Klinik für Kleintiermedizin, Abteilung Kardiologie
der Vetsuisse-Fakultät Universität Zürich

Direktorin Klinik: Prof. Dr. med. vet. Claudia Reusch
Leiter Abteilung: Prof. Dr. med. vet. Tony Glaus

Arbeit unter wissenschaftlicher Betreuung von
Prof. Dr. med. vet. Tony Glaus

Assessment of iron deficiency as comorbidity in dogs with advanced myxomatous mitral valve disease

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Angela Betschart

Tierärztin
von Muotathal, Schwyz

genehmigt auf Antrag von

Prof. Dr. med. vet. Tony Glaus, Referent
Prof. Dr. med. vet. Thomas Lutz, Korreferent

2020

Inhaltsverzeichnis

Abstract/ deutsche Zusammenfassung……..…….……4

Introduction…….……...5

Material and Methods……..……...6

Animals……..…….6

Echography…….……...6

Laboratory analyses……..…….7

Statistical methods……..……...…….……7

Results…….....……7

Discussion…….…….....….8

Study limitations…….……...8

Conclusions…….……...9

Conflict of interest…….……....9

Acknowledgements…….....9

Funding…….……9

References…….…….……...10

Anhänge…….……..13

Danksagung

CV

Zusammenfassung

Bei Menschen mit chronischer Herzinsuffizienz ist Eisenmangel eine häufige Komorbidität. Eine intravenöse Eisentherapie verbessert signifikant Belastbarkeit und Lebensqualität dieser Patienten. Die Richtlinien der European Society of Cardiology empfehlen deshalb, bei symptomatischen Herzpatienten mit reduzierter Ejektionsfraktion und Eisenmangel eine intravenöse Eisencarboxymaltose-Therapie in Betracht zu ziehen. Die vorliegende Studie untersuchte, ob bei Hunden in unterschiedlichen Stadien einer Mitralendokardiose (ME) ebenfalls ein vergleichbarer Eisenmangel als Komorbidität vorliegt.
Bei privaten Hunden mit einer ME wurden Hämatologie, Chemogramm und Eisenstatus, d.h. Serum-Eisen, Transferrinsättigung (Tsat), Ferritin und Gesamteisenbindungskapazität durchgeführt. Eingeschlossen wurden Hunde in den Stadien B2 (n= 33) und C (n= 37), gemäss ACVIM Konsensus Klassifikation. Als Kontrolle dienten 30 gesunde Hunde.
Hämatologisch unterschieden sich die Gruppen nicht. Das Serum-Eisen war bei C-Hunden signifikant niedriger als bei B2-Hunden ($p = 0,03$) und den Kontrollhunden ($p = 0,016$) und korrelierte negativ mit der Grösse der linken Ventrikel ($r = -0,23$); Tsat war bei C-Hunden signifikant niedriger als bei B2-Hunden ($p = 0,031$).
Wird Eisenmangel als erniedrigtes Serum-Eisen oder eine Tsat <20% definiert, wiesen zwei B2-Hunde und sieben C-Hunde einen Eisenmangel auf. Diese Daten implizieren, dass eine Untergruppe von Hunden mit fortgeschrittener ME an einem Eisenmangel leiden.

Schlüsselwörter: Serum Eisen, Transferrin Sättigung, Ferritin, C- reaktives Protein

Abstract

In humans with chronic heart failure, iron deficiency is a common comorbidity and intravenous iron therapy significantly improves exercise capacity and quality of life. The European Society of Cardiology Guidelines recommend considering intravenous ferric carboxymaltose therapy in symptomatic patients with heart failure with reduced ejection fraction and iron deficiency.
The objective of this study was to assess if iron deficiency is a comparable comorbidity in dogs at various stages of myxomatous mitral valve disease (MMVD). Hematology, biochemical analysis and iron status, i.e., serum iron, ferritin, transferrin saturation (Tsat) and total iron-binding capacity, were obtained in client-owned dogs with MMVD, in stage B2 (n=33) and C (n=37) heart failure, according to the ACVIM Consensus Classification. A control group of 30 healthy dogs was used as a comparison.
Hematological parameters were consistent between the groups. Serum iron was significantly lower in stage C compared to stage B2 ($p = 0.03$) and control dogs ($p=0.016$) and negatively correlated with left ventricular size ($r = -0.23$); Tsat was significantly lower in stage C compared to B2 dogs ($p = 0.031$). When using decreased serum iron or Tsat <20% as diagnostic criteria, two in stage B2 and seven in stage C were iron deficient. These data suggest presence of iron deficiency in a subset of dogs with advanced MMVD.

