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Diagnostic yield and accuracy of postmortem cytological sampling from the brain surface of animals with neurological abnormalities

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ABSTRACT

Clarification of central nervous system (CNS) disorders frequently requires pathological investigation via brain biopsy or postmortem examination. The use of cytology is usually restricted to diagnosis of mass lesions and septic meningitis. The value of brain cytology at postmortem examination has not been explored sufficiently. This study aimed to clarify the diagnostic value of meningeal imprint cytology at postmortem brain examination. Samples were taken from cerebrum and cerebellum and stained with the modified Wright stain and with haematoxylin–eosin. The slides were evaluated and findings were compared to brain histopathology with respect to resemblance, discrepancy and diagnostic validity. The study included 169 cases involving multiple animal species. Histopathology identified inflammatory disorders in 60/135 (44.4%) cases, neoplasia in 19/135 (14.1%) and non-infiltrative diseases in 56/135 (41.5%). Cytology revealed pathological changes in 79/135 (58.5%) of these cases. The histopathological diagnosis was reproduced in 57/135 (42.2%) cases, 43/57 (75.4%) of which were inflammatory. Non-diagnostic cases included 16/135 (11.9%) with sub-diagnostic cytological features and 3/135 (2.2%) with unclear phenomena. In 55/135 (40.7%) of brains with histological lesions, cytology proved inferior, providing negative results, including 40/55 (72.7%) cases with non-infiltrative diseases, 12/55 (21.8%) with inflammation and 3/55 (5.5%) with neoplasia. Conversely, 3/34 (8.8%) of controls showed cytological abnormalities. Cytological sampling from CNS adds to the sensitivity of neuropathological investigations, even if restricted to non-invasive surface imprints. The diagnostic accuracy exceeds 40%, with infiltrative diseases being five times more likely to be detected than non-infiltrative diseases.

Introduction

All too often, clarification of central nervous system (CNS) disorders requires morphological investigation carried out via brain biopsy or postmortem examination. Production of histological sections of brain requires a fully equipped histology laboratory and routine sections may not be available for evaluation earlier than 24 hours after sample arrival unless cryohistological sections are performed. Histological processing requires technical expertise and sparse biopsy material exposes the tissue to a significant risk of procedural artefacts that may mask or mimic pathological changes.

Imprint cytology of CNS lesions, as routinely performed in human medicine (Russell et al., 1937), could reduce the turnaround time of definite diagnoses (Long et al., 2002; De Lorenzi et al., 2006). Although providing a limited insight into the tissue context, brain cytology usually provides better opportunity for evaluation of cellular and subcellular details than cryosections. It is tissue sparing and hence comprises a non-interfering ancillary diagnostic tool that is quicker and easier to perform when compared to histopathology (Folkerth, 1994; Prayson and Napekoski, 2011).

Irrespective of these advantages, brain cytology has not been used widely in veterinary practice. Instead, it is restricted to diagnosis of mass lesions during brain surgery or biopsy and for diagnosis of suspected septic meningitis (De Lorenzi et al., 2006; Mishra et al., 2012). As to whether brain cytology may prove valuable in other disorders and how it performs in comparison to standard histopathology of brain lesions has not been systematically evaluated. This postmortem study aimed to investigate the diagnostic yield and accuracy of non-invasive brain surface cytology in veterinary investigations.

Materials and methods

Cases

This study was conducted on randomly chosen postmortem brain samples from animals of multiple species with neurological signs subjected to routine autopsy for diagnostic purposes unrelated to this investigation over a period of 3 years, including companion animals, horses, farm animals, zoo animals and wildlife. Samples of histopathologically confirmed normal brains taken at autopsy served as cytological controls.

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Tissue sampling and slide processing

All animals underwent full postmortem examination. The brains were removed as described by Matiasek et al. (2015). Upon removal of the dura mater and excessive fluids from the brain surface, touch preparations were taken from (1) the dorsolateral aspects of the cerebral convexity and (2) the dorsolateral cerebellar surface using standard slides (Star Frost Adhesive, Engelbrecht Medizin- und Labortechnik) (Fig. 1A). Additional cytological specimens were taken from focal brain lesions if previously identified via diagnostic imaging or external macroscopic evaluation. All slides were air-dried and fixed in 75% ethanol for 30 min for disinfection. The slides were routinely stained with modified Wright stain (modWS) (Long et al., 2002) and with haematoxylin–eosin (HE) (Jorundsson et al., 1999) (Fig. 1B).

Ancillary procedures

All cases were accompanied by full macroscopic and neurohistopathological investigation. Brains were fixed in 10% neutral-buffered formalin and trimmed as described by De Risio et al. (2012). Fixed tissues were processed in an automatic tissue processor, then underwent an ascending ethanol series and xylene treatment, after which the slides were embedded in Paraplast (Leica Biosystems Richmond). Sections (6 μm thick) were prepared and stained with HE, as well as various special stains depending on the case specific diagnostic request.

Neurocytological examination

The results of the microscopic evaluation were analysed by considering quality, representativity and cytopathological features (see Appendix: Supplementary Table S1).

After establishment of readability, preparations were assessed for components of the meninges (Fig. 2A), inner brain surfaces (ependyma, choroid plexus) (Fig. 2B), blood vessels (Figs. 2C, D), grey matter components (neurons; Figs. 2E, F) and white matter components (myelin figures, fibre bundles and tracts; Figs. 2G, H). Conclusions on the origin of the cells were drawn from (1) nerve cell size, morphology (granular, pyramidal, piriform, multipolar), arrangement and relative number of cells, as well as (2) the relationship between grey and white matter components and (3) vascularity.

