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Serum aluminum in 176 feline patients with application to the diagnostic approach to a tremoring patient with kidney disease receiving aluminum hydroxide therapy

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Abstract

Background Control of circulating phosphorus concentrations in patients with chronic kidney disease is a mainstay of treatment and may include use of aluminum hydroxide as an intestinal phosphate binder. Serious complications of excess aluminum reported in dogs and man include encephalopathy, microcytic anemia, osteomalacia, and regional myopathy at serum concentrations exceeding 100 ng/mL. Reports of aluminum toxicosis are not available for cats receiving aluminum hydroxide and circulating aluminum concentrations are poorly characterized. The aim of this study is to establish therapeutic and toxic serum aluminum concentrations in cats and apply this data to an intoxication case.

Results Of cats with CKD who received aluminum hydroxide, 9/21 serum samples exceeded aluminum concentrations of 100 ng/mL. After removal of outliers, 18 cats with kidney disease who received aluminum hydroxide had mean serum aluminum concentrations of 69 ng/mL [95% CI: 42–97 ng/mL], which was significantly higher than mean aluminum concentrations in cats not receiving aluminum hydroxide ($p = 0.0034$). The mean aluminum concentration of 141 feline serum samples not receiving aluminum hydroxide was 29 ng/mL [95% CI: 24–33 ng/mL]. Of the 141 samples, 16 cats presenting for wellness or dental procedures had mean concentrations of 36 ng/mL [95% CI: 15–56 ng/mL]. This data was applied to a case of a 16-year-old spayed female domestic shorthair with IRIS stage 2 chronic kidney disease with a 7-month history of mild hindlimb weakness and intermittent right forelimb myoclonus. The patient received oral aluminum hydroxide, and the serum contained 376 ng/mL of aluminum suggestive of toxicosis. Resolution of clinical signs was noted following a switch to an aluminum-free phosphate binding medication, and, at 5-month follow-up, the serum aluminum concentration was 71 ng/mL.

Conclusions Our data suggest that serum aluminum concentrations in cats exceeding 86 ng/mL can result in clinical aluminum toxicosis and is comparable to the 100 ng/mL toxic threshold described in humans. The data provided facilitate the diagnostic assessment of cats receiving aluminum hydroxide supplementation. Veterinarians must recognize the toxic effects of aluminum and pursue diagnostic testing in suspect cases to mitigate invasive and costly workup for aluminum-associated clinical signs or euthanasia due to deterioration of these patients.

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Keywords Renal disease, CKD, Cat, AlOH, Myoclonus, Neurologic, Toxicosis, Toxicity

Background

Aluminum (Al) is a non-essential element yet exposure through food, air, and pharmaceuticals is common. Aluminum hydroxide (AlOH) is used in veterinary patients as an intestinal phosphate binder for managing chronic kidney disease (CKD) and a gastroprotectant when combined with sucrose octasulfate as sucralfate. Accordingly, AlOH use as a phosphate binder and antacid has been approved by the Food and Drug Administration (FDA) in humans but has yet to be approved for companion animals despite widespread extra-label use by veterinarians [1]. While there may be little to no risk of Al intoxication in patients with normal renal function, patients with a decreased capacity to eliminate compounds through renal excretion are at an increased risk of developing adverse effects related to Al accumulation [2–5].

Cats with CKD represent a large percentage of the feline patient population and require life-long management by veterinarians. Of 86 randomly selected cats presenting for evaluation to a single veterinary clinic, 50% (43/86) were found to have CKD [6]. Loss of functional renal mass in kidney disease results in limited phosphorus excretion, increased phosphorus retention, development of renal secondary hyperparathyroidism, increased total and ionized calcium, and mineralization of soft tissues which is a major contributor to the progression of CKD [7]. According to the International Renal Interest Society (IRIS) guidelines, the control of intestinal phosphorus absorption and regulation of serum phosphorus concentrations is a critical component of effective treatment plans [8]. Due to the severe consequences of untreated hyperphosphatemia, AlOH remains a popular, affordable, and widely available option in controlling dietary phosphorus absorption from the gastrointestinal tract.

"Aluminum toxicity has not been found to be a concern in cats and dogs" is a claim included in the label for one AlOH product. Despite this label claim, data to support this statement is limited particularly in feline patients with renal disease receiving AlOH therapeutically [4, 9]. Al toxicosis remains poorly characterized in cats and generalized label claims can mislead pet owners and practitioners to assume there is no risk of adverse effects of AlOH use. Healthy companion animals with normal renal function can effectively eliminate absorbed Al though renal excretion, but patients with decreased glomerular filtration rates and renal dysfunction can accumulate Al in tissues, such as liver, brain, and bone, and develop signs of toxicosis [2, 4, 9–16].

In humans undergoing dialysis, Al exposure has been implicated in the pathogenesis of Alzheimer's and Parkinson's diseases and can cause encephalopathy, microcytic anemia, and bone disease [10, 16]. Clinical signs of Al toxicosis in veterinary species include microcytic anemia, weakness, tremors, convulsions, and obtundation, but the exact mechanism by which this occurs is unknown [3, 4, 14]. Because this condition remains poorly understood and described, these signs may be mistaken for uremic encephalopathy or other neurologic conditions resulting in extensive diagnostic workups and extended hospital stays. Since the description of Al toxicosis in two canine patients receiving AlOH therapy in 2008, limited data has been published to establish safe therapeutic serum or plasma concentrations of Al in patients with renal dysfunction [4].

Therapeutic monitoring of serum Al is an important part of a diagnostic work-up for cats with CKD receiving AlOH to assess for Al overexposure, but evaluation requires comparison to established reference ranges. No such reference data are currently available for cats. Therefore, when cases present to the veterinarian and veterinary diagnostic laboratory with clinical signs suggestive of Al toxicosis, interpretation of serum Al data can be challenging and requires comparison to published human or canine toxicity data or rodent studies. Carefully established therapeutic and toxic serum Al in cats will provide comprehensive and readily available information to veterinarians for adjusting treatment plans. The purpose of this study is to establish reference data for serum Al concentrations in cats with and without CKD and receiving or not receiving AlOH for application to a case for which Al toxicosis was a concern.

