

## Clinical and cytological staging of transmissible venereal tumour at the Botucatu Veterinary Hospital

### ARTÍCULO DE INVESTIGACIÓN

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**ABSTRACT:** The canine transmissible venereal tumour is a transplantable neoplasm of great scientific interest for its versatile biological behaviour and its complex mechanisms of evolution. The main objective of this research was to obtain information in order to carry out a better clinical and therapeutic approach of patients with this tumour, twenty dogs with clinical and cytological diagnosis of TVT were studied. The clinical staging (TNM) was carried out, the cytomorphologic classification and the nuclear malignancy and cytoplasmic criteria were obtained. The Chi-square test and the Mann-Whitney test were used for data analysis. Eighteen animals were mixed race with a 2 to 10 range of age, and the main location of the tumor was the external genital and the predominant cellular type was plasmacytoid. The malignancy nuclear and cytoplasmic criteria, with the exception of the perinuclear halo, were similar to those described in the literature. During the RNM classification, two tumours were categorized as T3M and one as T3N1; the other tumours were classified in different T degrees. Despite the lack of statistical significance for the employed method, in general, a good clinical relationship was seen between TNM staging and cytopathological findings, suggesting that both provide greater certainty about the degree of aggressiveness, progression and prognosis in the patients with TVT, as it occurs in other types of tumours. Use of staging in this tumour can serve as a criterion to suggest the possible evolution and type of therapy. Nevertheless we recommend future research to help define the benefits of using the TNM clinical staging in TVT.

**Key words:** cytopathology, dog, plasmacytoid

## Estadificación clínico y citológico del tumor venéreo transmisible en el hospital veterinario de Botucatu\*

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**RESUMEN:** El tumor venéreo transmisible canino es una neoplasia trasplantable que despierta gran interés científico por su comportamiento biológico versátil y sus complejos mecanismos de evolución; el objetivo de la investigación fue obtener información para realizar un mejor abordaje clínico-terapéutico de pacientes con este tumor, fueron utilizados 20 perros con diagnóstico clínico y citológico de TVT; fue realizada la estadificación clínica (TNM), la clasificación citomorfológica y la descripción de criterios de malignidad nuclear y citoplasmáticos. Para el análisis de los datos se utilizaron los test estadístico chi-cuadrado y el Mann-Whitney. 18 animales eran mestizos con un rango de edad entre los 2 a 10 años, la principal localización del tumor fue a nivel genital externo y el tipo celular más común fue el plasmocitóide; los criterios de malignidad nucleares y citoplasmáticos con excepción del halo perinuclear fueron similares a los descritos en la literatura. Durante la clasificación TNM, fueron categorizados dos tumores en estado T3M y uno en T3N1, los demás en diferentes grados de T. A pesar de la ausencia de significancia estadística por el método empleado, en general, fue vista una buena relación clínica entre la estadificación TNM y los hallazgos citopatológicos, lo que sugiere que ambos son complementarios para proveer mayor seguridad sobre el grado de agresividad, evolución y pronóstico en pacientes con TVT, como sucede con otros tipos de tumores. Emplear esta estadificación en este tumor, puede servir como criterio para decidir una posible evolución y tipo de terapia, sin embargo, como es el primer trabajo en este sentido, recomendamos futuras investigaciones que ayuden a definir las ventajas de utilizar la estadificación clínica TNM en el TVT.

**Palabras clave:** citopatología, plasmocitóide, perro

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### Introduction

The transmissible venereal tumour is a contagious and sexually transmissible neoplasia of unknown origin and, in natural conditions, only affects dogs, and experimentally, other species. It was first studied in 1876 by Novinsky, and later by Smith & Washbourn (Marchal et al., 1997; Rogers, 1997; MacEwen, 2001). TVT was first described as a transmissible and transplantable tumour (Booth, 1994) and has been transplantable since the first description, over 100 years ago (Hasler & Weber, 2000). The neoplasia has spread worldwide, but is prevalent in tropical and subtropical climates (Ferreira et al., 2000; Varaschin et al., 2001), mainly in countries with large populations of mongrel street dogs (Papazoglou et al., 2001).

