TITLE OF CASE

Cyclic neutropaenia in a basset hound

AUTHORS

Liza Köster*,[1,2] Cindy Harper,[3] Amelia Goddard[4]

[1]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng,
South Africa
[2]Department of Small Animal Internal Medicine, Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis
[3]Veterinary Genetics Laboratory, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng, South Africa
[4]Department of Clinical Pathology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng, South Africa
* Correspondence to Dr Liza Köster, lizakoster@gmail.com

SUMMARY

A 6 month old, male basset hound was referred for intermittent malaise, and pyrexia that repeated every 10-11 days. Sequential blood counts and bone marrow aspirates demonstrated severe neutropaenia and increased myeloid precursor cells in the bone marrow from day two of each cycle. By day five the bone marrow had predominantly mature neutrophils and band cells, and by day 10 the myeloid precursors had decreased with a myeloid:erythroid of 0.4:1. During the same period the blood neutrophils had rebounded to normal counts by day five, and were declining by day 9 and 10. The monocytes and platelets were oscillating in an opposite phase to the neutrophils.

The age of onset, regular cycle length, oscillation of neutrophil, platelet and monocyte counts and characteristic bone marrow cytology were diagnostic for cyclic neutropaenia. This syndrome has never before been described in any breed other than the grey collie. The grey collie gene mutation was not found in this dog. However, based on the clinical signs, and haematological and bone marrow results, it can be deduced that cyclic neutropaenia may develop as a result of other mutations in neutrophil elastase expression.

BACKGROUND

Cyclic neutropaenia is an unusual haematologic disease characterised by oscillation in blood neutrophil and monocyte counts in opposite phase to one another with an average of 21 days in humans and 14 days in dogs. Cyclical neutropaenic episodes occur where total neutrophil count is less than 0.2×10^9/L and can last 3 to 6 days during which time pyrexia, lymphadenomegaly and anorexia can be detected (Dale and others 2002, Ancliff and others 2003, Badolato and others 2004). The disease is transferable by bone marrow transplantation, suggesting a disorder of the haemopoietic stem cells (Weiden and others 1974). To date, cyclic neutropaenia in dogs has been reported to be an autosomal recessive disorder, in contrast to humans, where it is autosomal

dominant (Weiden and others, 1974). As is implied in the name, "grey collie syndrome", the disease is associated with hypopigmentation described only in the collie breed. Oscillations in haemopoietic cells occur approximately every two weeks as opposed to every three weeks as in humans and all stem cell lines are affected (Weiden and others 1974, Duan and others 2004). In affected collies, the mutation was found to be located on the *AP3B1* gene (Benson and others 2004). This *AP3B1* mutation is also responsible for Hermansky-Pudlak syndrome type 2 (HPS2), a heterogenous autosomal recessive disorder of mammals featuring albinism and bleeding because of defective platelet granule formation (Horwitz and others 2007).

This case report details the historical and clinical findings in a juvenile basset hound that presented on multiple occasions for complaints of lethargy, anorexia, pyrexia, lymphadenomegaly and rhinitis. In order to document the haemopoietic changes, three serial and paired complete blood counts, bone marrow aspirates and core biopsies were performed during one of the dog's 10 day cycle. This case report is the first documented report of canine cyclic neutropaenia in a breed of dog other than a grey collie

CASE PRESENTATION

A three month old, intact, male, basset hound was referred to the Onderstepoort Veterinary Academic Hospital (OVAH) for intermittent pyrexia, lethargy, lymphadenomegaly and bilateral, purulent nasal discharge. The referring veterinarian first saw the dog at two months of age and diagnosed pyoderma. The dog had visited the referring veterinarian for various inflammatory conditions approximately every 10-11 days thereafter, until the final diagnosis was made at the age of 6 months of age. The veterinarian had documented pyrexia (> 39.7°C) and mandibular lymphadenomegaly on each occasion.

INVESTIGATION

Prior to referral a complete blood count (CBC) performed by a human laboratory showed a mild hypochromic anaemia with a red cell count (RCC) of 5.0×10^{12}/L (5.5–8.5), HCT of 0.32 L/L (37-55), MCV of 63.7 fL (60-77) and MCHC of 30.8 g/dL (32-36). The results also showed a mature neutrophilia (21.5×10^9/L; 3–11.5) and monocytosis (6.5×10^9/L; 0.1 – 1.4).

On clinical examination, mandibular and prescapular lymphadenomegaly was palpable, and a malodorous purulent bilateral nasal discharge was found with moist crackles on lung auscultation. A repeat CBC (ADVIA 2120 automated haematology analyzer, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) performed at the OVAH showed the following results: RCC (5.4×10^{12}/L; 5.5-8.5), HCT (0.34 L/L; 0.37-0.55), MCV (63.1 fL; 60-77), MCHC (32.8 g/dL; 32-36), mature neutrophil count (0.8×10^9/L; 3.0-11.5), band neutrophil count (0.8×10^9/L; <0.5), lymphocyte count (11.3×10^9/L; 1.0-4.8), monocyte count (4.6×10^9/L; 0.15-1.35), eosinophil count (zero; 0.10-1.25),

and platelet count (601×10^9/L; 200-500). No abnormalities were detected by routine biochemistry and urinalysis collected by free flow. Cytology of the enlarged lymph nodes was consistent with reactive hyperplasia.

