J. Comp. Path. 2018, Vol. 163, 18–22

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Computerized Cytomorphometric and Cytomorphological Analysis of Canine Transmissible Venereal Tumours

C. Setthawongsin*, S. Tangkawattana†, A. Rungsipipat* and S. Techangamsuwan*

** Companion Animal Cancer Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok and † Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand*

Summary

Canine transmissible venereal tumour (CTVT) has three cytomorphological types: plasmacytic, lymphocytic and mixed type. Cytomorphometry, a method of computerized image analysis, has been used recently in veterinary medicine. This study compared the nuclear and cellular morphometric parameters (i.e. radius, diameter, perimeter and area) in different types of CTVT with other canine round cell tumours including lymphoma, mast cell tumour (MCT) and histiocytic tumour (HCT). We also evaluated the relationship between clinical information and the different CTVT cytomorphologic types. CTVT cells from 44 dogs revealed that the measured parameters were significantly different between different round cell tumours and among the CTVT cytomorphological types. CTVT had the largest cells, followed by HCTs, MCTs and lymphomas. The mixed type of CTVT had the largest nuclear and cellular size, followed by the plasmacytic and lymphocytic types. Lymphocytic CTVTs had less aggressive biological behaviour than the other types. Mixed type CTVTs were more likely to show malignant behaviour including metastasis and resistance to chemotherapy; however, there was no significant correlation between cytomorphological type of CTVT and response to chemotherapy.

Keywords: canine transmissible venereal tumour; chemotherapy; cytomorphology; cytomorphometry

Canine transmissible venereal tumour (CTVT) is named after its transmission, tumour location and cytomorphological features. CTVT normally arises in the external genital areas (genital TVT; GTVT), but it can also occur extragenitally and metastasize (extragenital TVT; ETVT). Cytomorphologically, CTVT is classified as a round cell tumour (Duncan and Prasse, 1979); however, ETVT might be confused with other skin tumours such as histiocytic tumour (HCT), mast cell tumour (MCT) and lymphoma (Ferreira *et al.*, 2000; Setthawongsin *et al.*, 2016). Therefore, a more definitive diagnosis by evaluation of cytomorphology could help to increase diagnostic accuracy.

Cytological evaluation of a tumour mass is of great value for rapid on-site diagnosis, especially for CTVT (Setthawongsin *et al.*, 2016). CTVT cytomorphology has been classified into three types according to the majority cell type: plasmacytic, lymphocytic and mixed type (Supplementary Table 1) (Amaral *et al.*, 2007). The plasmacytic type appears most frequent (Amaral *et al.*, 2007; Paranzini *et al.*, 2015) and is associated with greater malignancy (Amaral *et al.*, 2007; Silva *et al.*, 2007) and potential resistance to chemotherapy (Gaspar *et al.*, 2010). CTVT generally responds well to treatment with vincristine sulphate, which often leads to complete remission (Hantrakul

Correspondence to: S. Techangamsuwan (e-mail: somporn62@hotmail.com).

0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2018.07.003

et al., 2014). L-asparaginase combined with vincristine sulphate (Sudjaidee *et al.*, 2012) or a single doxorubicin protocol (Rogers *et al.*, 1998) are selected for vincristine-resistant CTVT.

Ki67 expression can be used to describe tumour activity, to determine responsiveness to chemotherapy or to monitor the proliferative status of tumour cells in cases of tumour recurrence (Tan *et al.*, 2005; Santos *et al.*, 2011; Melling *et al.*, 2016).

Cytomorphometry may be used to quantify the features of tumour cells by using image analysis to facilitate an objective measurement with standardization. This quantitative technique has been used to increase diagnostic reliability and avoid cytopathological interpretation bias (Chretien *et al.*, 1998; Tan *et al.*, 2001; Prvulović *et al.*, 2010). However, there are few studies of the morphometric characteristics of the subtypes of CTVT. The aim of this study was to analyze the nuclear and cellular cytomorphometric parameters of the subtypes of CTVT and of GTVT and ETVT and to compare these tumours with other canine round cell tumours. In addition, the correlations between the cytomorphological type, the site of the lesions, the phenotypic appearance and the response to treatment in CTVT cases were evaluated.

