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Hyperlipasemia in dogs with acute kidney injury treated with and without hemodialysis

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Contents

Summary

Hyperlipasemia has been reported in dogs with acute kidney injury (AKI) treated with and without hemodialysis (HD) but associations with AKI severity, treatment modality, and outcome have not been extensively evaluated.

Retrospective study including 125 client-owned dogs with AKI, with creatinine concentrations and 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methyresorufin) ester (DGGR) lipase activities measured within 24 hours of admission and during hospitalization. Dogs with a history of acute (AP) or chronic pancreatitis were excluded.

DGGR-lipase activity >3x upper reference limit (URL) was found in 28.8% and 57.5% of dogs at admission and during hospitalization, respectively, and severe hyperlipasemia (>10x URL) was seen in 34% of dogs during hospitalization. A diagnosis of AP was given to 8.8% and 16% of dogs at admission and during hospitalization, respectively. DGGR-lipase activity was higher in dogs with International Renal Interest Society (IRIS) grades 4–5 than in those with grades 1–3, but no correlation was found between DGGR-lipase activity and creatinine concentrations. Treatment with HD was not associated with hyperlipasemia independently of IRIS group. Severe AKI (IRIS 4–5) and high DGGR-lipase activity were associated with poor outcome.

Hyperlipasemia is frequent in dogs with AKI, and is associated with severity of AKI and death, but not independently with HD treatment. Further studies are needed to evaluate causes of hyperlipasemia in dogs with AKI.

Key words: Acute pancreatitis, Canine, DGGR-lipase, Pancreas-specific lipase.

Abbreviations

AKI	acute kidney injury
AP	acute pancreatitis
CI	confidence intervals
CKD	chronic kidney disease
cPL	canine pancreas-specific lipase (Spec cPL®, IDEXX laboratories)
DGGR-lipase	1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methyresorufin) ester lipase
HD	hemodialysis
IRIS	International Renal Interest Society
IQR	interquartile range
URL	upper reference limit

Introduction

Acute kidney injury (AKI) and acute pancreatitis (AP) are common disorders in dogs. Moderate to severe forms of both disorders have high mortality, reported between 45–57% for AKI (Harison et al. 2012, Legatti et al. 2018, Segev et al. 2008; Thoen et al. 2011, Vaden et al. 1997) and 23–40% for AP (Fabrès et al. 2019, Kuzi et al. 2020, Mansfield et al. 2008, Pápa et al. 2011, Ruaux and Atwell 1998). Both conditions can occur independently of each other, but may also arise concomitantly as a result of systemic conditions affecting both organs. In addition, AP may be both the cause of and a sequela to AKI. Indeed, AP was reported in 34% of dogs with acute on chronic kidney injury (Dunaevich et al. 2020), concurrent AKI was found in 26% of dogs with AP (Gori et al. 2019), and elevated pancreatic enzymes suggestive of pancreatitis were reported in 62% of dogs undergoing hemodialysis (HD) for AKI (Takada et al. 2018). The prognosis for AKI was found to be poorer with concomitant AP in both people and dogs in some studies although others found no difference in survival (Cridge et al. 2020, Perondi et al. 2018, Rimer et al. 2021, Zhou 2015).

In dogs, a diagnosis of AP generally relies on a combination of consistent clinical signs (including anorexia, vomiting, diarrhea and cranial abdominal pain), imaging findings (abdominal ultrasound and, less frequently, computed tomography), and measurement of pancreatic enzymes (Cridge et al. 2021). However, clinical signs are nonspecific and overlap with those of AKI. Furthermore, the sensitivity and specificity of abdominal ultrasound for a clinical diagnosis of AP varies between 43–89% and 43–92%, respectively, depending on disease severity and the ultrasonographic criteria used (Cridge et al. 2020).

Measurement of lipase activity using the enzymatic assay 1,2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin) ester (DGGR-lipase) and concentrations of canine pancreas-specific lipase (Spec cPL®, IDEXX laboratories (cPL)) are currently the most commonly used biomarkers to diagnose AP in dogs, whereby cPL is often considered superior because of its exclusive pancreatic origin (Graca et al. 2005, Mansfield et al. 2012, McCord et al. 2012). However, reported sensitivities and specificities for both assays are highly variable, further confounding a clinical diagnosis of AP. Reported sensitivity and specificity ranges between 86–93% and 53–74% for DGGR-lipase (Cridge et al. 2018, Graca et al. 2005), and 21–91% and 74–100% for cPL concentrations ≥400 µg/L (Cridge et al. 2018, Mansfield et al. 2012, McCord et al. 2012, Trivedi et al. 2011).

Elevated lipase activity above 3x the upper reference limit (URL) in the absence of pancreatic disease is common in people with critical illness (Muniraj et al. 2015, Manjuck et

al. 2005). Likewise, elevated cPL or DGGR-lipase activity have been reported in dogs without evidence of AP associated with a variety of nonpancreatic disorders (Rallis et al. 1996, Han et al. 2015, Mylonakis et al. 2014, Koster et al. 2015), administration of corticosteroids (Ohta et al. 2017), or critical illness (Prummer et al. 2020).

Moreover, elevated pancreatic enzymes commonly occur in people with both AKI and chronic kidney disease (CKD) (Masoero et al. 1996, Robitaille et al. 2006, Royse et al. 1987). The incidence of AP is higher in people with renal insufficiency than in the general population and elevated pancreatic enzymes without clinical evidence of pancreatitis are also observed in patients with AKI and CKD (Rani et al. 2015, Royse et al. 1987). Thus, the extent to which hyperenzymemia is due to reduced renal clearance of pancreatic enzymes or concomitant pancreatic injury remains unclear in many cases. Similarly, elevated cPL concentrations or lipase activity in dogs with renal disease has been reported (Jaensch 2013, Rosa et al. 2021, Takada et al. 2018), although correlation between creatinine concentrations and either lipase activity or cPL concentrations is poor (Hulsebosch et al. 2016, Takada et al. 2018, Steiner 2010). In addition, several studies found elevated pancreatic enzymes in people undergoing HD, and this has been variably attributed to reduced glomerular filtration, subclinical pancreatitis, hemodynamic factors, and heparin therapy during HD leading to heparin-associated increase in lipoprotein lipase activity (Chen et al. 2011, Masoero et al. 1996, Montalto et al. 1997, Shibasaki et al. 1996) and, in some studies, to an increased risk of AP in patients undergoing HD (Wang et al. 2021).

To date, there is scarce information about hyperlipasemia in dogs undergoing HD. One study showed a high prevalence of elevated cPL concentrations in dogs undergoing HD, but found no association with outcome (Takada et al. 2018), while others demonstrated a higher risk of AP in dogs with severe AKI (Rosa et al. 2021), and an association of high cPL concentrations and AP with poor prognosis for dogs with AKI managed with HD (Perondi et al. 2018). We have anecdotally observed severely elevated DGGR-lipase in some dogs undergoing HD at our institution but the clinical significance of this remains unclear. Thus, further investigations are needed to evaluate the diagnostic and prognostic value of hyperlipasemia in dogs with AKI and examine possible associations with HD treatment.

The aims of this study were thus to investigate the prevalence and clinical significance of hyperlipasemia based on DGGR-lipase activity in dogs with naturally-occurring AKI, treated with and without HD.