Thoracic radiographs revealed a mild interstitial pattern in the dorsocaudal lung field. A broncho-alveolar lavage (BAL) and nasal biopsies were performed; no macroscopic abnormalities were visible during bronchoscopy. The BAL cytology consisted predominantly of mononuclear cells, which included active macrophages (± 90%), some lymphocytes and the odd neutrophil. No micro-organisms were identified. Culture of the fluid was negative for any growth after 72 hours of incubation. Nasal brush cytology revealed non-degenerate neutrophils, without a prominent inflammatory reaction. Histopathologic examination of the nasal biopsy revealed lymphoplasmocytic infiltrates into the submucosa together with a moderate infiltrate of neutrophils, in some instances forming micro-abscesses within the respiratory epithelium. Resin processing electron microscopy of the nasal biopsy detected swollen cilia, compound cilia, blebs of axoneme membrane, and addition and deletion of peripheral doublets with many cells without cilia or internalised cilia. However, most cilia had a normal 9 + 2 microtubular configuration. The findings were consistent with secondary ciliary dyskinesia, the primary cause most likely a bacterial infection. Culture of a macerated nasal mucosal sample yielded scant growth of *Enterococcus spp.*, which was resistant to most antimicrobials except for floroquinolones, potentiated penicillins and sulfonamides. The dog was discharged with a 4-week course of amoxicillin-clavulonate (Synulox, Pfizer Laboratories [Pty] Ltd, Sandton, South Africa), 13 mg/kg twice daily.

One month later, the referring veterinarian again examined the dog for an owner complaint of lameness. Pyrexia (40°C) was detected and pain was elicited by manipulating the stifle, hip and elbow joints. A mild non-septic polyarthritis was diagnosed based on cytological findings. A tapering course of immunosuppressive doses of corticosteroids (Centaur-prednisolone, Centaur Labs, Bayer [Pty] Ltd, Animal Health Division, Isando, South Africa) was started (1 mg/kg, twice daily, orally) for two weeks and then tapered every other week thereafter. Once the dose was dropped to below anti-inflammatory doses two months later, the dog again became lethargic, anorexic and developed a generalised lymphadenomegaly, and was referred to the OVAH for the second time. The CBC conducted at the time of referral (two days after the start of the pyrexia) had the following abnormalities: mature neutrophil count (10.1×10^9/L; 3.0-11.5), band neutrophil count (6.3×10^9/L; <0.5), monocyte count (3.5×10^9/L; 0.15-1.35), and an eosinophil count of zero. A lymph node biopsy (mandibular) was performed under general anaesthesia. Culture of the lymph node after 72 hours revealed no growth of microorganisms. Histopathological examination revealed marked lymphoid hyperplasia, active germinal centres, and expansion of the medullary cords that were infiltrated with macrophages and plasma cells. The subcapsular and medullary sinuses were

dilated by red blood cells, degenerate neutrophils, and an increased number of macrophages. Ziehl-Nielson stain for mycobacteria was negative. A course of amoxicillin-clavulonate at 13 mg/kg orally twice daily was started and dispensed for additional 3 weeks.

