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Hypoglobulinemia in a dog with disseminated plasma cell neoplasia: Case report and review of the diagnostic criteria.

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Abstract

This is the first reported case of hypoglobulinemia in a dog with disseminated plasma cell neoplasia. A 6-year-old male intact Rottweiler was referred to the U-Vet Animal Hospital (Werribee, Vic, Australia) for weight loss, hyporexia, lethargy, vomiting, and soft stools. Examination of a buffy coat preparation and splenic and liver aspirates revealed a monomorphic population of plasmacytoid cells, and the same cells comprised approximately 90% of bone marrow samples submitted for cytologic and histologic evaluation. Biochemistry revealed a hypoglobulinemia, and the presence of an M-protein was not supported by serum and urine protein electrophoresis or serum immunofixation. Immunohistochemistry demonstrated strong nuclear labeling for MUM-1.

Keywords

Bone marrow; canine; electrophoresis; immunofixation; non-secretory; plasma cells

Case presentation

A 6-year-old male intact Rottweiler was referred to the U-Vet Animal Hospital (Werribee, Vic, Australia) for a 6-8-week history of weight loss, hyporexia, lethargy, vomiting, and soft stools. Physical examination revealed moderate tachypnea (RR 60-80 bpm), marked hepatosplenomegaly, mild pyrexia (103.6° F), and poor body condition (body condition score 3/9), with sarcopenia affecting the extraocular and masseter muscles.

Hematology was performed using EDTA-whole blood on the Sysmex XN-V (Sysmex Corporation, Kobe, Japan) (Table 1), revealing a mild normocytic normochromic non-regenerative anemia, marked thrombocytopenia, and marked leukopenia due to a severe neutropenia with occasional band neutrophils and mild toxic change, as well as a marked lymphopenia. Coagulation testing was

performed using citrated-whole blood on the STA Compact Max 2 (Diagnostica Stago, NJ, USA), and PT (9.2s, RI:6.9-8.8s) and aPTT (17.6s, RI:13.1-17.2s) were both mildly prolonged.

A Wright-Giemsa stained blood smear was evaluated, and confirmed the pancytopenia and demonstrated evidence of shear injury (acanthocytes, keratocytes, and schistocytes), as well as an inappropriate metarubricytosis (9/100 WBC). A small number of atypical round cells were identified at the feathered edge, and these comprised 30% of total leukocytes. Frequently, lysed cells were also present. A 1000-cell leukocyte differential count of a buffy coat preparation revealed 49.6% atypical round cells. The cells had moderate amounts of deep blue cytoplasm that frequently contained an area of paranuclear clearing, a round eccentric nucleus with clumped chromatin, and 1-3 distinct faint nucleoli (plasmacytoid appearance) (Fig. 1). Figure 2A shows the Sysmex white cell nucleated (WNR) channel scattergram, and Figure 2B demonstrates the cell populations after manual gating. The Sysmex WNR scattergrams from a dog with a metarubricytosis and a dog with stage V lymphoma are included for comparison and justification of the manual gating (Supp. 1).

The serum biochemistry panel was performed using the Cobas Integra 400 plus (Roche Diagnostics Ltd, Rotkreuz, Switzerland) (Table 2). It revealed a marked hypocholesterolemia and moderate hypoproteinemia due to a marked hypoglobulinemia with an albumin concentration within the reference interval (confirmed on repeat testing).

A free-catch urine sample was grossly dark brown to orange with a marked bilirubinuria (3+) (Multistix, Bayer Corp., Elkhart, IN, USA), pH 6.0, and USG >1.050. The protein reagent pad could not be interpreted due to gross urine discoloration; however, a sulfosalicylic acid test was negative.

Smears were prepared from bone marrow (BM) fine-needle aspirates (FNA), and a BM biopsy was obtained from the right humerus. The smears and biopsy were highly cellular with good cell preservation and staining. Approximately 90% of cells were monomorphic neoplastic round cells with similar morphologic features to those on the buffy coat preparation (Fig. 3). The cells had a moderate to high N:C ratios, deep blue cytoplasm that frequently contained an area of paranuclear clearing, and a single round eccentric nucleus with clumped chromatin and 1-3 distinct round nucleoli. The cells displayed moderate to marked anisocytosis (10-25µm), mild to moderate anisokaryosis (7-15µm), occasional binucleation, and occasional mitoses. Rarely, there was evidence of abnormal mitoses, including lag chromosomes, chromosome bridging, hyperchromatic

karyokinesis, abnormal chromosomal distribution, and satellite nuclei. Occasionally the cells contained a large clear to pale pink round intracellular inclusion (Fig. 4A and 4B). The remaining cells were a mixed population of hematopoietic cells, including occasional erythroid precursors (mostly metarubricytes and fewer rubricytes), occasional granulocytic precursors (mostly band and segmented neutrophils), and rare predominantly mature megakaryocytes. The erythroid series showed moderate dysplastic changes, including nuclear to cytoplasmic asynchrony, irregular nuclei (including satellite nuclei), and rare, atypical mitotic figures (lag chromosomes). The band and segmented neutrophils demonstrated moderate toxic changes (foamy light blue cytoplasm). Histiocytes and lymphocytes were rare.

On abdominal ultrasonography, the spleen was enlarged with a homogenous echotexture and multiple hypoechoic nodules of varying size, the liver was diffusely enlarged and hypoechoic, and there was a small volume peritoneal effusion. A mesenteric mass (1.7 cm diameter and isoechoic to the spleen) was considered to be an enlarged lymph node or accessory spleen. FNA smears of the liver and spleen revealed a large population of atypical round cells morphologically similar to those seen in the buffy coat preparation and BM samples. The amount of cytoplasm was variable, with some cells appearing more lymphoid (higher N:C ratio and prominent nucleoli), while most were plasmacytoid in appearance with abundant deep blue cytoplasm, clumped chromatin, indistinct nucleoli, and prominent Golgi. Survey thoracic radiographs revealed no significant abnormalities.

There was strong nuclear labeling for MUM-1 on immunocytochemistry (ICC) (Fig. 5A) and immunohistochemistry (IHC) (Fig. 5B) of the BM specimens using a monoclonal mouse anti-human MUM1 antibody (Dako, Protein clone MUM1p). Two different Pax-5 antibodies were used for IHC (BD Sciences Purified Mouse Anti-Pax-5; Dako Monoclonal Mouse Anti-Human B-Cell Specific Activator Protein Clone Dako Pax-5) and did not demonstrate any immunoreactivity on the cytologic or histologic specimens of BM (Fig. 6) or on cytologic smears from the spleen. CD3 (Dako FLEX Polyclonal – Rabbit Anti-Human CD3 Ready to Use), CD18 (University of California Davis Canine CD18 CA 16.3 C10 IgG1) and Iba-1 (Abcam AB 178847 Rabbit mAB to ABA-1) antibodies did not demonstrate immunoreactivity on histologic specimens (Supplementary figures. 2 and 3). Positive and negative control slides of canine tonsil were included for all antibodies. The cytoplasmic inclusions observed on BM histology did not stain with Periodic acid-Schiff (PAS).

Urine protein electrophoresis (UPE) showed a non-selective proteinuria with no atypical bands noted (external laboratory) (Fig. 7A, Table 3).