Materials and Methods

The medical records and laboratory database were retrospectively interrogated for dogs presented to the Small Animal Clinic, Department of Veterinary Clinical Medicine, Vetsuisse Faculty, University of Bern, Switzerland, between August 2018 and February 2021. During this period, DGGR-lipase activity was measured in all canine plasma samples submitted for biochemical analyses to the diagnostic laboratory but results were reported only if the assay was specifically requested by the attending clinician. Plasma DGGR-lipase activity was thus measured regardless of clinicians' suspicion of pancreatic disease. Dogs were included in the study if AKI was diagnosed at admission, and if both DGGR-lipase and creatinine were measured from the same blood sample within the first 24 hours of admission. Dogs presented for post-renal azotemia, CKD, or end-stage renal disease were not considered eligible. Dogs were also excluded if they had a prior history of AP, if they were given a primary diagnosis of AP at admission, or if medical records were incomplete. For dogs presented on more than one occasion for AKI, only data from the first admission were included. For dogs receiving plasma exchange therapy during hospitalization, only admission data were included, because plasma exchange therapy complicated the classification as to whether animals underwent HD or not.

A diagnosis of AKI was made by a board-certified internist or a primary clinician working under their direct supervision, and was generally based on history, acute onset of clinical signs, ultrasonographic findings, and laboratory evidence of AKI, such as progressive azotemia and consistent findings on urinalysisi. Likewise, a clinical diagnosis of secondary AP at admission or AP developing during hospitalization was made by a board-certified internist or clinician under their direct supervision, and was generally based on clinical signs, cPL and/or DGGR-lipase results and ultrasonographic findings. All ultrasonographic examinations were performed by a board-certified radiologist or radiology resident under their direct supervision. The decision to perform intermittent HD was made in consultation with a board-certified internist who is a fellow in veterinary nephrology and hemodialysis (TF, AS), and generally depended on clinical status, disease severity, response to conservative therapy, and owner agreement.

Data collected included signalment, cause of AKI, length of hospitalization, outcome to discharge and 30-day outcome, creatinine concentrations at admission, DGGR-lipase activities at admission and throughout the period of hospitalization, treatment modality

(conservative, HD, plasma exchange therapy), diagnosis of concurrent AP at admission or developing during hospitalization, and results of postmortem examinations where available.

Measurement of DGGR-lipase was performed by the Clinical Diagnostic Laboratory of the Vetsuisse Faculty Bern, Switzerland using a previously validated method (LIPC, Ref. 05401704, Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions using a clinica chemistry analyzer (Cobas c501, Roche Diagnostics, Basel, Switzerland) (Graca et al. 2005). The reference interval for DGGR-lipase activity (25–180 U/L) was previously established in-house using samples from 67 healthy adult dogs. Creatinine concentrations were measured on the same chemistry analyzer using a commercial enzymatic kit (CREP2, Ref. 03263991, Roche Diagnostics, Basel, Switzerland). All analyses were performed on heparinized plasma samples.

Grading of AKI at admission was performed according to guidelines of the International Renal Interest Society (IRIS) (http://www.iris-kidney.com, last accessed June 2022), and dogs were grouped into mild to moderate AKI (IRIS grades 1–3) or severe AKI (IRIS grades 4–5). Causes of AKI were grouped into infectious and noninfectious etiologies. Activities of DGGR-lipase were categorized into lipase grade 1 (≤ 3x URL; ≤ 540 U/L), lipase grade 2 (3–10x URL; 541–1,800 U/L), lipase grade 3 (10–30x URL;1,801-5,400 U/L) and lipase grade 4 (> 30x URL; >5,400 U/L). For measurements of DGGR-lipase activity subsequent to the initial measurement at admission, only the maximum of all subsequent DGGR-lipase measurements (MaxSub) was used for data analysis.

Statistical analysis

Data analysis was performed using commercial software (MedCalc® Statistical Software version 20.011, MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Data were analyzed for normality using Shapiro-Wilk tests and by examining normality plots. As most data were not normally distributed, nonparametric analyses were performed. Continuous variables were reported as median and interquartile range (IQR), and differences between groups were analyzed using Mann-Whitney tests. Categorical variables were reported as numbers and percentages, and the differences between groups were analyzed using χ^2 test or Fisher's exact tests. Wilcoxon's signed rank tests were used for comparisons between DGGR-lipase activity at admission and during hospitalization. Spearman's rank was used to assess the correlation between DGGR-lipase activity and creatinine concentrations. Odds ratios for 30-day survival were calculated for dogs in different IRIS groups and for dogs with different lipase grades for MaxSub. Statistical significance was set at $P<0.05$ throughout.

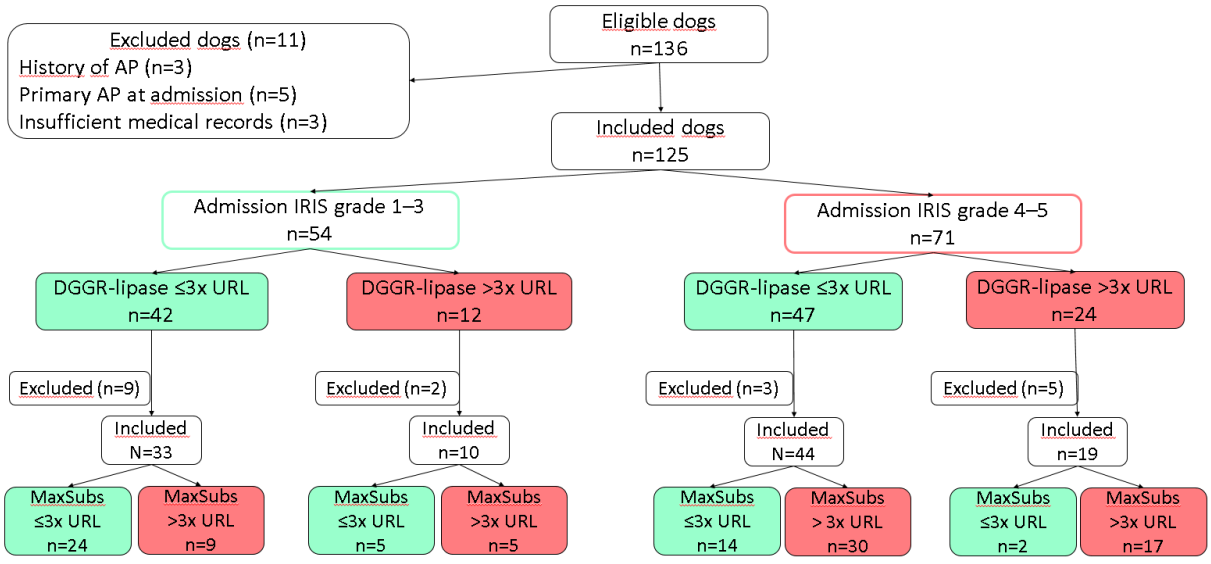
Results

Animals

An initial 136 dogs met the inclusion criteria. Dogs were excluded for a prior history of pancreatitis (n=3), a primary diagnosis of AP at admission (n=5), and insufficient data in the medical records (n=3). The final dataset therefore comprised 125 dogs (**Figure 1**). Dogs represented 58 breeds, the most common being mixed breed (n=22; 17.6%), Labrador Retriever (n=17; 13.6%) and Malinois (n=5; 4%) and less than five each of other breeds. The median age was 5.8 years (IQR, 2.9–9.6 years). There were 62 females (40 spayed) and 63 males (26 castrated).

