-Grünwald-Giemsa staining in only one of these tumours.

The final diagnosis could not be assessed on the basis of the results of the basic antibody panel in 5/60 cases (8.3%), as the tumour cells did not express MHCII, CD18, Iba1, CD3, CD79a, CD20 or mast cell tryptase.

In two of these cases (3.3%), tumour cells expressed vimentin and desmin and did not express the other markers, with the final diagnosis being alveolar rhabdomyosarcoma (Fig. 1E). In one of these dogs, due to the simultaneous occurrence of another tumour on the forearm, multiple round cell neoplasia was suspected. However, after the IHC assessment, the other tumour was diagnosed as a histiocytoma.

In one of the evaluated tumours, the tumour cells expressed vimentin, S-100 and melan-A and did not express the other evaluated markers, with a final diagnosis of amelanotic melanoma. No melanin granules were found in the cytoplasm of the tumour cells. The surface of the tumour was massively ulcerated, but distinct epidermal invasion was noted. In other tumour, the tumour cells expressed vimentin and S-100, but were negative to melan-A (as well as other evaluated markers), and therefore amelanotic melanoma was suspected. Unfortunately, in this case, the size of the sample was not sufficient for further evaluation.

In one tumour located in the area of the lips, the tumour cells expressed vimentin and α-SMA and did not express the other evaluated markers, and the tumour was finally diagnosed as a glomus tumour. Round to oval neoplastic cells formed small clusters surrounded by a PAS-positive basement membrane (Fig. 1F).