Key words: serum iron, transferrin saturation, ferritin, C-reactive protein

Assessment of iron deficiency as comorbidity in dogs with advanced myxomatous mitral valve disease

A. Betschart[a], M. Baron Toaldo[a,b], F. Dondi[b], I. Vannini[c], A. Kovacevic[d], B. Riond[e], M. Wenger[c], S. Burckhardt[f], M. Wyler[g], T.M. Glaus[a,*]

[a]*Division of Cardiology, Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland*
[b]*Department of Veterinary Medical Sciences, University of Bologna, Via Zamboni 33, 40126 Bologna, Italy*
[c]*Bessy's Kleintierklinik AG, Eichwatt 3, 8105 Watt/ Regensdorf, Switzerland*
[d]*Division of Cardiology, Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Berne, Länggassstrasse 120, 3012 Berne, Switzerland*
[e]*Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland*
[f]*Vifor Pharma AG, Flughofstrasse 61, 8152 Glattbrugg, Switzerland*
[g]*Department of Plant and Microbial Biology, Zollikerstrasse 107, 8008 University of Zurich, Switzerland*

* Corresponding author: Prof. T.M. Glaus, Division of Cardiology, Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland. Email: tglaus@vetclinics.uzh.ch

Introduction

Chronic heart failure (CHF) in dogs is a medical condition most commonly seen as the consequence of mitral regurgitation in myxomatous mitral valve disease (MMVD) (Buchanan, 1999; Kvart and Häggström, 2000; Baumgartner and Glaus, 2004). In human medicine, chronic heart failure CHF is a clinical problem of utmost importance with around 26 million people affected worldwide (Ponikowski et al., 2014a). Therefore, this condition is a medical and economic issue with epidemic qualities (Bui et al., 2011). Besides reduced life expectancy in humans (Cowie et al, 2000), impaired quality of life (QoL) is widely discussed (Hoekstra et al., 2011). Among other factors, iron deficiency (ID) is a proven and common comorbidity responsible for reduced QoL in humans, having a negative impact on the clinical situation and prognosis (Ponikowski et al., 2014b). Treatment of ID with intravenous ferric carboxymaltose (FCM) has been shown to significantly improve QoL and the New York Heart Association (NYHA) class (Anker et al., 2009). A follow-up trial further revealed that intravenous iron therapy had a positive impact on exercise capacity and reduced the risk of hospitalizations (Ponikowski et al., 2015). As a consequence of these findings, the 2016 guidelines of the European Society of Cardiology recommend screening symptomatic patients with heart failure with reduced ejection fraction for ID and, if present, considering treatment with FCM (Ponikowski et al., 2016). In humans, ID has been defined as serum ferritin <100 µg/L, or ferritin between 100–299 µg/L and transferrin saturation (Tsat) <20% (Ponikowski et al., 2016). Recently, this definition has been questioned and the diagnostic value of Tsat and serum iron to diagnose ID reconsidered (Grote Beverborg et al., 2018). In this study, Tsat <20% or low serum iron showed the best performance for detecting ID and best identified heart failure patients at the highest risk of death. Furthermore, Tsat and serum iron, but not ferritin, were independently
associated with mortality, which brings into question the diagnostic value of ferritin in the scenario of CHF (Grote Beverborg et al., 2018).

In dogs with CHF, the situation may be comparable; however, before translating treatment recommendations from human to veterinary cardiology, it should be confirmed that dogs diagnosed with CHF are similarly suffering from ID. Therefore, this study aimed to investigate whether, and to what extent, ID is a comorbidity in dogs suffering from CHF. We hypothesized that ID is common in dogs with MMVD and that the severity would increase as the disease progresses.

Materials and Methods

The objective of this multicenter, prospective case-controlled study was to test the hypothesis that dogs with CHF due to MMVD suffer from iron deficiency. The primary endpoint was iron status, the secondary endpoint was presence of anemia. Four centers participated in the study, the Divisions of Cardiology Vetsuisse Faculty Universities of Zurich and Bern, Department of Veterinary Medical Sciences University of Bologna, and Bessy's Kleintierklinik, CH-Watt. The study was approved by the State Veterinary Office of Zurich (Application number ZH 261/16).

