MYELOSUPPRESSIVE CANINE MONOCYTIC EHRLICHIOSIS *(EHRLICHIA CANIS):* AN UPDATE ON THE PATHOGENESIS, DIAGNOSIS AND MANAGEMENT

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ABSTRACT

Chronic severe canine monocytic ehrlichiosis (CME) due to *Ehrlichia canis* may appear from a few months and up to several years after the apparent recovery from the acute phase of the disease. It is typically characterized by severe bone marrow aplasia (myelosuppression), peripheral blood pancytopenia and high mortality due to septicemia and/or severe bleeding tendency. This review updates on the pathogenesis of the myelosuppressive CME, provides guidelines for the diagnosis and differentiation from other common canine pancytopenic diseases, and emphasizes the clinical management of this devastating disease.

Key words: Dog, bone marrow, *Ehrlichia canis*, myelosuppression, pancytopenia

INTRODUCTION

Canine monocytic ehrlichiosis (CME) is caused by tick-transmitted, gram-negative, obligate intracellular microorganisms of the genus *Ehrlichia* (family *Anaplasmataceae*). The species *Ehrlichia canis* is acknowledged as the primary cause of CME worldwide (1). Following an incubation period of 8-20 days, the clinical course of experimental CME can be sequentially divided into acute, subclinical and chronic phases, but the distinction between these phases is not straightforward in the naturally-occurring disease. Most untreated dogs recover spontaneously from the acute phase after 2-4 weeks, entering the subclinical phase that may last several months to years (2). Immunocompetent dogs may eliminate the infection during this period, but some will eventually develop the chronic phase of the disease, characterized by severe bone marrow (BM) aplasia (myelosuppression), peripheral blood pancytopenia and high mortality due to septicemia and/or severe bleeding (3). It is interesting to note that occasionally, myelosuppression may develop soon after the recovery from the acute phase of the disease, or without any prior signs of acute infection (3). Therefore the terms "non-myelosuppressive" and "myelosuppressive" CME, may better reflect the clinical severity of the disease, irrespective of its time progression (4). Since in endemic areas like Greece, CME is the major cause of persistent and life-threatening pancytopenia in the dog (5), this review emphasizes the current knowledge on the pathogenesis, diagnosis and therapeutic management of the myelosuppressive CME.

PATHOGENESIS

Unlike the acute form of CME, substantially less is known about the pathogenesis of the chronic severe CME, primarily due to the lack of a suitable experimental model for the induction of the chronic severe disease (6). Therefore, the potential role of various conditions in precipitating the myelosuppressive CME is currently being investigated in studies including more often naturally-infected dogs. Several clinical reports indicate that German shepherds are more susceptible to *E. canis* infection, presenting increased morbidity and mortality (3, 7), and a breed-specific defective cell-mediated immunity has been postulated based on experimental evidence (8). Simultaneous or sequential infections with other vector-borne pathogens (e.g. *Leishmania infantum*, *Babesia* spp., *Rickettsia* spp., *Bartonella* spp., *Hepatozoon canis*, *Anaplasma* spp., or other *Ehrlichia* species) have also been proposed as determinants of the severity of the disease. Although coinfections may affect the clinical and clinicopathological picture of the disease (9), there is currently lack of firm evidence that a certain coinfection pattern may be associated with myelosuppression or death (3, 10, 11). Virulence variation of the *E. canis* strains may also affect the severity of the disease. The results of a recent study indicated that *E. canis* strains involved in the non-myelosuppresive and myelosuppressive forms of CME shared an identical 16S rRNA genotype (4). Since the 16S rRNA gene has a high identity level in *E. canis*, additional sequence information from other genes (e.g. gp36 gene) would be interesting in order to more meaningfully determine if they potentially affect strain virulence and clinical outcome. Myelofibrosis has also been suspected as a contributing factor to aplastic pancytopenia

