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CASE REPORT

Megakaryocyte cytoplasmic fragments and ragocytes in the peripheral blood of a domestic cat infected with *Anaplasma*

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Abstract

In this case report, the author reports three strong and highly unusual changes in the peripheral blood of a domestic cat infected with *Anaplasma*. A freshly made blood smear from the cat *Anaplasma* had a shower of about 100 variably large to huge cytoplasmic fragments of megakaryocyte cytoplasm along the feathered edge. These had budding of platelets at their margins. Neutrophils with red cytoplasmic inclusions resembling ragocytes and neutrophils with ingested round inclusions of nuclear material were seen in a 1-day-old EDTA blood sample but not the freshly made blood smear. The cells with nuclear material resembled LE cells or alternatively Tart cells.

Keywords Domestic cat · Peripheral blood · Megakaryocyte cytoplasmic fragments · Ragocytes · LE cells · *Anaplasma*

Introduction

Anaplasma infection in cats often does not cause a reduced platelet count. A review of *Anaplasma* in cats indicated that thrombocytopenia was identified in only 20/34 cats (Schäfer and Kohn 2020). A platelet count within reference intervals does not eliminate the possibility that there was increased destruction of platelets caused by the *Anaplasma*, but that there is a great enough increase in platelet production to offset destruction of platelets.

Ragocytes in synovial fluid of human patients have been associated with rheumatoid arthritis (Cats et al. 1975). Ragocytes and LE cells have been reported in synovial fluid of dogs and a cat with immune-mediated polyarthritis (Anonymous 2022; Bolliger et al. 1994; Camus et al. 2010). Ragocytes seen in joint fluid are neutrophils which have variably sized and variably colored, round cytoplasmic inclusions that contain antibodies (IgM, IgG). Phagocytosis of antibodies has been stimulated experimentally in vitro by incubation of leukocytes together with synovial fluid or sera from human rheumatoid patients (Cats et al. 1975). Granulocytes from clinically well people phagocytized IgM, IgG, and C3 after incubation with sera from people with rheumatoid arthritis but even systemic lupus, Waldenström's macroglobulinemia, multiple myeloma, Hodgin's disease, and trypanosomiasis. Phagocytosis was positively associated with rheumatoid factor but occurred with sera and synovial fluid without rheumatoid factor from patients with the other diseases (Cats et al. 1975).

LE cells are phagocytes that ingested a LE body, which is a homogenous, round clump of magenta colored material. This nuclear material is from dead cells and has been acted upon by antinuclear antibodies. LE cells were reported once before in peripheral blood of a human lupus patient (Hepburn 2001). The LE phenomenon can be recreated by incubation of buffy coat leukocytes in vitro with plasma from patients with lupus. This is the basis of the LE test.

Various methods have been used for the LE test, but one method is to pass clotted venous blood through a wire mesh. This damages leukocytes and releases nuclear material to be exposed to autoantibodies against nuclear histones possibly in the serum. This mixture is centrifuged to obtain a buffy coat, which is then smeared on glass slides to allow a microscopic search for LE cells. It is important for the operator to differentiate LE cells from Tart cells (Hepburn 2001). LE cells must be differentiated from Tart cells which are only phagocytes which had eaten a nucleus from a dead cell (nuclear phagocytosis). Formation of LE cells supports the diagnosis of lupus. Tart cell formation does not support a diagnosis of lupus. Tart cells retain a distinct chromatin pattern in the round structure in the phagocyte. Tart was the

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name of a human patient who had many Tart cells in the bone marrow sample (Steensma 1999).

Case description and discussion

A tube of EDTA blood and one air-dried blood smear from a 16-year-old cat with depression and vomiting were submitted for laboratory analysis. The referring veterinarian's serum amyloid A concentration was reported to be > 100 mg/L with a reference value of < 7 mg/L and was thus very elevated. The referring veterinarian asked us to confirm there were *Anaplasma phagocytophilum* inclusions in the neutrophils in the blood smear. Hematology results were within reference intervals, including the platelet count (210×10^9/L) with a reference interval of 170–490×10^9/L. *Anaplasma* was identified in 14–18% of neutrophils on the blood smears.