Animals

Dogs were prospectively enrolled into the study from patients having cardiological examination for medical concerns, such as preventive cardiac checkup, pre-anesthesia assessment, heart murmur, weakness, exercise intolerance, syncope, cough, or dyspnea. Owners were thoroughly informed about the study via written statement and a client consent form was signed. All dogs underwent complete clinical and echocardiographic examination. In dogs with respiratory signs, thoracic radiographs in two perpendicular projections were performed to check presence of pulmonary infiltrates compatible with lung edema. Dogs with an echocardiographic diagnosis of MMVD were enrolled if they had cardiomegaly defined as left ventricular internal diameter at end diastole normalized to body weight (LVDDN) $\geq$1.7. They were classified as compensated (stage B2) or decompensated (stage C) in accordance with the ACVIM classification guidelines (Atkins et al., 2009). Systemic blood pressure measurement was carried out using an oscillometric method in each dog following published guidelines (Brown et al., 2007). Predefined exclusion criteria were any disease in addition to MMVD and any treatment in the previous four weeks besides cardiac drugs or routine antiparasitic drugs.

As controls, healthy dogs, based on history, physical and echocardiographic examination, were recruited from dogs attending for preventive cardiac checkup or pre-anesthesia assessment, and dogs owned by staff and students. These dogs were presented in the same period and similarly prospectively enrolled. The owners were thoroughly informed and signed a client consent form.

Echocardiography

Complete echocardiographic exams were performed with an Epiq 7 (Philips Medical, TG and MBT) or a Vivid 7 (GE Medical Systems, AK and IV) Ultrasound System in right and left lateral recumbency using standard views (Thomas et al., 1993). The diagnosis of MMVD was based on visible degenerative changes on two-dimensional (2-D) echocardiography and mitral valve insufficiency on color doppler examination (Boswood et al., 2016). The left ventricular internal diameter was measured from a 2-D guided M-Mode image obtained from a right parasternal short-axis view at the level of the papillary muscles (Boswood et al., 2016). The left atrial to aortic root ratio (LA/Ao) was also obtained from a short-axis view (Rishniw and Erb, 2000). Normal was defined as LVDDN $\leq$1.6 and LA/Ao $\leq$1.6.

Laboratory analyses

In order to evaluate the iron status, the following iron specific and ID-related parameters were analyzed: serum iron, Tsat, total iron binding capacity (TIBC), ferritin, hemoglobin (Hb), erythrocyte indices and the reticulocyte hemoglobin equivalent (RET-He). Four ml of blood was drawn either from the jugular or cephalic vein with caution to prevent hemolysis. Serum for analysis of iron specific parameters was immediately frozen and kept at -80° C; serum was carried on dry ice to the Laboratory at University of Bologna, where it was analyzed in batches. Serum iron and TIBC were measured using colorimetric methods (Iron Ferene, KAL 002, Olympus /Sentinel; UIBC OSR61205, Olympus/Beckman Coulter). Serum ferritin was measured using an assay validated for dogs (Ferritin OSR 61203, Olympus/Beckman Coulter) after standard internal validation.

To identify non-cardiogenic factors, e.g. inflammatory conditions, that might affect iron parameters like ferritin (Bohn, 2013; Grote Beverborg et al., 2018), a complete blood count, routine biochemical profile and serum C-reactive protein (CRP) were obtained. Hematological analyses were performed on an automated hematology analyzer (Sysmex-XT 2000iv). Biochemical analyses were performed on fully automated chemistry analyzers (Vetsuisse Zurich, Berne and Bessy's, Cobas 6000 c 501, Roche; Bologna, Olympus AU 480, Olympus/Beckman Coulter). C-reactive protein was measured using an immunoturbidimetric assay (CRP OSR6147, Olympus/Beckman Coulter).

Statistical methods

Data processing and statistical evaluation were performed with R (v3.5, R Core Team 2013). The normal distribution of measurement errors was ascertained through visual inspection using QQ-plots from the Car Package (v.3.0.2, Fox & Weisberg, 2019) and by inspecting the standardized residuals. All data were normally distributed. Therefore, analysis of variance was performed to test effect of disease stage on performed measurements. For significant differences, subsequent pairwise comparisons of group means were performed using Tukey's Range Test with the Agricolae Package (v1.3.0, https://CRAN.R-project.org/package=agricolae).

Pairwise monotonic relationships between measurements were calculated with Spearman correlation, using the Hmisc Package (v.4.1.1, https://CRAN.R-project.org/package=Hmisc) and significance was set at $P < 0.05$.

Results

Seventy dogs with CHF and 30 control dogs were included. Of the dogs with CHF, 33 (47%) were classified as stage B2, 37 (53%) as stage C. Control dogs were younger than dogs with CHF and body condition score was significantly lower in stage C dogs compared to both control and stage B2 dogs (table 1). There was a large variety of breeds in each class with only Chihuahua (n= 12), Cavalier King Charles (n= 8) and Jack Russel Terrier (n=6) appearing more than twice in total. The LVDDN and LA/Ao values were, by definition, smaller in control dogs than those with heart disease, and significantly larger in stage C compared to stage B2 dogs ($p < 0.01$, table 1).

