Anemia in a dog

U. Falkenö[1], H. Tvedten[2]

[1] Clinical Pathology Laboratory, University Animal Hospital, Swedish University of Agricultural Sciences, Box 7040, 750 07 Uppsala, Sweden

[2] Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Box 7054, 750 07 Uppsala, Sweden

Phone: +46 18 671611

Fax: +46 18 672883

E-mail: ulrika.falkeno@uds.slu.se

Signalement

A 1.5-year-old, intact female, Border collie dog

Case History

The dog was presented to the referring veterinarian with a two-week history of being lethargic. It had attended a herding training camp two weeks previously. It had never been outside of the northern part of Sweden and had not had any ectoparasites according to the owner. There was no suspicion of intoxication and the there was no history of previous illness or medication.

Physical exam

On presentation the dog was lethargic and had pale mucous membranes, poor body condition and a poor hair coat.

Laboratory findings

A blood sample was submitted by mail to the Clinical Pathology Laboratory, University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala, Sweden, for a complete hematological profile. The hematology analysis was performed on an Advia 2120 hematology instrument (Siemens Healthcare Diagnostics, Erlangen, Germany) and by evaluation of a May-Grünwald Giemsa stained blood smear. The blood sample was run twice on the hematology instrument because the results from the first run came with many flags. The results from the second run differed from the results from the first run but the flags were similar.

A second blood sample was submitted by mail 17 days later.

Table 1: Hematology results obtained on May 21st with the Advia 2120.

Parameter	Result, 1st run	Result, 2nd run	Unit	Reference Interval
Hematocrit	0.31	0.30[1]	L/L	0.38 - 0.57
Reticulocytes	406[1]	286	10^9/L	<50
Reticulocytes %	10.4[1]	7.6	%	0 - 1.5
Platelets	329*[1]	300*[1]	10^9/L	170-490
WBC reported	7.7 [1]	21.7[1]	10^9/L	5.8 - 16
WBC Baso	22.5[1]	21.7[1]	10^9/L	5.8 - 16
WBC Perox	7.7[1]	8.1[1]	10^9/L	5.8 - 16
Neutrophils	1.6	1.6[1]	10^9/L	3 - 11.5
Eosinophils	0.1	0.1[1]	10^9/L	0.1 - 1.2
Basophils	0.5[1]	1.5[1]	10^9/L	<0.1
Lymphocytes	5.0[1]	19.3[1]	10^9/L	1.4 - 4.8
Monocytes	0.6	0.6[1]	10^9/L	0.2 - 1.4
340 metarubricytes/100 WBCs were seen on blood smear evaluation				

[1] Results with flags

*Marked platelet clumping

Table 2: Hematology results obtained on June 7th with the Advia 2120.

Parameter	Result	Unit	Reference Interval
Hematocrit	0.33	L/L	0.38 - 0.57
Reticulocytes	31	10^9/L	<50
Reticulocytes %	0.7	%	0 - 1.5
Platelets	173*[1]	10^9/L	170-490
WBC reported	13.6 [1]	10^9/L	5.8 - 16
WBC Baso	13.6[1]	10^9/L	5.8 - 16
WBC Perox	8.6[1]	10^9/L	5.8 - 16
Neutrophils	3.6[1]	10^9/L	3 - 11.5
Eosinophils	0.1[1]	10^9/L	0.1 - 1.2
Basophils	0.9[1]	10^9/L	<0.1
Lymphocytes	9.1[1]	10^9/L	1.4 - 4.8
Monocytes	0.7[1]	10^9/L	0.2 - 1.4
147 metarubricytes/100 WBCs were seen on blood smear evaluation			

[1] Results with flags

*Marked platelet clumping

Figure 1: Blood Smear Morphology, May 21st (May Grünwald-Giemsa stain, original magnification was about 500X).