Seven days later the owners noted the dog was hot to the touch, anorexic and depressed again and the dog was brought to the OVAH for a third visit. At this time the CBC revealed a severe neutropaenia (mature neutrophils of zero and band neutrophils 0.1×10^9/L), a monocytosis $(2.8 \times 10^9$/L), and an eosinophil count of zero. A bone marrow aspiration and deep core biopsy, using a 3.5 inch, 9-gauge, Jamshidi bone marrow biopsy-aspiration needle (Biopsy Needle, GTA®,
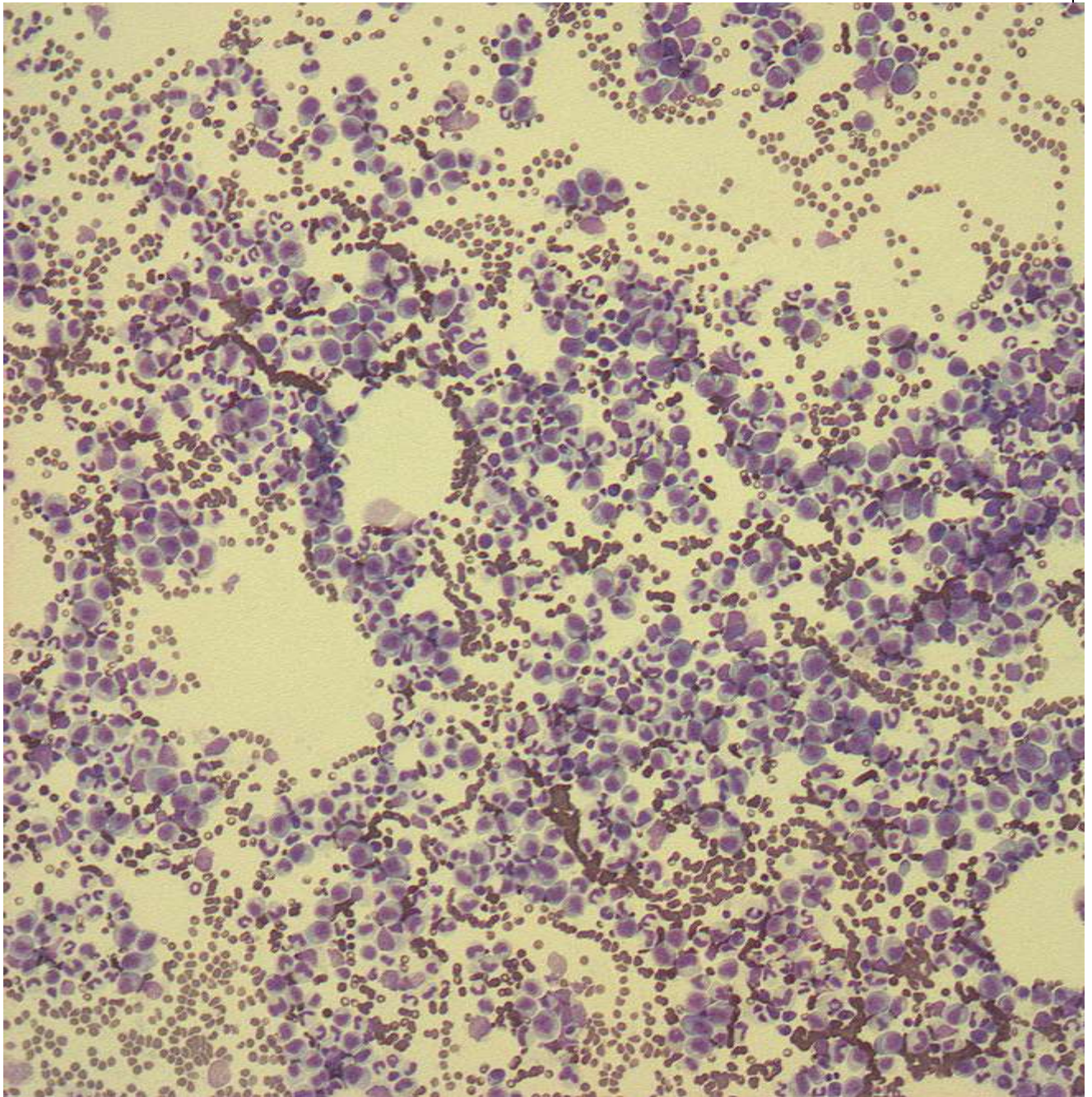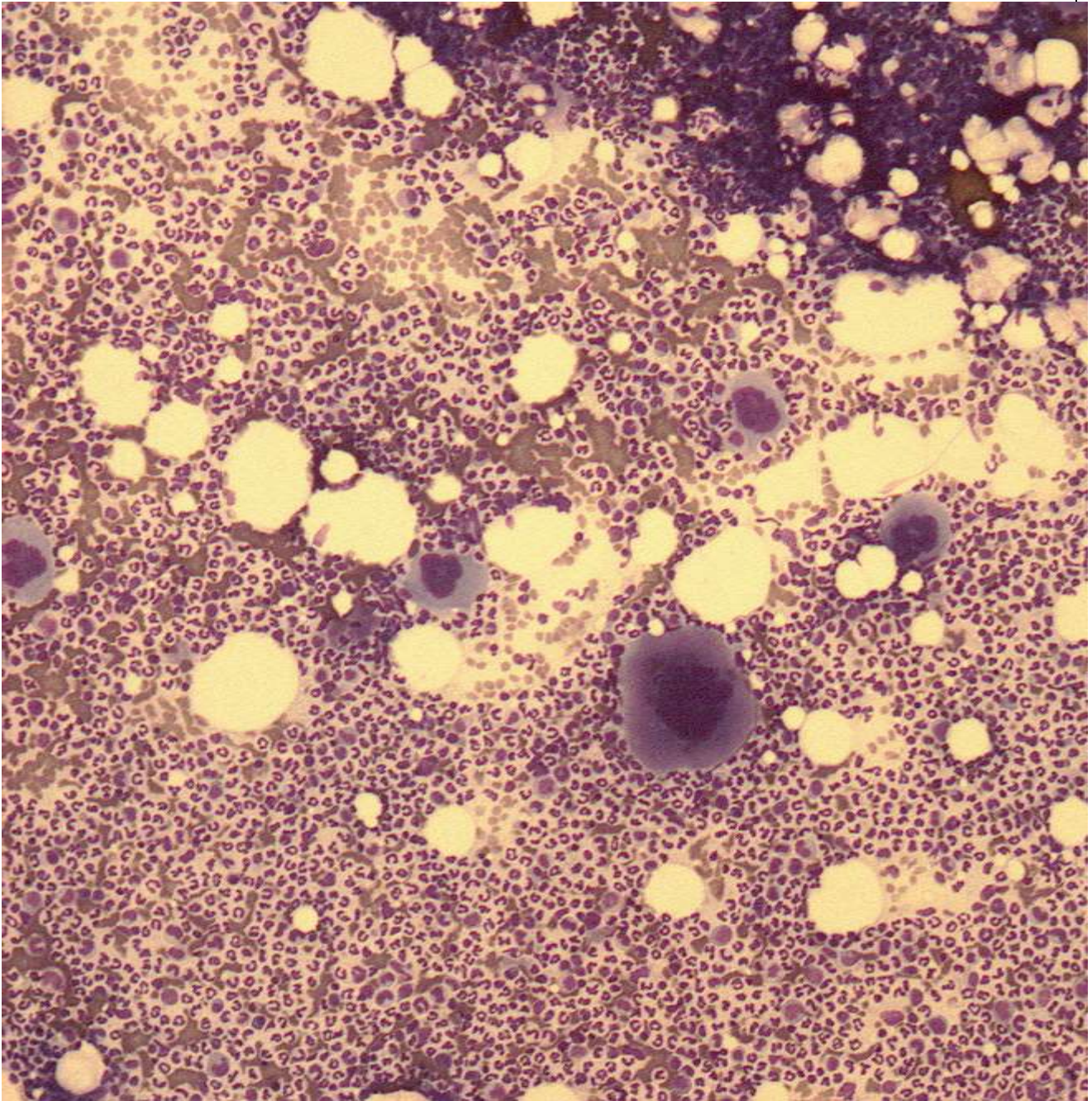