(12), but no substantial BM fibrosis could be found in dogs with *E. canis*-induced myelosuppression, suggesting that myelofibrosis is probably not an important contributor to *E. canis*-associated BM failure (13). Importantly, recent evidence in canine experimental studies, suggests that the clinical severity in CME may be related to the mode of patient's immunologic response (i.e. Th1 versus Th2) (14) and the cytokine profile induced post-inoculation; high levels of IFN-γ have been associated with mild disease, while persistently elevated IL-1β and IL-8 have been found in the severely-affected dogs (15, 16). While cellular immunity is pivotal for the protection against *E. canis*, the exuberant humoral response appears to confer no protection, and in fact, may be detrimental to the host (2). Interestingly, BM plasmacytosis is significantly more common in the myelosuppressive compared to the non-myelosuppressive CME (17). The possibility that destruction of BM stem cells/progenitor cells has an immune-mediated pathogenesis in CME may warrant further investigation (18), as it might have significant therapeutic and prognostic implications (e.g. inclusion of steroids in the treatment plan of pancytopenic dogs). Finally, persistent reinfection and *E. canis* inoculum size may increase the chances for severe disease, emphasizing the importance of implementing a comprehensive acaricidal preventive strategy (6, 19).

DIAGNOSTIC APPROACH OF THE MYELOSUPPRESSIVE CME

Diagnosis of myelosuppressive CME is based on the historical (living in or traveling to endemic areas) and clinical compatibility, consistent clinicopathological abnormalities, *Ehrlichia*-specific testing and exclusion of other common causes of canine pancytopenia (e.g. drug-induced BM aplasia, myelophthisic disorders) (20).

Clinical findings

Depression, anorexia, mucosal pallor, bleeding tendency, fever or hypothermia, lymphadenomegaly, splenomegaly and ocular abnormalities are prominent clinical manifestations in the spontaneous myelosuppressive CME (Table 1). Overall, the spectrum of clinical manifestations of the myelosuppressive overlaps substantially with that of the non-myelosuppressive CME or other disorders causing pancytopenia (20); however, the frequency and severity of certain manifestations differ between these two forms. For instance, bleeding diathesis is more common and severe in the chronic phase of CME (17). It is mainly expressed as superficial bleeding such as cutaneous and mucosal petechiae and ecchymoses, hyphaema, epistaxis, haematuria, melena and prolonged bleeding from venipuncture sites due to the impairment of primary haemostasis (3) (Figures 1-3). Haematomas (e.g. subcutaneous, sublingual) may uncommonly be seen in the chronic CME, presumably due to secondary haemostasis abnormalities (3, 21). Finally, ulcerative stomatitis and necrotic glossitis, hind limb and/or scrotal edema, icterus and central nervous system signs have been occasionally reported in the chronic CME.

Haematological and biochemical findings

Pancytopenia in the context of an aplastic BM is the hallmark of myelosuppressive CME, although mono- or bi-cytopenias may be seen in the transition period preceding terminal BM suppression. Importantly, pancytopenia with normocellular BM is occasionally seen in the acute CME, and unlike aplastic pancytopenia, is well responsive to treatment. Due to the shorter lifespan of canine neutrophils (4-8 hours) and thrombocytes (5-7 days) compared to erythrocytes (120 days), severe anaemia is usually the last abnormality witnessed in the progression to aplastic pancytopenia (22). Although lymphopenia is more common in chronic CME, granular lymphocytosis may also appear, usually prior to BM aplasia (23). It is most probably associated with a homogeneous or clonal expansion of CD8+ T-lymphocytes, a unique feature of CME among the canine infectious diseases (24). Well-differentiated lymphocytes do not usually exceed 20,000-30,000/μl, and they return to normal range following anti-ehrlichial chemotherapy (23). Thus, in endemic areas, CME should be a top differential for mild-to-moderate persistent lymphocytosis, before considering a diagnosis of a neoplastic lymphoproliferative disorder (i.e. chronic lymphocytic leukaemia or small cell lymphoma) (24). In the latter cases, peripheral blood lymphocytosis is non-responsive to anti-ehrlichial medication while the BM is typically packed with mature-appearing lymphocytes frequently exceeding 30% of all nucleated cells (25).

Bone marrow aspiration cytology (review of at least 4 smears) and/or core biopsy is an integral part of the diagnostic approach in myelosuppressive CME (13). In both instances, a marked reduction of haematopoietic tissue is noticed, occupying less than 25% of the marrow flecks and usually consists of adipocytes, stromal and plasma cells (Figures 4a, b and c) (22). Occasionally, mild-to-moderate mature mast cell and/or plasma cell hyperplasia may be seen and should not be confused with systemic mastocytosis or multiple myeloma, respectively (17). In the latter, mast cells and plasma cells tend to form aggregates and frequently present cytomorphological atypia (26, 27). Bone marrow cytology and/or histology is also valuable for ruling out other causes of pancytopenia that mimic aplastic pancytopenia, including myelonecrosis, myelofibrosis and myelodysplastic syndromes (22, 25, 28).