The laboratory parameters and correlations are summarized in tables 2-4. The hematological parameters were consistent between the groups, even though hemoglobin concentration correlated with each parameter of iron status. No significant differences in RET-He were identified between the groups. The CRP concentration was significantly higher in stage C compared to stage B2 and control dogs ($P < 0.01$).

Serum iron was significantly lower in stage C dogs (118 $\pm$ 56 ug/dl) compared to control (156 $\pm$ 45 ug/dl, $p = 0.016$) and stage B2 (151 $\pm$ 57 ug/dl, $p= 0.030$) dogs (figure 1, table 3). Tsat was significantly lower in stage C dogs (30 $\pm$ 12%) compared to control dogs (39 $\pm$

12%, p = 0.031), but not compared to stage B2 (37 + 14%, p = 0.078) dogs (figure 2). There were no differences in TIBC and ferritin between the groups.
Seven dogs had a serum iron concentration below the reference range; Tsat was <30% in all seven and <20% in six of these. One of these seven dogs was in class B2 (LVDDN 2.4); the other six were in class C (LVDDN 2.1-2.6). Five of these seven dogs had an elevated CRP of 17-193 mg/l. An additional two dogs had a Tsat below 20%; one of these was in stage B2 (LVDDN 2.0) with a TSAT of 18%, serum iron of 58 ug/ml and low CRP <10 mg/l; the other was in stage C (LVDDN 2.3) with a TSAT of 19%, serum iron of 90 ug/dl and CRP <10 mg/l. An additional 26 dogs had a TSAT <30%, eight of these were control dogs, and nine each in stage B2 and C. In these, the CRP was elevated in five stage C dogs, one stage B2 dog and two control dogs.
The following correlations were found: The serum iron weakly and negatively correlated with LVDDN (r = -0.23, P = 0.03), body condition score (r = -0.35, P = <0.01) and CRP (r = -0.28, p < 0.01). The CRP also weakly and negatively correlated with Tsat (r = -0.27, P = 0.01) and body condition score (r = -0.3, P = 0.01), and it weakly correlated with LVDDN (r = 0.21, P = 0.05) and LA/Ao (r = 0.22, P = 0.05). The body condition score also negatively correlated with LVDDN (r = -0.39, P <0.01) and LA/Ao (r = -0.45, P = <0.01).

Discussion
In human patients with CHF, ID - with or without associated anemia - is an important comorbidity which receives therapeutic attention. Our study failed to provide any evidence for anemia in dogs even with advanced MMVD. Furthermore, if ID is diagnosed based on serum ferritin, this study also failed to identify ID without anemia. However, several dogs had low serum iron concentration; all in stage C heart failure with one exception, indicating ID could be a potential comorbidity of CHF in dogs. If the criteria for diagnosing ID in veterinary hematology (Naigamwalla et al., 2012), or recently suggested in human cardiology (Grote Beverborg et al., 2018) are applied; low serum iron or Tsat <20%, ID was present in 6% of dogs in stage B2 and 19% of dogs in stage C.
A newer parameter to diagnose ID is RET-He. This marker has been suggested as reliable reflecting iron availability for Hb synthesis (Fuchs et al., 2017). In the present study, RET-He was similar in all groups. This parameter might only be decreased when iron deficiency becomes severe enough to affect hematopoiesis.
Factors implicated in ID in people with CHF are ischemic insults initiating inflammatory processes (Dick and Epelman, 2016). Whilst ischemic injury plays a minor role in canine MMVD, inflammatory processes do occur in advanced CHF (Domanjko et al., 2018). Similarly, in our study, CRP was higher in dogs in stage C and negatively correlated with serum iron concentration. Further factors implicated in ID in humans are inadequate nutrition, micro-bleeding, gastritis and ulcers due to concomitant medication, low-protein diet and proteinuria because of additional renal disease, malabsorption because of intestinal mucosal edema and impaired gastrointestinal blood circulation (Sica, 2003; Okonko and Anker, 2004; Ather et al., 2012; McDonagh and Macdougall, 2015). In dogs, weight loss as a visible result of malnutrition has most recently been described as a typical sign of reaching the end stage in MMVD (Boswood et al., 2020). Likewise, in this study, the body condition score was significantly lower in dogs in stage C and negatively correlated with serum iron.