Results

Reference data generation

Serum Al was determined in 21 cats with CKD and which were reported to be receiving AlOH therapy. Of the 21 CKD cats, three outliers (334, 376, and 2,159 ng/mL) were identified and removed from descriptive statistical analysis. Descriptive statistics for the remaining 18 samples are described in Table 1. Of the 175 submitted samples from patients not receiving AlOH, 20 submissions were excluded to remove serial sample submissions from animals already included in the study. Serum Al was assessed in 155 cats with serum urea nitrogen (SUN) and creatinine within the reference intervals suggestive of no clinically significant functional renal impairment or dehydration. Samples were separated into the following groups: Wellness and Pre-dental ($n = 17$), Non-renal/Non-urinary Illness ($n = 71$), Lower Urinary Tract Disease ($n = 27$), Renal Disease or Injury no Al ($n = 24$), and Medical Oncology ($n = 16$) based on the presenting complaint

Table 1 Descriptive statistics of serum aluminum concentrations in feline populations

	Wellness and Pre-Dental	Cats without AlOH Therapy	CKD Cats with Al Therapy
Minimum	< 2	< 2	< 2
5th Percentile	< 2	< 2	< 2
25th Percentile	5	2	15
Median	23	24	57
75th Percentile	52	47	119
95th Percentile	132	86	176
Maximum	132	100	176
95% CI of Median	[4–53]	[17–29]	[15–115]
Mean	26	29	69
Standard Deviation	38	27	55
95% CI of Mean	[15–56]	[24–33]	[42–97]
n	16	141	18

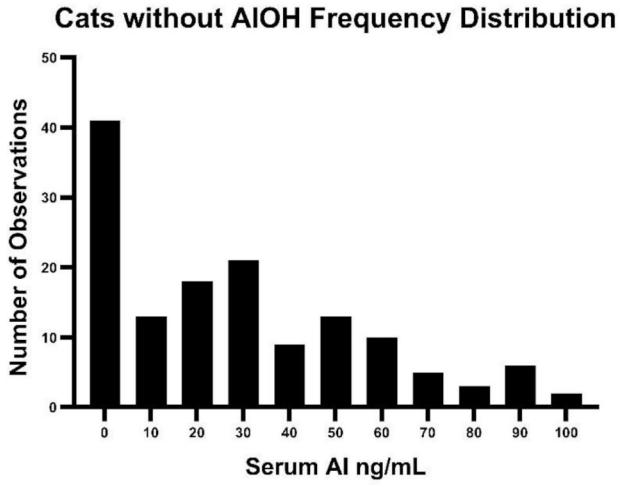

Fig. 1 Distribution of serum Al concentrations in cats not receiving AlOH ($n = 141$)

and major medical history provided at the time of serum collection. Analysis of 155 samples for outliers identified 14 outliers which were removed for descriptive statistics analysis and reference data generation. Descriptive statistics for serum Al (see Table 1) and frequency distributions (see Figs. 1, 2 and 3) are shown for the 141 cats not receiving AlOH. Additionally, descriptive statistics from the 17 cats presenting for wellness and pre-dental bloodwork are also shown isolated from the category "cats without AlOH therapy"; after removal of one outlier, this subgroup has 16 cats (see Table 1).

Data for cats not reported to receive AlOH was not normally distributed ($p < 0.0001$ for Shapiro Wilk and Kolmogorov-Smirnov tests) whereas the CKD with AlOH group was normally distributed ($p = 0.078$ for Shapiro Wilk and $p > 0.100$ with Kolmogorov-Smirnov test). The lack of a Gaussian distribution in cats not receiving AlOH is likely due to the large number of animals for which serum Al was below the reporting limit of the assay resulting in a left skew of the distribution as shown in Fig. 1. The "Wellness and Pre-dental" group was normally distributed by Kolmogorov-Smirnov test ($p > 0.100$) but not by Shapiro-Wilk test ($p = 0.0110$). When the cats without AlOH data was grouped by sex, there was an approximately equal representation of spayed female and castrated male cats for Al less than 60 ng/mL as shown in Fig. 3. Assessment of sex-based differences in Al concentration were beyond the scope of this study; however approximately equal representation of spayed female and castrated male cats was necessary to mitigate sex-based variabilities in the reference data.

When comparing cats with CKD receiving AlOH to cats presenting apparently healthy for wellness and dental procedures, or to all cats surveyed not reported to be receiving AlOH, statistically significant differences were reported and are shown in Fig. 4. When compared to cats presenting for wellness exam or pre-anesthetic bloodwork prior to dental examination, cats with CKD receiving AlOH had significantly higher serum Al concentrations (student's t-test, one-tailed, $F = 2.120$ ($p = 0.1478$), $t = 2.036$ ($p = 0.0251$)). When compared to all cats surveyed not receiving AlOH, the serum Al concentrations of cats with CKD receiving AlOH were significantly greater (Unequal variances t-test (Welch's test), one-tailed, $F = 4.329$ ($p < 0.0001$), $t = 3.061$ ($p = 0.0034$)). Additional comparisons between the CKD with AlOH group and the cats without AlOH subgroups are included in Fig. 5 (after removal of outliers) and Fig. 6 (with the inclusion of outliers) by both parametric and non-parametric statistical methods.

A reference interval for serum Al concentrations was established using a modification of the methods described in the American Society for Veterinary Clinical Pathology position statement for the determination of reference intervals in veterinary species [17]. Based on the data from all cats not reported to receive AlOH included in the study, the reference range for the middle 90% of the population is < 86 ng/mL. The middle 90% of the wellness and pre-dental group had serum Al concentrations < 132 ng/mL whereas the CKD with AlOH group had Al concentrations < 176 ng/mL. Due to the subgroup sample sizes of less than 20 individuals per group, specific reference ranges for these subpopulations could not be established.

