Morphological patterns and malignancy criteria of transmissible venereal tumor in cytopathological and histopathological exams

Padrões morfológicos e critérios de malignidade do tumor venéreo transmissível aos exames citopatológico e histopatológico

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Abstract

The aim of this study was to identify morphological patterns and malignancy criteria of the TVT in cytopathological and histopathological evaluations and relate these characteristics to clinical evolution and response to chemotherapy. Regarding studied animals, sixteen dogs were female and four were male. The age of the animals ranged between one and ten years old. Considering breed, 80% of the dogs were mongrel dogs and 20% were of other breeds. It was found that the cytological samples allowed a better characterization of the cell type than histological ones. The plasmacytoid was the most common morphological type of TVT, followed by the lymphocytoid and mixed standards. There was no difference among the scores for the malignancy criteria and morphological types of TVT. Regarding response to chemotherapy, no morphological type of the TVT showed any difference, but the TVT presents morphological peculiarities that may interfere with tumor behavior, especially those related to increased aggressiveness and that are observed in the plasmacytoid TVT.

Keywords: Dog. Round cell tumor. Morphology.

Resumo

Este estudo teve por objetivo identificar os padrões morfológicos e os critérios de malignidade do TVT aos exames citológico e histopatológico e relacionar essas características à evolução clínica e à resposta à quimioterapia. Dos animais estudados, dezesseis cães eram fêmeas e quatro machos. A idade dos animais variou entre um e dez anos de idade. Quanto à raça, 80% dos cães eram sem raça definida e 20% de outras raças. Constatou-se que as amostras citológicas permitiram melhor caracterização do tipo celular do que as histológicas. O TVT tipo plasmocitoide foi o de maior ocorrência, seguido pelos padrões linfocitoide e misto. Não houve diferença entre os escores estabelecidos para os critérios de malignidade e os tipos do TVT. Nenhum tipo morfológico do TVT diferiu quanto à resposta quimioterápica, mas o TVT apresenta particularidades morfológicas que podem interferir no comportamento tumoral, especialmente aquelas relacionadas à maior agressividade, e que são observadas no TVT plasmocitoide.

Palavras-chave: Cão. Neoplasia de células redondas. Morfologia.

Introduction

The transmissible venereal tumor (TVT) is characterized as an undifferentiated neoplasm of round cells, whose histological origin is still controversial (MOUTINHO et al., 1995; DAS; DAS, 2000). TVT has a worldwide distribution and in natural conditions, it affects only dogs (BASSANI-SILVA et al., 2007).

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However, its occurrence was already described in phylogenetic-related species as result of experimental tumor transplantation (COCKRILL; BEASLEY, 1979; HARMELIN et al., 2002). It is a neoplasm commonly transmitted during copulation, by means of a rare deployment mechanism involving viable tumor cells in the genital mucosa of dogs, especially in presence of tissue injury and discontinuity (SCARPELLI; VALLADÃO; METZE, 2010). The social habits inherent to the dogs, such as sniffing and licking, also favor the primary development of neoplasm in extra genital sites (SANTOS et al., 2008).

In general, tumor masses are characteristic and easily recognized by clinical examination, exhibiting friable aspect, reddish appearance, easy bleeding, constant signs of ulceration and inflammation, and diameter in the range 0.5-10cm (STOCKMANN et al., 2011). Deformation in the genital region, offensive odor and licking the genital area are also signs frequently reported. On the other hand, the overall physical condition is usually preserved (DAS; DAS, 2000).

Diagnosis of TVT is based on clinical signs, and additional exams are necessary to the diagnostic confirmation (SCARPELLI; VALLADÃO; METZE, 2010). Cytopathological and histopathological exams stand out among the tests employed in diagnostic routine (STOCKMANN et al., 2011; SPUGNINI; DOTSINSKY; MUDROV, 2008; SÁNCHEZ-SERVÍN et al., 2009). Even though the TVT presents peculiar characteristics, studies have shown morphological differences between cell types that compose the neoplasm. Thus, Amaral et al. (2007) and Floréz et al. (2012) proposed morphological classification of TVT in three types, according to the predominant cell pattern, and described the lymphocytoid, plasmacytoid and mixed forms, which comprises an intermediate pattern between the first two.