Hyperglobulinaemia, hypoalbuminaemia and mildly elevated alkaline phosphatase and alaninoaminotransferase activities are common biochemical abnormalities in chronic severe *E. canis* infection (Table 1). Hypeglobulinaemia does not correlate with anti-*E. canis* titers, and on serum electrophoresis appears to be polyclonal, or rarely, oligoclonal or monoclonal in nature. Pancytopenic dogs tend to have lower γ-globulin concentrations compared to their non-pancytopenic counterparts (29).

Ehrlichia-specific testing

Unlike the acute CME, cytological demonstration of ehrlichial morulae is a notoriously insensitive method (lower than 10%) in the myelosuppressive CME and should not be

relied upon to establish the diagnosis of the disease (3, 30). However, cytology of various tissues (i.e. blood, lymph node, BM) may document coinfections (e.g. *Babesia* spp., *H. canis*, *L. infantum*) which may have therapeutic and prognostic implications (31).

Serology is the most frequently used diagnostic modality for the confirmation of *E. canis* infection in the dog. Indirect fluorescent antibody (IFA) testing is considered the "gold standard" for the detection and titration of antibodies, although enzyme-linked immunosorbent assays (ELISA) are also commonly used (32). For most laboratories, an IgG IFA titer equal to or greater than 1:80 is considered indicative of prior exposure to *E. canis*. Antibody titers do not reliably correlate with the duration of infection, the current carrier status, or the presence and severity of clinical disease (2). Because of the prolonged latent period and the persistent seropositivity following therapy or self-eradication of the infection, clinicians should be well aware that seroreactivity to *E. canis*, especially in an endemic area, does not unequivocally confirm that the clinical manifestations and the aplastic pancytopenia are due to *E. canis* infection. For example, bleeding tendency and profound aplastic pancytopenia in a dog seropositive to *E. canis* may be due to exogenous or endogenous (testicular or ovarian tumors) estrogen toxicity (33). On the other hand, *E. canis*-infected moribund animals may rarely demonstrate very low or undetectable antibody titers (34). Several in-house ELISA tests are commercially available for *E. canis* antibody testing. In general, these screening tests have been calibrated to be positive at an antibody level corresponding to an IFA titer of approximately 1:320 or higher; thus, their sensitivity is expected to be satisfactory in the chronically infected dogs which usually have high anti-*E. canis* antibody titers (3).

Polymerase chain reaction (PCR) is a highly sensitive and specific technique for the detection, molecular characterization and quantification (real-time PCR) of the ehrlichial organisms (19, 32). Provided that it is subjected to stringent quality control, PCR may overcome several diagnostic limitations pertaining to serology and cytology. It is also more useful than serology, for the detection of concurrent ehrlichial infections and for the post-treatment monitoring (1, 10, 35, 36). Several PCR assays have been developed targeting an array of genes, such as the 16S rRNA or the p30 genes, to specifically detect *E. canis* (32, 35-37). Successful amplification of ehrlichial DNA may be accomplished from several tissues, including whole blood, BM, spleen, liver, kidney, lung and lymph nodes (30, 38, 39). Unlike the non-myelosuppressive CME, the diagnostic sensitivity of PCR appears to decline considerably in the myelosuppressive CME (especially when blood is examined), as concluded in a recent clinical study in which PCR was applied in BM specimens (3). Optimization of the PCR techniques and/or selection of more suitable tissues for PCR testing may improve the overall diagnostic performance in the myelosuppressive CME (1,3).

Ancillary testing

Suspicion of endogenous estrogen toxicity, may necessitate fine needle aspiration of testicular masses, abdominal ultrasound to confirm the presence of ovarian tumors, serum measurement of estrogen concentration and/or cytological demonstration of vaginal or preputial mucosa keratinization (5,33).