Case progression

A 16-year-old 4 kg spayed female domestic shorthair cat with a five-year history of IRIS stage 2 CKD presented with a seven-month history of mild hindlimb weakness and intermittent right forelimb myoclonus. Serum biochemistry assessment did not indicate uremic encephalopathy and suggested stable CKD with SUN

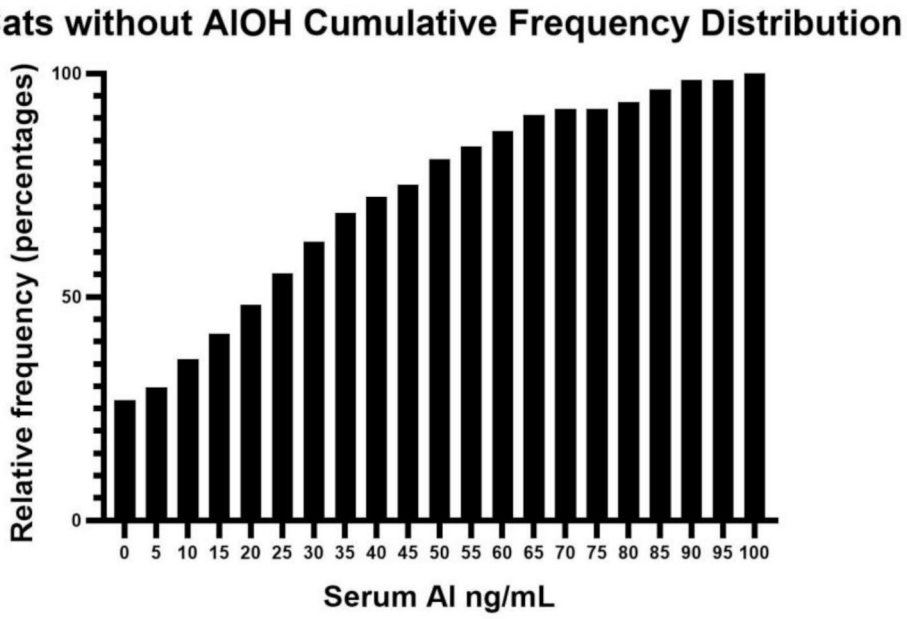

Fig. 2 Cumulative distribution of serum Al concentrations in cats not receiving AlOH ($n = 141$)

49 mg/dL (16–37), creatinine 2 mg/dL (0.9–2.3), symmetric dimethylarginine (SDMA) 11 µg/dL (0–14) and phosphate 5.3 mg/dL (2.9–6.3) mostly within reference intervals. The patient had been managed on maropitant citrate (6 mg PO SID), mirtazapine (0.47 mg PO q24h), prednisolone (5 mg PO EOD), gabapentin (12.5 mg PO q12h), cobalequin (1 chewable), chlorambucil (2 mg q7d) and Phos-Bind (AlOH) (500 mg PO q12h). Chlorambucil was discontinued to rule out the tremor as an adverse effect of this pharmaceutical with no resolution of clinical signs. Serum Al was reported as 376 ng/mL. The patient was switched to Epakitin (Vetoquinol, Fort Worth, TX, 76137, USA), an Al-free phosphate binder formula, and the AlOH supplementation was discontinued. At follow-up, there was a complete resolution of the right forelimb myoclonus. Serum Al at 5-month follow-up after discontinuation of AlOH therapy was 71 ng/mL and the owner reported a complete resolution of the neurologic signs.

Discussion

Evaluation of Al toxicosis with CKD presents unique challenges for veterinarians and diagnosticians due to the limited availability of published case reports and lack of available reference data and toxic thresholds, especially for cats. Based on the presented serum Al concentrations, cats can experience elevated circulating Al which can increase the risk of developing clinical toxicosis. Eighteen cats with CKD receiving AlOH had mean serum Al of 69 ng/mL [95% CI: 42–97 ng/mL]. These Al concentrations were significantly higher than those in cats without azotemia who did not receive Al-containing phosphate binders. Three animals were statistically identified as outliers with serum Al reported as 334, 376, and 2159 ng/mL. The cats with Al reported as 334 and 2159 ng/mL presented to the diagnostic endocrinology service for new onset of hypercalcemia and additional history and follow up information were not available for these two patients. The serum Al in the presented feline patient was 376 ng/mL following chronic exposure to AlOH as an intestinal phosphate binder therapy for CKD management. When cases, such as the one presented herein, are submitted to the toxicology laboratory for evaluation of suspected Al toxicosis, the toxicologist is tasked with interpreting values for which little to no reference data is available. Toxicologists are left to extrapolate data from humans, rabbits, rodents, or dogs to make a determination of the significance of the result with the referring veterinarian.

Toxicity studies in healthy young beagle dogs did not find significant toxicosis with dietary Al of 30 mg/g diet for 26 weeks [2]. However, the proportion of free Al versus protein-bound Al increases with increased renal dysfunction associated with age and renal pathology. Therefore, it is inappropriate to assess the potential for Al to cause intoxication in CKD patients with feeding trials in otherwise healthy individuals [9, 18]. Additionally, the lack of finding significant toxicosis in this study suggests aluminum is not of toxicologic significance in veterinary species when aluminum toxicosis has been documented in dogs. A swift resolution of neurologic signs and return to normal serum Al was reported in an otherwise healthy, young dog following removal of an Al razor blade foreign body [14]. Serum Al was observed to decrease over time from the initial 140 ng/mL and 250 ng/mL on the second- and third post-operative days to 85 ng/mL and 13