Cytopathological features were sorted into (1) presence of non-residential cells or endogenous material (e.g. red blood cells, leucocytes including macrophages, tumour cells, contaminating cells, blood clots), (2) cytomorphological and numerical abnormalities of residential cells (e.g. inclusions, vacuolation, chromatolysis, neuronal degeneration, demyelination, astrogliosis, microglial activation), (3) tissue necrosis and (4) presence of microorganisms (e.g. bacteria, fungi, parasites) or foreign bodies. In cases of suspected haemorrhage, efforts were taken to identify (1) erythrophagocytosis and (2) siderophages (with or without Perl's stain). Evaluation for cellular atypia was performed using standard algorithms for oncocytology (Sharma and Deb, 2011). The presence of bacteria was categorised into (1) intracellular or extracellular organisms, (2) Gram positive or Gram negative organisms and (3) coccoid, rod-shaped, fusiform or mixed morphologies.

Examples for cytological abnormalities are provided in Fig. 3. The most important changes were check-listed in a purpose designed evaluation form (see Appendix: Supplementary Table S1).

All cases were investigated by two blinded investigators. Six diagnostic levels were distinguished: (1) not evaluable, (2) inconspicuous, (3) unclear phenomena, (4) distinct but sub-diagnostic cytopathological features, (5) phenomenological diagnosis possible and (6) aetiological diagnosis possible. The outcome of these examinations was compared to the 'gold standard' histology with regards to their resemblance or discrepancy and comparative diagnostic validity. Equal outcome was graded 0, lower diagnostic value was rated –1 and surplus information +1.

Distribution rating and data analysis

For the assessment of diagnostic value, four factors were considered: (1) localisation, (2) distribution and (3) degree of a lesion, as well as (4) degree of cellular exfoliation. Localisation considered the proximity of any lesion (1) to the leptomeninges (with or without direct meningeal involvement or involvement of the ventricles) and (2) to the sampling site, namely the dorsolateral convexity. Lesions were separated in those affecting the meninges, parenchyma and/or ventricles. Positive cytology slides further were rated with regards to the histological lesion involving the same fossa as the imprinted brain site (Fig. 4). The degree of exfoliation comprised (1) highly infiltrative and exfoliative lesions (such as diffuse inflammation and round cell tumours), (2) less exfoliative lesions (such as solid granulomas and cohesive neoplasms) and (3) non-infiltrative disorders.

A correlation analysis using Spearman's rank sum test was performed to evaluate the effects of (1) evidence of pathological cytology, (2) diagnostic value and (3) diagnostic accuracy on the likelihood of detection by non-selective sampling. χ^2 and Fisher's exact tests were performed for comparison of groups. Statistical analyses were conducted using GraphPad PRISM software. A P value ≤ 0.05 was considered to indicate a significant difference.

Results

Demographics

The study included 169 cases from 29 vertebrate species, including 168 eutherians and 1 marsupial (see Appendix: supplementary Table S2); 87 were companion animals, 11 were horses, 56 were farm animals, 13 were zoo animals and two were wildlife. Age and sex distribution are depicted in the Appendix (Supplementary Table S2). Neurolocalisation on the basis of clinical records is indicated in Table 1.

Brain histopathology and disease classification

Thirty-four cases (20.1%) were histopathologically normal, while 135 cases (89.9%) presented with histopathological abnormalities after consideration of species-specific features and the state of maturity. The distribution of affected regions is shown in Table 1. Brain lesions were classified on the basis of histopathology as infiltrative (79/135, 58.5%), inflammatory (60/135, 44.4%) and neoplastic (19/135, 14.1%). The remaining 56/135 (41.5%) of cases comprised non-infiltrative diseases (NID), including degenerative, metabolic, vascular and traumatic disorders (Table 2). Seventy-four lesions involved the meningeal (59) or ventricular (15) surfaces and 61 cases were localised to the parenchyma. Forebrain involvement alone was seen in 46 cases, whilst eight cases selectively affected the

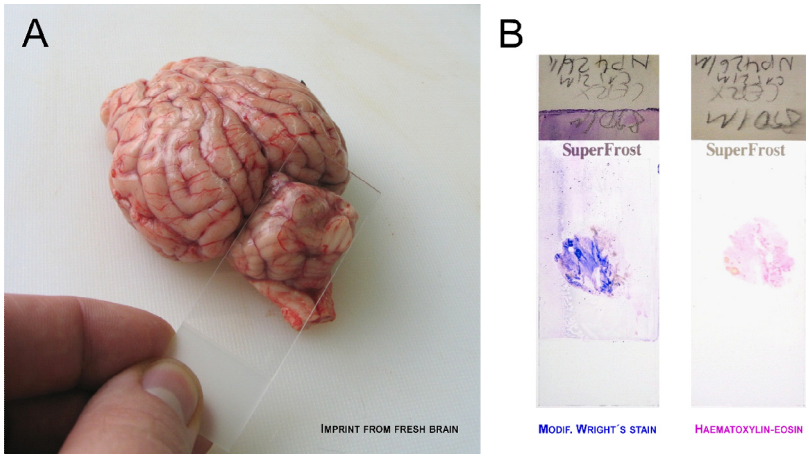

Fig. 1. (A) Imprint from the dorsolateral cerebellar surface. (B) Slides stained with modified Wright's stain and haematoxylin and eosin.