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SPONTANEOUSLY ARISING DISEASE

Automated Analysis of Bone Marrow Aspirates from Dogs with Haematological Disorders

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Summary

Automated analysis of bone marrow (BM) aspirates is a useful 'pre-microscopical' screen to identify hypocellular samples and those with potentially abnormal cells. In order to determine whether automated analysis could also be used to identify haemopoietic abnormalities, EDTA-anticoagulated BM aspirates from 43 dogs were analysed using the Advia 2120 instrument. Corresponding Wright-stained BM smears were evaluated microscopically to determine smear quality, cell composition and 500-cell differential counts, and correlation to automated analysis parameters was computed. Leucocyte cytograms generated by the automated analyzer were scrutinized and compared with those of 'normal' BM. Twenty-three neoplastic and 20 non-neoplastic samples were analysed, including samples from 10 cases of acute myeloid leukaemia, four cases of acute lymphocytic leukaemia, four cases of chronic lymphocytic leukaemia, one case of chronic neutrophilic leukaemia, three cases of multiple myeloma, one case of myelodysplastic syndrome, five cases of non-regenerative immune-mediated haemolytic anaemia, one case of immune-mediated neutropenia, three cases of immune-mediated thrombocytopenia, six cases of inflammatory disease, three samples with myelotoxicity and two samples analysed for staging of neoplasia. Automated white blood cell (WBC) counts correlated significantly with smear cellularity, particle cellularity and particle number. There was a significant difference in WBC counts of samples with insufficient versus sufficient particles. Significant correlations between Advia percent neutrophils and microscopical determination of marrow segmented neutrophils/neutrophilic granulocyte reserve, Advia percent lymphocytes and microscopical determination of lymphocytes/rubricytes, Advia percent large unstained cells and microscopical determination of myeloblasts/promyelocytes and between Advia percent eosinophils and manual determination of eosinophils were identified. This suggested that Advia WBC counts may be used to approximate BM sample quality and that Advia differential counts may predict marrow granulocyte reserve and lymphocyte/rubricyte stores. Distinct and consistent alterations in cytogram patterns were observed in cases of acute leukaemia, but were less obvious in chronic leukaemia. Complete automated BM analysis was performed in approximately 2 min, while staining and coverslipping of BM slides required approximately 30 min. Hence, although automated analysis should not supplant microscopical evaluation of BM, it can provide useful ancillary information in a short time and flag potentially inadequate or abnormal samples.

Keywords: Advia 2120; haematology analyzer; haemopoiesis; leukaemia

Introduction

Bone marrow (BM) assessment is an important component of diagnostic and toxicological investigation of haematological disorders. In clinical settings, BM aspirate and core biopsy are indicated when persistent or unexplained abnormalities in the peripheral blood are present, such as cytopenia, cytosis or presence of atypical cells, or for staging of neoplasia (Harvey, 2012). In addition to qualitative assessment of slides by a pathologist, differential counting of 200–1,000 cells is recommended, in particular for toxicological assessment (Provencher-Bollinger, 2004; Travlos, 2006; Riley *et al.*, 2009). However,

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differential counting appears to be performed less frequently in diagnostic settings, possibly due to the labour-intensive and time-consuming nature of this procedure (Harvey, 2012). Automated haematological analysis of BM aspirates can provide valuable pre-microscopical information concerning the quality of samples, presence of cell types and the potential need for further analysis. For example, Fan *et al.* (1999) found that BM analysis with the Cell Dyn 4000 instrument compared with microscopical analysis was sensitive and specific for detection of granulocytic hyperplasia (86% and 88%, respectively), lymphocyte-predominance (97% and 92%), increased blast cell proportion (89% and 98%) and non-diagnostic samples (68% and 96%).

Previously, we demonstrated the utility of automated analysis of BM aspirates from healthy dogs using the Advia 2120 haematology analyzer (Siemens Healthcare Diagnostics, Tarrytown, New York, USA; Tan *et al.*, 2013). In that study, automated results were available within approximately 2 min of the sample arriving in the laboratory, while staining and coverslipping slides required approximately 30 min. Automated results correlated well with microscopical evaluation of BM smears and provided useful ancillary information regarding the potential diagnostic quality of samples and the adequacy of marrow granulocyte and rubricyte reserves. We now wished to determine whether automated analysis would prove similarly useful in the analysis of BM samples from dogs with haematological abnormalities. The specific aims of this study were: (1) to determine whether results of automated analysis correlated with microscopical assessments of a BM smear; and (2) to investigate whether Advia cytograms could provide useful information about haematological disorders.

Materials and Methods

Bone Marrow Samples

BM samples were obtained from dogs admitted to the Ontario Veterinary College Health Sciences Centre (OVC-HSC) for investigation of haematological abnormalities (e.g. cytopenia or cytosis, atypical cells detected on blood smears) or staging of neoplasia. BM was aspirated from the manubrium, iliac crest or humerus as deemed appropriate by the attending clinician. BM fluid (approximately 0.5 ml) was aspirated into a syringe containing 0.6 ml saline with 1% sterile ethylenediaminetetraacetic acid (EDTA). One drop of the aspirate was placed on each of three to five glass slides, blood was removed by tilting the slides onto absorbent paper, smears were prepared by squash preparation and stained twice with modified Wright's stain using an automated stainer (Hema-Tec® 2000, Siemens Healthcare Diagnostics, Tarrytown, New York, USA; Bienzle, 2012; Defarges *et al.*, 2013). The remainder of the BM aspirate was placed into a 3 ml EDTA tube (Becton Dickinson, Franklin Lakes, New Jersey, USA) for automated analysis. BM smears and fluid were processed within 2 h of collection. Any clots apparent prior to automated analysis were removed manually with a pipette and if clots were multiple or large, the sample was excluded from automated analysis. Samples without detectable particles on microscopical analysis were also excluded from analysis.

Bone Marrow Automated Analysis

The Advia 2120 is a multichannel, optical plus cytochemical, automated haematology analyzer that generates a five-part white blood cell (WBC) count and enumerates reticulocytes. The differential WBC count in the Advia is based on basophil/lobularity and peroxidase analyses in different channels. In the basophil/lobularity channel, cells are exposed to a reagent that lyses red blood cells (RBCs), platelets and the cytoplasm of most leucocytes. Basophil enumeration in this channel is based on cluster analysis of size (forward low-angle light scatter) versus nuclear lobularity, since human basophils are more resistant to lysis than other leucocytes (Gibbs, 2011). Basophils from dogs and cats do not appear to be lysis resistant and are not enumerated accurately with this method (Lilliehöök and Tvedten, 2011). In the peroxidase channel, leucocytes are categorized by cluster analysis based on size (forward low-angle light scatter) versus cytoplasmic peroxidase content. This method discriminates five unique clusters in canine samples: neutrophils, eosinophils, monocytes, lymphocytes and large unstained cells (LUCs; Fig. 1A). This latter cell type refers to cells that are large, but lack peroxidase activity (PA), and may include blast cells of various lineages. Basophils are not enumerated separately in the Advia peroxidase channel, since they appear to cluster with lymphocytes or LUCs (Gibbs, 2011). The Advia 2120 reticulocyte concentration is derived from analysis of RNA content of isovolumetrically sphered RBCs through binding of oxazine 750 to RNA, which reduces light transmission in anucleate cells.

BM aspirates were analysed in the Advia 2120 using the manufacturer's haematology settings for dogs, software version 5.9 MS. Parameters collected included the following: total WBC counts (from the basophil/lobularity channel), results of the automated

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