The transference between dogs is found equally in female as well male dogs, and occurs by implantation of viable tumoral cells in the mucous membranes during coitus, or by scratching, licking, biting or smelling a carrier animal (Varaschin et al., 2001). Besides the genital contact, TVT also may have an extra-genital localization (Pereira et al. 2000; Rodrigues et al., 2001; Albanese et al., 2002). TVT usually is restricted to its points of origin and implantation, except in rare cases (around 2,5%) (Pandey et al., 1989), where it has invaded adjacent tissues, lymphatic and/or blood circulation and reached distant areas such as lungs, liver, spleen, brain and other organs as metastatic deposits (Meuten, 2000; Jubb et al., 2007). There are also reports of multiple subcutaneous masses (Kroger et al., 1991). This tumour has a behaviour that is unique, as shown by occurrence of spontaneous regression in adults, but in newborns and immunocompromised dogs, it is metastatic and fatal (Cohen, 1973; Yang & Jones, 1973).

The cytological evaluation of superficial injuries is an extremely useful, simple, quickly done test with low cost and minimal risk to patients (Cowel & Tyler, 1989; Wright & Parry, 1989; Kroger et al., 1991).

The cytological diagnosis must be precise and able to differentiate TVT from other round cell cancers, especially lymphoma and histiocytoma; Cytological examination has also been considered essential for monitoring of treatment of transmissible venereal tumors (Amaral et al., 2007).

Amaral et al. (2007) demonstrated different cytomorphological types in cytological samples of Transmissible Venereal Tumour. There is a predominance of round cells, scarce cytoplasm, and high nucleus to cytoplasm ratio in the lymphocytoid pattern. There is a predominance of ovoid cells, ample cytoplasm, and an eccentric nucleus in the plasmacytoid pattern. The presence of both morphological types does not show predominance of either in the mixed pattern. According to Bassani-Silva et al. (2007) when TVT cells of lymphocytoid and plasmacytoid morphologies were exposed to extracts of propolis in vitro, the plasmacytoid phenotype was more resistant.

This could be an indication that the plasmacytoid TVT is more aggressive than the lymphocytoid (Fonseca et al., 2012) and make us conclude that this neoplasia has varying degrees of aggressiveness and different TVT cellular lineages (Roger et al., 1998; Montoya et al., 2012), with variable biological behaviour (Roger et al., 1998). This demonstrates the need for a specific treatment for each type of tumour, which would minimize cost and collateral effects by avoiding excessive application of chemotherapy. The TNM system was established by the International Union Against Cancer (UICC), is the most widely used means for classifying the extent of cancer spread. The TNM system consists of: the size of the primary lesion (T), the extent of its spread to regional lymph nodes (N) and the presence or absence of distant metastases (M). Determining the clinical stage defines the extension of

the tumor. The TNM allows a prognosis to be established and treatment to be planned, giving precise information to the pathologist concerning the material submitted for analysis, and in comparing clinical observations from different sources (Owen, 1980; Morrison, 2002; Amaral, 2005).

As it is known that some TVTs are resistant to chemotherapy (Brandão et al., 2002; Bassani-Silva et al., 2007; Gaspar et al., 2010). Thus, the aim of this study was to use the TNM clinical system in TVT, to evaluate a possible relationship between the tumour cytological classification criteria and clinical stage (TNM), that allowed in a future provide a system for prediction of the clinical treatment.

## **Material and Methods**

This study was approved by the Ethics Committee on Animal Experimentation of the Faculdade de Medicina Veterinária UNESP-Botucatu, São Paulo, Brazil. Samples were taken from 20 dogs with cytological and clinical diagnosis of TVT; none of them had received any prior therapy. The animals were selected independently of breed, sex and age, and they originated from the Hospital of Veterinary Medicine - UNESP at Botucatu.

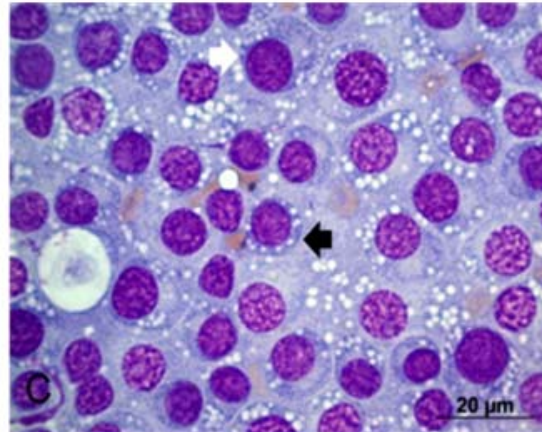
### ***Triage and clinical staging of the animals***

On initial exam a history was done to obtain information on the evolution, followed by a physical examination of the animal to decide on the TNM staging. For this, we used the following criteria; (T1 = Tumor less than or equal to two centimetres; T2 = Tumor larger than two centimetres but less than five centimetres; T3 = Tumor larger than five centimetres; N0 = absence of metastasis; N1 = metastasis to regional lymph nodes; M, metastasis to other organs except the regional lymph nodes) (Hataka, 2004).