Serum samples for electrophoretic tracing and gel and immunofixation studies were sent to Colorado State University (CO, USA), using previously validated reagents.[1] A distinct hypogammaglobulinemia was confirmed with mild decreases in β2 and α2 globulins (Fig. 7B, Table 4). Minimal IgG was found by routine immunofixation (Fig. 8), and no atypical restricted bands were seen. Immunofixation did not support the presence of serum-free light chains (Fig. 8).

Outcome

Based on cytomorphology, the interpretation was round cell neoplasia with marked infiltration of the liver, spleen, and BM. Prior to performing immunophenotyping, the primary differentials were B-cell lymphoma with plasma cell differentiation and disseminated plasma cell neoplasia (commonly referred to as multiple myeloma), with histiocytic sarcoma and acute myeloid leukemia considered less likely. The owner elected euthanasia of the dog, given the poor prognosis associated with multicentric round cell neoplasia. No necropsy was performed.

Discussion

The term multiple myeloma (MM) refers to diffuse plasma cell neoplasia and represents up to 3.6% of all canine bone tumors.[2] It is comprised of a clonal population of terminally differentiated plasma cells and is generally considered a primary tumor of bone.[3] Clinical signs include lameness and skeletal pain associated with focal or multifocal lytic bone lesions, signs associated with hypercalcemia (eg, polydipsia), and complications due to hyperglobulinemia (including blindness, neurological signs, hyperviscosity, and renal insufficiency).[4,5]

Hyperglobulinemia due to the production of a monoclonal immunoglobulin (most commonly IgG or IgA) by neoplastic plasma cells is a characteristic of secretory MM[5] and is often used as a criterion for diagnosis.[2] Monoclonal gammopathies with normoglobulinemia can also occur,[6,7] and this is relatively common in human MM cases (31-59%).[4,8,9] The neoplastic cell products are referred to as 'M-proteins' (monoclonal proteins), and might include polypeptide subunits of the immunoglobulin (heavy or light chain).[4] M-protein production can also be seen in other B cell lineage neoplasms.[10]

The current diagnostic definition for MM in dogs requires the fulfillment of two to three of the following criteria: radiographic evidence of osteolysis, plasma cells comprising greater than 20% of the BM population, a monoclonal gammopathy on serum protein electrophoresis, and/or Bence-Jones (light chain M-protein) proteinuria.[5,6]

Human criteria for the diagnosis of MM have been recently reviewed.[11,12] The current criteria no longer require documentation of complete M-proteins; diagnosis is made based on clonal BM plasma cells of ≥10% or biopsy-proven bony or extramedullary plasmacytoma and one or more 'myeloma defining events' of either end-organ damage (ie, CRAB lesions – Calcium, Renal, Anemia, Bony lysis) or presence of a biomarker of malignancy (>60% BM plasma cells, altered κ:λ ratio, or > 1 focal lesion on magnetic resonance imaging).[12] The presence or absence of an M-protein is used to subdivide MM into secretory and non-secretory myeloma (NSM).[12]

NSM is comparatively rare, comprising 1-5% of all cases of MM in people[3,13] and is only rarely described in dogs.[14–17] As the name suggests, NSM is characterized by the absence of a secretory product.[18] Diagnosis of NSM is more challenging than MM, and several criteria have been suggested for use in human medicine.[19] The International Myeloma Working Group[11] has proposed NSM be used for patients with no serum or urine M-component and BM containing ≥10% clonal plasma cells. Therefore, the absence of M-proteins, in this case, does not exclude it from the current human diagnostic definition of MM.

To further complicate the diagnostic process, NSM can be further subcategorized into two entities that might be diagnostically indistinguishable. 'True' NSMs are those with neoplastic cells that do not synthesize any products, while 'false' NSMs are those in which the secretory product is either retained intracellularly due to excretion issues or cannot be measured by routine methods.[3,20] The latter could be due to an abnormal and thus undetectable product,[21] or due to product levels that are too low to be detectable by current methods (also called oligosecretory).[3,18,20] In most human cases, a secretory product can be identified by electron microscopy, suggesting that the majority of NSMs are not 'true' NSMs[3,22] and many human NSM cases have now been reclassified as secretory MM with the use of immunochemical identification of serum-free light chains (FLCs).[23]

Proposed theories concerning the mechanism behind these 'false' NSMs include rapid intracellular or extracellular degradation of immunoglobulin, abnormal glycosylation, a faulty release mechanism or release of products in secretory vesicles, deposition of the secretory product in tissues, or unfavorable environmental conditions limiting secretion.[24–26]

198 The limitations of the current canine inclusion criteria for MM have almost certainly resulted in the
199 exclusion of NSM cases from multiple myeloma studies, and thus the incidence of NSM is largely
200 unknown in dogs. In patients with no discernible hyperglobulinemia, clinicians might be less likely to
201 pursue testing for the presence of M-proteins.[6] Additionally, commercial electrophoresis, and
202 particularly immunofixation, are not widely available.
203
204 *Comments on this case*
205 After reviewing the literature, several differential diagnoses remained. While other lineages could
206 demonstrate MUM-1 labeling (including histiocytes[27]), the cytomorphology of the cells was
207 consistent with a lymphoid origin, and positive labeling for MUM-1 thus confirmed either late B-cell
208 or plasma cell neoplasia. A specific immunophenotype for plasma cell neoplasia in dogs has not yet
209 been determined; however, the high sensitivity and specificity of MUM-1 labeling in canine plasma
210 cells has been demonstrated.[28] There was no antibody labeling for Pax-5, meaning that either no
211 normal B-lymphocytes remained in the tissue due to complete effacement by the neoplastic cells or
212 that our marker was unsuccessful. IHC was repeated with another Pax-5 antibody clone, and the
213 controls for both clones were positive, suggesting effacement was the more likely cause.
214
215 Given the strongly positive MUM-1 labeling, lack of antibody labeling for Pax-5, and the significant
216 proportion of plasmacytoid cells in the BM (approximately 90%), disseminated plasma cell neoplasia
217 was considered the most likely diagnosis, despite not fulfilling the current diagnostic criteria for
218 canine MM.
219
220 Hypogammaglobulinemia is seen in approximately 10% of human patients with secretory multiple
221 myeloma (often associated with Bence-Jones proteinuria);[4,29,30] however, hypoglobulinemia has not
222 been reported in dogs with myeloma-related disease. It has been postulated that the increased
223 production of M-protein has a depressive effect on normal immunoglobulin synthesis,[31] however
224 hypogammaglobulinemia is also seen in cases of NSM,[30] suggesting that this explanation is an
225 oversimplification. Several studies have proposed an altered immunoregulatory mechanism that
226 results in impaired B-cell maturation (and thus reduced immunoglobulin production) by specific T-
227 cell subsets.[32] The decreased serum alpha 2 and beta 2 globulin fractions could suggest non-specific
228 renal protein loss as a mechanism for hypogammaglobulinemia; however non-specific protein loss
229 should also induce hypoalbuminemia and a distinct albumin band in the urine (features not seen in
230 this case)[33]. Given the large proportion of plasma cells effacing the BM, the marked peripheral
231 lymphopenia, and the negative Pax-5 labeling on both the BM and spleen specimens, reduced B-cell

numbers (secondary to myelophthisis), and impaired B-cell maturation and function were the most likely mechanisms for hypoglobulinemia in this case.

