Abnormal WDF and WNR scattergrams from Sysmex XN-V in a dog

Contributors

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Specimen

EDTA whole blood and abdominal effusion

Signalment

4-month-old intact male, Australian shepherd dog

History

The dog was referred to the emergency unit at the veterinary teaching hospital of Toulouse, France, for the medical care of a parvovirus infection diagnosed by a positive SNAP test (SNAP Parvo, Idexx Laboratories, Westbrook, USA) in the context of vomiting and diarrhoea evolving for 2 days.

Clinical findings

Clinical examination revealed pale mucous membranes and palpable fluid accumulation in the abdomen.

A CBC performed at the emergency unit with the ProCyte Dx (Idexx, Westbrook, USA)) (Table1) revealed a marked normocytic normochromic regenerative anemia, and leukocytosis with neutrophilia, monocytosis, and thrombocytopenia with a flag and an increased MPV. The thrombocytopenia was suspected to be true despite the observation of few platelet-fibrin clots on the blood smear.

An abdominal point-of-care ultrasound (POCUS) confirmed the presence of an abdominal effusion. The dog was transfused with compatible blood.

The next day, a complete abdominal ultrasound was performed by a specialist and revealed a large amount of abdominal effusion and a hyperechoic mass with ill-defined contours located between the liver and the stomach and consistent with a hematoma.
Blood and effusion were sampled and analyzed with the Sysmex XN-V (Sysmex, Kobe, Japan) (Figure 1; Tables 1 and 2) and smears were reviewed. Very few platelets with no clumps were observed in blood and effusion. Hemostasis panel was performed on STA Compact Max3 (Stago, Asnières-sur-Seine, France) and was unremarkable (Table 3).