Figure 1A: Cytology of the bone marrow aspirate (10× objective), 24-hours after the neutropenia was detected on CBC, stained with Romanowsky stain (Rapidiff stain, Clinical Sciences Diagnostics CC, South Africa). A high percentage of myeloid precursors were seen (90%), and only a few erythroid precursors were noticed (10%).
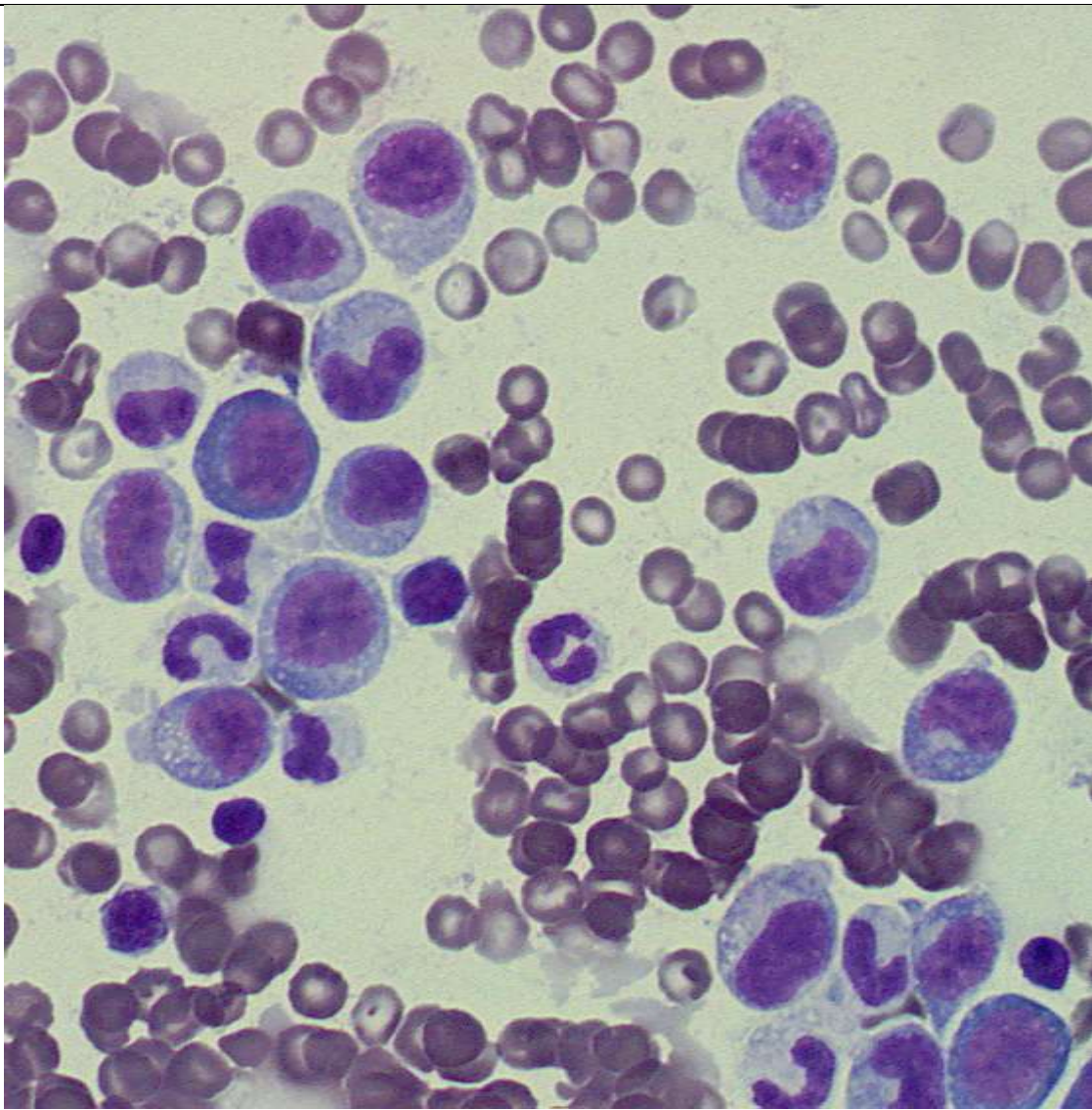
Figure 1B: The myeloid precursor cells seen under high power magnification (100× objective). The myeloid precursors (thick black arrows) are large, round cells with basophilic cytoplasm, fine to finely stippled chromatin and prominent and multiple nucleoli. They are difficult to differentiate from early erythroid precursors (thin black arrow).

International Medical Devices, La Caleta, Dominican Republic) was collected from the head of the humerus 24 hours after detecting the neutropaenia. A CBC was performed at the same time and a rebound in the neutrophils (mature neutrophil count 1.4×10^9/L; band neutrophil count 5.5×10^9/L) and marked monocytosis (5.26×10^9/L) was evident. The platelet count (299×10^9/L) had decreased substantially from the previous day (389×10^9/L). Bone marrow cytology showed highly cellular material with both mature and immature megakaryocytes. There were many myeloid precursor cells present (71%), few erythroid precursors (9%), and undifferentiated cells (20%). These undifferentiated cells were immature, round cells with granular basophilic cytoplasm, coarse chromatin, prominent and multiple nucleoli, and were believed to be very early myeloid precursor cells (Fig 1). Histopathology changes included a proportional increase of promyelocytes with lesser numbers of erythroid precursors. The leucocyte precursor cells were characterised by large round cells with basophilic cytoplasm and bean-shaped to segmented nuclei. Due to the cyclical nature of