Study limitations
The CRP was higher in dogs with advanced heart failure and negatively correlated with serum iron concentration. Even though inflammation is known to be present in CHF in humans as well as in dogs, and inflammation is one postulated cause of ID in human CHF, inflammation is also known to affect iron metabolism, which might be a confounding factor.

Bone marrow iron staining, the gold standard for assessing iron stores (Burns et al., 1990; Grote Beverborg, 2018), was not performed due to its invasiveness. However, in view of the fact that any disease other than MMVD was an exclusion criterion, and comparing our data to recent human data suggests that ID was, indeed, present in these dogs (Grote Beverborg et al., 2018). With this perception, inflammation associated with CHF seems more likely to be causative both for higher CRP concentrations and ID, rather than inflammation causing only iron sequestration.
Cardiac patients and normal dogs were prospectively enrolled irrespective of sex, age and body weight, i.e. they were not matched for these parameters. However, hematological and iron parameters should not be affected by age in adult dogs within the ranges used, and did not correlate in this study.

Conclusions
This study suggests ID to be a potential comorbidity in dogs with advanced MMVD. Further studies are necessary to prove the development of ID in the course of this disease, and, if present, if iron supplementation will have similarly positive effects on life span and quality, as in humans.

Conflict of interest
At the time of this study, Dr. Burckhardt was working at Vifor Pharma AG, Glattbrugg, Switzerland.

Acknowledgements
The data were presented as an abstract at the 28th Annual ECVIM-CA Congress in Rotterdam, 6-8 September 2018.
We thank Vifor Pharma AG, Glattbrugg, Switzerland for supporting our study with a research grant.

Funding
This study was supported with a research grant by Vifor Pharma AG, Glattbrugg, Switzerland.

References

Anker, S.D., Comin Colet, J., Filippatos, G., Willenheimer, R., Dickstein, K., Drexler, H., Lüscher, T., Bart, B., Banasiak, W., Niegowska, J., et al., 2009. Ferric carboxymaltose in patients with heart failure and iron deficiency. New England Journal of Medicine 361, 2436-2448.

Atkins, C., Bonagura, J., Ettinger, S., Fox, P., Gordon, S., Haggstrom, J., Hamlin, R., Keene, B., Luis-Fuentes, V., Stepien, R., 2009. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. Journal of Veterinary Internal Medicine 23, 1142-1150.

Ather, S., Chan, W., Bozkurt, B., et al., 2012. Impact of noncardiac comorbidities on morbidity and mortality in a predominantly male population with heart failure and preserved versus reduced ejection fraction. Journal of the American College of Cardiology 59, 998-1005.

Baumgartner, C., Glaus, T.M., 2004. Acquired cardiac diseases in the dog: a retrospective analysis. Schweizer Archiv fur Tierheilkunde 146, 423-430.

Bohn, A., 2013. Diagnosis of disorders of iron metabolism in dogs and cats. Veterinary Clinics of North America: Small Animal Practice 43, 1319-1330.

Boswood, A., Häggström, J., Gordon, S.G., Wess, G., Stepien, R.L., Oyama, M.A., Keene, B.W., Bonagura, J., MacDonald, K.A., Patteson, M., et al., 2016. Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiomegaly: The EPIC Study – A randomized clinical trial. Journal of Veterinary Internal Medicine 30, 1765-1779.

Boswood, A., Gordon, S.G., Häggström, J., Vanselow, M., Wess, G., Stepien, R.L., Oyama, M.A., Keene, B.W., Bonagura, J., MacDonald, K.A., et al., 2020. Temporal changes in clinical and radiographic variables in dogs with preclinical myxomatous mitral valve disease: The EPIC study. Journal of Veterinary Internal Medicine 34, 1108-1118.

Brown, S., Atkins, C., Bagley, R., Carr, A., Cowgill, L., Davidson, M., Egner, B., Elliott, J., Henik, R., Labato, M., et al., 2007. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. Journal of Veterinary Internal Medicine 21, 542-558.

Buchanan, J.W., 1999. Prevalence of cardiovascular disorders. In: Textbook of canine and feline cardiology. W.B. Saunders, Philadelphia, PA, USA, pp. 457-470.

Bui, A.L., Horwich, T.B., Fonarow, G.C., 2011. Epidemiology and Risk Profile of Heart Failure. Nature Reviews Cardiology 8, 30-41.

Burns, E.R., Goldberg, S.N., Lawrence, C., Wenz, B., 1990. Clinical utility of serum tests for iron deficiency in hospitalized patients. American Journal of Clinical Pathology 93, 240-245.
Cowie, M.R., Wood, D.A., Coats, A.J., Thompson, S.G., Suresh, V., Poole-Wilson, P.A., Sutton, G.C., 2000. Survival of patients with a new diagnosis of heart failure: a population based study. Heart 83, 505-510.