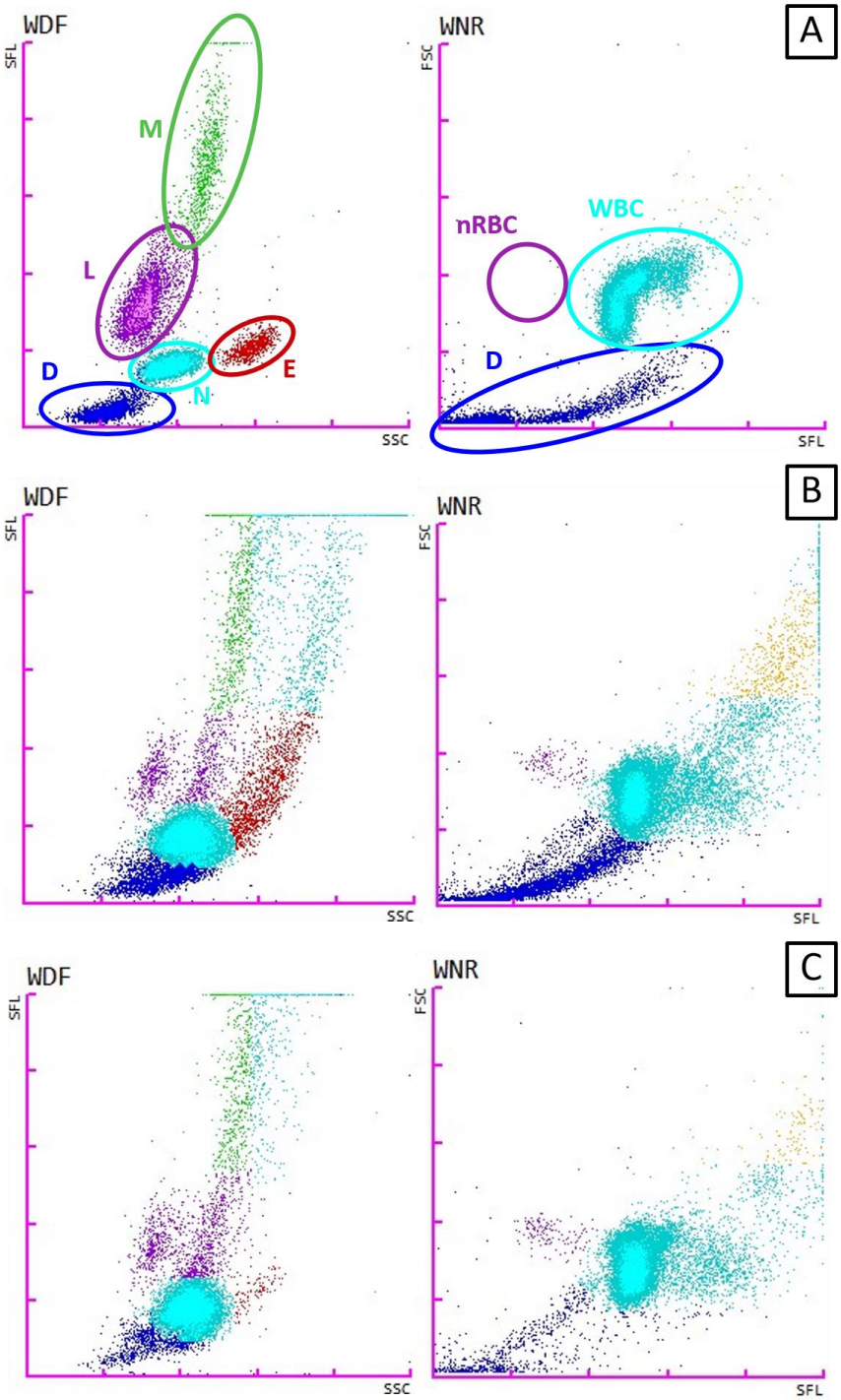

Figure 1 : Sysmex XN-V WBC differential (WDF) and white cell nucleated (WNR) scattergrams of EDTA-blood specimen from a healthy 3-year-old dog (A) and EDTA-blood (B) and EDTA-effusion (C) specimens from a 4-month-old Australian shepherd dog with Parvovirus infection.
Abbreviations: D, debris; E, eosinophils; FSC, forward scatter; L, lymphocytes; M, monocytes; N, neutrophils; nRBC, nucleated red blood cells; SFL, side fluorescence light; SSC, side scatter; WBC, white blood cells

Table 1: Hematological numerical results for EDTA-blood specimens with ProCyte Dx (before transfusion) and Sysmex XN-V (after transfusion) and EDTA-effusion specimen with Sysmex XN-V.

	ProCyte Dx Blood specimen		Sysmex XN-V Blood specimen		Sysmex XN-V Effusion
Variable	Result	RI	Result	RI	Result
RBC ($x10^{12}$/L)	**2.41**	5.65-8.87	**3.46**	5.20-7.90	3.44
