Department for Small Animals and Horses
Unit of Small Animal Internal Medicine of the University of Veterinary Medicine Vienna
(Head: Univ. Prof. Dr.med.vet. Iwan Burgener, PhD)

Carboxyhemoglobin levels in dogs with immune mediated hemolytic anemia compared to non-hemolytic anemia and acute hemorrhagic diarrhea syndrome

Diploma thesis
University of Veterinary Medicine Vienna

Submitted by
Noemi Kermann

Vienna, June 2024

Thesis supervisor and first Reviewer:

Univ.-Prof. Dr.med.vet. Iwan Burgener, PhD, Dipl. ECVIM-CA, Dipl. ACVIM

Second Reviewer:

Univ.-Prof. Dr. med. vet. Jessika-Maximiliane Cavalleri, Dipl. ECEIM

Table of contents

Declaration of Autonomy

I hereby declare that I have written the submitted thesis independently, and have not used any other sources and aids than those indicated. All text passages taken from external sources have been identified. I carried out the decisive work myself and listed all the contributors with their contribution to the work. The present work has not been submitted or published elsewhere.

Vienna, June 2024
Noemi Kermann

Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorgelegte Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Alle übernommenen Textstellen aus fremden Quellen wurden kenntlich gemacht. Ich habe die entscheidenden Arbeiten selbst durchgeführt und alle zuarbeitend Tätigen mit ihrem Beitrag zur Arbeit angeführt. Die vorliegende Arbeit wurde nicht an anderer Stelle eingereicht oder veröffentlicht.

Wien, Juni 2024
Noemi Kermann

Acknowledgments

I would like to thank my parents, without whose financial support this course of study would never have been possible. They never pushed me and made it possible for me to continue my studies despite the occasional lack of motivation and self-doubt to put one foot in front of the other to be able to complete my veterinary degree. This would not have been possible without your support and goodwill.

Thanks also to my supervisor Univ.-Prof. Dr.med.vet. Iwan Burgener for his support, to Dr. Pavlos Doulidis for his expertise, and time and to Dr. Alexander Tichy for his patient help with the statistical analysis.

I am deeply thankful to the people who believed in me and were convinced that I would be able to muster the necessary stamina to complete this study. First and foremost, my boyfriend Michael, who has the biggest and most loving heart I know and who has never let me down for a second of the time. Especially noteworthy are also my long-standing friends, my brothers and my pets, who have always been by my side and have lifted my spirits on melancholic days.

To my dearest fellow students, who are now very important to me as friends. I would like to thank them for their good encouragement, their faith in my knowledge and memory when I often thought I had lost it and for getting through this hellish ride together. Without you, it would have been difficult for me to succeed!

Abstract

Introduction:

Carboxyhemoglobin forms when carbon monoxide binds to hemoglobin. Carboxyhemoglobin can be a reliable indicator of blood carbon monoxide levels. Critically ill patients often tend to form carboxyhemoglobin. Immune-mediated hemolytic anemia is a severe disease and the most common form of hemolytic anemia in dogs.

Hypothesis:

This thesis aims to assess carboxyhemoglobin levels in dogs with immune-mediated hemolytic anemia in contrast to those with non-hemolytic anemia and dogs suffering from acute hemorrhagic diarrhea syndrome. The hypothesis is that measurement of carboxyhemoglobin is higher in dogs with immune-mediated hemolytic anemia than in animals with non-hemolytic anemia and dogs with acute hemorrhagic diarrhea syndrome.

Material and Methods:

Retrospective study based on medical records at the clinic for small animals of the University of Veterinary Medicine in Vienna, Austria. The data from patients hospitalized for immune-mediated hemolytic anemia (n=28), non-hemolytic anemia (n=29), or for acute hemorrhagic diarrhea syndrome (Control, n=44) were analyzed retrospectively.

Results and Conclusion:

Carboxyhemoglobin significantly differed between dogs with immune-mediated hemolytic anemia and dogs with non-hemolytic anemia. Also, there was a significant difference in dogs with immune-mediated hemolytic anemia in comparison to those with acute hemorrhagic diarrhea syndrome. There was no significant difference in carboxyhemoglobin levels between the patients with non-hemolytic anemia and the ones with acute hemorrhagic diarrhea syndrome.

Zusammenfassung

Einleitung:
Carboxyhämoglobin wird gebildet, wenn sich Kohlenmonoxid an Hämoglobin bindet. Die Messung von Carboxhämoglobin kann ein zuverlässiger Indikator für die Kohlenmonoxid-Konzentration im Blut sein. Schwerkranke Patienten neigen häufig zur vermehrten Bildung von Carboxyhämoglobin. Die immunvermittelte hämolytische Anämie ist eine schwere Erkrankung und die häufigste Form der hämolytischen Anämie bei Hunden.

Hypothese:
Ziel dieser Arbeit ist es, die Carboxyhämoglobinwerte bei Hunden mit immunvermittelter hämolytischer Anämie im Vergleich zu Hunden mit nicht-hämolytischer Anämie und Hunden mit akutem hämorrhagischem Diarrhöe-Syndrom zu bestimmen. Die Hypothese lautet, dass die Carboxyhämoglobin-Werte im Blut bei Hunden mit immunmediierter hämolytischer Anämie höher sind als bei Tieren mit nicht-hämolytischer Anämie und bei Hunden mit akutem hämorrhagischem Diarrhöe-Syndrom.

Material und Methoden:
Retrospektive Studie auf der Grundlage von Krankenakten aus der Klinik für Kleintiere der Veterinärmedizinischen Universität Wien in Österreich. Die Daten von Patienten, die wegen einer immunvermittelten hämolytischen Anämie (n=28), einer nicht-hämolytischen Anämie (n=29) oder wegen eines akuten hämorrhagischen Diarrhöe-Syndroms (Kontrolle, n=44) hospitalisiert wurden, wurden retrospektiv analysiert.

Ergebnisse und Schlussfolgerung:
Der Carboxyhämoglobinanteil unterschied sich signifikant zwischen Hunden mit immunvermittelter hämolytischer Anämie und nicht-hämolytischer Anämie. Zudem bestand ein signifikanter Unterschied zwischen Hunden mit immunvermittelter hämolytischer Anämie und solchen mit akutem hämorrhagischem Diarrhöe-Syndrom. Bei den Carboxyhämoglobinwerten gab es keinen signifikanten Unterschied zwischen den Hunden mit nicht-hämolytischer Anämie und den Hunden mit akutem hämorrhagischen Diarrhöe-Syndrom.

List of abbreviations

AHDS	acute hemorrhagic diarrhea syndrome
AST	aspartate-aminotransferase
CBC	complete blood count
CO	carbon monoxide
COHb	carboxyhemoglobin
cCRP	canine C-reactive protein
DAT	direct antiglobulin test
HA	hemolytic anemia
Hb	hemoglobin
Hct	hematocrit
HGE	hemorrhagic gastroenteritis
IAT	indirect antiglobulin test
IMHA	immune-mediated hemolytic anemia
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
Non-HA	non-hemolytic anemia
RBC	red blood cell
RDW	red cell distribution width
SAT	saline agglutination test
TIS	Tierspitalinformationssystem

1 Introduction

The term anemia refers to a medical condition marked by a reduced level of hemoglobin (Hb) in the blood, typically linked with a decreased count of red blood cells (RBC) and/or hematocrit (Hct) (1). Anemia can develop from two main causes. The first is a decrease in the production of RBCs, which can occur due to various illnesses affecting RBC production indirectly and can also be categorized as aregenerative anemia. These can include intoxications, infectious or bone marrow diseases, as well as metabolic and cancerous disorders. The second cause is a shortened lifespan of mature RBCs, typically resulting from sudden blood loss or hemolysis (2). The duration of RBCs' lifespan is shortened in two situations: significant acute bleeding and hemolytic anemia. In both scenarios, the bone marrow operates normally and produces young RBCs to replenish the erythrocyte count, resulting in what is categorized as regenerative anemia (1).

Hemolytic anemia (HA) is a type of anemia that occurs when RBCs are destroyed faster than they can be replaced. This can be due to a variety of causes, including inherited disorders, autoimmune diseases, infections, reactions to blood transfusions, and exposure to certain medications or toxins (3,4). Immune mediated hemolytic anemia (IMHA) is an autoimmune disease (5), the most common form of HA in dogs, and is linked to high morbidity and mortality (3,4,6–8). In dogs suffering from IMHA, pathogenic autoantibodies exhibit a specific affinity for erythrocyte membrane epitopes, creating a mechanism by which macrophages participate in extravascular hemolysis (3,8). Moreover, complement activation occurs when antibodies bind to erythrocytes, facilitating either extravascular hemolysis or intravascular hemolysis through the formation of the membrane attack complex. Intravascular hemolysis pertains to the breakdown of RBCs within the blood vessels (3,8).

