The Veterinary Journal 236 (2018) 96–101

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Original Article

Cytomorphological description and intra-observer agreement in whole slide imaging for canine lymphoma

F. Bonsembiante[a,*], V. Martini[b], U. Bonfanti[c], G. Casarin[a], D. Trez[a], M.E. Gelain[a]

[a] Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] Department of Veterinary Medicine, University of Milan, Via Celoria 10, 20133 Milan, Italy
[c] La Vallonèa Veterinary Diagnostic Laboratory, Via Sirtori 9, 20017 Passirana di Rho, MI, Italy

ARTICLE INFO

Article history:
Accepted 30 April 2018

Keywords:
Canine
Cytology
Digital pathology
Lymphoma
Whole slide imaging

ABSTRACT

Whole slide imaging (WSI) uses robotic microscopes for computerising entire slides into digital images. The aim of this study was to assess the agreement between WSI and optical microscopy for evaluating canine lymphoma cytological samples. Forty-four slides were computerised using a WSI scanner and the digital and glass slides were examined by three observers with different levels of expertise. Morphology and grade of lymphoma were scored on the basis of the updated Kiel classification and intra-observer agreement was assessed. The accuracy of determining the grade of lymphoma with digital and glass slides based on the results of flow cytometry (FC) was established. The overall intra-observer agreement for cytomorphological features was fair to moderate ($\kappa = 0.34–0.52$) for the three observers and moderate ($\kappa = 0.44–0.53$) for the evaluation of grade of malignancy. The diagnostic agreement between FC and digital slides was slight ($\kappa = 0.16$) for the inexperienced observer, fair ($\kappa = 0.32$) for the mildly experienced observer and moderate ($\kappa = 0.50$) for the very experienced observer. The diagnostic agreement between FC and glass slides was fair ($\kappa = 0.37$) for the inexperienced observer, substantial ($\kappa = 0.63$) for the mildly experienced observer and moderate ($\kappa = 0.50$) for the very experienced observer. These findings underline the importance of observer experience in determining the grade of malignancy, especially if digital slides are used. The study also identifies some technical limitations of the WSI scanner used in this study, mainly linked to image quality, which might affect the morphological evaluation of neoplastic cells.

Introduction

Digital pathology is a branch of pathology in which images are visualised on a computer monitor rather than directly through a microscope (Weinstein, 1986). Telepathology has evolved from the transmission of static images captured using microscope-mounted cameras to the use of robotic microscopes controlled by pathologists at distant sites and, more recently, to whole-slide imaging (WSI) (Webster and Dunstan, 2014). A WSI scanner is a robotic microscope capable of digitising an entire cytology glass slide (GS) by using software to merge the individually captured images into a composite digital image (Pantanowitz et al., 2013). The digital images can be viewed by using a specific software designed to emulate a light microscope (Webster and Dunstan, 2014), locally or at a distant site via a high-speed internet connection (Steinberg and Ali, 2001). WSI maintains the relative simplicity of static image transfer and eliminates its limitations by making the entire specimen available for review (Wilbur, 2011). It also allows the magnification of the digital slide (DS) to be changed (Wilbur et al., 2009). Moreover, the most updated scanners have the capability to perform multiple line scans of the same area at different fields of focus (Webster and Dunstan, 2014). This function, the so-called 'z-stack' mode, is important when acquiring images of cytological specimens, in which cells are often arranged in multiple layers (El-Gabry et al., 2014).

In recent years, researchers have shown increasing interest in both human and veterinary digital pathology (Maiolino et al., 2006; Kelly, 2007; Al-Janabi et al., 2012; Webster and Dunstan, 2014; Bertram et al., 2018). In human medicine, WSI is used mainly for digital diagnostics and teleconsultation (Al-Janabi et al., 2012). In veterinary medicine, despite the increased use of WSI instruments in reference laboratories, few reports on digital pathology are present in the literature. To the authors' knowledge, no studies focusing on morphological descriptive capability using WSI have been published. Moreover, the use of WSI has never been validated and its capability to replace the conventional microscope has not been assessed using cytological samples.

