An Appraisal of Bone Marrow Biopsy in Assessment of Sick Dogs

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ABSTRACT

Dogs were classified into a number of disease categories according to hematological, cytological and serochemical changes. Aspiration and core bone marrow biopsies were examined in 128 dogs in the various disease categories and compared to marrow samples in 36 dogs which appeared clinically normal. Differential cell counts on bone marrow smears were examined in relation to the blood variables in all animals. Blood and bone marrow data (group means) were compared among the normal and disease groups.

Anemia, responsive and poorly responsive was the most frequent blood abnormality. Most dogs in the thrombocytopenia group had increased numbers of megakaryocytes in the marrow but two dogs had a marked decrease. The frequency of serious alteration of marrow production of the erythroid, myeloid and megakaryocytic series was less than anticipated. Marrow hemopoiesis was not significantly compromised in dogs with lymphoma or in dogs with other types of cancer.

Bone marrow examination was necessary for the diagnosis of myelofibrosis and pancytopenia and was very helpful in the groups with insufficient change in the blood to permit a definitive diagnosis to be made.

The myeloid-erythroid ratio was a useful indicator of marrow response while the erythroid maturation index and the myeloid maturation index were useful for identification of altered patterns of maturation (ineffective hemopoiesis). The reticulocyte response in absolute numbers is the most efficient and clinically relevant measure of erythroid response.

Key words: Dogs, bone marrow, blood, hematology, hematological diseases, hematopoiesis, myeloid-erythroid ratio.

RÉSUMÉ

Cette étude portait sur 128 chiens atteints de diverses maladies, que les auteurs divisèrent en un certain nombre de groupes, d'après les changements hématologiques, cytologiques et sérologiques qu'ils affichaient. Ils examinèrent des biopsies osseuses de tous ces chiens et les comparèrent à celles de 36 témoins. Ils analysèrent les numérations cellulaires différentielles des biopsies osseuses en tenant compte des paramètres sanguins, chez tous les chiens. Ils comparèrent aussi la moyenne des résultats des examens du sang et de la moelle osseuse des chiens des divers groupes avec celle des témoins.

Une anémie normalement ou médiocrement régénératrice s'avéra l'anormalité sanguine la plus fréquente. La plupart des chiens atteints de thrombocytopénie affichaient une augmentation du nombre de leurs mégakaryocytes médullaires, tandis que deux en présentaient une diminution marquée. Une altération sérieuse de la production médullaire des cellules des lignées érythroïde et myéloïde, ainsi que des mégakaryocytes, se révéla en deçà des prévisions. Par ailleurs, l'hémopoïèse médullaire ne s'avéra pas sérieusement compromise, chez les chiens atteints de lymphome malin ou d'autres cancers.

L'examen de la moelle osseuse s'avéra nécessaire pour le diagnostic de la myélofibrose et de la pancytopénie; il se révéla aussi très utile dans les groupes qui souffraient d'un changement sanguin insuffisant pour permettre de poser un diagnostic définitif.

Le rapport entre les cellules myéloïdes et érythoïdes se révéla un indice utile de l'activité médullaire, tandis que l'indice de maturation des cellules érythroïdes et celui des cellules myéloïdes facilitèrent l'identification des altérations des profils de maturation, lors d'hémopoïèse insuffisante. Le nombre absolu de réticulocytes se révéla la mesure clinique la plus efficace et la plus significative de l'érythropoïèse.

Mots clés: chiens, moelle osseuse, sang, hématologie, maladies du sang, hémopoïèse, rapport entre les cellules myéloïdes et érythroïdes.

INTRODUCTION

Bone marrow biopsy is considered a valuable adjunct to the study of diseases of the hemopoietic tissues. There are many reports regarding the use of bone marrow examination in dogs but there are few detailed clinical studies of marrow changes in specific disease conditions. The technique has been safe in almost all circumstances, even in thrombocytopenic states (1-17), but veterinarians have been hesitant to use marrow biopsy routinely as an aid to diagnosis. The reasons for this resistance are not clear but may include lack of training and practice in collection, evaluation and interpretation of bone marrow biopsies.

The objective of this work was to evaluate the peripheral blood and bone marrow findings in clinically normal dogs and dogs with various diseases. We felt that the changes in the blood would be reflected by changes in the bone marrow that would be of diagnostic and prognostic significance.

MATERIALS AND METHODS

Samples of peripheral blood, serum

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and bone marrow from 36 dogs that were considered clinically normal and 128 dogs with various diseases were studied from June 1981 to May 1982.

The 36 clinically normal dogs used as reference animals were of mixed breed and estimated to be one to three years of age. These dogs were divided into two groups based upon hematological information, primarily related to low erythrocyte values. Twenty one were assigned to the reference group normal hematology and 15 to reference group abnormal hematology (Table II). Twenty-nine of the 36 dogs were strays (Animal Care Committee approval) whereas seven dogs were preconditioned for six weeks.

The clinically abnormal dogs were divided into ten disease groups (Table I). Some of the animals were included in more than one group. Dogs were classified as anemic on the basis of hematocrit (Hct), hemoglobin (Hb) and red blood cell count (RBC) (Hct $<$ 0.37 L/L, Hb $<$ 126 g/L and RBC $<$ 5.5 x 10^{12}/L) (20). The Hct was the main criteria for the classification of anemia; in some instances the other parameters did not parallel the Hct and for that reason two dogs with RBC count and Hb of 6.2 x 10^{12}/L and 134 g/L respectively, were classified as anemic. Other criteria such as reticulocyte count, mean corpuscular volume (MCV $<$ 66 fL) and adequate erythroid response over a period of weeks were also considered for the purposes of subclassification into responsive and poorly responsive anemia groups. One dog with an MCV of 79 fL and another with a reticulocyte count of 118 x 10^9/L were classified in the poorly responsive anemia group due to failure of adequate response over several weeks repeated sampling. The responsive anemias were defined on the basis of reticulocyte counts of $>$80 x 10^9/L as evidence of effective erythropoiesis. Dogs with immune mediated anemia were selected on the basis of responsive erythropoiesis and a positive direct antiglobulin test.