Treatment and prognosis

Doxycycline, a semi-synthetic tetracycline, has been the first-line drug in the treatment of CME. While in the acute CME doxycycline has been shown to be very effective in eliminating the infection (39, 40), its effectiveness in the suclinical and chronic *E. canis* infection is still controversial (35, 41-43). Current dosing recommendation for doxycycline in CME is 5 mg/Kg, orally, twice daily, for at least 28 days (1), although a more prolonged administration may be required for chronically infected dogs (41). Nausea and vomiting seeing occasionally with oral doxycycline can be ameliorated by mixing it with the food. There is currently limited evidence-based justification for using other tetracyclines (minocycline, tetracycline, oxytetracycline), chloramphenicol, enrofloxacin, or imidocarb dipropionate in the treatment of *E. canis* infection. The latter was recently found to be ineffective in eliminating natural and experimental *E. canis* infections (44, 45); thus, it is no longer indicated, unless a coinfection amenable to this drug (e.g. *Babesia canis*) is documented or suspected. Interestingly, in a recent report, rifampin at 15 mg/kg, orally, twice daily, for 7 days, was as effective as doxycycline in eliminating experimental subclinical *E. canis* infection in two dogs (46). Further research is warranted to investigate if rifampin can be an alternative to doxycycline in CME.

In the pancytopenic severely ill dog, supportive treatment is crucial if the limited chances for survival are to be pursued and includes the administration of balanced crystalloid solutions and/or the periodic blood-typed and cross-matched packed red blood cells or whole blood (20 ml/kg) transfusions. Platelet components may also be given, but they are impractical in the clinical setting. A fresh whole blood unit may increase the platelet count of a 20 Kg dog by approximately 20,000-30,000/µl and may be of help to stop the ongoing haemorrhage (47). Iron sulphate supplements (100-300 mg, daily, per os, x 3-5 months, at least 2 hours prior to, or after oral doxycycline), are also indicated, as in a subset of dogs with chronic CME there is iron depletion, presumably due to the chronic haemorrhagic tendency (13).

In dogs with moderate-to-severe (neutrophil count <1,000/µl) and persistent (more than 2 weeks) neutropenia, the prophylactic use of antimicrobials, may reduce the risk for life-threatening bacterial infections that may arise from disrupted physical barriers (i.e. the gastrointestinal tract) or from hospital-acquired organisms (48). In CME-associated pancytopenia, the initial antibiotic choice is influenced by its effectiveness to achieve "selective intestinal decontamination" (i.e. selectively reducing Gram negative and Gram postive aerobic, but leaving relatively unaffected

the anaerobic intestinal flora), its minimal effect on platelet function and the lack of toxicity to an already compromised BM (48). In this respect, sulfonamides, chloramphenicol, and penicillins, are to be avoided in the myelosuppressive CME (22, 48, 49); of comparative interest, in patients with human monocytic ehrlichiosis due to *E. chaffeensis*, an exacerbation of the clinical disease has been associated with the long-term administration of sulfa-containing antibiotics (50). The authors routinely treat the asymptomatic neutropenic dogs, using fluoroquinolones (e.g. enrofloxacin, 10 mg/Kg, orally, once daily) in addition to doxycycline, until the neutrophil count exceeds 1,000/μl and suggest home confinement and periodic temperature measurement. If the dog becomes febrile while being on prophylactic treatment or is febrile on first admission, antibiotic selection should be based on culture and sensitivity testing (e.g. blood and/or urine culture), or a combination of an intravenous fluoroquinolone and a β-lactame (e.g. cefazolin, 30 mg/Kg, every 8 hours, i.v. or i.m.) should be given and the dog is hospitalized. If fever does not abate within 2 days, the addition of metronidazole (15 mg/Kg, every 8 hours, i.v.) is advisable to strengthen the anaerobic spectrum of the antimicrobial regimen.

Haematopoietic growth factors (recombinant human granulocyte-colony stimulating and recombinant human erythropoietin) have been used successfully in a small number of pancytopenic or anaemic CME dogs (51, 52). Although the authors have limited experience with these drugs in CME, the administration of filgrastim (Granulokine, Genesis, Greece), at 5 μg/Kg, s.c., SID, for 5 days in three severely pancytopenic dogs with CME, resulted in the complete resolution of neutropenia (and eventually of pancytopenia) in one dog, while two other dogs failed to respond and succumbed to the disease (Mylonakis, unpublished observations).

Glucocorticosteroids have been advocated in CME to attenuate the immune-mediated component of the disease manifestations. In the authors, opinion, there is currently no evidence-based justification to use glucocorticosteroids in the myelosuppressive form of CME, since an immune-mediated destruction of marrow haematopoietic cells has yet to be documented, while their use did not seem to be of benefit in a recent retrospective study (21).