* Corresponding author.
E-mail address: federico.bonsembiante@unipd.it (F. Bonsembiante).

https://doi.org/10.1016/j.tvjl.2018.04.020
1090-0233/

One of the most commonly obtained cytological samples in canine medicine is a fine needle aspirate (FNA) of lymph nodes. This technique is used to discriminate between inflammatory and neoplastic disorders, among which, non-Hodgkin's lymphoma is the most common primary neoplasia affecting the lymph node (Richards and Suter, 2015). The identification of a clonal expansion of neoplastic lymphocytes is essential for lymphoma diagnosis. Histology, immunocytochemistry, immunohistochemistry, flow cytometry (FC) and PCR to detect clonal antigen receptor gene rearrangement (PARR) are tools that can be used to confirm the diagnosis of lymphoma and to identify the phenotype for prognostic purposes (Burkhard and Bienzle, 2013). These diagnostic procedures can be performed only in specialised laboratories. Commonly, the cytological examination of lymph node samples obtained via FNA is the first step in the diagnosis of lymphoproliferative diseases in dogs, because it is less invasive than biopsy, as well as inexpensive and rapid (Amores-Fuster et al., 2015). Cytological features can be used to classify different lymphoma subtypes with prognostic significance (Ponce et al., 2004). However, the morphological evaluation of neoplastic lymphocytes is complex and the opinion of a skilled clinical pathologist is often required for the correct diagnosis and classification of different lymphoma subtypes.

WSI has the potential improve the quality of cytological services and can be used to share cytological images of canine lymphomas with more experienced clinical pathologists. The three-dimensional architecture of cells in FNA samples has limited the application of digital pathology to cytological samples to date. However, with the introduction of WSI scanners with a z-stack function, FNA samples have become more suitable for digitalisation and visualisation on a monitor (El-Gabry et al., 2014). There is a need to evaluate the reliability of cytological evaluation and the technical aspects of the scanning process before this technology can be applied routinely in veterinary cytology. The aims of this study were to assess: (1) the intra-observer agreement (IOA) between WSI and optical microscopy in the evaluation of cellular morphology in canine lymphoma samples, using the updated Kiel classification (Ponce et al., 2010); (2) the IOA between WSI and optical microscopy in the assessment of lymphoma grading; and (3) whether the accuracy of grading assessment varied between WSI scanner and optical microscopy, using FC as a reference method. In addition, the influence of the level of observer expertise on performance was evaluated.

Materials and methods

Cytological samples

The data base of the Flow Cytometry Service of the Department of Veterinary Medicine, University of Milan, Italy, was searched to select consecutive canine lymphoma samples diagnosed on the basis of clinical, clinicopathological, cytological and FC data from January 2015 to June 2015. Only cases with good-quality lymph node cytological smears were enrolled in the study, to allow for a detailed evaluation of the morphological features of the neoplastic cells.

Processing of samples for FC analysis and criteria for lymphoma diagnosis has been described previously (Gelain et al., 2008). The following antibodies were used: CD45 (clone YKIX716.13, Bio-Rad), CD3 (clone CA17.2A12, Bio-Rad), CD5 (clone YKIX322.3, Bio-Rad), CD4 (clone YKIX302.9, Bio-Rad), CD8 (clone YCATE55.9, Bio-Rad), CD21 (clone CA21D6, Bio-Rad), CD79a (clone MCA1298F, Bio-Rad), and CD34 (clone 1H6, Pharmingen, BD Bioscience). Neoplastic cells were identified on the morphological cytogram, using forward scatter (FSC) versus side scatter, or on the CD45 versus FSC cytogram. The percentage of neoplastic cells, the phenotype and the mean FSC (mean cellular size) were used to identify the cell type (B cell or T cell) and cell size. Cytology slides were stained by the May–Grünwald–Giemsa technique and, for each case, the slide with higher cellularity and better preservation was selected.