In primary or nonassociative IMHA, autoantibodies develop against the RBC membranes without a known cause, often diagnosed through a process of elimination of infectious, neoplastic, or toxic causes (3,8,9). In secondary or associative IMHA, there's a trigger for antibody production. Potential underlying causes include infections, lympho- and myeloproliferative disorders, certain medications, and inflammatory conditions (3). When immune-related thrombocytopenia also occurs concurrently, this condition is termed Evans syndrome (9).

The clinical manifestation of hemolysis and its severity dictate symptomatology. Common presentations include lethargy, decreased appetite, paleness of mucous membranes, rapid heart rate (tachycardia), occasional rapid breathing (tachypnea), jaundice, fluctuation in body temperature, and vomiting. Additionally, symptoms of any underlying conditions may also be evident (10).

Canine IMHA prognosis is poor, and additional immunomodulatory treatments with glucocorticoids lack clear benefits. The use of immunosuppressive treatment leads to various negative impacts on patient health, caused by both the medications themselves and the extended length of treatment, which collectively increase patient morbidity (11). Certain risk factors, including icterus and increased creatinine, greatly raise the risk of death in IMHA (5). Researchers proposed a risk stratification system based on various parameters, enabling the prediction of death likelihood in dogs with IMHA. The risk is highest during the initial two weeks after diagnosis, largely due to coagulation issues, as well as liver and kidney failure from anemia-induced tissue hypoxia. Collaborative efforts, standardized criteria, and a mortality risk scoring system are needed to improve IMHA prognosis and treatment (5).

When RBCs break down, they release hemoglobin into the bloodstream. This hemoglobin is metabolized by an enzyme called heme-oxygenase 1, resulting in the production of iron, biliverdin, and carbon monoxide (CO) (12–15). CO binds to hemoglobin about 220 times more strongly than oxygen does. (16) After this Carboxyhemoglobin (COHb) is formed when CO binds to the iron in hemoglobin. This process prevents hemoglobin from carrying oxygen to the body's tissues (12) and therefore is one of the several mechanisms of toxicity of CO (17). RBCs breakdown resulting in increased CO production and an associated increase in COHb levels is seen in various pathological conditions, such as anemias, hematomas and a variety of other hematologic malfunctions and diseases (18).

COHb, this stable compound of CO and hemoglobin, can indicate blood CO levels effectively (4). COHb blood levels remain relatively constant and can be reliably assessed using a blood sample that has been transported (19). In clinical practice, arterial and venous COHb levels are typically regarded as interchangeable (17).

In human medicine, the measurement of COHb is particularly important for the finding of CO poisoning. The standard procedure for identifying and measuring CO poisoning involves analyzing blood samples, whether from veins or arteries, to determine the percentage of COHb (16). The final diagnosis of CO poisoning requires CO exposure, the occurrence of symptoms, and an elevated COHb level (20).

HA is a common condition observed in both humans and dogs in intensive care units, posing a risk of life-threatening organ failure, particularly in severe cases (4,5,12). During hemolysis, the breakdown of hemoglobin is followed by CO binding to free hemoglobin. The so formed COHb can be routinely assessed in ICU settings, offering rapid results (12). In earlier studies from human medicine as well as in a veterinarian study in Israel 2022 COHb differs between individuals with HA and non-HA and between non-HA and nonanemic individuals and did so proof as a promising predictor of hemolytic anemia (4,12,21).

While certain characteristics like hyperbilirubinuria, hemoglobinemia, hemoglobinuria and reticulocytosis are common in hemolytic anemias in general (3,22), three specific markers - persistent autoagglutination, marked spherocytosis, and a positive direct antiglobulin test (DAT, Coombs) - aid in diagnosing IMHA (22). To this day, there is no universal standard for diagnosing IMHA in veterinary patients (3). Tests such as a positive DAT, spherocytosis, and true autoagglutination are widely used to detect anti-erythrocyte antibodies, but standardization is still lacking (4,12). Spherocytes should only be considered a diagnostic factor in dogs, as feline RBCs don't consistently show a central light area (3).

The Coombs' test identifies autoantibodies that are attached to RBCs directly (known as direct antiglobulin test or DAT) or are present unattached in the serum or plasma (known as indirect antiglobulin test or IAT) (22). More precisely it can identify both, immunoglobulin and complement substances present on the surface of erythrocytes. Therefore, the Coombs test can be useful in assisting with the diagnosis of IMHA (23). The British Society for Hematology recommends the DAT for diagnosing canine IMHA, but it's not entirely specific or sensitive (3). It's essential to interpret positive test results in conjunction with other clinical and hematologic indicators of IMHA (23) and all diagnostic tests should be interpreted along with other tests and response to immunosuppression (3).

Acute hemorrhagic diarrhea syndrome (AHDS) was formerly known as canine hemorrhagic gastroenteritis (HGE). AHDS in dogs is a gastrointestinal disorder characterized by sudden and severe vomiting and bloody diarrhea, often accompanied by notable hemoconcentration (24,25). There are potentially life-threatening symptoms present, and it exhibits a notable prevalence within the canine population (25).

In the course of this diploma thesis, results of blood samples from dogs with IMHA, Non-HA and AHDS were analyzed retrospectively for their COHb levels using spectrophotometric methods. The results are to be compared to determine if there is a significant difference in COHb levels between the groups.

There is a pressing need to establish standardized diagnostic tests and criteria, and clinical trials may benefit from categorizing dogs based on their risk of mortality (5). The development of a reliable diagnostic tool could have significant implications for the treatment and management of IMHA in veterinary patients.

2 Hypothesis

The objective of this thesis is to evaluate the levels of COHb in dogs with IMHA in comparison to animals with other forms of anemia and dogs suffering from AHDS. IMHA is a common and serious disease, and the diagnosis often remains a challenge. Therefore, this diploma thesis is dedicated to the following hypothesis based on a previous study (4): Measurement of Carboxyhemoglobin is higher in dogs with hemolytic anemia then in animals with other forms of anemia and dogs with acute hemorrhagic diarrhea syndrome.

3 Materials and Methods

3.1. Literature Review

Two databases (PubMed/Medline, Mendeley/Elsevier) were systemically searched for relevant references using appropriate keywords and filters. References captured by the search algorithm with one or more of the keywords “anemia”, “dog” “immune”, “IMHA”, “carboxyhemoglobin” and “AHDS” were sighted. Then they were imported into reference management software (Mendeley, Elsevier, New York; EndNote X8, Clarivate Analytics, Philadelphia) before manual screening based on inclusion criteria.

Abstracts of all papers were reviewed leading to the rejection of some due to their non-reliability and relevance for this study. Review papers on the diagnosis and treatment of IMHA in dogs and human, studies on the diagnostic value of COHb in veterinary and human medicine, and general articles on hemolytic anemia in dogs and human were used as references. Data therefore were extracted from 53 papers.

3.2. Online dictionary

As this work was not written in the author's native language, the online dictionary Linguee with the online service for machine translation DeepL (DeepL SE, Köln, Germany) was used as required to support the scientific formulation.

3.3. Study design

The study is a retrospective study and was conducted at the clinic for small animals of the University of Veterinary Medicine in Vienna, Austria. Using the internal electronic medical databases of the teaching hospital (TIS; Tierspitalinformationssystem, Application Orbis VetWare; Agfa HealthCare) data of the animals was filtered with key words “IMHA”, “anemia” and “AHDS”.

3.4. Animals

The IMHA-group (n=28) includes primarily dogs diagnosed with either nonassociative (primary) or associative (secondary) IMHA and one dog with concomitant thrombocytopenia (Evans syndrome). Depending on the presence of a previous blood transfusion and/or prior treatment with cortisone preparations, the IMHA group was further divided into subgroups regarding the statistical analysis. Other treatments with immunosuppressants were also recorded, but did not affect the parameters studied, as they were only administered after COHb has been measured.

The group of anemic dogs without hemolysis (non-HA, n=29) is composed of animals with anemia secondary to blood loss (n=28) or to renal disease (n=1). The third group as reference consists of dogs with AHDS (n=44), formerly known as HGE.

3.4.1. Inclusion and exclusion criterions

IMHA patients were searched for in the TIS using the search terms "IMHA" and "anemia". It was then checked whether at least one blood gas measurement had been carried out, and whether a suspected diagnosis of IMHA had been made in the medical records. After a review of the medical records, the animals were included in the IMHA group or excluded based on exclusion criteria.