TABLE I. Group Classification for 128 Dogs

Disease Groups	No. of Dogs
Anemia poorly responsive	60
Anemia responsive	15
Immune hemolytic anemia	8
Thrombocytopenia likely immune, ITP	10
Neutrophilia	54
Neutropenia	12
Lymphoma	34
Cancer — other than lymphoma	37
Renal failure	5
Myelofibrosis	5

Ten dogs with thrombocytopenia (Table I) were classified on the basis of number of megakaryocytes per high power field, morphological abnormalities in the megakaryocytes or dysthrombopoiesis (vacuolation, immaturity and basophilia in cytoplasm) and response to therapy (steroids and/or vincristine). The platelet factor 3 (PF-3) test was not available and in addition all dogs had received prior therapy with steroids.

The neutropenia and neutrophilia groups were defined as outside of limits of neutrophil reference values used at the University of Guelph Veterinary Teaching Hospital. Thus dogs with neutrophil counts less than 3.9 x 10^9/L were classed in the neutropenic group and those with counts greater than 12.0 x 10^9/L in the neutrophilic group.

Allocation to the lymphoma group was based upon diagnosis established using accepted cytological and/or histological criteria (32). The main cytological criteria were high cellularity, monomorphism, fine chromatin distribution and variation in the number, size and shape of nucleoli. These animals were sampled at various stages of the disease.

The diagnosis of myelofibrosis was suspected on finding a "dry tap" during the marrow aspiration, and confirmed on the histological section of the core biopsy. The renal failure group was classified on a clinical basis as having chronic azotemia. The final group consisted of animals with a diagnosis of cancer other than lymphoma and included animals with mast cell tumors (8), sarcoma (9), carcinoma (9), adenoma (3), myeloma (4), melanoma (2), acute lymphocytic leukemia (ALL) (1) and chronic lymphocytic leukemia (CLL) (1).

Bone marrow examinations were undertaken for a variety of reasons, including investigation of anemia and thrombocytopenia, confirmation of clinical suspicion of metastatic cancer and staging of animals with tumor prior to therapy. Some of these dogs were examined sequentially, others only once prior to postmortem. Seventy-five of the 128 animals were included in the two broad groups of responsive and poorly responsive anemia.

PERIPHERAL BLOOD

Blood samples of each dog were drawn from the jugular vein at the time of bone marrow sampling using standard technique and were placed in vacutainer tubes (Becton, Dickinson Co. Columbus, Ohio) containing ethylenediamine tetraacetic acid (EDTA). Total red (RBC) and white blood cell (WBC) counts, hemoglobin (Hb), hematocrit (Hct), MCV, MCH and MCHC determinations were determined using the Coulter Model-S (Coulter Electronics, Hialeah, Florida). Packed cell volumes were confirmed using capillary-tube microhematocrit. Blood smears for white cell differential counts (one hundred cells per smear) were made within 30 minutes of blood collection and were stained with Wright's stain (Ames Hema-Tek, Fisher, Toronto, Ontario).

SERUM IRON AND TOTAL IRON BINDING CAPACITY

Serum samples were collected and frozen at the time of bone marrow sampling using standard techniques. Serum iron (SI) and unbound iron binding capacity (UIBC) were measured by the AMB-610 method using a biochemical analyzer (KDA., American Monitor Corporation, Indianapolis, Indiana). Total iron binding capacity (TIBC) and percent saturation (% sat.) were calculated.

BONE MARROW COLLECTION

Bone marrow aspiration and core biopsies were collected from all dogs using a Jamshidi bone marrow biopsy needle (Kormed Inc., St. Paul, Minnesota) either 11 or 13 gauge depending on the size of the dog. The site of choice in all dogs was the dorsal wing of the ileum (3,15,16). The biopsy site was prepared as for surgery and infiltrated with local anaesthetic. A small stab incision was made in the skin and the samples were collected for cytological and histological evaluation as previously described (3,7,12-16). The histological sampling included both particle sections as described by Rywlin *et al* (12) and core biopsies as described by Jamshidi (8). In a few cases,

only core biopsies were obtained.

All reference dogs and 24 dogs of the diseased group were biopsied within 24 hours preceeding death or euthanasia. Biopsies were also collected from the femur of these dogs within one hour following death. The femur was removed and hemisectioned for ease of sampling. The bone marrow particle smears and squash preparations were routinely stained using Wright's stain and the Prussian blue reaction for iron.

A differential count on 1,000 nucleated cells were made from the Wright's stained bone marrow smears on each of the 164 dogs. Nomenclature and criteria for cell identification are those of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs (1948-1950), as described by Schalm (20). Reticuloendothelial cells as used herein refers to bare nuclei with cribriform chromatin pattern and prominent nucleoli (20).

The bone marrow aspiration sample (1-2 mL) was fixed in 10% formalin for particle sectioning (12,14,15). Paraffin sections, 3-6 μm thick, were prepared using Petirfi's double embedding method and were stained using hematoxylin and eosin, Giemsa, Prussian blue reaction to demonstrate hemosiderin, Masson-trichrome for collagen and Gordon-Sweet' method for reticulin fibres.

The myeloid-erythroid (M:E) ratio, the erythroid maturation index (EMI) and the myeloid maturation index (MMI), were calculated in an attempt to evaluate the erythroid and myeloid marrow response. The erythroid maturation index (EMI) is the sum of erythroid maturation phase cells (polychromatic rubricytes, normochromic rubricytes and metarubricytes) divided by the sum of erythroid proliferative phase cells (rubriblasts, prorubricytes, basophilic rubricytes). The myeloid maturation index (MMI) is the sum of myeloid maturation phase cells (metamyelocytes, band and segmented neutrophils) divided by the sum of myeloid proliferative phase cells (myeloblasts, promyelocytes and myelocytes). These ratios (EMI and MMI) were determined in order to detect asynchrony of maturation and to test the value of these indices in interpretation of various disease conditions.

