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Canine bone marrow cytological examination, classification and reference values: A retrospective study of 295 cases

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ABSTRACT

Cytologic assessment of bone marrow with knowledge of the hemogram represents an effective method to investigate hemic tissue and its function. To determine the spectrum and prevalence of canine bone marrow disorders over a 2 year period in a diagnostic laboratory setting achieved through a standard approach to cytologic bone marrow assessment. A retrospective study of bone marrow fine needle aspirates sample preparations, blood smears, hemogram data and case records. Of the 295 bone marrow samples evaluated, 90 (30.5%) were nondiagnostic samples. Of the remaining samples, 25.1% were classified as hyperplasia of which most were granulocytic hyperplasia (58.1% of the total hyperplasia), 19.3% had no cytological abnormalities, 12.9% had malignant hemopathy and 7.8% had hypo-aplastic conditions. Only a small proportion of cases involved dysplasia (1.7%) and metastatic disease was detected in only one case (0.3%). Reference values of nucleated cells and the M/E ratio were calculated for normal and erythroid and granulocytic hyperplastic bone marrow. This study provides the spectrum and the prevalence of canine bone marrow disorders as well as a differential bone marrow cell counting and determination of reference intervals for diseases.

1. Introduction

Cytological bone marrow examination with knowledge of the hemogram, is an invaluable diagnostic test of best utility when investigating certain peripheral blood abnormalities (e.g. persistent cytopenias, leukocytoses, morphologic atypia of blood cells). It can also be beneficial for staging certain neoplastic conditions, in investigating particular parasitic infections (e.g. leishmaniasis), or in establishing cause of fever of unknown origin (FUO), weight loss or unexplained malaise (Turinelli et al., 2012; Weiss and Smith, 2000).

Few retrospective studies of bone marrow disorders have been published and the prevalence of such disorders has been rarely investigated. Furthermore, bone marrow differential cell counting has not been provided for disease affecting this tissue malaise (Turinelli et al., 2012; Weiss and Smith, 2000). The aim of the present study was to provide a general overview regarding the prevalence of canine bone marrow disorders encountered in a private laboratory setting, to classify them and to assess the differential cell counts relating to such disorders.

2. Materials and methods

Bone marrow samples were retrospectively evaluated over a 2 year period. Two hundred and ninety-five bone marrow samples were classified using cytological and numerical criteria. Samples were collected from female and male dogs referred for several disorders originating from Italy (71%), Germany (22%), Netherland, Switzerland, Spain, Poland, Norway and Sweden (7%). For the majority of studied dogs a blood smear evaluation and measurement of cell concentrations (CBC) were also performed using ADVIA 120 Hematology System®, Siemens. Blood was collected the same day as the bone marrow sample. Bone marrow sampling was performed from various sites: iliac crest, trochanteric fossa of the proximal femur, proximal humerus, cost-condrale junction of the 7th, 8th, 9th ribs and the 3rd, 4th, 5th sternebra. Bone marrow aspirate smears were prepared immediately following sampling from live animals. Aspirates were stained with May Grünwald Giemsa. The cellularity of bone marrow samples was estimated by examining the proportion of cells versus fat presence in particles. Normal cellularity varies between 25% and 75% cells. A differential cell count was performed by evaluating 500 nucleated cells in a monolayer within the preparations using a 100× objective lens. For the erythroid lineage the cells were separated into: rubriblasts, prorubricytes, rubricytes, and metarubricytes. For the myeloid lineage the cells were separated into: myeloblasts together with promyelocytes, neutrophil

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myelocytes, neutrophil metamyelocytes, band/segmented neutrophils, total eosinophilic cells and total basophilic cells. Lymphocytes, plasma cells, monocytes, macrophages and blasts of uncertain lineage were counted separately. Megakaryocytes and their precursors were not included in the differential cell count.

The megakaryocytic lineage was evaluated at low magnification (10 objective lens) and the megakaryocytes were counted in large spicules.

The myeloid-to-erythroid ratio (M:E) was calculated as previously described (Harvey, 2001; Harvey, 2012, Mischke and Busse, 2002).

Iron stores were also assessed: hemosiderin within macrophages and as stained particles.

Bone marrow was considered unremarkable/normal when: bone marrow cell morphology, cellularity and the hemogram were normal compared to literature and laboratory established reference limits, the number of megakaryocytes in the unit particles were 3 to 7, the M:E was 0.75–2.5:1, the rubriblasts plus prorubricytes were <5% of all nucleated cells and myeloblasts plus promyelocytes were <5% of all nucleated cells (Harvey, 2001; Harvey, 2012).

Aplastic bone marrow was defined by the presence of pancytopenia in blood and the presence of spicules composed of >75% adipose tissue with all cell lines underrepresented or absent (Brazzell and Weiss, 2006; Harvey, 2001; Harvey, 2012).

Megakaryocytic hypoplasia was defined when, in a thrombocytopenic dog, the bone marrow cellularity, the granulocytic and erythrocytic lineage were normal and there were <3 megakariocytes per unit particle or low power field (Brazzell and Weiss, 2006; Grindem et al., 2002; Fournel-Fleury et al., 1994).

Erythroid hypoplasia was reported when an anemia was present, as well as normal or decreased bone marrow cellularity and absolute blood neutrophil count, M:E > 2.5:1, and the rubriblasts plus prorubricytes were rare or absent (Brazzell and Weiss, 2006; Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012; Weiss, 2008).