For the non-HA group, patients were searched for using the search term "anemia". The dogs were then assigned to the non-HA group based on the suspected diagnosis of bleeding anemia or renal anemia together with the remaining medical history, a COHb measurement present and the results of further examinations.

The animals in the AHDS group were filtered from the medical database using the search term "AHDS". Only dogs with a suspected diagnosis of AHDS and a measured COHb value were included. Some of the dogs in this group were part of a prospective study on AHDS and the diagnosis was underlying precisive and strict criteria (26).

In the IMHA group, the dogs had to have a suspected diagnosis of IMHA or Evans syndrome - in the presence of concomitant thrombocytopenia - in their medical records. Usually, a

diagnosis of IMHA relies on the presence of anemia (Hct <37 %), coupled with at least one of the following results that was shown at the Department of Laboratory Diagnostics at the Vetmeduni Vienna. A positive result in the DAT (Coombs' test), the identification of spherocytosis through a peripheral blood smear examination conducted by a clinical pathologist or in some cases a positive saline agglutination test (SAT). In addition, the response to immunosuppressive therapy played a decisive role. The result of the Coombs' test was considered positive if the reaction was positive at 1:8 dilution or higher.

Only data from values that were measured in enough dogs to ensure comparability were included in the discussion of the results. Pre-treatment with cortisone and transfusions with blood products prior to the measurement must be considered when evaluating the data. Both, dogs with associative and nonassociative IMHA were included in the IMHA-group.

3.5. Sample Collection and Laboratory Equipment

The data from patients hospitalized for IMHA, non-HA or for AHDS were analyzed retrospectively including data from 2018 to 2024. Sex, age, breed, and spay/neuter status of the animals were documented, as well as treatment with cortisone or other immunosuppressants as mycophenolic acid or cyclosporine and blood transfusion.

Data of blood samples taken to perform complete blood count (CBC), blood biochemistry and a blood gas analysis including COHb quantification in percentage of hemoglobin were retrospectively analyzed. Where relevant, the results of urinalysis, tests for endoparasites and tests for canine vector-borne diseases were also included in the data collection.

The level of venous COHb was determined using the *Cobas® b 123* (Roche Diagnostics Automation Solutions GmbH, Ludwigsburg, DE) blood gas analyzer. CBC was performed using *Advia 2120* (Siemens Healthcare, Munich, DE). Blood chemistry values were performed on heparinized blood using *Cobas C501* (Roche Diagnostics Automation Solutions GmbH, Ludwigsburg, DE).

All blood analyses were carried out with the devices available at the university hospital of the University for Veterinary Medicine in Vienna.

3.6. Statistical Analysis

All analyses were performed using the statistical software SPSS (Statistics for Windows, Version 29, IBM Corp, Armonk, New York). A professional statistician from the Institute of Bioinformatics and Biostatistics at the University of Veterinary Medicine, Vienna was involved in the statistical analysis and interpretation of the data.

First, an exploratory data analysis was carried out to gain an impression of the data set. Descriptive statistics (Table 1) were calculated for all demographic parameters and all groups analyzed as an overview. Box and whisker plot diagrams were created for the relevant values to illustrate the most important robust measures of position and dispersion. The minimum, the lower quartile, the median, the upper quartile and the maximum are shown.

Based on these analyses, other values in addition to COHb were analyzed in more detail using further statistical tests. In particular, the values COHb (%), hemoglobin (g/dL) and log leucocytes (/mL) were analyzed in more detail regarding possible differences between the three groups, as well as within the IMHA-group depending on a previous cortisone therapy and blood transfusions before measurement of relevant blood values.

Within the IMHA group, a further distinction was made between the groups "Cortisone treatment, blood transfusion", "Cortisone treatment, no blood transfusion", "No cortisone treatment, blood transfusion" and "No cortisone treatment, no blood transfusion". The Levene's test was used as a significance test for equality of variances to see if there were differences within the IMHA group depending on pretreatment.

The Kolmogorov-Smirnov test was carried out as a non-parametric test to test the normal distribution assumption in all groups. The three groups were tested for differences in COHb levels using a one-sided ANOVA (analysis of variance) and subsequent post-hoc tests, to show between which groups the differences were significant.

For qualitative (e.g. yes or no) variables of the IMHA-group and the non-HA group, cross-tabulations and then following chi-square tests were performed.

4 Results

4.1 Descriptive statistics

Table 1 Descriptive statistics (Minimum, Maximum, Median, Standard deviation); Overview.

Group	Parameter	N	Minimum	Maximum	Median	Standard Deviation
IMHA	COHb (%)	28	1,30	11,10	7,568	2,082
	Hemoglobin (g/dL)	27	2,20	11,20	6,174	2,189
	Hct (%)	27	7,40	32,60	18,511	6,309
	MCV (fL)	27	42,10	95,40	74,263	11,109
	MCH (pg)	27	14,60	37,30	24,667	3,558
	MCHC (g/dL)	27	26,20	45,00	33,367	3,888
	Reticulocytes (%)	22	0,20	25,90	8,068	6,535
	Reticulocytes (abs.)	24	7035,00	417473,00	177787,292	124621,374
	Thrombocytes (10^3/Mikrog)	27	2,00	434,00	142,480	126,895
	Leucocytes (/mL)	27	2780,00	77689,00	24316,070	16008,233
	Log Leucocytes (/mL)	27	3,44	4,89	4,289	0,320
	RDW (%)	23	12,20	32,00	19,087	5,001
	Segmental granulocytes (%)	23	12,00	88,30	70,535	17,518
	Lymphocytes (%)	27	1,00	6530,00	418,685	1488,791
	PTT	14	9,80	107,00	33,321	36,456
	PTZ	14	7,00	83,00	14,636	19,780
	TZ	9	9,60	17,00	12,533	2,031
	Urea (mg/mL)	20	15,90	110,80	51,700	20,960
	Creatinine (mg/mL)	25	0,20	1,40	0,678	0,246
	TP (g/dL)	25	4,07	8,00	5,973	0,908
	Albumin (g/dL)	25	1,28	3,57	2,750	0,548
	AP (U/L)	23	41,00	7615,00	832,700	1604,012
	AST (U/L)	11	21,00	457,00	177,270	172,501
	ALT (U/L)	24	19,00	3419,00	511,000	955,011
	GLDH (U/L)	6	1,28	220,40	56,185	88,542
NonHA	COHb (%)	29	4,20	8,20	6,324	1,042
	Hemoglobin (g/dL)	26	2,90	19,30	7,985	3,356
	Hct (%)	26	9,00	59,00	23,835	10,038
	MCV (fL)	25	50,80	75,80	66,192	5,967
	MCH (pg)	24	16,90	26,20	22,313	2,370
	MCHC (g/dL)	25	29,10	39,50	33,860	2,590
	Reticulocytes (%)	12	0,50	9,90	3,992	3,136
	Reticulocytes (abs.)	18	3,80	440300,00	117946,006	132643,176
	Thrombocytes (10^3/Mikrog)	25	0,00	555,00	186,880	159,312
	Leucocytes (/mL)	26	850,00	33250,00	15637,040	8709,329
	Log Leucocytes (/mL)	26	2,93	4,52	4,102	0,341
	RDW (%)	22	12,40	23,20	16,423	3,046
	Segmental granulocytes (%)	22	56,90	92,00	75,432	9,198
	Lymphocytes (%)	26	3,90	440,00	41,973	100,109
	PTT	10	11,60	143,00	46,190	50,729
	PTZ	11	6,50	16,00	9,673	3,102
	TZ	7	11,30	18,00	13,629	2,860
	Urea (mg/mL)	16	12,20	615,30	112,531	167,331
	Creatinine (mg/mL)	22	0,30	11,40	2,186	2,963
	TP (g/dL)	22	2,80	9,04	5,580	1,554
	Albumin (g/dL)	24	0,85	4,26	2,410	0,745
	AP (U/L)	20	20,00	1913,00	246,100	429,786
	AST (U/L)	6	25,00	481,00	142,000	174,338
	ALT (U/L)	19	14,00	1037,00	171,470	285,990
	GLDH (U/L)	8	1,54	9,65	5,312	2,825
AHDS	COHb (%)	44	4,70	7,50	6,350	0,737
	Hemoglobin (g/dL)	44	8,50	26,40	18,805	3,563
	Hct (%)	44	24,50	77,90	53,907	9,771
	MCV (fL)	43	63,80	77,50	69,361	2,760
	MCH (pg)	43	21,00	27,10	24,130	1,338
	MCHC (g/dL)	44	32,10	38,30	34,839	1,454
	Reticulocytes (%)	4	0,60	129,00	33,125	63,922
	Reticulocytes (abs.)	3	37440,00	192200,00	89026,667	89350,728
	Thrombocytes (10^3/Mikrog)	40	54,00	511,00	175,350	83,280
	Leucocytes (/mL)	44	2890,00	28560,00	11520,230	5553,733
	Log Leucocytes (/mL)	44	3,46	4,46	4,005	0,239
	RDW (%)	44	10,80	14,30	12,355	0,697
	Segmental granulocytes (%)	44	43,00	91,10	74,264	11,085
	Lymphocytes (%)	44	1,00	36,50	14,225	8,345
	PTT	20	11,10	35,60	15,215	5,520
	PTZ	20	6,30	11,10	7,560	0,980
	TZ	20	9,70	15,50	13,015	1,426
	Urea (mg/mL)	43	1,90	161,40	32,674	25,814
	Creatinine (mg/mL)	44	0,30	4,90	0,757	0,672
	TP (g/dL)	44	2,13	7,54	5,609	1,025
	Albumin (g/dL)	44	1,10	4,64	3,059	0,689
	AP (U/L)	44	1,00	324,00	77,410	69,323
	AST (U/L)	24	15,00	101,00	43,080	21,847
	ALT (U/L)	44	12,00	167,00	49,340	31,355
	GLDH (U/L)	2	3,92	6,65	5,285	1,930

