Abnormal peroxidase scatterplot and marked large unclassified cell counts on the ADVIA 2120i in a mixed-breed dog

Contributors

Alessandro Tirolo[1], Oriana Falcone[1], **Greta Colombo[1]**, Saverio Paltrinieri[2], Maria Chiara Sabetti[1]

[1]Department of Veterinary Science, University of Parma, Parma, Italy

[2]Department of Veterinary Science, University of Milan, Milan, Italy

Greta Colombo – greta.colombo@unipr.it

Specimen

EDTA whole blood

Signalment

Mixed-breed dog, 13-year-old, castrated male

History

The patient was evaluated after acute smoke exposure from a residential fire. The dog developed acute respiratory distress syndrome (ARDS) secondary to smoke inhalation requiring intensive care; the condition resolved with appropriate treatment. Previous medical history included a mild increase in hepatic transaminase activity managed with nutraceutical therapy. Five months after initial presentation, the dog was re-admitted for recurrence of dyspnea.

Clinical findings

At the initial presentation (Day 1, following smoke exposure), the dog exhibited severe inspiratory dyspnea accompanied by obtunded mentation and marked mucosal cyanosis.

Arterial blood gas analysis identified severe carboxyhemoglobinemia (18.7%). Three hours after admission, following oxygen supplementation via nasal cannula (estimated FiO_2 0.5), repeat arterial blood gas analysis showed a reduction in carboxyhemoglobin concentration to 7,6% and a ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2) of 168 mmHg. In conjunction with the acute smoke inhalation injury, diffuse pulmonary infiltrates identified by thoracic imaging and exclusion of cardiogenic pulmonary edema these findings fulfilled the updated ARDS Vet criteria for non intubated mild/moderate ARDS (PaO_2/FiO_2 >100 to ≤300 mmHg) (Balakrishnan et al., 2025).

Point-of-care ultrasound (POCUS) and thoracic radiographs revealed a severe interstitial pulmonary pattern, which progressed during the initial hours of hospitalization. Supplemental oxygen therapy was initially administered via nasal cannula; however, persistent hypoxemia prompted escalation to non-invasive ventilation using continuous positive airway pressure (CPAP) (positive end-expiratory pressure (PEEP) 5–7 cmH_2O, FiO_2 100%). Following initiation of CPAP, the dog demonstrated rapid improvement in carboxyhemoglobin concentration, accompanied by a slower but progressive clinical recovery, paralleled by serial improvement in both POCUS findings and thoracic radiographic abnormalities. The patient was successfully weaned from CPAP after 36 hours and was discharged from the hospital four days later.

A complete blood cell count (CBC) was performed using an ADVIA 2120i (Siemens Healthineers, Germany) on five occasions over a 5-month period (Day 1–3, Week 6, and Month 5). Results from the initial presentation (Day 1, following smoke exposure) are summarized in Table 1; results from subsequent evaluations are summarized in Table 2.

On all five evaluations, the automated WBC differential showed a markedly elevated large unclassified cell (LUC) count (range: 26.1–75.3%). The peroxidase (Perox) scatterplot demonstrated a downward shift of the neutrophil population into the LUC, monocyte and

lymphocyte areas, whereas the basophil/lobularity (Baso) channel showed a normal leukocyte distribution (Figure 1).

Manual blood film examination revealed markedly discrepant neutrophil counts: true segmented neutrophil percentages ranged from 61% to 76%, in stark contrast to the 0.1–1.7% reported by the analyzer. Mild to moderate neutrophil toxic changes were present during both hospitalizations (initial admission and the Month-5 readmission) (Figure 2). In addition to the neutrophil toxic changes, blood film review revealed reactive (activated) lymphocytes on several evaluations and occasional activated monocytes at the Day 2 and Month-5 timepoints, graded semiquantitative as detailed in the table footnotes. No dysplastic or atypical features were observed in any leukocyte population.

Table 1. Automated CBC (ADVIA 2120i; Siemens Healthineers, Germany) and manual differential results from Day 1 evaluation.