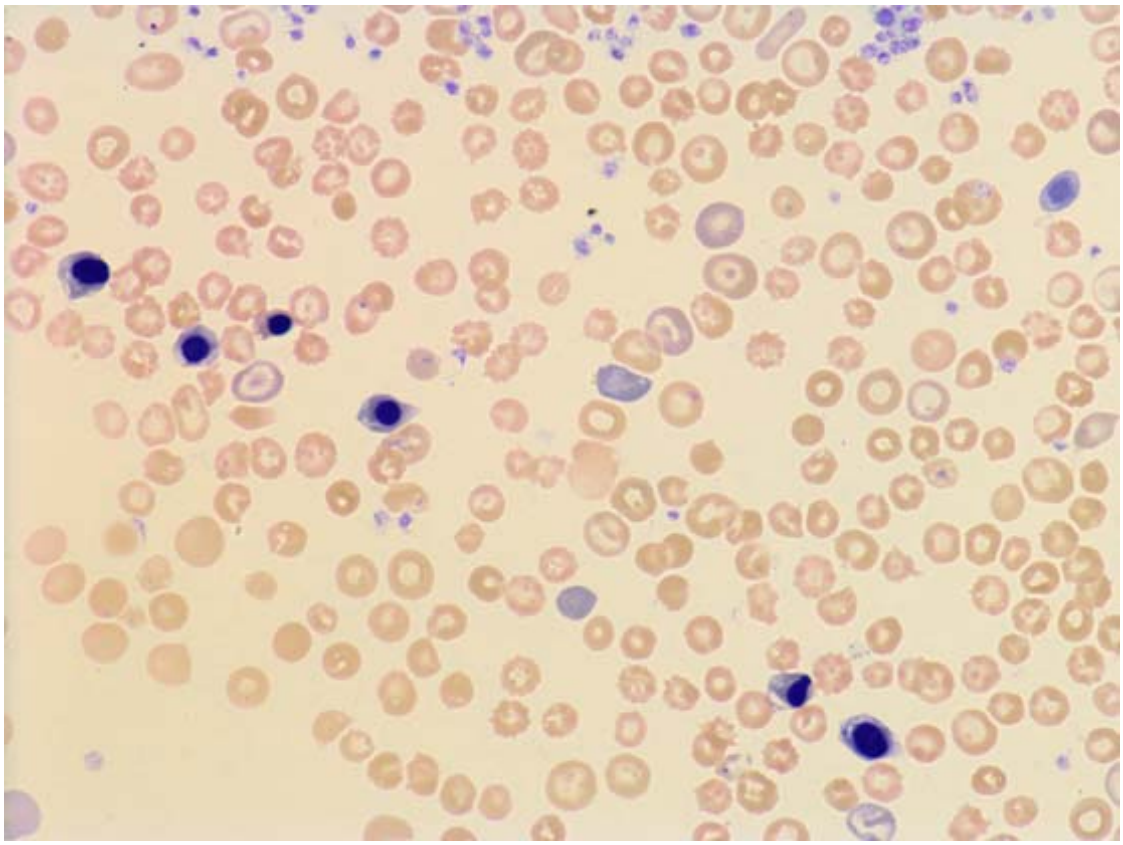


Figure 2: Advia 2120 Graphics, May 21st.