4.2. Group demographics

Overall, 101 dogs were included in the retrospective study, including 28 IMHA patients, 29 dogs diagnosed with another form of anemia, and 44 dogs suffering from AHDS. Breeds that were represented more than once in the anemic dogs (IMHA and Non-HA) included: mixed breed dog (14), Labrador Retriever (3), Malteser (2), Malinois (2), Poodle (2), Cavalier King Charles Spaniel (2), German Short Hair Pointer (2) and Pomeranian (2).

Females were overrepresented in the IMHA group, and most of the dogs were neutered. The IMHA group included 18 females (15 neutered, 3 intact) and 10 males (7 neutered and 3 intact). The age of the dogs in the IMHA group varied between 4 months and 14 years. The median age of the IMHA patients was 7,34 years.

4.3. Carboxyhemoglobin concentration

In the group of dogs diagnosed with IMHA, COHb was significally increased compared to both, the non-HA group (P = 0.002; Figure 1) as well as the AHDS-group (P < 0.001; Figure 1). The dogs with hemolytic anemia had the highest COHb values, and there was no significant difference between the other two groups.

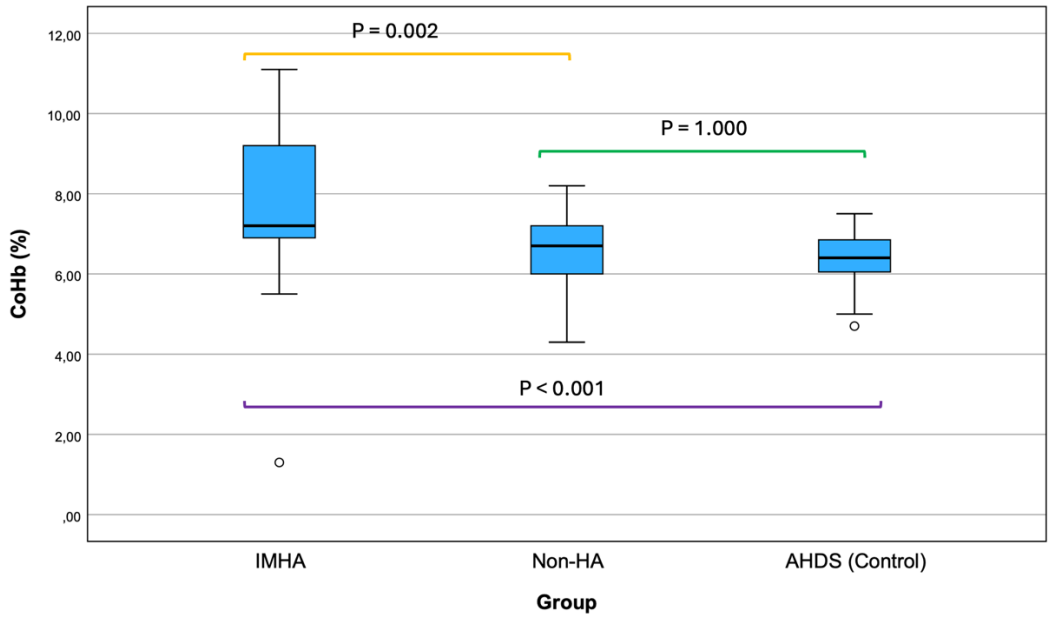

Figure 1 Box plots for carboxyhemoglobin concentrations, presented as % of total hemoglobin, in dogs with immune mediated hemolytic anemia (IMHA), dogs with non-hemolytic anemia (non-HA), and nonanemic acute hemorrhagic diarrhea syndrome (AHDS) dogs. °… statistical outlier

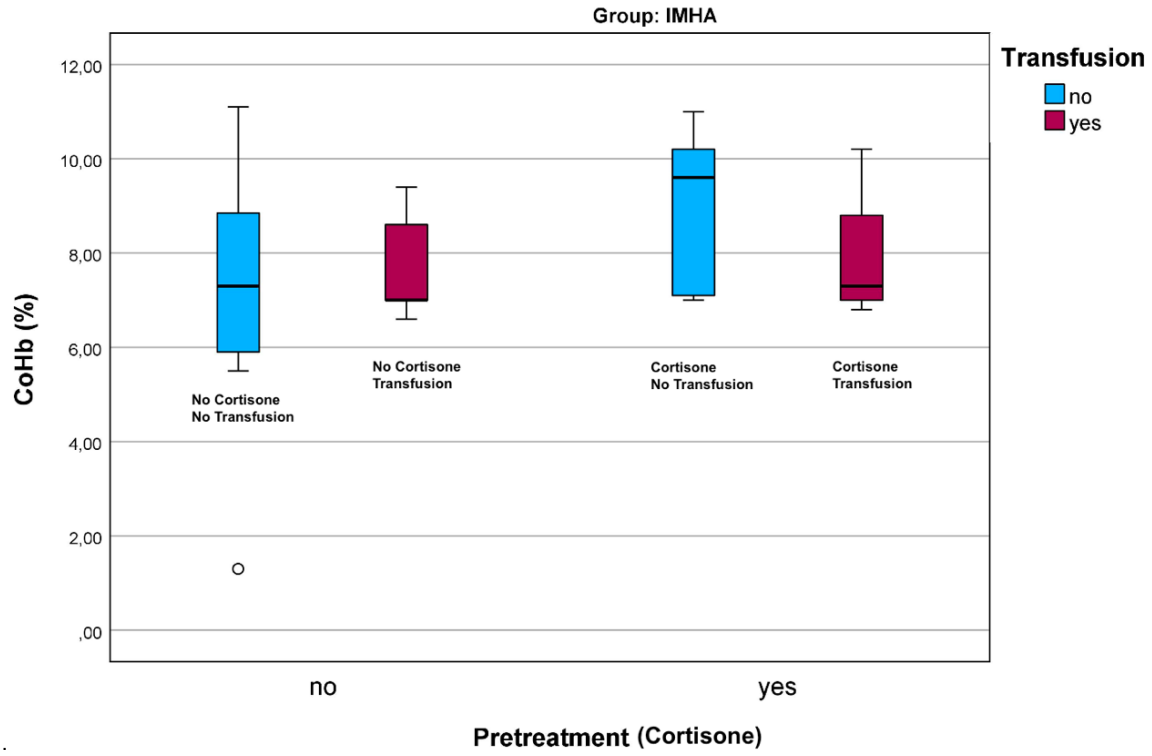

Figure 2 Box plots for carboxyhemoglobin concentrations, presented as % of total hemoglobin in the immune mediated hemolytic anemia (IMHA) dogs, depending on pretreatment with cortisone and/or previous blood transfusion.; °… statistical outlier

There was no significant difference in COHb levels between IMHA patients without prior treatment with cortisone and/or blood transfusion and those with prior cortisone treatment or transfusions with blood products (Figure 2).

4.4. Hemoglobin concentration

The group of animals with IMHA as well as the group of animals with non-HA had a significantly lower hemoglobin content than the AHDS dogs (P = 0.001; Figure 3). There was no significant difference between the non-HA and the IMHA group. There was a significantly higher hemoglobin content in IMHA patients who had received a transfusion with blood products before the measurement (P = 0.017; Figure 4) in comparison to those IMHA patients, who did not receive a transfusion.