HCT (%)	**16.5**	37.3-61.7	**25.6**	35.0-52.0	25.1
HGB (g/dL)	**5.8**	13.1-20.5	**8.5**	12.4-19.2	8.4
MCV (fL)	68.5	61.6-73.5	**74.0**	60.0-71.0	73.0
MCH (pg)	24.1	21.2-25.9	24.6	21.9-26.3	24.4
MCHC (g/dL)	35.2	32.0-37.9	**33.2**	34.4-38.1	33.5
RDW (%)	14.9	13.6-21.7	**12.1**	13.2-19.1	12.0
RET (%)	3.7	-	1.45	-	1.44
Corrected RET (%)	**1.4**	0-1	0.83	0-1	-
RET ($x10^9$/L)	89.4	10.0-110.0	50.2	19.1-150.1	49.5
WBC ($x10^9$/L)	**18.23**	5.05-16.76	18.88[a]	5.60-20.40	17.03[a]
Neutrophils ($x10^9$/L)	**14.72**	2.95-11.64	**15.45**	2.90-13.60	15.12
Lymphocytes ($x10^9$/L)	2.06	1.05-5.10	**0.78**	1.10-5.30	1.01
Monocytes ($x10^9$/L)	**1.35**	0.16-1.12	0.71	0.40-1.60	0.65
Eosinophils ($x10^9$/L)	0.09	0.06-1.23	1.44	0.10-1.50	0.13
Basophils ($x10^9$/L)	0.01	0.00-0.10	0.50	Rare	0.12
PLT[b] ($x10^9$/L)	**2***	148-484	**12***	108-562	5
MPV (fL)	**23.0**	8.7-13.2	7.7*	-	10.5