The cause of AKI was unknown (n=29; 23.2%), leptospirosis (n=23; 18.4%), intoxication (n=14; 11.2%), pyelonephritis (n=13; 10.4%), sepsis (n=8; 6.4%), post-operative AKI (n=6; 4.8%), and five or less cases of other etiologies. There were 74 dogs (40.8%) in the non-infectious and 51 dogs (59.2%) in the infectious etiology group. Eleven dogs (8.8%) had a diagnosis of secondary or concurrent AP at the time of admission and seventeen dogs (13.6%) developed secondary AP during hospitalization.

Figure 1: Flow diagram of dogs stratified by IRIS grade at admission, with admission and maximum subsequent (MaxSubs) DGGR-lipase activity.



Admission DGGR-lipase activities and creatinine concentrations

At admission, DGGR-lipase activity ranged from 13–13,788 U/L (**Table 1**). DGGR-lipase activity was ≤ 3 xURL (lipase grade 1) in 89 (71.2%) dogs. Hyperlipasemia > 3 xURL was

present in 36 (28.8%) dogs. These were 25 (20.0%) dogs with lipase grade 2 (3–10x URL), 10 (8.0%) dogs with lipase grade 3 (10–30x URL), and 1 dog with lipase grade 4 (>30x URL).

Creatinine concentrations ranged from 43–1,746 µmol/L (median 476; IQR 287–748) at admission, with 13 dogs (10%) classified as IRIS grade 1, 15 (12%) as grade 2, 26 (21%) as grade 3, 50 (40%) as grade 4, and 21 (17%) as grade 5. There was a significant difference (P=0.034) in DGGR-lipase activity between dogs in IRIS 1–3 and those in IRIS 4–5 groups (**Table 1**). However, the correlation between DGGR-lipase activity and creatinine concentrations was poor (ρ=0.22; 95% confidence intervals (CI), 0.04–0.38). There was no significant difference (P=0.215) in DGGR-lipase activities between dogs with infectious and those with noninfectious causes of AKI (**Table 1**).

Table 1: DGGR-lipase activity and creatinine concentrations at admission in 125 dogs with AKI

Dog groups	N (%)	DGGR-lipase (U/L) median (IQR)
All dogs	125 (100%)	177 (82–674)
IRIS 1–3	54 (43.2%)	146 (64–424)*
IRIS 4–5	71 (56.8%)	240 (93–801)*
Noninfectious causes of AKI	74 (59.2%)	149 (78–507)
Infectious causes of AKI	51 (40.8%)	249 (86–789)
Survival to discharge	82 (70.7%)	144 (79–484)†
Non-survival to discharge	43 (34.4%)	268 (114–880)†

The same superscript symbols denote data that differed significantly

Length of hospitalization and follow-up to discharge

The length of hospitalization ranged from 1–26 days (median 6 days; IQR 3–10 days). Dogs survived to discharge in 82/125 cases (65.6%). These were 64/89 (71.9%) dogs with DGGR-lipase grade 1, 14/25 (56%) dogs with grade 2, and 4/10 (40%) with grade 3. The only dog admitted with lipase grade 4 died within 24 hours of admission. Median DGGR-lipase activity was significantly higher (P=0.016) in dogs that did not survive to discharge than in those that did (**Table 1**).

Subsequent DGGR-lipase measurements

There were 108 dogs that had at least one subsequent DGGR-lipase activity measurement during hospitalization, of which data for two dogs receiving plasma exchange therapy were

excluded. The remaining 17/125 dogs either died or were discharged without subsequent measurements of DGGR-lipase. For the 106 dogs with subsequent measurements, the median number of subsequent measurements was 3.5 (IQR 2–8 measurements); a total of 61 dogs (57.5%) had hyperlipasemia >3x URL on at least one subsequent measurement. The maximum of all DGGR-lipase activities measured occurred at admission in 20 (18.9%) dogs, of which two dogs were in the HD group, and subsequently in 85 (80.2%) dogs; in one dog, admission DGGR-lipase and MaxSub activities were the same. The range of DGGR-lipase activities for these 106 dogs was 13–5,186 U/L and 25–19,857 U/L for admission and MaxSub, respectively (**Table 2**). In total, 36 (34%) dogs received HD and 70 (66%) did not. The highest post-admission DGGR-lipase (MaxSub) was measured after initiation of HD in 34/36 (94%) dogs undergoing HD.

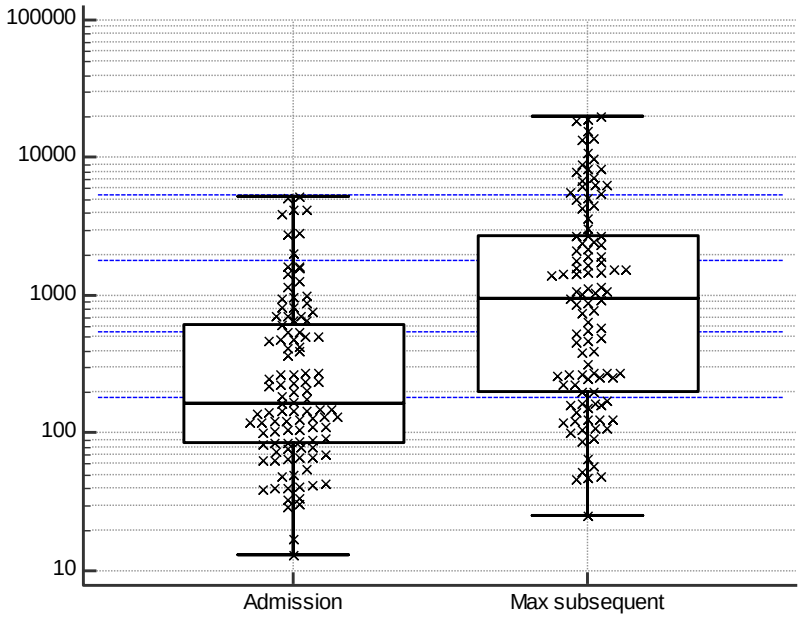
Table 2: DGGR-lipase activity at admission and during hospitalization (MaxSub) in 106 dogs with acute kidney injury

Dog groups	N (%)	DGGR-lipase (U/L), median (IQR)	
		Admission	MaxSub
All dogs	106 (100%)	166 (85–621)*	944 (202–2719)*
IRIS 1–3	43 (40.6%)	145 (65–460)	254 (130–1113)†
IRIS 4–5	63 (59.4%)	234 (90–695)	1562 (541–4878)†
No hemodialysis	70 (66.0%)	195 (85–718)	390 (159–2157)‡
Hemodialysis	36 (34.0%)	136 (84–381)	1445 (768–5298)‡
Survival at 30 days	58 (58.6%)	132 (79–472)†	821 (165–1807)§
Non-survival at 30 days	41 (41.4%)	274 (119–830)†	1929 (439–6804)§

The same superscript symbols denote values with a significant difference

DGGR-lipase activity at admission was significantly (P<0.001) lower than MaxSub activity (**Table 2**, **Figure 2**). Likewise, the grades of MaxSub differed significantly (P=0.003) from those at admission, whereby the lipase grade increased from admission in just over one half of dogs but decreased in only 6.6% (**Table 3**, **Figure 3**). In total, MaxSub activity was > 3 xURL in 61 (57.5%) dogs and > 10 xURL (grade 3) in 36 (34.0%) dogs (**Table 3**).