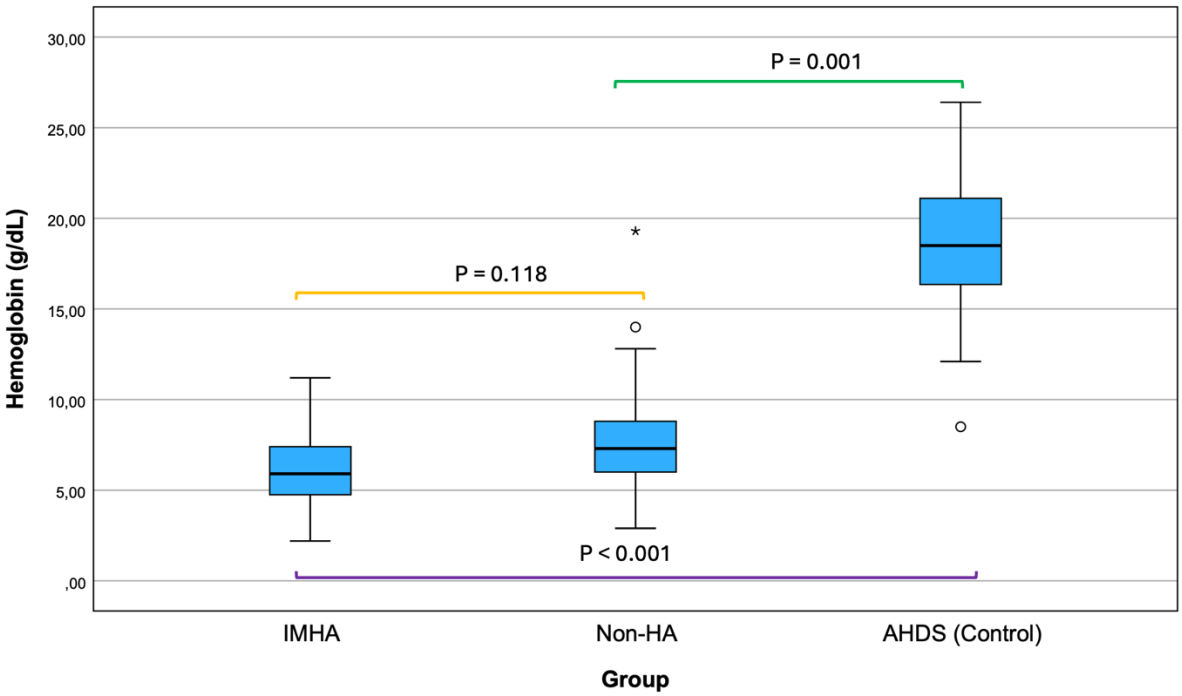

*Figure 3 Box splots of Hemoglobin (g/dL) concentration in dogs with immune mediated hemolytic anemia (IMHA), dogs with non-hemolytic anemia (non-HA), and nonanemic acute hemorrhagic diarrhea syndrome (AHDS) dogs. °… statistical outlier; * … extreme value.*

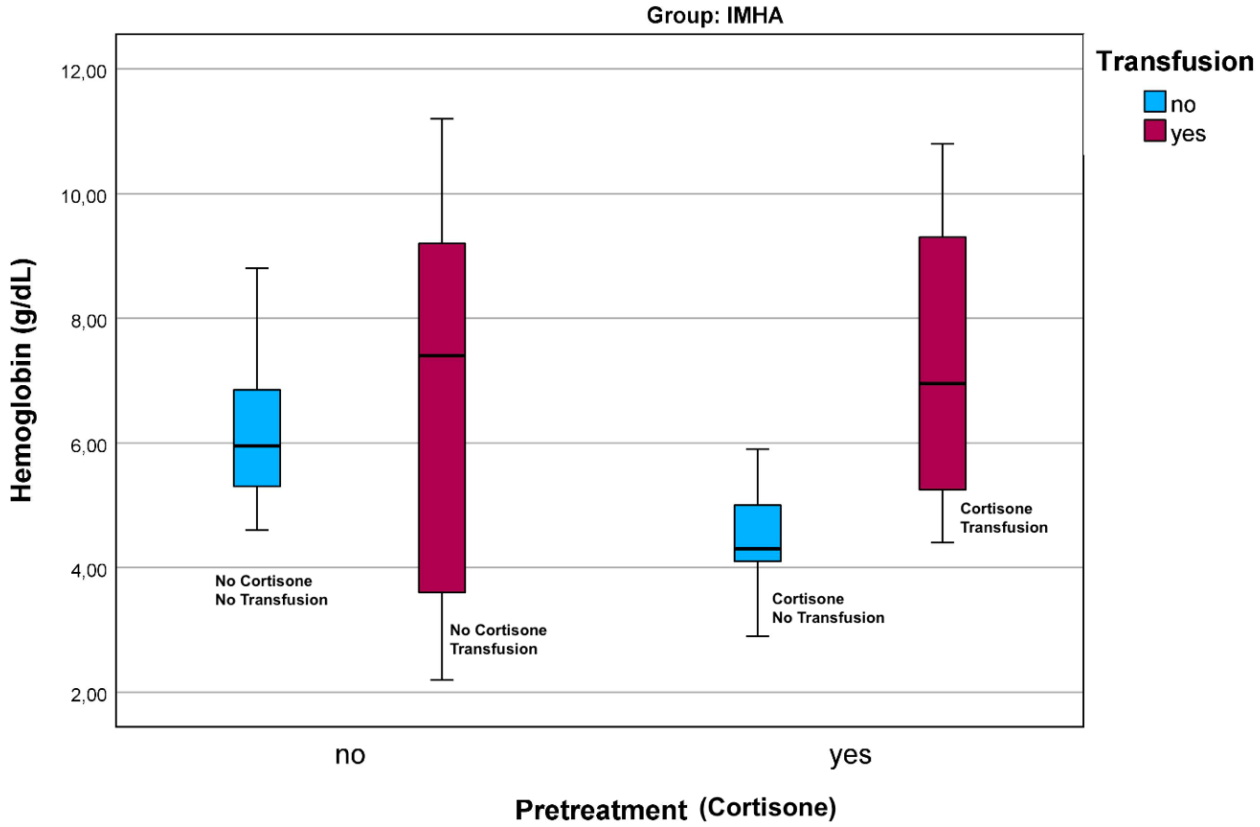

Figure 4 Box splots of Hemoglobin (g/dL) concentration in the immune mediated hemolytic anemia (IMHA) dogs, depending on pretreatment with cortisone and previous blood transfusion.

4.5. Leucocytes

There was a significantly higher amount of leucocytes (/ml) in the IMHA-group compared to the AHDS group (P = 0.001; Figure 5). There was no significantly higher count of leucocytes in the IMHA group compared to the non-HA group. Pre-treatment with cortisone or a previous blood transfusion had no significant influence on the leukocyte count (Figure 6).