Dick, S.A., Epelman, S., 2016. Chronic heart failure and inflammation: What do we really know? Circulation Research 119, 159-176.

Domanjko, Petric A., Lukman, T., Verk, B., Nemec Svete, A., 2018. Systemic inflammation in dogs with advanced-stage heart failure. Acta Veterinaria Scandinavica 60, 20.

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, Third Edn. Sage, Thousand Oaks, CA.

Fuchs, J., Moritz, A., Grussendorf, E., Lechner, J., Neuerer, F., Nickel, R., Rieker, T., Schwedes, C., DeNicola, D.B., Russell, J., Bauer, N., 2017. Canine reticulocyte hemoglobin content (RET-He) in different types of iron-deficient erythropoiesis. Veterinary Clinical Pathology 46, 422-429.

Grote Beverborg, N., Klip, I.T., Meijers, W.C., Voors, A.A., Vegter, E.L., van der Wal, H.H., Swinkels, D.W., van Pelt, J., Mulder, A.B., Bulstra, S.K., et al., 2018. Definition of Iron Deficiency Based on the Gold Standard of Bone Marrow Iron Staining in Heart Failure Patients. Circulation Heart Failure 11, e004519.

Hoekstra, T., Lesman-Leegte, I., van Veldhuisen, D.J., Sanderman, R., Jaarsma, T., 2011. Quality of life is impaired similarly in heart failure patients with preserved and reduced ejection fraction. European Journal of Heart Failure 13, 1013-1018.

Kvart, C., Häggström, J., 2000. Acquired valvular disease. In: Textbook of veterinary internal medicine. W.B. Saunders, Philadelphia, PA, USA, pp. 787-800.

McDonagh, T., Macdougall, I.C., 2015. Iron therapy for the treatment of iron deficiency in chronic heart failure: intravenous or oral? European Journal of Heart Failure 17, 248-262.

Naigamwalla, D.Z., Webb, J.A., Giger, U., 2012. Iron deficiency anemia. The Canadian veterinary journal 53, 250-256.

Okonko, D.O., Anker, S.D., 2004. Anemia in chronic heart failure: pathogenetic mechanisms. Journal of Cardiac Failure 10, 5-9.

Ponikowski, P., Anker, S.D., AlHabib, K.F., Cowie, M.R., Force, T.L., Hu, S., Jaarsma, T., Krum, H., Rastogi, V., Rohde, L.E., et al., 2014a. Heart failure: preventing disease and death worldwide. European Society of Cardiology Heart Failure Association 1, 4-25.

Ponikowski, P., van Veldhuisen, D.J., Comin-Colet, J., Ertl, G., Komajda, M., Mareev, V., McDonagh, T.A., Parkhomenko, A., Tavazzi, L., Levesque, V., et al., 2014b. Rationale and design of the CONFIRM-HF study: a double-blind, randomized, placebo-controlled study to assess the effects of intravenous ferric carboxymaltose on functional capacity in patients with chronic heart failure and iron deficiency. European Society of Cardiology Heart Failure Association 1, 52-58.

Ponikowski, P., van Veldhuisen, D.J., Comin-Colet, J., Ertl, G., Komajda, M., Mareev, V., McDonagh, T., Parkhomenko, A., Tavazzi, L., Levesque, V., et al., 2015. Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency. European Heart Journal 36, 657-668.

Ponikowski, P., Voors, A.A., Anker, S.D., Bueno, H., Cleland, J.G.F., Coats, A.J.S., Falk, V., González-Juanatey, J.R., Harjola, V.P., Jankowska, E.A., et al., ESC Scientific Document Group, 2016. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal 37, 2129-2200.

Rishniw, M., Erb, H.N., 2000. Evaluation of Four 2-Dimensional Echocardiographic Methods of Assessing Left Atrial Size in Dogs. Journal of Veterinary Internal Medicine 14, 429-435.

Sica, D.A., 2003. Pharmacotherapy in congestive heart failure: drug absorption in the management of congestive heart failure: loop diuretics. Congestive Heart Failure 9, 287-292.

Thomas, W.P., Gaber, C.E., Jacobs, G.J., Kaplan, P.M., Lombard, C.W., Moise, N.S., Moses, B.L., 1993. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. Journal of Veterinary Internal Medicine 7, 247-252.

Table 1
Demographic and selective echo data (mean ± SD) in 30 healthy dogs and 70 dogs at different stages of myxomatous mitral valve disease.

