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Filling the gap between histology and cytology: description of an innovative technology (Cytomatrix) to increase the diagnostic effectiveness of fine needle aspirates data

Fine needle aspiration (FNA) is a marginally invasive, fast and cost-effective technique to diagnose malignancy as well as other pathologies in different anatomical sites. In the last few years, there has been a significant increase in the demands on sensitivity and specificity of cytodiagnosis as well as in the requirement to produce further biological data to better determine treatment and prognosis.[1] Unfortunately, cytology specimens often are not suitable for ancillary studies both for the quality and the amount of the material. Furthermore, carrying out more aspirations may not be doable. Another point to be underlined is the fact that many times pathologists adapt ancillary techniques learnt in histology to cytology specimens without bearing in mind main differences in the specimen preparation that could alter the final interpretation of the data and ultimately the diagnosis for patient care.[2] This is particularly true in the case of immunohistochemistry, where the great majority of the commercially available antibodies have been selected for their reliability when used in tissues fixed in formalin and paraffin-embedded.[3 4] In this article, we propose a novel approach for the immunohistochemical characterisation of tumour fine needle aspirates by taking advantage of Cytomatrix, a recently defined synthetic matrix that counts among its various characteristics the property to capture and store inside its three-dimensional structure, the biological material (micro-macro cells and cell aggregates) from needle withdrawal samples. The cytological material collected in this way can be processed as a histology specimen, allowing to perform immunohistochemical analysis with routine protocols.[5] As experimental in vivo model, we chose two different spontaneous malignancy in pets, where the possibility to carry on specific diagnostic tests from fine needle aspirates would be of great support to clinicians: visceral lymphoma in dogs and cats and widespread mast cell tumour in dogs. For both tumours, immunohistochemical characterisation carries a prognostic and therapeutic value: in the case of lymphoma the B cell and T cell types carry a different prognostic value and also a potentially different therapeutic approach, for mast cell tumours, CD117 expression is predictive of response to CD117 inhibitor drugs.[6 7]

EXPERIMENTAL DESIGN, MATERIALS AND METHODS

Collection of the cytological specimens

For the purpose of this preliminary investigation, samples from 25 patients with spontaneous neoplastic disorders were collected. In detail, there were 8 canine lymphomas, 13 feline lymphomas and 4 canine mast cell tumours (see table 1). Briefly, privately owned companion animals (specifically dogs and cats), referred for cancer staging and therapy, were enrolled in the study and treated in the Clinica Giaconella in Rome. Pets were staged accordingly to the current guidelines, with complete blood cell count, biochemical profile, urinalysis, thoracic radiographs, abdominal ultrasonography and tumour tissue biopsy for histology (haematoxylin-eosins and immunohistochemistry). As a complementary test to standard procedures, fine needle aspirates of tumour lesions were performed and matched to diagnostic microscopy tests. Informed consent was obtained by pet owners. The samples were collected with a 5 mL syringe mounting a 27-gauge needle, by applying negative pressure. For deep or visceral lesions, the procedure was performed under ultrasonography guidance. Histopathology confirmation was performed on samples obtained either through surgical excision or tru-cut biopsy. Fine needle aspirates were squeezed on the CytoMatrix sponge. In detail, it is made up of chitosan, a biocompatible material characterised by high ion affinity for cell samples. Chitosan is able to successfully increase the process of retaining small amounts of biological material taken up by needle aspirates, inside its three-dimensional structure. The porous support is allocated into a plastic bio-cassette permitting to immediately proceed with the following steps of classical histological technique. The sponge was processed as follows: fixation in formalin for at least 12 hours; processing and paraffin embedding of the aspirated complex CytoMatrix-material as any tissue sample; application to the inclusion obtained; the various diagnostic techniques used in the histopathology laboratory.