The referring veterinarian asked us to evaluate what they called large platelets. The freshly made blood smear had about 104 large cytoplasmic fragments along the feathered edge which were interpreted to be fragments of megakaryocyte cytoplasm (Figs. 1 and 2). About 46 of these fragments of megakaryocyte cytoplasm were extraordinarily huge (Fig. 2). Some of the largest fragments looked like a complex of 2–4 smaller units. There was budding along the margins of the structures suggesting the active formation of platelets.

The appearance of neutrophils in a blood smear made from the 1-day-old EDTA blood was different than that neutrophils in the freshly made blood smear. About 24% of the neutrophils in a smear made from the 1-day-old EDTA blood had variably sized, reddish cytoplasmic inclusions as stained with a May Grunewald Giemsa stain (Fig. 3). No reddish inclusions were seen in neutrophils in the freshly

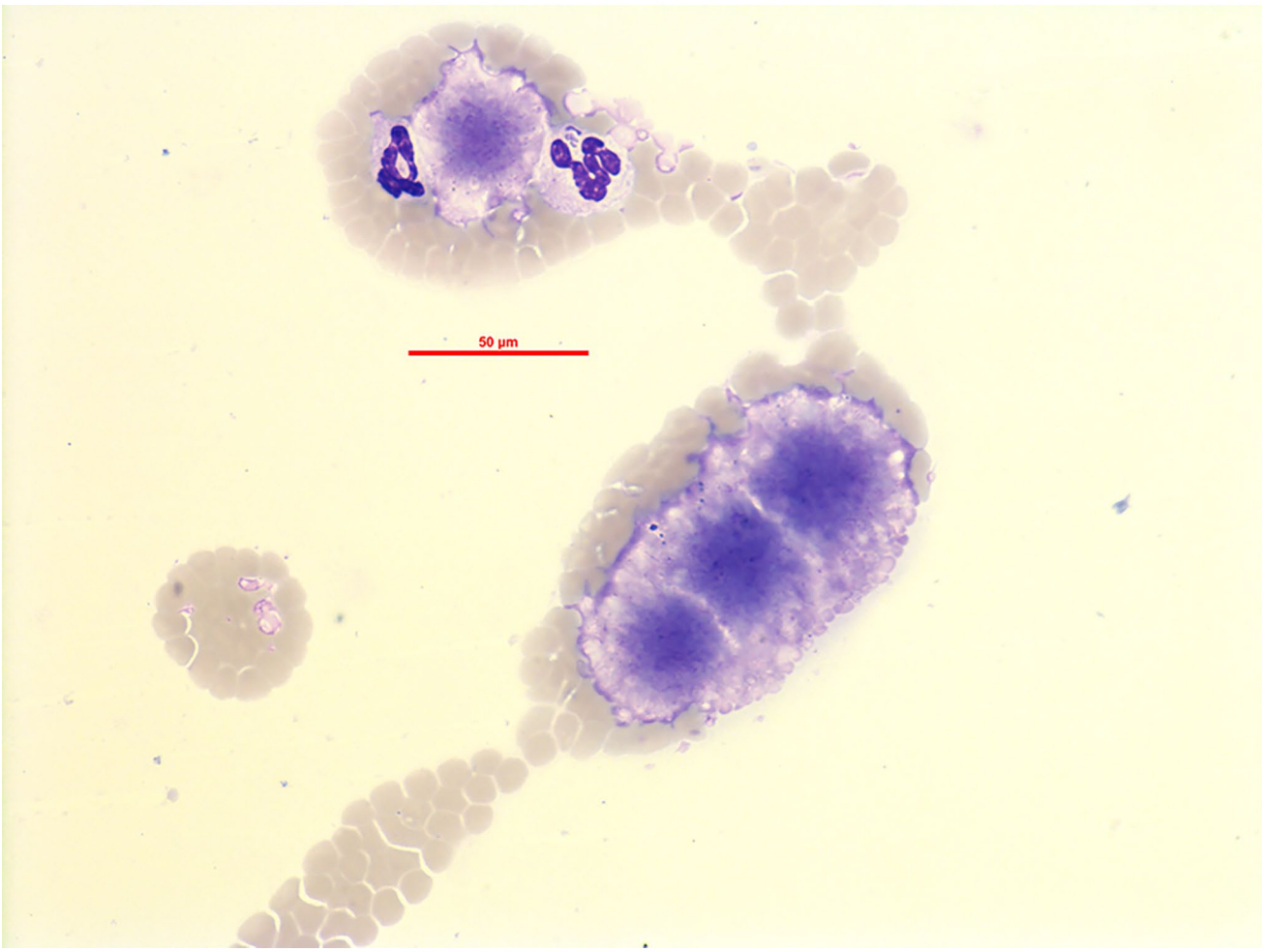

Fig. 1 Large fragments of megakaryocyte cytoplasm at the feathered edge of a blood smear. Use a neutrophil (about 15 µm) or erythrocytes (about 6 µm) to indicate size of the larger structures. The diameters of these fragments were about 2 times the diameter of a neutrophil. The largest structure was a complex of three fragments. One neutrophil contains *Anaplasma*. May Grunewald Giemsa stain, 60× objective