Pure red cell aplasia (PRCA) was defined as the presence of severe nonregenerative anemia and a M:E > 75:1 (Weiss, 2008).

Granulocytic-neutrophilic hypoplasia was defined when the bone marrow cellularity was normal or decreased, the hemoglobin level was normal, the M:E < 0.75:1 and myeloblasts plus promyelocytes were rare or absent (Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012; Weiss, 2008).

Generalized bone marrow hyperplasia was reported when >75% of the hemopoietic space in unit particles consisted of hemopoietic cells (Weiss, 2008).

Megakaryocytic hyperplasia was defined by the presence of >7 megakaryocytes per unit particle or low power field. The proportion of large mature thrombocytopenic megakariocytes with granular eosinophilic cytoplasm compared to earlier stages of development was also evaluated (Cowell et al., 2008; Fournel-Fleury et al., 1994; Grindem et al., 2002).

Erythroid hyperplasia was reported when the bone marrow cellularity was normal or increased, the absolute blood neutrophil count was normal or increased, the M:E was <0.75:1 and the rubriblasts plus prorubricytes were >5% of all nucleated cells (Brazzell and Weiss, 2006; Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012; Weiss, 2008).

Granulocytic-neutrophilic hyperplasia was defined when the bone marrow cellularity was normal or increased, the hematocrit was normal or increased, the M:E was >2.5:1 and myeloblasts plus promyelocytes were >5% of all nucleated cells (Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012; Weiss, 2008).

Criteria for diagnosis of myelodysplastic syndrome (MDS) included hypercellular bone marrow, blood cytopenias and qualitative anomalies of the three cell lineages and less than 20% of blasts in the bone marrow and blood (Fournel-Fleury et al., 1994; Grindem et al., 2002; Harvey, 2001; Harvey, 2012; Weiss, 2005). No subclassification of myelodysplastic syndrome was performed.

Dysmegakaryocytopoiesis was reported when maturation and/or morphological abnormalities were observed in the megakaryocytic series (Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012).

Dyserythropoiesis was defined when abnormal erythroid maturation and/or morphology were observed in association with ineffective erythropoiesis (Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012).

Dysgranulopoiesis was defined by the presence of abnormal granulocyte maturation and/or morphology, along with ineffective granulopoiesis resulting in neutropenia in peripheral blood (Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012).

Plasma cells hyperplasia was reported when plasma cells exceed 3–5% of total nucleated cells (Grindem et al., 2002; Harvey, 2001; Harvey, 2012).

Lymphocytic hyperplasia was reported when small, normal lymphocytes exceed 10% of total nucleated cells (Grindem et al., 2002; Harvey, 2001; Harvey, 2012).

In the broad group of malignant hemopathy were included acute and chronic lymphoid and myeloid leukemia as well as lymphoma infiltrating bone marrow and multiple myeloma (Fournel-Fleury et al., 1994; Harvey, 2001; Harvey, 2012; Valli, 2007). A diagnosis of acute lymphoid leukemia (ALL) was based on >20% of total nucleated cells being lymphoid blasts. Lymphoid blast cells could be observed also in the peripheral blood.[9] Chronic lymphocytic leukemia (CLL) was diagnosed when small mature lymphocytes exceeded 20% of total bone marrow nucleated cells and a permanent peripheral lymphocytosis was reported after excluding other possible causes such as ehrlichiosis or other chronic infections with appropriate diagnostic tests (Fournel-Fleury et al., 1994). The large granular lymphocytes (LGL) variant was included in this group. Acute myeloid leukemia (AML) was reported when >20% of total nucleated cells in the bone marrow were myeloblasts. Myeloid blasts could be also observed in the peripheral blood (Grindem et al., 2002). The diagnosis of chronic myeloid leukemia (CML) was based on the presence of a persistent peripheral blood leukocytosis ($>100 \times 10^9$/L), hypercellular bone marrow with extreme myeloid hyperplasia (M/E > 23/1), orderly myeloid maturation and after having excluded other possible causes (e.g. severe inflammation or paraneoplastic syndromes). The diagnosis of multiple myeloma (MM) was based on the summation of several criteria (two or more of the following): >30% plasma cells in bone marrow, morphological abnormalities of plasma cells, presence of a high level of monoclonal immunoglobulin detected by serum protein electrophoresis, presence of Bence-Jone's proteinuria, presence of osteolytic lesions (Fournel-Fleury et al., 1994; Grindem et al., 2002; Harvey, 2001; Harvey, 2012; Valli, 2007).

The bone marrow metastasis group included mast cell tumor, carcinoma, histiocytic sarcoma and sarcoma.

In the broad group of nondiagnostic bone marrow specimen were included the poor cellular samples, the formalin fixed samples, the excessive scratched smears, etc.

Statistical analysis was performed using a software package (MedCalc Version 13.0.2.). Reference intervals (RI) were defined by the 2.5 and 97.5% percentiles if the number of measured values were adequate. In addition mean, median, standard deviation, maximum and minimun values were determined (CLSI, 2008; Geffrè et al., 2009a; Geffrè et al., 2009b).

3. Results

Two-hundred ninety-five bone marrow aspirates were analyzed. The indications for each bone marrow cytological exam, provided by the practitioners, are summarized in Table 1.

The results of bone marrow cytological and numerical classification are reported in Table 2. The large groups of hyperplasia and hypoaplasia were subclassified into smaller classes including: generalized, megakaryocytic, erythroid and granulocytic. The dysplasia group was