Myeloperoxidase deficiency in dogs observed with the ADVIA®120
A retrospective study

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Key words
Myeloperoxidase Index, MPXI, neutrophils, hematology, canine, WBC, leukocytes

Summary
Objective: In contrast to humans, neutrophil myeloperoxidase deficiency (MPOD) has been rarely investigated in dogs. The hematology analyzer ADVIA®120 differentiates leukocytes based on the cellular volume and their myeloperoxidase concentration. The aim of this study was the characterization of myeloperoxidase deficiency in dogs and the evaluation of the diagnostic use of the ADVIA®120 Myeloperoxidase Index (MPXI).

Material and methods: ADVIA® peroxidase scatter plots indicative of MPOD were reviewed. Severity of MPOD was classified semiquantitatively in three groups (MPOD grade 1–3): MPOD grade 1 (MPOD-1): neutrophils showing an abnormal shift of the population, <25% extending in the monocyte cluster and therefore misclassified, MPOD-2: ~25–50% of neutrophils classified, MPOD-3: 50–100% of the neutrophils misclassified due to their location in the monocyte cluster. Sex, age, and breed of the dogs as well as diagnosis, and MPXI were recorded. Results: 29 dogs (nine females and 20 males belonging to 23 breeds) with 38 analyses consistent with MPOD were found. Diseases were characterized by severe leukocyte consumption and included mainly parvovirose (8/29), DIC/sepsis (3/29), pyometra, pyothorax, pneumonia, pancreatic abscess, and cystitis. A significantly lower mean MPXI in MPOD-3 was present in comparison to the mean MPXI of MPOD-1 (p < 0.05), however, there was a great overlap between the groups. Conclusion: Diseases associated with neutrophil consumption may show an acquired MPOD in dogs. High standard deviation limits the diagnostic use of the MPXI for detection of MPOD.

Clinical relevance: The ADVIA®120 cytograms are a good screening tool for detection of MPOD in dogs, but the use of the MPXI is impaired in this species.

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Schlüsselwörter
Myeloperoxidaseindex, MPXI, neutrophile Granulozyten, Hämatologie, kanin, WBC, Leukozyten

Zusammenfassung
Gegenstand und Ziel: Ein Myeloperoxidaseemangel (myeloperoxidase deficiency, MPOD) neutrophiler Granulozyten wurde beim Hund im Gegensatz zum Menschen nur selten untersucht. Das Hämatologiegerät ADVIA®120 differenziert Leukozyten anhand ihrer Größe sowie des intrazellulären MPO-Gehalts. Ziel der Studie war die Beschreibung von Hunden mit MPOD sowie die Evaluierung des ADVIA®-spezifischen Myeloperoxidaseindexes (MPXI) zur Diagnostik des MPOD bei Hunden.


Detektion des Myeloperoxidasemangels bei Hunden mit dem ADVIA®120.
Eine retrospektive Studie
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Introduction

Myeloperoxidase (MPO) is one of the bactericidal proteins of myeloid cells, especially of neutrophil granulocytes and to a lesser extent of monocytes (10). It is part of the powerful MPO-hydrogen peroxide-chloride system, whereby hydrogen peroxide interacts with MPO and a halide, predominantly chloride, to generate the powerful antibacterial substance hypochlorous acid (7). Using this system, neutrophils are able to destroy invading pathogens e. g., bacteria, fungi, and viruses. Additionally, MPO leads to increased integrin (CD11b) surface expression on neutrophils, which is critical for neutrophil adherence before extravasation (5).

In humans, MPO deficiency (MPOD) occurs as an acquired as well as a hereditary disease, known as Grignaschi anomaly (10). Diseases associated with MPOD in humans include hematological neoplasms, disseminated cancers, drug-induced diseases (e. g. cytotoxic agents), or severe infectious diseases (10). The clinical importance of MPOD is under debate. Some authors detected a significantly higher frequency of life-threatening infections and an increase in chronic inflammatory diseases in patients with MPOD (9). Lanza (10), however, observed that the majority of the patients affected by MPOD do not suffer from infections and routine antibiotic therapy is usually not indicated.

