Veterinary Clinical Pathology ISSN 0275-6382

CASE REPORT

Spurious reticulocyte profiles in a dog with babesiosis

Laetitia Piane[1], Marie-Laure Théron[2], Marcel Aumann[2], Catherine Trumel[1]

[1]Equipe de Biologie Médicale-Histologie, CREFRE, INSERM, UPS, ENVT; and [2]Unité d'Urgence et soins intensifs, UPS, INP, ENVT, Université de Toulouse, Toulouse, France

Key Words
Babesia, pseudoreticulocytosis, reticulocyte fluorescence ratios, scattergram

Correspondence:
L. Piane, Department of Clinical Sciences, National Veterinary School of Toulouse, 23 Chemin des Capelles, BP 87614, Toulouse Cedex 3 31076, France
E-mail: laetitia.piane@gmail.com

DOI:10.1111/vcp.12395

Abstract: A 9-year-old, female Maltese dog was referred to the Veterinary School of Toulouse with a 2-day history of anorexia and weakness. On clinical examination, the dog had hyperthermia (39.7°C), abdominal discomfort, and polypnea. Significant laboratory findings included pigmenturia, hyperbilirubinemia, hypercreatininemia, hyperfibrinogenemia, abnormal Snap canine pancreas-specific lipase, and pancytopenia with a nonregenerative anemia. A peripheral blood smear revealed numerous intraerythrocytic large *Babesia* but no polychromasia. There was a discrepancy between the absolute automated reticulocyte count (Sysmex reticulocyte count: 60 × 10^9/L; RI 19.4–150.1 × 10^9/L) and the manual reticulocyte count (3.6 × 10^9/L) as well as the absence of polychromasia. The optical red blood cell scattergram showed an abnormal isolated reticulocyte cluster at the location of low-fluorescence ratio cells. These findings were interpreted as erythrocytes parasitized by large *Babesia*. The discrepancy between the Sysmex reticulocyte count and the manual reticulocyte count has been reported previously in people with falciparum malaria and numerous intra-erythrocytic *Plasmodium falciparum* organisms. This spurious reticulocyte profile and reticulocyte count were observed with the Sysmex XT-2000iV and the ProCyte using the same fluorescent dye polymethine but not with the LaserCyte using new methylene blue which does not stain *Babesia* organisms on a blood smear performed for manual reticulocyte counting.

Case Presentation

A 9-year-old, female Maltese dog was referred to the Veterinary School of Toulouse with a 2-day history of anorexia and weakness. On clinical examination, the dog had hyperthermia (39.7°C), abdominal discomfort, and polypnea without significant changes on thoracic auscultation. A urine sample was obtained and revealed red-colored urine. Pigmenturia was suspected as the supernatant was also red colored after centrifugation and no erythrocytes were observed in the urinary sediment. Plasma biochemistry (Vitros 350; Ortho-Clinical Diagnostics, Issy les Moulineaux, France) abnormalities revealed marked hypercreatinemia (522 μmol/L, RI 44–133 μmol/L) and hyperbilirubinemia (34.8 μmol/L, RI 1.7–12 μmol/L), mildly elevated ALP activity (164 U/L, RI: 20–155 U/L) and mild hyperfibrinogenemia (7.11 g/L, RI: 1.3–4.8 g/L), and an abnormal SNAP canine pancreas-specific lipase activity measured with a rapid assay (Idexx, Westbrook, ME, USA). Other serum analytes (concentration of proteins, albumin, glucose, and ALT activity) were within RI. The CBC determined with the Sysmex XT-2000iV Hematology analyzer (Sysmex Corporation, Kobe, Japan), revealed moderate leucopenia (3.9 × 10^9/L, RI 5.6–20.4 × 10^9/L) due to mild neutropenia (2.7 × 10^9/L, RI 2.9–13.6 × 10^9/L) and lymphopenia (0.8 × 10^9/L, RI 1.1–5 × 3.10^9/L), marked thrombocytopenia (6 × 10^9/L, RI 108–509 × 10^9/L), and moderate normocytic normochromic anemia (HCT 25%, RI 35–52%; HGB 91 g/L, RI 124–192 g/L; RBC 3.6 × 10^{12}/L, RI 5.1–7.6 × 10^{12}/L). The relative reticulocyte count was 1.66% with an absolute count of 60 × 10^9/L (RI 19.4–150.1 × 10^9/L), including 73.8% low-fluorescence ratio (LFR; RI 63.7–93.8%), 22.6% medium-fluorescence ratio (MFR; RI 4.1–23.6), and 3.6% high-fluorescence ratio (HFR; RI 6.2–36.3) reticulocytes, and a 26.2% immature