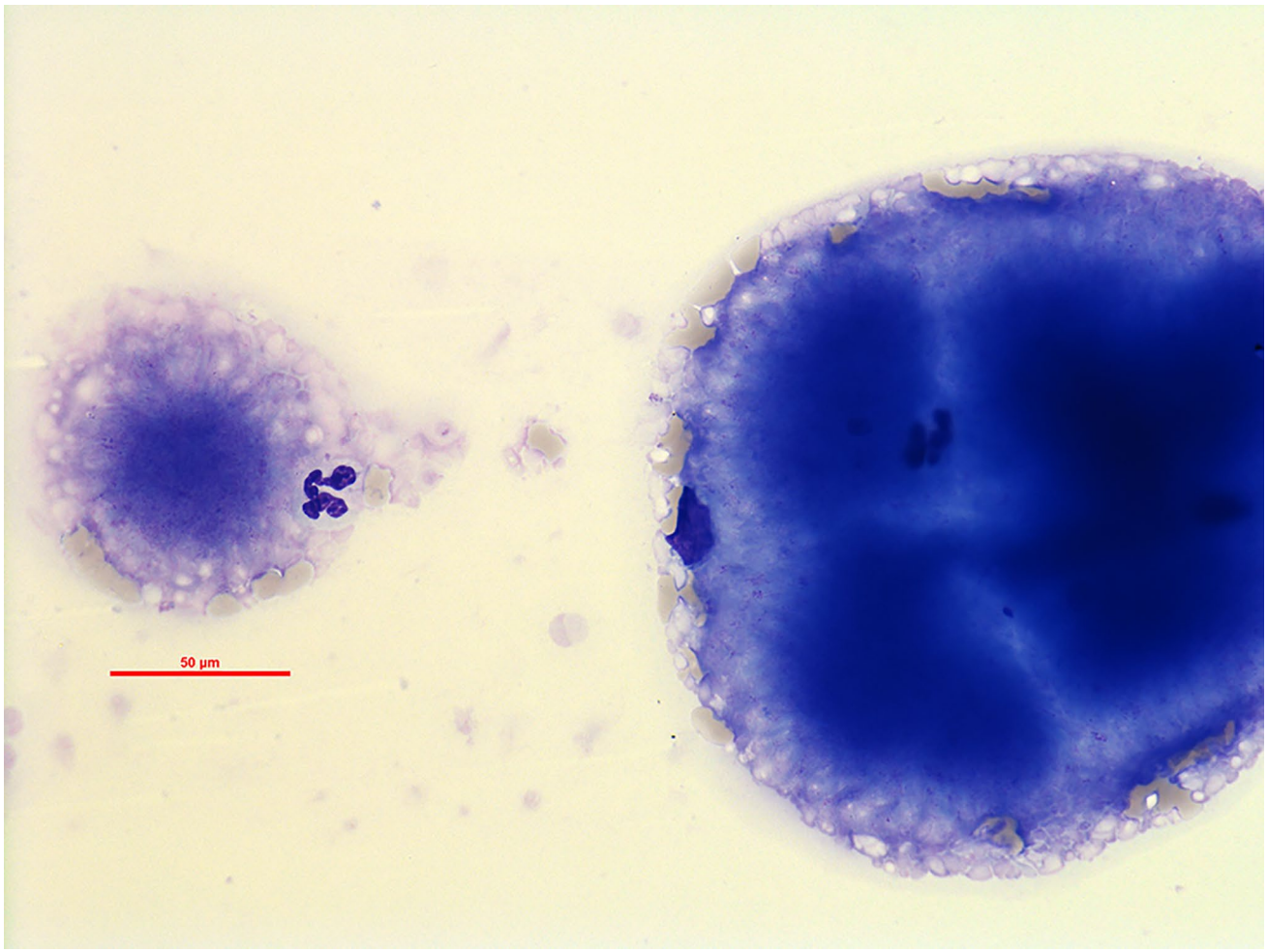

Fig. 2 Huge fragments of megakaryocyte cytoplasm of the feathered edge of a blood smear. The diameter of the left fragment was about 4 times the diameter of a neutrophil, and the right fragment was about 9 times the diameter of a neutrophil. The right fragment resembles a complex of four smaller fragments. May Grunewald Giemsa stain, 40×objective

made blood smear. Some monocytes also had the reddish inclusions. These cells resemble ragocytes reported previously in joint fluids of cats and dogs with immune-mediated polyarthritis (Anonymous 2022; Bolliger et al. 1994; Camus et al. 2010). Fewer neutrophils contained a round, dark blue structure resembling an altered nucleus (Fig. 3). Ragocytes and nuclear phagocytosis were only seen in the smear made from the day-old EDTA blood sample, which indicated they formed in vitro. Similar numbers of neutrophils in both the fresh and the 1-day-old blood smears had *Anaplasma* (14 and 18%). Similar numbers of neutrophils had one or more small blue inclusions resembling Döhle bodies in both smears (18–22%).

The cat had three remarkable changes not previously described in peripheral blood of a cat with *Anaplasma* infection. The very large number of very large and variably large to huge cytoplasmic fragments of megakaryocytes has not been described before even though *Anaplasma* is a common disease of cats and dogs in certain regilons. The fragments did not include a nucleus and were not megakaryocytes. Because it is a new observation, it is difficult to suggest a unique name for the fragments which are intermediate in size between megakaryocytes and platelets.

Megakaryocytes produce platelets by extending very thin, almost thread-like extensions of cytoplasm with thicker buds. These are termed proplatelets (Thon et al. 2010). Proplatelets from megakaryocytes in the bone marrow and lung extend out into the bloodstream. These then break into individual platelets. Proplatelets have been studied in mouse fetal liver megakaryocyte cultures. Proplatelets could be subdivided into two groups by their perimeter (Thon et al. 2010). The perimeter of large proplatelets was > 50 µm and about 30–50 µm for small proplatelets. Another intermediate stage is termed a preplatelet (Thon et al. 2010). The preplatelet is round-to-discoid form and about 2–10 µm in diameter. The size previously reported for proplatelets or