Figure 2: Box-and-whisker plots showing log DGGR-lipase activities at admission and during hospitalization in 106 dogs with acute kidney injury differing significantly (P<0.001).



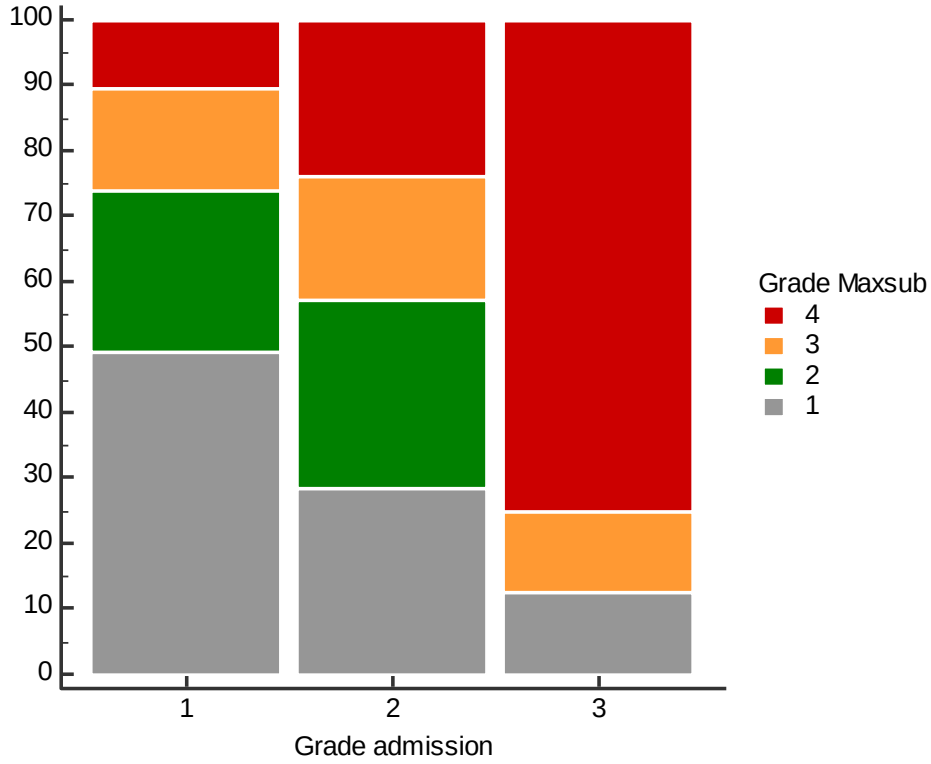
Horizontal blue dashed lines show the upper reference limit (URL) of DGGR-lipase (180 U/L), 3x URL (540 U/L), 10x URL (1800 U/L) and 30x URL (5400 U/L), respectively.

Table 3: Grades of DGGR-lipase activity at admission and during hospitalization (MaxSub) in 106 dogs with acute kidney injury

Variable		Admission	MaxSub
Lipase grades, N (%)	Grade 1 (≤3x URL)	77 (72.7%)	45 (42.5%)
	Grade 2 (3–10x URL)	21 (19.8%)	25 (23.6%)
	Grade 3 (10–30x URL)	8 (7.5%)	17 (16.0%)
	Grade 4 (>30x URL)	0 (0.0%)	19 (17.9%)
Change in lipase grade, N (%)	Increase from admission	54 (50.9%)	
	Equal to admission	45 (42.5%)	
	Decrease from admission	7 (6.6%)	

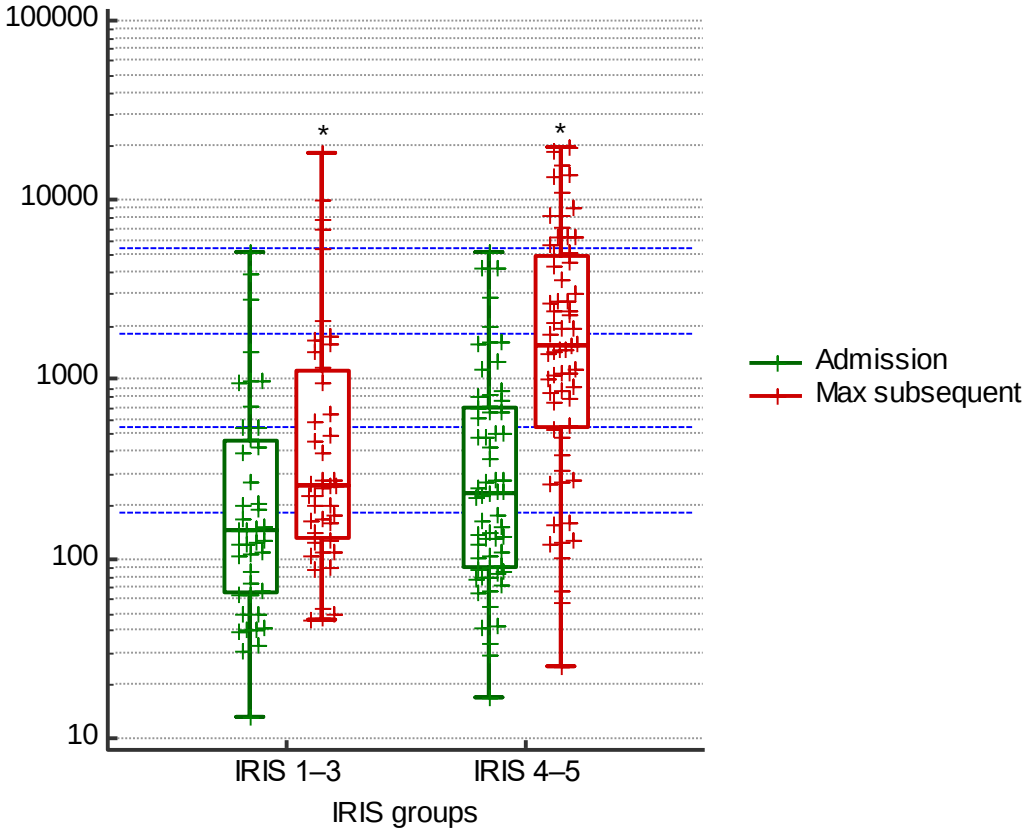
MaxSub, maximum of all measurements subsequent to admission; URL, upper reference limit

Figure 3: DGGR-lipase grades at admission compared to grades during hospitalization (MaxSub) in 106 dogs with acute kidney injury



There was a significant difference between dogs in IRIS grade 1–3 and those in IRIS grade 4–5 for DGGR-lipase activity at MaxSub (P<.001) but not at admission (P=0.123) (**Table 2**, **Figure 4**).