Parameters	RI	Automated CBC (ADVIA2120i)	Manual differential
WBC (×10³/µL)	*5.0–14.1*	9.34	—
Metamyelocytes (%)	—	—	0
Band neutrophils (%)	—	—	2
Segmented neutrophils (%)	—	**0.8**	**67**
Lymphocytes (%)	—	**67.8**	**15**
Monocytes (%)	—	**1.9**	**15**
Eosinophils (%)	—	3.2	1
Basophils (%)	—	0.2	0
LUC (%)	*0–3.5*	**26.1**	N/A
Metamyelocytes (×10³/µL)	*0*	—	0
Band neutrophils (×10³/µL)	*0–0.3*	—	0.19
Segmented neutrophils (×10³/µL)	*2.9–11.1*	**0.08**	6.26
Lymphocytes (×10³/µL)	*1.0–4.8*	**6.33**	1.40
Monocytes (×10³/µL)	*0.15–1.35*	0.18	**1.40**
Eosinophils (×10³/µL)	*0.1–1.25*	0.30	**0.09**
Basophils (×10³/µL)	*0–0.1*	0.02	0
LUC (×10³/µL)	*0–0.4*	**2.43**	N/A
MPXI	—	22.5	—
RBC (×10⁶/µL)	*5.5–8.5*	8.08	—
HGB (g/dL)	*12.0–18.0*	16.5	—
HCT (%)	*37–55*	52.6	—
MCV (fL)	*62–72*	65.1	—
PLT (×10³/µL)	*170–400*	416	—
RBC morphology	—	—	WNL
WBC morphology	—	—	Act. lymph; neutro tox +/−−
PLT morphology	—	—	Adequate; clumps

Abbreviations: *Act. lymph = activated lymphocytes; HGB, hemoglobin; HCT, hematocrit; LUC, large unclassified cell; MCV, mean cell volume; MPXI = myeloperoxidase index; neutro tox = neutrophil toxic change; N/A = not applicable; PLT, platelet count; RBC, red blood cell count; WBC, white blood cell count; WNL = within normal limits. Morphological findings were graded semiquantitative according to the proportion of affected cells: +/−− (grade 1) = 25–50% of cells affected; ++/− (grade 2) = 50–75%; +++ (grade 3) = >75%. Findings below 25% were recorded as absent/occasional.*

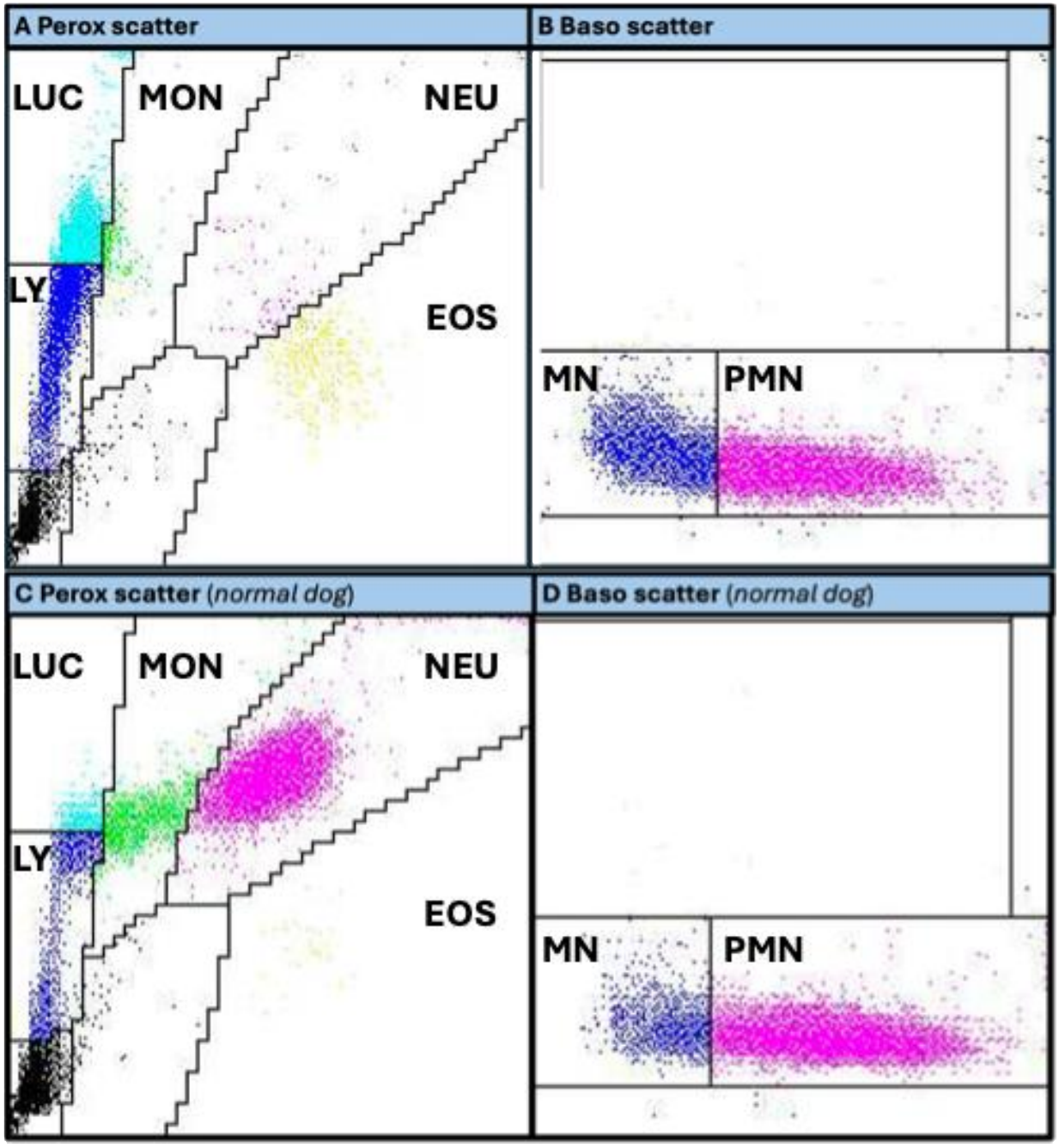
Table 2. Automated CBC (ADVIA 2120i; Siemens Healthineers, Germany) and manual differential results from four subsequent evaluations (Day 2, Day 3, Week 6, and Month 5).

