Correlation between separate findings in 60 dogs suffering from CKD in IRIS stage 1

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Abstract

Background The International Renal Interest Society (IRIS) reports guidelines for classification and therapy for dogs suffering from chronic kidney disease (CKD). Dogs in stage 1 show no elevated serum creatinine, but some other sign of kidney disease like elevated Symmetric Dimethylarginin (SDMA), proteinuria, low urine specific gravity or abnormalities in the sonography of the kidneys. Objective of this study was to access the correlation between inclusion criteria and to give an estimation whether a more detailed staging or a substaging for patients with elevated SDMA levels might be useful regarding possible treatment recommendations.

Results Sixty patients in IRIS stage 1 were included into the study. Most of these dogs were included due to raised SDMA level (n=22) or sonographic abnormalities of the kidneys (n=16). In order to rank and compare results of ultrasonography, a sonography score was developed. Additionally, results from blood work, urinalysis, ultrasonography and in some cases glomerular filtration rate (GFR) were examined. Correlation analysis showed positive correlation between creatinine and urea and negative correlation between creatinine and urinary protein-to-creatinine ratio (Up/c). Between SDMA, phosphate, urine specific gravity and sonographic findings there is no dependency with any other examined parameter.

Conclusion Results showed that patients in IRIS stage 1 are a heterogeneous group and giving precise treatment recommendation might be challenging. If future studies will suggest treatment in this stage of CKD, such as renal diet, a more detailed classification is needed.

Background

Chronic kidney disease (CKD) is characterized by the progressive and irretrievable loss of functioning nephrons in one or both kidneys for at least three months or longer (1). The disease is the most common kidney disease and as various studies showed, most relevant in older dogs (2-4). The most recent study by O'Neill et al. reported a prevalence of 0.37% (5), older studies even referred to a prevalence up to 3.74% (6). While aetiology remains unknown in most cases, therapy concentrates on symptomatic treatment. Hypertension, nausea, polyuria and polydipsia are the most relevant signs shown by patients (7).

Gold standard for the evaluation of kidney function is the measurement of glomerular filtration rate (GFR) (8). Direct measurement of GFR is costly and time-consuming, as it involves hospitalization and the collection and analysis of multiple blood samples (8, 9). In everyday clinical practice, it is common to rely on endogenous serum biochemical markers like serum creatinine, which is known to be influenced by the patient's muscle mass and to be unable to detect early decrease of kidney function (10).

The International Renal Interest Society (IRIS), a group of veterinarian experts, developed a four-stage system to classify dogs suffering from CKD (11). The four-stage system is based on the results of blood chemistry, urinalysis and systemic arterial blood pressure measurement (11). The initial staging is based on serum creatinine. While stages two to four are composed of patients with different levels of raised creatinine, stage one includes non-azotaemic patients, which exhibit other signs of CKD. Possible criteria

for the inclusion into IRIS stage 1 are: Polyuria, polydipsia, proteinuria, poor urine concentration, abnormalities in sonography and increased symmetric dimethylarginine (SDMA) (11).

Current treatment recommendations given by the IRIS, suggest starting to feed a renal diet, which is one of the cornerstones of CKD treatment, in stage 2 of the disease (11-14). For stage 1, only diagnostic procedures are indicated, unless signs of dehydration, hypertension or proteinuria are detected. A recent study, however, suggested that patients might benefit from receiving a renal diet as early as IRIS stage 1 (determined by raised serum SDMA level) (15).

SDMA is the methylated form of arginine and primarily eliminated by renal excretion. In previous studies, it showed a high correlation to glomerular filtration rate (GFR) in dogs suffering from kidney disease and was increased in average 9.4 months earlier compared to creatinine (16). Therefore, SDMA seems to be a promising biomarker for an early detection of CKD (16-19).

While correlation of serum SDMA and GFR as the Gold standard for the evaluation of renal function has already been established, correlation between abnormalities in sonography of the kidneys and GFR is still inconclusive. Regarding ultrasonography, a scoring system considering several sonographic parameters of the kidneys was developed, since single criteria of the sonographic appearance of the kidneys have shown little correlation to kidney function in past studies (20-23).

Regarding the wide variety of symptoms that lead to inclusion into stage 1, it is obvious that giving precise treatment advice is challenging.

Due to the possible positive effect of receiving a renal diet in patients with raised SDMA levels (24), further differentiation of IRIS stage 1 might be necessary to improve treatment recommendations for early CKD.

In the current study, blood results, reports of abdominal sonography and results of urinalysis of 60 patients that were presented to the Small Animal Clinic of the University of Veterinary Medicine Hanover from 2017 to 2019 and who were classified as belonging to IRIS stage 1 were reviewed.

The objectives of this study were to

1. record the different causes that lead to inclusion into IRIS Stage 1 in clinical practice (regarding primary care and referral cases)
2. assess the correlation between the numerous criteria associated with CKD and lead to inclusion into the study
3. give an estimation whether a more detailed differentiation between patients, which are classified as IRIS Stage 1 right now, might be necessary regarding possible treatment recommendations and

Materials And Methods

Study design

A retrospective analytical study design was used. The medical record database at the Small Animal Clinic of the University of Veterinary Medicine Hanover was searched to identify dogs with chronic kidney disease in IRIS stage 1.

Patients

All the animals included in this study were presented to the clinic from January 2017 to December 2018. For each recorded case, the following data was analysed: age, breed, cause of presentation, presence of polyuria and polydipsia, feed of renal diet, outcome of ultrasonography of the urinary tract, lab work including blood count, clinical chemistry and urinalysis, and cause for inclusion into IRIS stage 1 (Table 1). As very young dogs, as well as special breeds, such as greyhounds, are known to have higher SDMA levels, patients had to be at least one year old to be included into this study. In addition to this dogs of breeds known to have divergent SDMA reference ranges were not included into the study. Patients with proteinuria or low specific urine gravity were only included into IRIS stage 1, if additional diagnostics were performed to rule out non-renal reasons for the observed changes. Patients showing polyuria and polydipsia for any other reason than renal disease were excluded from the study. There was no data on patients' muscle condition score available for the examined cases.

inclusion criteria	IRIS stage 1 patients	reference range
creatinine*	≤ 1.5 mg/dl (rounded)	< 1.5 mg/dl
SDMA	> 14 µg/dl	< 14 µg/dl
clinical symptoms	polyuria/polydipsia	No clinical symptoms
spezific urine gravity	< 1016	1016 - 1040
Up/c	> 0.5	< 0.5
sonography oft the kidneys	> 2 score points	0 score points
serial creatinine samples	increasing creatinine levels	Creatinine samples on one level

Table 1: inclusion criteria for the study. Criteria for Creatinine had to be met (*), as well as one additional criteria for patients to be included into the study.

Laboratory Parameters

Blood analysis including blood count (Advia2120i Hematology System, Siemens Healthcare, Eschborn / Germany) and complete blood chemistry as well as electrolytes (Rapidlab 1260, Siemens Healthcare Diagnostics GmbH, Eschborn / Germany) was performed in house at the Small Animal Clinic. Blood samples for SDMA measurement were stored at 4 °C and sent to IDEXX-laboratories® to be measured the day after blood sampling. The reference interval for serum SDMA concentrations used in this study was ≤ 14 µg/dl.

Urinalysis was performed in house at the clinic for small animals by utilizing urine sticks, refractometer and cytology.