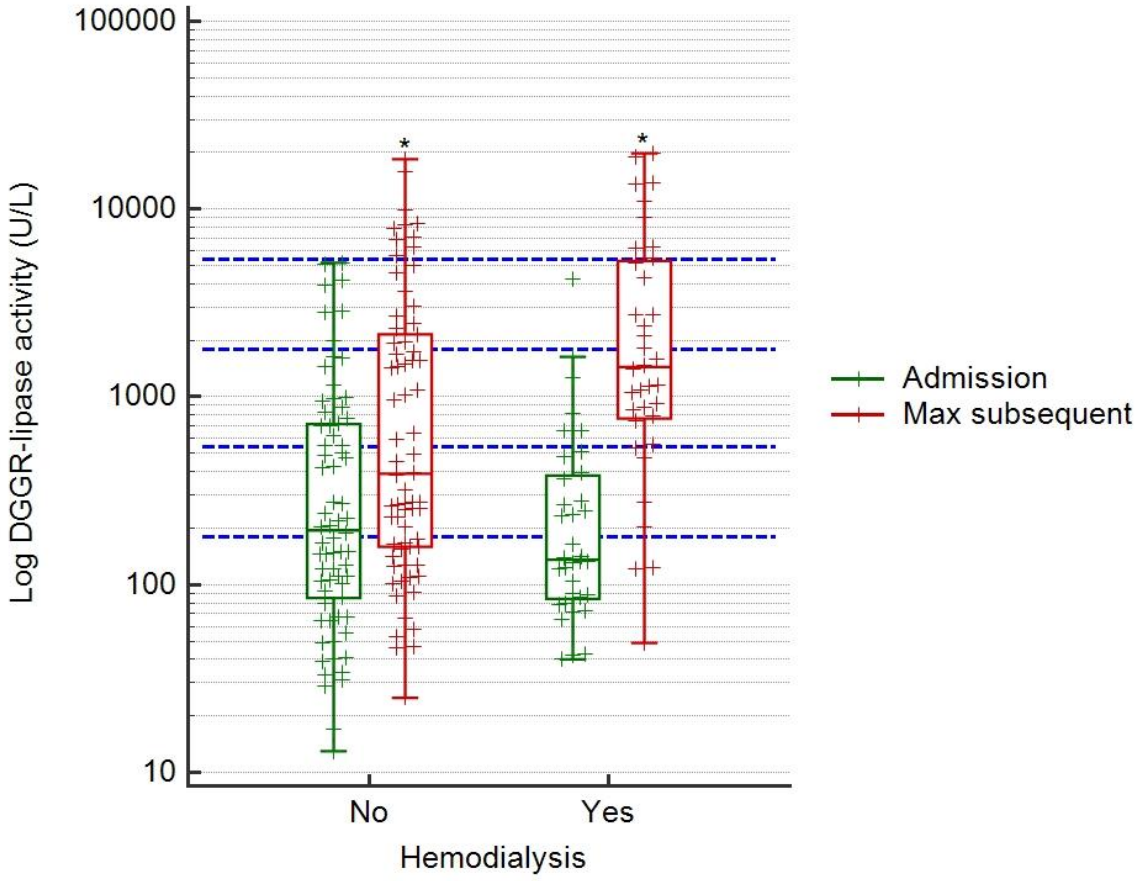
Figure 4: Box-and-whisker plots showing DGGR-lipase activities at admission and during hospitalization in 106 dogs with acute kidney injury with IRIS grades 1–3 compared to IRIS grades 4–5.



Horizontal blue dashed lines show the upper reference limit (URL) of DGGR-lipase (180 U/L), 3x URL (540 U/L), 10x URL (1800 U/L) and 30x URL (5400 U/L), respectively. The same superscript symbols denote values with a significant difference.

There was no difference in admission DGGR-lipase activities (P=0.397) or grades (P=0.206) between dogs treated with HD and those treated without (**Table 2, Figure 5**). There was a significant difference in MaxSub activities (P=0.005) and grades (P=0.004) between dogs treated with and without HD (**Table 2**, **Figure 5**). However, no difference between dogs treated with or without HD for MaxSub activity or grades was found within each of the two IRIS groups evaluated separately (**Table 4**, **Figure 6**).

Figure 5: Box-and-whisker plots showing DGGR-lipase activities at admission and during hospitalization in 106 dogs with acute kidney injury treated with or without hemodialysis

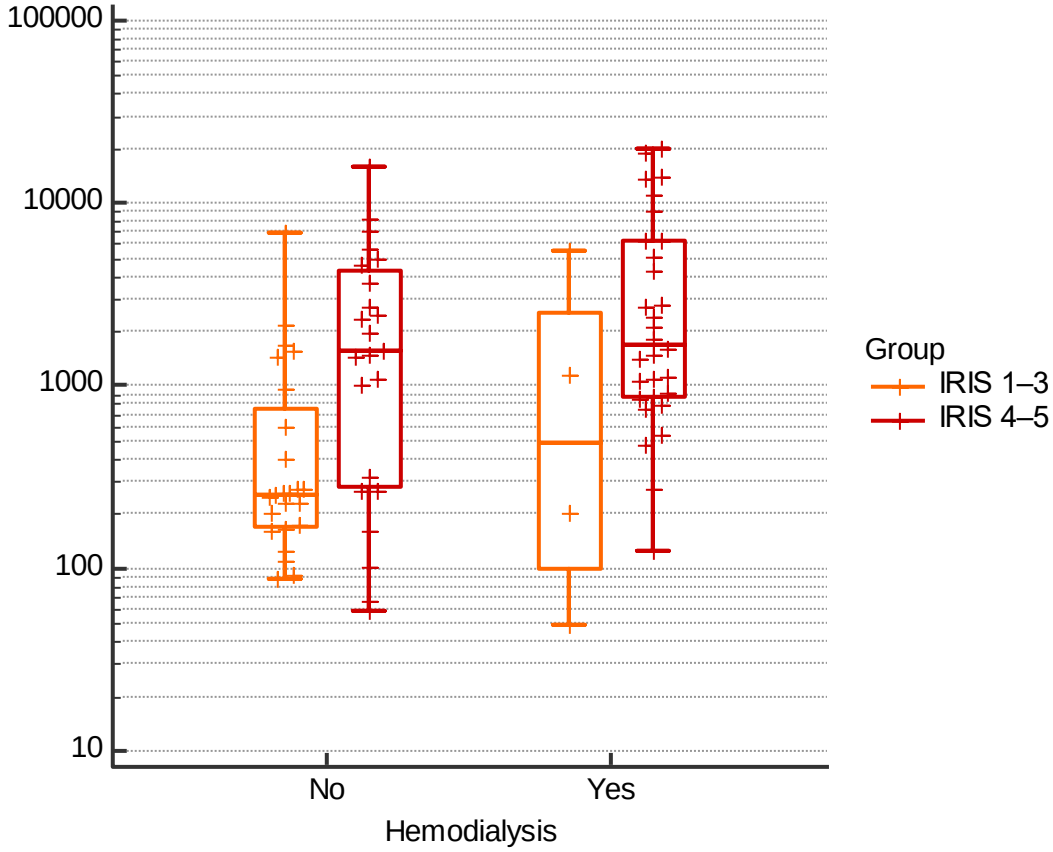


Horizontal blue dashed lines show the upper reference limit (URL) of DGGR-lipase (180 U/L), 3x URL (540 U/L), 10x URL (1800 U/L) and 30x URL (5400 U/L), respectively.