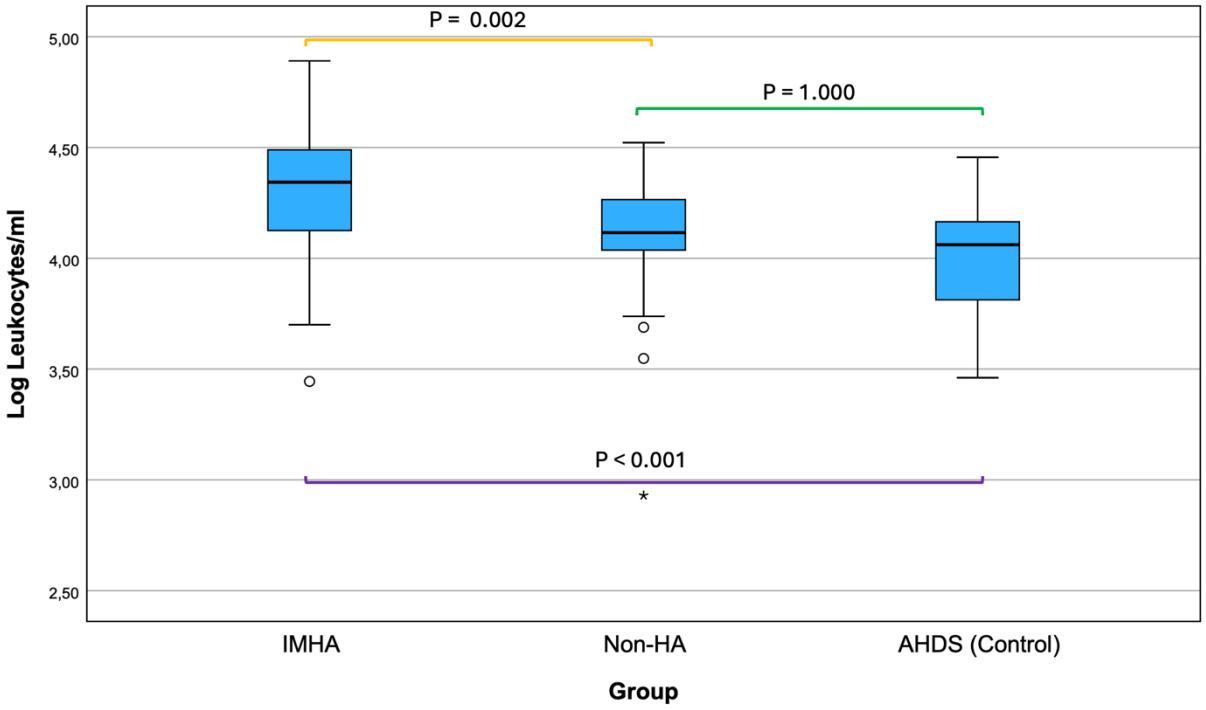

*Figure 5 Box splots of Log Leukocytes (/mL) concentration in dogs with immune mediated hemolytic anemia (IMHA), dogs with non-hemolytic anemia (non-HA), and nonanemic Acute hemorrhagic diarrhea syndrome control dogs. °… statistical outlier; * … extreme value.*

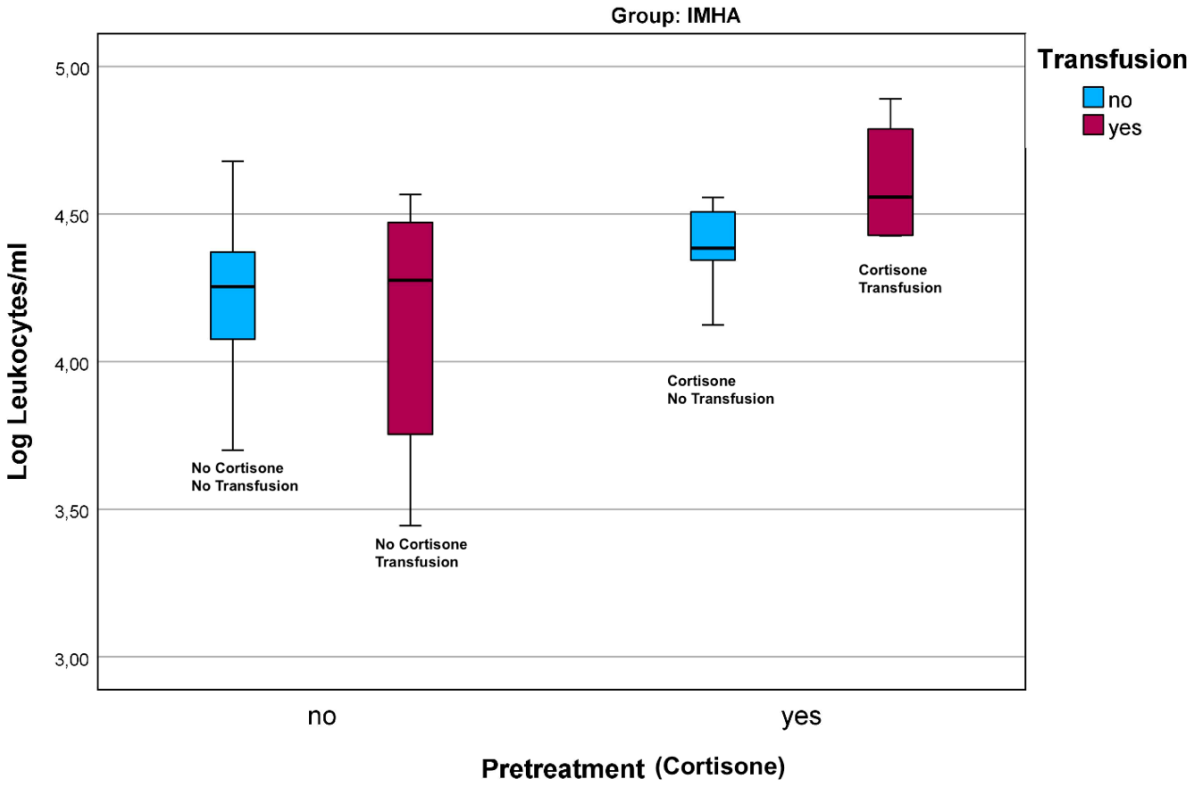

Figure 6 Box splots of Log Leucocytes (/mL) concentration in the immune mediated hemolytic anemia (IMHA) dogs depending on pretreatment with cortisone and previous blood transfusion.

4.6. Others

The red cell distribution width (RDW), the levels of aspartate aminotransferase (AST), mean corpuscular hemoglobin concentration (MCHC), and the canine C-reactive protein (cCRP) were also slightly elevated in the IMHA group. No statistical statements were made, as it would have gone beyond the scope of the study to go into all the differences.

4.7. Qualitative parameters

Apart from the blood values, there were other qualitative (e.g. yes/no) variables, that were analyzed in more detail for differences between the IMHA and non-HA dogs. It should be noted that the AHDS control group was not included in the comparison due to lack of data. Furthermore, these were tests and deviations in blood analysis, which serve to differentiate between IMHA and non-HA. It must be mentioned, however, that not enough data was available for all values in the non-HA dogs to carry out meaningful statistical evaluations.

4.7.1. Coombs

Due to the lack of testing of the non-HA dogs, not enough data was available to draw statistically meaningful conclusions. A Coombs test was carried out on 25 of the 28 IMHA dogs, but only on 7 of 29 non-HA dogs.

4.7.2. Spherocytes

There was a significant (P = 0.001) difference between the IMHA-group and the non-HA-group regarding spherocytosis. Spherocytosis refers to the condition of increased spherocytes, i.e. sphere-shaped erythrocytes.

4.7.3. Polychromasia

A significant difference (P = 0.016) was found between the two groups (IMHA, non-HA) regarding the polychromasia - i.e. the different stainability of the individual RBCs - of the blood smears examined. Polychromasia was more common in the IMHA dogs.

4.7.4. Anisocytosis

Regarding anisocytosis, i.e. the non-uniform size of the individual RBCs, there was again a significant difference (P = 0.019) between the dogs with IMHA and those without hemolytic anemia. In the dogs with IMHA, anisocytosis was more often present.

4.7.5. Bilirubinuria

No significant difference was found in the two anemic groups regarding bilirubinuria, which is caused by an increased bilirubin content in the urine. However, this result is not statistically significant, as urinalysis was undertaken too rarely in the group of animals with non-hemolytic anemia to draw any conclusions (9 out of 29 dogs).

4.7.6. Hemoglobinuria

There was no significant result due to an insufficient amount of data regarding this parameter. Hemoglobinuria is a condition in which there is a higher proportion of the red blood pigment hemoglobin in the urine. While 23 of the 28 dogs in the IMHA group had their urine examined, only 9 of the 29 dogs in the non-HA group had their urine looked at.

4.7.7. Hemolytic Plasma

There was no significance regarding the difference when it comes to hemolytic plasma in the anemic patients. Hemolytic plasma is present, when there is a higher amount of hemoglobin in the blood plasma, but before interpreting it as evidence of hemolysis, artifactual hemolysis must be eliminated (3).

4.7.8. Icteric Plasma

In the group with dogs with present hemolysis a significally higher ($P = 0.005$) amount of bilirubin in the plasma was morphologically detectable.

5 Discussion

Immune-Mediated Hemolytic Anemia (IMHA) is a common and often life-threatening condition in dogs characterized by the immune system attacking and destroying red RBCs. (3,4,6) The findings of this thesis indicate significant elevations in COHb levels in the IMHA group compared to the other groups, corroborating earlier research (4).

Females were overrepresented in the IMHA group, and most of the dogs were neutered with the median age of the dogs being 7.34 years. This result reflects earlier study findings, in which IMHA patients were more often female, middle-aged dogs (27–29).

In the group of dogs with IMHA the levels of COHb were significantly elevated compared to both the non-HA group and the AHDS group. Although the dogs with hemolytic anemia exhibited the highest COHb values, there was no significant difference between the other two groups. This discovery mirrors the findings of a study conducted in Israel in 2021 (4), where the HA group also showed significantly higher COHb levels, although the disparity was not as pronounced in the present study. Also, this study looked in difference to the study from 2021 only on the usefulness of COHb as a marker of hemolysis in IMHA and not hemolytic anemia in general.