Bolded values are outside the reference interval (RI).
Abbreviations: HGB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean cell volume; MPV, mean platelet volume; nRBCs, nucleated red blood cells; PLT, platelets; RBC, red blood cells; RET, reticulocytes; RDW, red cell distribution width; WBC, white blood cells; *, error flag.
[a] Leukocyte count obtained with the XN-V analyzer WNR channel
[b] Platelet counts were obtained with the impedance channel with the ProCyte Dx and with the optical channel with the Sysmex XN-V

Table 2: Additional results for abdominal effusion and EDTA-whole blood

Variable	Abdominal effusion	EDTA- whole blood
Macroscopic appearance	Red, opaque	Normal
TNCC[a] ($x10^9$/L)	16.69	-
Total proteins[b] (g/L)	33	-
Packed cell volume[c] (L/L)	25	25
Manual cell differential[c] (%)		
Neutrophils	69	90
Lymphocytes	4	3
Monocytes/Macrophages	27	5
Eosinophils	0	2

Abbreviations: TNCC, Total nucleated cells
[a] Leukocyte count obtained with the XN-V analyzer WDF channel
[b] Obtained on supernatant with a refractometer
[c] Obtained by manual methods

Table 3: Hemostasis panel

Variable	Result	Reference interval
Antithrombin III (%)	111	102 – 191
FDP (mg/L)	< 5	0 – 5
Fibrinogen (g/L)	3.7	1.3 – 4.7
PT (s)	8.0	7.3 – 9.9
aPTT (s)	15.8	12.9 – 16.9

Abbreviations: aPTT, activated partial thromboplastin time; FDP, Fibrin degradation product ; PT, prothrombin time

Questions

1/ Give your interpretation and the most probable cause for the abdominal effusion.

2/ Concerning the CBC performed on Sysmex XN-V (Figure 1), what is the main anomaly in the scattergrams from the case compared to the ones of a healthy dog? What does it imply regarding numerical results?

3/ What could be the cause of the abnormal scattergrams and how would you investigate it?

Interpretation/Diagnosis

Given the macroscopic aspect and the very similar results between effusion and blood, effusion was interpreted as hemoperitoneum. Few platelets were observed in the hemoperitoneum which is in favor of an active phenomenon and/or iatrogenic blood contamination.
Given the results of the hemostasis panel, the most probable cause in this case was hemoperitoneum secondary to the severe thrombocytopenia which is itself probably secondary to parvovirus infection.

The main anomaly in scattergrams from Sysmex XN-V is the presence of abnormal additional clusters on both WDF and WNR.
On the WDF scattergram of the blood specimen (Figure 1.B), an abnormal additional banana-shaped cluster was seen. It reached from the debris at the lower left of the scattergram, crossed the neutrophil cluster and extended to the top right of the scattergram. It was mostly identified as eosinophils and partially as neutrophils and debris by the analyzer, thus the differential count given by the analyzer is erroneous with an overestimation of the eosinophils.
On the WNR scattergram (Figure 1.B), an abnormal banana-shaped cluster was also observed ranging from the debris and unlysed RBC cluster, crossing the WBC cluster, and reaching the upper right of the scattergram. The cluster was partially recognized as WBC by the analyzer resulting in a probable overestimation of the WBC count.
This interference could be secondary to the presence of abnormal cells, abnormal cell distribution or an interfering substance of unknown origin.

Additional information

During hospitalization, two additional CBCs performed with the Sysmex XN-V revealed no abnormal clusters. On the blood smear of the specimen with the abnormal scattergrams, a bright purple granular amorphous material was present in the background or superposed on blood cells (Figure 2). This material closely resembles the ultrasound gel which is occasionally seen on cytologic preparations in our laboratory.