Table 4: Grades DGGR-lipase activity during hospitalization (MaxSub) in 106 dogs treated with and without hemodialysis for acute kidney injury

		Grades of MaxSub DGGR-lipase activity (n)				
Dog groups	Group	Grade 1	Grade 2	Grade 3	Grade 4	*P*
All dogs	No hemodialysis	38	12	10	10	**0.004**
	Hemodialysis	7	13	7	9	
IRIS 1–3	No hemodialysis	27	7	1	4	0.423
	Hemodialysis	2	1	0	1	
IRIS 4–5	No hemodialysis	11	5	9	6	0.140
	Hemodialysis	5	12	7	8	

Figure 6: DGGR-lipase activities during hospitalization in 106 dogs with AKI treated with or without hemodialysis in IRIS groups 1–3 and 4–5



A clinical diagnosis of concurrent AP at admission was given in 7 (6.6%) dogs and development of AP during hospitalization in a further 17 (16%) dog. A clinical diagnosis of AP at admission as given to 0/77, 3/21, and 4/8 dogs with admission DGGR-lipase grades of 1, 2, and 3, respectively. A diagnosis of AP developing during hospitalization was given to 1/45, 3/25, 4/17 and 9/19 dogs with Maxsub lipase grades 1, 2, 3, and 4, respectively.

There was no difference between lipase grades in dogs with an infectious and those with a noninfectious etiology of AKI at admission (P=0.531) or at MaxSub (P=0.098).

Outcome

The 30-day outcome for dogs with at least one subsequent DGGR-lipase measurement was available in 99 dogs. Of these, 58 (58.6%) dogs survived, and 41 (41.4%) dogs died or were euthanized within 30 days.

DGGR-lipase activity both at admission (P=0.018) and at MaxSub (P=0.002) were significantly associated with 30-day outcome (**Table 2**). Lipase grades also differed significantly at MaxSub (P=0.003) but not at admission (P=0.193) between survivors and

non-survivors at 30 days (**Figures 7, 8**), whereby 54/80 (67.5%) dogs with MaxSub lipase grades of 1, 2 or 3 survived but only 4/19 (21.1%) dogs with a MaxSub lipase grade 4.

In addition, a significant difference in 30-day survival (P=0.021) was found between dogs in IRIS 1–3 compared to IRIS 4–5 groups, whereby 29/40 (72.5%) and 29/59 (55.8%) survived, respectively. The Odds ratio for death within 30 days was 2.7 (95% CI, 1.2–6.5) in dogs with IRIS 4–5 compared to those with IRIS 1–3 (P=0.022). The Odds ratio for death within 30 days was 7.8 (95% CI, 2.4–25.8) for dogs with MaxSub grade 4 compared to dogs with other MaxSub grades (P<0.001).

Figure 7: 30-day outcome in 99 dogs with acute kidney injury and different grades of DGGR-lipase activities at admission

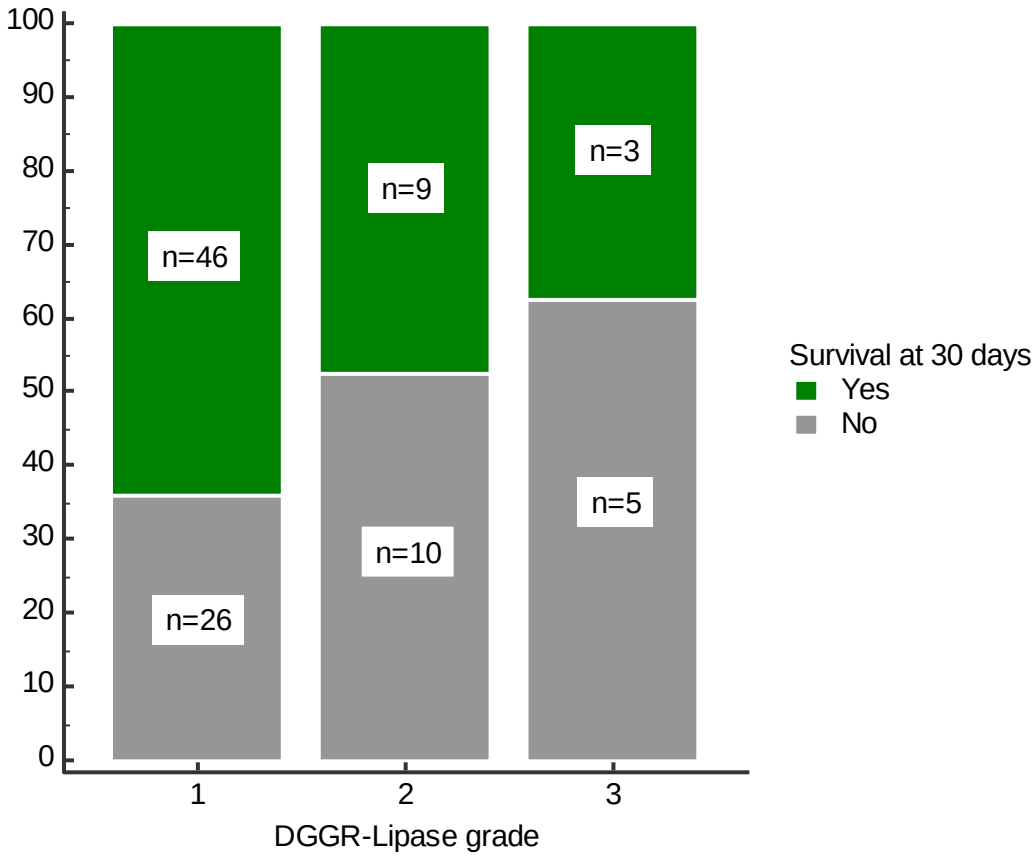
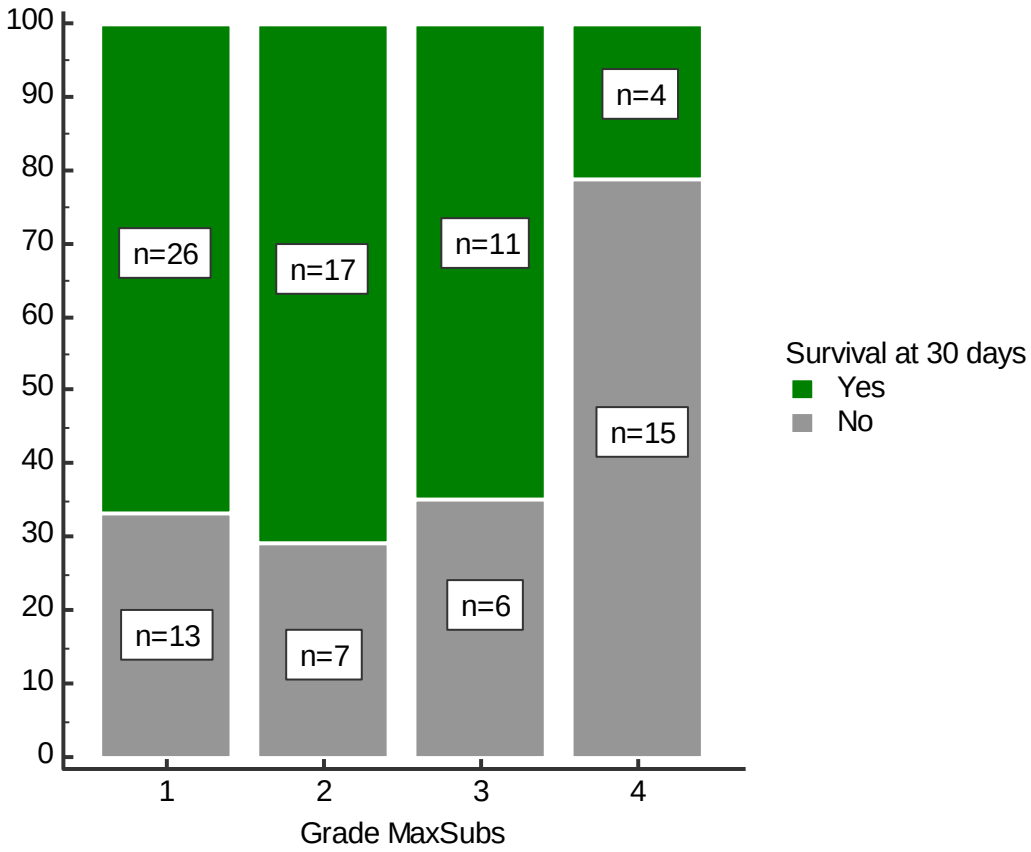
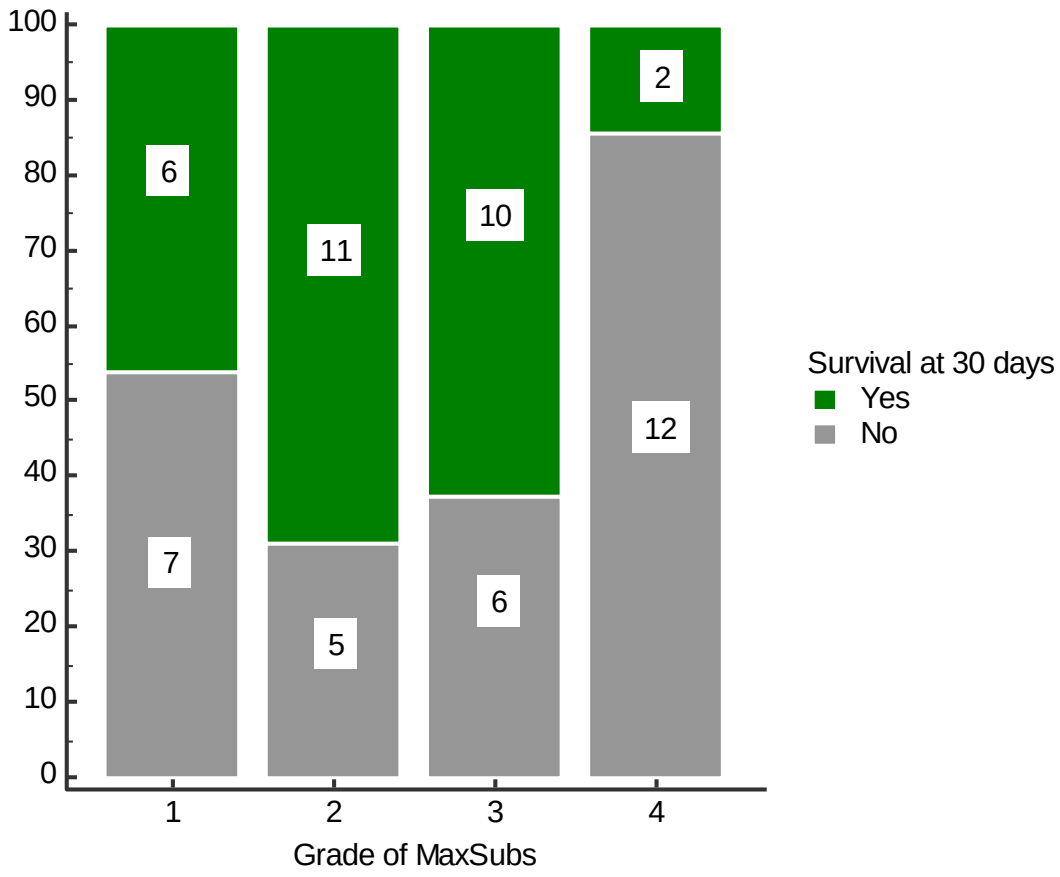