### ***Cytopathological examination***

The cytopathological samples were collected using a gynaecology brush (external genitalia) and fine needle aspiration, (FNA) depending on the site of the mass. Two slides were prepared from each tumor, fixed and stained, one by Giemsa staining and the other by the method of Shorr. Counting was performed using a common optical microscope at a magnification of 10X to observe the entire extension of the slide and evaluate the quality and quantity of the material. At 400X magnification, the cells were observed individually for counting and verification of the characteristics of malignancy. A careful and detailed cytomorphological analysis was done using a reticulated eye piece (100/25 grid integration with 25 mm<sup>2</sup> area) which delimited the field of cell count, for cellular morphology, plasmocytic type (60% or more of TVT neoplastic cells with ovoid morphologic standard, abundant cytoplasm and nucleus eccentrically) or lymphocytoid morphology

(60% or more of typical TVT cells, with round morphology, rare cytoplasm and central nucleus) and mixed cellularity between lymphocytic and plasmacytic cell types, in which none surpassed 59% of the total (Amaral et al., 2007), (Figure 1).



**Figure 1.** 20x. Different cytomorphological types in cytological samples of Transmissible Venereal Tumor: lymphocytic (black arrow) cells with round morphology, rare cytoplasm and central nucleus and plasmacytic (white arrow) cells with ovoid morphologic standard, abundant cytoplasm and eccentric nucleus.

As well, the smears were analyzed for the presentation of nuclear malignancy criteria as evidenced by moulding, denuded, nuclear inclusions, evident nucleoli, halo around the nucleolus, slit and binucleation, and cytoplasmatic characteristics such as tadpole, signet ring, projections and cannibalism.

### *Statistic analysis*

For the statistical analysis the chi-square test was utilized, with significance level set at  $p < 0.05$ . The animals were divided into 2 groups: Group I: animals with a single mass; Group II: animals with metastasis. The comparison of the malignancy criteria between groups I and II was done using the Mann-Whitney test at level of  $p < 0.05$ .

## **Results**

### *Clinical and epidemiological data of the animals*

Eighteen of the 20 dogs were mixed breed, with one French Poodle and one Belgian Shepherd. Dog ages ranged from 2 to 10 years with a mean age of 4.1 years. The number of males and females were equal (50% from each sex). The tumour was most commonly found in the external genitalia (20 cases); the extra-genital area was not a site for any primary tumour. Three cases (15%) of metastatic tumour were found in which the primary tumour was localized in the external genitalia of the dogs. The tumour evolution time was: early tumours (up to three weeks of evolution) (2 cases), established (three to eight weeks of evolution) (5 cases) or older (more than eight weeks) (9 cases). There were 4 cases in

which owners did not know the evolution time because they had recently acquired the animal from the street. Tumor sizes were as follows: less than two centimetres in diameter (8 animals), between two and five centimetres (5 animals); and above five centimetres (7 animals). These data are summarized in Table 1.