Presence of three or more malignancy criteria indicates the malignant potential of a neoplasm (ACKERMAN, 2007), which also applies to the TVT. Moreover, some studies address possible correlations between the predominant morphological type, and its malignant potential (STOCKMANN et al., 2011; FLORÉZ et al., 2012; GASPAR, 2005; SALAMANCA et al., 2008) and resistance to chemotherapy (FLORÉZ et al., 2012; GASPAR et al., 2010) of this neoplasm in dogs. Therefore, this study aimed to identify morphological patterns and malignancy criteria of TVT using cytopathological and histopathological exams, and relate these characteristics to clinical evolution and response to chemotherapy with vincristine sulfate.

Material and Methods

This study was conducted after approval of the Committee for Research Ethics of the Federal University of Goiás (COEP/UFG - 123/2011). The experimental protocol included obtaining clinical data from dogs with TVT, samples from these animals for cytopathological and histopathological analyzes, and data related to monitoring of chemotherapy.

From August to December 2011, 20 dogs with TVT of primary genital location and attended at the ambulatory routine of a veterinary hospital were included in the study. There were no restrictions to breed, sex and age. In order to obtain the clinical data, each animal underwent a complete physical examination, and the information was recorded on an individual clinical evaluation protocol. Only dogs in their first occurrence of the TVT, which had not received previous chemotherapy, were admitted into the study.

The clinical evaluation protocol included data on breed, age and sex of the animals, as well as breeding habits, reproductive history and contact with other dogs. Information related to neoplasm was also recorded, such as appearance of the tumor mass, duration of clinical evolution, tumor size, evaluation of the overall physical condition of the animals, and presence or absence of apparent metastasis by visual inspection, palpation and detailed physical examination of dogs with TVT.

For microscopic evaluations, two clinical specimens were collected from the genital tumor of each dog. One of them was used for the preparation of the cytological sample and the other for the preparation of the histological sample. Impression cytology was performed by gentle compression of a slide against the neoplastic fragment, allowing the sample to dry at room temperature. Each slide was identified, fixed in methanol (5 min), and subjected to Giemsa stain. The tumor fragment for histological analysis, which was obtained from the border region between the neoplasm and normal tissue, was fixed (48 hours) in 10% buffered formalin (pH 7,0), processed, and included in paraffin. After that, sections of 4 μm were obtained, stretched on histological slides, stained with hematoxylin and eosin (HE), and evaluated concerning histomorphological aspects. Cytological and histological exams were performed by two researchers, who were blinded to identification of samples and followed the criteria described by both, Floréz et al. (2012) for cytomorphological classification (Table 1), and Gaspar et al. (2010) for malignancy criteria of TVT. Slides were evaluated for checking the sample cellularity and stain standard (5x), and for analysis of cellular details and malignancy criteria (10x, 20x and 40x) (Table 1).

Criteria for cell malignancy were divided into general, cytoplasmic and nuclear. All of them were classified as absent, mild, moderate or severe, according to either intensity or amount. Among the general ones, anisocytosis, anisokaryosis, macrokaryosis, basophilia, eosinophilia, cytoplasmic vacuoles, and lymphoglandular bodies were considered. Cytoplasmic criteria comprised changes in cell morphology, such as spindle cell, tadpole cells, phagocytosis, and cytoplasmic projections. Regarding nuclear criteria, characteristics such as hyperchromasia, binucleation, multinucleation, pseudo-inclusion and mitosis were included.

After diagnosis confirmation, the animals underwent chemotherapy with vincristine sulfate (0.025 mg/kg, intravenous), every seven days, in at least four applications. The end of treatment was determined when total regression in the apparent tumor mass was verified and a second cytopathological exam showed absence of viable neoplastic cells.

In order to analyze the results related to clinical data and response to therapy, descriptive statistic was used. For classification of the TVT (cytomorphological standard), the statistical test based on χ^2 was used. The general, cytoplasmic, and nuclear malignancy criteria and comparison between the morphological type and response to therapy underwent a nonparametric statistical comparison test by using the Kruskal-Wallis ranks. A 5% significance level was used for all tests (SAMPAIO, 1998).