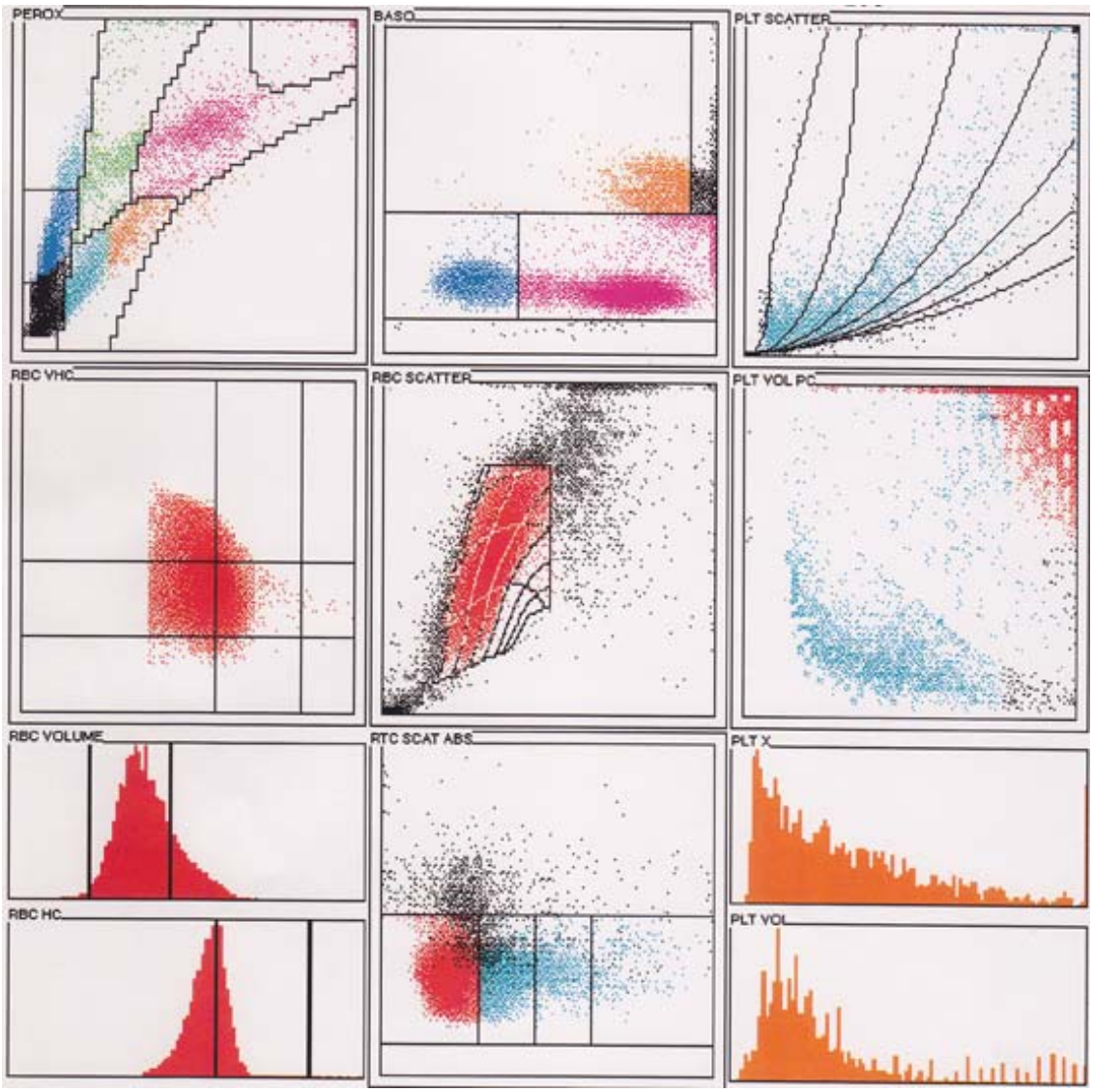
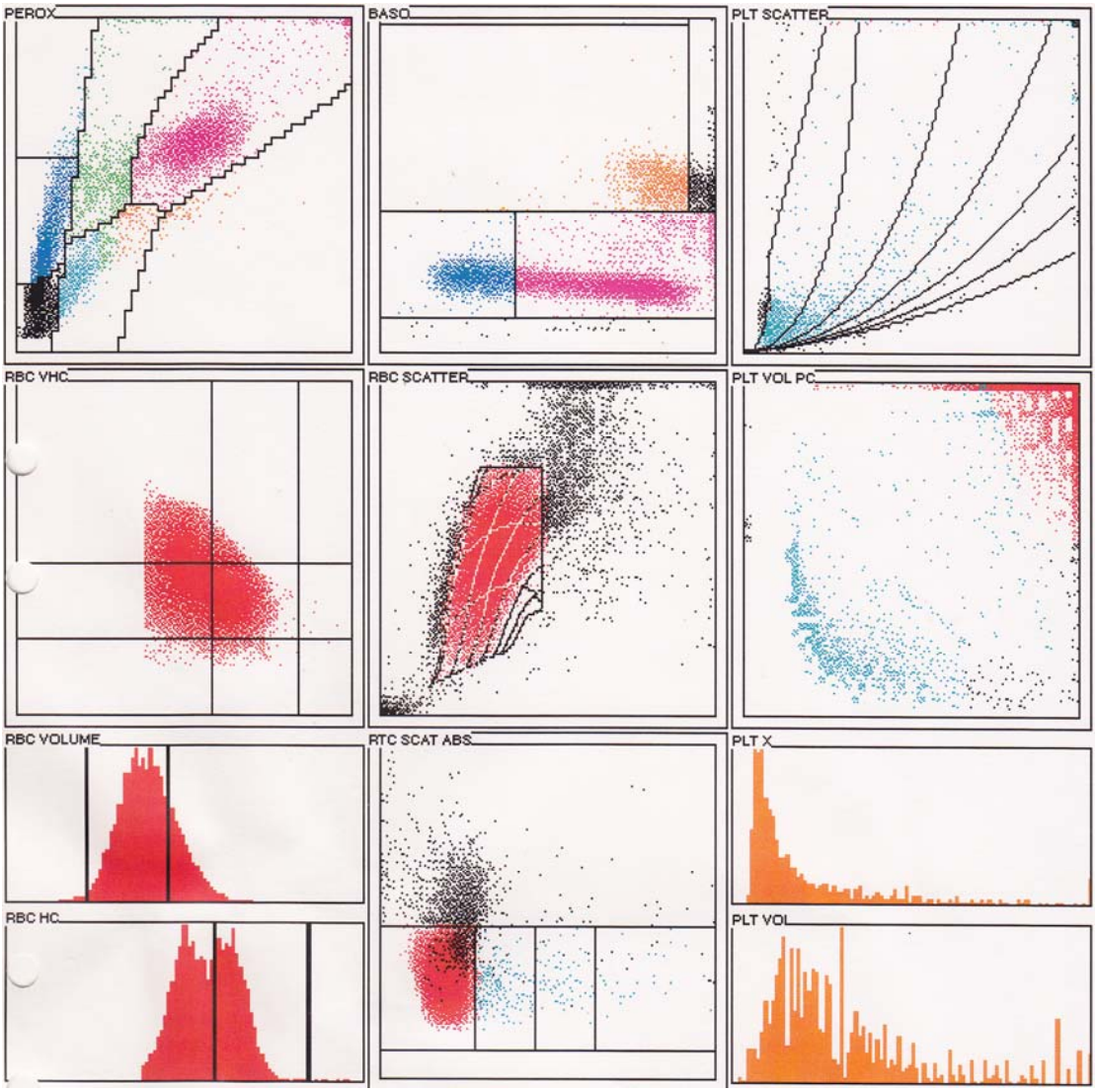