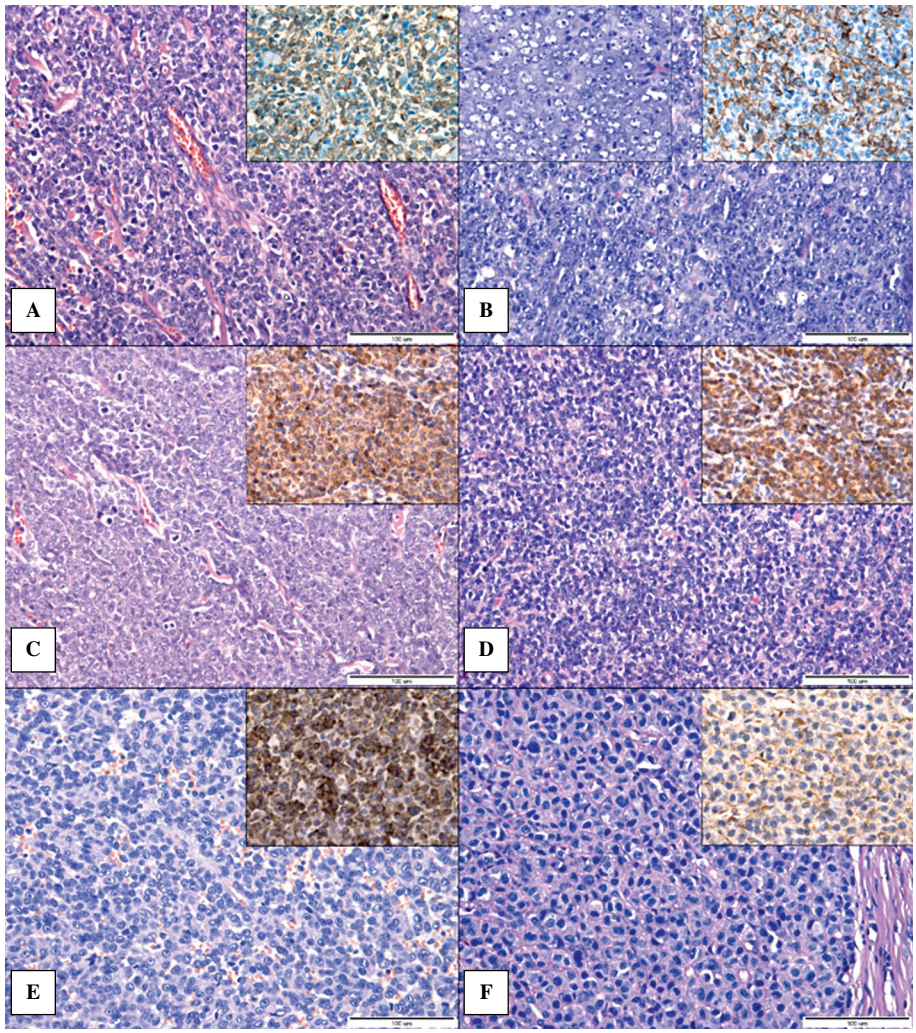

Figure 1. Microphotographs show undifferentiated canine cutaneous round cell tumours with similar histologic presentations and various definitive diagnoses. **(A)** Histiocytoma. Tumour comprised uniformly polygonal cells, with a moderately abundant, slightly eosinophilic cytoplasm (HE). Inset: Tumour cells show cytoplasmic and membranous expression of Iba1 (IHC, DAB). **(B)** Histiocytic sarcoma. Round to polygonal tumour cells with high mitotic and apoptotic rates. Morphologically, the tumour cells are similar to the testicular tumour (left inset), but these cells are packed more densely with nuclear crowding (HE). Right inset: Tumour cells showing the cytoplasmic and membranous expression of Iba1 (IHC, DAB). **(C)** Epitheliotropic T-cell lymphoma. Round to polygonal tumour cells with low-to-moderate anisocytosis and anisokaryosis and a high mitotic rate (HE). Inset: Tumour cells show the cytoplasmic expression of CD3 (IHC, DAB). **(D)** Nonepitheliotropic B-cell lymphoma. Tumour comprised uniformly round cells with low anisocytosis and anisokaryosis and small-to-moderate amounts of cytoplasm (HE). Inset: Tumour cells show the cytoplasmic expression of CD79a (IHC). **(E)** Alveolar rhabdomyosarcoma. Polygonal tumour cells with moderate anisocytosis and anisokaryosis form clusters separated by thin, highly vascularized fibrous septa (HE). Inset: Tumour cells show the cytoplasmic expression of desmin (IHC, DAB). **(F)** Glomus tumour. Small clusters of tumour cells are surrounded by a PAS-positive basement membrane (PAS). Inset: Immunoreactivity to ^SMA concentrated near the cellular membrane of the tumour cells (IHC, DAB)

Discussion

In the present study, histiocytomas and cutaneous lymphomas constituted the vast majority (75%) of the evaluated tumours. Therefore, histiocytomas and cutaneous lymphomas should be considered at the beginning of the differential diagnosis of CCRCTs. Histiocytomas are very common in dogs [2, 20], while cutaneous lymphomas occur less commonly and represent 1% of all canine skin tumours [12, 21]. In the present study, only tumours posing diagnostic difficulties were included; therefore, the number of detected tumours does not reflect their occurrence in the population.

In the present study, multiple tumours were seen in 4 cases of epitheliotropic T-cell lymphoma and in

one case of histiocytoma. While epitheliotropic T-cell lymphoma has a wide range of clinical presentations, and primary skin lesions, even if solitary, mostly progress into disseminated or generalized disease [12], multiple histiocytoma is very rare and occurs in less than 1% of the cases [22]. However, other CCRCTs, including non-epitheliotropic lymphoma, mast cell tumour and plasmacytoma, can also present as multiple tumours [21, 23, 24].

Epitheliotropism is an important diagnostic feature of some types of CCRCTs, allowing clinicians to narrow the list of possible diagnoses. The ability of tumour cells to invade and infiltrate the epidermis and/or adnexa results from the expression of specific adhesion molecules [25, 26]. Distinct epitheliotropism is highly indicative for epitheliotropic lymphoma and histiocytoma, but can be also seen in melanoma [2], and was reported in a few cases of mast cell tumour [26]. In the present study, epitheliotropism was a distinct feature of epitheliotropic lymphoma, but was also noted in most cases of histiocytoma and one case of amelanotic melanoma. However, the presence *versus* the lack of epitheliotropism should be interpreted with caution, as some authors claim that epitheliotropism of the epitheliotropic lymphoma may be reduced in association with progressive and severe dermal invasion [12]. Nevertheless, epitheliotropism is usually still prominent in canine tumour-stage mycosis fungoides, unlike its human equivalent [25]. Furthermore, the characteristic packets of tumour cells within the epidermis are not easy to detect in massively ulcerated tumours, as seen in the present study and in previous reports [8, 27].

The results of the present study indicate, that expression of Iba1 is specific for canine histiocytic tumours, what was also reported previously [11]. In the present study, expression of both MHCII and CD18 was not specific for histiocytoma, and was seen also in 4 cases of epitheliotropic T-cell lymphoma and one case of plasmacytoma. Furthermore, in 4 cases of histiocytoma and a histiocytic sarcoma, tumour cells were negative to CD18. The previous report revealed, that significant number of histiocytomas showed CD18 expression in only a small number of the tumour cells [8]. Therefore, we suggest, that Iba1 can easily replace both MHCII and CD18 in the basic antibody panel for the diagnosis of CCRCTs.