The decision to treat a dog with myelosuppressive CME should be made on the clear understanding from the part of the owner that it may be cost-prohibitive and eventually ineffective. Profoundly pancytopenic, leucopenic, neutropenic or anaemic dogs, especially of the German shepherd breed have a poor prognosis (3, 7, 21).

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TABLES

Table 1 - Common clinical, haematological and biochemical abnormalities in 19 dogs with chronic (myelosuppressive) naturally-occurring canine monocytic ehrlichiosis[3]

Clinical manifestation	No. with finding/No. tested (%)
Depression	19/19 (100)
Bleeding diathesis	19/19 (100)
Mucosal pallor	18/19 (95)
Anorexia	18/19 (95)
Fever	10/19 (53)
Lymphadenomegaly	9/19 (47)
Weight loss	6/19 (32)
Splenomegaly	6/19 (32)
Hypothermia	5/19 (26)
Ocular discharge	2/19 (11)
Respiratory distress	4/19 (21)
Uveitis	4/19 (21)
Tick infestation	3/19 (16)
Haematology	
Thrombocytopenia	19/19 (100)
Anaemia	19/19 (100)
Lymphopenia	18/19 (95)
Leucopenia	17/19 (89)
Neutropenia	17/19 (89)
Pancytopenia	17/19 (89)
Serum biochemistry	
Elevated ALT*	13/18 (72)
Hypoalbuminaemia**	10/19 (53)
Elevated ALP**	6/17 (35)
Hyperglobulinaemia	6/19 (32)

ALT: Alanine aminotransferase, APL: Alkaline phosphatase

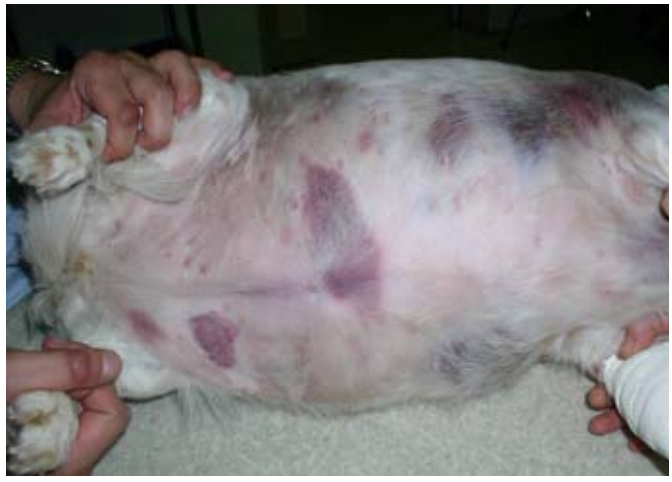


Figure 1- Several cutaneous echymoses in a dog with canine monocytic ehrlichiosis (*Ehrlichia canis*).

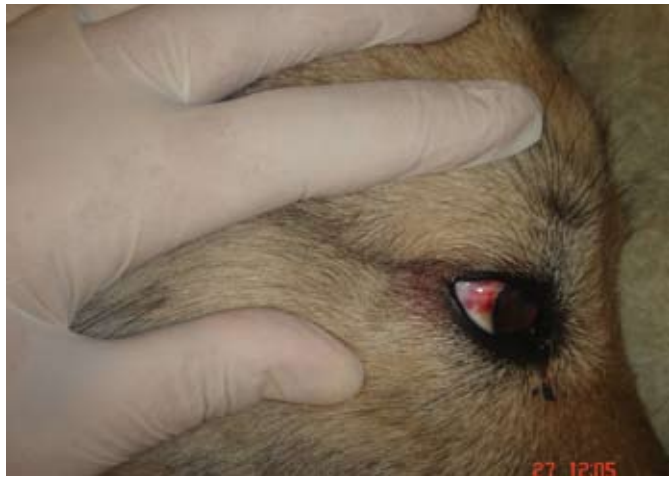


Figure 2- Scleral echymoses in a dog with canine monocytic ehrlichiosis.