the neutropaenia and pyrexia and the rebound of the cell lines (neutrophils, monocytes and platelets), cyclic neutropaenia was suspected, although in an atypical breed.
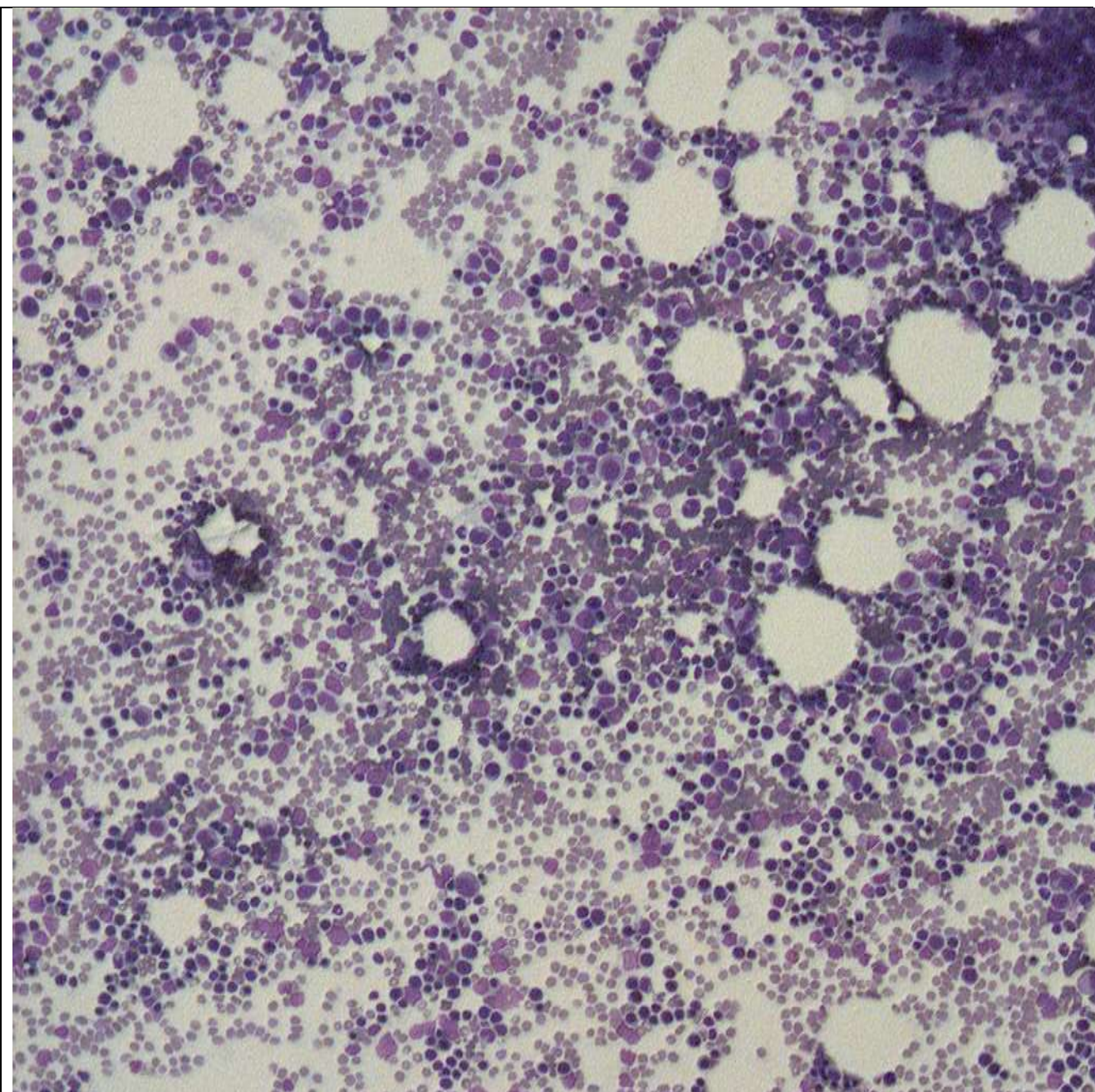