First, the diagnostic use of the MPXI and ADVIA®120 parameters characterizing the location of the neutrophil population in relation to the x-axis and y-axis (neu_x_mean; neu_y_mean) was evaluated in dogs with three different grades of MPOD.

In a second part of the study, ADVIA®120 findings indicative of MPOD were confirmed with cytochemical staining and third, diseases associated with MPOD were further characterized by the severity of MPOD as well as the behavior of MPXI, neu_x_mean and neu_y_mean.

Material and methods

Evaluation of the ADVIA®120 parameters for detection of MPOD

Raw data obtained with ADVIA®120 multispecies software, version 3.1.8.0-MS in the central laboratory, faculty of veterinary medicine (from July 2004 to July 2007) was retrospectively reviewed. The majority of specimens were obtained from in-house patients and hematological analysis was performed within 4 hours after sampling. During the study period daily internal quality control was performed using three levels of control material (Testpoint, three levels, Siemens Dx, Fernwald, Germany). In addition external quality controls were run every second week (Riquas, Randox Laboratories Ltd., Crumlin, UK) using human control material. Every 3 months, specific veterinary controls were applied as external quality control (VLA Quality Assurance Unit, Sutton Bonington, UK).

Results were sorted according to their percentage monocyte count based on the automatic differential cell count. Afterwards, all peroxidase scattergrams of patients with > 17% “monocytes” were evaluated by one of the authors (SK). The cutoff of 17% “monocytes” was arbitrarily chosen, as such a high monocytosis cannot be easily explained by an unspecific response of monocytes to corticosteroid effect or inflammatory and neoplastic diseases. Analyses were categorized in three stages of MPOD. This classification was performed semiquantitatively as follows:

- MPOD-1 with < 25% neutrophils misclassified in the peroxidase scattergram and the whole neutrophil cluster shows an abnormal shift of the population,
- MPOD-2 with approximately ~25 to 50% of the neutrophils extending in the monocyte cluster and therefore misclassified as “monocytes”,
- and MPOD-3 with misclassification of 50 to 100% of neutrophils due to their location in the monocyte cluster (Fig. 2).

To further describe the location of the neutrophil cluster in the peroxidase scattergram, total leukocyte count, MPXI, dimensionless mean x and y values of the neutrophils in the cytogram
(neu_x_mean, neu_y_mean) were evaluated for the different groups of MPOD. Finally, it was evaluated in how many analyses a manual differential count (MDiff) was requested, if the MDiff confirmed, that the neutrophils were misclassified as monocytes and if a left shift (increased amount of absolute number of band neutrophils) was present. A manual differential count was requested by the clinical pathologist during routine medical validation if there was evidence of a left shift based on the morphology of the cellular population in the basophilic channel (i.e., indistinct separation between mononuclear and polymorphonucleated cells).

**Confirmation of MPOD**

One blood smear of a male castrated 13-year-old mongrel dog with transient MPOD-3 due to aspiration pneumonia and a blood smear of the same dog 2 days later with normal distribution of the neutrophils in the peroxidase scattergram were stained with a special peroxidase stain (Leucognost®POX, Merck KGaA, Darmstadt, Germany) to assure diminished truly intracellular peroxidase concentration in the myeloperoxidase deficient neutrophils. Due to the retrospective nature of the study and the fact that the myeloperoxidase stain has to be performed on fresh (<3 days old) blood smears, the blood samples used for performance of the peroxidase stain did not belong to a dog of the study population.

**Description of patients with MPOD**

In addition to the parameters described above, signalment and final diagnosis were recorded for all MPOD patients included in the study. For patients with DIC or sepsis, exact definition could not be followed retrospectively and was adopted from the diagnoses finally made from the responsible clinician. To characterize the severity of MPOD based on the aetiology of disease, patients were sorted in three groups (1. Parvovirus, 2. other gastrointestinal diseases, 3. miscellaneous diseases). MPOD, MPXI, neu_x_mean, and neu_y_mean were evaluated for the different groups. To investigate if MPOD was a permanent or transient condition in dogs, results of additional follow-up examinations were reviewed if available and the presence of MPOD based on the ADVIA®120 cytogram, MPXI, neu_x_mean, as well as neu_y_mean were recorded and compared with the baseline values. These follow-up examinations are not the same as the “repeated measurements” included in the analyses above as these examinations did not match the inclusion criteria of this study.