reticulocyte fraction (IRF; RI 6.2–36.3%). The reticulocyte area of the dog's optical RBC (RBC-O) scattergram showed a higher density of dots in the central part of the "tail of the comet" without a continuum of dots from the mature RBC plot (Figure 1).

On a blood smear stained with May–Grünwald–Giemsa (MGG; Figure 2), the diagnosis of large form babesiosis was based on a high number of parasitized erythrocytes (manual count > 1000 parasitized RBC). There was a markedly heterogeneous distribution of parasitized erythrocytes throughout the smear, with 1.4% parasitized RBC in the center to 30% at the feathered edge of the smear, and a mean of 2.1% count in 10 randomly selected fields. The reticulocyte count obtained with the Sysmex contrasting with the absence of polychromasia on the MGG blood smear (Figure 2) and a manual reticulocyte count on a new methylene blue (NMB)-stained blood smear with just 0.1% reticulocytes (absolute count 3.6×10^9/L). Intra-erythrocytic parasites were not stained by NMB (Figure 3).

For further evaluation, a CBC was performed each on a LaserCyte Dx (Idexx) and a ProCyte Dx hematology analyzer (Idexx) within 3 hours of the first CBC. The reticulocyte scattergram from the ProCyte was similar to the Sysmex in spite of an erroneous RBC mass (HCT 41.5%, RI 37.3–61.7%; HGB 153 g/L, RI 131–205 g/L; RBC 6.59×10^{12}/L, RI 5.65–8.87×10^{12}/L), and reticulocytes represented 0.8% of RBC with an absolute count of 53×10^9/L (Idexx; RI 10–110×10^9/L). The reticulocyte scattergram from the LaserCyte was normal (Figure 4), and reticulocytes represented 0.4% of RBC with an absolute count of 16×10^9/L (no RI available).

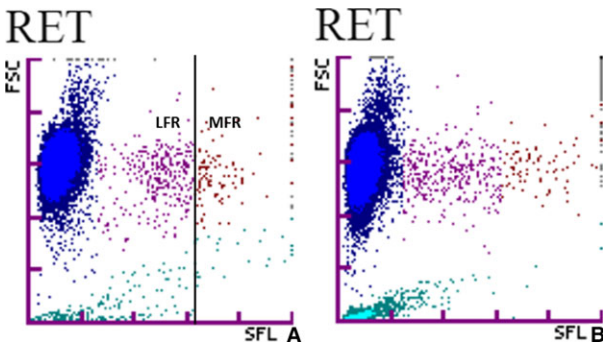

Figure 1. Sysmex XT-2000iV optical red blood cell (RBC-O) scattergram of blood from a Maltese dog with babesiosis and nonregenerative anemia (**A**) and a dog with regenerative anemia (**B**). Notice the separation between the reticulocyte dot plot (in purple and red) and the RBC plot (in blue) in the scattergram of the Maltese dog compared with the continuum between the reticulocyte plot and the RBC plot in the dog with regenerative anemia (low- and medium-fluorescence ratio reitculocytes are in purple and red, respectively; high-fluorescence ratio reticulocytes are not represented in this graph). RET indicates reticulocyte; MFR, medium-fluorescence ratio; LFR, low-fluorescence ratio; FSC, Forward-Scattered Light; SFL, Side-Fluorescence Light.