Ultrasound

Ultrasound of the urogenital tract was performed by ultrasonography specialists working at the Small Animal Clinic, using the standard ultrasonography machine of the clinic (Logiq 7, GE Healthcare, Solingen / Germany). Images were acquired with either a curvilinear or a linear array transducer with frequencies ranging from 8 to 14 MHz according to clinician preference. All images were retrospectively reviewed by the primary investigator and scored considering the following criteria: kidney size in relation to aorta, shape, cortex thickness and echogenicity, proportion of cortex and medulla, kidney pelvis, medullary rim sign, calcifications, cysts and surface of each kidney (table 2). Scoring was performed for both kidneys independently and the results of both kidneys were summed up to build the finale score. To be included into the study due to sonographic abnormalities of the kidneys, patients had to meet at least two points of the score.

sonographic abnormality	scoring points
size (in relation to aorta)	1
proportion cortex : medulla	1
kidney pelvis dilatation (>2 mm)	1
medullary rim sign	1
cortical thickness	1
reduced corticomedullary demarcation	1
abnormality in shape *mild* *moderate* *highly*	 1 2 3
increased echogenicity of cortex *mild* *moderate* *highly*	 1 2 3
calcification *mild* *moderate* *highly*	 1 2 3
cysts *Single* *few* *multiple*	 1 2 3

Table 2: Sonographic signs can reach one or, where declared, up to three points, depending on the severity of the sign. Each kidney's points are charged individually and summed up for final evaluation. Patients were included in IRIS stage 1 when they reached > 2 points.

Glomerular filtration rate

In six dogs, GFR measurement was available in addition to the aforementioned parameters. Measurement was performed using endogenous creatinine clearance. 5%-creatinine solution in a dosage of 5 gr creatinine/m^2 body surface was mixed and filtrated in an in house laboratory 24 hours prior to application. After measuring basal serum creatinine, 5% creatinine solution was applicated subcutaneously. Additional measurements of serum creatinine were performed three, five and eight hours after application. During the whole time, fluids were given intravenously in a dosage of 2 ml/kg/h.

Statistics

For statistical analysis, SAS Enterprise 7.1 guide (SAS institute, North Carolina, USA) was used. The Shapiro-Wilk-test was used to test for standard distribution of the data. Seeing only data for creatinine showed standard distribution, Spearman rang correlation was used to analyze correlation between results. The minimal level of significance was set to <0.05.

Results

60 dogs met the inclusion criteria for the study. The average age was 9.5 (± 3.3) years (fig. 1), medium weight was 18.6 (± 12.5) kg. 35 dogs were male (26 neutered) and 25 were female (23 neutered). Most of the dogs were mixed breeds (18/60), but the study also encompassed breeds like Yorkshire Terrier (n=4), Retrievers (n=4), Galgo Espanol (n=3), Jack Russel Terrier (n=3), Australian Shepherd (n=2), Collies (n=2), Dachshund (n=2), German Shepherd (n=2), Bull Terrier (n=2) and Pinscher (n=2). Additionally, one patient of each of the following breeds was present: Beagle, Bernese mountain dog, Border Collie, Boxer, Brittany Dog, Burgundy Mastiff, Dalmatian, Havanese, Hovawart, Husky, Maltese, Miniature Schnauzer, Parson Russel Terrier, Poodle, Pug and Small Munsterlander. Detailed information on weight, age, breed and gender of the included dogs is given in table 4.

Results of blood analysis were available in all dogs. Overall, SDMA was measured in 51 patients and showed a mean value of 14.7 (± 4.62) µg/dl. Serum creatinine (1.01 ± 0.27) mg/dl and urea (42.7 ± 24.73) mg/dl were measured in all patients (n=60). Phosphate was measured in 52 patients and had an average value of 1.1 (±0.39) mmol/l.

GFR measurement using exogenous creatinine clearance was successful in all six patients. Mean GFR level was 60.02 % (±8.6%). Patients that were available for GFR testing showed SDMA levels in a range from 14 to 16. In three patients, SDMA was within the reference range, three patients showed elevated SDMA levels.

Cystocentesis was performed in 22 patients, 34 times urinalysis was performed from spontaneous urine samples. Mean urine specific gravity was 1028.3 (±14.58), Up/c was 0.2 (±0.3). Mean values are shown in table 3, detailed information in patients' data is shown in table 5.

laboratory parameter	arithmetical mean (standard deviation)	number of patients
SDMA	14.7 (± 4.62) µg/dl	51
creatinine	1.01 (± 0.27) mg/dl	60
urea	42.7 (± 24.73) mg/dl	60
phosphate	1.1 (±0.39) mmol/l	52
urine specific gravity	1028.3 (±14.58)	56
Up/c	0.2 (±0.3)	56

Table 3: arithmetical mean and standard deviations of laboratory parameters in this study and number of investigations that were available for analysis.

Ultrasonographic examination was available in 58 patients. Retrospectively converted into a scoring system, 25 patients achieved zero to two scoring points and 30 patients three to six points in the ultrasonography score. Only three patients had a score of 7 or more points (fig. 2). The most common feature seemed to be reduced corticomedullary demarcation (n=41).

22 dogs were originally included into the study due to increased SDMA. Initially, 16 dogs were included in the study because of sonographic changes. Nine dogs showed increased urea in fasted trials, seven dogs showed decreased urinary specific weight. Proteinuria was present in four dogs and only two dogs were included because of polyuria and polydipsia (fig. 3). There were 25 patients who showed two of the examined signs to be included into IRIS stage 1, eight patients showed three signs, three patients showed four signs and one patient showed five signs.

At the time of presentation, seven dogs were fed a renal diet by the owners, following their veterinarians' advice. Three of these dogs were included in the study because of increased urea, two dogs were presented with elevated SDMA. Proteinuria and low urinary specific gravity were detected in one dog each.

Correlation analysis showed that phosphate, urine specific gravity and sonographic findings (fig. 4; fig. 5) did not correlate with any other examined attribute. Up/c was negatively correlated to creatinine ($p<0.0001$) (fig. 6). Creatinine did correlate with urea ($p=0,0067$) (fig. 7) but not with SDMA (fig. 8). No correlation between GFR and SDMA was found.

Discussion

Patients classified as IRIS Stage 1 for the purposes of this study were a heterogenous group of dogs with increased SDMA levels, sonographic abnormalities of the kidneys or abnormal findings in urinalysis. 43% of patients suffering from CKD in this study was included into IRIS stage 1 due to elevated SDMA-levels. Since in some patients all other blood parameters examined in this study were in the reference range and no sonographic abnormalities were detected, SDMA seems to be a sensitive marker of early loss of renal function. This fits the results of prior studies, where an increased SDMA level was detected up to 9.8 months earlier compared to a raise in creatinine (16, 25).

16 dogs were included in this study because of sonographic kidney abnormalities. Sonographic kidney abnormalities are a common finding, especially in older patients (29). As IRIS does not define any details about the kind or extent of sonographic abnormalities needed for inclusion in IRIS stage 1 and many seemingly healthy dogs show slight abnormalities of the kidneys in abdominal sonography (20), a scoring system was used in this study. As previous studies have already shown, there is a poor relation between single sonographic findings and kidney function in dogs (20, 21, 29) as well as in humans (30). Thus, patients in this study had to achieve at least two points to be included. Since sonographic scoring points did not correlate to creatinine or SDMA, it is still questionable if sonographic signs should be used to draw inferences about kidney function at all or if different inclusion criteria are needed or more severe findings are required to find a correlation between sonography and renal function. At this point, no long-term data is available to examine the progress of ultrasonographic kidney abnormalities or the impact of the sonographic appearance on progression of the disease.

CKD is reported to be a disease mostly occurring in older dogs, which was confirmed in the present study with a medium age of 9.5 years. In total, 24 different breeds but mostly mongrels were seen, which fits the results of prior studies and shows that mixed breeds are as likely as pure breeds to develop CKD (5). Prior studies reported age and breed as well as several comorbidities to have an increasing effect on

SDMA (17, 31). Therefore, only dogs older than one year were included in this study and dogs of breeds that were reported to show increased SDMA were excluded from the study. If patients showed any comorbidity that is known to influence SDMA levels, these patients were not included in the study as well (32, 33). Regarding patients' neuter status, a striking number of patients in this study were neutered. This partly meets the findings of other studies which demonstrated a high number of neutered patients suffering from CKD, but could not find a significant correlation between neuter status and the prevalence of kidney disease (5). Still, further studies might be needed to evaluate whether a connection between development of CKD and neuter status exists.