The presence of M-proteins cannot be entirely excluded in this case for several reasons. Firstly, negative urine electrophoresis testing is insensitive for M-protein detection, and elevated acute phase proteins may mask small increases in M-protein.[4] Secondly, immunofixation on the urine for free light chains was not performed due to sample volume limitations. Lastly, at the point of testing, the serum-free light chain immunofixation assay performed on this patient used anti-human FLC reagents, which, while they have been shown to accurately label canine serum and urine FLCs, might not label all canine FLCs.[1] Given the prevalence of light chain myeloma in people (16% in one study)[29], secretory MM is considered a differential for this case. A previous case of suspected NSM in a dog with normoglobulinemia had negative UPE and serum protein electrophoresis (SPE), however, immunofixation was not performed, and thus, the absence of an M-protein was not confirmed.[17]

In people, a quantitative serum FLC assay has been found to have greater sensitivity than immunofixation in detecting clonal FLCs,[23] and this might be a promising area for investigation in dogs. Further, human patients with NSM have developed M-proteins in the serum and/or urine during follow-up;[29] therefore, serial testing is also indicated in future suspect cases of NSM.

There was a discrepancy between the positive urine protein analysis performed at the external laboratory and the negative sulfosalicylic acid (SSA) test. Given the subjectivity of the latter, the proteinuria was likely true; however, it is challenging to explain. Some urinary proteins are soluble in SSA but react with Pyrogallol red, including alpha 1-acidoglycoprotein (variable) and Tamm-Horsfall mucoprotein.[34] Precipitation of Ig light chains in the renal tubules, renal amyloidosis, glomerulonephropathy, or renal infiltration with neoplastic cells are also possibilities.[35] Unfortunately, a post-mortem examination was declined; however, histologic evaluation and special staining for the presence of amyloid would be warranted in future suspect cases.

The neoplastic cells, in this case, demonstrated cytoplasmic inclusions, which could suggest a 'false' NSM. The inclusions were PAS negative; however, abnormal glycosylation of immunoglobulins cannot be excluded, and PAS negative Russell bodies have been reported in human cases of MM.[36] Erythrophagia was considered unlikely given the size and color of the inclusions. A previous suspected canine NSM case with normoglobulinemia and no detectable M-protein used ultrastructural examination to demonstrate flocculent cisternal contents in the rough endoplasmic

reticulum, suggesting synthesis, but not release, of immunoglobulin or other protein product.[14] Another study demonstrated a lack of cisternal dilation by ultrastructural analysis to confirm a non-productive (or 'true') NSM in a dog.[16] Antisera specific to kappa light chain Ig can also be used to demonstrate intracellular M-proteins in dogs.[35]

We cannot entirely exclude the presence of osteolytic lesions as full skeletal survey radiographs and computed tomography were not performed, although there was no lameness or evidence of bone pain on physical examination. Human cases of NSM with no osteolytic lesions have been reported,[22] so this remains a possibility.

Additional immunophenotyping might have assisted in excluding B-cell lymphoma with plasmablastic differentiation (eg, CD79a, CD21).[4] VLA-4 is highly expressed in canine T-cell lymphomas and not typically expressed in B-cell lymphomas,[37] however, expression of VLA-4 has been demonstrated in the majority of human MM cases,[38] suggesting another possible avenue for further immunophenotyping.

The hypocholesterolemia was most likely attributable to the disseminated plasma cell neoplasia. Alterations in cholesterol occur in various malignancies in both animals and people, including histiocytic sarcoma and lymphoma,[39–41] and lower cholesterol concentrations have been reported in people with MM compared with healthy controls.[41] The mechanism is likely related to increased use by neoplastic cells that require cholesterol for growth and replication.[41]

The thrombocytopenia and leukopenia were attributed to myelophthisis, with disseminated intravascular coagulation (DIC) also a consideration given the prolonged PT and aPTT and evidence of shear injury, though testing for D-dimer and fibrinogen and fibrin degradation products (FDPs) was not performed. Shear injury could also have been secondary to the altered marrow and splenic stromal environments, as well as increased erythrocyte fragility due to dyserythropoiesis and the concurrent hepatopathy. The inappropriate metarubricytosis likely reflected dyserythropoiesis due to the altered marrow environment and splenic dysfunction.

In people, the prognosis for NSM is better than for secretory MM[42,43] and is directly associated with the amount of damage caused by the neoplastic cells (including bony lysis, renal dysfunction, and anemia). Human NSM patients have reduced age at presentation, reduced incidence of anemia and

renal dysfunction, and reduced BM involvement compared with patients with MM.[44] So far, there is no evidence concerning the prognostic indicators specifically for NSM in dogs; however, the monitoring of serum and urine globulins have been suggested to identify potential progression from non-secretory to secretory disease.[13] Until further data is available, it seems reasonable to treat NSM with protocols used to treat classically defined canine MM; this requires accurate distinction of NSM from lymphoma, as the treatment protocols for each are significantly different.

Conclusions

While this case did not fulfill the published criteria for the diagnosis of canine MM, it is strongly supported by the extensive infiltration of BM, spleen, and liver by a large population of round cells with strong nuclear labeling for MUM-1 and lack of labeling for both CD3 and Pax-5. It is the first reported case of hypoglobulinemia in a dog with disseminated plasma cell neoplasia, and reduced production of immunoglobulins by B cells with impaired maturation and/or function is proposed as a likely mechanism. The diagnostic criteria for canine MM have remained unchanged since its introduction in 1986.[5] Using the current human definition, this case fulfilled the criteria for the diagnosis of MM, suggesting the need for a review of the current canine MM diagnostic criteria. Given the difference in the prognosis of NSM and MM in people, it is important to differentiate these conditions in dogs to determine if outcomes are similarly affected.

In the pursuit of an NSM diagnosis, normal serum and UPE results do not exclude the presence of an M-protein. The hypoglobulinemia and lack of evidence of a monoclonal gammopathy in the serum and urine suggest that this case represents either NSM (which may be 'true' or 'false') or light chain (Bence-Jones) myeloma (with light chain proteinuria or deposition of light chains in tissue). In people, true NSM is extremely rare,[3] and the presence of unidentified intracellular inclusions in the neoplastic cells, in this case, is supportive of a 'false' non-secretory mechanism. Future investigations might, therefore, consider extrapolation of the human model distinguishing 'true' NSMs from 'false' or oligosecretory NSMs, for use in dogs, which could be done by ultrastructural evaluation of neoplastic cells or with special stains. Finally, this case highlights the need for further research on the immunophenotypic profile of disseminated plasma cell neoplasia in dogs.

Acknowledgments

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Tables

Table 1. Hematologic results from a dog with disseminated plasma cell neoplasia (Sysmex XN-V; Sysmex Corporation, Kobe, Japan)

	Unit	Result	Reference Interval
Red cell count	X 10^{12}/L	5.6	5.5-8.5
Hemoglobin	g/dL	12.6	12-18
Hematocrit	%	**36**	37-55
MCV	fL	64	60-75
MCH	pg	23	19-24
MCHC	g/dL	35.2	32-38
Platelets	x 10^9/L	**46**	200-500
White cell count	x 10^9/L	**0.9**	6.0-17.0
Bands	x 10^9/L	0	0-0.3
Neutrophils	x 10^9/L	**0.2**	3.0-11.5
Lymphocytes	x 10^9/L	**0.1**	1.0-4.8
Monocytes	x 10^9/L	0.3	0.2-1.4
Eosinophils	x 10^9/L	**0**	0.1-1.3
Basophils	x 10^9/L	0	<0.1
Atypical cells	X 10^9/L	**0.3**	0
NRBC	/100 WBC	**9**	Rare
Reticulocytes	X 10^3/µL	61	10-110

Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NRBC, nucleated red blood cells.