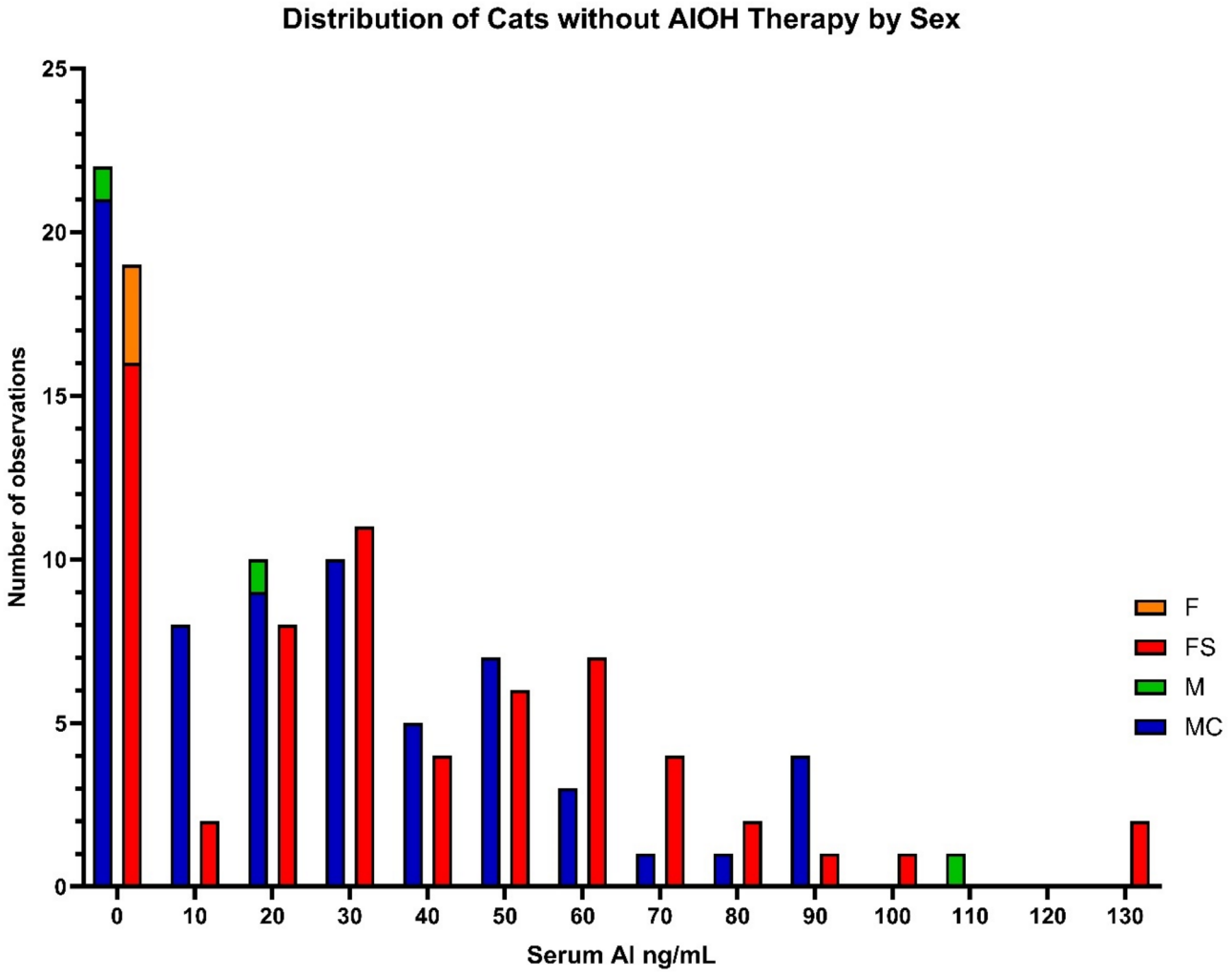

Fig. 3 Distribution of control serum Al by sex: female = F, female spayed = FS, male = M, and male castrated = MC

ng/mL at two- and twelve-week follow-up [14]. Serum Al may have returned to baseline prior to final follow-up, however data between the two- and twelve-week assessments was not available in this study. Additionally, this dog was not reported to have a history of renal disease or renal injury secondary to the foreign body, therefore the elimination kinetics for this case are difficult to extrapolate to patients with elevated serum Al and renal function impairment.

When comparing the presented feline case to published data in dogs, the serum Al concentration at presentation is consistent with previous reports of intoxications in canine patients with CKD. In 18/52 dogs with suspected Al toxicosis undergoing AlOH therapy, serum Al ranged from 31 to 520 ng/mL (reference range of < 80 ng/mL) [3]. The serum Al in the presented feline patient was 376 ng/mL and is comparable to two dogs who developed Al toxicosis while receiving AlOH for which serum Al was reported as 520 and 318 ng/mL [3, 4]. Reference data and diagnostic criteria for Al toxicosis in cats to assess potentially toxic concentrations of serum Al were not available in the literature for application to the presented case at the time of submission to the diagnostic laboratory. Additionally, there are no published reports of clinical cases of Al toxicosis in cats. Reference intervals utilized by diagnostic laboratories are established as the range of values for a particular analyte in the central 90 to 99% in a population of at least 120 individuals [17, 19]. The data established here suggests serum Al that exceeds 86 ng/mL in cats should prompt evaluation for Al toxicosis and consideration of Al-free therapies to mitigate neurologic and hematologic clinical signs. Further, the clinical signs of tremors reported in this cat were consistent with previous reports of Al intoxicated patients [2, 4, 6, 10–12]. Additionally, the marked clinical improvement following discontinuation of AlOH therapy provided added support for the diagnosis of Al intoxication in this patient.