Figure 2A: Low power magnification (10× objective) of the bone marrow on day 5 after the neutropenia, stained with Romanowsky stain (Rapidiff stain, Clinical Sciences Diagnostics CC, South Africa). The myeloid precursors had decreased, and high numbers of mature neutrophils and band neutrophils, as well as mature and immature megakaryocytes were seen.
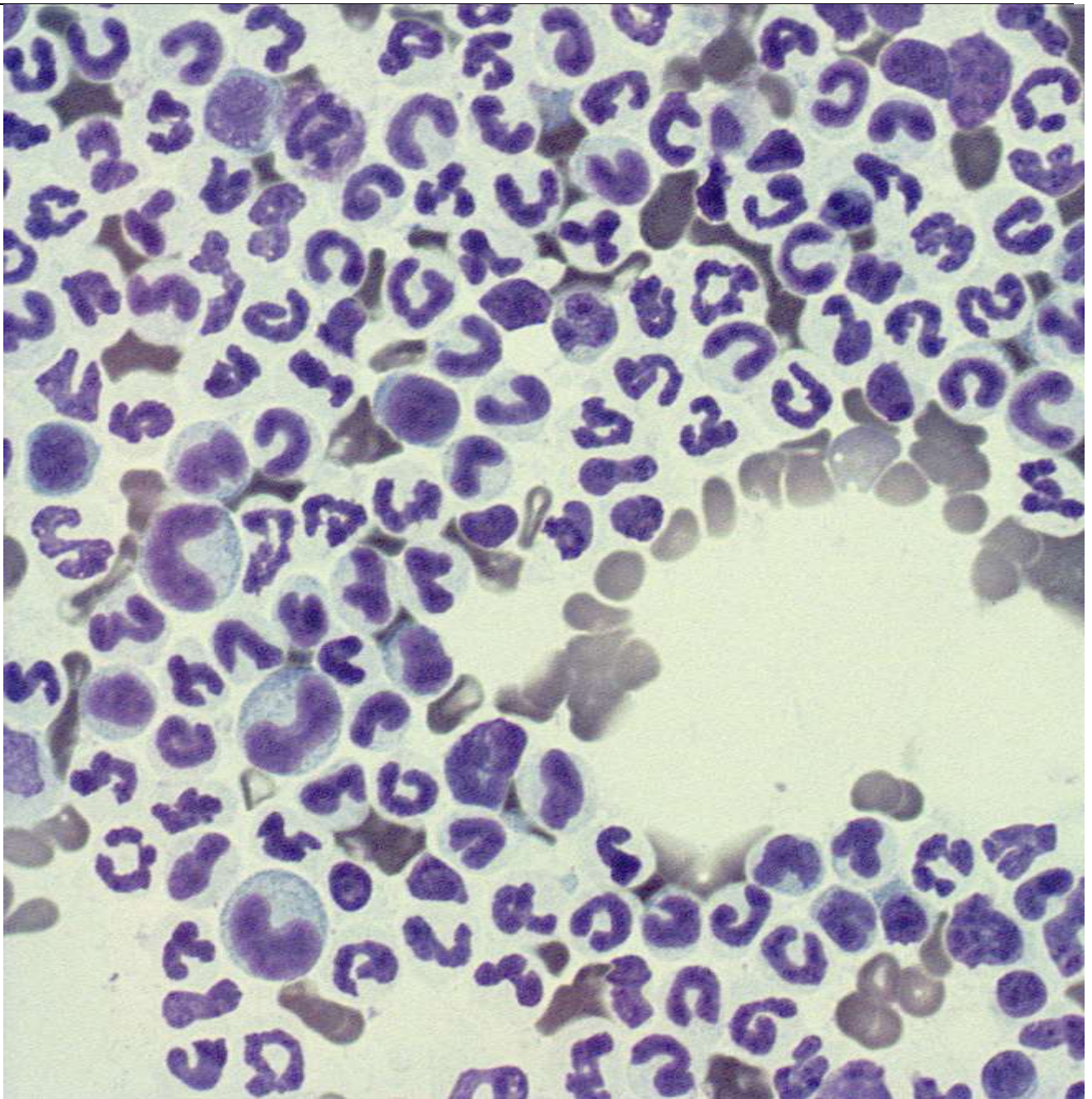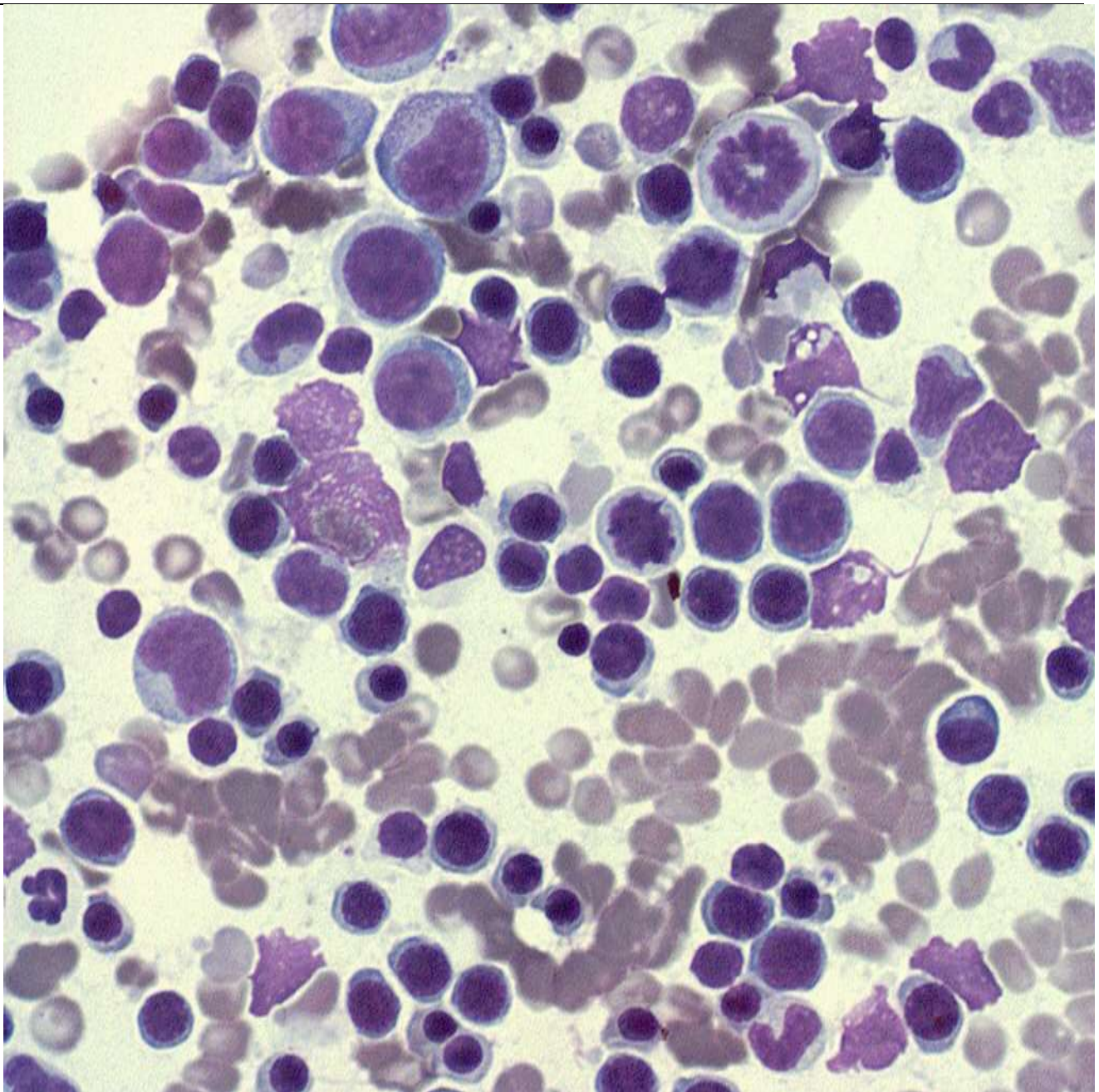
Figure 2B: High power maginification (50× objective) of the bone marrow aspirate on day 5 after the neutropenia. The myeloid precursors had decreased to 14%, and erythroid precursors to 1%, but mature neutrophils (42%) and immature neutrophils (39%) had increased. All the different stages of the RBC and WBC precursors were present.

Two subsequent bone marrow aspirates and concurrent haematology analysis were conducted over the following 10 days to better describe the cyclical nature of this disease. The day of presentation was considered the nadir point of the cycle. A second sample was scheduled 4 days later and the third at the anticipated decline, 10 days later. The CBC on day four (post neutropaenia) demonstrated a mature neutrophilia (8.1×10^9/L), increased band neutrophils (0.7×10^9/L) and normal monocyte count (0.3×10^9/L). The platelet count was slightly lower in number since the day of neutropaenia (268×10^9/L). Significant changes had also occurred in the bone marrow by day four. The myeloid precursors had decreased to 14%, and erythroid precursors to 1%, but mature

Figure 3A: Low power magnification (10× objective) of the bone marrow on day 10 after the neutropenia, stained with Romanowsky stain (Rapidiff stain, Clinical Sciences Diagnostics CC, South Africa). The image shows a reduced number in myeloid precursors with an M:E ratio of 0.4:1.