Cellularity was assessed by subjective evaluation of aspirated granules and recorded as percent cellularity (Table II-IV).

The peripheral blood and bone marrow results for each dog were recorded by group using a record administration program (Mabra, I.P. Sharp Associates, Toronto, Ontario), examined for Gaussian distribution, log transformed if non-Gaussian, analyzed using analysis of variance (ANOVA) (APL Sharp University of Guelph statistics program, J. Hines, Department of Mathematics and Statistics, University of Guelph) and least significant difference (LSD) (38) to indicate the presence of significant differences between mean values for the designated groups. Descriptive statistics were determined for each group (APL Sharp University of Guelph statistics program, #22 DSTAT).

RESULTS

The observations from peripheral blood and bone marrow studies are recorded in Tables II, III and IV. The serum iron, unbound iron-binding and total iron binding capacity of the various groups are recorded in Table V. A comparison with the literature on cytological examination of bone marrow of the dog is presented in Table VI.

Increases in M:E were found for the poorly responsive anemia, renal failure and myelofibrosis groups (groups 3, 7 and 8 respectively). Group mean bone marrow total granulocytes were decreased only in animals with responsive anemia (group 4). A lower mean M:E was not found for the responsive anemia and IHA groups, although there was an increase in total erythroid cells. However, in the responsive anemias (group 4 and 5) the M:E was below one. The M:E ratio for the thrombocytopenia group was 2.7 with a peripheral reticulocytosis. The M:E was increased for animals with myelofibrosis (group 8) and was higher but not significantly different for animals with neutropenia, neutrophilia, lymphoma and cancer other than lymphoma (groups 9, 10, 11 and 12 respectively).

The total erythroid cells are increased in the responsive anemia groups (4 and 5) but reduced in the animals with myelofibrosis, lymphoma and cancer other than lymphoma (groups 8, 11 and 12 respectively). The numbers of late rubricytes are reduced in all but the responsive anemia and thrombocytopenia groups.

The renal failure group and the myelofibrosis groups had increased bone marrow neutrophils and increased MMI. A decrease was noted in the bone marrow eosinophils in all groups.

A poor prognosis associated with marrow metastasis could be demonstrated only in animals with lymphoma and mast cell tumors where there were sufficient cases for comparison. Sixteen of 34 dogs with lymphoma had tumor cells in the bone marrow. Fifteen of these 16 dogs lived <3 months and eight dogs either died or were euthanized in <2 weeks. Of the 18 dogs without tumor cells in the marrow, 14 of 15 lived >6 months.

In the ITP group there were six of ten dogs with increased numbers of small immature megakaryocytes with cytoplasmic vacuolation, increased basophilia and decreased granulation. In two dogs no megakaryocytes were visible in the bone marrow biopsies. One of these dogs died in a bleeding diathesis and the other had a protracted recovery lasting several weeks. The eight dogs with megakaryocytes all recovered in two to five days after therapy with steroids and/or vincristine.

The dogs with myelofibrosis all had "dry" taps on bone marrow aspiration. Diagnosis was confirmed on the histological section of the core biopsy. On retrospective examination, all dogs with myelofibrosis had ovalocytes in the peripheral blood smears. Four dogs recovered and the fibrosis decreased with steroid and/or cyclophosphamide therapy. One dog had a transfusion reaction and was euthanized. The remaining four dogs were clinically normal one year postdiagnosis. Three were lost to follow up.

Of the ten dogs with mast cell tumor, four had clusters of mast cells in the bone marrow. These four animals either died or were euthanized within four days. The remaining six dogs were clinically normal at 90 days postdiagnosis.

TABLE II. Results of Analysis on Peripheral Blood and Bone Marrow from Normal and Diseased Dogs

