The controversial histologic classification of canine subcutaneous whorling tumours: The path to perivascular wall tumours.

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Abstract

Subcutaneous spindle cell tumors characterized by whorling growth patterns are common in dogs and are identified as a distinct entity. These tumors were misnamed as hemangiopericytomas (HPCs) due to some minor morphological parallels with their human counterparts. In veterinary medicine, the cell of origin of HPC has been under debate for a long time. Some authors have suggested a perivascular origin while others a perineural one. The evidence of the orientation of the neoplastic cells around the vessels and the expression of contractile proteins supported a perivascular origin while S100 expression and an inconsistent vascular connection supported a perineural origin. Despite the morphological similarities with peripheral nerve sheath tumors in humans, the perineural origin was supported mainly by the expression of markers with low specificity. On the contrary, the majority of studies have supported the perivascular origin of “old” canine HPC. Since a variable degree of myoid-pericytic differentiation was described, the term perivascular wall tumors (PWTs) was suggested to substitute HPC. Once the diagnostic criteria of PWTs were defined, the clinical behavior and prognostic variables were investigated, demonstrating differences as compared with the group of canine soft tissue sarcomas (STSs) in general. PWTs are less aggressive, mostly locally invasive, and rarely metastasizing. Their behavior seems to be less influenced by histological grade, suggesting that canine STSs are heterogeneous. The study of the biological behavior of specific STS tumor types may be valuable in detecting differences which have passed unnoticed when STSs have been studied concomitantly.

Keywords: canine soft tissue sarcoma, hemangiopericytoma, peripheral nerve sheath tumor, perivascular wall tumor, histologic classification, prognosis

Introduction

Subcutaneous spindle cell tumors characterized by whorls and interlacing bundles growth patterns are common in dogs.[1-3] These tumors were originally called hemangiopericytomas (HPCs) because perivascular whorling was described in the human tumor counterpart, despite the fact that this pattern was neither the major nor its most characteristic morphological feature.[1,4-6] Following the misnomers, wherein the pericytic origin of human HPCs was questioned and the majority of HPCs were reclassified as solitary fibrous tumors,[7] true perivascular myoid differentiation was determined for other entities, such as myofibromas, myopericytomas, glomus tumors and angioleiomyomas, representing a spectrum of lesions of mural cell origin (the non-endothelial component of blood vessels).[25,29]

In the meantime, in veterinary medicine, the debate focused on pericytic or perineural HPC origin.[8] The dispute divided the community of veterinary pathologists, and the same canine neoplasm, whose unicity was not a matter of discussion,[1] was diagnosed as an HPC by some pathologists and as a schwannoma/peripheral nerve sheath tumor (PNST) by others. Pericytic origin of canine HPC was supported by the perivascular orientation of the whorls and by the immunohistochemical expression of contractile proteins recapitulating the phenotype of the vascular mural cells.[9-12] On the other hand, perineural origin was claimed on the basis of the inconsistent finding of patent blood vessels at the center of the whorls which often surrounded amorphous eosinophilic material or collagen fibers.[13,14] Furthermore, the storiform arrangement of the neoplastic cells, and the mixture of hypercellular and hypocellular areas (which may share some similarities with Verocay bodies, and Antoni A and Antoni B patterns, respectively) in association with S100

expression reinforced the perineural hypothesis.[13,14] It is nevertheless important to note that S100 immunohistochemistry is problematic in dogs since this marker has not been validated in canine species, and its expression has been reported in a variety of mesenchymal and non-mesenchymal cells.[18-22]

This debate was so animated that the WHO classification of tumors of domestic animals published in 1998 included HPC in the group of unclassified tumors, and the canine HPC/PNST debate was pointed out as an example of the unsolved questions regarding the diagnosis of mesenchymal tumors, stating that "*the controversy over the differentiation between canine hemangiopericytoma and peripheral nerve sheath tumor rages on, despite hosts of investigations using immunohistochemical and ultrastructural techniques*".[8]

After the publication of the WHO classification,[8] several studies were carried out in order to better describe the features of this common entity and to clarify the cellular derivation. One of the first steps of this process was the description of its cytological features which were considered distinctive when compared with other spindle cell tumors. Features included the procurement of highly cellular fine needle aspiration (FNA) samples, some degree of cohesivity, the finding of plump spindle cells with variably distinct borders, occasional cytoplasmic vacuolation, the presence of bi- and multinucleated neoplastic cells with peripheralized nuclei (so-called insect head cells and crown cells, respectively) and prominent nucleoli.[15] These features were confirmed to be highly diagnostic in a subsequent study which demonstrated that the cytologic evaluation clearly separated "old" canine HPC from other canine spindle cell tumors.[16]