Figure 3B: High power magnification (50× objective) of the bone marrow aspirate on day 10 after the neutropenia. The image shows a reduced number in myeloid precursors with a myeloid:erythroid ratio (M:E) of 0.4:1. Most of the WBC precursors were in the promyelocyte and myelocyte stage of development.

neutrophils (42%) and band neutrophils (39%) had increased. The previously seen undifferentiated myeloid precursors had decreased. All the different stages of the RBC and WBC precursors were present (Fig 2). On day nine (post neutropaenia), the total neutrophil count (mature neutrophils 5.8×10^9/L, band neutrophils zero) was still within normal limits although decreasing, and the monocyte- (1.6×10^9/L) and platelet count (531×10^9/L) were increasing in number. The decision was to post-pone the bone marrow aspirate one more day. By day 10, the mature neutrophil count (2.3×10^9/L) had decreased substantially and the monocyte- (2.6×10^9/L) and platelet count (585×10^9/L) had continued to increase. The bone marrow aspirate collected at the same time showed a myeloid:erythroid (M:E) of 0.4:1 (normal 1–2.5:1). Most of the WBCs were in the

promyelocyte and myelocyte stages of development. Many megakaryocytes (mature and immature) were present (Fig 3). A diagnosis of cyclic neutropaenia was made based on the dynamics of the serial bone marrow aspirates and haematology.

DIFFERENTIAL DIAGNOSIS

On all occasions, except for the initial referral consultation, the dog had severe neutropaenia documented, which resolved completely after a few days of antibiotic treatment. This most likely correlated to the natural rebound of neutrophil numbers after the maturation arrest of the neutrophils resolved rather than treatment success. The severe neutropaenia and regularity of cyclical events made cyclic neutropaenia most likely.

The differential diagnoses for neutropaenia include: cyclic neutropenia, severe congenital neutropaenia, haematologic malignancy, drug-induced agranulocytosis, viral infections, ehrlichiosis and juvenile leukaemia. The greatest difficulty is differentiating cyclic neutropaenia from severe congenital neutropaenia or Kostmann syndrome. In both diseases there is neutropaenia with elevated peripheral monocyte counts. Cyclic neutropaenia may eventually become a chronic non-cyclic form. Clinical signs overlap, but in the case of cyclic neutropaenia, the pyrexia precedes the neutropaenia in contrast to severe congenital neutropaenia. In this case, pyrexia and listlessness preceded neutropaenia by a few days. In humans, cyclic neutropaenia is classified into 5 major groups based on the age of onset, association with malignancy or other cyclic phenomenon. The case in this report would fall into the human class IA i.e. childhood onset without family history.

TREATMENT

Due to lack of commercially available canine granulocyte-colony stimulating factor (G-CSF), the recommended therapy, the medical management comprised of prednisolone (Centaur-prednisolone, Centaur Labs, Bayer [Pty] Ltd, Animal Health Division, Isando, South Africa) at 0.5 mg/kg, once daily during periods of pyrexia (day 1-2), with doses tapering to 0.25 mg/kg daily for the next 4 days and then every other day until the next cycle (approximately 10-11 days later). Antibiotics were recommended only if purulent infections could be demonstrated. Rigorous dental hygiene was recommended. It was recommended that the dog not be used for breeding and castration was advised to reduce the risks of prostatitis associated with benign prostatic hyperplasia later in life.

OUTCOME AND FOLLOW-UP

At follow-up consultation at one-year of age, the owner reported more subtle cyclical clinical signs (every 10 days) but not severe enough to affect the dog's appetite or cause a pyrexia.

This dog was without colour coat dilutions and thus grey collie syndrome was considered unlikely. DNA was extracted from a whole blood sample, using a Phenol-Chloroform-Isoamylalcohol method (Budowle and others, 2000) for the extraction of DNA from blood routinely used in the Veterinary Genetics Laboratory at the Faculty of Veterinary Science, Onderstepoort. An aliquot of

extracted DNA was sent to the Healthgene Laboratory in Toronto, Canada for testing (Cyclic Neutropenia or Grey Collie Syndrome, test code 136). Test results were negative for the mutation. It is unknown if the siblings, parents or related dogs were similarly affected and as a result the authors cannot comment on a possible mode of inheritance of this defect in the basset hound.

DISCUSSION

DNA testing confirmed that this dog did not have the specific mutation for the *AP3 adapter protein* which is responsible for grey collie syndrome, further supported by the lack of typical phenotype which characterises the syndrome in collies. Apoptosis or programmed cell death is an important regulatory mechanism in normal haemopoiesis and in myelodysplastic syndromes. It has been demonstrated that ineffective production of neutrophils is due to an accelerated apoptosis of bone marrow myeloid progenitor cells in cyclic neutropaenia with normal circulatory lifespan of peripheral blood neutrophils (Aprikyan and others 2001). It has been reported that missense or deletion mutations in the gene encoding for neutrophil elastase (NE) and expression of mutant NE may lead to accelerated apoptosis in myeloid-committed progenitor cells (Aprikyan and Dale 2001). The cyclic neutropaenia disease phenotype in dogs is similar to that of humans, but with a different mutation, the grey collie syndrome being an *AP3* adapter protein mutation and human cyclic neutropaenia being an *ELA2* mutation. Neutrophil elastase trafficking is reliant upon *AP3* and, although *ELA2* genes are normal in grey collies, there is nearly undetectable NE protein due to excessive re-routing of NE to granules (Horwitz and others 2007). The proposed hypotheses for the pathophysiologic mechanism of cyclic neutropaenia in humans include: loss of enzymatic properties of NE; mistrafficking of NE by *AP3,* a cytoplasmic cargo protein; mislocalisation of NE on the membrane causing proteolyses of hemopoeitic regulatory hormones (G-CSF) and receptors (G-CSF, and c-KIT); apoptosis of progenitor cells; promyelocyte arrest and lastly that myeloid progenitors can produce monocytes instead of neutrophils (Benson and others 2003, El Ouriaghli and others 2003, Hunter and others 2003, Carter and others 2004, Massullo and others 2005, Kollner and others 2006).