Parameter	RI	Automated CBC (ADVIA2120i)				Manual differential			
		Day 2	Day 3	Week 6	Month 5	Day 2	Day 3	Week 6	Month 5
WBC (×10³/µL)	*5.0–14.1*	**18.20**	**17.37**	9.56	13.89	—	—	—	—
Metamyelocytes (%)	—	—	—	—	—	0	0	0	1
Band neutrophils (%)	—	—	—	—	—	17	4	0	3
Segmented neutrophils (%)	—	0.1	0.2	0.6	1.7	63	76	76	76
Lymphocytes (%)	—	27.1	22.9	37.2	28.9	9	8	10	15
Monocytes (%)	—	0.3	0.6	0.2	1.3	10	10	6	2
Eosinophils (%)	—	0.0	0.6	4.7	4.2	1	2	8	3
Basophils (%)	—	0.3	0.4	0.2	0.3	0	0	0	0
LUC (%)	*0–3.5*	**72.2**	**75.3**	**57.1**	**63.6**	N/A	N/A	N/A	N/A
Metamyelocytes (×10³/µL)	*0*	—	—	—		0	0	0	**0.13**
Band neutrophils (×10³/µL)	*0–0.3*	—	—	—	—	**3.09**	**0.69**	0	0.39
Segmented neutrophils (×10³/µL)	*2.9–11.1*	**0.02**	**0.03**	**0.05**	**0.23**	**11.47**	**13.20**	7.27	10.10
Lymphocytes (×10³/µL)	*1.0–4.8*	**4.93**	3.98	3.56	4.01	1.64	1.40	**0.96**	1.99
Monocytes (×10³/µL)	*0.15–1.35*	**0.05**	**0.10**	**0.02**	0.19	**1.82**	**1.74**	0.57	0.26
Eosinophils (×10³/µL)	*0.1–1.25*	**0.01**	0.10	0.45	0.59	0.18	0.35	0.76	0.39
Basophils (×10³/µL)	*0–0.1*	0.06	0.08	0.01	0.04	0	0	0	0
LUC (×10³/µL)	*0–0.4*	**13.13**	**13.07**	**5.46**	**8.83**	N/A	N/A	N/A	N/A
MPXI	—	**3.2**	**24.0**	**20.8**	**16.8**	—	—	—	—
RBC (×10⁶/µL)	*5.5–8.5*	6.83	6.82	7.23	7.10	—	—	—	—
HGB (g/dL)	*12.0–18.0*	14.4	14.2	15.4	15.3	—	—	—	—
HCT (%)	*37–55*	46.1	45.0	47.2	48.9	—	—	—	—
MCV (fL)	*62–72*	67.4	66.0	65.3	68.8	—	—	—	—
PLT (×10³/µL)	*170–400*	396	395	406	414	—	—	—	—
RBC morphology	—	—	—	—	—	Aniso +/−−	Aniso +/−−	Aniso +/−−	Aniso +/−−
WBC morphology	—	—	—	—	—	Act. lymph; neutro tox ++/−; act. mono +/−−	neutro tox +/−−	WNL	Act. lymph; neutro tox ++/−; act. mono +/−−
PLT morphology	—	—	—	—	—	Adequate; clumps	Adequate; clumps	Adequate	Adequate; clumps

Abbreviations: *Act. lymph = activated lymphocytes; Act. mono = activated monocytes; Aniso = anisocytosis; HGB, hemoglobin; HCT, hematocrit; LUC, large unclassified cell; MCV, mean cell volume; MPXI = myeloperoxidase index; neutro tox = neutrophil toxic change; N/A = not applicable; PLT, platelet count; RBC, red blood cell count; WBC, white blood cell*