One purpose of this study was to identify the context between the numerous criteria that lead to inclusion into IRIS stage 1. No significant relationship between serum SDMA concentrations and serum creatinine, urea, phosphate, specific gravity, urine protein/creatinine or sonographic findings was observed. In the data of this study there was only a correlation between serum creatinine and urea and creatinine and Up/c. This is a discrepancy to the results of a former study that revealed a significant association between SDMA and creatinine in dogs affected with CKD caused by X-linked hereditary nephropathy (17), as well as in cats suffering from CKD and humans (34, 35). This might be due to the heterogenous inclusion criteria of the patients participating in the study, many of which did not show elevated SDMA levels. Additionally, only patients in a very early stage of kidney disease were analysed, whereas the dogs in the study of Nabity et al. affected with X-linked hereditary nephropathy developed severe renal insufficiency in the course of the study (17). In the healthy control group, no correlation between SMDA and GFR was found in the study by Nabity et al. either.

Correlation between serum creatinine and urea was shown in this study population. Although urea is influenced by patients' food composition as well as liver function, it is known to be a marker for kidney function and therefore was expected to correlate with creatinine (5). As patients were in an early state of CKD, it is obligatory that creatinine was within the reference range and in most cases, urea was not increased either. The negative relation between Up/c and creatinine might have occurred as a result of few individuals showing very low creatinine but Up/c above the reference range. Most Up/c values were within the reference range (n=47) and as figure 6 shows, the majority clustered at a low value between 0.1 and 0.3.

Regarding the question whether the IRIS staging in its current form would be suitable as the basis for therapeutic measures (like the decision to feed a renal diet) in non-azotemic patients, the inclusion criteria in their current iteration seem rather diverse, leading to a very heterogenous group of IRIS stage 1 patients. Right now, IRIS recommends treatment for patients that are at least stage 2 of chronic kidney disease. In this stage, dietary intervention is indicated to slow progression of the disease (13-15, 36). At this time, no treatment recommendations are given in the IRIS therapy recommendations for patients in stage 1 of the disease, unless dogs are dehydrated, suffer from hypertension or are diagnosed with proteinuria (11). Within the six patients that were tested to estimate GFR in this study, reduced GFR up to 45.45 % was detected, but only three of these patients showed increased SDMA levels at the time of GFR measurement. Due to this decrease in renal function, these patients might benefit from receiving a

protein-reduced diet. Hall et al. reported patients in IRIS stage 1 to benefit from a special diet (high quality protein, fish oil, antioxidants, L-carnitine, controlled sodium) in comparison to an owner's choice diet (24). However, these patients all showed elevated SDMA levels. At this point, it cannot be concluded if this effect can be shown in all patients of IRIS stage 1. Regarding the patients in this study, it seems unlikely that uniform treatment recommendations should be given for such a heterogeneous group of patients. Therefore, it might be beneficial to substage patients into a group only for patients showing increased SDMA levels and another subgroup for patients with less concrete signs such as changes in sonography of the kidneys. Initiation of a renoprotective diet might be considered in patients, who exhibit reduced GFR or increased SDMA levels, in contrast to patients included into IRIS stage 1 for other signs of CKD.

Conclusion

Since only diagnostic measures are recommended for patients in IRIS stage 1, the current heterogenous inclusion criteria seem adequate at the moment. If future research should suggest the initiation of therapeutic measures like feeding a renal diet for patients in the non-azotemic stage of CKD, further differentiation for these patients (e. g. subgrouping regarding SDMA levels) might become necessary.

Limitations

One limitation of this study is the number of patients. Regarding the inclusion criteria into IRIS stage 1, some were only fulfilled by a few patients, while other were achieved by a higher number of patients. Regarding laboratory results, it would have been desirable to have a full data including a detailed follow-up in each patient, which in this case could not be ensured due to the retrospective study design. Additionally, interpretation of urine specific gravity should be done carefully due to day-to-day variation and dependence on the patients' hydration status. In this study, the study design followed the relevant IRIS guidelines at the time of study inclusion, so urine specific gravity was regarded as inclusion criterion. Changes in sonography were ranked in a scoring system that was developed for this study. Ideally, it would have been reasonable to work with an internationally approved score. The lack of international consensus on sonographic criteria for CKD led to the development of a new score. Since blood pressure measurements were not recorded in most patients, blood pressure results were not included in our study. As hypertension is an important consequence of CKD and blood pressure measurement is part of the IRIS substaging system, it should be performed in patients showing signs of kidney disease.

Abbreviations

IRIS	International Renal Interest Society
CKD	Chronic kidney disease
SDMA	Symmetric Dimethylarginin
GFR	Glomerular filtration rate

Up/c	Urinary protein-to-creatinine ratio

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent to publish:

Not applicable.

Availability of data and material:

All data generated or analyzed during this study are included in this published article.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

MGS made substantial contribution to acquisition of data, analysis and interpretation. MGS wrote the original draft. IN was involved in revising the manuscript critically for important intellectual content and made substantial contributions to conception and design. JPB made substantial contributions to conception and design and was involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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References

1. Polzin, J D. Chronic kidney disease in small animals. Veterinary Clinics: Small Animal Practice. 2011;41(1):15-30.
2. Macdougall DF, Cook T, Steward AP, Cattell V. Canine chronic renal disease: prevalence and types of glomerulonephritis in the dog. Kidney International. 1986;29(6):1144-51.
3. Müller-Peddinghaus R, Trautwein G. Spontaneous glomerulonephritis in dogs: II. Correlation of glomerulonephritis with age, chronic interstitial nephritis and extrarenal lesions. Veterinary pathology.

1977;14(2):121-7.
4. Vaden SL. Glomerular disease. Topics in companion animal medicine. 2011;26(3):128-34.
5. O'Neill DG, Elliott J, Church DB, McGreevy PD, Thomson PC, Brodbelt DC. Chronic Kidney Disease in Dogs in UK Veterinary Practices: Prevalence, Risk Factors, and Survival. Journal of Veterinary Internal Medicine. 2013;27(4):814-21.
6. Sosnar M, Kohout P, Růžička M, Vrbasova L. Retrospective Study of Renal Failure in Dogs and Cats Admitted to University of Veterinary and Pharmaceutical Sciences, Brno During 1999-2001. Acta Veterinaria Brno. 2003;72(4):593-8.
7. Bartges JW. Chronic kidney disease in dogs and cats. Veterinary Clinics: Small Animal Practice. 2012;42(4):669-92.
8. Von Hendy-Willson VE, Pressler BM. An overview of glomerular filtration rate testing in dogs and cats. The Veterinary Journal. 2011;188(2):156-65.
9. Braun J, Lefebvre H, Watson A. Creatinine in the dog: a review. Veterinary Clinical Pathology. 2003;32(4):162-79.
10. Grauer GF. Early detection of renal damage and disease in dogs and cats. Veterinary Clinics: Small Animal Practice. 2005;35(3):581-96.
11. www.iris-kidney.com. website International Renal Interest Society. wwwiris-kidneycom.
12. Roudebush P, Polzin DJ, Ross SJ, Towell TL, Adams LG, Forrester SD. Therapies for feline chronic kidney disease: What is the evidence? Journal of feline medicine and surgery. 2009;11(3):195-210.
13. Jacob F, Polzin DJ, Osborne CA, Allen TA, Kirk CA, Neaton JD, et al. Clinical evaluation of dietary modification for treatment of spontaneous chronic renal failure in dogs. Journal of the American Veterinary Medical Association. 2002;220(8):1163-70.
14. Polzin DJ. Evidence-based step-wise approach to managing chronic kidney disease in dogs and cats. Journal of veterinary emergency and critical care. 2013;23(2):205-15.
15. Hall JA, MacLeay J, Yerramilli M, Obare E, Yerramilli M, Schiefelbein H, et al. Positive impact of nutritional interventions on serum symmetric dimethylarginine and creatinine concentrations in client-owned geriatric dogs. PLoS One. 2016;11(4):e0153653.
16. Hall J, Yerramilli M, Obare E, Yerramilli M, Almes K, Jewell D. Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidney disease. Journal of Veterinary Internal Medicine. 2016;30(3):794-802.
17. Nabity M, Lees G, Boggess M, Yerramilli M, Obare E, Yerramilli M, et al. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the early detection of chronic kidney disease in dogs. Journal of Veterinary Internal Medicine. 2015;29(4):1036-44.
18. Hall J, Yerramilli M, Obare E, Yerramilli M, Jewell D. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. Journal of Veterinary Internal Medicine. 2014;28(6):1676-83.