Observers

Three observers with different levels of expertise participated in the study; all were blinded to the FC results. The inexperienced observer, a PhD student, had the lower experience in cytological evaluation; the mildly experienced observer, a postdoctoral researcher, had intermediate experience in cytological evaluation; and the very experienced observer, a board-certified clinical pathologist, had extensive experience in cytological evaluation. None of the observers had previously used the WSI technology for routine diagnostic procedures.

Digital slides

All cytology GSs were scanned using the 40× objective with the z-stack modality using a WSI scanner (D-sight, A. Menarini Diagnostics) to obtain the DSs. The DSs were scanned using automated tissue detection and focus point assignments with seven-line scans of the same area at different fields of focus. The research case numbers assigned to the DSs were different from those assigned to the corresponding GSs to minimise recall bias. Digital slides were subsequently uploaded to a server to be evaluated by the three observers using an online software (Telepathology, Visia Imaging) (Fig. 1). The monitors used ranged from 14 to 15.6 inches (355.6 to 396.2 mm), with a screen resolution of at least 1366 × 768 pixels; no special monitor or setting was used and all observers used the screen of their own laptop (notebook) computer to evaluate all DSs. A period of at least one month was imposed between the dates of evaluation of GS and DS by the same observer to ensure the observers did not remember the cases from the previous evaluations.

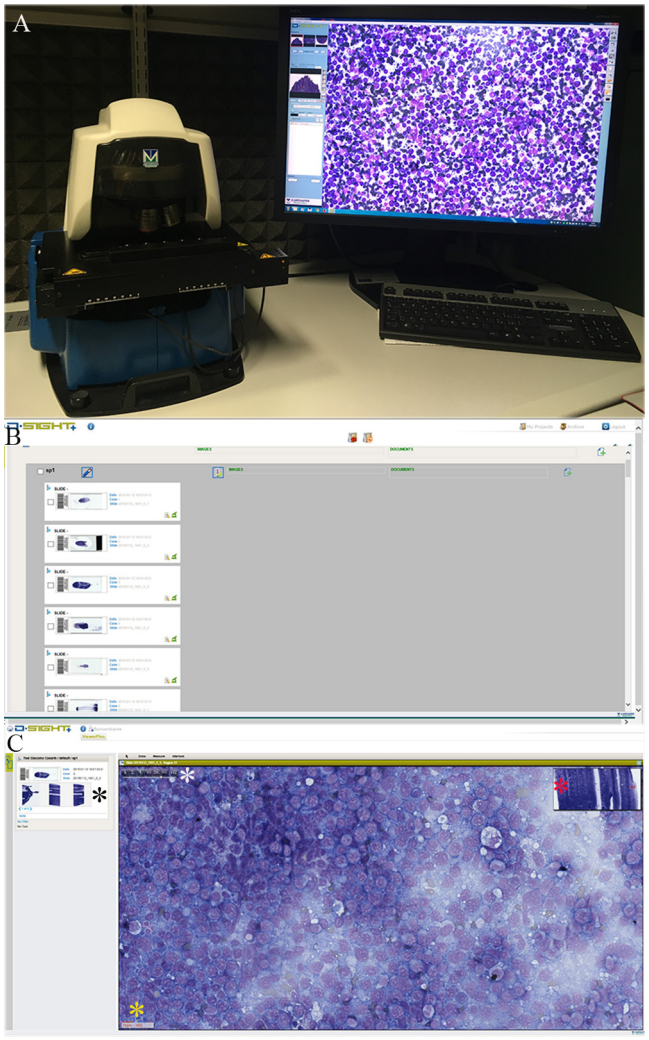

Fig. 1. Whole slide imaging microscope imaging workstation (D-sight, A. Menarini Diagnostics). (a) The system consists of a five-slide capacity scanner with four objectives (4×, 10×, 20× and 40×) and a high-performance desktop computer. (b) The main page of the online software used for analysis (Telepathology, Visia Imaging). The system can be used to digitise, store and preview all digitalised slides; the user can scroll through the archive and select the slide of interest. (c) Navigation page of the online software. Using the image multi-preview, the user can select and edit any area of interest (black asterisk). Information is provided for the different magnifications available (white asterisk), the current magnification (yellow asterisk) and the navigation map of the area selected (red asterisk).