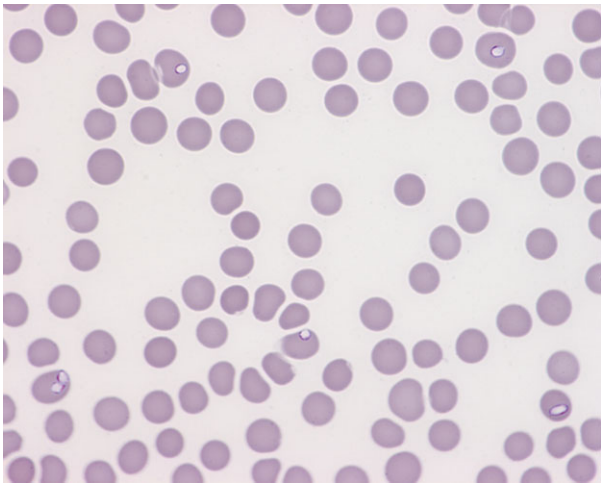

Figure 2. Blood smear from a Maltese dog. Note the presence of numerous *Babesia* in erythrocytes and absence of polychromasia. May–Grünwald–Giemsa, 9100 oil immersion objective.

The dog received imidocarb (6.6 mg/kg, IM; Carbesia, Intervet) and infusion of crystalloïds by intravenous infusion for 3 days. After improvement of the clinical status and discharge the dog was lost to follow-up.

Discussion

To the best of our knowledge, this is the first report of a spurious reticulocyte profile and reticulocyte count in a dog with large form babesiosis. Sysmex and ProCyte atypical reticulocyte profiles and false reticulocyte counts were thought to result from the presence of large *Babesia* in mature erythrocytes. Specifically, the automated reticulocyte count from the Sysmex was higher than the manual count. The discrepancy between automated reticulocyte count obtained with different fluorescent dyes and the manual reticulocyte count based on a NMB-stained blood smear has been previously reported in people with falciparum malaria infection, and the difference between the 2 was described as proportional to the parasitemia in some cases.[1,2] The Sysmex and the ProCyte perform an optical reticulocyte count using the same fluorescent dye polymethine, whereas the LaserCyte uses the classical NMB to stain ribonucleic acid (RNA) in cells. Reticulocytes with increased RNA content are displayed on the right of the reticulocyte scattergram as the "tail of the comet" and the resulting reticulocyte count has been

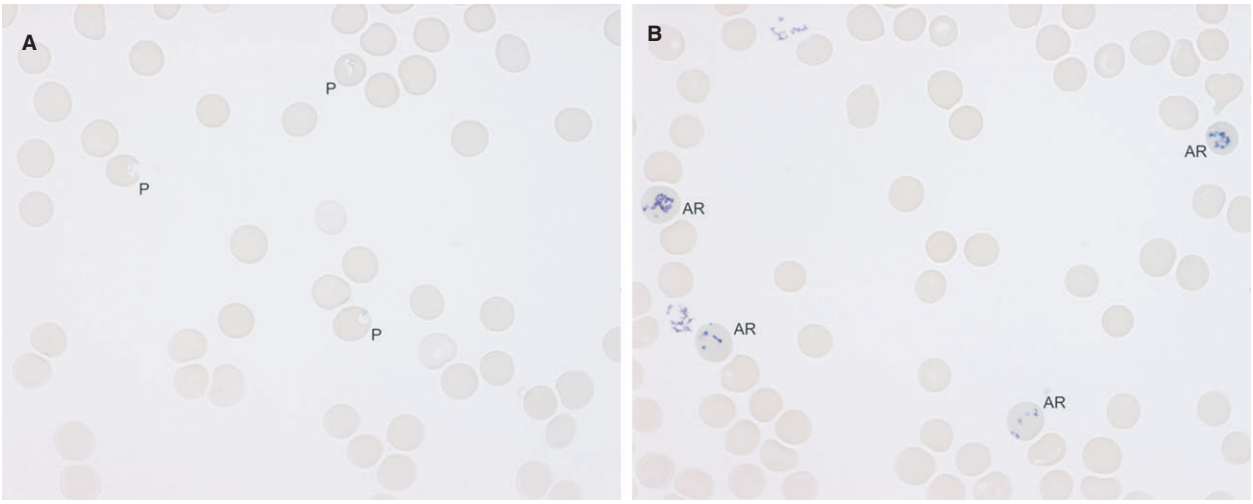

Figure 3. Blood smear from a Maltese dog with babesiosis (**A**) and a dog with regenerative anemia (**B**). New methylene blue (NMB), ×100 oil immersion objective. Note the absence of reticulocytes and the lack of intraerythrocytic parasite staining with NMB (A) in contrast to the dog with regenerative anemia (B). AR indicates aggregate reticulocytes; P, parasite.