**Table 1.** Individual list of 20 dogs (♂ ♀): results of clinical and epidemiological presentation

N / CASE	ANIMAL				PARAMETERS			
	Breed	Sex	Age	Weeks evol.	Loc.	Type	Origin	TNM*
01	WDB	F	2	3	EG	P	Primary	T <sub>2</sub>
02	WDB	F	4	3	EG	P	Primary	T <sub>2</sub>
03	WDB	F	5	2	EG	P	Primary	T <sub>3</sub>
04	WDB	F	6	3	EG	P	Primary	T <sub>3</sub>
05	WDB	M	5	1	EG	P	Primary	T <sub>2</sub>
06	WDB	M	5	1	EG	P	Primary	T <sub>1</sub>
07	WDB	F	7	2	EG	P	Primary	T <sub>1</sub>
08	WDB	F	2	2	EG	P	Primary	T <sub>3</sub>
09	WDB	M	10	3	EG	P	Primary	T <sub>1</sub>
10	WDB	M	2	3	EG	L	Primary	T <sub>1</sub>
11	WDB	M	9	2	EG and Lymph nodes	P	Primary / Metastasis	T <sub>3</sub> N <sub>1</sub>
12	WDB	F	2	2	EG	P	Primary	T <sub>2</sub>
13	WDB	M	2	3	EG	P	Primary	T <sub>1</sub>
14	WDB	M	2	3	EG	P	Primary	T <sub>1</sub>
15	WDB	F	3	?	EG and Spleen	P	Primary / Metastasis	T <sub>3</sub> M
16	Poodle	M	3	?	EG	P	Primary	T <sub>1</sub>
17	WDB	M	2	?	EG	P	Primary	T <sub>1</sub>
18	Belgian Shepherd	F	4	3	EG and Breast	P	Primary / Metastasis	T <sub>3</sub> M
19	WDB	M	5	3	EG	P	Primary	T <sub>3</sub>
20	WDB	F	2	?	EG	P	Primary	T <sub>2</sub>

\* T<sub>1</sub> ≥ 2 cm; T<sub>2</sub> > 2 to 5 cm; T<sub>3</sub> > 5 cm; N<sub>1</sub> = metastasis to regional lymph nodes; M = metastasis to other organs except lymph nodes; ? = Without information. Were mixed-breed dogs (MB), EG: External Genitalia, P: plasmacytoid, L: lymphocytoid.

### Cytopathological analysis

The slides were analyzed qualitatively and quantitatively according to the malignancy criteria observed. Those stained by the Giemsa method were used for cytoplasmatic and nuclear classification; the Shorr method was used to determine specific nuclear malignancy criteria. Ten fields from each slide were analyzed to give average values for each characteristic. The predominant morphology was the plasmocytoid type which occurred in 19 animals and the lymphocytoid pattern was found in 1 animal.

### TNM classification

According to the TNM classification, three dogs had metastases. Two cases were stage T3M, in other words, beyond the primary mass: one had metastasis in the spleen and the other in the breast. The third case was stage T3N1 with metastases in the lymph nodes. The other 17

animals were classified into different T stages according to size of the mass (data summarized in Table 1).

**Statistical analysis**

There was no statistically significant difference in terms of results of clinical and epidemiological presentation. Listed in Table 1. In the statistical analysis between the groups for one of the malignancy criteria (halo around the nucleoli), the difference was significant, with higher values found in specimens of animals with metastases. Table 1 and 2.

**Table 2.** Cytological criterion in TVT, average comparisons between Groups I and II, slides stained by Giemsa

Group	Nuclear		Morphological Criterion				Cycle Phase
	moulding	denuded	Tadpole	Signet ring	Projections	Cannibalism	Mitoses
G I	4.04 (a)	1.98 (a)	0.94 (a)	0.02 (a)	0.84 (a)	0.05 (a)	1.57 (a)
G II	6.87 (a)	1.40 (a)	0.23 (a)	0.00 (a)	1.87 (a)	0.03 (a)	1.03 (a)

There was no statistically significant difference in any of the evaluated characteristics between the two groups (p<0,05).

**Discussion**

In the cytological service in the Veterinary Hospital-UNESP Botucatu-SP, TVT is the second most common neoplasm found in dogs, following mammary cancer. Thus, TVT deserves special attention by pathologists, oncologists and surgeons (Amaral et al., 2004).

The use of cytological exam for diagnosis of round cell neoplasia was recommended by Duncan and Prasse in 1979, as these authors considered it more accurate than the histopathological exam. Since then, the cytological exam has been considered essential for diagnosis and monitoring of treatment of transmissible venereal tumors (Amaral et al., 2007).

The predominant morphologic cell type cell in both the secondary and primary TVTs was the plasmacytic pattern, found in 95% of the cases in our study. These results are similar to those described by Fonseca et al. (2012), who found this cell type in 82% of their Cases. In two studies, one with 132 and the other with 576 TVT samples, the relevant percentages of plasmacytic cells found were 53% and 74% (Amaral et al., 2004; Amaral, 2005).

One possible analysis of these results may suggest a probable tumour progression to more aggressive forms, in the patients treated at the hospital.