Results

Among the 20 animals with TVT studied, 16 of them were females (80%) and 4 were males (20%). Age of the animals ranged from one to ten years, and the highest

Table 1 - Criteria for cytomorphological classification of TVT in cytopathological and histopathological exams (Jataí, 2012)

Types	Characteristics
Lymphocytoid	Prevalence (≥60%) of typical TVT cells with rounded morphology, scanty and finely granular cytoplasm, vacuoles in the periphery of the cell, round nuclei with rough chromatin, and one or two protruding nucleoli.
Plasmacytoid	Prevalence (≥60%) of TVT cells with ovoid morphology, more abundant cytoplasm (lower nucleus:cytoplasm ratio), and eccentric nucleus.
Mixed	Mixed cellularity in lymphocytoid and plasmacytoid cell types, (Prevalence ≤59% of the total)

Source: Floréz et al. (2012)

percentage of dogs affected was concentrated in the four-to-six years age group (50%). Regarding breed, 80% of the dogs were mongrel dogs and the other 20% were of other breeds (Table 2). In most cases, the dogs had a reproductive history of 3 or more copulas and no animal in the study had been neutered. Regarding the breeding habits, it was found that the animals had free access to the streets, being impractical to control the origin of the contacts. Despite the negative impact of the neoplastic process on health, only one dog showed poor general physical condition, with obvious signs of dehydration and weight loss. The remaining dogs were in apparently good condition, except for the presence of tumor mass. Concerning size of the tumor mass, small diameter was found in 9 (45%), medium size in 5 (30%), and large size in 6 (40%) animals. The owners could not provide exact information about the evolution time of the neoplastic lesions.

About comparison between cytological and histological analyzes, cytological samples allowed a better characterization of the cell type, as well as the malignancy criteria, with less image distortion when compared to the histological samples (Figure 1). When determining the morphological pattern of TVT, plasmacytoid type was the most frequent (45%), followed by the lymphocytoid (30%) and mixed (25%) cell-type patterns (Figure 2). In evaluating the relationship between the morphological pattern and neoplasm size, the lymphocytoid and mixed cell types were predominant in medium- and large-size tumors, and the plasmacytoid cell type was predominant in the small-size masses. It was not possible to evaluate the relationship between clinical evolution and morphological pattern, since the owners could not provide exact information about the evolution time of lesions. Furthermore, apparent metastases were not

Table 2 - Cases of TVT in dogs by age, gender, and breed (Jataí, 2012)

	Gender and Breed of Dogs with TVT							
	Female				Male			
Age Range (years)	Boxer	Pit Bull	Poodle	Mongrel	Boxer	Pit Bull	Poodle	Mongrel
0-3	1	1	-	4	-	1	-	-
4-6	-	-	-	7	-	-	1	2
7-10	-	-	-	3	-	-	-	-

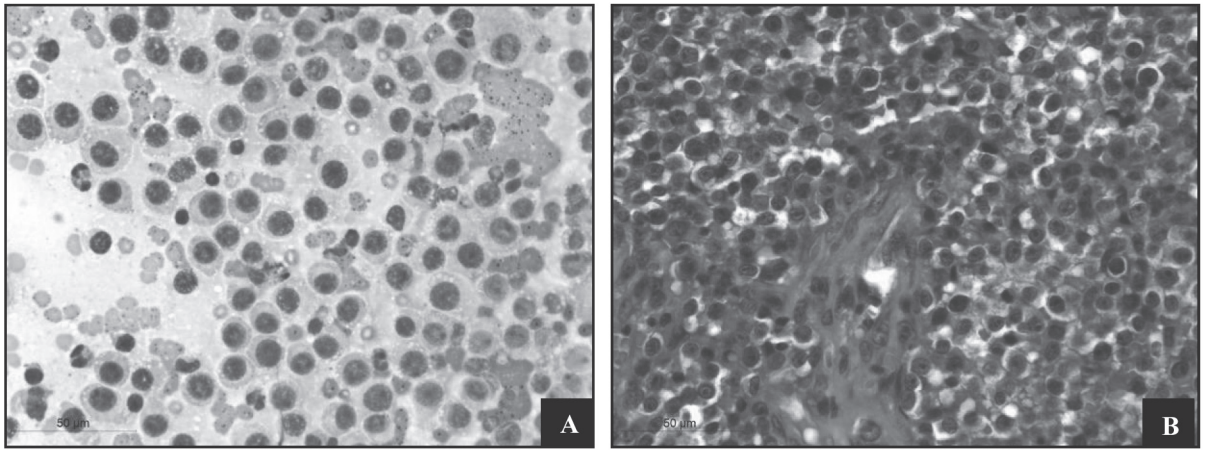

Figure 1 - Canine transmissible venereal tumor (TVT). Preservation of cellular morphology in the cytological sample **(A)**, Giemsa, 40x, when compared to histological sample **(B)**, HE, 40x