Table 2. Biochemistry results from a dog with disseminated plasma cell neoplasia (Cobas Integra 400 plus; Roche Diagnostics Ltd, Rotkreuz, Switzerland)

	Unit	Result	Reference Interval
Sodium	mmol/L	155	144-160
Potassium	mmol/L	5.2	3.5-5.8
Chloride	mmol/L	118	109-122
Calcium	mmol/L	2.40	2.30-3.00
Phosphate	mg/dL	4.34	3.10-8.06
Urea	mg/dL	19.0	8.4-24.4
Creatinine	mg/dL	0.8	0.5-1.6
Glucose	mmol/L	6.4	3.4-7.4
Cholesterol	mg/dL	**15.4**	150.6-301.2
Total Bilirubin	µmol/L	20	0-20
ALT	IU/L	**91**	3-83
ALP	IU/L	**217**	0-170
GGT	IU/L	**0**	1-12
Amylase	IU/L	780	180-1200
Lipase	IU/L	73	0-395
CK	IU/L	167	50-400
Total Protein	g/dL	**4.3**	5.1-7.2
Albumin	g/dL	3.5	3.1-4.4
Globulin	g/dL	**0.8**	1.4-3.7

Abbreviations: ALT, alanine aminotransferase; ALP, Alkaline Phosphatase; CK, creatine kinase; GGT, gamma-glutamyl transferase.

Table 3. Urine Protein Electrophoresis results from a dog with disseminated plasma cell neoplasia (external laboratory). See Figure 7A for tracing.

Fractions	%	g/L
Unknown	7.6	0.10
Unknown	24.7	0.32
Unknown	53.7	0.70
Unknown	14.0	0.18
Total Protein		1.31

Table 4. Serum Protein Electrophoresis results from a dog with disseminated plasma cell neoplasia (Colorado State University, CO, USA). See Figure 7B for tracing.

Fractions	%	g/dL	Canine Reference Interval
Albumin	67.9	3.05	2.77-3.64
Alpha 1	6.0	0.27	0.23-0.38
Alpha 2	9.4	0.42	0.67-1.16
Beta 1	8.4	0.38	0.37-0.81
Beta 2	5.6	**0.25**	0.39-0.81
Gamma	2.7	**0.12**	0.33-0.76
Total Protein		4.49	

Figure Captions

Figure 1. Photomicrograph of a buffy coat preparation from a dog with disseminated plasma cell neoplasia showing the neoplastic round cell population with plasmacytoid features. (Wright-Giemsa stain, original magnification 100x)

Figure 2. A, A Sysmex white cell nucleated (WNR) channel scattergram from whole blood in a dog with disseminated plasma cell neoplasia. The original scattergram shows one cloud on the left, consistent with metarubricytes (confirmed on the blood smear), and two clouds on the right that are merging. B, The cloud on the right ('Atypical cells') was considered likely to reflect the atypical lymphoid population. Once gated, the percentage of atypical cells (52.7%) was consistent with the manual 1000-cell differential count of the buffy coat preparation (49.6%).

Figure 3. Photomicrograph of a bone marrow cytology smear from a dog with disseminated plasma cell neoplasia. The cytology showed high cellularity and large numbers of neoplastic round cells. (Wright-Giemsa stain, original magnification, 50x)

Figure 4. A, Photomicrograph of a bone marrow cytologic smear from a dog with disseminated plasma cell neoplasia showing the neoplastic round cell population. A cell with a cytoplasmic inclusion is shown. Wright-Giemsa stain, original magnification, x100 objective B, Photomicrograph of a bone marrow histologic specimen showing the neoplastic round cell population. Two cells with cytoplasmic inclusions are shown (arrows). Wright-Giemsa stain, original magnification, x100 objective

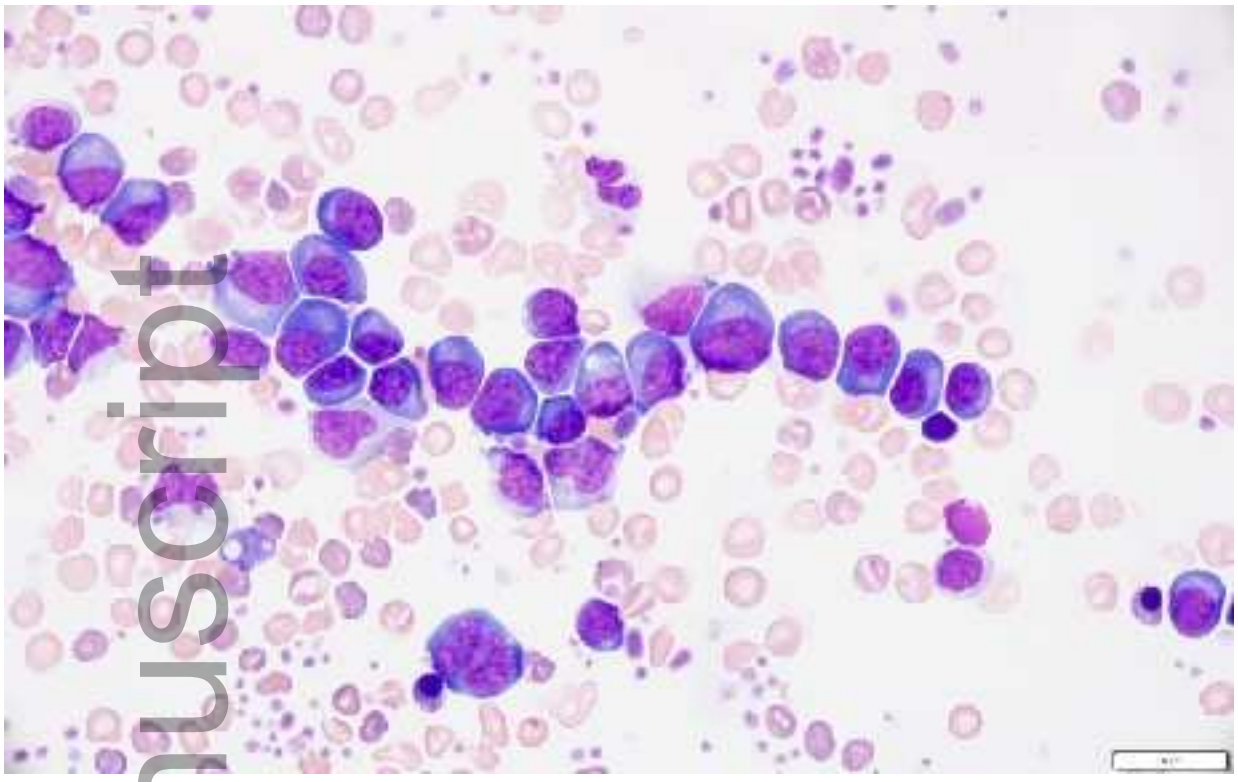
Figure 5. A, Photomicrograph of a bone marrow cytology smear from a dog with disseminated plasma cell neoplasia showing round cells with strong nuclear labeling for MUM-1

(immunocytochemistry staining with monoclonal mouse anti-human MUM-1, Dako Protein clone MUM1p; original magnification, x50 objective B, Photomicrograph of a bone marrow histologic specimen showing neoplastic cells with strong nuclear labeling for MUM-1 (immunohistochemistry staining with monoclonal mouse anti-human MUM-1, Dako Protein clone MUM1p, original magnification, x50 objective

Figure 6. Photomicrograph of bone marrow histologic specimen from a dog with disseminated plasma cell neoplasia showing neoplastic cells with no antibody labeling for Pax-5. (immunohistochemistry staining with BD Sciences Purified Mouse Anti-Pax-5, original magnification, x50 objective

Figure 7. A, Urine protein electrophoresis (external laboratory) using urine from a dog with disseminated plasma cell neoplasia. Note the lack of a discrete narrow peak. B, Serum protein electrophoresis tracing, with control (pink). (Colorado State University, CO, USA).