Figure 8: 30-day outcome in 99 dogs with acute kidney injury and different grades of in-hospital DGGR-lipase activities (MaxSub)



For dogs admitted in IRIS 4–5 group, there was a significant difference (P=0.013) in survival between dogs with MaxSub grade 4 compared to those with MaxSub grades 1 to 3 (**Figure 9**).

Figure 9: 30-day outcome in 59 dogs with IRIS grades 4–5 and different grades of in-hospital DGGR-lipase activities (MaxSub)



Necropsy results were available in nine dogs. Of these, gross abnormalities of the pancreas were observed in three dogs, but histopathological examination revealed no evidence for AP. Of these three dogs, one had a clinical diagnosis of AP at admission and one during hospitalization.

Discussion

In the present study, hyperlipasemia >3xURL at admission and during hospitalization was found in 28.8% and 57.5%, respectively, of dogs presenting with AKI, and was associated with both high IRIS grades and a negative outcome. To our knowledge, this is the first study examining the prevalence and development of hyperlipasemia, measured by the DGGR-lipase assay, in dogs with AKI undergoing HD.

We found prevalences of hyperlipasemia similar to that reported in a previous study, which found cPL concentrations ≥400 µg/L in 62% of 53 dogs with AKI undergoing intermittent HD (Takada et al. 2018). However, cPL concentrations in the previous study were measured at any time within 7 days prior to or after admission and an association with HD or admission IRIS grades cannot therefore be inferred (Takada et al. 2018). Moreover, cPL concentrations were measured at the request of clinicians, presumably because AP was suspected in the latter study, but DGGR-lipase was measured in all dogs in our study regardless of clinicians' suspected diagnoses. Similarly, another study found cPL concentrations ≥400 µg/L in 71% of 28 dogs with AKI in IRIS grades 3–5 either at admission or within the first 8 days of hospitalization (Rosa et al. 2021). Lastly, cPL concentrations ≥400 µg/L were measured in 2/5 dogs with gentamicin-induced AKI and serum lipase activity (using the 1,2-diglyceride-based enzymatic/colorimetric assay) also increased in 2/5 of these dogs (Hulsebosch et al. 2016).

Studies in people mainly focus on pancreatic enzymes in patients with CKD or end-stage renal disease undergoing HD with prevalences of increased lipase reported in up to 80%, but enzyme elevations only rarely exceed 3–5x URL (Aatif & Akka 2019, Chen et al. 2011, Lin et al. 1988, Masoero et al. 1996, Ventrucci et al. 1995, Wang et al. 2021). Many of these studies found enzyme elevation in the absence of clinical evidence of AP (Chen et al. 2011, Lin et al. 1988, Masoero et al. 1996, Ventrucci et al. 1995). Similarly, AP was only diagnosed in 8.8% of dogs at admission and only 13.6–16.0% of dogs were considered to have developed AP during hospitalization in our study despite a high proportion of dogs with hyperlipasemia. This finding is similar to previous studies in dogs reporting concurrent pancreatitis in 14% and 15% of dogs presenting with AKI (Vaden et al. 1997, Perondi et al. 2018), but somewhat lower than two studies describing AP in 22% and 34% of dogs with AKI and acute on chronic kidney disease, respectively (Rimer et al. 2022, Dunaevich et al. 2020). However, a diagnosis of AP in the face of AKI is challenging as clinical signs overlap and may be concealed by the administration of anti-emetics and analgesics. Additionally, the

suboptimal sensitivity of abdominal ultrasound limits its use as a diagnostic tool and, in our clinic, ultrasound is often not repeated during hospitalization after an initial examination at admission. The true prevalence of AP may therefore have been underestimated in our study, particularly since ultrasonographic changes in AP may occur after the onset of clinical signs (Leoni et al. 2020). Moreover, as results of DGGR-lipase activity were not reported unless the measurement was specifically requested, clinicians were often blinded to results, which may otherwise have increased the prevalence of a clinical diagnosis of AP. As histopathological examination of the pancreas was only rarely performed post-mortem, the extent to which elevated DGGR-lipase activity was associated with pancreatic lesions in the present study is not known.