		Reference with Normal Hematology (group 1)		Reference Abnormal Hematology (group 2)		Anemia Poorly Responsive (group 3)		Anemia Responsive (group 4)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
		(n = 21)		(n = 15)		(n = 60)		(n = 15)	
Peripheral Blood	**Units**								
WBC	10^9/L	9.4	6.6-14.4	8.2	3.4-18.7	16.9	2.1-128	30.6[a]	6.8-97.4
RBC	10^{12}/L	6.9	5.9-8.1	5.6[a]	4.7-6.8	4.1[a]	1.2-6.2	3.3[a]	1.6-4.9
Hb	g/L	163	140-199	132[a]	99-160	90[a]	30-134	80[a]	49-117
HCT	L/L	0.45	0.38-0.54	0.36[a]	0.27-0.43	0.26[a]	0.10-0.36	0.24[a]	0.13-0.35
MCV	fL	66	57-72	64	59-69	64	49-79	74[a]	61-85
MCH	pg	24	21-26	23	21-26	23[a]	15-26	25	22-30
MCHC	g/L	364	355-382	370	356-375	349[a]	300-411	341[a]	292-479
Segs	μL	5922	3483-10512	5262	1496-14585	10600	164-59300	18000[a]	4600-38000
Bands	μL	68	0-436	26	0-196	1010[a]	0-20580	2900[a]	0-11700
Lymphocytes	μL	2053	1241-3068	1368	327-2875	3400	0-120000	1538	147-3896
Monocytes	μL	794	219-1456	595	0-1870	1142	0-5401	2433	0-9072
Eosinophils	μL	545	0-2618	838	183-2490	328	0-1582	345[a]	0-1479
Basophils	μL	4	0-81	0	0	0	0-0	0	0-0
Rubricytes	μL	5	0-109	4	0-60	126	0-2940	4[a]	0-4
Immature granulocytic	μL	0	0	46	0-561	312	0-16464	333	0-3896
Disintegrated	μL	43	0-654	0	0	95	0-3840	108	0-888
Platelets	x 10^9/L	—	—	—	—	105	7-575	135	8-400
Platelet estimate	x 10^9/L	250	175-400	262	150-400	289	50-700	184[a]	20-300
Protein[b]	g/L	65	57-73	62	53-70	66	38-108	67	5-92
Polychromasia	%	0.1	0-1	0.1	0-0.5	0.7	0-1	9.8[a]	0.5-29
Reticulocytes	%	0.6	0-2.4	0.6	0-2.4	1.7	0-3.2	12.1	2.3-30
Absolute reticulocytes	10/L	50	30-80	50	30-80	65	0-118	290	87-540
Bone Marrow Differential 1000 Cells									
Rubriblast-prorubricyte		12.2	0-23	13.1	3.0-23.6	12.2	0-29	23.6[a]	5-58
Early rubricyte		68.3	40-94	62.2	35-92	70.2	0-202	153.5[a]	58-360
Late rubricyte		251.2	78-341	224.8	128-364	172.8[a]	0-496	310.5	110-467
Metarubricyte		30.9	8-73	39.5	7-96	29.8	0-203	48.1	15-81
Total erythroid		362.7	159-522	339.6	221-483	281.6	7-745	535.6[a]	265-711
Lymphocyte		60.6	23-129	67.1	30-109	96.9	2-957	33.7	18-88
Plasma		20.5	6-44	16.7	3-34	14.0[a]	1-64	10.6[a]	5-41
Monocyte/macrophage		14.9	6-40	15.3	1-30	24.5	0-484	15.1	2-41
Reticuloendothelial		3.4	0-11	1.5	0-7	6.7	0-208	2.7	0-11
Myeloblast-promyelo-myelocyte		55.9	19-134	57.1	15-104	60.9	0-281	41.7	15-68
Metamyelocyte		111.7	49-260	141.9	57-242	121.5	0-380	92.3	28-150
Band		117.6	71-162	134.3	71-220	122.2	0-341	103.9	44-171
Neutrophils		185.9	85-304	163.2	63-263	221.4	2-564	130.5	26-245
Eosinophils		46.4	12-90	54.7	30-77	26.6[a]	0-168	20.3[a]	7-45
Basophils		0.8	0-3	0.7	0-3	0.8	0-16	1.1	0-11
Unidentified cells		0.7	0-9	0.1	0-2	0.2	0-5	0.1	0-1
Bare nuclei		19.3	0-52	18.7	0-70	22.6	0-149	13.7	0-74
Total granulocytes		518.2	328-692	551.8	435-703	553.6	2-923	389.3[a]	203-615
M:E		1.6	0.6-4.4	1.7	0.9-2.8	6.4[a]	0.1-96	0.8	0.3-2.2
EMI		3.6	1.3-4.9	3.7	2.2-6.5	2.7[a]	0-10	2.5[a]	0.7-4.4
MMI		8.8	3.6-26.7	10.4	4.1-39.2	13.6	0-80.5	9.8	2.8-27.5
Cellularity		50.9	40-60	53.3	45-65	63.8[a]	8-100	71.1[a]	50-85

[a]Superscript indicates significant difference from reference mean ($P<0.05$)
[b]TS-Meter, American Optical, Buffalo, N.Y. 14215

DISCUSSION

The goal of this study was to determine the diagnostic and possibly prognostic value of examining the bone marrow in sick dogs. We attempted to correlate the peripheral blood and bone marrow data from 128 dogs with a variety of diseases. The dogs were placed into disease categories according to hematological and cytological data and a number of characteristics of bone marrow were compared among the disease groups. The system of classification is far from perfect, but it is an attempt by the authors to show the various disease groups as clearly as possible. Some of these groups overlapped.

Dogs with poorly responsive anemia formed the largest group and represents a very common clinical condition. This group included a variety of diseases and 48 of the 60 animals had neoplasia, thrombocytopenia, renal disease or myelofibrosis. A few dogs had endocrine disorders, blood loss and other immune mediated diseases. In a case of histoplasmosis, the parasites were first seen in marrow

TABLE III. Results of Analysis of Peripheral Blood and Bone Marrow from Normal and Diseased Dogs

