

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

Contributors

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Answers to 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?](#)

The near-complete absence of neutrophils in the automated differential, contrasting with a true neutrophil percentage of 61–76% on manual review, is explained by a functional defect in myeloperoxidase (MPO) activity (Siraki 2021). The ADVIA 2120i peroxidase (Perox) channel classifies leukocytes by staining cells with an MPO substrate and measuring the resulting colorimetric signal. In the absence of functional MPO, neutrophils take up the substrate but cannot oxidize it, generating no signal; they therefore migrate into the low-peroxidase region of the scatterplot overlapping with monocyte and lymphocyte gates, and are reported as large unclassified cells (LUC). This artifact results in a spurious severe neutropenia, which, if unrecognized, could prompt unnecessary antimicrobial treatment or invasive diagnostic workup.

[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?](#)

The ADVIA 2120i classifies leukocytes through two independent channels. The Perox channel relies on MPO-mediated substrate oxidation, while the Baso channel classifies cells by nuclear volume and resistance to acid lysis, entirely independently of peroxidase activity. In this case, the Perox channel is grossly abnormal (LUC 26–75%) while the Baso channel remains completely normal, correctly resolving all leukocyte populations. This Perox/Baso dissociation is the instrumental signature of myeloperoxidase deficiency (MPOD), hereditary or not, on the ADVIA platform. Recognizing it immediately redirects the clinician toward manual blood film review and genetic testing and prevents misinterpretation of the CBC.

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

The Perox/Baso dissociation pattern is indicative of MPOD in general, which can be acquired or hereditary. In this case, the persistence of the pattern across five evaluations and, crucially, after complete resolution of the acute inflammatory disease, was the key observation that raised suspicion for the hereditary form and prompted genetic testing.

The diagnosis is therefore hereditary myeloperoxidase deficiency (HMD). The confirmatory test is genetic sequencing of the MPO gene. In this case, sequencing identified a homozygous c.1936C>T variant, consistent with the pathogenic nonsense mutation previously described in dogs with HMD (Gentilini et al. 2016).

Interpretation/Diagnosis

Hereditary myeloperoxidase deficiency, confirmed by identification of a homozygous c.1936C>T pathogenic variant in the MPO gene. This is the first genetically confirmed case reported in a mixed-breed dog.

Follow up and clinical outcome

The dog was discharged after four days of hospitalization, with serial follow-up examinations demonstrating progressive and complete radiographic resolution of the pulmonary interstitial pattern. Five months later, the dog was re-admitted for hyperacute progressive dyspnea. Diagnostic imaging revealed a non-cardiogenic pulmonary edema pattern consistent with suspected vasculitis, although pneumonia could not be excluded. Despite intensive treatment, including empirical antimicrobial therapy, the patient developed cardiocirculatory arrest and died within a few hours. Post-mortem examination was declined by the owners.

Discussion

Mechanism of detection on the ADVIA 2120i

In health, neutrophils express high MPO activity and occupy well-defined clusters in the Perox scatterplot, while monocytes, which also contain MPO at lower levels, form a separate cluster. In HMD, the absence of functional MPO prevents colorimetric signal generation: neutrophils produce no peroxidase signal and migrate downward in the scatterplot, overlapping with the monocyte and lymphocyte gates, and are consequently reported as LUC.

Because the Baso channel is peroxidase-independent, it continues to resolve leukocyte populations correctly. The resulting dissociation, a grossly abnormal Perox scatterplot alongside a normal Baso pattern, is therefore a highly characteristic instrumental finding of MPOD on the ADVIA platform.

Role of the MPXI

The myeloperoxidase index (MPXI), an instrument-derived parameter quantifying MPO staining intensity, has been proposed as a screening tool for MPOD. Although markedly negative in humans with total HMD on older Bayer-Technicon systems (Lanza 1998), its diagnostic utility is both species- and platform-dependent. On the ADVIA 120, MPXI showed wide variability and

considerable overlap with the reference interval in dogs with MPOD (Klenner et al. 2010). On the ADVIA 2120i, MPXI remains within the normal range even in cases of total MPOD, both hereditary and acquired (Gleghorn et al. 2020), as confirmed in this case (range: 3.2–24.0). These findings confirm that MPXI is not a reliable screening criterion for HMD on the ADVIA 2120i, and that recognition of the Perox/Baso dissociation combined with manual blood film review remains essential.