19. Hall JA, Yerramilli M, Obare E, Yerramilli M, Melendez LD, Jewell DE. Relationship between lean body mass and serum renal biomarkers in healthy dogs. Journal of Veterinary Internal Medicine. 2015;29(3):808-14.
20. Mantis P, Lamb CR. Most dogs with medullary rim sign on ultrasonography have no demonstrable renal dysfunction. Veterinary Radiology & Ultrasound. 2000;41(2):164-6.
21. Banzato T, Bonsembiante F, Aresu L, Zotti A. Relationship of diagnostic accuracy of renal cortical echogenicity with renal histopathology in dogs and cats, a quantitative study. BMC veterinary research. 2016;13(1):24.
22. Mareschal A, D'ANJOU MA, Moreau M, Alexander K, Beauregard G. Ultrasonographic measurement of kidney-to-aorta ratio as a method of estimating renal size in dogs. Veterinary Radiology & Ultrasound. 2007;48(5):434-8.
23. Platt J, Rubin J, Bowerman R, Marn C. The inability to detect kidney disease on the basis of echogenicity. American Journal of Roentgenology. 1988;151(2):317-9.
24. Hall J, Fritsch D, Yerramilli M, Obare E, Yerramilli M, Jewell D. A longitudinal study on the acceptance and effects of a therapeutic renal food in pet dogs with IRIS-Stage 1 chronic kidney disease. Journal of animal physiology and animal nutrition. 2018;102(1):297-307.
25. Dahlem D, Neiger R, Schweighauser A, Francey T, Yerramilli M, Obare E, et al. Plasma symmetric dimethylarginine concentration in dogs with acute kidney injury and chronic kidney disease. Journal of Veterinary Internal Medicine. 2017;31(3):799-804.
26. Höchel J, Finnah A, Velde K, Hartmann H. Evaluation of a modified exogenous creatinine clearance as a suitable renal function test for the small animal practice. Berliner und Munchener tierarztliche Wochenschrift. 2004;117(9-10):420-7.
27. Hartmann H, Mohr S, Thüre S, Höchel J. Routine use of a renal function test for quantitative assessment of glomerular filtration rate (GFR) including determination of a cut-off value for azotemia in the dog. Eur J Companion Anim Pract. 2008;18:29-36.
28. Cortadellas O, Del Palacio MF, Talavera J, Bayón A. Glomerular filtration rate in dogs with leishmaniasis and chronic kidney disease. Journal of Veterinary Internal Medicine. 2008;22(2):293-300.
29. Moghazi S, Jones E, Schroepple J, Arya K, Mcclellan W, Hennigar RA, et al. Correlation of renal histopathology with sonographic findings. Kidney international. 2005;67(4):1515-20.
30. Emamian SA, Nielsen MB, Pedersen JF, Ytte L. Kidney dimensions at sonography: correlation with age, sex, and habitus in 665 adult volunteers. AJR American journal of roentgenology. 1993;160(1):83-6.
31. Liffman R, Johnstone T, Tennent-Brown B, Hepworth G, Courtman N. Establishment of reference intervals for serum symmetric dimethylarginine in adult nonracing Greyhounds. Veterinary clinical pathology. 2018;47(3):458-63.
32. Peterson M, Varela F, Rishniw M, Polzin DJ. Evaluation of serum symmetric dimethylarginine concentration as a marker for masked chronic kidney disease in cats with hyperthyroidism. Journal

of veterinary internal medicine. 2018;32(1):295-304.
33. Cirera S, Moesgaard SG, Zois NE, Ravn N, Goetze JP, Cremer SE, et al. Plasma proANP and SDMA and microRNAs are associated with chronic mitral regurgitation in a pig model. Endocrine connections. 2013;2(3):161-71.
34. Braff J, Obare E, Yerramilli M, Elliott J, Yerramilli M. Relationship between serum symmetric dimethylarginine concentration and glomerular filtration rate in cats. Journal of Veterinary Internal Medicine. 2014;28(6):1699-701.
35. Kielstein JT, Salpeter SR, Bode-Boeger SM, Cooke JP, Fliser D. Symmetric dimethylarginine (SDMA) as endogenous marker of renal function—a meta-analysis. Nephrology Dialysis Transplantation. 2006;21(9):2446-51.
36. Hall J, Yerramilli M, Obare E, Yu S, Jewell D. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in healthy geriatric cats fed reduced protein foods enriched with fish oil, L-carnitine, and medium-chain triglycerides. The Veterinary Journal. 2014;202(3):588-96.

Tables

Table 4: Characteristics of the included dogs, f= female, m=male, n=neutered

patient	weight (kg)	age (years)	breed	gender
1	57,2	8,20	mixed breed	fn
2	9,4	13,06	miniature Schnauzer	m
3	20,8	12,07	miniature bullterrier	mn
4	35,3	7,14	boxer	f
5	27,8	2,55	collie	m
6	46,50	7,89	german shepherd	mn
7	7,20	13,33	dachshund	fn
8	40,80	7,89	bernese mountain dog	mn
9	27,00	8,43	galgo espanol	fn
10	24,50	12,07	border collie	mn
11	25,00	10,41	german shepherd	fn
12	12,00	13,50	jack russel	mn
13	11,70	8,93	brittany dog	mn
14	22,40	7,61	mixed breed	mn
15	24,40	12,15	mixed breed	fn
16	20,00	12,15	collie	mn
17	13,80	10,96	mixed breed	mn
18	2,90	15,88	yorkshire terrier	mn
19	30,00	11,94	mixed breed	mn
20	17,20	10,84	mixed breed	mn
21	23,60	14,71	mixed breed	fn
22	16,20	10,44	mixed breed	mn
23	22,80	14,37	mixed breed	fn
24	14,20	3,43	dalmatian	f
25	7,50	15,97	parson russel terrier	mn
26	21,20	8,29	australian shepherd	fn
27	12,40	1,68	beagle	m
28	24,30	9,71	australian shepherd	mn
29	28,40	12,49	retriever	mn
30	11,80	9,75	mixed breed	fn
31	13,70	9,58	mixed breed	fn
32	23,70	5,98	galgo espanol	mn
33	27,50	7,93	galgo espanol	mn
34	9,40	8,87	mixed breed	mn
35	27,60	10,03	retriever	fn
36	17,00	9,37	mixed breed	fn
37	14,70	5,95	pinscher	fn
38	40,50	6,31	mixed breed	fn
39	15,30	15,71	mixed breed	fn
40	55,00	9,41	burgundy mastiff	fn
41	20,80	4,68	mixed breed	fn
42	40,80	8,43	retriever	m
43	13,00	6,56	mixed breed	mn
44	8,00	11,46	poodle	mn
45	11,40	13,50	dachshund	m
46	4,40	14,26	yorkshire terrier	fn
47	30,60	4,86	small munsterlander	mn

48	6,90	7,69	jack russel terrier	mn
49	4,10	7,68	maltese	m
50	7,40	15,94	jack russel terrier	fn
51	28,70	9,16	mixed breed	fn
52	5,30	7,90	havananese	fn
53	33,70	9,66	retriever	mn
54	3,60	12,95	yorkshire terrier	m
55	12,20	8,74	pug	m
56	4,00	2,08	yorkshire terrier	fn
57	17,30	8,99	bull terrier	mn
58	14,50	10,03	pinscher	fn
59	28,00	11,76	husky	mn
60	41,30	7,07	hovawart	m