CKD patients receiving oral AlOH have chronic exposures to, decreased clearance of, and age-related

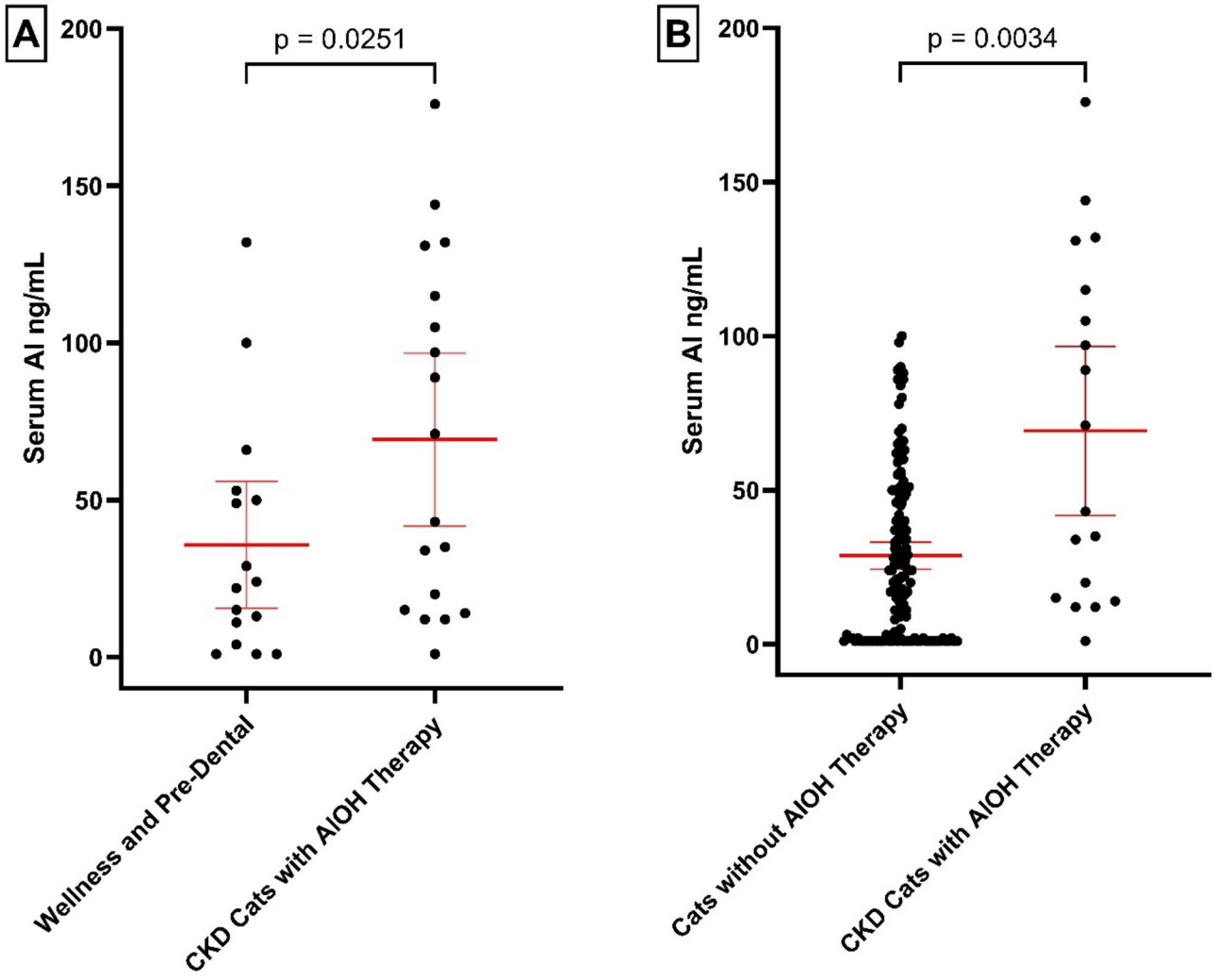

Fig. 4 Effect of AlOH supplementation in CKD cats on serum Al compared to cats not reported to receive AlOH therapeutically. **(A)** Comparison of cats with CKD receiving AlOH therapeutically versus patients presenting for wellness and pre-dental assessment analyzed by student's t-test (one-tailed, $F = 2.120$ ($p = 0.1478$), $t = 2.036$ ($p = 0.0251$)). **(B)** Comparison of cats with CDK receiving AlOH therapeutically versus all cats not reported to be receiving AlOH analyzed by unequal variances t-test (Welch's test) (one-tailed, $F = 4.329$ ($p < 0.0001$), $t = 3.061$ ($p = 0.0034$))

sensitivity to Al resulting in Al accumulation in tissues and increased risk for clinical toxicosis. Current recommendations include AlOH doses of 30–100 mg/kg/day [7]. For an average 5.5 kg cat at the highest recommended dose, this equates to a 550 mg AlOH oral daily dose. A study comparing renally intact and renally impaired rabbits found that renal impairment significantly decreased systemic clearance of Al following single intravenous Al lactate administration [9]. Rabbits with chronic Al exposures of 400 µmol/kg by subcutaneous injection five days weekly for five weeks displayed significantly elevated brain, adrenal, bone, heart, kidney, liver, lung, spleen, stomach, and serum Al compared to sodium dosed controls [18]. Additionally, significant elevations in Al were demonstrated in serum, kidney, liver, lung, and spleen following 200 µmol/kg intravenous infusion. Serum and kidney showed the greatest deviations from controls and are appropriate ante- and postmortem samples for Al toxicosis assessment when reference data are available. Half-lives for serum, liver, lung, and spleen were estimated to be 42, 74, 44, and 113 days respectively with the kidney retaining Al above control levels beyond the 128 days of the study [20]. Due to the long half-lives reported in rabbits with renal impairment, monitoring of serum Al concentrations over several weeks to months may be required for monitoring of clinically intoxicated patients.