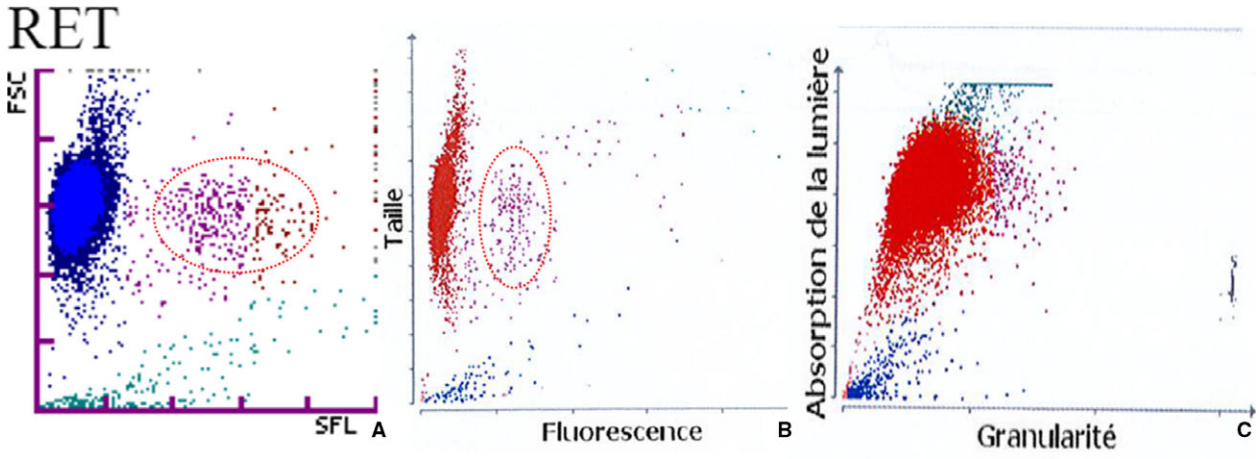

Figure 4. Sysmex XT-2000iV (**A**), ProCyte (**B**) and LaserCyte (**C**) optical RBC (RBC-O) scattergram from a Maltese dog with babesiosis. Sysmex (A) and ProCyte scattergram (B) show an atypical reticulocyte cluster separated from the RBC cluster (red ellipses), not observed on LaserCyte scattergram (C).

shown to be more or less correlated with the manual count depending on the analyzer and the concentration of reticulocytes.[3–6]

In our case, parasite RNA was most probably falsely interpreted as reticulocyte RNA, explaining the incorrect reticulocyte count using the Sysmex and the Procyte.[1,7] Careful gating of reticulocytes is crucial to determine accurate counts, because polymethine also stains RNA in platelets and WBC. Therefore, giant PLT, PLT clumps, abnormal WBC, abnormal numbers of WBC, WBC fragments, Heinz bodies, or spherocytes are potential sources of interference with automated methods of reticulocyte analysis.[7–12] In addition, cytoplasmic particles including Howell–Jolly bodies, Pappenheimer bodies, or basophilic stippling may be confused with reticulum granules using automated as well as manual techniques.[7,10,11,13]

Reticulocytes are classified by Sysmex as LFR, MFR or HFR depending on the amount of RNA. The intensity of the fluorescence is correlated with the amount of RNA with the most immature reticulocytes being the brightest.[3,7] In a typical regenerative anemia, the reticulocyte profile is a continuum of dots highly suggestive of the tail of a comet. In our case, an abnormal isolated reticulocyte cluster was found at the location of LFR cells (73.8%) and MFR (22.6%). Other

cases with abnormal reticulocyte profiles have been described in cases of canine and human acute myeloid and lymphoid leukemia, and in human beings with malaria.[1,2,14,15] In leukemia, the discrepancy has been proposed to be due to leukemic cells and cell fragments.[14,15] But, in such cases, the atypical fluorescent cells are in the HFR, where 66% HFR reticulocytes were detected on an extended reticulocyte scattergram as an isolated cluster to the right of the normal location of HFR cells, and WBC can display young reticulocyte-like fluorescence leading to an erroneous estimation of the HFR reticulocyte count.[7,16] Leukemic cells were speculated to stain less intensely with polymethine than normal leukocytes and thus be spuriously identified as reticulocytes.[15] More similar to our case, an erroneously high IRF was reported in a cases of high intra-erythrocytic parasitemia in human malaria.[1,2]

The Sysmex and the ProCyte produced similar false reticulocyte numbers probably because they both use polymethine supposedly staining *Babesia* organisms. The results with the LaserCyte which uses NMB were different, likely because NMB did not stain *Babesia* organisms which has been shown in blood smears manually stained with NMB.