Table 5: Laboratory results, sonography score points and anamnestic detail of the included dogs

patient	creatinine	urea	phosphate	SDMA	Up/c	urine sprecific gravity	sonography	Pu/Pd
1	1,2	28	1,4	**20**			**2**	no
2	0,42	24		11	**2,44**	1016	**4**	no
3	1,33	**53**	1,03	13	0,2	1047	1	no
4	1	**57**	0,91	11	0,15	1056	1	no
5	0,69	18	1,23		**1**	**1015**	1	no
6	1,1	**108**	1,51	13	0,09	1028	1	no
7	0,81	45	0,66	**20**	0,12	1024	0	**yes**
8	1,27	**61**	0,92	**15**	0,18	1022	**3**	no
9	1,37	49	1,25	**19**	0,19	1020	**5**	**yes**
10	1,34	**70**	0,97	11	0,34	1022	**6**	no
11	0,93	27	1,2	**16**	0,11	1040	**11**	no
12	0,93	**52**	0,95	**19**	0,9	1025	**4**	**yes**
13	0,75	22	1,07	**15**	0,13	1044	0	no
14	1,08	16	0,79	12	0,08	1026	1	**yes**
15	1,38	38	1,11	**16**	0,1	1018	**2**	**yes**
16	1,2	29	1	**18**	0,1	1046	**5**	no
17	1,51	41	0,99	**22**	0,07	1022	**4**	no
18	1,41	**112**	**1,66**	**36**	0,4	**1006**	**6**	no
19	0,99	**70**	1,33	14	0,12	1036	**4**	no
20	0,71	16	0,6	14	0,1	**1015**	**2**	no
21	1,13	**77**	1,14	**19**	0,07	**1009**	**8**	**yes**
22	0,87	**51**	1,19	**15**	0,11	**1007**	**4**	no
23	0,76	31	1,05	14	0,1	1050	**6**	no
24	1,07	38	1,25	14	0,07	1022	**6**	no
25	1,1	35	1,1	**19**	0,18	1028	**2**	no
26	1,28	**52**	0,74	13	0,06	1050	**2**	no
27	0,7	49	1,29	10	**1,03**	1021	**4**	no
28	1,03	24	0,92		0,1	**1007**	**2**	**yes**

29	1,21	39	1,09	**15**	0,11	1029	**2**	no
30	0,94	31	0,76	8	0,09	1050	**2**	no
31	1,53	45	0,97	14	0,07	**1014**	**4**	no
32	1,04	29	1,07	6	0,07	1032	**6**	no
33	1,06	16	1,04	14	0,05	1029	**4**	no
34	1,23	42	0,91	14	0,06	1050	**4**	no
35	0,86	21	1	14	0,08	1012	**4**	no
36	1,38	**55**	**1,69**	11	0,06	1050	**2**	no
37	1,22	42	1	**15**	0,08	1019	0	no
38	1,19	49	0,99	12	0,07	1042	**2**	no
39	0,67	**56**	0,85	**21**	**0,55**	1024	**2**	no
40	1,49	27	1,28	**15**	0,07	1030	**2**	no
41	1,39	29	1,1	**15**	0,07	1050	0	no
42	0,9	23	0,93	10	0,09	1048	**4**	no
43	0,81	27	1,09	14	0,08	1037	**2**	no
44	1,04	**63**	0,8	14	0,32	1022	**3**	no
45	0,77	29	1,23	12	0,29	**1006**	**4**	no
46	0,91	28		**21**	0,48	1030	0	**yes**
47	0,91	17				**1006**	**4**	no
48	0,92	29	0,93				**2**	no
49	0,28	16				1036	**4**	no
50	0,95	**132**	**3,39**				**4**	no
51	0,68	39	1,5		0,5	1017	**4**	**yes**
52	0,93	16	1,21	**15**	0,08	**1014**		**yes**
53	1,27	27		14		1017		**yes**
54	0,41	**54**	0,91		**1**	1028	**10**	no
55	0,77	36		7			**4**	no
56	0,85	33	0,96	**15**		1050	**4**	no
57	0,74	28		10	0,5	1050	**2**	no

58	0,87	29		11	0,41	**1008**	**4**	no
59	1,13	**113**	1,3		0,09	1018	**4**	no
60	1,19	47	0,95	12	0,09	1044	**4**	no

Figures

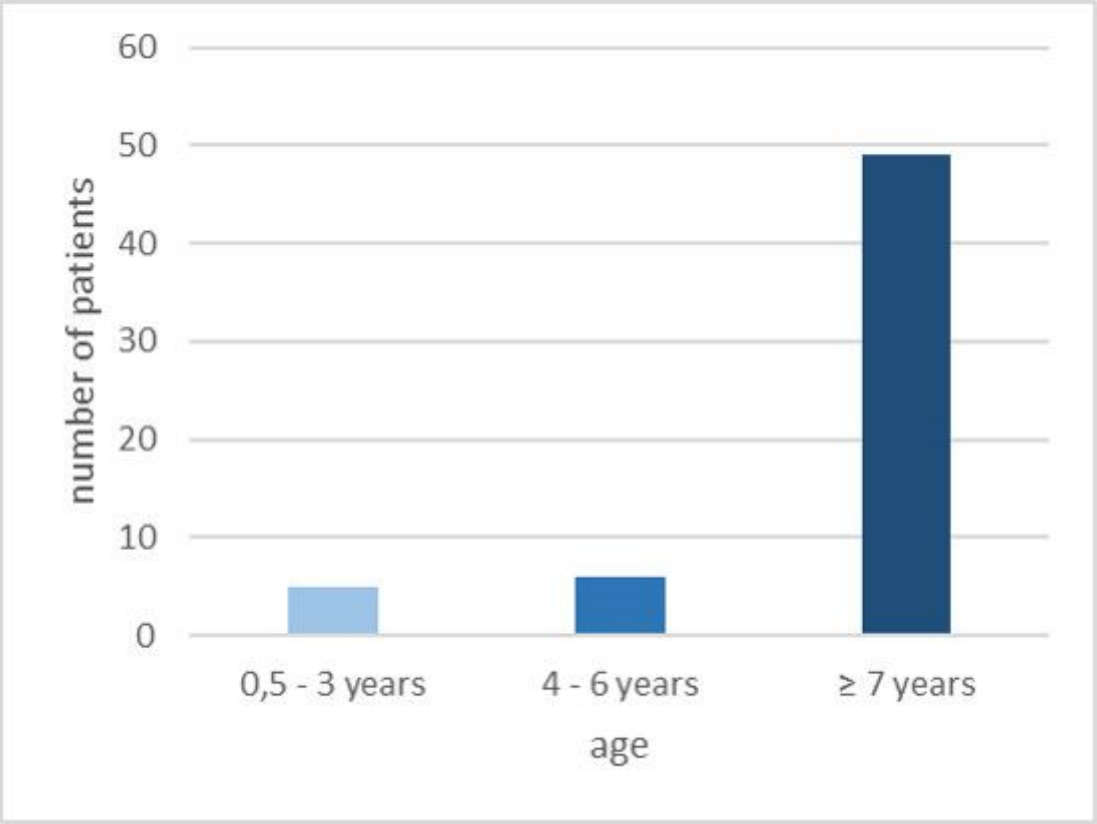

Figure 1

Age of patients, graded into three groups. Five Patients were included into the group of younger patients (6 months-3 years), six patients were 4-6 years old. The majority of patients (n=49) were 7 years of age or older.