To confirm our hypothesis, we added 1µL of ultrasound gel (Supragel, LCH medical product, Paris, France) to 1mL of a fresh canine EDTA-blood specimen left over from a blood donation.
A CBC and a smear examination of both the unaltered specimen and the specimen mixed with the gel were performed. The CBC of the unaltered blood specimen revealed a mild erythrocytosis and

examination of the blood smear only revealed few small platelet aggregates. However, a banana-shaped cluster very similar to the one described in our case was observed on both WDF and WNR scattergrams (Figure 3) of the specimen mixed with gel, resulting in a slight overestimation of WBC and eosinophil counts. The other scattergrams were normal. On the smear, a granular material similar to the one observed in our case was visualized, as expected (Figure 4).

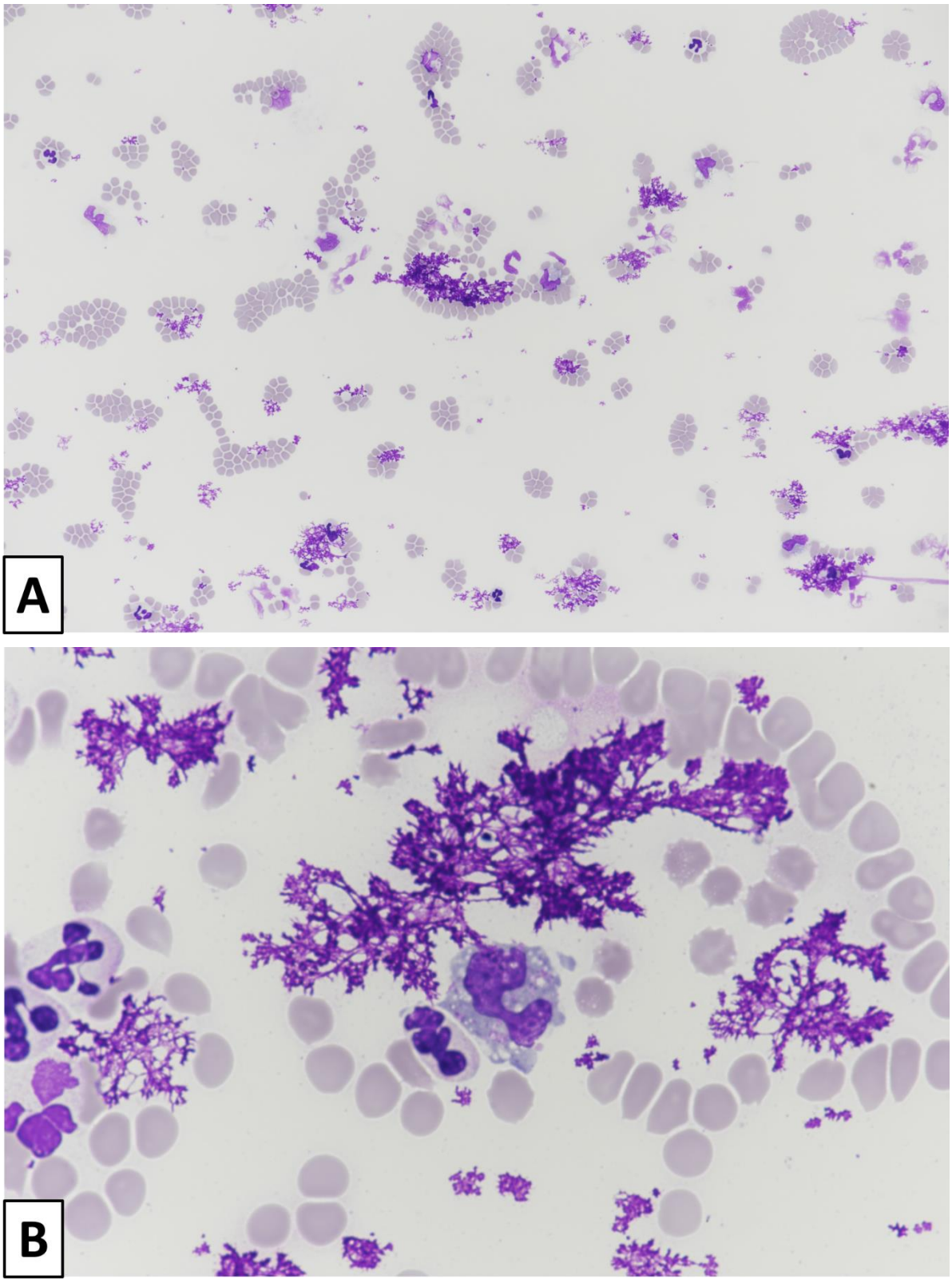

Figure 2: Peripheral blood smear from a 4-month-old Australian shepherd dog with parvovirus infection and hemoperitoneum suspected to be secondary to a thrombocytopenia. Magnifications 200x (A) and 1000x (B), May-Grünwald Giemsa.