Variable	Control N = 30	Stage B2 N = 33	Stage C N = 37	P-value ANOVA[§]	P-value Tukey's
Age (years)	7.9 ± 3.7	10.3 ± 2.4	10.9 ± 3.2	<0.001	<0.01[ab]
Sex (f / m)	13 / 17	16 / 17	11 / 26	0.167	na
Weight (kg)	14.3 ± 9.6	11.5 ± 8.9	11.5 ± 11.1	0.46	na
BCS	5.4 ± 0.9	5.4 ± 1.0	4.6 ± 1.4	0.01	0.02[b] 0.05[c]
LVDDN	1.49 ± 0.08	1.94 ± 0.22	2.22 ± 0.27	<0.01	<0.01[abc]
LA/AO	1.40 ± 0.17	1.87 ± 0.33	2.32 ± 0.54	<0.01	≤0.01[abc]

[§]one-way ANOVA, except Chi Square test for sex; BCS, body condition score; LVDDN, left ventricular diameter in diastole normalized to body weight; LA/Ao, left atrial to aortic root ratio; na, not applicable.
[a]control versus stage B2, [b]control versus stage C, [c]stage B2 versus stage C.

Table 2
Selective laboratory parameters (mean ± SD) in 30 healthy dogs and 70 dogs at different stages of myxomatous mitral valve disease.

Variable	Unit	Control N = 30	Stage B2 N = 33	Stage C N = 37	Reference range	P-value ANOVA
Hct	%	48.2 ± 4.7	45.2 ± 7.4	46.4 ± 5.6	42-55	0.15
Hb	g/dl	16.8 ± 1.6	15.5 ± 2.7	16.0 ± 2.1	14.4-19.1	0.07
RET-He	pg	27.4 ± 1.7	27.1 ± 1.4	26.7 ± 1.9	26-30	0.38
MCH	pg	23.2 ± 1.1	23.3 ± 1.1	23.1 ± 1.1	23-26	0.75
MCV	fl	66.6 ± 4.6	67.9 ± 4.2	67.5 ± 4.7	64-73	0.51
MCHC	g/dl	35.2 ± 1.2	34.4 ± 1.7	34.2 ± 1.9	34-36	0.05
Tc	10^3/ul	302 ± 118	400 ± 140	374 ± 192	130-394	0.04
CRP	mg/l	3 ± 9	4 ± 11	27 ± 49	<10	<0.01*

Hct, hematocrit; Hb, hemoglobin; RET-He, reticulocyte hemoglobin equivalent; Tc, platelets; CRP, C-reactive protein.
* Tukey's range test: stage C versus control, P <0.01; stage C versus B2, P <0.01

Table 3
Parameters of iron metabolism (mean ± SD) in 30 healthy dogs and 70 dogs at different stages of myxomatous mitral valve disease.

Variable	Unit	Control (30)	Stage B2 (33)	Stage C (37)	Reference range	P-value ANOVA	P-value Tukey's
Ferritin	ng/ml	159 ± 59	174 ± 73	198 ± 188	60-190	0.46	Na
Iron	ug/dl	156 ± 45	151 ± 57	118 ± 56	50-230	0.007	0.016[b] 0.030xy[c]
Tsat	%	39 ± 12	37 ± 14	30 ± 12	30-68	0.026	0.031[b] 0.078[c]
TIBC	ug/dl	414 ± 82	410 ± 79	392 ± 98	240-440	0.306	Na

Iron, serum iron concentration; Tsat, Transferrin saturation; TIBC, total iron binding capacity; na, not applicable.
[a]control versus stage B2, [b]control versus stage C, [c]stage B2 versus stage C.

Table 4

Selective correlations in 100 dogs, 30 control dogs and 70 dogs with degenerative mitral valve disease at ACVIM stage B2 and C.