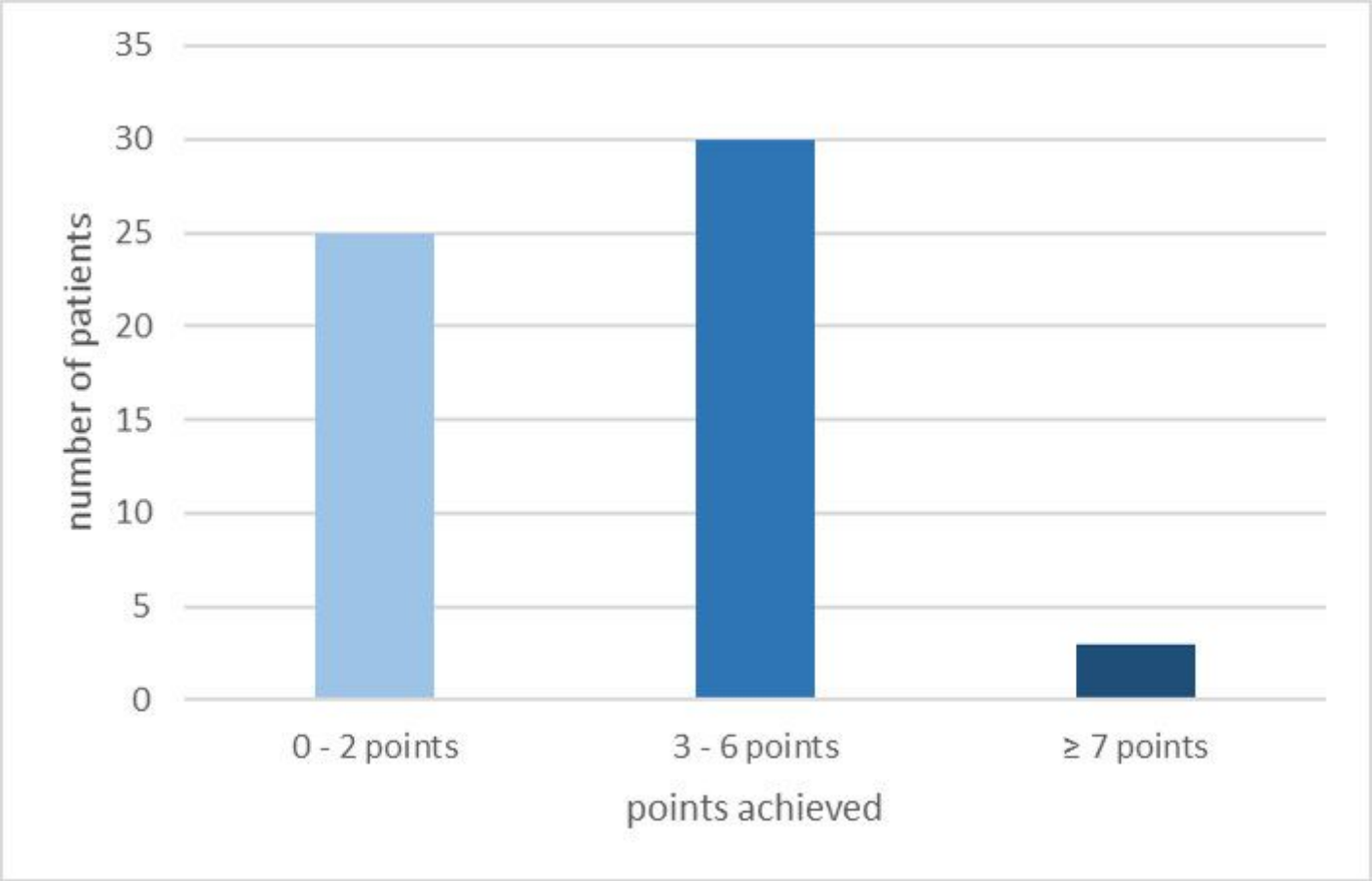

Figure 2

sonography score of patients participating in the study. Half of the study population achieved 3 to 6 points, 25 patients achieved 2 points or less, 3 patients achieved 7 or more points.

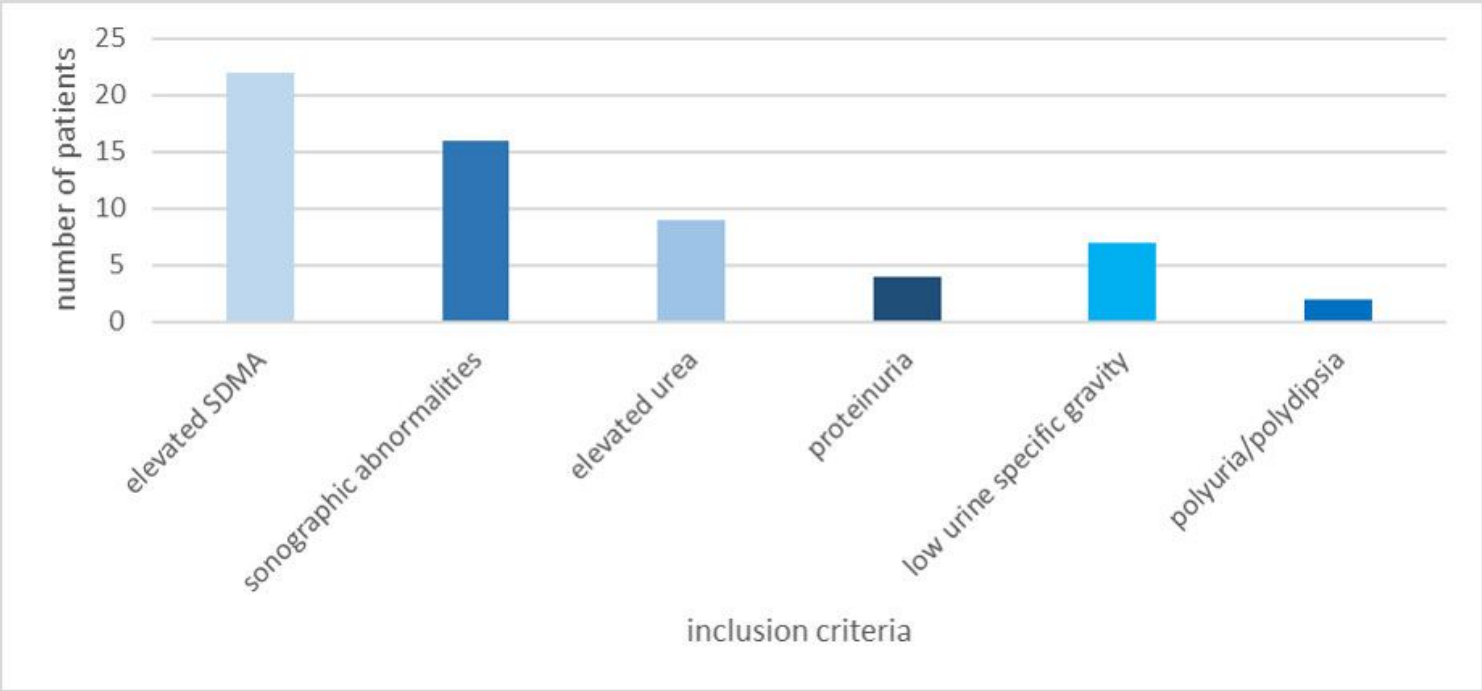

Figure 3

inclusion criteria met by patients included in the study.