Figure 8. Serum immunoglobulin immunofixation (Colorado State University) using serum from a dog with disseminated plasma cell neoplasia. There is decreased IgG-FC labeling, consistent with the hypogammaglobulinemia in the electrophoretic study. There is also a lack of labeling in all other heavy chains (IgA, IgM, IgG4), anti-canine light chain (LC), and anti-human free κ and λ light chains (fκ, fλ).



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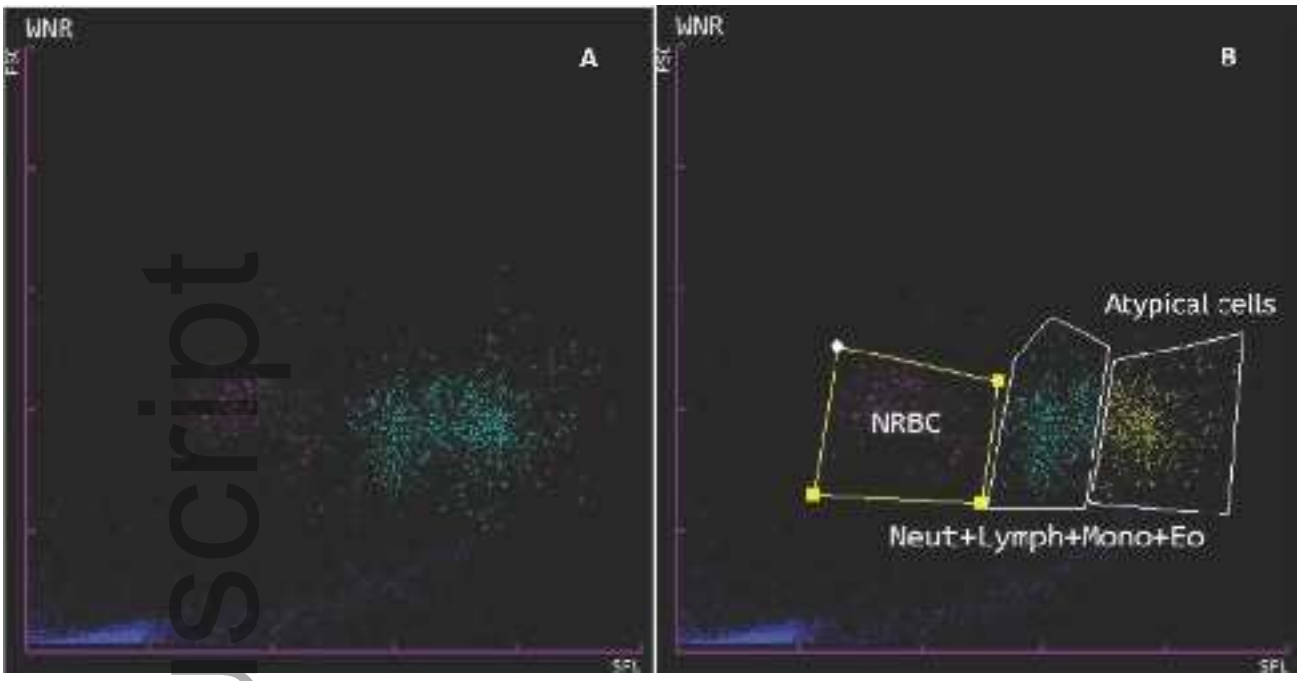

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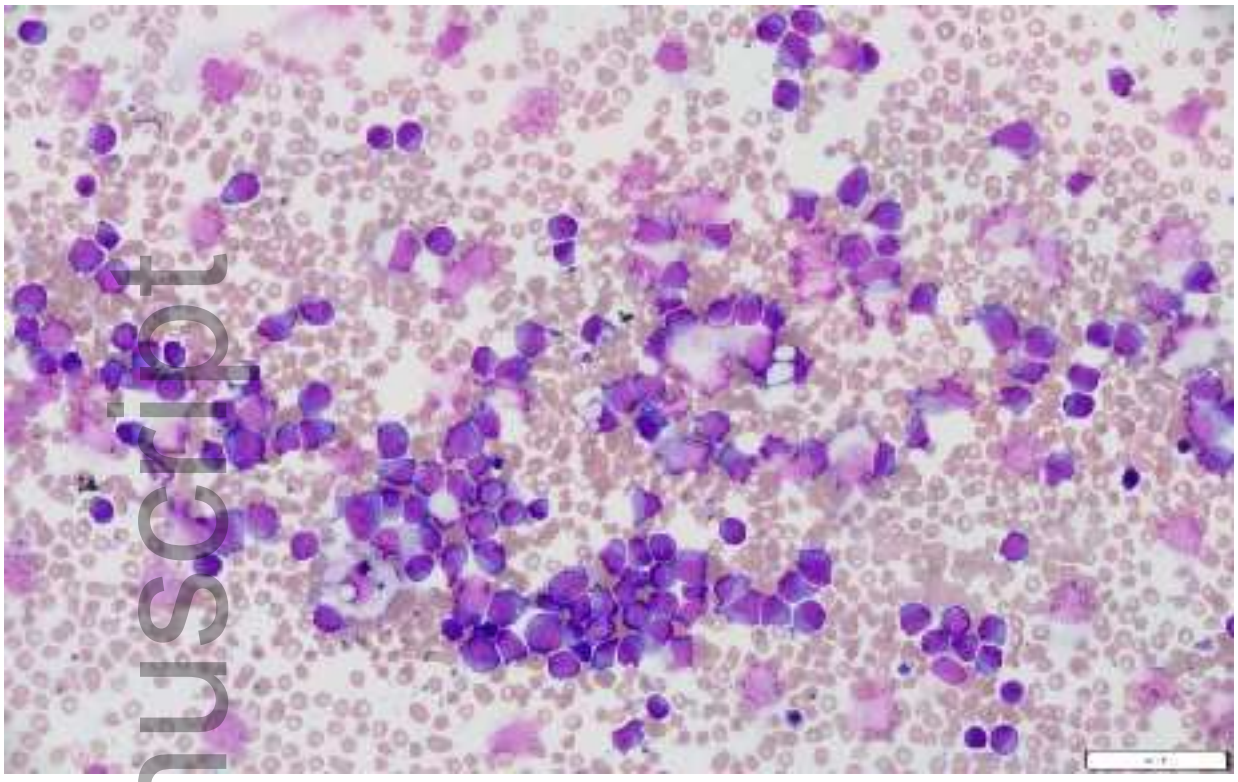