Diagnosis of cyclic neutropaenia relies on the demonstration of cyclical fluctuations in serial haematologies , regular episodes of neutropaenia ($<0.2\times10^9$/L) together with cyclical fluctuations of monocytes and reticulocytes, and many patients are chronically anaemic, particularly if there are associated severe infections (Dale and others 2002). Bone marrow examinations demonstrate striking changes within each cycle. This is characterised by a period of neutropaenia, which is followed by a marked increase in the number of neutrophil precursors (promyelocytes and myelocytes). This was evident in the bone marrow aspirate of this case collected 24 hours after the absolute neutrophil count of zero, consistent with maturation arrest. By the time the rebound in peripheral neutrophils occurred, the bone marrow had an abundance of late neutrophil precursor

cells. This was clearly seen in the bone marrow aspirate collected 4 days after the neutrophil nadir. By approximately 8-10 days after the neutrophilia, there is a paucity of neutrophil precursors, inverting the myeloid to erythroid ratio. The third bone marrow aspirate of this case showed that the erythroid precursors outnumbered the myeloid precursors substantially. The erythroid and platelet precursors also cycle and hence the term "periodic" or "cyclic haematopoiesis" has also been used (Dale and others 2002). In this case oscillations in the peripheral neutrophil, monocyte and platelet counts were obvious.

In humans, long-term care is necessary to reduce the impact that episodes of neutropaenia can have on quality of life. Clinical signs become less severe as the person gets older, with mild clinical signs experienced in the late teens (Dale and others 2002). This dog was described as having good quality of life once he reached maturity, with less intense clinical episodes. Antibiotics are not required for every neutropaenic episode, as antibiotic use can be associated with the development of super-infections, but antibiotics are often prescribed when otitis, stomatitis and abdominal pain are detected (Horwitz and others 2004, Yanay and others 2006). This dog required antibiotics for the treatment of the purulent rhinitis. On all occasions, the dog had severe neutropaenia when treated at the OVAH, which resolved completely after a few days of antibiotic treatment. This most likely correlated to the natural rebound of neutrophils after the maturation arrest of the neutrophils resolved rather than treatment success.

A number of therapeutics have been proposed to ameliorate the clinical signs associated with cyclic neutropaenia. These include lithium carbonate, prednisolone, androgenic steroids, plasmapharesis, interleukin-3 (IL-3), granulocyte-macrophage colony stimulating factor (GM-CSF), G-CSF and recombinant DNA technology (Hammond and others 1990, Colijn and others 2007, Horwitz and others 2004, Wright and others 1978, Yanay and others 2006). It has however been demonstrated that lithium is ineffective in treating cyclic neutropaenia (Hammond and others 1983). Humans are managed with prednisolone therapy at anti-inflammatory doses (0.5 mg/kg) every other day (Horwitz and others 2004, Wright and others 1978). One study reported that the use of recombinant interleukin 3 (rhIL-3) in grey collies was shown to have no effect on recurrent neutropaenia, but was associated with eosinophilia. Granulocyte-CSF has been used in humans and dogs at a dose of 3-5 μg/kg subcutaneously daily to combat the effects of apoptosis on myeloid-progenitor cells and has reduced the number and severity of infectious episodes, and prolonging survival by reducing the period that the person or dog is susceptible to life-threatening infections (Colijn and others 2007, Yanay and others 2006). Recombinant human GM-CSF caused neutrophilia and eosinophilia in dogs with cyclic neutropenia, but cycling haematopoiesis persisted, whereas recombinant human G-CSF caused neutrophilia and obliterated cyclic neutropaenia and periodic fluctuations of

monocytes, eosinophils, reticulocytes and platelet counts (Hammond and others 1990). In the same study it was shown that dogs developed antibodies to the G-CSF and GM-CSF, and the response to factors was decreased by 2 to 3 weeks, most likely due to the antibodies reducing the *in vivo* effect of the exogenous factors. In contrast, canine recombinant G-CSF had a sustained effect on bone marrow over months. Canine recombinant G-CSF was not commercially available at the time of diagnosis and the use of human recombinant G-CSF was not an option long-term due to the predictable development of antibodies and treatment failure after a couple of weeks. The only therapeutic option available for the dog was prednisolone, which resulted in significant amelioration of clinical signs according to owner observation.

This case report highlights a rare haemopoietic condition that is familial in the collie breed, and, is the first reported case, to the authors' knowledge, describing cyclic neutropaenia in the basset hound. The mutation in this dog could be in the *AP3* adapter protein but is not the same mutation that causes grey collie syndrome. The mutation is most likely in a gene responsible for the regulation of NE. Further studies to demonstrate the familial occurrence of this condition in the basset hound and genetic studies to isolate the mutation in this breed are required. This dog was successfully managed with prednisolone, which is described as a treatment option in the human form of cyclic neutropaenia. This case report highlights the importance of considering cyclic neutropaenia as a differential diagnosis in immature animals of breeds other than grey collies.

LEARNING POINTS/TAKE HOME MESSAGES

- This case report highlights a rare haemopoietic condition that is familial in the collie breed, and is the first reported case, to the authors' knowledge, describing cyclic neutropaenia in the basset hound.
- The mutation in this dog could be in the *AP3* adapter protein but is not the same mutation that causes grey collie syndrome.
- Further studies to demonstrate the familial occurrence of this condition in the basset hound and genetic studies to isolate the mutation in this breed are required.
- This dog was successfully managed with prednisolone, which is described as a treatment option in the human form of cyclic neutropaenia.
- This case report highlights the importance of considering cyclic neutropaenia as a differential diagnosis in immature animals of breeds other than grey collies.

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