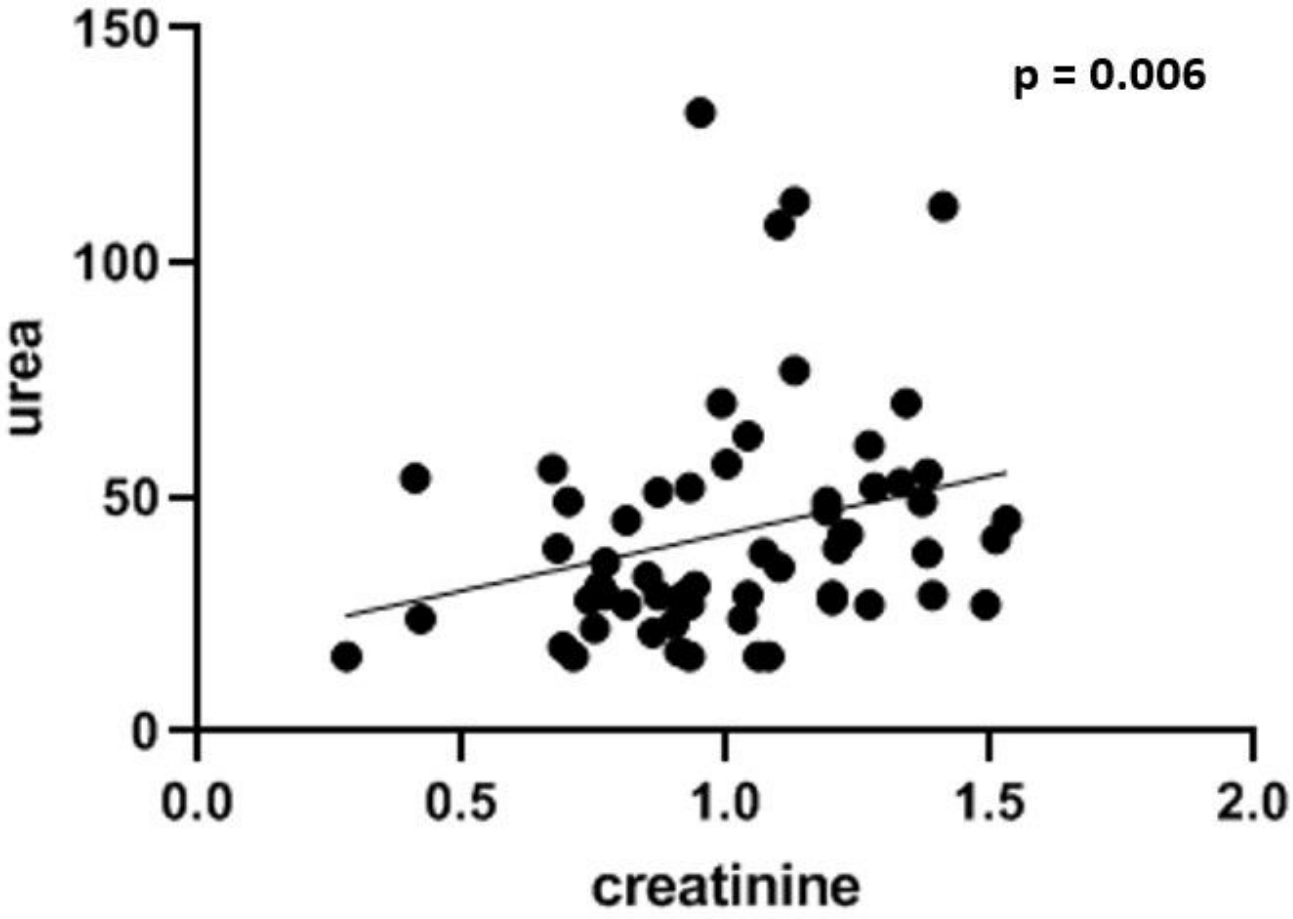

Figure 4

Scatter plot displaying the relation between measured creatinine and sonography. Most patients showed serum creatinine between 0.7 mg/dl and 1.4 mg/dl and sonographic score points between 2 and 6. Creatinine did not correlate significantly to sonographic score points (p= 0.33).

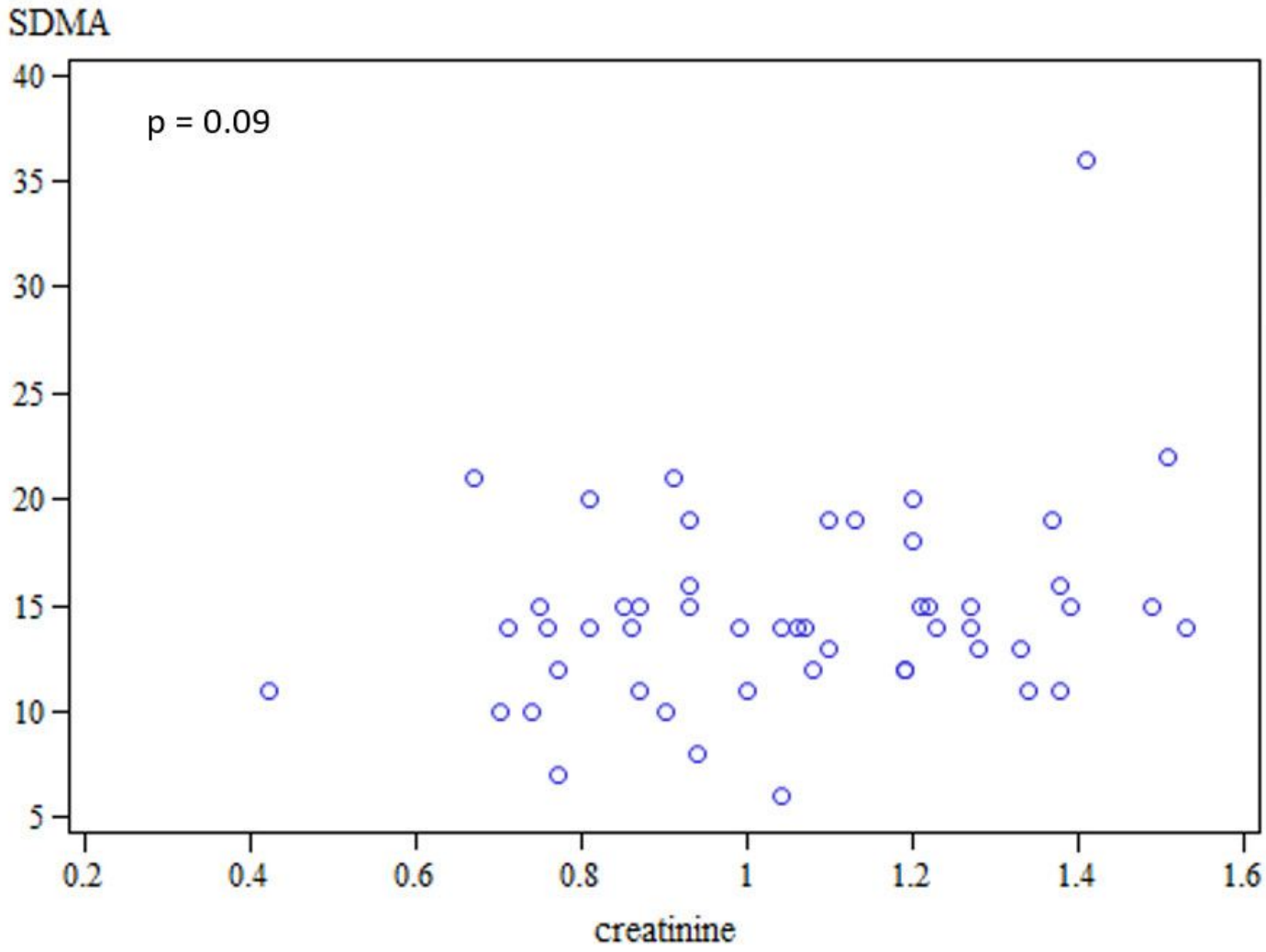

Figure 5

Scatter plot displaying the relation between measured SDMA and sonography. Most patients showed SDMA between 10 µg/dl and 20 µg/dl and sonographic score points between 2 and 6. SDMA and sonographic score points were not significantly correlated (p = 0.64).