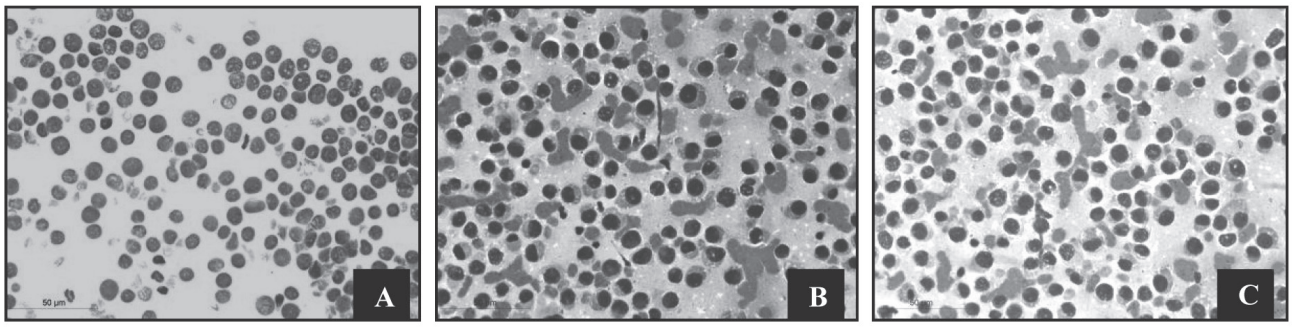

Figure 2 - Morphological patterns of TVT in cytopathological exam. **(A)** Lymphocytoid pattern. Round cells with large nucleus and scanty cytoplasm, similar to lymphocytes. **(B)** Plasmacytoid pattern. Cells with abundant cytoplasm and eccentric nucleus, with low nucleus/cytoplasm ratio. **(C)** Mixed pattern. Cells with lymphocytoid and plasmacytoid morphology. Giemsa, 20x

observed, including the skin, lymph nodes, and oral, nasal, and ocular mucous membranes.

Regarding malignancy criteria, including general, cytoplasmic and nuclear ones, no significant difference ($P < 0.05$) was observed between morphological types of TVT and the scores set for each criteria. Therefore, the malignancy criteria and the ratio between the highest and lowest frequency are described in terms of relative numbers. Among the general criteria, anisocytosis was observed in 14 (70%) samples with a discreet score and in 6 (30%) with a moderate score (30%). Anisokaryosis was found in 100% of the samples, with discreet and moderate scores in 15 (75%) and 5 samples (25%), respectively. Macrokaryosis was not present in 10 (50%) samples of TVT, but it was mild or moderate in 5 (25%), respectively. The cytoplasmic vacuoles criterion was found in discrete degree in 8 (40%) samples, moderate in 7 (35%) and severe in 3 (35%). However, this criterion was not observed in 2 samples (10%). The cytoplasmic characteristics of basophilia and eosinophilia were analyzed simultaneously, since in the presence of one the absence of the other was considered. Cytoplasm predominance was found to be basophilic and eosinophilic in 6 (30%) and 14 (70%) of all samples. In both characteristics, the scores were concentrated on mild or moderate degrees. Lymphoglandular corpuscles were not observed in the total (100%) of the samples analyzed (Table 3).

Concerning cytoplasmic criteria, spindle cells were not found in any sample. Phagocytosis was the second cytoplasmic criterion among those with low occurrence, being observed in only 6 (30%) samples, with a discreet score. Among these, 4 (20%) exhibited plasmacytoid morphological pattern. The tadpole cells and the cytoplasmic projections also showed low frequency. Eight (40%) samples were positive for both criteria, being 6 (30%) with a discrete and 2 (10%) with a moderate score. Although no significant difference was found, the findings reported for these criteria were more frequent in samples of the plasmacytoid cell type (Table 3).