While this study provides much needed data to facilitate the diagnostic assessment and monitoring of cats receiving AlOH supplementation as a therapeutic component in the management of CKD, efficacious and safe treatment protocols for cats with Al toxicosis have yet to be established. Hemodialysis with deferoxamine

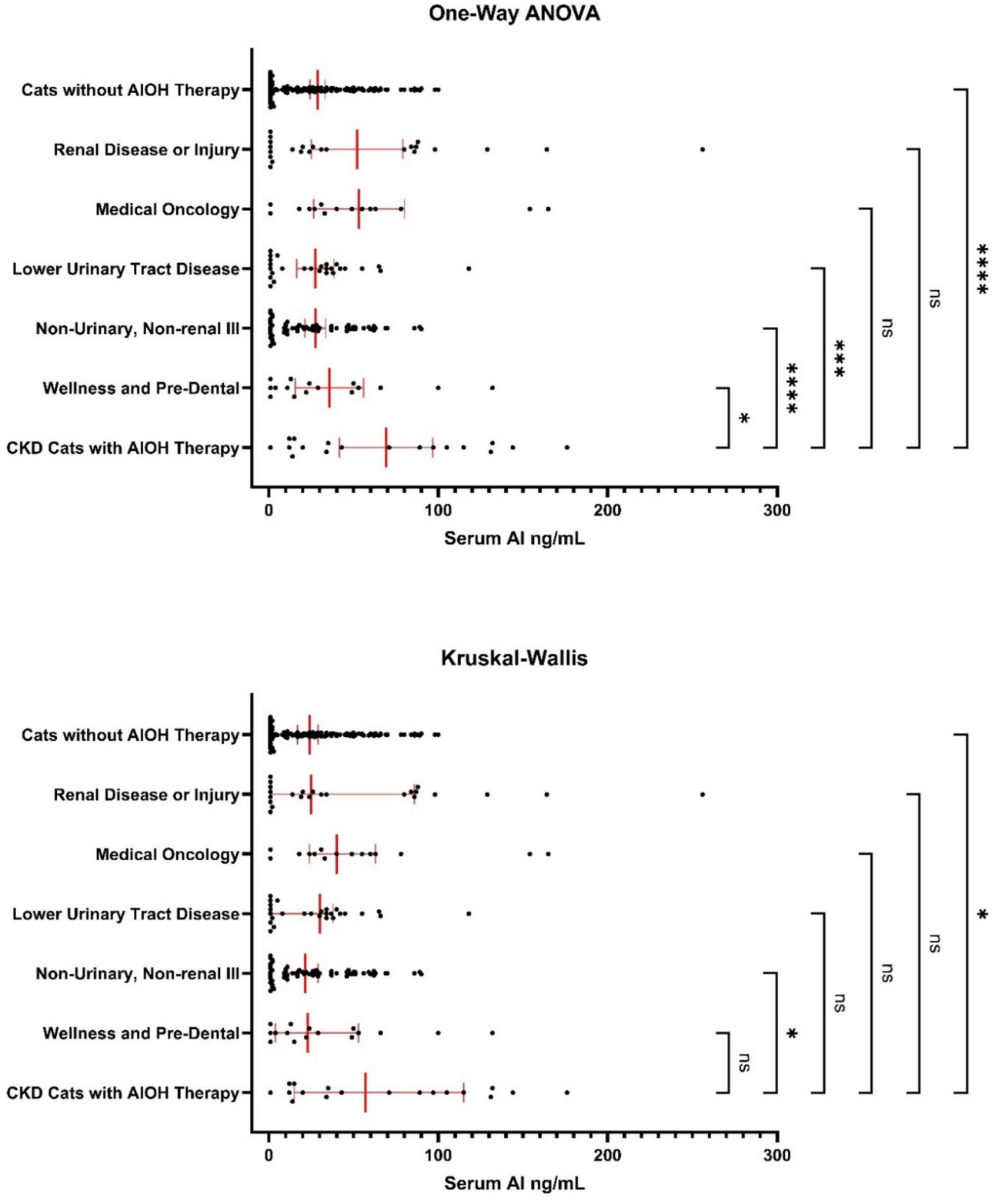

Fig. 5 Comparison of parametric (one-way ANOVA) and non-parametric (Kruskal-Wallis) tests for differences of means across all study sub-groups after ROUT analysis and exclusion of outliers