The definition of cytologic hallmarks in a context in which, for the same neoplasm, the histologic diagnosis depended on the pathologist rather than on the tumor, led to the false assumption that the same cytologic features could be consistent with two distinct neoplastic entities: "old" HPC and PNST. At this point, it was, and still is, pivotal to stress that cytomorphology allows the distinction of spindle cell tumors with whorls of the subcutis of

dogs, and that the focus of the discussion was still the origin of this entity, not the distinction of two separate neoplasms with similar histologic appearances.

A second step in the process of the characterization of ”old” canine HPC was an immunohistochemical study investigating the expression of several markers in order to verify the perivascular rather than the perineural origin of the neoplastic cells and to exclude other spindle cell tumor derivations.[11] The study provided two key facts: first, the immunohistochemical profile was consistent with origin from the vascular wall rather than the nerve sheath, several contractile proteins being expressed simultaneously while perineural markers were consistently negative and second, the microscopic as well as the immunohistochemical features were suggesting, for the first time, that this entity encompassed a spectrum of lesions.[11]

On the other hand, some studies supported the perineural origin of various series of canine spindle cell tumors with whorls, based on the expression of S100 (the main immunohistochemical marker for schwannoma in humans) and the identification of collagen fibers rather than vessels at the center of the whorls.[13,14,17] Interestingly, the expression of myoid markers, such as smooth muscle actin (sma), was reported in conjunction with S100 in several cases or was not tested in other reports.[13,14,17] These data were worthy of mention since S100 expression was progressively demonstrated in several normal tissues and in different types of canine spindle cell tumors, including leiomyosarcomas, liposarcomas, histiocytic sarcomas and hemangiosarcomas,[18-22] thus reducing its diagnostic relevance.

Based on these premises, an additional immunohistochemical study with the aim of clarifying the origin of the canine tumor entity was carried out on a different caseload which was selected on the basis of the cytologic features, applying a wider immunohistochemical panel on snap-frozen tumor tissues. The major relevance of the case selection lay in evaluating, for the first time, a homogeneous group of tumors and studying marker expression on frozen samples, avoiding the bias of formalin fixation, and enzymatic or heat-

induced antigen retrieval, potentially affecting the immunohistochemical findings.[23] This study confirmed that the marker expression was consistent with a vascular wall rather than a nerve sheath origin.[23] It should be noted that the degree of myoid differentiation was highly variable, resembling the transition from pericytes to smooth muscle cells which can be found along the peripheral capillary beds.[23] As a matter of fact, at this level, the vascular wall is composed of the endothelial lining and a non-endothelial component consisting of mural cells.[24] Vascular mural cells are heterogenous and are represented by pericytes around the capillaries, smooth muscle cells around the arterioles and venules, and an intermediate cell type, called myopericyte, in the transition between the vascular beds (arterioles to capillaries and capillaries to venules).[24] The same study identified four major morphological patterns indicative of a vascular wall origin (perivascular pattern) of a tumor: perivascular whorls, placentoid growth, bundles from the vascular wall and staghorn vessels.[23] Staghorn is the least common growth pattern observed in “old” canine HPC[23] while it was considered the most notable diagnostic histologic feature of human HPC.[4-6] Moreover, perivascular whorls, considered to be the most common aspect of “old” canine HPC , were seldom described in human HPC but, on the contrary, were consistently observed in association with the expression of contractile proteins, to a recently described and rare human tumor called myopericytoma.[25,26]

It then appeared clear that the most typical histological pattern and immunohistochemical reactivity of this common canine tumor paralleled the histological features of a more recently described and much less frequently found tumor called myopericytoma.[23,25,26]