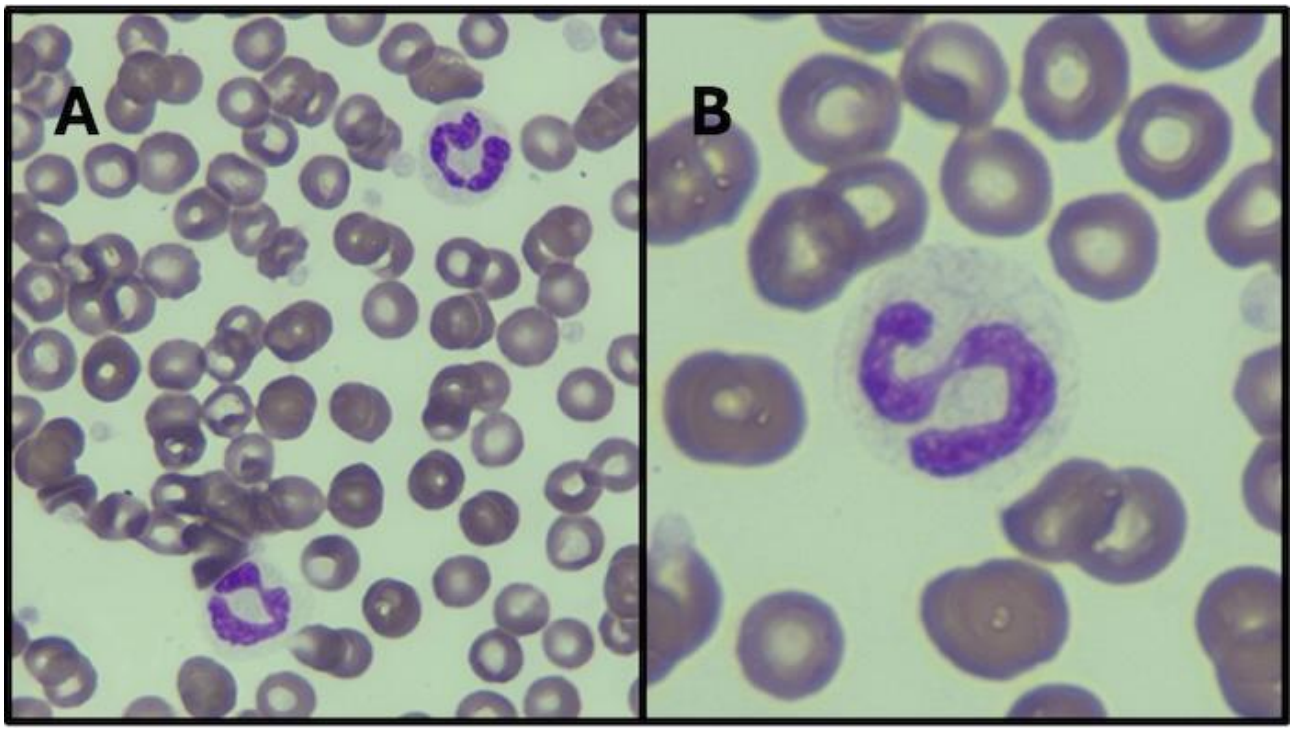
count; WNL = within normal limits. Morphological findings were graded semiquantitative according to the proportion of affected cells: +/−− (grade 1) = 25–50% of cells affected; ++/− (grade 2) = 50–75%; +++ (grade 3) = >75%. Findings below 25% were recorded as absent/occasional.

Figure 1. Representative ADVIA 2120i scatterplots from the affected dog (Day 1, following smoke exposure) and normal dog.



ADVIA 2120i leukocyte scatterplots from the affected dog (A, B) and a healthy control dog (C, D). In the affected dog, the Perox channel (A) shows loss of the normal neutrophil cluster, with downward displacement into the low-peroxidase region and marked expansion of the LUC area, whereas the Baso channel (B) remains unremarkable. In the control dog, both the Perox (C) and Baso (D) scatterplots show normal leukocyte distribution. Abbreviation: LUC = large unclassified cells, LY = lymphocytes, MON = monocytes, NEU = neutrophils, EOS = eosinophils, MN = mononuclear, PMN = polymorphonuclear.

Figure 2. Segmented neutrophils with mild toxic changes at blood smear (Day 1, following smoke exposure).



Blood film from the affected dog (May-Grünwald Giemsa stain, ×100 oil immersion objective; panel B is a higher-magnification detail). Segmented neutrophils display normal nuclear segmentation with mild cytoplasmic toxicity, evidenced by slight basophilia and vacuolation.

Questions

1 - How do you explain the striking discordance between the automated neutrophil count (0.1–1.7%) and the manual differential (61–76%) across all five evaluations? What analytical or pathological mechanism could account for this finding?

2 - The ADVIA 2120i uses two independent optical channels to classify leukocytes: the Perox channel and the Baso channel. In this case, one channel is grossly abnormal while the other remains normal. What does this dissociation tell you about the underlying mechanism, and what is its diagnostic significance?

3 - Based on the clinical presentation, the CBC findings, and the scatterplot pattern, what is your diagnosis and what confirmatory test would you recommend?