Figure 3: Advia 2120 Graphics, June 7th.



Questions:

1. What caused the difference in WBC and reticulocyte counts?

2. What diseases would you consider?

3. What additional tests would you consider?

Answers and Interpretation:

Hematology results obtained on May 21st showed a mild, moderately regenerative anemia and a moderate mature neutropenia. Microscopic evaluation of a blood smear revealed a moderate polychromasia, moderate anisocytosis, presence of some basophilic stippling and Howell Jolly bodies and a marked metarubricytosis. There was mild hemolysis in the EDTA plasma but there was no autoagglutination, less than 5 % spherocytes and no signs of oxidative damage (no Heinz bodies or eccentrocytes were seen).

Many platelet clumps were observed on blood smear examination, precluding an accurate automated platelet count but the amount of platelets was estimated to be at least normal on a visual count.

There were great variations in the different total WBC counts obtained by Advia 2120. The WBCB (baso channel) was much greater than the WBCP (peroxidase channel). This was apparently due to inclusion of rubricytes and metarubricytes in the WBCB count. The usual position of metarubricytes is in the "body of the worm" (Figure 4). The dense chromatin of metarubricytes usually places them among the neutrophils. In the first two blood samples collected from this dog there was a distinct, round population of lyse resistant cells in the right side of the basophil counting area. These were interpreted to also be rubricytes or metarubricytes. The WBC count from the first run came with the system flags WBC SUB and B-SUB. When those two flags are present, the WBCB is substituted with the WBCP. The second analysis of the sample did not have these flags and that explains why the WBCP was reported the first time and the WBCB the second time. WBCB from both runs came with the system flag BSUSP, which means that there are lyse resistant cells in the baso channel, located in an unusual position. The lyse resistant cells are not included in the WBCB count but they are reported as basophils in the differential. The most correct WBC count n this case was interpreted to be sum of the WBCB count, with the Advia basophil count added which was then corrected for the 340 NRBC/100 WBC.

The difference in reticulocyte counts from the two runs on May 21st (406 x 10^9/L and 286 x 10^9/L respectively) is more difficult to explain. The result from the first analysis came with the system flag RTC-FS which, among other things, can be caused by large numbers of metarubricytes. The reticulocyte count from the second run was not flagged and was interpreted to be the most correct. The polychromasia observed in the blood smear was also more consistent with the reticulocyte count from the second run. The mean cell volume of the reticulocytes (MCVr) and the mean cell hemoglobin concentration of the reticulocytes (CHCMr) from the first and second run were very similar as were % low, medium and high fluorescence reticulocytes. The RTC volume and HC curves suggest that a greater percentage of reticulocytes were included in the first analysis (Figure 5). This suggests that there is a variable threshold to the amount of RNA signal between erythrocytes and reticulocytes.