This may be explained by previous studies done at the Laboratory of Investigative Pathology FMVZ Botucatu, where TVTs of different degrees of aggressiveness have been under evaluation (Amaral, 2005; Bassani-Silva, 2005; Gaspar, 2005). According to Bassani-Silva et al.

(2007) when TVT cells of lymphocytoid and plasmacytoid morphologies were exposed to extracts of propolis in vitro, the plasmacytoid phenotype was more resistant. Comparing the results of response to chemotherapy among the 2 morphological groups, it can be noted that the plasmacytoid group was significantly less sensitive to chemotherapy in relation to the lymphocytoid (Gaspar et al., 2009; Gaspar et al., 2010).

It has been shown that TVTs with plasmacytoid morphology have fewer DNA breaks (Amaral et al., 2011), larger nucleolar sizes (Amaral, 2005), a high reactivity of Ki67 (MBI-1) (Gaspar, 2005), a higher proportion of metastases (Amaral et al., 2007), and increased expression rates of p-glycoprotein (Gaspar et al., 2010). A direct consequence of this is the higher number of chemotherapy applications required to obtain initial regression (Gaspar et al., 2010). This results in increasing costs for owners and of side effects in animals such as anorexia, myelosuppression and nephrotoxicity. However, further research on this condition is required to confirm this hypothesis.

Similarly, in the three metastatic cases seen, the predominant morphology pattern was plasmacytoid, a result that is corroborated by a previous study, in which plasmacytoid cell type was more commonly found in metastases and recurrent lesions (Amaral, 2005), providing additional evidence that tumours with higher possibility of aggression, have cells with plasmacytic morphology.

The evaluation of nuclear and cytoplasmic malignancy provides information on changes in the cell cycle, division, maturation, behaviour and cellular function, all important characteristics predicting the degree of benignity or malignancy of the tissue under consideration.

In general the nuclear and cytoplasmic malignancy criteria observed in different TVTs analyzed, did not differ from the literature (Table 1 through 3); however, the perinucleolar halo found in 45% of samples was different between the groups ( $p < 0,05$ ), with higher values in samples of animals with metastases. Amaral (2005) reported the a perinucleolar halo in 87% of the cases, without differences between groups.

**Table 3.** Cytological criteria in TVT, average comparisons between Group I and II, slides stained by Shorr

Group	Nuclear criterion				Binucleation
	Pseudo-inclusion	Evident nucleoli	Halo	slit	
G I	0.77 (a)	5.45 (a)	1.19 (a)	0.35 (a)	0.51 (a)
G II	0.10 (a)	24.23 (a)	9.80 (b)	0.17 (a)	0.30 (a)

Different letters indicate statistically significant difference ( $p < 0,05$ ).

The scientific literature does not refer to this finding in transmissible venereal tumour, perhaps because in the routine veterinary practice, looking for the cytologic characteristics of malignancy which were considered in this study, is infrequently done (Greenings, 1993).



However based on our results, further studies should be undertaken to validate this characteristic as indicating a more aggressive, malignant and metastatic type of TVT.

Our study showed that use of the TNM staging as well as the quantitative and qualitative assessment of cytoplasmic and nuclear changes can both contribute more information on cases of TVT to the clinical veterinarian.

TNM staging, established by the International Union Against Cancer (UICC), consists in separating the tumour cases into staging groups. The UICC believes that it is important to determine disease extension to each anatomic location, because the accurate clinical description and microscopic classification of the neoplasm can contribute to better planning of therapy, a more accurate indication of prognosis; improvement in the evaluation of the treatment results; facilitate the exchange of information between the treatment centers; and contribute to cancer research.

Likewise, this classification is a dual system that includes a clinical (This is based on evidence acquired before treatment) and a pathological (postsurgical histopathological) classification. It is imperative to differentiate between these classifications because they are based on different methods of examination and serve different purposes.

Thus, due to the increasing number and increasing aggressiveness of cases of naturally occurring TVT, and the advantages of the cytological exam in this tumour, we decided to use clinical staging to provide a system for prediction of the clinical treatment based on the tumour cytological classification.

Despite the fact that clinical TNM has not been used for this tumour, our results are similar to described for other tumours in humans and animals, such as lung (Mountain, 2002), breast (Karayannopoulou et al., 2005), prostate (Cheng et al., 2012).