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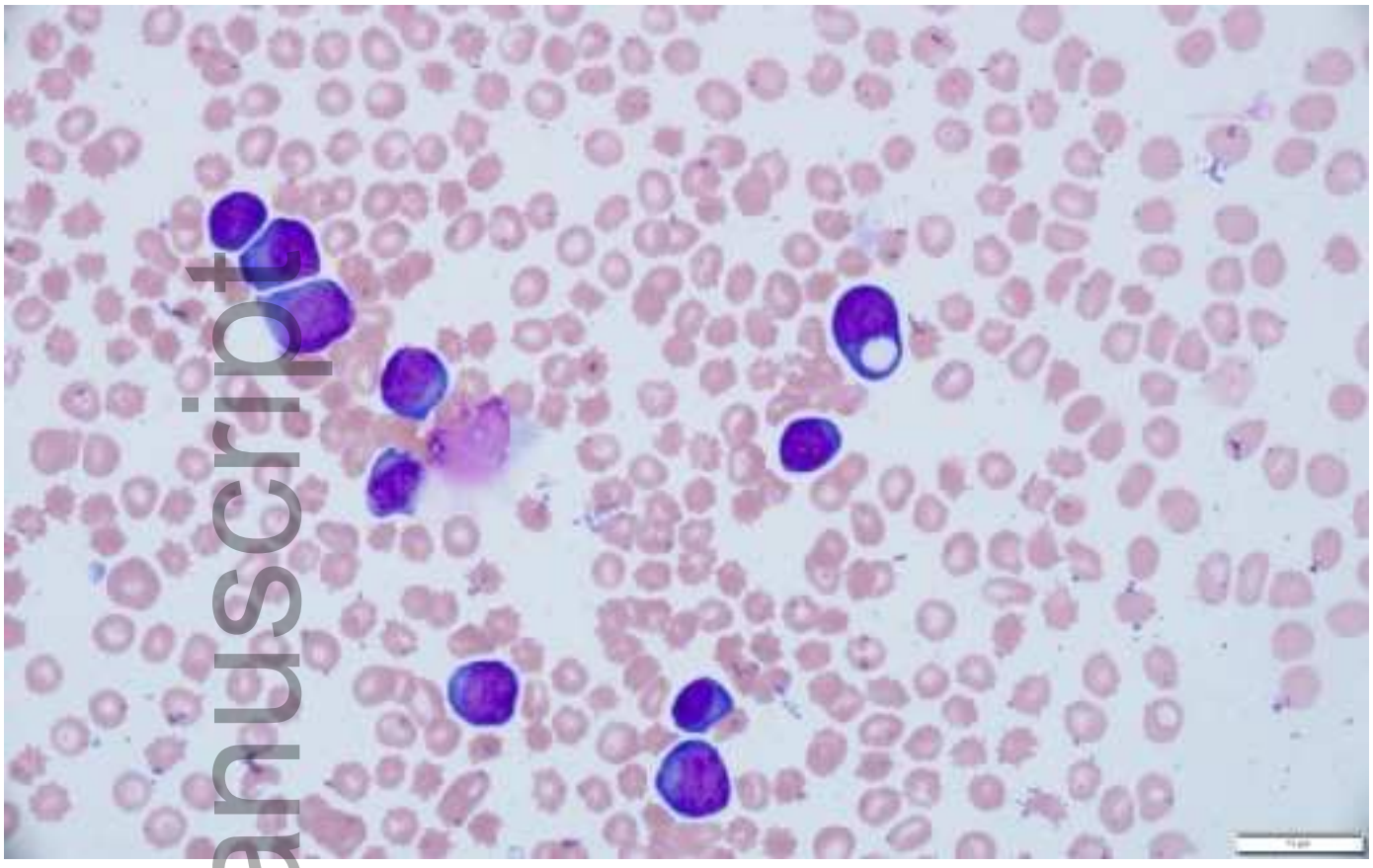

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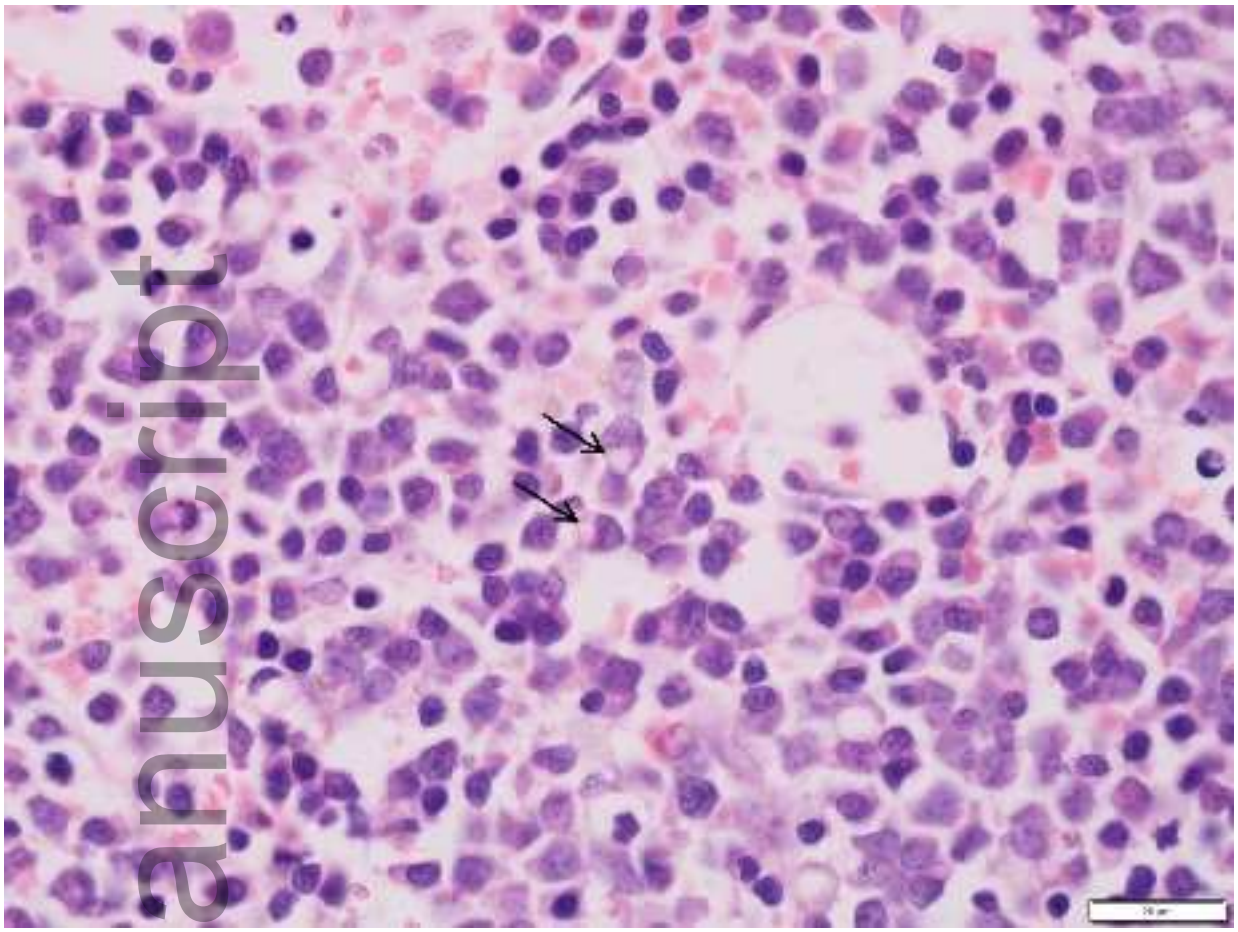