Binucleation was the most frequent criteria of nuclear malignancy, with 7 (35%) samples exhibiting discreet score and 2 (10%) moderate score. Hyperchromasia and nuclear pseudo-inclusions were found in discreet score in 2 (10%) samples of plasmacytoid type. Regardless of the predominant cell pattern, these criteria were not observed in other samples. Multinucleation was present with a discreet score in 5 samples of the plasmacytoid (3), lymphocytoid (1) and mixed (1) cell types. Mitosis were detected in 5 (25%) samples of TVT. Among these, discrete and moderate scores were exhibited by 4 (80%) and 1 (20%) samples, respectively. When cases of discreet score for mitosis were analyzed, they were equally distributed between the lymphocytoid (2) and plasmacytoid (2) cell types. On turn, mitosis

with moderate degree was found in the plasmacytoid cell type, although with no significant difference as compared to other cell types ($p > 0.05$) (Table 3).

Among the 20 dogs with the TVT, 13 were monitored from the beginning of treatment until the complete remission of neoplasm, which was determined by the clinical absence of tumor mass and tumor cells in the cytopathological exam. However, no significant difference was observed between responses to chemotherapy and the morphological pattern of TVT (Table 4).

Five owners opted to interrupt the treatment of their animals before complete regression and confirmation of absence of the TVT. Two animals died during the study period due to other complications. Necropsies of the animals were not performed because they were not authorized by the owners.

Table 3 - Distribution of samples among the general, cytoplasmic, and nuclear malignancy criteria according to scores and morphological types of TVT (Jataí, 2012)

Criteria	**Absent**			**Discreet**			**Moderate**			**Severe**		
	L	**P**	**M**	**L**	**P**	M	L	P	M	L	P	M
General Criteria (n=20)												
Anisocytosis	-	-	-	5a	6a	5a	1a	3a	1a	-	-	-
Anisokaryosis	-	-	-	5a	6a	4a	1a	3a	1a	-	-	-
Macrocariose	4a	4a	2a	1a	2a	2a	1a	3a	1a	-	-	-
Basophilia	-	-	-	0a	3a	0a	1a	2a	1a	-	-	-
Eosinophilia	-	-	-	3a	2a	2a	2a	2a	2a	-	-	-
Cytoplasmic Vacuoles	0a	2a	0a	3a	4a	1a	2a	3a	2a	1a	0a	2a
Lymphoglandular Bodies	-	-	-	-	-	-	-	-	-	-	-	-
Cytoplasmic Criteria (n=20)												
Spindle Cell	6a	9a	5a	-	-	-	-	-	-	-	-	-
Tadpole Cell	4a	5a	3a	2a	2a	2a	0a	2a	0a	-	-	-
Phagocytosis	5a	5a	4a	1a	4a	1a	-	-	-	-	-	-
Cytoplasmic Projections	5a	4a	3a	1a	3a	2a	0a	2a	0a	-	-	-
Nuclear Criteria (n=20)												
Hyperchromasia	6a	7a	5a	0a	2a	0a	-	-	-	-	-	-
Binucleation	4a	4a	3a	2a	4a	1a	0a	1a	1a	-	-	-
Multinucleation	6a	4a	5a	1a	3a	1a	-	-	-	-	-	-
Pseudo-inclusions	6a	7a	5a	0a	2a	0a	-	-	-	-	-	-
Mitosis	5a	4a	6a	2a	2a	0a	0a	1a	0a	-	-	-

The same overwritten letters in the same column (a) do not differ among each other by nonparametric test of Kruskal-Wallis ranks ($P > 0.05$). Numerical values correspond to the number of samples with correspondent findings for each score and morphological type. L: lymphocytoid; P: plasmacytoid; M: mixed.

Table 4 - Relationship between the number of chemotherapy sessions and morphological pattern prevalent in dogs with TVT (Jataí, 2012)

Number of chemotherapy sessions	**Morphological standard of the TVT**		
	Lymphocytoid	**Plasmacytoid**	**Mixed**
Four (n=9)	3a	4a	2a
Five (n=3)	1a	2a	0a
Seven (n=1)	0a	1a	0a

The same overwritten letters in the same column (a) do not differ among each other by nonparametric test of Kruskal-Wallis ranks ($P > 0.05$).

Discussion

The study of the TVT arouses interest in the scientific community because it is a common tumor in dogs, mainly in Brazil, and genital location is one of its main characteristics. Amaral et al. (2007) evaluated 138 dogs with this tumor and showed that 89.6% of the neoplasms were located in genital area. In the present study, a comparable situation was observed, in which all animals selected presented primary TVT in their genitals.