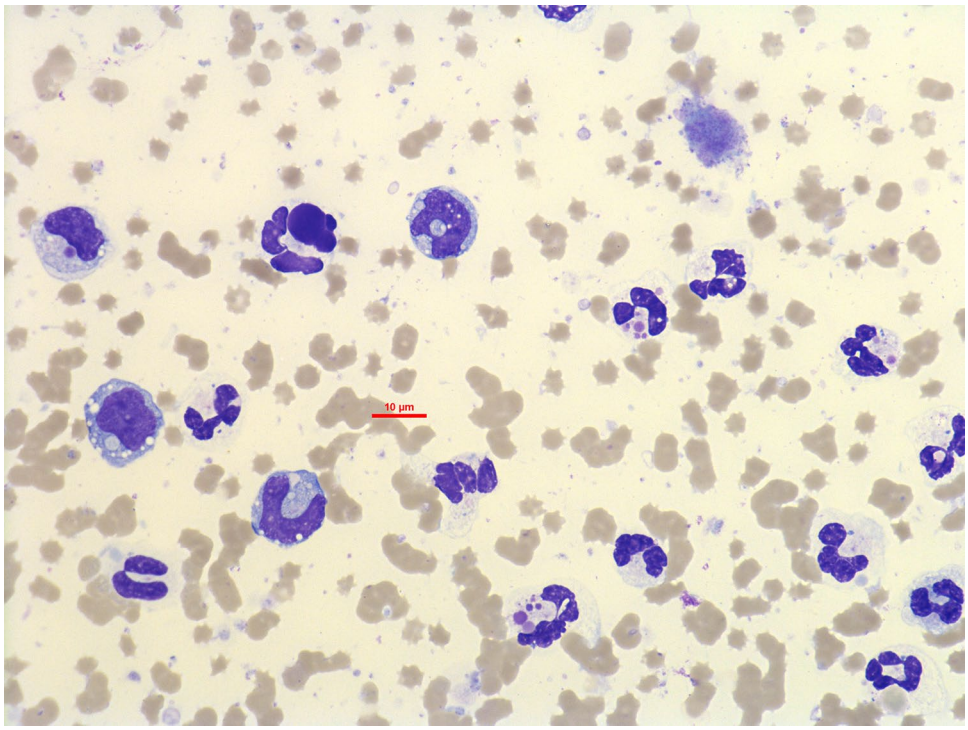

Fig. 3 Blood smear from day-old EDTA blood. One neutrophil (upper center) contains a round dark body resembling an altered nucleus. Several neutrophils contain various sized red inclusions and resembled ragocytes. May Grunewald Giemsa stain, 60 × objective

pre-platelets is much smaller than the structures seen in this cat. An erythrocyte should be about 6 µm in diameter. Neither the previously described proplatelets or pre-platelets are as large or round as the structures in this cat's blood, which had diameters of 2 to 10 times the diameter of a neutrophil (Fig. 2).

Ragocytes and LE cells have been reported in joint fluid but not in peripheral blood. The numerous ragocytes in the blood smear can support a conclusion that the neutrophils containing an altered nucleus were LE cells because formation of both is stimulated by a similar immune process. Morphology is used for differentiation of LE cells from Tart cells (Hepburn 2001; Steensma 1999). LE cells have a LE body, which is a homogenous, round clump of magenta-colored material. Identification of LE cells in the LE test was earlier one of the criteria used to diagnose lupus. Tart cells are only phagocytes which had eaten a nucleus from a dead cell (nuclear phagocytosis) and are not diagnostic for lupus. Tart cells retain a distinct chromatin pattern in the round structure in the phagocyte. Cells in this cat were not light magenta colored but were often quite darkly stained. Some had a granular texture suggesting a retained chromatin pattern and which would indicate that they were more likely Tart cells and not typical LE cells.

This cat recovered well to antibiotic treatment for *Anaplasma* and appeared normal after a few days of treatment. No follow-up testing was performed. Therefore, it was not determined if the cat had similar cytoplasmic fragments before or after this hematological evaluation.

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Declarations

Ethics approval This is not applicable to a case report. No procedures were performed except for routine patient care.

Consent to participate This is not applicable to a case report. No procedures were performed except for routine patient care. But in addition, the University Animal Hospital of the Swedish University of Agricultural Sciences obtains written consent from all patient owners to use information from routine patient care for publications.

Consent for publication The author consents for Comparative Clinical Pathology to publish this manuscript and may take ownership of it after doing so.

Conflict of interest The author declares no competing interests.

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