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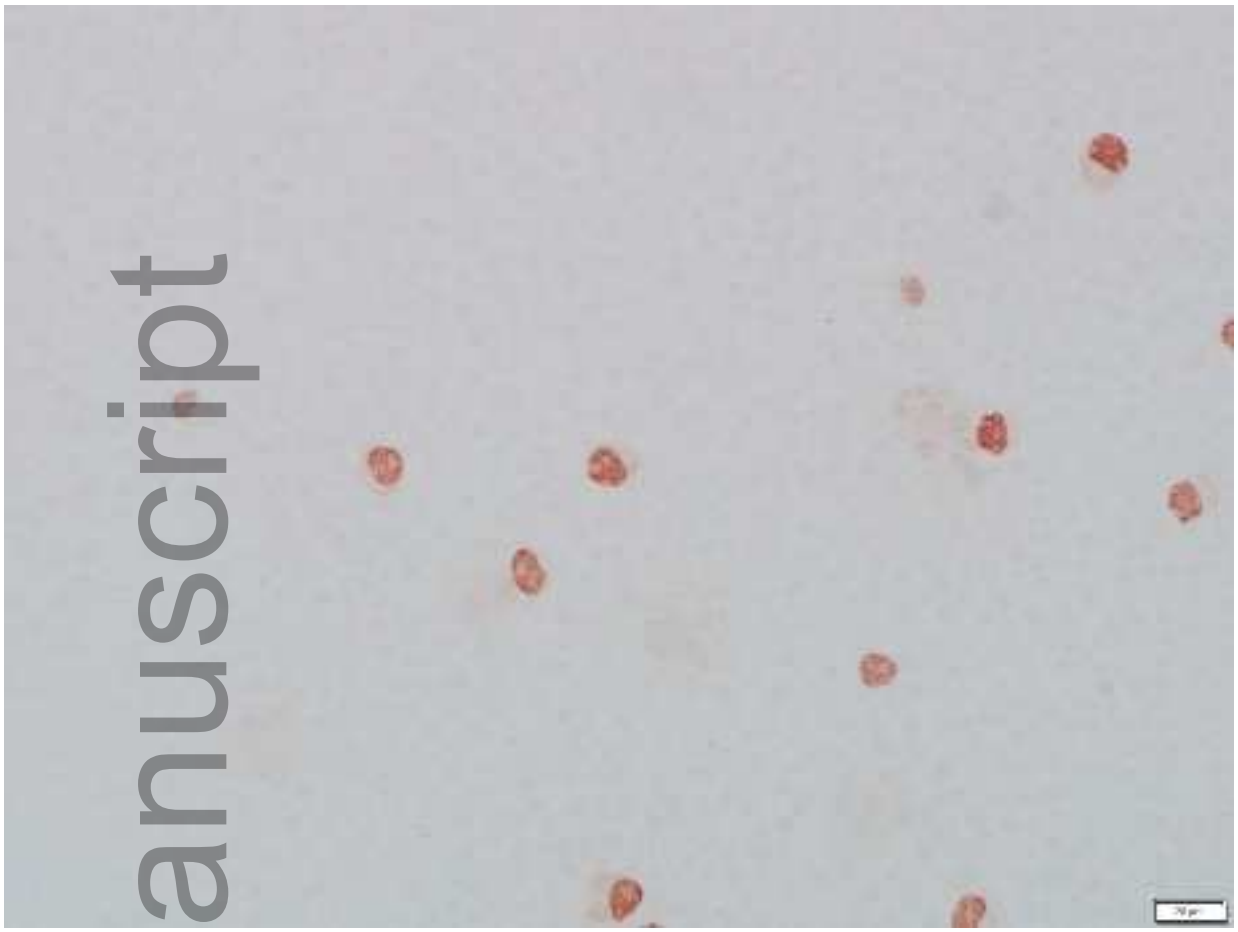

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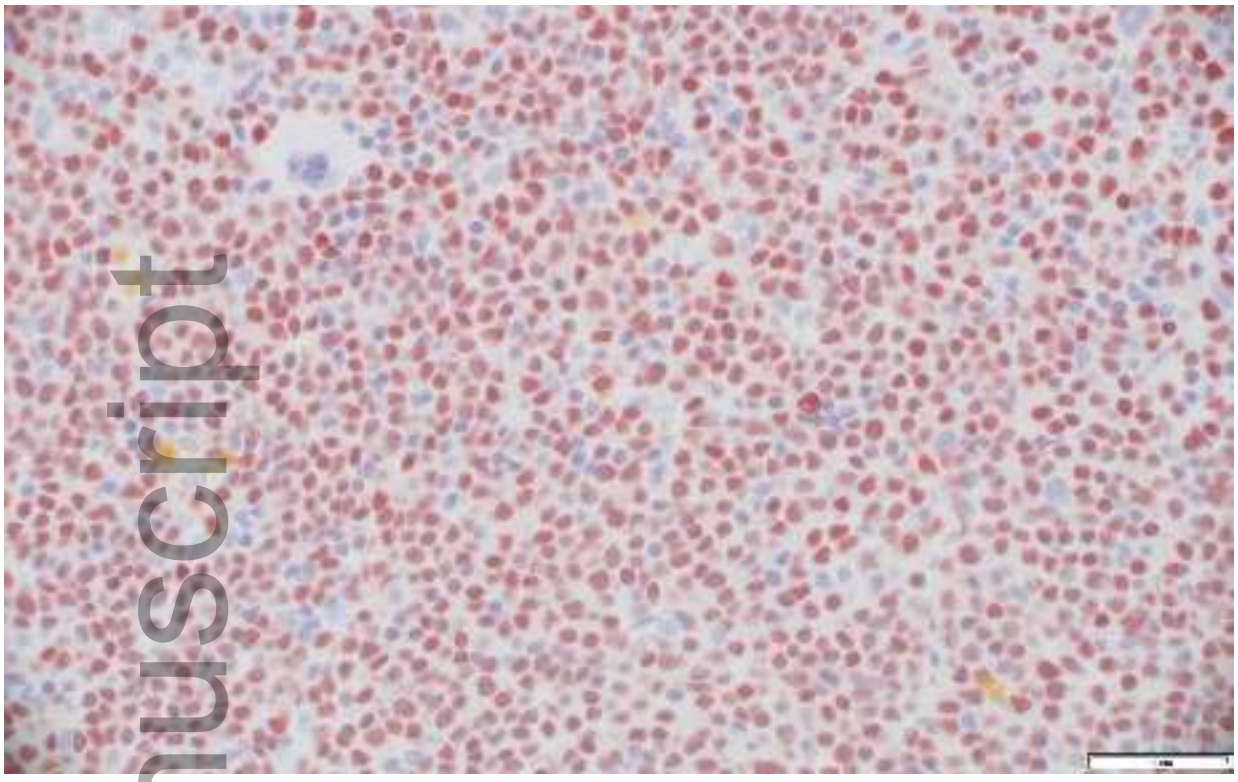