We found a significant increase in DGGR-lipase activity during hospitalization compared to admission, which was >3x URL in 58% of dogs. Moreover, DGGR-lipase activity was severely elevated (>10x URL) in 34% of dogs at some point during hospitalization but only in 8.1% of dogs at admission. This prevalence of in-hospital increase in DGGR-lipase activity was higher than the 23% previously demonstrated in dogs presenting with critical illness of any cause at our institution, which included dogs presenting with primary AP (Prümmer et al. 2020). However, renal disease was the most frequent diagnosis in dogs with in-hospital DGGR-lipase elevations in that study (Prümmer et al. 2020). Moreover, of dogs with renal disease, approximately one third had hyperlipasemia >3x URL at admission and half had significant increases during hospitalization, paralleling findings of the present study (Prümmer et al. 2020).

We found that high DGGR-lipase activity at admission and particularly during hospitalization was associated with high IRIS grades, although correlation of DGGR-lipase and creatinine concentrations at admission was poor. One previous study in dogs, in which cPL concentrations ≥400 µg/L were found within 1–8 days of admission in 71% of 28 dogs with IRIS grades 3–5, found a positive correlation between cPL and creatinine concentrations, which was interpreted as evidence of predisposition to AP in dogs with severe AKI (Rosa et al. 2021). However, dogs with AKI were compared to healthy controls and the extent to which the severity of AKI was associated with elevated cPL concentrations was not evaluated (Rosa et al. 2021). We also found higher DGGR-lipase activity during hospitalization in dogs receiving HD treatment compared to those treated conservatively. However, there was no difference in DGGR-lipase activity between the treatment groups when evaluated separately in dogs with IRIS 1–3 or IRIS 4–5, respectively. These finding suggests that only the severity of AKI but not the treatment modality was associated with high activity of DGGR-lipase.

However, the number of dogs in the IRIS 1–3 group undergoing HD was low, and thus the statistical analysis within this IRIS group may have been underpowered, potentially leading to a type II error. The only previous study evaluating lipase in dogs undergoing HD also found no association between cPL ≥400 µg/L and dialysis-dependency at 30 days, however measurements were performed within 7 days prior to or after admission and the numbers of analyses performed prior to or after HD was unclear (Takada et a1. 2018). Similarly, in a study of uremic people, 80% were found to have hyperlipasemia with higher enzyme activity in patients with more severe renal impairment but no difference was found between pre- and post-dialysis activities (Lin et al. 1988).

Some studies have demonstrated an increase in post-dialysis lipase activity in people when heparin was used as an anticoagulant for HD (Montalto et al. 1997). This is likely due to heparin-induced release of lipoprotein lipase and/or hepatic lipase. In cats and dogs, the use of heparin also leads to increases of DGGR-lipase activity but these were short-lived (<20 minutes) and of minimal magnitude with a median increase from 49.8 U/L to 54.1 U/L in dogs (Lim et al. 2020). As we almost exclusively use citrate as an anticoagulant in our institution and reported heparin-induced increases in lipase are minimal compared to increases we observed during hospitalization, heparin-induced release of nonpancreatic lipases was likely not a significant factor affecting findings in the present study.

Previous studies have demonstrated elevated lipase activity or cPL concentrations in dogs with a variety of infectious disorders, including ehrlichiosis, babesiosis and parvovirosis (Kalli et al. 2017, Koster et al. 2048, Mylonakis et al. 2014). Infectious disease may therefore have contributed to hyperlipasemia in some dogs in our study, but we found no difference in DGGR-lipase activity between dogs with and without an infectious cause of AKI.

Overall survival (66% to discharge and 59% 30-day) was similar to or higher than that previously reported for dogs with AKI both with and without HD, which ranges from 27–76% (Perondi et al. 2018, Rimer et al. 2021, Rosa et al. 2021, Segev et al. 2008, Takada et al. 2018). However, survival was defined disparately between studies, and some studies only included dogs undergoing HD whilst others only looked at dogs not offered HD. In addition, some studies included only dogs with IRIS grades of 3 and above. Thus, direct comparison between studies is difficult. We found that hyperlipasemia was associated with lower survival to discharge and 30-day survival. Moreover, only 20% of dogs with extreme in-hospital hyperlipasemia (MaxSub >30x URL) survived to 30 days, representing an almost 8x higher odds of negative outcome compared to lower lipase grades. Negative outcome at 30 days was

also associated with IRIS grades. However, extreme in-hospital DGGR-lipase activity was associated with poor outcome for dogs within the IRIS group 4–5, suggesting that extreme hyperlipasemia may be an independent predictor of survival in dogs with severe AKI, rather than merely mirroring IRIS grades. Similarly, DGGR-lipase activity was significantly higher in non-survivors in a study of 100 dogs with acute on chronic kidney disease (Dunaevich 2020).

Our study had several limitations. The timing and frequency of measurements of DGGR-lipase was not standardized and assays were performed only if clinicians submitted samples for biochemical analyses, presumably to monitor patient progress. Dogs with higher IRIS grades and those receiving HD are more likely to have frequent samples analyzed than those in lower IRIS grades or those not receiving HD. As only the maximum measured DGGR-lipase after admission (MaxSub) was used for data analysis, this may have favored increased DGGR-lipase activity in these dogs as peak lipase activity would more easily be found. Likewise, the maximum of in-hospital measurements (MaxSub) was compared to a single analysis at admission, favoring higher measurements during hospitalization compared to admission. Additionally, the decision to perform HD was not only based on medical indication but also on owners' financial constraints and ethical perceptions. Therefore, not all dogs for which HD was considered indicated were treated with HD. Furthermore, the review of medical records to determine whether dogs were considered to have concurrent AP or AP developing during hospitalization may have been biased by a lack of standardized criteria for a diagnosis of AP. In absence of histopathology, a panel composed of several board-certified internal medicine specialists reviewing set diagnostic criteria, including lipase activity and imaging, would have been beneficial. As this was not possible with the data available, the degree to which hyperlipasemia reflected AP in our study is unclear.

In conclusion, hyperlipasemia is frequent in dogs with AKI at admission and during hospitalization, despite only a minority considered clinically to have AP. Hyperlipasemia is associated with the severity of AKI but is not independently associated with HD treatment. Severe hyperlipasemia is associated with increased odds of nonsurvival at 30 days. Further studies are needed to evaluate possible underlying causes for hyperlipasemia in dogs with AKI and to assess the extent to which clinically relevant AP is a contributing factor.

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