Further investigations are needed to determine if the atypical reticulocyte cluster described in this case report is characteristic and common in babesiosis, if it is observed in other pathologic conditions, and if it could give an erroneous diagnostic of regenerative anemia. This finding could be a useful tool to screen patients for *Babesia* infections.

References

1. Laurencet FM, Martinez T, Beris P. Spurious extreme reticulocytosis with an automated reticulocyte analyzer. *N Engl J Med*. 1997;337:1922–1923.
2. Scott CS, Van Zyl D, Ruivo L, Kunz D, Coetzer TL. Patterns of pseudo-reticulocytosis in malaria: fluorescent analysis with the Cell-Dyn CD4000. *Clin Lab Haematol*. 2002;24:15–20.
3. Lilliehöök I, Tvedten H. Validation of the Sysmex XT-2000iV hematology system for dogs, cats, and horses. I. Erythrocytes, platelets, and total leukocyte count. *Vet Clin Pathol*. 2009;38:163–174.
4. Collicutt NB, Grindem CB, Neel JA. Comparison of manual polychromatophilic cell and automated reticulocyte quantification in evaluating regenerative response in anemic dogs. *Vet Clin Pathol*. 2012;41:256–260.
5. Welles EG, Hall AS, Carpenter DM. Canine complete blood counts: a comparison of four in-office instruments with the ADVIA 120 and manual differential counts. *Vet Clin Pathol*. 2009;38:20–29.
6. Wenger-Riggenbach B, Hässig M, Holfmann-Lehmann R, Lutz H. Evaluation of the LaserCyte: an in-house hematology analyzer for dogs and cats. *Comp Clin Pathol*. 2006;15:117–129.
7. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analyzers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticuolcytes. *Int J Lab Hematol*. 2007;29:21–41.
8. Espagnol I, Pedro C, Remacha AR. Heinz bodies interfere with automated reticulocyte count. *Haematologica*. 1999;84:373–374.
9. Hinchliffe RF. Errors in automated reticulocyte counts due to Heinz bodies. *J Clin Pathol*. 1993;46:878–879.
10. Ghevaert C, Fournier M, Reade V, Mehiaoui L, Goudemand J. Laboratory evaluation of the Coulter STKS reticulocyte method in a children's hospital. *Lab Hematol*. 1997;3:92–97.
11. Pappas AA, Owens RB, Flick JT. Reticulocyte counting by flow cytometry. A comparison with manual methods. *Ann Clin Lab Sci*. 1992;2:125–132.
12. Oyamatsu T, Shimizu N, Takeuchi K, et al. Automated measurement of reticulocyte count by flow cytometry. II: analysis of the blood containing abnormal erythrocytes or giant platelets. *Rinsho Byori*. 1989;37:807–812.
13. Riley RS, Ben-Ezra JM, Goel R, Tidwell A. Reticulocytes and reticulocyte enumeration. *J Clin Lab Anal*. 2001;15:267–294.
14. Tvedten H. What is your diagnosis? Discrepancy between Sysmex XT-2000iV reticulocyte count and polychromasia. *Vet Clin Pathol*. 2011;40:275–276.
15. Huh J, Moon H, Chung W. Erroneously elevated immature reticulocyte counts in leukemic patients determined using a Sysmex XE-2100 hematology analyzer. *Ann Hematol*. 2007;86:759–762.
16. Villamor N, Kirsch A, Huhn D, Vives-Corrons JL. Interference of blood leucocytes in the measurements of immature red cells (reticulocytes) by two different (semi-) automated flow-cytometry technologies. *Clin Lab Haematol*. 1996;18:89–94.