Together with the perivascular patterns, in the canine tumor entity, non-perivascular patterns were also described and included storiform, solid, interlacing bundles and myxoid.[23] Some of the tumors, despite the typical cytological features, were mainly characterized by lack of specific perivascular patterns or by the low to absent expression of myoid markers; therefore, their origin was difficult to define.[23] Regarding those unclassified cases, a

subsequent ultrastructural study provided results which paralleled a previous ultrastructural study on canine HPC,[9] further demonstrating the perivascular origin of this entity.[27] Interestingly, the cases evaluated by electron microscopy (EM) were characterized by previously described “specific” cytological features, and the demonstration of their perivascular differentiation, despite the lack of clear perivascular histological patterns, confirmed once again the high diagnostic value of cytological sampling for this specific soft tumor entity.[15,23,27] Nevertheless, the heterogeneity of the histomorphology and immunohistochemical profile suggested that the term HPC, indicating an origin from pericytes, might not be appropriate and it was then substituted by the term perivascular wall tumors (PWTs) to better reflect the origin from the morphological and phenotypical continuum represented by the mural cells of the peripheral microcirculation.[23]

Despite the publication of these results, a large proportion of the veterinary scientific community was still reluctant to abandon the diagnosis of PNST in favor of PWT because some histomorphologic features resembled those of PNSTs.[23] In fact, there are several similarities, especially regarding the three classical morphological features described for human PNSTs, including whorls, Verocay bodies, and Antoni A and Antoni B areas.

Whorls are the histological hallmark of perineurioma, a rare benign PNST in which the neoplastic cells are arranged in a storiform pattern and in whorls, surrounding nerve fibers and vessels.[28,29] Thus, perineurioma seems to share a high degree of morphological similarity with canine PWTs. The demonstration of nerve fibers entrapped in the whorls of neoplastic cells, which can be observed in a subset of soft tissue perineurioma, may aid the diagnosis but, to the best of the knowledge of the Authors, it has not yet been confirmed with the exception of two cases of intraneural perineuriomas.[30,31] Furthermore, in canine PWTs, the vessels at the center of whorls may collapse and simulate nerve fibers on hematoxylin and eosin staining (Figure 1).[2] The demonstration of the vascular nature of these structures is feasible and can been obtained by immunohistochemistry[2] (Figure 1) and

electron microscopy.[9,27] In addition, S100 expression, which has been used as the immunohistochemical basis of a PNST diagnosis in cases of canine spindle cell tumors with whorls, is inconsistent with the diagnosis of perineurioma because S100 is expressed by Schwann cells but not by perineurial cells and perineurioma is definitely S100 negative.[28,29] On the contrary, a schwannoma is consistently S100 positive in humans, but usually lacks perineural whorls since its most typical histological features are Verocay bodies and Antoni A and Antoni B areas.[28,29]

Verocay bodies are structures formed by two rows of palisading nuclei separated by an anuclear area composed of the aligned cytoplasmic cell processes (Figure 2).[28,29] In canine PWTs, the storiform arrangement of neoplastic cells can mimic this feature with the relevant difference being that nuclei do not palisade, and the central area is not anuclear (Figure 2).[23] Antoni A and Antoni B areas are defined as areas composed of compact spindle cells arranged in bundles, with storiform and palisading patterns, and areas of loosely arranged spindle cells within a myxoid background, respectively.[28,29] These alternating hyper- and hypocellular areas also represent a common finding in canine PWTs in which myxoid is one of the most frequent non-perivascular patterns,[23] and comparison with schwannomas is therefore necessary. Nevertheless, the lack of true Verocay bodies and of entrapped nerve fibers, and the immunohistochemical diagnosis, for the most part based mostly on the expression of an unspecific marker, such as S100, do not support the nerve sheath origin of this tumor in dogs (either perineurial or schwannian). It is worth mentioning that the association of palisading and myxoid areas with the concurrent expression of S100 and myoid markers has been reported in 44 bovine primary cardiac spindle cell tumors which were shown to have had vascular smooth muscle rather than perineural differentiation.[32] A second study additionally supporting low S100 specificity in canine PNST was able to differentiate malignant PNSTs from PWTs on the basis of the application of a panel of antibodies, including multiple myoid markers to assess the perivascular origin of PWTs, and

S100, nerve growth factor receptor (NGFR) and oligodendrocyte transcription factor (Olig2), to assess the perineural origin of PNSTs.[33] This study supported the previously published results,[9,11,23,27] confirming that S100 may be useful for diagnosing perineural spindle cell tumors only if associated with positivity to other perineural markers as well as with negativity to myoid markers.[33] For all the above reasons, S100 should no longer be used as the only marker to demonstrate a peripheral nerve sheath origin in canine spindle cell tumors.