The increased COHb levels in the blood of animals with hemolytic anemia can be explained by the fact that there is increased hemoglobin catabolism during hemolysis, leading to this phenomenon (4,13,18,21,30). Furthermore, in the course of inflammatory diseases - such as IMHA - an increased cytokine concentration is normal (6). These pro-inflammatory messengers can subsequently cause CO metabolism and the formation of COHb (6).

Earlier studies in human medicine showed, that COHb was increased when hemolysis was present (12,21,31–36). In Veterinary medicine, there is only the mentioned study (4) and one focusing on dogs with Babesia canis infection from 1991 (37). In the field of pediatrics, there are publications that have investigated a possible connection between elevated COHb levels and various pathologies (38–41).

The hemoglobin content in animals with IMHA was notably lower than in AHDS animals. The anticipated increase in hemoglobin content due to hemoconcentration in the AHDS group was

confirmed by the results. In earlier studies, the hematocrit level of AHDS patients was notably elevated compared to that of the healthy control dogs. This increase indicates a concentration of blood due to the loss of fluid in the intestines. The term "hemorrhagic" may not be completely accurate, as it suggests the loss of whole blood. The fluid lost into the intestinal lumen is mainly plasma water, with only a small number of RBCs (42). The difference in hemoglobin between the IMHA-group and the non-HA group was not significant.

Another prominent finding was the higher count leukocyte count in the IMHA group compared to AHDS. While IMHA usually involves a regenerative response in the bone marrow along with an increase in mature and immature neutrophils, recent findings have shown a link between higher mortality rates and the severity of leukocytosis and the left shift (29). Further studies with strictly controlled study conditions could provide more information in the future regarding the white blood cell content and composition in IMHA patients compared to other dogs and the implied consequences.

Moreover, the RDW, the levels of AST and cCRP were slightly elevated in the IMHA group. No statistically significant statements could be made, due to limitations of this thesis. An increased RDW is usually a sign of enhanced regeneration (43). AST is often used as hemolysis marker, as it is frequently elevated in patients with hemolysis (44,45). This is because AST is found particularly in erythrocytes and is released during hemolysis. (46,47) Further studies should be initiated that focus more specifically on these parameters and have comparable data in the different study groups.

There was a significant difference between the IMHA-group and the non-HA-group regarding spherocytosis. Most dogs with IMHA had spherocytosis in previous studies (29). Therefore, an elevated spherocyte count is often considered a diagnostic criterion for IMHA (3,29). Nonspherocytic, nonagglutinating IMHA is rare in dogs (29). Spherocytes should be used as a diagnostic criterion only in dogs, assessed in the monolayer of a well-made blood smear to avoid artifacts and should be interpreted cautiously after transfusions (3).

The IMHA group yielded significantly higher polychromasia. This phenomenon points towards the presence of more immature RBCs (48). These stain bluish-purple in the Wright’s or rapid (e.g. Diff-quick) smears due to the higher RNA content. A higher presence of reticulocytes is observed in regenerative anemias (48), such as hemolytic anemia.

In the dogs with IMHA, anisocytosis was more often present. Severity of anisocytosis (RDW) were correlated with grade of reticulocytosis in earlier studies (49). According to the RDW, increased anisocytosis is therefore a sign of regeneration.

The IMHA group provided a significally higher amount of bilirubin in the plasma. This can be an important diagnostic value for IMHA according to some studies (50), but it has also been indicated in previous studies as a possible predictor of mortality in severely ill dogs (29,51,52). Hyperbilirubinemia may indicate hemolysis if there is no evidence of decreased functional hepatic mass, obstructive cholestasis, or sepsis (3).

5.1. Limitations

The most significant limitation of the present study is its retrospective nature.
As to this fact, the groups to be compared were not exposed to the same study conditions. This limits the significance of the study somewhat, as neither the treatments and examinations carried out within the individuals of the groups nor within the different groups were all the same.

Another important limitation is the small number of patients with IMHA and measured COHb and the lack of a control group with healthy animals.
The diagnosis of IMHA in dogs is still a diagnosis based on various clinical and laboratory diagnostic parameters, but largely on the exclusion of other causes. This is certainly an important limitation. One constraint of this study pertains to the diagnostic approach for AHDS as well, which is also primarily a diagnosis reached by eliminating other possible causes.

COHb measurement is still unavailable in most private veterinary practices. However, it is highly stable in heparinized blood for up to 4 weeks (19) and can be measured in many emergency and referral centers. Internal bleeding could lead to elevated COHb levels due to the formation of hematomas and the breakdown of hemoglobin, as observed in a previous study (53). This factor should be considered when evaluating COHb concentrations in anemic dogs.

The absence of a control group in the statistical analyses for the qualitative variables should be addressed in future prospective studies under controlled conditions. Overall, these limitations highlight the need for further research with more standardized study conditions, larger sample sizes, and inclusion of control groups to better understand and validate the findings related to IMHA in dogs.

6 Conclusion

In Conclusion, COHb-levels in dogs with IMHA are increased in this retrospective study in comparison to the non-HA group, as well as to the AHDS group. Future studies with a prospective case-control design are needed to further investigate the significance and reproducibility of this phenomenon. It is crucial for these studies to incorporate rigorous criteria for identifying IMHA and accurately diagnosing any comorbidities to ensure the reliability and validity of the findings. By addressing these factors, future research can provide a clearer understanding of the role of COHb as a diagnostic marker in IMHA and potentially improve diagnostic and therapeutic approaches.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Paltrinieri S. The diagnostic approach to anaemia in the dog and cat. Journal of the Hellenic Veterinary Medical Society. 2014;65(3).
2. Tvedten H. Schalm's Veterinary Hematology. 6th ed. Weiss DJ WK, editor. Ames: Blackwell Publishing; 2010. 152–161 p.
3. Garden OA, Kidd L, Mexas AM, Chang YM, Jeffery U, Blois SL, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. J Vet Intern Med. 2019;33(2):313–34.
4. Nivy R, Sutton G, Bruchim Y. Carboxyhemoglobin as a diagnostic and prognostic biomarker of hemolytic anemias in dogs. J Vet Intern Med. 2022 Jan 1;
5. Piek CJ. Canine idiopathic immune-mediated haemolytic anaemia: A review with recommendations for future research. Vol. 31, Veterinary Quarterly. 2011.
6. Swann JW, Woods K, Wu Y, Glanemann B, Garden OA. Characterisation of the immunophenotype of dogs with primary immune-mediated haemolytic anaemia. PLoS One. 2016;11(12).
7. Weng J, Levy NA, Abbott HY, Mix JA, Wills RW, Mackin AJ, et al. Retrospective analysis of immunosuppressive and anti-thrombotic protocols in nonassociative immune mediated hemolytic anemia in dogs. J Vet Intern Med. 2023 Mar 1;37(2):528–36.
8. Balch A, Mackin A. Immunvermittelte hämolytische Anämie beim Hund: Pathophysiologie, klinische Symptome und Diagnose. Tierärztliche Praxis Ausgabe K: Kleintiere / Heimtiere. 2007;35(04).
9. Bestwick JP, Skelly BJ, Swann JW, Glanemann B, Bexfield N, Gkoka Z, et al. Splenectomy in the management of primary immune-mediated hemolytic anemia and primary immune-mediated thrombocytopenia in dogs. J Vet Intern Med. 2022 Jul 1;36(4):1267–80.
10. Lutz H, Kohn B, Forterre F. Krankheiten der Katze. Lutz H, Kohn B, Forterre F, editors. Vol. 5. Stuttgart: Georg Thieme Verlag KG; 2015. 21.2.5.1.
11. Swann JW, Garden OA, Fellman CL, Glanemann B, Goggs R, LeVine DN, et al. ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs. J Vet Intern Med. 2019;33(3).
12. Hariri G, Hodjat Panah K, Beneteau-Burnat B, Chaquin M, Mekinian A, Ait-Oufella H. Carboxyhemoglobin, a reliable diagnosis biomarker for hemolysis in intensive care unit: a retrospective study. Crit Care. 2021;25(1):7–9.
13. Wu L, Wang R. Carbon monoxide: Endogenous production, physiological functions, and pharmacological applications. Vol. 57, Pharmacological Reviews. 2005.
14. Wu L, Wang R. Carbon monoxide: Endogenous production, physiological functions, and pharmacological applications. Vol. 57, Pharmacological Reviews. 2005. p. 585–630.
15. Ryter SW, Otterbein LE, Morse D, Choi AMK. Heme oxygenase/carbon monoxide signaling pathways: Regulation and functional significance. Mol Cell Biochem. 2002;234–235:249–63.
16. Yu J, Lee J, Cho Y, Oh J, Kang H, Lim TH, et al. Correlation between Carboxyhemoglobin Levels Measured by Blood Gas Analysis and by Multiwave Pulse Oximetry. J Pers Med. 2024;14(2).