		Reference Normal Hematology Mean (n = 21)	IHA (group 5) Mean (n = 8)	IHA (group 5) Range	ITP (group 6) Mean (n = 10)	ITP (group 6) Range	Renal Failure (group 7) Mean (n = 5)	Renal Failure (group 7) Range	Myelofibrosis (group 8) Mean (n = 5)	Myelofibrosis (group 8) Range
Peripheral Blood	**Units**									
WBC	10^9/L	9.4	31.8[a]	6.8-97.4	18.3[a]	7.4-31.2	14.1	8.2-23.5	6.2	3.6-9.9
RBC	10^{12}/L	6.9	3.1[a]	1.6-4.6	4.1[a]	1.8-7.2	3.4[a]	1.8-5.47	2.3[a]	1.2-3.8
Hb	g/L	163	77[a]	49-117	94[a]	38-181	81[a]	44-127	50[a]	30-82
HCT	L/L	0.45	0.23[a]	0.13-0.35	0.27[a]	0.13-0.49	0.23[a]	0.12-0.36	0.15[a]	0.10-0.24
MCV	fL	66	76[a]	61-85	67	49-85	68	65-71	65	57-79
MCH	pg	24	26[a]	22-30	23	15-29	25	23-24	22[a]	20-25
MCHC	g/L	364	338[a]	292-479	331[a]	255-364	348	338-362	331[a]	313-342
Segs	μL	5922	19000[a]	4600-38000	12000	590-28000	11500	5570-19200	4111	2052-5246
Bands	μL	68	2900[a]	0-12000	1266[a]	0-3984	80	0-235	243	0-990
Lymphocytes	μL	2053	1696	662-3896	1294	147-2715	1275	1012-1722	1604	885-2970
Monocytes	μL	794	595	231-3490	2318[a]	750-4706	1054	164-2585	356	144-693
Eosinophils	μL	545	361	0-1324	342	0-1086	102	0-326	287	0-946
Basophils	μL	4	0	0-0	0	0-0	0	0-0	0	0-0
Rubricytes	μL	5	5600[a]	0-36000	436	0-3850	0	0-0	43	0-177
Immature granulocytic	μL	0	487	0-3896	110	0-1100	0	0-0	0	0
Disintegrated	μL	43	92	0-738	36	0-362	147	0-574	76	0-172
Platelets	x 10^9/L	—	204	39-400	29	8-94	—	—	575	
Platelet estimate	x 10^9/L	250	200	100-300	—	—	330	250-450	267	220-350
Protein[b]	g/L	65	71	64-92	62	50-71	71	55-108	66	61-71
Polychromasia	%	0.1	9.6	0.5-29	4.2	0-13	—	—	0.2	0-1
Reticulocytes	%	0.6	10.4	5.5-23.5	10.9	0.6-30	0.1	0.1-0.1	0	0
Absolute reticulocytes	x 10 /L	50	290	120-510	230	11-540	25	0-40	0	0
Bone Marrow Differential 1000 Cells										
Rubriblast-prorubricyte		12.2	25.1	5-58	15.0	2-33	9.4	1-15	6.4	4-8
Early rubricyte		68.3	153.6	58-360	89.4	33-190	49.4[a]	0-94	63.4	28-129
Late rubricyte		251.2	309.0	110-467	209.0	50-374	171.0[a]	0-402	98.6[a]	38-163
Metarubricyte		30.9	45.9	15-68	27.7	1-52	29.4	0-31	20.8	2-58
Total erythroid		362.7	533.6[a]	265-711	341.1	96-644	259.2	8-516	189.2[a]	90-358
Lymphocytes		60.6	31.9	18-63	51.7	8-139	92.6	13-226	118.0	78-204
Plasma		20.5	13.8	5-41	11.7	4-28	7.6[a]	2-19	10.0[a]	4-26
Monocytes/macrophages		14.9	19.3	5-41	16.4	1-96	10.6	0-22	14.8	8-32
Reticuloendothelial		3.4	3.4	0-11	1.5	0-5	5.8	0-19	6.0	0-14
Myeloblast-promyelo-myelocyte		55.9	45.5	18-68	85.8	34-281	60.6	15-121	31.0	8-60
Metamyelocyte		111.7	82.9	42-135	134.0	64-380	102.4	33-172	79.6	32-144
Band		117.6	104.4	44-171	117.7	29-194	114.8	70-163	76.0	18-152
Neutrophils		185.9	131.4	26-245	189.3	23-387	325.2[a]	185-480	388.0[a]	242-564
Eosinophils		46.4	20.6[a]	8-45	24.5[a]	7-56	14.8[a]	7-230	22.8[a]	0-68
Basophils		0.8	1.9	0-11	2.8	0-16	0.2	0-1	0.4	0-2
Unidentified cells		0.7	0.1	0-1	0.6	0-5	0	0-0	0.2	0-1
Bare nuclei		19.3	13.0	0-48	26.3	0-149	7.0	0-19	63.8	0-134
Total granulocytes		518.2	387.5	203-615	558.1	304-839	620.0	451-843	597.8	461-676
M:E		1.6	0.9	0.3-2.2	2.7	0.5-7.1	23.8[a]	1.0-96	6.1[a]	2.7-11.3
EMI		3.6	2.6	0.7-4.4	2.3[a]	1.5-3.9	4.0	0-10	1.8[a]	0.8-2.8
MMI		8.8	8.8	2.8-24.6	7.4	1.1-12.4	16.6	5.3-54.1	30.4	9.0-80.5
Cellularity		50.9	71.9[a]	50-80	69.5[a]	50-85	57.0	50-70	48.0	30-75

[a]Superscript indicates significant difference from reference mean ($P < 0.05$)
[b]TS-Meter, American Optical, Buffalo, N.Y. 14215

macrophages. In cases with pancytopenia and myeloid dysplasia a marrow sample was necessary for a diagnosis.

MYELOID ERYTHROID RATIO

The M:E reports relative myeloid versus erythroid hemopoiesis and must be interpreted in relation to the absolute number of blood erythrocytes and granuloctyes. Increases in M:E were observed in the poorly responsive anemia, renal failure and myelofibrosis groups (groups 3, 7 and 8 respectively) where this change apparently indicates reduced erythropoiesis rather than increased myelopoiesis (Tables II and III). Group mean bone marrow total granulocytes were not increased for any group while marrow total erythroid cells were decreased in the poorly responsive anemia and myelofibrosis groups (groups 3 and 8 respectively). Although not significant, M:E less than one was found for the responsive anemia and IHA groups, apparently due to relatively increased numbers of erythroid cells.

The reference group M:E values differ slightly from the values of Schalm (20) but compare well with the values of Melveger and Vanloon (29) and Meyer and Bloom (28) (Table VI). The M:E ratio in clinically normal dogs is generally between 1.0 and 2.0. Melveger *et al* (29) compared the cytology of aspirates from the sternum and the rib of five Beagle dogs, 1 to 1.5 years of

TABLE IV. Results of Analysis on Peripheral Blood and Bone Marrow from Normal and Diseased Dogs