	Tsat*	TIBC	Ferrritin	LVDDN	LA/Ao	BCS	CRP	RET-He	Hb
Iron	**r = 0.8** **P = <0.01**	**r = 0.44** **P = <0.01**	r = 0.01 P = 0.93	**r = -0.23** **P = 0.03**	r = - 0.21 P = 0.06	**r = -0.35** **P = <0.01**	**r = -0.28** **P < 0.01**	R = 0.18 P = 0.12	**r = 0.31** **P = <0.01**
Tsat		r = -0.11 P = 0.26	r = 0.04 P = 0.72	r = -0.18 P = 0.1	r = -0.08 P = 0.47	r = 0.18 P = 0.13	**r = -0.27** **P = 0.01**	**r = 0.3** **P = 0.01**	**r = 0.23** **P = 0.02**
TIBC			r = -0.06 P = 0.58	r = -0.13 P = 0.23	r = 0.21 P = 0.06	**r = 0.34** **P = <0.01**	r = -0.08 P = 0.42	r = -0.17 P = 0.16	**r = 0.24** **P = 0.02**
Ferritin				r = 0.06 P = 0.6	r = 0.01 P = 0.92	r = -0.06 P = 0.61	r = -0.05 P = 0.65	r = -0.11 P = 0.34	**r = 0.28** **P = 0.01**
LVDDN					**r = 0.83** **P = <0.01**	**r = -0.39** **P = <0.01**	**r = 0.21** **P = 0.05**	r = 0.02 P = 0.89	r = -0.17 P = 0.12
LA/Ao						**r = -0.45** **P = <0.01**	**r = 0.22** **P = 0.05**	R = 0.01 P = 0.94	r = 0 P = 0.99
BCS							**r = -0.3** **P = 0.01**	r = 0.1 P = 0.48	r = 0.18 P = 0.13
CRP								r = -0.21 P = 0.07	r = -0.18 P = 0.08
RET-He									r = 0.12 P = 0.3

*for abbreviations see tables 1, 2, 3

Figure captions

Fig. 1: Serum iron concentration in control dogs and dogs with degenerative mitral valve disease at ACVIM stage B2 and C. The serum iron is significantly lower in stage C dogs compared to the other two groups.

Fig. 2: Transferrin saturation (Tsat) in control dogs and dogs with degenerative mitral valve disease at ACVIM stage B2 and C. The Tsat is significantly lower in stage C compared to control dogs

Danksagung

Ich möchte mich herzlich bei folgenden Personen für ihre Unterstützung bedanken:

Prof. Tony Glaus für die Zusammenarbeit und Realisierung meiner Doktorarbeit

Prof. Thomas Lutz für seine Unterstützung als Korreferent

Dr. Susanna Burckhardt, Dr. Felix Funk und Cyrill Blättler von der Vifor Pharma AG für die gute und freundschaftliche Kollaboration

Dr. med. vet. Marco Baron Toaldo, Prof. Dr. med. vet. Francesco Dondi, Dr. med. vet. Imelda Vannini, Dr. med. vet. Alan Kovacevic, Dr. med. vet. Barbara Riond, Dr. med. vet. Monique Wenger und Michele Wyler für ihre höchst geschätzten Beiträge zur Datengenerierung

Prof. Tony Glaus, Med. vet. Frane Ivasovic, Dr. med. vet. Urszula Bartoszuk, Dr. med. vet. Marco Baron Toaldo, Dr. med. vet. Jose Novo Matos und Michaela Schira für die tolle Zeit und Zusammenarbeit während meines Internships in der Kardiologie am Tierspital Zürich

Meinen Eltern, Nicole Joller, Mirjam Pewsner, Regula Wuhrmann, Julia Zellweger, Anna Schildknecht und Ignacio Sanz Martin

Curriculum Vitae

Vorname Name	Angela Betschart
Geburtsdatum	12. Oktober 1980
Geburtsort	Münsterlingen, TG
Nationalität	Schweizerin
Heimatort bei Schweizer/-in	Muotathal, SZ
August 1988- Juli 2002	**Schulausbildung** Grundschule/ Sekundarschule/ Pädagogische Hochschule, Kreuzlingen, Schweiz
Juli 2001	**Höchster Schulabschluss** Matura, Pädagogische Hochschule, Kreuzlingen, Schweiz
August 2003 – Oktober 2008	**Studium** Veterinärmedizin, Universität Zürich, Zürich, Schweiz
Oktober 2008	**Abschlussprüfung med. vet.** Universität Zürich, Zürich, Schweiz
Januar 2017- September 2020	**Anfertigung der Dissertation** unter Leitung von Prof. Dr. med. vet. Tony Glaus, an der Klinik für Kleintiermedizin der Vetsuisse-Fakultät Universität Zürich Direktorin: Prof. Dr. med. vet. Claudia Reusch
März 2009- Januar 2011	Tierärztin, Tezet AG, Müllheim, Schweiz
Januar 2011- September 2012	Clinical Science Manager, Geistlich Pharma AG, Root, Schweiz
August 2014- September 2016	Marketing Communications Manager, Vifor Pharma AG, Zürich, Schweiz
Januar 2017- März 2018	Intern/ Doktorandin, Tierspital Zürich, Schweiz
Februar 2019- Jetzt	Tierärztin/ FVH Kandidatin Medi-Vet SA, Lausanne, Schweiz