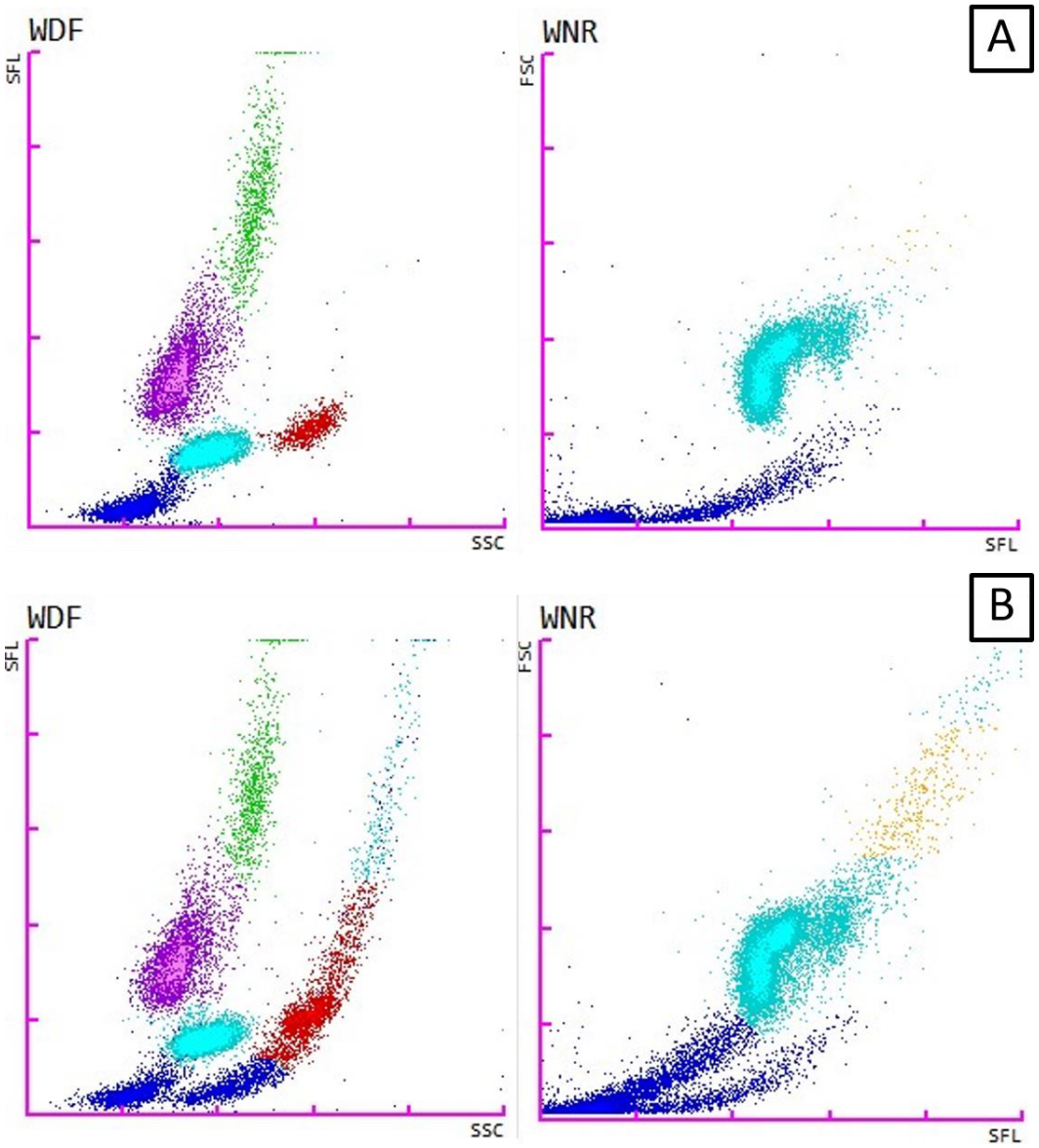

Figure 3: Sysmex XN-V WBC differential (WDF) and white cell nucleated (WNR) scattergrams from unaltered EDTA-blood specimen (A) and 1mL of the same EDTA-blood specimen mixed with 1µL of ultrasound gel (Supragel) (B) obtained with leftovers from a blood donation by/from a healthy 3-year-old dog.