The hematology results that were interpreted to be the most correct were WBCB with the Advia basophil count added and after that corrected for the metarubricytes, a manual differential and reticulocyte count from the second run (Table 3).

Possible differentials regarding a marked metarubricytosis are hypoxia, heat stroke, a disorder affecting the spleen or bone marrow or lead poisoning. There was mild hemolysis in the EDTA plasma. The hemolysis could have occurred as an artifact in vitro during shipping, but hemolytic anemia caused by zinc or copper poisoning, hypophosphatemia, PFK deficiency, PK deficiency or blood parasites were part of our differentials. There were no signs of oxidative damage (no Heinz bodies or eccentrocytes were seen) or immune mediated hemolytic anemia such as autoagglutination or more than occasional spherocytes.

On June 7th (17 days later) a second, mailed in, blood sample was analysed (Table 2). There was still a mild anemia, prominent metarubricytosis and mild hemolysis in the EDTA plasma. There was no longer a reticulocytosis but a large group of macrocytic hypochromic erythrocytes was observed as a double peak

in the RBC HC histogram (Figure 3). These macrocytic hypochromic cells were likely reticulocytes that had lost their RNA but remained as larger cells. This shows evidence of a regenerative response despite the normal number of reticulocytes, usually interpreted to indicate a nonregenerative response.

Because this was a young Border collie and a mutation in the cubilin gene in that breed can cause metarubricytosis, we analyzed serum cobalamin and folate concentrations on an Immulite 2000 (Siemens Healthcare Diagnostics, Erlangen, Germany). Cobalamin concentration was below the measurement range (< 111 pmol/L: reference 135-670) and folate concentration was unremarkable (18.6 nmol/L, reference 15-45).

Genetic testing showed that the dog was homozygous for the mutation in the cubulin gene that causes Imerslund-Gräsbeck syndrome in Border collies.

Diagnosis: Imerslund-Gräsbeck syndrome (I-GS) or hereditary selective cobalamin malabsorption of Border collies

The dog received parenteral cyanocobalamin supplementation (1 mg) subcutaneously twice with a 13 day interval and a follow-up blood sample was submitted 27 days later (July 4th). Hematology results and serum cobalamin concentration were unremarkable (Table 4, Figure 6).

Discussion

Cobalamin (vitamin B_{12}) is a water-soluble vitamin that is absorbed in the intestine by a complex process [1-3]. In the gastrointestinal tract cobalamin binds to the carrier protein intrinsic factor (IF), which is produced by the gastric mucosa and, in dogs, also by the pancreatic duct epithelium [1, 3, 4]. The cobalamin-IF complex attaches to the cubam receptor in the distal small intestine (ileum) and undergoes receptor-mediated endocytosis. The cubam receptor consists of the protein subunits cubilin and amnionless and is expressed in epithelial brush borders of the distal small intestine and renal proximal

tubules. A mutation in either the cubilin or amnionless gene causes Imerslund-Gräsbeck syndrome and loss of cubam function in both ileum and renal tubules [2, 5].

Cobalamin is an essential cofactor for the activity of methylmalonic-CoA mutase and methionine synthase in mammals. Decreased activity of methylmalonic-CoA mutase causes increased serum and urine methylmalonic acid concentrations. The result of reduced methionine synthase activity is homocysteinemia and inhibition of the conversion of 5-methyl-tetrahydrofolate to tetrahydrofolate, necessary for purine, pyrimidine and amino acid synthesis. The decreased synthesis of nucleic acids inhibits hematopoiesis by impairing the maturation of the rapidly dividing cells in the bone marrow [6, 7].

Hereditary cobalamin malabsorption has previously been reported in Border collies, giant schnauzers, beagles, Australian shepherd dogs and Chinese shar pei dogs [1, 2, 4, 8-12]. Imerslund-Gräsbeck syndrome in giant schnauzers and Australian shepherds is caused by mutations in the amnionless gene [13]. Recent studies have identified a mutation in the cubilin gene as most likely causative for selective cobalamin malabsorption in Border collies, which is inherited as an autosomal recessive trait [2, 5].