**Statistical analyses**

The results of the ADVIA®120 analyses were compared with laboratory internal reference intervals which were established as described previously (12). Briefly, 46 dogs with an unremarkable physical examination were included in the evaluation of the reference range. Age ranged from 0.5 to 11 years and sex included 45% females and 55% males. None of the dogs received any medication despite anthelmintic treatment at the time of sampling.

All data were evaluated for normality using the Anderson Darling test (Analyse-it for Microsoft Excel [version 2.00, Analyse-it Software, Ltd., Leeds, UK]). This included assessment of normality in the total leukocyte count, MPXI, neu_x_mean and neu_y_mean in the three different stages of MPOD and in the three different groups of diseases, the MPXI, neu_x_mean, and neu_y_mean in patients with MPOD analysis and a follow-up analysis. Parametric 1-way ANOVA with post hoc Bonferroni test or nonparametric Kruskall-Wallis test with post hoc Dunn’s multiple comparisons test were applied to detect a possible difference between the groups (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA). The paired t-test was performed with the same software, to evaluate a possible difference between the MPXI in patients with MPOD...
and the corresponding follow-up examination. Nonparametric Wilcoxon signed rank test was used to assess significance in neu_x_mean and neu_y_mean between the MPOD analysis and the follow-up analysis. P < 0.05 was considered significant.

Results

Evaluation of the ADVIA®120 parameters for detection of MPOD

Twenty-nine patients with 38 analyses consistent with MPOD were found including nine analyses consistent with MPOD-3, 16 grouped in MPOD-2, and 13 classified as MPOD-1. Median time between the first and second analysis in the individuals with repeated measurements in this study was 3 days with a range of 1 to 15 days (data not normally distributed). Figure 3 depicts the total leukocyte count in the different groups of MPOD. Statistically, no significant difference between the groups could be found. Mean and standard deviation of the MPXI in the different groups were 16.95 ± 2.23 for MPOD-1, 10.08 ± 2.32 for MPOD-2, and 6.84 ± 3.74 for MPOD-3. A significantly lower MPXI (p < 0.05) was found in analyses with MPOD-3 as compared to analyses with MPOD-1 (Fig. 3). Large range of the MPXI as well as wide overlap with the reference interval was obvious in all three groups. Surprisingly, numerous analyses revealed a result of 0.00 for neu_x_mean and neu_y_mean. In the group of MPOD-1 6/13 (46%) results were 0.00, in MPOD-2 13/16 (81%) results, and in MPOD-3 6/9 (67%) results (Fig. 3).

A manual differential count was available for 31 of 38 analyses. All MDiffs of the analyses categorized as MPOD-2 and MPOD-3 confirmed that the neutrophils were misclassified as monocytes. As expected, results of the MDiffs of the MPOD-1 group were similar to the results of the ADVIA®120, as < 25% of misclassification in association with an abnormal shift of the neutrophil population was used to define this group. In 10/13 analyses categorized as MPOD-1 an MDiff was requested showing a left shift in 6/10 (60%) specimens. For the MPOD-2 group, an MDiff was available in 13/16 analyses showing a left shift in 5/13 (38%) MDiffs. An MDiff was present in 8/9 analyses grouped in as MPOD-3. An increased absolute number of band neutrophils was visible in 3/8 (38%) MDiffs in this category.

Confirmation of MPOD

In the Leucognost®POX stain, decreased peroxidase staining intensity was visible in a sample with MPOD-3 in contrast to a sample with a normal scattergram and confirmed the findings obtained with the automated analyzer (Fig. 4).

Description of patients with MPOD

Twenty-nine dogs (20 males, nine females) of 23 different breeds were included (Table 1). Figure 5 depicts the age pattern as well as the MPOD grade of the different patients. It is clearly visible, that patients with 2 months of age dominated. 34% of the patients were younger than 1 year. MPOD-3 was present in very