The cases of TVT were selected regardless of gender, breed and age of the animals; although some studies state that there is no predisposition to neoplasia related to these variables (BRANDÃO et al., 2002; SALAMANCA et al., 2008). However, female dogs were predominant, reflecting the proper deployment of TVT cells in the genital tract of bitches. Regarding breed, most tumors occurred in mongrel dogs, confirming the description of Brandão et al. (2002), who attributed a higher occurrence of TVT in mixed-breed dogs. Added to this, Souza et al. (2000) complement that TVT is more common in areas with stray dogs. The age of animals affected by TVT ranged from one to ten years, and the highest frequency occurred in the age group four to six years old. In addition, these dogs had reproductive history of three or more copulations. Similarly, Das and Das (2000) mentioned that dogs from three to six years of age are most affected by TVT because they are in the period of the highest sexual activity of adult dog. One animal had physical weakness, with signs of weight loss and dehydration. On the other hand, the general condition was good in most dogs, confirming the descriptions by Amaral (2005), that physical condition of dogs with TVT is usually preserved.

Regarding the microscopic analyzes, the cytopathological exam allowed better visualization of the cell type and better evaluation of malignancy criteria of the TVT, with less image distortion when compared to histological slides (GASPAR, 2005). In this context, Duncan and Prasse (1979), who discussed the use of cytology for diagnosis of round cell neoplasias, pointed out that in many cases cytological characteristics are definitive for diagnosis as compared to the histological ones. This was also observed in the present study. Thus, the cytopathological analysis is suggested for morphological classification of TVT and evaluation of malignancy criteria.

The predominant morphological pattern of TVT followed the criteria of Floréz et al. (2012), with higher frequency of plasmacytoid type, followed by the lymphocytoid and mixed patterns, respectively, and this condition was also observed by Gaspar et al. (2010). It is noteworthy that there was a relationship between morphological pattern and the size of the tumor, confirming that the plasmacytoid cell type is predominant in small masses whereas the others cell types are predominant in medium and large masses, which is also reported by Salamanca et al. (2008). On the other hand, it was not possible to establish the relationship between the morphological pattern and duration of clinical evolution, as most owners could not provide information about the time of onset of the neoplasm. Amaral et al. (2007) also did not succeed regarding this parameter. A similar situation occurred in the relationship between morphological pattern and presence of metastasis, since no apparent metastatic focus was observed in those study animals. Regarding this situation, it is worth noting the low occurrence of metastases in dogs with TVT, what according to Tinucci-Costa (2008), is less than 5%, hindering studies involving such relationship.

Among the general malignancy criteria, including anisocytosis, anisokaryosis, macrokaryosis, basophilia and eosinophilia, there are relevant data about the cytomorphological changes. According to Meinkoth, Cowell and Cowell (2002), these criteria for evaluation of malignancy in cell samples are well established. However, some studies report that some degree of anisocytosis is present in any tissue (ACKERMAN, 2007; MEINKOTH, COWELL; COWELL, 2002). In addition, macrokaryosis results from increase in the content of nuclear DNA, and presence of

this characteristic in moderate to severe scores is considered clinically important (ACKERMAN, 2007). In this research, 25% of samples showed moderate macrokaryosis, indicating an important finding on the malignant potential of TVT.

Regarding the cytoplasmic vacuoles, Meinkoth, Cowell and Cowell (2002) describe that this finding is the morphological characteristic that distinguishes TVT from the other round cells neoplasias, and its absence is rare. In our study, only two samples showed cytoplasmic vacuoles in tumor cells. Despite that, TVT diagnosis was confirmed based on clinical and microscopic characteristics. Lymphoglandular bodies were not found in the samples analyzed. Although Amaral et al. (2007) describe them as a consistent finding, they are small and may be unnoticed, fact which may have occurred in this study. Spindle cells were not observed in this study. In contrast, tadpole cells and cytoplasmic projections were found in 40% of tumors, which is in accordance with the finds of Gaspar et al. (2010). These authors relate tadpole cells with action of microtubules, rigidity of the membrane, and pressure of contiguous cells, which allows inferring high cellularity of neoplastic masses that present this cytomorphological criterion.