Table 1 Characteristics of the patients enrolled and immunohistochemical data produced

Patient	Tumour type	Immunohistochemistry	Phenotype
Dog	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Dog	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Dog	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Dog	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Dog	Lymphoma	CD 3– CD20– CD 117– PANCK–	Null cell
Dog	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Dog	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Dog	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3+ CD20 CD 117– PANCK–	T cell
Cat	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Cat	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Cat	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Cat	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Cat	Lymphoma	CD 3–CD20+ CD 117– PANCK–	B cell
Dog	Mast cell tumour	CD 3– CD20– CD 117+ PANCK–	CD117+
Dog	Mast cell tumour	CD 3– CD20– CD 117– PANCK–	CD117+
Dog	Mast cell tumour	CD 3– CD20– CD 117+ PANCK–	CD117+
Dog	Mast cell tumour	CD 3– CD20– CD 117– PANCK–	CD117–

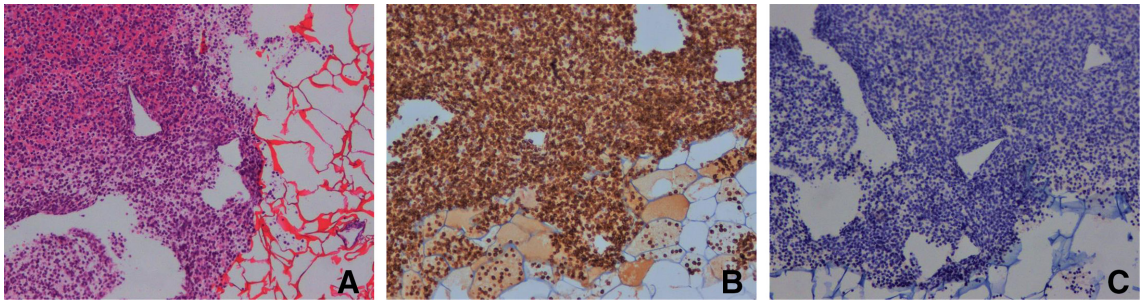

Figure 1 (A) Histological aspect of the material collected from a fine needle aspirate from a cutaneous lymphoma and treated with Cytomatrix (H&E, original magnification ×10). (B) The neoplastic cells are strongly positive for CD3 (ABC, original magnification ×10). (C) The neoplastic cells are consistently negative for CD20 (ABC, original magnification ×10).

Immunohistochemistry

Immunohistochemistry on formalin fixed paraffin-embedded tissue sections obtained from tissue biopsy of tumour samples and immunocytochemistry on formalin fixed paraffin embedded cellular sections obtained from FNA cytological material entrapped in CytoMatrix were performed on an automated immunostainer (Bond-III, Leica, Biosystems, Italy), as previously described.[8] Briefly, paraffin sections were cut at 5 µm using a microtome LEICA SM 2000R (Advanced Research Systems, Macungie, Pennsylvania, USA), dewaxed in xylene, rehydrated through a series of graded ethanol solutions and stained with Gill's Haematoxylin and Eosin (Bio-Optica, Milan). A citrate buffer, pH 6 or pH 8 was used to unmask the antigens. The primary antibodies used were, respectively, CD3 (clone LN10), CD20 (clone L26) and CD117 (clone EP10) (Leica, Biosystems, Italy). Images were obtained by using a light microscope (Microscope Nikon ECLIPSE 55i) equipped with a Digital Image Capture software (Leica Application Suite V.4.8).

DATA DESCRIPTION

Table 1 summarises the immunohistochemical data produced on the cytological material entrapped in the synthetic matrix through matching with histopathology on surgical specimens derived from the same cases. Data effectiveness and repeatability were significant. All the specimens, collected with the synthetic matrix, were suitable for morphological and immunohistochemical analysis, thus allowing the correct diagnosis of the tumours, as well as the immunophenotypes of the lymphomas included in the study (B-cell or T-cell lymphoma) and, form the other side, the definition of CD117 expression in the mast cell tumours analysed.

In figure 1A, paradigmatic example is reported, where the immunophenotype of a T-cell lymphoma, analysed by immunohistochemistry, is depicted (CD3 positive and CD20 negative).

VALUE OF THE DATA

- The synthetic matrix allows an easy and fast analysis of morphological and immunohistochemical characteristics of fine needle aspirate material from various malignancies.
- Pathologists can include, among the different techniques used to analyse cytological specimens, the one described in this article, to make the specimens suitable to morphological analysis and also to immunohistochemical analysis.
- Further experimentations are ongoing to demonstrate the suitability of the cytological material collected with this technique for more sophisticated assays of molecular biology.

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