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**Fig. 3**

Total leukocyte count (WBC), Myeloperoxidase Index (MPXI), and parameters describing the location of the neutrophils cluster in the peroxidase scattergram (neu_x_mean and neu_y_mean) for patients classified as grades 1–3 of myeloperoxidase deficiency (MPOD). The black horizontal lines represent the mean (WBC, MPXI), or the median (neu_x_mean and neu_y_mean). A significantly lower MPXI is found in MPOD-3 in comparison to MPOD-1. For explanation of MPOD grade see Fig. 2. The grey areas depict the reference interval.
young patients of 2 to 4 months of age as well as in patients between 8 to 11 years (Fig. 5). The patients were diagnosed with variable diseases. The majority of dogs were infected with parvovirus (8/29, 28%). In 5/29 (17%) dogs other gastrointestinal diseases than parvovirosis were present, and 16/29 (55%) patients were suffering from miscellaneous diseases. Table 1 details the signalement, final diagnoses, MPXI, neu_x_mean as well as neu_y_mean of all patients. In 8/12 (67%) analyses, neu_x_mean and neu_y_mean were set zero by the hematology system. Median MPOD grades were not significantly different in the three groups of diseases. In contrast to this, a significantly lower MPXI was present in patients with parvovirus infection than in individuals suffering from other gastrointestinal diseases or miscellaneous inflammatory diseases (Fig. 6).

In 12/29 patients, a follow-up hematological examination was available to verify the transient nature of MPOD. Based on the criteria described above (i.e., the location of the neutrophil population in the peroxidase scattergram), an MPOD was absent in 8/12 individuals, indicating transient MPOD and thus an acquired condition. In 4/12 (34%) analyses definite exclusion of MPOD-1 was not possible. Mean time between the first analyses and the follow-up examination showing a normal ADVIA®120 cytogram was 3 days (range 1 to 20 days). Compared to the follow-up examination, there was no significant difference between the MPXI at the time of MPOD (mean 11.8 ± 10.0) and at the time of re-evaluation (mean 14.2 ± 9.3) (Fig. 7). In contrast to the first analyses classified as MPOD, none of the follow-up analyses showed a result of 0.00 for neu_y_mean and neu_y_mean. Significantly lower median neu_x_mean and neu_y_mean were present in face of MPOD in comparison to the follow-up examination (Fig. 7).

Discussion

To the authors’ knowledge, this is the first study diagnosing MPOD in dogs using the ADVIA®120 technology which directly stains the intracellular myeloperoxidase. MPOD was diagnosed based on the degree of misclassification of neutrophils as “monocytes” or an abnormal shift of the neutrophil population in the peroxidase scattergram of the ADVIA®120. Up to now, only three reports about MPOD in dogs are present in the literature (3, 6, 14). All publications describe cases of acquired MPOD. One investigation focused on MPOD in English Setters with ceroid-lipofuscinosis (14). Caldwell and coworkers (3) performed an experimental study investigating MPOD in dogs intoxicated with lead, and finally, Ibrahim and coworkers (6) observed myeloperoxidase depleted neutrophils in dogs with *Hepatozoon canis* infection. In numerous patients in our study, MPOD was absent in a follow-up hematological examination indicating transient MPOD and thus an acquired condition similar to the three reports present in the literature. In 4/12 follow-up analyses permanent MPOD-1 could not be definitively excluded.
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<th>Neu_y_mean</th>
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Abbreviations: DIC = disseminated intravascular coagulation, f = female, m = male, mc = male castrated, Am. = American, CKCS = Cavalier King Charles Spaniel, WHWT = West Highland White Terrier
In the current study numerous different diseases have been observed in patients with MPOD. Dominantly, these diseases were associated with severe leukocyte consumption. Infectious and inflammatory diseases have also been observed in approximately 50% of human patients evaluated (9). Hematologic neoplasms were a further differential diagnose in human patients with MPOD (2, 4). In the current study, just one patient suffered from lymphoma. Studies in human people demonstrated that patients with acquired as well as hereditary MPOD do not show increased incidence of infectious disease, which most likely results from MPO-independent antimicrobial systems in the neutrophil (e. g., nicotinamide adenine dinucleotide phosphate [NADPH]-oxidase system) (10). If MPOD-neutrophils of dogs display an impaired function, has not been elucidated in this work and should be a subject for future research.