Acquired versus hereditary MPO deficiency: clinical discriminants

The Perox/Baso dissociation pattern is not exclusive to the hereditary form of MPO deficiency. A secondary, acquired form has been described in association with a wide range of conditions in humans, including diabetes mellitus, pregnancy, iron deficiency, lead poisoning, renal transplantation, severe infections, cytotoxic agents, and hematological neoplasms such as acute and chronic myeloid leukemia, myelodysplastic syndrome, polycythemia vera, and Hodgkin lymphoma (Lanza 1998). In veterinary medicine, acquired MPO deficiency has been documented in dogs with lead intoxication (Caldwell 1979) and MPO consumption during severe systemic disease, such as babesiosis, has been proposed as a potential mechanism for transient MPO depletion in canine neutrophils (Celliers et al. 2020). Unlike the hereditary form, acquired MPO deficiency is typically partial, affecting only a fraction of neutrophils, and transient, resolving once the underlying condition improves (Klenner et al. 2010). In this case, the persistence and completeness of the pattern after clinical recovery directed the workup toward genetic testing.

Differential diagnosis of LUC elevation

On the ADVIA platform, elevated LUC counts may reflect a variety of conditions beyond MPO deficiency, including the presence of reactive or atypical lymphocytes, circulating blasts, and left shift, all of which can cause cell populations to spill into the LUC gate of the Perox

scatterplot (Stirn et al. 2014). The key discriminating feature of HMD is the Perox/Baso dissociation: in neoplastic or reactive conditions causing LUC elevation, the Baso channel is also expected to show abnormalities, such as a blast nose or dispersion of cells into the lyse-resistant area, whereas in HMD it remains entirely normal (Stirn et al. 2014). Furthermore, in HMD the cells identified as LUC on manual blood film review correspond exclusively to morphologically normal neutrophils, without any atypical or blast cells, confirming the purely artifactual nature of the finding.

Analyzer-dependent nature of the artifact

The Perox/Baso dissociation described in this report is specific to hematology analyzers that classify leukocytes using a peroxidase-based optical channel, such as the ADVIA platform (Stirn et al. 2014). Analyzers belonging to the Sysmex XN series, in contrast, classify leukocytes in the white blood cell differential channel (WDF) channel through fluorescent staining of nucleic acids and light scatter parameters, entirely independently of peroxidase activity (Bauer et al. 2012); MPO deficiency would therefore not be expected to cause neutrophil misclassification on these platforms. This has important practical implications: a dog with HMD analyzed on a Sysmex instrument would likely yield a normal automated differential, with no flag and no prompt for manual review, potentially delaying recognition of the underlying condition entirely. Conversely, on the ADVIA, the artifact itself becomes the diagnostic clue. Awareness of this platform-specific behavior is therefore essential for any laboratory using peroxidase-based analyzers.

Clinical significance and breed considerations

HMD in dogs was previously linked to a nonsense MPO mutation originally described in Italian Hounds (Gentilini et al. 2016). The identification of the same pathogenic variant in a mixed-breed dog raises the possibility that HMD may occur beyond this breed; however, Italian Hound

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ancestry cannot be excluded given the unknown genetic background of mixed-breed dogs (Gentilini et al. 2016, Pallotti et al. 2022).

Throughout the 5-month observation period, no infectious complications or immune dysfunction were recorded, consistent with the subclinical phenotype reported in previous canine cases (Caldwell et al. 1979, Gentilini et al. 2016). The recurrence of dyspnea and the fatal outcome five months after the initial presentation warrant careful consideration as to whether they might reflect an infectious complication or an underlying immune dysfunction attributable to MPOD. Several lines of reasoning argue against this interpretation. First, diagnostic imaging at readmission was consistent with a non-cardiogenic pulmonary edema pattern suggestive of vasculitis rather than with a primary infectious pneumonia, although the latter could not be entirely excluded and post-mortem examination was declined by the owners. Second, MPOD in dogs is generally regarded as a subclinical laboratory finding rather than a clinically significant immunodeficiency, because MPO-independent microbicidal mechanisms, primarily the NADPH oxidase system, provide effective compensation through an increased and prolonged oxidative burst with greater hydrogen peroxide accumulation (Klebanoff et al. 1971, Klenner et al 2010, Kutter et al. 1998, Stendahl et al. 1984). Third, and consistent with this, the dog had reached 13 years of age with no prior history of recurrent or opportunistic infections; a clinically meaningful immunodeficiency would be expected to manifest with a pattern of recurrent infections well before this age. Taken together, these considerations indicate that the terminal event is more plausibly explained by a process unrelated to the MPOD and support the view of canine HMD as a subclinical trait rather than a cause of clinical immunocompromise.