The results of multiple studies carried out by different groups on different caseloads has led to the conclusion that canine spindle cell tumors with whorls arise from the non-endothelial component of vessels and, specifically, from mural cells of the vascular wall rather than from nerve sheaths, and should therefore be called PWTs.[9,11,23,27]

Despite the long debate, and still with some disagreement, and the fact that a common result has been achieved regarding the classification and histogenesis, the clinical-pathological characterization of PWTs remains controversial. One of the main pitfalls in studying the biological behavior and prognostic variables of canine soft tissue sarcomas (STSs) is the absence of stratification of the results according to histotype,[34-38] based on the assumption that the majority of canine STSs share the same behavior.[38,39] On the one hand, if there is no specific evidence of the prognostic impact of the histotypes, it is also true that studies demonstrating the same prognosis for different STSs are still lacking.

Several clinical and pathological variables have been investigated in numerous studies in order to describe and anticipate the clinical behavior and prognosis of STSs; however, the heterogeneity of the caseloads hampers the interpretation of the results and their application in clinical practice.[34-38] In this background, the specific diagnosis of PWT as a subtype of canine STS, has allowed the evaluation of its clinical behavior, eliminating the confounding effect of other tumor types. In one study, canine PWTs at first presentation were reported to have a lower metastatic rate (4%) as compared with canine STSs in general (8-17%). Furthermore, the recurrence rate was only moderate (20%) despite the fact that numerous

cases (33%) were removed with a surgical margin at risk (clean but close or infiltrated) and only a few (7%) underwent adjuvant therapy. These results suggested that PWTs seemed to represent a less aggressive form of STSs.[40]

This finding supported the previously proposed hypothesis that, even if a wide excision is always desirable, more conservative surgery could be applied to PWTs arising in the extremities in which a 3-cm lateral margin cannot be achieved.[45] Moreover, in this anatomical location, PWTs often display expansile growth, probably allowing for a higher rate of non-infiltrated margins as compared with other anatomical regions in which infiltrative and/or satellite growths are more frequent.[46]

Although PWTs seem to display more favorable behavior, the definition of specific prognostic variables may aid clinicians in the selection of dogs requiring adjuvant therapy to reduce the risk of local recurrence. The two main prognostic variables identified for PWTs are tumor size and the histological infiltration of the underlying muscular layer. Specifically, each cm of increase in tumor size has been associated with a greater risk of relapse and a size larger than 5 cm or involvement of muscular layer increases the risk of recurrence 7 and 8 fold, respectively.[40,46]

The microscopic involvement of the muscular layer is also generally reported for PWTs with an infiltrative and satellite growth pattern, and in tumors not located on the extremities.[46] This combination of features defines a profile which is associated with the highest risk of recurrence while dogs with PWTs not located at the extremities but with the sole involvement of the subcutis had the lowest risk.[46]

Despite the fact that histologic grading is the most significant prognostic factor for canine cutaneous STSs,[34,35,41,47] neither the histologic grade nor any of its components (mitotic index, percentage of necrosis or tumor differentiation) were statistically associated with the outcome of PWTs, supporting the distinctive behavior of this entity.[40]

This finding was probably justified by the fact that PWTs mainly recur locally while histologic grade better predicts the probability of metastasis.[29,34] Furthermore, PWTs at first emergence are usually low grade;[46] thus, grade may not be informative of the probability of relapse, that can be better predicted by other parameters.[40,46]

Finally, canine PWTs have been reported to express proteins of vascular endothelial growth factor (VEGF)-, platelet-derived growth factor subunit B (PDGFB)-, and basic fibroblast growth factor (bFGF)-mediated pathways, suggesting the possible role of these molecules as targets for adjuvant therapy.[48] Nevertheless, studies regarding the efficacy of adjuvant therapies for PWTs, such as the administration of commercially available tyrosine-kinase inhibitors, are lacking, and oncologists should follow the therapy suggested for the generic group of STSs, such as radiation therapy and/or metronomic chemotherapy.[35,41,42-44,48]

In summary, the studies published in the last decade have led to a better definition of the origin of “old” HPC and PWTs clarifying many of the diagnostic features and their peculiar clinical behavior.[11,23,27,33,40,46,48] Several data are still lacking for the complete understanding of canine PWTs, and additional studies are required and should be encouraged to analyze its pathogenesis and therapy.

The authors believe that, as has occurred for canine PWTs, improvement in the definition of the specific neoplastic entities is a necessary step in researching any canine STS in order to carry out studies on homogeneous caseloads, allowing the description of the differences (or similarities) between STS subtypes.

Conflict of Interest

The author has no potential sources of conflict of interest related to this work to declare.

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