Abnormalities previously reported in cobalamin deficient border collies include stunted growth, lethargy, anorexia, non-regenerative anemia, neutropenia, mild proteinuria and elevated aspartate aminotransferase activity [1, 8]. The dog in this case had vague clinical signs such as lethargy, poor body condition and poor hair coat.

Anemia of cobalamin deficiency has been described as nonregenerative, normocytic normochromic [4, 11]. This case had a clearly regenerative anemia. Cobalamin deficient dogs have both macrocytic and microcytic erythrocytes, which results in an increased red cell distribution width (RDW), while mean cell volume (MCV) remains unremarkable [4, 7]. In this case the RDW, MCV and MCHC were not reliable because the blood sample was received by mail one day after blood collection and MCV, MCHC and

RDW were erroneous attributable to EDTA-caused erythrocyte swelling. This dog had prominent reticulocytosis on the first hematologic examination and a large percentage of macrocytic hypochromic erythrocytes 17 days later. Despite errors in the MCV and MCHC in an older sample, two distinct erythrocyte populations were seen on the RBC HC histogram. A marked metarubricytosis was observed in blood samples collected at both occasions. Metarubricytosis has been noted in a border collie with Imerslund-Gräsbeck syndrome previously [8]. The cause for the regenerative response and metarubricytosis in our dog is not known. Interestingly though, hematology results from a littermate of the dog presented here also showed a mild, moderately regenerative anemia with metarubricytosis and cobalamin concentration below the reportable range. It apparently also had hereditary selective cobalamin malabsorption and a regenerative anemia. These are only 2 cases of I-GS affected Border collies with regenerative anemia, but they may indicate that this disease may cause an intermittent regenerative anemia.

The dog presented here had a moderate neutropenia without a left shift, which is a commonly reported finding in dogs with cobalamin deficiency [1, 4, 8].

The cubilin gene is expressed in renal tubules, and I-GS in Border collies is previously reported to be associated with a mild proteinuria [1, 5, 8]. The dog in this case had a 2+ proteinuria but this was, at least in part, attributable to a urinary tract infection with hemoglobinuria (2+) and a bacterial culture positive for *Staphylococcus spp*.

Other findings in dogs with hereditary cobalamin malabsorption are homocysteinemia and methylmalonic aciduria [4, 8]. These analyses were not performed in this dog or its littermates.

The age of this dog (1.5 years) is consistent with previous reports where Border collies with IG-S have been diagnosed at variable ages as late as 42 months [1, 8, 11]. This is in contrast to the much earlier age (8 weeks) when I-GS affected giant schnauzers and Australian shepherds become inappetent and

exhibit failure-to-thrive [4, 13]. The reason behind the later recognition of disease in Border collies is not fully understood.

After the diagnosis of Imerslund-Gräsbeck syndrome was made in this dog, two of its littermates have also tested positive for the same mutation in the cubilin gene.

Figure 4: Advia Baso WBC dot plot, May 21st.

The usual position of metarubricytes is in the "body of the worm" (red oval). The dense chromatin of metarubricytes usually places them among the neutrophils. In both of the blood samples from this dog there was a population of lyse resistant cells to the right in the basophil counting area (green oval).