After TNM staging, the neoplasm was evaluated microscopically looking for cytological indications of tumour aggressiveness which could be correlated to the TNM stage. Although our study included only twenty animals, our microscopic findings, were similar to those previously reported (Amaral, 2005). These cytologic criteria of malignancy are important to note, because one of these animals in which they were found had one of the highest TNM stages (T3N1), and the three cases with a secondary metastatic mass always had a primary mass greater than 5 cm (T3). Our data suggest that the clinical TNM and cytopathological information are complementary, giving better diagnostic and prognostic accuracy, and helping guide the choice of appropriate therapy, and monitoring.

The TNM staging was easily done in applied in all animals in the present study, demonstrating that this classification method is possible and should be used routinely to improve TVT diagnosis, and for better determination of tumour extension.

By using the TNM staging system for naturally occurring TVTs, the oncologist will be able to exchange information more readily with others. This would enable a more rapid switch of therapeutics in the case of non responders.

The epidemiologic data we obtained agrees with literature reports stating no predilection for sex or dog breed (Nieslsen & Kennedy, 1990; Cowell & Tyler, 1999; Das & Das, 2000). Eighteen (90%) of the 20 animals studied were mixed-breed dogs (MB), in agreement with descriptions by Brandão et al. (2002), Amaral et al. (2004), Lefebvre et al. (2007) and Fonseca et al. (2012). These authors grouped the MB dogs into a population characterized as homeless and therefore more exposed to the transmission of this tumor. The ranged of age in affected animals was from 2 to 10 years, the sexually active stage of the lives of these animals, which is as expected, given TVT is a venereal disease (Amaral et al., 2004; Amaral, 2005).

The predominant tumor localization was genital in comparison with extra genital sites, which is the most common site for naturally occurring TVT in male and female dogs (Nieslsen & Kennedy, 1990; Rogers, 1997) corroborating the results by (Das & Das, 2000; Nak et al., 2005; Amaral et al., 2007; Fonseca et al., 2012). Tumor metastasis to other organs has been described (Ferreira et al., 2000; Hasler & Weber, 2000; Park et al., 2006). Metastases were found in three cases in our study (15%), with the primary tumor being found in the external genital area.

The occurrence of metastasis in TVT has been estimated by various authors, according to Ferreira et al. (2000), may vary between 1.5% and 6%, both (Roger, 1997; MacEwen, 2001) found an incidence between 0% to 17%. These studies agree with our findings, however, this is less than the incidence of 25% reported by Amaral (2005). TVT metastases sites include: lung, spleen, liver, kidneys and lymph nodes (Park et al., 2006). In our study of three metastatic cases, the sites were the lymph nodes in a male, and spleen and breast in the females. In TVT, metastasis to regional lymph nodes is well established (Rogers et al., 1998; Das & Das, 2000) and metastasis in breast (Amaral, 2005) and spleen (Romero et al., 2010) already have been described.

With respect to time of evolution of the masses, 10% took 3 weeks, 25% appeared between 3 and 8 weeks, while 45% of the tumors had been present for eight weeks or more at clinical examination. In 4 cases the date of appearance was unknown. According to the literature, tumor growth appears 15-60 days after implantation (Duarte et al., 2006).

However, it remains difficult to infer the time evolution in cases of TVT (Santos, 2008).

With respect to the size of the masses, there were approximately equal numbers of tumors smaller than 2 cm and larger than 5 centimeters, with seven and eight observations respectively. The remaining tumors were between 2 and 5 cm. Our tumor measurements were within the range cited in the literature which reports lesions less than 1 cm diameter to large, multilobular nodules of 10 cm diameter (Cohen, 1985; Das & Das, 2000; Goldshmidt & Hendrick, 2002).

### **Conclusion**

This is the first report on the use of TNM staging in TVTs. While more studies need to be done to confirm its practicality, we found it easy and accurate to use. We found only one statistically significant difference (halo around the nucleolus) between the group without metastatic disease and the group with metastases. Despite the lack of statistical significance, in general, a good clinical relationship was seen between TNM staging and cytopathological findings, suggesting that use of both together provide greater certainty regarding degree of aggressiveness, progression and prognosis in the patients with TVT, as occurs in other tumour types. We believe that more extensive use of the TNM staging system will assist in a number of objectives such as: estimating survival times, evaluating treatment results, and aiding in choice of therapies for patients with TVTs.

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