In the present study, the expression of CD20 was reported in two cases of nonepitheliotropic B-cell lymphoma and one case of plasmacytoma, so its sensitivity in identifying tumours of B-cell origin was much lower, than CD79a. Although CD20 is a valuable aid in immunophenotyping of canine lymphomas [16], the utility of this marker in diagnosing CCRCTs seems to be limited. Furthermore, it has been shown recently, that expression of CD20 in canine epitheliotropic T-cell lymphoma is not uncommon [28]. In the present study, one of the epitheliotropic lymphomas expressed CD79a and did not express CD3. Although epitheliotropic lymphomas are regarded to be exclusively of T-cell origin [21, 29], there is one report describing a B-cell epitheliotropic lymphoma [1].

The diagnosis of plasmacytoma in the present study was based on the immunoexpression of CD79a and the morphology, as the expression of CD79a alone cannot be used to distinguish B-cell lymphomas from plasmacytomas [1, 7]. All evaluated plasmacytomas expressed CD79a, and most of them (except for one) stained positive for MGP. Therefore, MGP staining can be used as an adjunct to the diagnosis of plasmacytomas, but cannot replace immunohistochemical markers. In a previous study, the antibody Mum-1p, used to detect multiple myeloma 1/interferon regulatory factor 4 (MUM1/IRF-4) antigen, was proven to be specific for canine plasmacytomas, superior in both sensitivity and specificity to CD79a [15]. However, the expression of MUM1/IRF-4 was also recently described in canine cutaneous histiocytomas, and therefore, this immunolabelling is recommended to be used only as a part of the round cell tumour panel, not alone [30].

Mast cell tumours frequently occur in dogs, and their diagnosis is usually straightforward due to the presence of characteristic, metachromatic granules in the cytoplasm of the tumour cells, which are easily identifiable with histochemical stains [31]. However, these granules are occasionally difficult to detect in some subsets of poorly differentiated mast cell tumours [32]. In the present study, the metachromatic granules were detected by histochemical staining (MGG) in only one of the four poorly differentiated mast cell tumours, but all of these tumours showed positive findings for mast cell tryptase. Therefore, mast cell tryptase immunolabelling should be considered superior in sensitivity to histochemical stainings for the identification of canine mast cell tumours. However, mast cell tumours that did not express tryptase but showed positivity for toluidine blue were previously described [1, 33]. Thus, the combination of mast cell tryptase immunolabelling and one of the histochemical stainings for metachromatic granule visualization would be the most appropriate method for the diagnosis of poorly differentiated mast cell tumours.

Amelanotic melanomas can be diagnostically challenging, as the cellular features of neoplastic cells may mimic poorly differentiated carcinomas, soft tissue sarcomas, and lymphomas [27, 34]. In the

present study, one case of CCRCT was diagnosed as amelanotic melanoma based on the immunoexpression of vimentin, S-100 and melan-A. In the other case, tumour cells expressed vimentin and S-100, but were melan-A negative. The expression of both vimentin and S-100 is not specific for melanocytes [34], and therefore the final diagnosis in that case was not obtained. However, the amelanotic melanoma can be suspected, as other possible differential diagnoses were excluded by other immunohistochemical stainings. In this case, other melanocytic markers should be applied, such as antibody PNL2, which was shown to be more sensitive than melan-A in the identification of canine melanocytic neoplasms [27, 35].

Other round cell tumours included in the present study, *i.e.*, alveolar rhabdomyosarcoma and glomus tumour, are not usually included in the differential diagnosis of CCRCTs. However, alveolar rhabdomyosarcoma can manifest as a cutaneous lesion, as described in a previous study [36]. In contrast to alveolar rhabdomyosarcoma, which, in our opinion, should be always included in the differential diagnosis of poorly differentiated CCRCTs (negative to basic antibody panel), glomus tumours seem to be extremely rare. Glomus tumours are benign neoplasms derived from glomus cells and are found frequently in humans but have been described in only single case reports in dogs [37–40].

In conclusion, histiocytoma and cutaneous lymphoma should be considered at the beginning of differential diagnosis of poorly differentiated CCRCTs. The expression of Iba1 is specific for canine cutaneous histiocytic tumours (including histiocytoma and histiocytic sarcoma), and more sensitive than CD18. The utility of CD20 in the diagnosis of CCRCTs is limited. Although most undifferentiated CCRCTs can be diagnosed immunohistochemically using 1–4 basic antibodies, some require a broad antibody panel, including mesenchymal, epithelial, myogenic, and melanocytic markers. Clinicians should also take into account the fact that many different types of CCRCTs can be morphologically indistinguishable from each other, and immunohistochemistry should be routinely recommended to confirm the morphological diagnosis, especially when there are discrepancies between the diagnosis and the clinical picture of the disease.

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Conflict of Interest

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