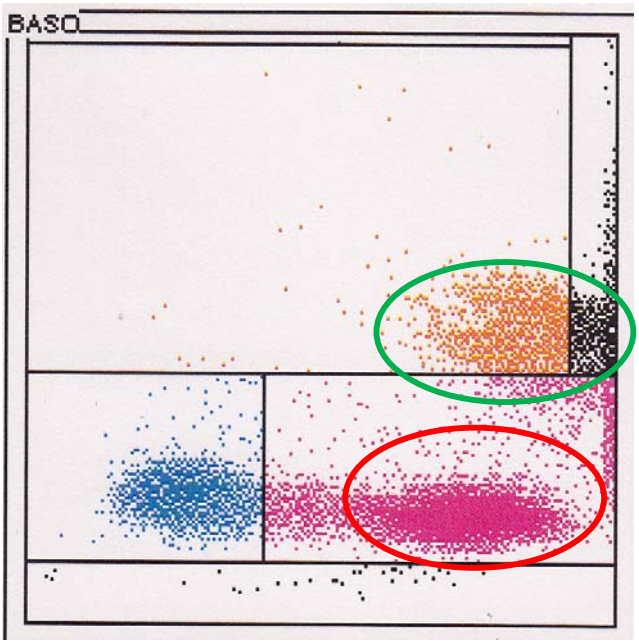
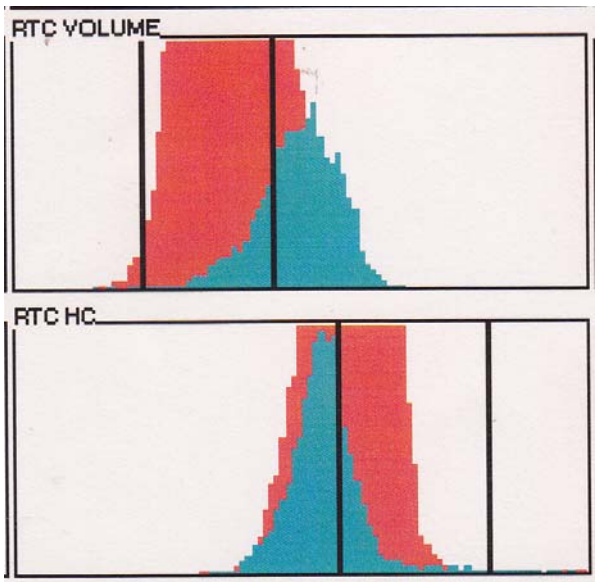


Figure 5. Advia Reticulocyte volume and hemoglobin histograms of first and second analysis (upper and lower respectively) of the same blood sample on May 21st. Erythrocytes are red and reticulocytes are blue. The upper histograms show that a higher percentage of erythrocytes were identified as reticulocytes on the first analysis.



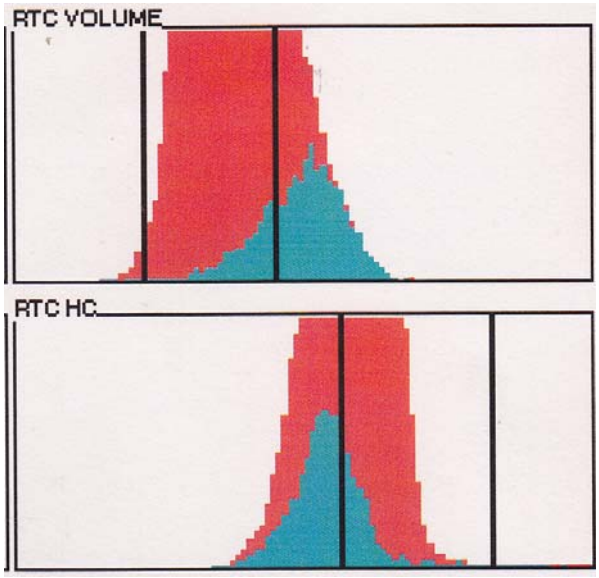


Table 3: Hematology results interpreted to be the most correct, May 21st.

Parameter	Result	Unit	Reference Interval
Hematocrit	0.31	L/L	0.38 - 0.57
Reticulocytes	286	10^9/L	<50
Reticulocytes %	7.6	%	0 - 1.5
WBC	5.2[1]	10^9/L	5.8 - 16
Band neutrophils	0.1	10^9/L	0 - 0.3
Segmented neutrophils	1.4	10^9/L	3 - 11.5
Eosinophils	0.0	10^9/L	0.1 - 1.2
Basophils	0.0	10^9/L	<0.1
Lymphocytes	2.7	10^9/L	1.4 - 4.8
Monocytes	1.1	10^9/L	0.2 - 1.4
Metarubricytes	340	/100 WBC	