		Reference Normal Hematology Mean (n = 21)	Neutrophilia (group 9) Mean	Range (n = 54)	Neutropenia (group 10) Mean	Range (n = 18)	Lymphoma (group 11) Mean	Range (n = 34)	Cancer Other than Lymphoma (group 12) Mean	Range (n = 37)
Peripheral Blood	**Units**									
WBC	10^9/L	9.4	29.9[a]	6.5-128	4.5[a]	2.1-7.4	18.6	4.1-128	23.3[a]	4.5-128
RBC	10^{12}/L	6.8	4.8[a]	1.4-9.3	5.4[a]	3.9-6.9	5.7[a]	2.0-8.9	5.8[a]	3.6-8
Hb	g/L	163	111[a]	35-212	121[a]	82-163	134[a]	48-206	137[a]	81-206
HCT	L/L	0.45	0.31[a]	0.10-0.62	0.34[a]	0.24-0.47	0.37[a]	0.13-0.56	0.38[a]	0.23-0.57
MCV	fL	66	66	57-85	63	57-68	65	20-57	65	57-70
MCH	pg	24	23	20-30	22[a]	20-24	23	20-26	23	20-26
MCHC	g/L	364	355	292-479	354	329-377	363	330-388	364	333-388
Segs	μL	5922	19700[a]	12000-59000	1897[a]	164-3710	9200	2400-19000	12000[a]	2800-34000
Bands	μL	68	1900[a]	0-21000	85	0-370	352[a]	0-2960	423	0-2960
Lymphocytes	μL	2053	4100	0-120000	1094	252-1850	1344	67-5766	9100	440-120000
Monocytes	μL	794	2009	0-9072	1092	25-4218	977	0-4320	1243	0-432
Eosinophils	μL	545	510	0-6666	158	0-588	219	0-960	265[a]	0-1320
Basophils	μL	4	4	0-240	0	0-0	7	0-240	9	0-240
Rubricytes	μL	5	1100[a]	0-36000	91	0-852	233	0-6600	294	0-660
Immature granulocytic	μL	0	448	0-16464	0	0-0	0	0-0	59	0-888
Disintegrated	μL	43	159	0-3840	3	0-36	213	0-3840	279	0-384
Platelets	x 10^9/L	—	96	8-380	211	7-575	—	—	47	13-80
Platelet estimate	x 10^9/L	250	254	20-450	306	100-500	239	62-450	244	62-450
Protein[b]	g/L	65	69	51-98	66	48-91	70	57-103	72	57-103
Polychromasia	%	0.1	2.3	0-29	0.2	0-1.5	0.5	0-7.5	1.5	0-7.5
Reticulocytes	%	0.6	5.0	0-23.5	1.3	0-2.5	0.1	0-0.1	0.1	0-0.1
Absolute reticulocytes	x 10 /L	50	150	0-510	65	0-120	25	0-65	25	0-55
Bone Marrow Differential 1000 Cells										
Rubriblast-prorubricyte		12.2	15.2	0-58	8.3	0-15	10.6	0-54	9.9	0-54
Early rubricyte		68.3	79.1	0-360	75.2	28-314	53.4	2-233	46.1[a]	2-135
Late rubricyte		251.2	189.7[a]	0-421	186.3[a]	12-476	153.3[a]	1-355	145.7[a]	1-344
Metarubricyte		30.9	29.7	0-203	30.1	1-70	28.6	2-129	26.6	2-129
Total erythroid		362.7	309.9	7-711	299.8	88-820	247.6[a]	7-640	230.5[a]	7-512
Lymphocytes		60.6	110.9	12-957	55.6	2-160	211.4	12-557	235.6[a]	12-957
Plasma		20.5	13.8[a]	0-47	14.8	3-40	31.1	1-220	31.9	1-220
Monocytes/macrophages		14.9	12.7	0-41	66.4	7-484	12.4	3-66	11.3	3-35
Reticuloendothelial		3.4	3.7	0-16	20.8	0-208	4.4	0-34	3.4	0-14
Myeloblast-promyelo-myelocyte		55.9	59.5	6-160	71.9	6-281	43.4	0-130	41.7	0-109
Metamyelocyte		111.7	128.5	28-327	132.8	14-380	99.4	0-216	101.7	0-227
Band		117.6	133.1	20-341	120.1	16-236	115.5	0-220	124.4	0-300
Neutrophils		185.9	185.1	26-476	157.2	19-268	193.7	2-534	170.4	2-534
Eosinophils		46.4	25.1[a]	3-62	27.6[a]	0-77	26.1[a]	0-168	27.7[a]	0-168
Basophils		0.8	0.8	0-16	0.9	0-3	0.5	0-3	0.4	0-3
Unidentified cells		0.7	2.3	0-109	0.1	0-1	0.1	0-2	8.7	0-116
Bare nuclei		19.3	12.9	0-149	30.8	0-134	13.0	0-62	10.4	0-62
Total granulocytes		518.2	530.4	122-923	510.5	57-839	481.8	2-750	470.8	2-783
M:E		1.6	5.1	0.3-96	2.7	1.1-6.8	4.7	0.3-53.8	4.9	0.3-53.3
EMI		3.6	2.7[a]	0-6.4	2.9	0.8-4.6	3.3	0.4-6.4	3.5	1.9-6.4
MMI		8.8	9.8	0-36.1	12.6	1.9-49.5	13.2	0-44.4	14.0	0-44.3
Cellularity		50.9	67.2[a]	35-100	60.3[a]	8-90	66.8[a]	35-90	70.6[a]	55-90

[a]Superscript indicates significant difference from reference mean ($P<0.05$)
[b]TS-Meter, American Optical, Buffalo, N.Y. 14215

age. Their results were 1.66 ± 0.38 for sternal marrow and 1.53 ± 0.23 for rib marrow. Rekers and Coulter (24) compared marrows from the rib, femur, tibia and humerus. Mean M:E for rib marrow from 36 mongrel dogs, 9 to 24 months of age of both sexes, was 1.89 while the M:E for long bones varied between 1.4 and 2.9. Schalm (20) described iliac crest samples from a normal dog on three occasions a few days apart when the M:E ratios were 1.5, 1.3 and 1.8 demonstrating repeatability of results. This dog had fewer prorubricytes (0.6%) and lower metamyelocytes (5.8%) than the mean reference values derived from counts on a number of dogs. The values reported for this single dog (20) are very similar to those of the dogs seen in this study which is probably because our dogs are from "all walks of life" whereas the reference dogs from other studies were confined laboratory animals.