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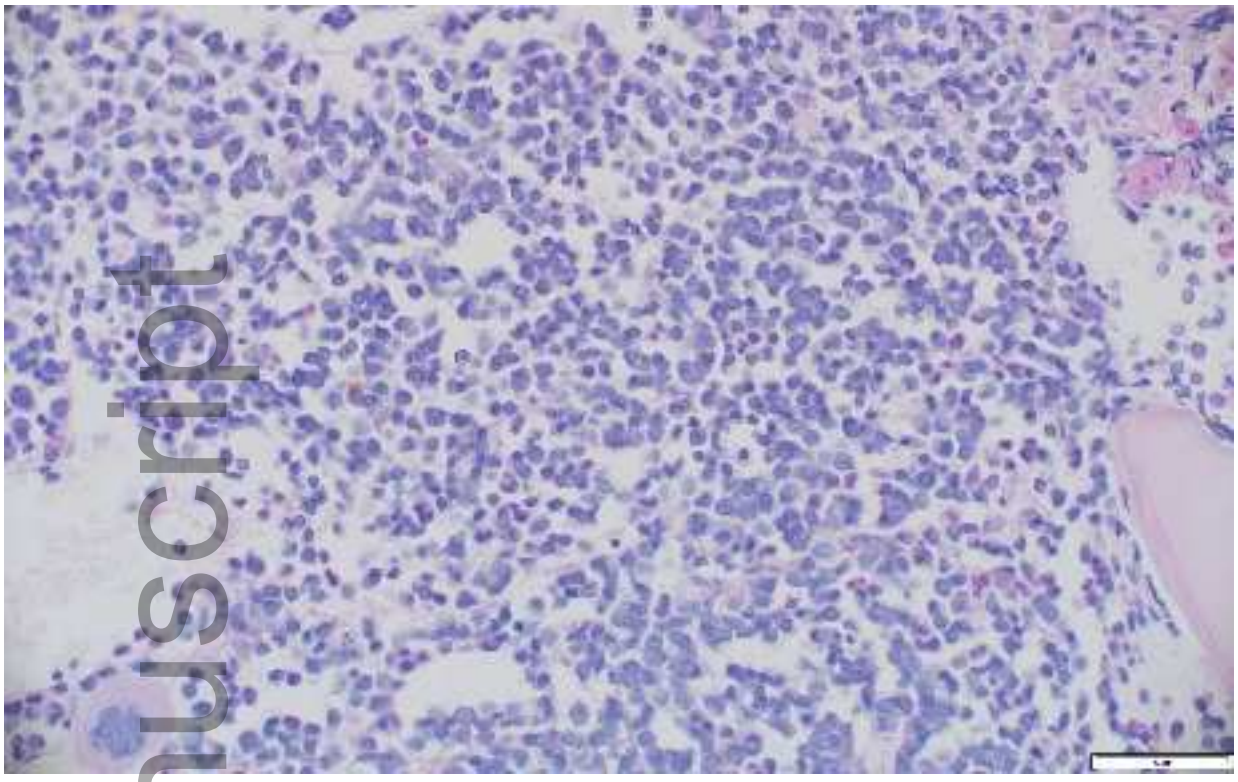

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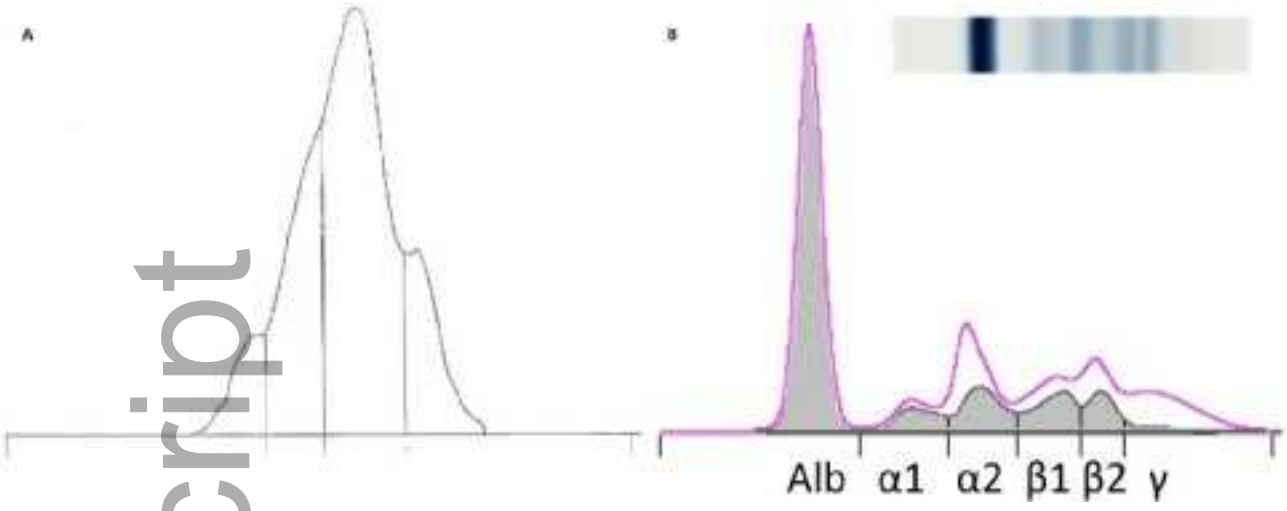

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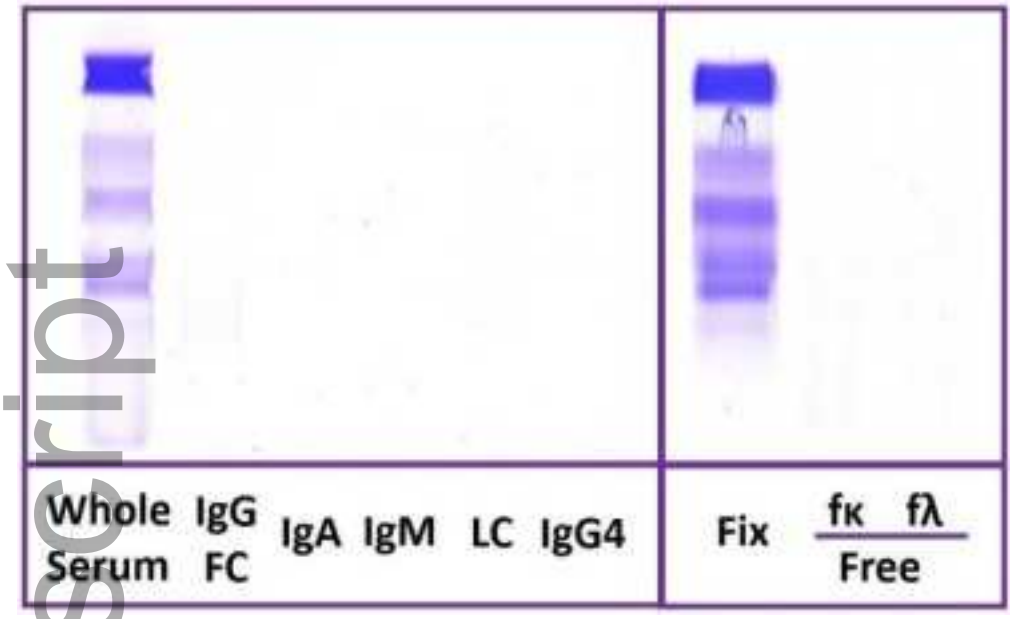

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