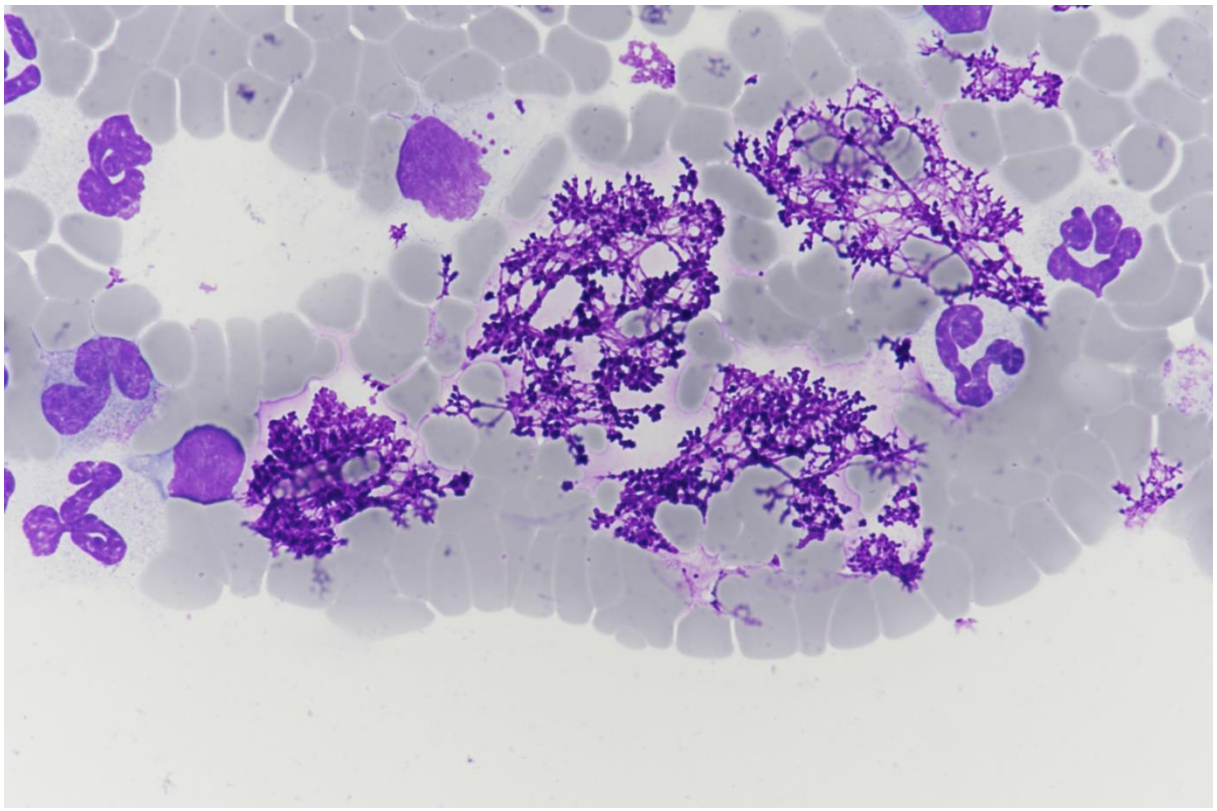

Figure 4: Peripheral blood smear from EDTA-blood specimen mixed with 1µL of ultrasound gel obtained with leftovers from a blood donation from a healthy dog. Magnification 1000x, May-Grünwald Giemsa.

Follow up and clinical outcome

The dog was discharged 5 days after presentation, due to significant clinical improvement. A CBC at time of discharge revealed a persistent severe thrombocytopenia and a mild monocytosis, and no abnormal cluster was present.

The dog was discharged with antibiotic therapy, a recheck appointment was recommended but the dog was lost to follow-up.

Discussion

To our knowledge, there are only few reports of interfering factors on Sysmex WDF and WNR scattergrams in human and veterinary medicine.

Grebert et al. described modifications of WNR and/or WDF scattergrams in dogs with abnormal leukocytes (band cells and acute leukemias) leading to an extended cluster from the modified population and arbitrary separation of clusters on the WDF scattergram, rather than the presence of an additional banana-shaped cluster originating from debris and unlysed RBC[1].

A similar banana-shaped pattern on the WDF scattergram was observed using the Sysmex XN-1000 in body fluid mode in a cerebrospinal fluid specimen from a human patient. This patient had lymphoma metastasized to the brain and was treated with intrathecal DepoCyt®, a drug composed of cytarabine in liposomal particles[2]. After experimentally mixing DepoCyt® with blood, the authors suggested that liposomal particles are similar in size, have similar physical properties to WBCs, and fall into similar areas of WDF scattergram, overestimating cell count.

Another banana-shaped cluster was also observed with the Sysmex XN-9000 in blood specimens from two human patients with circulating giant platelets and megakaryocytes secondary to type 2-refractory anemia with excess blasts[3].

Layssol-Lamour et al. also reported a banana-shaped cluster using the ProCyte Dx in mice and rats with prominent platelet aggregation[4]. This is routinely observed by the authors with the Sysmex XN-V and is usually more prominent in cats and goats than in dogs, but never as pronounced as in this case (personal observation). In the validation study of the Sysmex XN-V in dogs, no interference with platelet aggregation was described[1].

In our case, none of the previously reported interference were present. Moreover, the other CBCs performed did not show interference or granular material on the smears. Experimental contamination confirmed that the interference was secondary to specimen contamination with ultrasound gel or a similar substance. Indeed, we can also imagine that this kind of interference could be seen with a contamination of a specimen with another gel such as lubricating gel used for catheterization or antibacterial gel for example. Because the blood was collected after an ultrasound examination and a very small amount of gel can cause significant interference, contamination of equipment (e.g., gloves used due to the infectious status of the patient) is suspected to have caused the specimen contamination, probably by contaminating the sampling area or material.

While some ultrasound gels have clearly been associated with artifactual structures on slides stained with Romanowsky stains, not all have[5]. It would be interesting to know if other gels cause interference with the Sysmex XN-V scattergrams and WBC counts as observed in this case.

This case is the first report of a banana-shaped additional cluster on WDF and WNR scattergrams of Sysmex XN-V secondary to a contamination of canine blood with ultrasound gel. This emphasizes the fact that in case of abnormal clusters, blood smear examination is mandatory to validate the analyzer's results, and that ultrasound gel must be carefully removed before sampling a biological fluid to avoid potential interference leading to erroneous white blood cell differential and spuriously high WBC counts with the Sysmex XN-V.

References

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