Schalm (20) described a dog with otitis externa and a peripheral blood leukocytosis of 25 x 10^9/L, with a M:E of 3.3 which is similar to the dogs with neutrophilia (group 9) with a mean M:E of 5.1. In the renal failure and myelofibrosis group, a depression of erythrogenesis as indicated by a Hct of 0.23 and 0.15 respectively and low reticulocyte counts was associated with an increased M:E of 23.8 and 6.1 respectively. The M:E of 4.7 for dogs with

lymphoma and 4.9 for dogs with cancer other than lymphoma (groups 11 and 12 respectively) suggests a depression of erythrogenesis. Myelophthisis may have been significant in the 16 of 34 dogs with lymphoma that had tumor cells in the bone marrow (31). However, decreased erythropoiesis is characteristic of all malignancies and is not limited to hemopoietic cancer (14). Further, inhibition of stem cell function is more a feature of the myeloproliferative rather than the lymphoproliferative diseases (39).

TABLE V. Serum Iron, Unbound Iron-binding and Total Iron Binding Capacity[b] of 164 Dogs

	Reference dogs Normal Hematology (n = 21)		Reference dogs Abnormal Hematology (n = 15)		Anemia Poorly Responsive (n = 60)		Anemia Responsive (n = 15)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
SI	20	10-43	17	7-30	25	4-68	40[a]	9-113
UIBC	32	5-54	35	19-58	24	19-78	23	0-70
TIBC	52	36-64	53	37-68	46	0-58	61	33-113
% Sat.	40	20-89	36	14-56	51	8-100	61	15-100
	Immune Hemolytic Anemia (n = 8)		Thrombocytopenia (n = 10)		Renal Failure (n = 5)		Myelofibrosis (n = 5)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
SI	40[a]	13-83	24	9-37	18	12-24	55[a]	45-64
UIBC	21	0-70	39	1-51	17	0-44	—[c]	—
TIBC	56	33-83	60	35-78	35	19-68	—	—
% Sat.	65	16-100	40	15-97	62	35-100	—	—
	Neutrophilia (n = 55)		Neutropenia (n = 12)		Lymphoma (n = 34)		Cancer Other than Lymphoma (n = 37)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
SI	23	4-112	22	5-38	28[a]	5-68	22	5-37
UIBC	25	0-70	31	19-45	20	0-60	18	0-36
TIBC	46	19-113	52	37-67	48	5-74	40	5-68
% Sat.	48	9-100	40	13-62	59	18-100	59	33-100

[a]Superscript indicates significant difference from reference mean ($P<0.05$)
[b]μmol/L
[c]No results available

ERYTHROID RESPONSE

In dogs with poorly-responsive anemia (group 3) the RBC, Hb, MCV, reticulocyte count and total erythroid bone marrow counts had upper limits within the range of the reference dogs but when examined individually the high values were found to originate from two dogs. These dogs had been classified in the poorly responsive group because of low hematocrits and reticulocyte counts. Both dogs had neoplasia. Another dog with an MCV of 79 (range 49-79) had early myelofibrosis demonstrated on repeated biopsies. The erythrocytes were consistently low at 1.2×10^{12}/L while the MCV remained high. Another dog had a reticulocyte count which varied from 0 to 118×10^{9}/L with 3.2×10^{12}/L RBC's and was monitored for a period of one month without an increase in hematocrit. The dog eventually did recover from an immune mediated anemia (Coombs positive), but was placed in this group because of poor response.

The erythroid maturation index (EMI) was similar in both the responsive and poorly responsive anemia groups but lower than the reference group (Table II). This would be expected in responsive anemias with normal stem cell input and premature release of rubricytes causing an apparent shift to increased immaturity. The group with responsive anemia had approximately twice as many immature erythroid precursors as the other groups. Comparing these results to the peripheral blood, it can be seen that the lower maturation index (EMI) in the two groups of anemic animals (group 3 and 4) does not suggest the marked variation in production that can be seen in their absolute mean reticulocytes (65×10^{9}/L for poorly-responsive and 290×10^{9}/L for responsive anemia). Thus as a group the anemic dogs with poor reticulocyte response can be concluded to have normal maturation of too few erythroid precursors.

It is apparent that the mean absolute number of peripheral blood reticulocytes is the most reliable index of effective erythropiesis. Further review of five individual cases indicated that those dogs with the highest reticulocyte counts ($> 250 \times 20^{9}/\mu$L) also had the lowest EMI. Therefore a very active peripheral reticulocyte response indicates erythroid hyperplasia with synchronous maturation.

As would be expected the MCV of the groups with poorly responsive anemia was similar to the controls while the mean MCV of the responsive anemia group was 10.2 fL higher. In the group with poorly responsive anemia the MCH was similar to the control group. Thus, the red cells in the responsive group was larger and contained more hemoglobin on an absolute basis (MCH) than the red cells in the nonresponsive group.

There was some variation in reference values between authors (Table V), especially in regard to the proliferative stages of the rubricytic and granulocytic series. The larger numbers of dogs examined in this study (21) together with the uncertain background of the reference animals may explain some of the differences.

It was noted that significant thrombocytosis did not occur with the responsive anemia group. Thrombocytosis is usually reported in responsive anemias (20,25,31). The range of platelet numbers in the responsive anemia group ($8\text{-}400 \times 10^{9}$/L) was very wide and included four cases of thrombocytopenia. Therefore some of the animals included in the responsive anemia group were likely complicated by concurrent ITP (Evans Syndrome) (31). The mean platelet count of the IHA group without the four animals with probable ITP is much higher (320×10^{9}/L), which is in agreement with the response reported in immune mediated anemia.