Recommended diagnostic approach

When the characteristic Perox/Baso dissociation pattern is encountered, three steps are recommended:

1. Manual blood film examination, which is indispensable to establish true leukocyte counts, exclude genuine neutropenia, and identify any additional morphological findings.
2. Genetic testing of the MPO gene for definitive confirmation.
3. Breeding counseling, where applicable, given the autosomal recessive mode of inheritance.

References

1. Balakrishnan, A., Silverstein, D. C., Bedenice, D., Bersenas, A., Bourgeois, J. P., Carroll, C. L., ... & Yehya, N. (2025). Acute Respiratory Distress Syndrome in Veterinary Medicine—The ARDSvet Definitions. *Journal of Veterinary Emergency and Critical Care*, 35(4), 327-338.
2. Bauer, N., Nakagawa, J., Dunker, C., Failing, K., & Moritz, A. (2012). Evaluation of the automated hematology analyzer Sysmex XT-2000 i V™ compared to the ADVIA® 2120 for its use in dogs, cats, and horses. Part II: Accuracy of leukocyte differential and reticulocyte count, impact of anticoagulant and sample aging. *Journal of Veterinary Diagnostic Investigation*, 24(1), 74-89.
3. Caldwell, K. C., Taddeini, L., Woodburn, R. L., Anderson, G. L., & Lobell, M. (1979). Induction of myeloperoxidase deficiency in granulocytes in lead-intoxicated dogs.
4. Celliers, A., Rautenbach, Y., Hooijberg, E., Christopher, M., & Goddard, A. (2020). Neutrophil myeloperoxidase index in dogs with babesiosis caused by *Babesia rossi*. *Frontiers in veterinary science*, 7, 72.
5. Gentilini, F., Zambon, E., Mancini, D., & Turba, M. E. (2016). A nonsense mutation in the myeloperoxidase gene is responsible for hereditary myeloperoxidase deficiency in an Italian hound dog. *Animal Genetics*, 47(5).
6. Gleghorn, D. M., & Thomas, W. (2020). Prevalence of myeloperoxidase deficiency determined using an ADVIA 2120i. *International Journal of Laboratory Hematology*, 42(5).

7. Klebanoff, S. J., & Pincus, S. H. (1971). Hydrogen peroxide utilization in myeloperoxidase-deficient leukocytes: a possible microbicidal control mechanism. *The Journal of Clinical Investigation*, 50(10), 2226-2229.
8. Klenner, S., Richartz, J., Bauer, N., & Moritz, A. (2010). Myeloperoxidase deficiency in dogs observed with the ADVIA® 120. *Tierärztliche Praxis Ausgabe K: Kleintiere/Heimtiere*, 38(03), 139-146.
9. Kutter, D. (1998). Prevalence of myeloperoxidase deficiency: population studies using Bayer-Technicon automated hematology. *Journal of molecular medicine*, 76(10), 669-675.
10. Lanza, F. (1998). Clinical manifestation of myeloperoxidase deficiency. *Journal of molecular medicine*, 76(10), 676-681.
11. Pallotti, S., Piras, I. S., Marchegiani, A., Cerquetella, M., & Napolioni, V. (2022). Dog-human translational genomics: state of the art and genomic resources. *Journal of Applied Genetics*, 63(4), 703-716.
12. Siraki, A. G. (2021). The many roles of myeloperoxidase: from inflammation and immunity to biomarkers, drug metabolism and drug discovery. *Redox biology*, 46, 102109.
13. Stendahl, O., Coble, B. I., Dahlgren, C., Hed, J., & Molin, L. (1984). Myeloperoxidase modulates the phagocytic activity of polymorphonuclear neutrophil leukocytes. Studies with cells from a myeloperoxidase-deficient patient. *The Journal of clinical investigation*, 73(2), 366-373.
14. Stirn, M., Moritz, A., & Bauer, N. (2014). Rate of manual leukocyte differentials in dog, cat and horse blood samples using ADVIA 120 cytograms. *BMC veterinary research*, 10(1), 125.