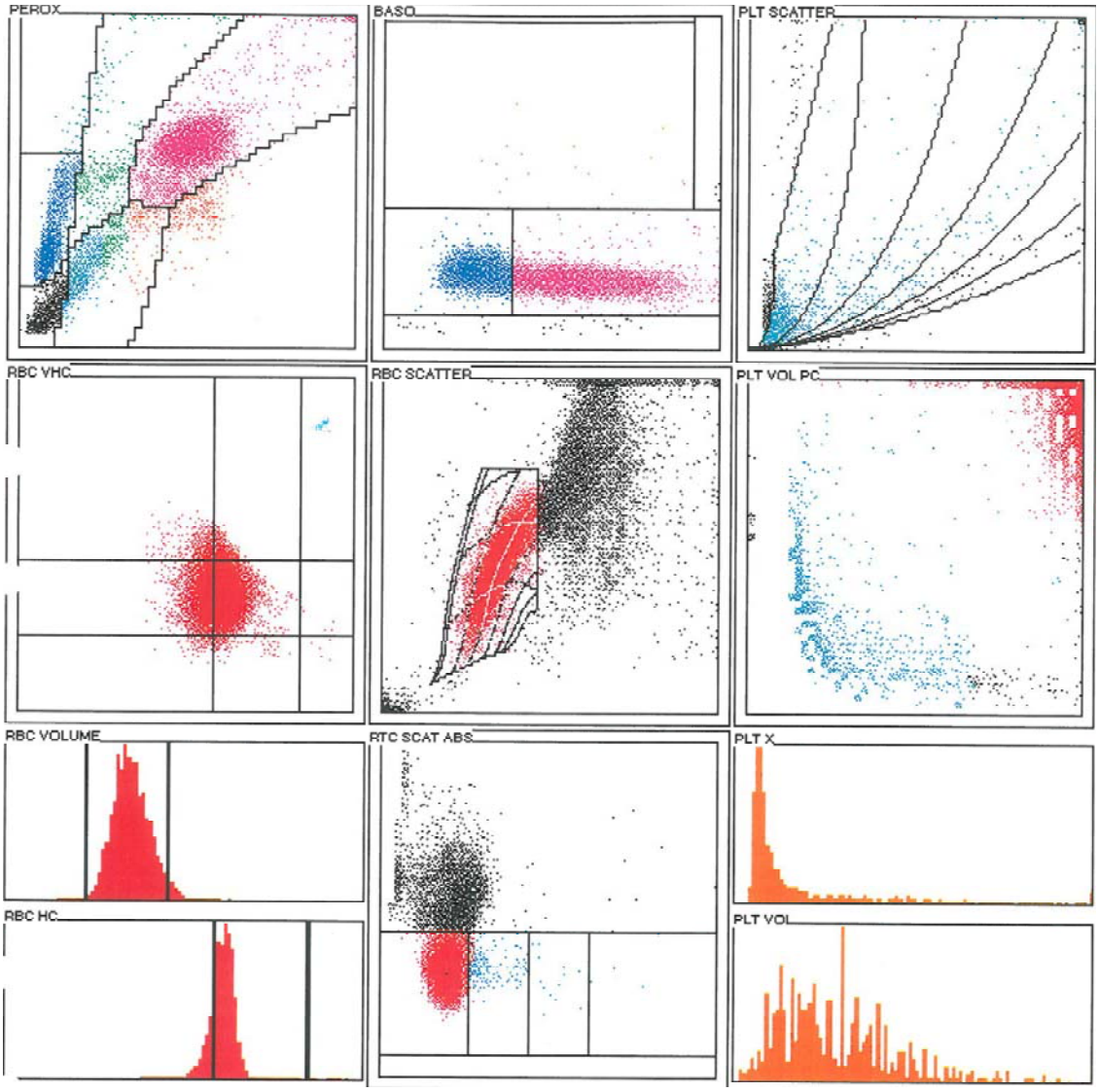
[1]WBCB + Advia basophil count and then corrected for metarubricytes

Table 4: Hematology and serum cobalamin results, July 4th.

Parameter	Result	Unit	Reference Interval
Hematocrit	0.49	L/L	0.38 - 0.57
Reticulocytes	22	10^9/L	<50
Reticulocytes %	0.3	%	0 - 1.5
Platelets	190*	10^9/L	170-490
WBC	8.3	10^9/L	5.8 - 16
Band neutrophils	<0.1	10^9/L	0 - 0.3
Segmented neutrophils	5.1	10^9/L	3 - 11.5
Eosinophils	0.6	10^9/L	0.1 - 1.2
Basophils	0.2	10^9/L	<0.1
Lymphocytes	2.2	10^9/L	1.4 - 4.8
Monocytes	0.2	10^9/L	0.2 - 1.4
Cobalamin	682	pmol/L	135-670

*Marked platelet clumping

Figure 6: Advia 2120 Graphics, July 4th. Patient after treatment. Results are fairly normal except for the artifacts of platelet clumping and swelling of erythrocytes in a mailed in sample.



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