17. Hampson NB. Carboxyhemoglobin: A primer for clinicians. Undersea and Hyperbaric Medicine. 2018;45(2).
18. Owens EO. Endogenous carbon monoxide production in disease. Vol. 43, Clinical Biochemistry. 2010.
19. Hampson NB. Stability of carboxyhemoglobin in stored and mailed blood samples. American Journal of Emergency Medicine. 2008;26(2).
20. Papin M, Latour C, Leclère B, Javaudin F. Accuracy of pulse CO-oximetry to evaluate blood carboxyhemoglobin level: a systematic review and meta-analysis of diagnostic test accuracy studies. Vol. 30, European Journal of Emergency Medicine. Lippincott Williams and Wilkins; 2023. p. 233–43.
21. Karabulut B, Arcagok BC. A Neglected and Promising Predictor of Severe Hyperbilirubinemia Due to Hemolysis: Carboxyhemoglobin. Fetal Pediatr Pathol. 2020;39(2).
22. Idalan N, Müller E, Giger U. The Indirect Antiglobulin (Coombs') Test Is Specific but Less Sensitive Than the Direct Antiglobulin Test for Detecting Anti-Erythrocytic Autoantibodies and Thereby Immune-Mediated Hemolytic Anemia in Dogs. Vet Sci. 2023 Jul 1;10(7).
23. Wardrop KJ. Coombs' Testing and Its Diagnostic Significance in Dogs and Cats. Vol. 42, Veterinary Clinics of North America - Small Animal Practice. 2012.
24. Burrows CF. Canine hemorrhagic gastroenteritis. J Am Anim Hosp Assoc. 1977;13(4).
25. Dupont N, Jessen LR, Moberg F, Zyskind N, Lorentzen C, Bjørnvad CR. A retrospective study of 237 dogs hospitalized with suspected acute hemorrhagic diarrhea syndrome: Disease severity, treatment, and outcome. J Vet Intern Med. 2021;35(2).
26. Huber L, Kuropka B, Doulidis PG, Baszler E, Martin L, Rosu A, et al. Plasma proteome signature of canine acute haemorrhagic diarrhoea syndrome (AHDS). PLoS One. 2024;19(2 February).
27. Balch A, Mackin A. Canine immune-mediated hemolytic anemia: Pathophysiology, clinical signs, and diagnosis. Compendium: Continuing Education For Veterinarians. 2007;29(4).
28. Woolhead VL, Szladovits B, Chan A, Swann JW, Glanemann B. Breed predispositions, clinical findings, and prognostic factors for death in dogs with nonregenerative immune-mediated anemia. J Vet Intern Med. 2021 Jan 1;35(1):252–60.
29. Weinkle TK, Center SA, Randolph JF, Warner KL, Barr SC, Erb HN. Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 Cases (1993-2002). J Am Vet Med Assoc. 2005;226(11).
30. Fazekas AS, Wewalka M, Zauner C, Funk GC. Carboxyhemoglobin levels in medical intensive care patients: A retrospective, observational study. Crit Care. 2012;16(1).
31. Hampson NB. Carboxyhemoglobin Elevation Due to Hemolytic Anemia. Journal of Emergency Medicine. 2007;33(1).
32. Widness JA, Lowe LS, Stevenson DK, Vreman HJ, Weiner CP, Hayde M, et al. Direct relationship of fetal carboxyhemoglobin with hemolysis in alloimmunized pregnancies. Pediatr Res. 1994;35(6).
33. Kai Man C, Koon Ngai L. Endogenous carbon monoxide production in extracorporeal membrane oxygenation-related hemolysis: potential use of point-of-care CO -oximetry carboxyhemoglobin to detect hemolysis . Clin Case Rep. 2018;6(2).
34. Bemtgen X, Rilinger J, Holst M, Rottmann F, Lang CN, Jäckel M, et al. Carboxyhemoglobin (CO-Hb) Correlates with Hemolysis and Hospital Mortality in

Extracorporeal Membrane Oxygenation: A Retrospective Registry. Diagnostics. 2022;12(7).
35. Hoffman KR, Burrell AJC, Diehl A, Butt W. Elevated carboxyhaemoglobin as a novel indicator for extracorporeal membrane haemolysis and oxygenator exchange. Crit Care. 2021;25(1).
36. Awad HA, Shehata BM, Fouad MA. Correlation between Non-invasive Measurement of Carboxyhemoglobin and Bilirubin Level Measurement in Near- Term and Term Neonates as a Predictor of Neonatal Hemolysis. QJM: An International Journal of Medicine. 2021;114(Supplement_1).
37. Taylor JH, Guthrie AJ, Leisewitz A. The effect of endogenously produced carbon monoxide on the oxygen status of dogs infected with Babesia canis. J S Afr Vet Assoc. 1991;62(4).
38. Jenkinson A, Zaidi S, Bhat R, Greenough A, Dassios T. Carboxyhaemoglobin levels in infants with hypoxic ischaemic encephalopathy. J Perinat Med. 2023;51(9).
39. Dani C, Remaschi G, Monti N, Pratesi S. Carboxyhemoglobin as biomarker of late-onset sepsis in preterm infants. Eur J Pediatr. 2023 Oct 1;182(10):4523–8.
40. Vardar G, Ozek E. Carboxyhemoglobin Levels in Preterm Neonatal Late-Onset Sepsis: to Predict or not to Predict. Mediterr J Hematol Infect Dis. 2023;15(1).
41. Alakaya M, Arslankoylu AE. Prognostic value of carboxyhemoglobin in pediatric intensive care unit. Pak J Med Sci. 2022 Aug 20;38(7):1911–7.
42. Mortier F, Strohmeyer K, Hartmann K, Unterer S. Acute haemorrhagic diarrhoea syndrome in dogs: 108 cases. Veterinary Record. 2015;176(24).
43. Neiger R, Hadley J, Pfeiffer DU. Differentiation of dogs with regenerative and non-regenerative anaemia on the basis of their red cell distribution width and mean corpuscular volume. Veterinary Record. 2002;150(14).
44. Ghaleb S, Reagor JA, Tarango C, Benscoter A, Smith R, Byrnes JW. Correlation among Hemolysis Biomarkers in Pediatric Patients Undergoing Extracorporeal Membrane Oxygenation. Journal of Extra-Corporeal Technology. 2021 Jun 1;53(2):125–9.
45. Nsiah K, Dzogbefia VP, Ansong D, Akoto AO, Boateng H, Ocloo D. Pattern of AST and ALT changes in relation to hemolysis in sickle cell disease. Clin Med Insights Blood Disord. 2011;4:1–9.
46. Koller PU, Tritschler W, Carstensen CA. Untersuchungen von Störeinflüssen am Reflotron® System. LaboratoriumsMedizin. 1989;13(11).
47. Koseoglu M, Hur A, Atay A, Çuhadar S. Effects of hemolysis interferences on routine biochemistry parameters. Biochem Med (Zagreb). 2011 Feb;21(1):79–85.
48. Hodges J, Christopher MM. Diagnostic accuracy of using erythrocyte indices and polychromasia to identify regenerative anemia in dogs. J Am Vet Med Assoc. 2011;238(11).
49. Hodges J, Christopher MM. Diagnostic accuracy of using erythrocyte indices and polychromasia to identify regenerative anemia in dogs. J Am Vet Med Assoc. 2011 Jun 1;238(11):1452–8.
50. Bennett D, Finnett SL, Nash AS, Kirkham D. Primary autoimmune haemolytic anaemia in the dog. Vet Rec. 1981;109(8):150–3.
51. Swann JW, Skelly BJ. Systematic Review of Prognostic Factors for Mortality in Dogs with Immune-mediated Hemolytic Anemia. Vol. 29, Journal of Veterinary Internal Medicine. 2015.
52. Piek CJ, Junius G, Dekker A, Schrauwen E, Slappendel RJ, Teske E. Idiopathic immune-mediated hemolytic anemia: Treatment outcome and prognostic factors in 149 dogs. J Vet Intern Med. 2008;22(2).

53. Ziemann-Gimmel P, Schwartz DE. Increased carboxyhemoglobin in a patient with a large retroperitoneal hematoma. Anesth Analg. 2004;99(6).