The criteria of hyperchromasia and nuclear pseudoinclusion malignancy occurred in 10% of samples that exhibited the plasmacytoid morphological pattern. Ackerman (2007) also observed such criteria in cytological samples of TVT and its occurrence was associated with increase of DNA content in preparation for cell division, which supports the findings related to mitosis in this study, especially the largest mitotic index in samples with plasmacytoid cytomorphology. Mitosis is a common and well-established characteristic of TVT. However, MacLachlan and Kennedy (2002) do not consider this criterion a strong indicator of malignancy for TVT, since the majority of mitosis is typical. Nevertheless, this criterion was considered in the evaluation of malignancy in this study, since malignant cells replicate with increased speed.

Treatment with vincristine sulfate was effective in all animals treated in the present study and, in general 93%, the dogs showed complete remission of the neoplasm with four or five sessions of chemotherapy. Only in one animal with plasmacytoid TVT diagnosis was healed at the seventh session. According to Amaral (2005) and Gaspar (2005), the plasmacytoid morphological pattern exhibits a more aggressive behavior and increased expression of P-glycoprotein, responsible for tumor resistance, which could be related to that observed in this study. Given this finding and others described, was verified that aggressive characteristics are higher in plasmacytoid TVT than other morphological types. Thus, it is possible that plasmacytoid TVT shows a higher resistance to the vincristine sulfate. It is necessary to add that disregard of the treatment, which occurred in five cases, precluded calculating a correlation between the therapeutic success and morphological pattern of TVT. Furthermore, as described by Amaral (2005), treatment interruption comprises factor that contributes to resistance of the neoplastic cells to antineoplastic medications.

Conclusions

The cytopathological exam allows better characterization of the morphological patterns and malignancy criteria of the TVT compared to the histopathological exam, being possible to identify the plasmacytoid, lymphocytoid and mixed types. The TVT presents morphological peculiarities that may interfere with tumor behavior, especially those related to increased aggressiveness and that are observed in the plasmacytoid TVT, the most common morphological type of this neoplasia.

References

ACKERMAN, M. R. Acute inflammation. In: McGAVIN, M. D.; ZACHARY, J. F. **Pathologic basis veterinary disease**. Philadelphia: Mosby Elsevier, 2007. chap. 3-4, p. 101-191.

AMARAL, A. S. **Tumor venéreo transmissível canino**: critérios de malignidade e caracterização citomorfológica correlacionada à imunocitoquímica e lesões de DNA. 2005. 225 f. Tese (Doutorado em Ciência Animal) - Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, 2005.

AMARAL, A. S. do; BASSANI-SILVA, S.; FERREIRA, I.; FONSECA, L. da; ANDRADE, F. H. E. de; GASPAR, L. F. J.; ROCHA, N. S. Caracterização citomorfológica do tumor venéreo transmissível canino. **Revista Portuguesa de Ciências Veterinárias**, v. 102, n. 563/564, p. 253-260, 2007.

BASSANI-SILVA, S.; SFORCIN, J. M.; AMARAL, A. S.; GASPAR, L. F. J.; ROCHA, N. S. Propolis effect in vitro on canine transmissible venereal tumor cells. **Revista Portuguesa de Ciências Veterinárias**, v. 102, n. 563/564, p. 261-265, 2007.

BRANDÃO, C. V.; BORGES, A. G.; RANZONI, J. J. T.; RAHAL, S. C.; TEIXEIRA, C. R.; ROCHA, N. S. Tumor venéreo transmissível: estudo retrospectivo de 127 casos (1998-2000). **Revista Educação Continuada - CRMV-SP**, v. 5, n. 1, p. 25-31, 2002.

COCKRILL, J. M.; BEASLEY, J. N. Transmission of transmissible venereal tumor of the dog to the coyote. **American Journal of Veterinary Research**, v. 40, n. 3, p. 409-410, 1979.

DAS, U.; DAS, A. K. Review of canine transmissible venereal sarcoma. **Veterinary Research Communications**, v. 24, n. 8, p. 545-556, 2000.

DUNCAN, J. R.; PRASSE, K. W. Cytology of canine cutaneous round cell tumors: mast cell tumor, histiocytoma, lymphosarcoma and transmissible venereal tumor. **Veterinary Pathology**, v. 16, n. 6, p. 673-679, 1979.

FLORÉZ, M. M.; PEDRAZA, F.; GRANDI, F.; ROCHA, N. S. Cytological subtypes of canine transmissible venereal tumor. **Veterinary Clinical Pathology**, v. 41, n. 1, p. 3-5, 2012.