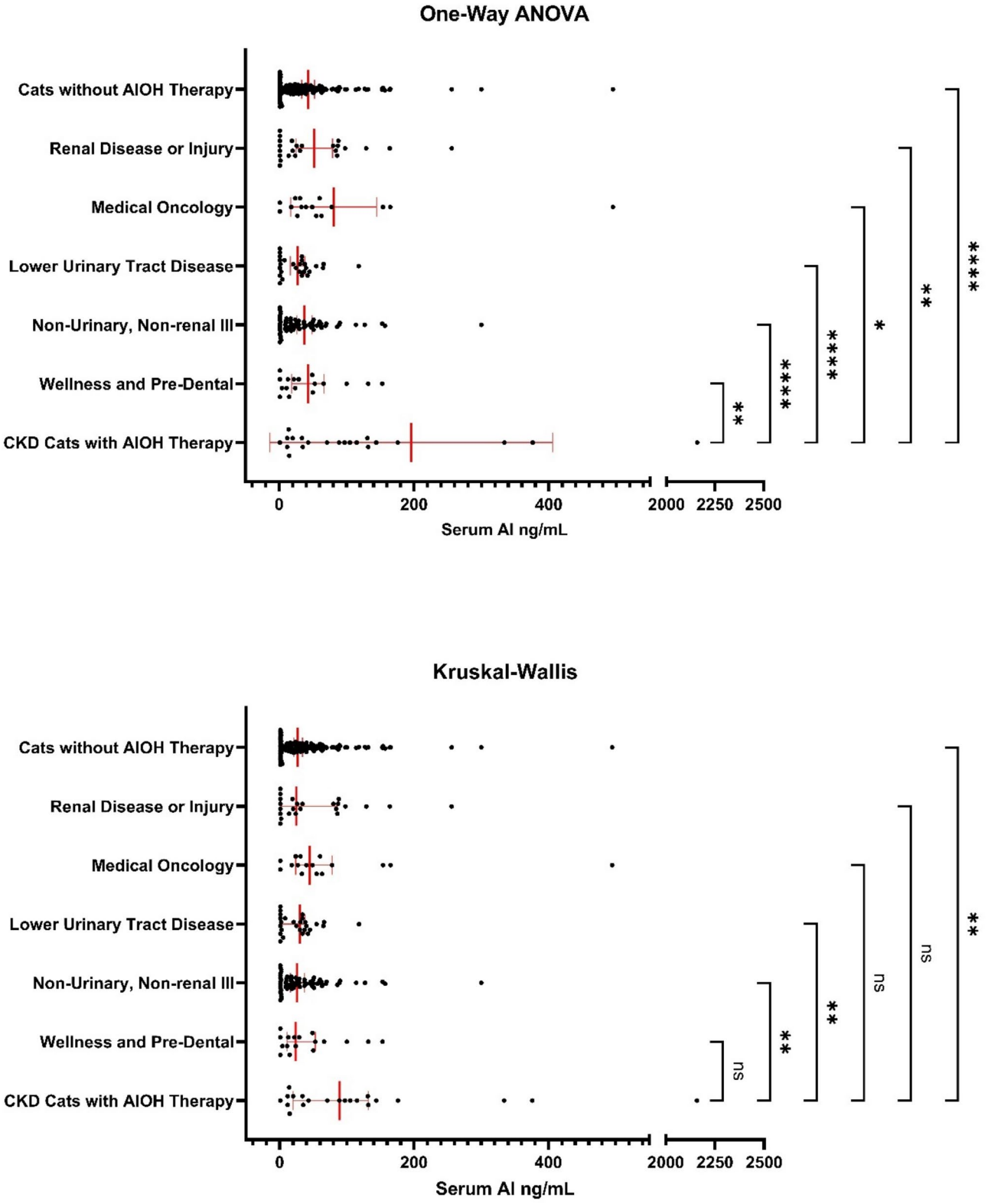

Fig. 6 Comparison of parametric (one-way ANOVA) and non-parametric (Kruskal-Wallis) tests for differences of means across all study sub-groups including identified outliers

chelation has been used with some success and has been recommended to eliminate accumulated Al in tissue in severe cases [4]. The return of serum Al to background levels is expected to be delayed in geriatric and renally impaired patients compared to the previously described young healthy dog at twelve-week follow-up. For the feline case described here, serum Al was 71 ng/mL at five-month follow-up supporting tissue redistribution and prolonged serum Al elevations in CKD patients after discontinuing Al therapy. The time to resolution of Al toxicosis in renal patients with mild clinical signs, such as a mild or moderate tremor, after discontinuation of Al therapeutics from treatment protocols has yet to be established; however, our data suggest serum Al assessment over the subsequent 4–6 months until concentrations fall below the proposed 86 ng/mL threshold as an appropriate monitoring strategy.

While this study presents a large population of 176 cats for serum Al assessment, not every cat assessed was healthy and free from injury or chronic disease. Of the patients assessed for serum Al, 17 were healthy animals, 71 had illness or injury not involving the urinary system, 27 had existing lower urinary tract disease, 24 had CKD or were being monitored for AKI following ingestion of nephrotoxic substances, 16 were medical oncology patients, and 21 had CKD and were receiving AlOH. Of these, 14 outliers were identified in the cats without AlOH group and 3 were identified in the CKD cats receiving AlOH. For cats not receiving AlOH, outliers were found to be serum Al greater than 114 ng/mL whereas in the cats with CKD receiving AlOH, outliers were identified as exceeding 334 ng/mL. When compared to the reported toxic threshold in humans of 100 ng/mL or previous data in dogs that concentrations greater than 80 ng/mL are of concern, these identified outliers could represent cases for which additional evaluation is warranted [3, 11]. Early clinical signs such as changes in speech pattern and forgetfulness or dementia are noted in humans, however these subtle changes are difficult to assess in veterinary patients [3]. Only the presented case was submitted for evaluation with a clinical suspicion of Al intoxication yet these additional individuals with elevated serum Al may be cases of intoxication that have yet to be recognized. For individuals not receiving AlOH, other sources of Al should be considered as a source for Al exposure such as pet foods, water, and other medications for which Al may be present [21, 22]. Patients with underlying disease present unique challenges when assessing trends of exposure due to the complexity of underlying disease that can affect Al kinetics and the multitude of medications that a patient may be receiving such as with medical oncology patients or post-operative patients that receive rigorous treatment protocols. Further evaluation of patients with elevated serum Al will help assess the transient or chronic nature of the Al elevations and evaluate sources of Al and the potential for tissue accumulation and clinical toxicosis in ill and renally compromised patients.

Ideally, a reference range would be established for at least 120 healthy individuals across various ages and equal representation of male and female patients. Here, we present data from 16 individuals for which serum was submitted as a part of a wellness examination or for pre-anesthetic bloodwork prior to a dental procedure. Availability of serum from healthy animals is limited at the diagnostic laboratory and tertiary care hospital as samples are submitted for a specific diagnostic purpose in animals experience illness, injury, or disease. Additionally, samples from primary care veterinarians may be limited as compliance for wellness visits is often lower for cats, especially those with fear and anxiety associated with veterinary visits. Owners may decline routine bloodwork if their cat is not currently experiencing any health changes and presents an additional barrier in sample acquisition. Young animals may be overrepresented in reference range populations if serum is submitted as pre-anesthetic bloodwork prior to spay and neuter procedures and most older cats will develop some form of chronic illness excluding them from the reference data population. Future studies will aim to expand the available data from healthy cats and those with CKD. Prospective studies assessing the fluctuation and accumulation over time of serum Al in cats receiving AlOH at increasing doses and advancing stages of renal disease will provide critical data in understanding Al kinetics in renal compromised cats. This data will provide further evidence of interactions of Al with calcium and phosphorus homeostasis, the cause and occurrence of anemias in cats with renal disease, and characterization of neurologic signs and better characterize the incidence of Al toxicosis in veterinary patients.

Conclusion

Al toxicosis is likely underdiagnosed in cats due to lack of awareness of this condition by veterinarians, uncharacterized pathogenesis and mechanism of action of Al, and decreased utilization and availability of Al assessment by veterinary diagnostic laboratories. To the authors' knowledge, this is the first case report of Al toxicosis in a cat after receiving Al-containing phosphate binders. Veterinarians should recognize the potential risk in using these binders in an extra-label manner and consider therapeutic monitoring, particularly in geriatric and advanced stage kidney disease patients, to mitigate Al accumulation in tissues and ensure serum concentrations remain in an acceptable range.