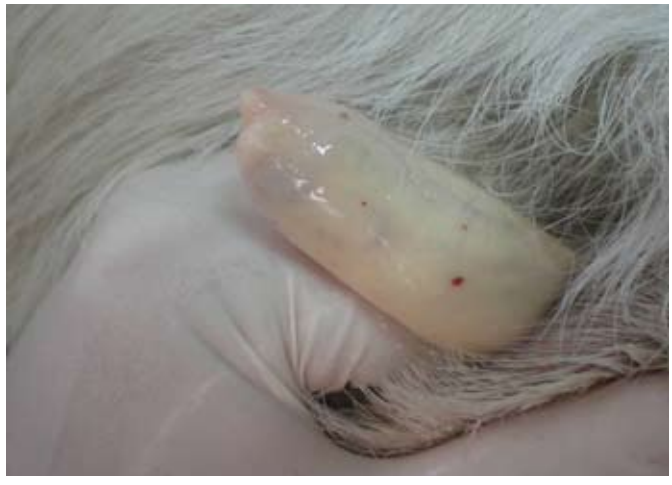


Figure 3- Paleness and petechiation of the penile mucosa in a dog with myelosuppressive CME.

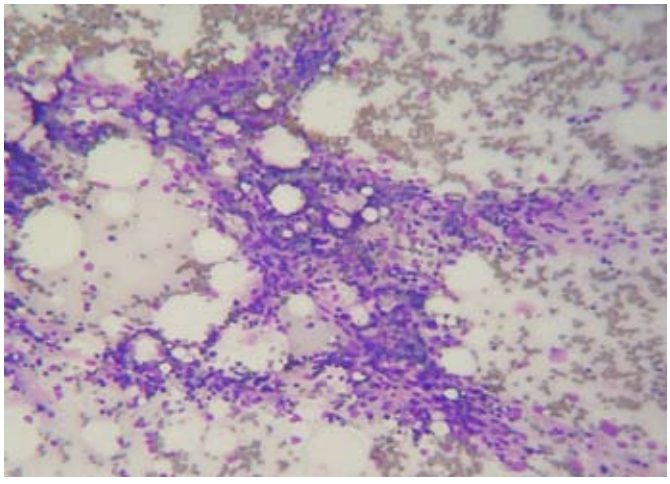
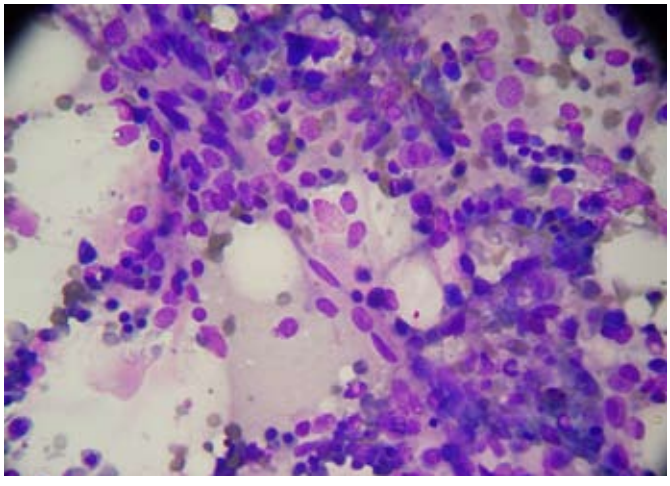
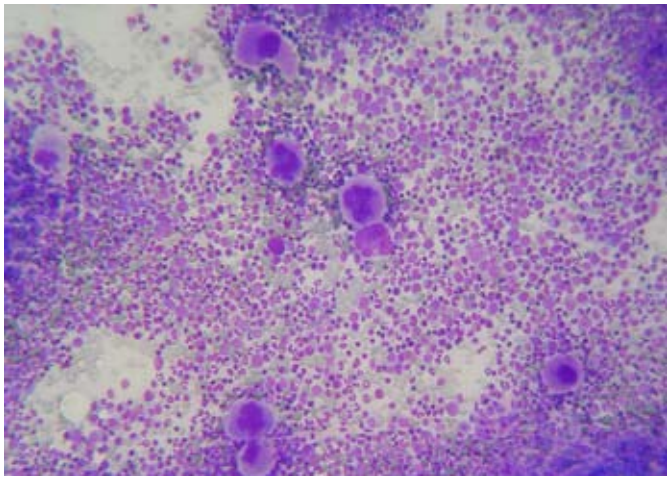


Figure 4 - a) Hypocellular bone marrow due to chronic CME (Giemsa, objective 10x).



b) Same dog: the flecks consist mostly of adipocytes, stromal and plasma cells (Giemsa, objective 40x)



c) normocellular bone marrow in a dog with acute CME. Several megakaryocytes are also visualized (Giemsa, objective 10x).