GASPAR, L. F.; FERREIRA, I.; COLODEL, M. M.; BRANDÃO, C. V.; ROCHA, N. S. Spontaneous canine transmissible venereal tumor: cell morphology and influence on p-glycoprotein expression. **Turkish Journal of Veterinary and Animal Sciences**, v. 34, n. 5, p. 447-454, 2010.

GASPAR, L. F. J. **Caracterização citomorfológica do tumor venéreo transmissível canino correlacionada com danos citogenéticos, taxa de proliferação e resposta clínica à quimioterapia**, 2005. 157 f. Tese (Doutorado em Ciência Animal) – Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, 2005.

HARMELIN, A.; PHINTUS, J. H.; FRIEDMANN-MORVINSKI, D.; KAUFMAN, K.; BRENNER, O. Lack of MHC expression and retention of ultrastructural characteristics by xenograft transmissible venereal tumor cells in SCID mice. **Veterinary Immunology and Immunopathology**, v. 63, n. 3, p. 245-249, 2002.

MacLACHLAN, N. J.; KENNEDY, P. C. Tumors of genital systems . In: MEUTEN, D. J. (Ed.). **Tumors in domestic animals**. 4. ed. Ames: Lowa Lowa State , 2002. chap. 3, p. 547-573.

MEINKOTH, J. H.; COWELL, J. H. Recognition of basic cell types and criteria of malignancy. **The Veterinary Clinics of North America, Small Animal Practice**, v. 32, n. 6, p. 1209-1235, 2002.

MOUTINHO, F. Q.; SAMPAIO, G. R.; TEIXEIRA, C. R.; SEQUEIRA, J. L.; LAUFER, R. Tumor venéreo transmissível com metástases cutâneas em um cão. **Ciência Rural**, v. 25, n. 3, p. 469-471, 1995.

SALAMANCA, S.; SANTADER-BAQUERO, A.; TRIANA-GARCÍA, P. A.; ROMERO, S.; RONDÓN- BARRAGÁN , I. S. Tumor venéreo transmisible (TVT) con metástasis pulmonar: reporte de caso. **Orinoquia**, v. 12, n. 2, p. 162-170, 2008.

SÁNCHEZ-SERVÍN, A.; MARTINÉZ, S.; CÓRDOVA-ALARCON, E.; FAJARDO, R. TP53 Polymorphisms allow for genetic sub-grouping of the canine transmissible venereal tumor. **Journal of Veterinary Science,** v. 10, n. 4, p. 353-355, 2009.

SANTOS, F. G. A.; VASCONCELOS, A. C.; NUNES, J. E. S.; CASSALI, G. D.; PAIXÃO, T. A.; MARTINS, A. S.; SILVA, S. S.; MARTINS, R. F.; MORO, L. Apoptosis in the transplanted canine transmissible venereal tumor during growth and regression phases. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 60, n. 3, p. 607-612, 2008.

SCARPELLI, K. C.; VALLADÃO, M. L.; METZE, K. Predictive factors for the regression of canine transmissible venereal tumor during vincristine therapy. **The Journal Veterinary**, v. 183, n. 3, p. 362-363, 2010.

SOUSA, J.; SAITO, V.; NARDO, A.; RODASKI, S.; GUÉRIOS, S.; BACILA, M. Características e incidência do tumor venéreo transmissível (TVT) em cães e eficiência da quimioterapia e outros tratamentos. **Archives of Veterinary Science**, v. 5, n. 1, p. 41-48, 2000.

SPUGNINI, E. P.; DOTSINSKY, I.; MUDROV, N. Biphasic pulses enhance bleomycin efficacy in a spontaneous canine genital tumor model of chemoresistance: sticker sarcoma. **Journal of Experimental & Clinical Cancer Research**, v. 26, n. 4, p. 483-487, 2008.

STOCKMANN, D.; FERRARI, H. F.; ANDRADE, A. L.; LOPES, R. A.; CARDOSO, T. C.; LUVIZOTTO, M. C. R. Canine transmissible venereal tumors: aspects related to programmed cell death. **Brazilian Journal of Veterinary Pathology**, v. 4, n. 1, p. 65-75, 2011.

TINUCCI-COSTA, M. Tumor venéreo transmissível canino. In: DALECK, C. R.; NARDI, A. B.; RODASKI, S. **Oncologia em cães e gatos**. São Paulo: Roca, 2008. p. 539-556.