Forty-four CTVTs were included in the study. These tumours had been characterized by cytology, histology and polymerase chain reaction (PCR) for detection of the specific LINE1-*cmyc* rearrangement characteristic of CTVT cells (Setthawongsin *et al.*, 2016). The GTVT group ($n = 30$) included only dogs with a CTVT on the penis or vulva, while the ETVT group ($n = 14$) comprised of dogs with a CTVT elsewhere on the body, regardless of genital organ involvement. None of the dogs had received chemotherapy prior to sample collection. They were assigned randomly to one of two treatment protocols: (1) vincristine sulphate (VCS, Boryung Pharm, Seoul, South Korea), 0.025 mg/kg, intravenously, weekly; (2) combination L-asparaginase (Leunase, Hyowa Hakko Kirin, Tokyo, Japan), 200 IU/kg or 5,000 IU/mm^2, subcutaneously in weeks 1 and 3 and vincristine sulphate, 0.025 mg/kg, intravenously in weeks 2 and 4 for at least one cycle. VCS-resistant cases were defined when dogs received more than eight cycles of chemotherapy without complete remission (Sudjaidee *et al.*, 2012).

Biopsy samples of the tumours were collected under local anaesthesia (ethical approval from Institutional Animal Care and Use Committee, number 133100077). A cytological preparation was made by direct impression of the mass onto two glass slides and staining by Giemsa. Cytology slides from cases of lymphoma ($n = 5$), MCT ($n = 5$) and HCT ($n = 5$) were evaluated similarly.

Image digitization was by use of a Zeiss Primo Star microscope (Zeiss, Oberkochen, Germany) with a Canon EOS 550D camera (Canon, Tokyo, Japan). Cytomorphometric analysis for CTVT type classification was performed by evaluation at least 300 cells from each slide at ×400 magnification (Fig. 1 and Supplementary Table 1) (Amaral *et al.*, 2007). Nuclear and cellular parameters were analyzed by i-solution software (IMT, Burnaby, British Columbia, Canada). The cell perimeter^2 and nuclear perimeter^2 were calculated by Excel software (Microsoft, Redmond, Washington, USA).

The tissue sample was then processed routinely for histopathology and immunohistochemistry (IHC) to evaluate Ki67 expression. The primary antibody used in IHC was mouse monoclonal anti-Ki67 (clone MM1, Leica Biosystems, Milton Keynes, UK; 1 in 100 dilution) and secondary detection was with the polymer-based EnVision™ system (Dako, Glostrup, Denmark). The chromogen was 3, 3′-diaminobenzidine tetrahydrochloride (DAB, Dako) and counterstaining was with Mayer's haematoxylin (Supplementary Fig. 1). Negative control sections omitted the primary antibody and positive controls were sections of lymphoma tissue. The Ki67-positive cells (nuclear labelling) were counted in at least 1,000 cells over 10 fields at ×400 magnification (Santos *et al.*, 2011). The percentage of Ki67 positivity was calculated and then converted to a proliferative index (PI). Subsequently, CTVT masses were classified by the PI as being of either low or high grade (cut-off index = 40).

Tumour volume (cm^3) was calculated by use of the formula: (3.14 × width × length × thickness)/4 (Hsiao *et al.*, 2002; Spugnini *et al.*, 2008). TNM

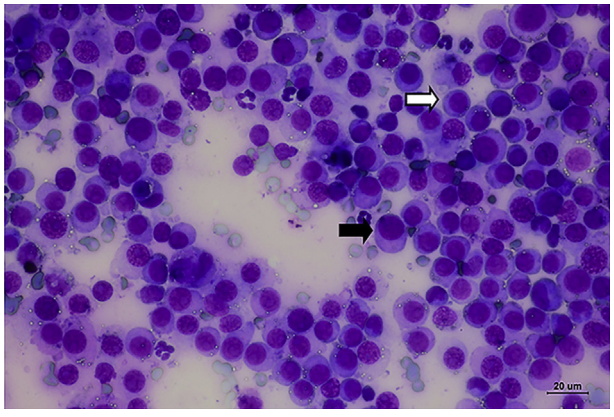

Fig. 1. Cytomorphological feature of CTVT cells: lymphocytic cell (white arrow) and plasmacytic cell (black arrow). Giemsa. Bar, 20 µm.