Methods

Sample acquisition

Serum for the presented case was submitted to the Michigan State University Veterinary Diagnostic Laboratory (MSU-VDL) Toxicology Service for quantitative Al assessment to confirm clinical suspicion of Al toxicosis. Additional serum samples were acquired from submissions to the MSU-VDL Clinical Pathology and Endocrinology Services. For reference data establishment, samples were collected from cats with normal SUN and creatinine concentrations within the reference interval. Inclusion criteria for assessment included SUN and creatinine within the reference range on serum biochemistry assessment of the analyzed sample. Exclusion criteria for samples included elevations in SUN or creatinine indicating azotemia or significant dehydration and exclusion of duplicate samples from animals already included in the study population. Samples were collected from cats with CKD submitted to the Endocrinology service with a reported history of AlOH administration. Sample collection and utilization was exempt from IACUC protocol guidelines as samples were collected from remaining serum submitted for diagnostic tests performed by private practitioners or diagnostic laboratories.

Patient grouping

Of the 155 cats assessed, 17 presented otherwise healthy for wellness examination or for pre-anesthetic bloodwork prior to a dental procedure. The remaining patients were divided based on relevant medical history and presenting complaint for veterinary evaluation into four additional groups. Seventy-one cats presented for veterinary evaluation for reasons not related to the urinary or renal systems (such as an abscess or gastrointestinal disease) and were classified as "non-urinary/non-renal ill". Twenty-seven cats with lower urinary tract disease (such as resolved urethral obstruction, feline lower urinary tract disease, or monitoring of their subcutaneous ureteral bypass systems) were classified as "lower urinary tract disease". Medical oncology patients ($n = 16$) were separated from all other groups even if their specific neoplasia did not have direct renal involvement as these patients receive aggressive treatment protocols that may or may not contain other Al therapies such as sucralfate as a gastroprotectant. An additional 24 cats with a history of CKD that were not receiving AlOH or cats presenting for potential nephrotoxicity (e.g. ibuprofen and lily ingestion) were characterized as "renal disease or injury".

Serum aluminum assessment

Serum Al was quantitated utilizing inductively coupled plasma mass spectrometry (ICP-MS) methodology. Samples were prepared as a 25-fold dilution in a solution containing 0.5% EDTA and Triton X-100, 1% ammonium hydroxide, 2% 1-butanol, 5 ppb of scandium and 7.5 ppb of rhodium, indium, and bismuth as internal standards. The ICP-MS was tuned to yield a minimum of 7500 cps sensitivity for 1 ppb yttrium (mass 89), less than 1.0% oxide level as determined by the 156/140 mass ratio and less than 2.0% double charged ions as determined by the 70/140 mass ratio. Al concentrations were calibrated using a 6-point linear curve of the analyte-internal standard response ratio. Standards are from Inorganic Ventures (Christiansburg, VA). A source calibration check standard from High Purity Standards (North Charleston, SC) was utilized.

Statistical analysis

Data were collected then analyzed using GraphPad Prism software for statistical analysis (version 10.4.1., GraphPad Software, Boston, MA, USA). Samples were recorded by medical record number and duplicate submissions from the same animal were removed. Values recorded as less than the reporting limit of the assay (< 2 or < 4 ng/mL) were assigned a value of half the reporting limit (1 or 2 ng/mL). Outliers were assessed by the robust regression and outlier removal (ROUT) method (Q = 1%). Quantitative data were shown as mean, standard deviation of the mean, 95% confidence interval (CI) of the mean, minimum, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, maximum, and 95% CI of the median. Shapiro-Wilk and Kolmogorov-Smirnov tests for normality were utilized. Reference intervals were generated from the 5th to the 95th percentile using a modification of the method described by the position statement by the American Society for Veterinary Clinical Pathology [17]. A student's t-test (one-tailed) was used to compare the means between groups with equal variances and an unequal variances t-test (one-tailed t-test with Welch's correction) was used to compare means of groups for which variances were significantly different (F-test). Parametric (one-way analysis of variance (ANOVA)) and non-parametric (Kruskall-Wallis) tests for differences in distributions were utilized to compare each control subgroup to cats with CKD receiving AlOH. Dunnett's tests (ANOVA) and Dunn's tests (Kruskall-Wallis) were utilized to assess statistically significant differences between the CKD cats receiving AlOH group and other groups of interest with adjusted p-values reported. Analysis was complete with and without the inclusion of outliers. An adjusted P-value ≤ 0.05 was considered to be of statistical significance.

Abbreviations

Al	Aluminum
AlOH	Aluminum hydroxide
CKD	chronic kidney disease
FDA	Food and Drug Administration
SUN	serum urea nitrogen
SDMA	symmetric dimethylarginine

PO	per os (by mouth)
SID	semel in die (once a day)
EOD	every other day
IACUC	International Animal Care and Use Committee
ICP	MS–Inductively coupled plasma mass spectrometry
IRIS	International Renal Interest Society
MSU VDL	Michigan State University Veterinary Diagnostic Laboratory
ROUT	Robust regression and outlier removal
ANOVA	analysis of variance

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Author contributions
RS analyzed and interpreted acquired data, acquired funding for analysis, and is the major contributor to the writing of the manuscript. SK provided diagnostic samples and historical information for the case report. SR provided technical support in preparation and analysis of samples. VM provided diagnostic samples and historical information for samples for patients receiving aluminum hydroxide. JPB provided technical support in acquiring serum samples for control populations. BP was responsible for the supervision, execution, and funding acquisition for this project. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All samples utilized are exempt from International Animal Care and Use Committee (IACUC) protocols and approval. Samples were acquired from left-over serum submitted to the diagnostic laboratory for other diagnostic testing. Informed consent for serum aluminum testing was acquired by the referring veterinarian for the case presented herein.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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