It would have been interesting to underline the findings of this study by applying additional techniques like flow cytometry using an anti-myeloperoxidase antibody (15), photometry, or immunohistochemistry (11). 34% of dogs < 1 year old and 28% of dogs infected with parvovirus could be detected in this study. Dogs with an age < 1 year represented up to 20% of the hospital’s patient population in the years 1993 to 1997 (17). Therefore young dogs in our study are slightly overrepresenting the regular clientele of the hospital, although current numbers of the hospital’s patients age distribution for the timeframe here evaluated were not available. In patients suffering from MPOD, parvovirus infection was markedly overrepresented compared the clinic population in which the diagnosis of parvoirosis was made in 2.3% of the cases during 1993 to 1997 (17). If patients suffering from parvoirosis really are more prone to MPOD than patients with other diseases should be a topic for prospective studies.

As this is a retrospective study, the confirmation of the ADVIA®120 results was just exemplarily performed on one normal sample, which was compared to a MPOD-3 sample. Like all retrospective studies, this investigation has several limitations. Delayed sample processing might affect location of the cells in the cluster of the hematology analyzer and one might suspect impaired MPOD classification therefore. However, a significant effect of sample aging appears very unlikely in this study, as in the authors’ laboratory, the majority of specimens were obtained from in-house patients and were analyzed within 4 hours after sampling. Only analyses with a monocyte count of > 17% were retrospectively examined for MPOD. Analyses showing myeloperoxidase deficient neutrophils not matching this inclusion criterion might
Conclusion for practice
Acquired MPOD occurs in dogs with diseases associated with severe leukocyte consumption – and thus life-threatening conditions – and should be taken into account in face of a misclassification of neutrophils as monocytes by the ADVIA®120 hematology analyzer. The ADVIA®120 peroxidase scattergram provides a good screening tool to detect MPOD easily during the routine diagnostic. However, unlike the situation in humans, the MPXI cannot be used to diagnose MPOD in dogs as the analyte displays a wide range and overlap with the reference interval so that the ADVIA®120 scattergrams have to be evaluated.

have been missed by the authors. One additional limitation of the study was the semiquantitative – and thus subjective – classification of the three MPOD grades based on the degree of an abnormal shift of the neutrophil population and/or the percentage of misclassification of neutrophils as monocytes by the ADVIA®120 hematology analyzer. However, as there are just three reports about MPOD in dogs in the literature, this is a first insight in this disease condition evaluated with the ADVIA®120 technology.

The study was further limited by the fact that the ADVIA®120 results were not compared with a reference method i. e., quantification of the myeloperoxidase protein by flow cytometry. Nucleotid sequencing can give additional information about the etiology of MPOD, e. g. about missense mutations leading to impaired function of the protein. However, performance of these techniques was not possible in a retrospective study and MPOD was transient in all dogs, which would not be expected in a permanent mutation. Nevertheless, this point may be an interesting aspect of future studies. Furthermore detailed evaluation of neutrophil morphology e. g., the presence of toxic or dysplastic changes, might be interesting in future research as one might suspect decreased myeloperoxidase staining intensity in morphologically abnormal neutrophils. A limitation of the study is the fact that a manual differential was not requested in all cases of MPOD. An increased number of band neutrophils has been detected in some of the analyses in our study. There seems to be a tendency of a higher number of analyses showing a left shift in MPOD-1 than in MPOD-2 or MPOD-3. However, this should be verified in a higher number of analyses.

This study finally evaluated the MPXI, as this parameter is routinely used in human patients to detect MPOD automatically during the hematological analysis e. g. with the ADVIA®120 precursors from the Technicon H series (8, 13). In contrast to human medicine, wide range of the parameter and considerable overlap with the reference range seems to impair the diagnostic use of this parameter in dogs. Unlike in our study, the MPXI was increased in dogs 6 to 8 hours after extraction of a pyometra in comparison to healthy dogs (16). However, this study did not mention if a MPOD was visible in the peroxidase scattergrams at time of the analysis. Diagnostic use of the parameters neu_x_mean and neu_y_mean for the detection of MPOD was hampered by the fact, that the hematologysystem automatically sets these parameters zero if morphologic abnormalities are detected (Personal communication to Renate Schiller, Technical Support Hematology, Siemens Healthcare Diagnostics GmbH). Despite that, significantly higher neu_x_mean and neu_y_mean were present in the follow-up examination of patients previously showing MPOD.

References