MYELOID RESPONSE

The myeloid maturation index (MMI — maturation phase/proliferative phase) was not different in any of the disease processes, but the group means varied from a low of 7.4 with

ITP to a high of 30.4 with myelofibrosis. This lack of significant difference between means is in contrast with the erythroid system and is likely due to a lack of storage pool cells in the erythroid system and a great variation between animals within disease groups in release of granulocytes in response to peripheral demands. A significant difference was noted between the mean bone marrow neutrophil counts in the renal failure and the myelofibrosis groups (groups 7 and 8) and the reference group. This increase in bone marrow neutrophils may be due to impaired release as no peripheral neutrophilia was noted in these animals. Part of this change may be due to shifts in M:E, however, both groups 7 and 8 have increased MMI which suggests that ineffective myelopoiesis may be partially responsible for the marrow neutrophils (late asynchrony) (42,43). The MMI should be useful to identify ineffective myelopoiesis in animals with myelodysplasia resulting in neutropenia with hyperplastic marrow and increased marrow granulocyte reserves.

There was a difference between the means of peripheral blood eosinophils for the reference and other groups. The reference group mean of 4.6% is higher than other workers report (Table VI). This may be due to the source of animals since laboratory dogs were employed in other studies (28,29). The bone marrow eosinophil levels were low in all groups of diseases animals. With prolonged steroid administration, eosinophil production is reduced, as shown by lowering of the total marrow eosinophils and decreased mitotic rate in the precursors (36). The bone marrow eosinophils may be increased after brief stress due to a reduced release coincident with peripheral blood eosinopenia (36). The high level of steady of state eosinopoiesis in our reference dogs may be related to lack of preconditioning.

LYMPHOMA

Bone marrow involvement was found in 16 of the 34 dogs with lymphoma (47%). The tumor involvement in the marrow appears to have prognostic significance since extensive bone marrow involvement was associated with a poor prognosis (14). The level of detection of marrow infiltration was greater with core biopsies than with aspirate smears and could be further increased by bilateral biopsies. Other marrow stromal reactions also appeared to have prognostic value.

MYELOFIBROSIS

Five dogs with nonresponsive anemia were found to have myelofibrosis when examined by core biopsy (Table III, group 8). No underlying myeloproliferative disorder could be identified in these animals and marrow aspirates were poorly cellular. Therapy with steroids and cyclophosphamide was followed by a decrease in marrow stroma and improved marrow function in three dogs. In most cases the cause of myelofibrosis is unknown. In myeloproliferative disorders, particularly of the megakaryocytic series, there is a rapid development of myelosclerosis (40,41). This association has not been well defined in animals.

MEGAKARYOCYTIC RESPONSE

Megakaryocyte numbers varied from markedly increased in six dogs to normal in two dogs and decreased in two dogs. Other workers (34) report an absence or decreased number of megakaryocytes in the bone marrow during the early phase of ITP and increased numbers during later compensatory phase (34). A very low number or absence of megakaryocytes has been associated with very poor prognosis by some workers possibly due to antibodies directed against megakaryocyte precursors rather than platelets (34). In our study, of the two dogs with decreased megakaryocytes one recovered over a period of three weeks. The other died with a bleeding diathesis after a prolonged illness. It appears that those cases of ITP with hypoplasia of early precursors have a poorer prognosis than cases where megakaryocyte numbers are normal or increased. In the myelofibrosis group, megakaryocyte numbers were normal or increased in four of the five dogs, while the morphology of the megakaryocytes appeared to be normal.

HEMOSIDERIN AND SERUM IRON

In our study marrow hemosiderin was evaluated with respect to quantity (increased, normal, or decreased) and distribution (predominantly fine granules, both fine and coarse or predominantly coarse granules). On the basis of subjective evaluation the reference dogs all had adequate amounts of hemosiderin present in the marrow, mostly as fine granules with or without some coarse granules. In general both the amount and coarseness of marrow hemosiderin increases with age. The animals with neoplasia or chronic inflammatory conditions had adequate marrow iron stores but a marked predominance of coarse hemosiderin in the marrow macrophages. This finding is consistent with decreased turnover and apparent availability of iron for erythropoiesis (37).

Serum iron levels were elevated in the responsive anemia, IHA, myelofi-

TABLE VI. Cytological Examination of Canine Bone Marrow

	Present Study Normal hematology n = 21		Melveger and VanLoon, 1969 n = 5		Schalm, 1975 n = ?	Meyer and Bloom, 1943 n = 10
	x̄	S.D.	x̄	S.D.	x̄	x̄
Rubriblasts					0.2	1.0
Prorubricytes	1.2	±0.6	6.5	±0.45	3.9	2.5
Rubricytes	31.9	±1.9	27.6	±4.4	27.0	35.2
Metarubricytes	3.1	±1.8			15.0	
Total Erythroid Cells	36.3	±7.8	34.7	±4.7	46.4	38.7
Myeloblasts			0.9	±0.2	0.0	0.6
Progranulocytes	5.6	±2.4	2.1	±0.4	1.3	
Myelocytes			6.3	±1.0	9.0	3.8
Metamyelocytes	11.2	±4.4	7.9	±2.4	7.5	23.5
Band neutrophils	11.8	±2.6	11.3	±2.2	13.6	
Segs	18.6	±5.7	23.5	±1.3	18.4	18.5
Eos	4.6	±2.2	2.5	±0.3	0.3	1.7
Total Myeloid Cells	51.8	±8.6	55.2	±5.9	53.4	48.5
M:E ratio	1.6	±0.8	1.7	±0.4	1.2	1.4
Lymphocytes	6.1	±2.7	8.1	±2.7	0.2	9.8
Monocytes	1.5	±0.8	0.0	±0.0	0.0	1.2
Plasma cells	2.1	±0.8	0.7	±0.3	0.0	0.8

brosis and lymphoma groups. In the responsive anemia and immune hemolytic anemia groups (Table V) elevated serum iron levels (40 μmol/L) were most likely due to recycling of hemoglobin from destroyed or damaged red cells. With marrow damage, ineffective erythropoiesis or parenchymal iron overload, the serum iron tends to rise, leading to full saturation in more severe cases (35). This may explain the rise seen with the myelofibrosis and lymphoma groups in our study (Table V).

It is hoped that the data presented here will encourage others to more effectively use bone marrow examination in clinical diagnosis.

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