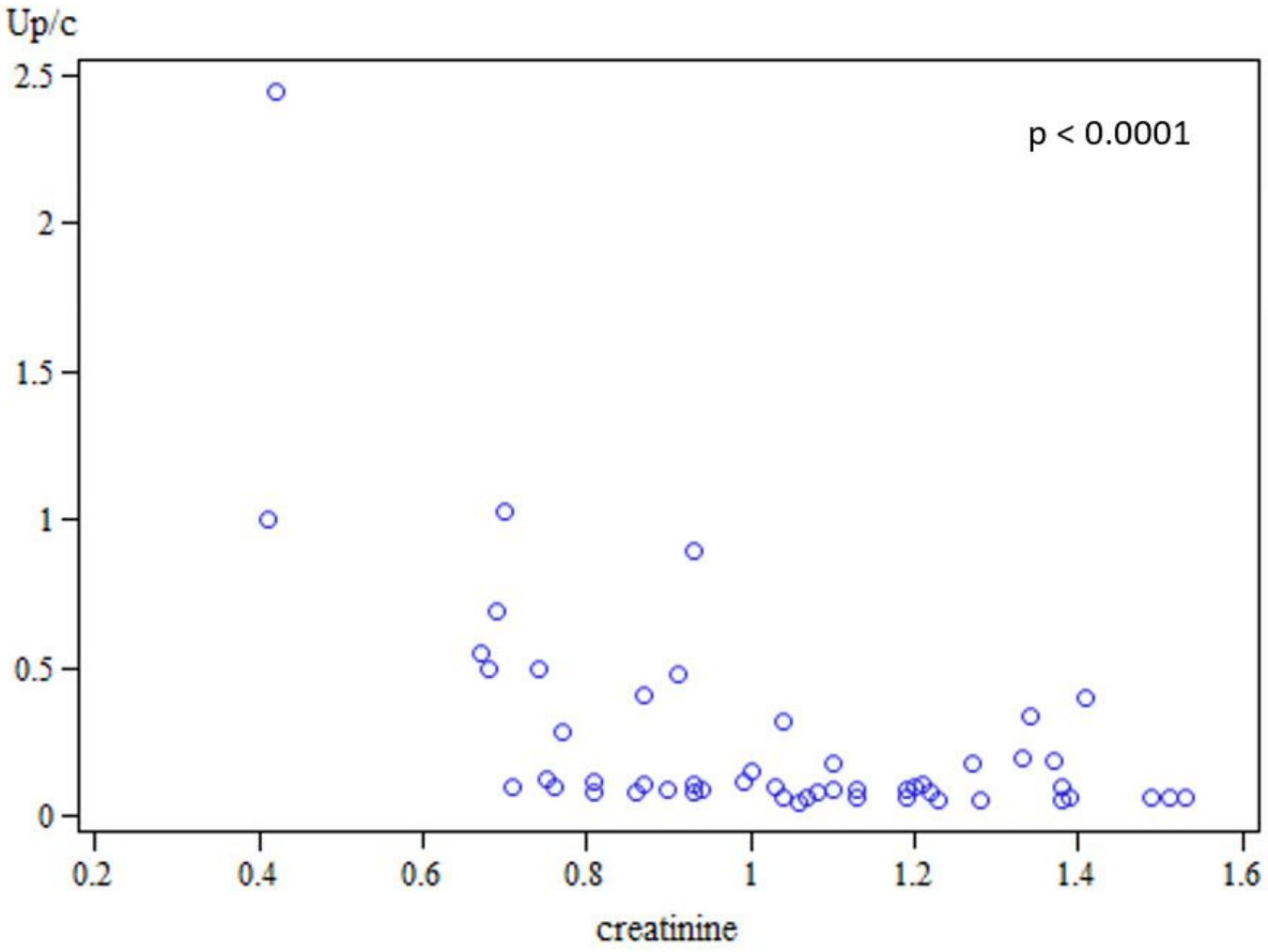

Figure 6

Scatter plot displaying the relation between measured creatinine and Up/c. Most patients showed serum creatinine between 0.7 mg/dl and 1.4 mg/dl and Up/c inside the reference range of 0.1 and 0.5. Up/c and creatinine were negatively correlated (p= <0.0001), even though most Up/c values clustered within the reference range (< 0.5).

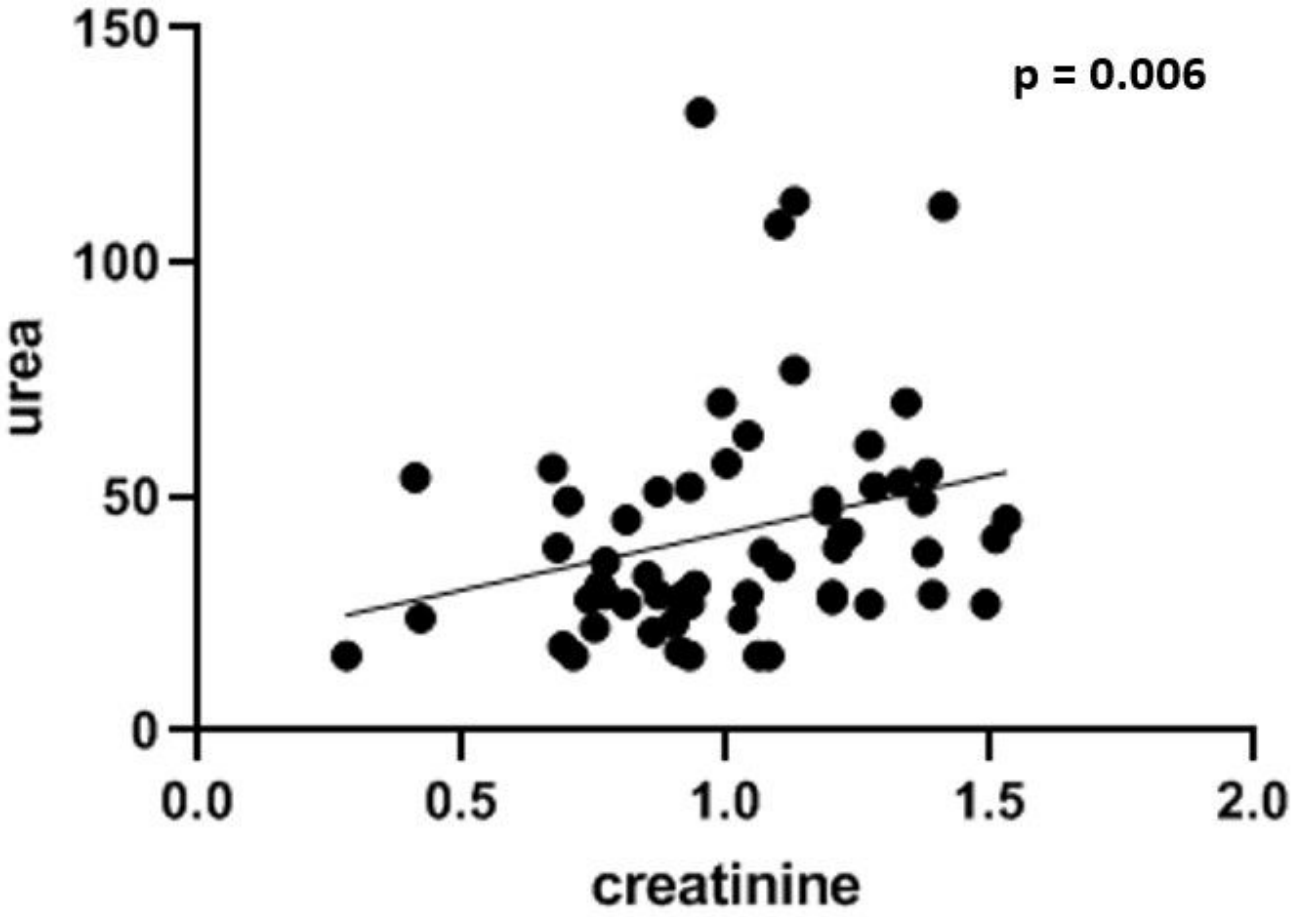

Figure 7

X-axis showing creatinine levels, Y-axis showing urea levels. Most patients showed serum creatinine between 0.7 mg/dl and 1.4 mg/dl and urea between 20 mg/dl and 60 mg/dl. Creatinine and urea were positively correlated (p = 0.006).

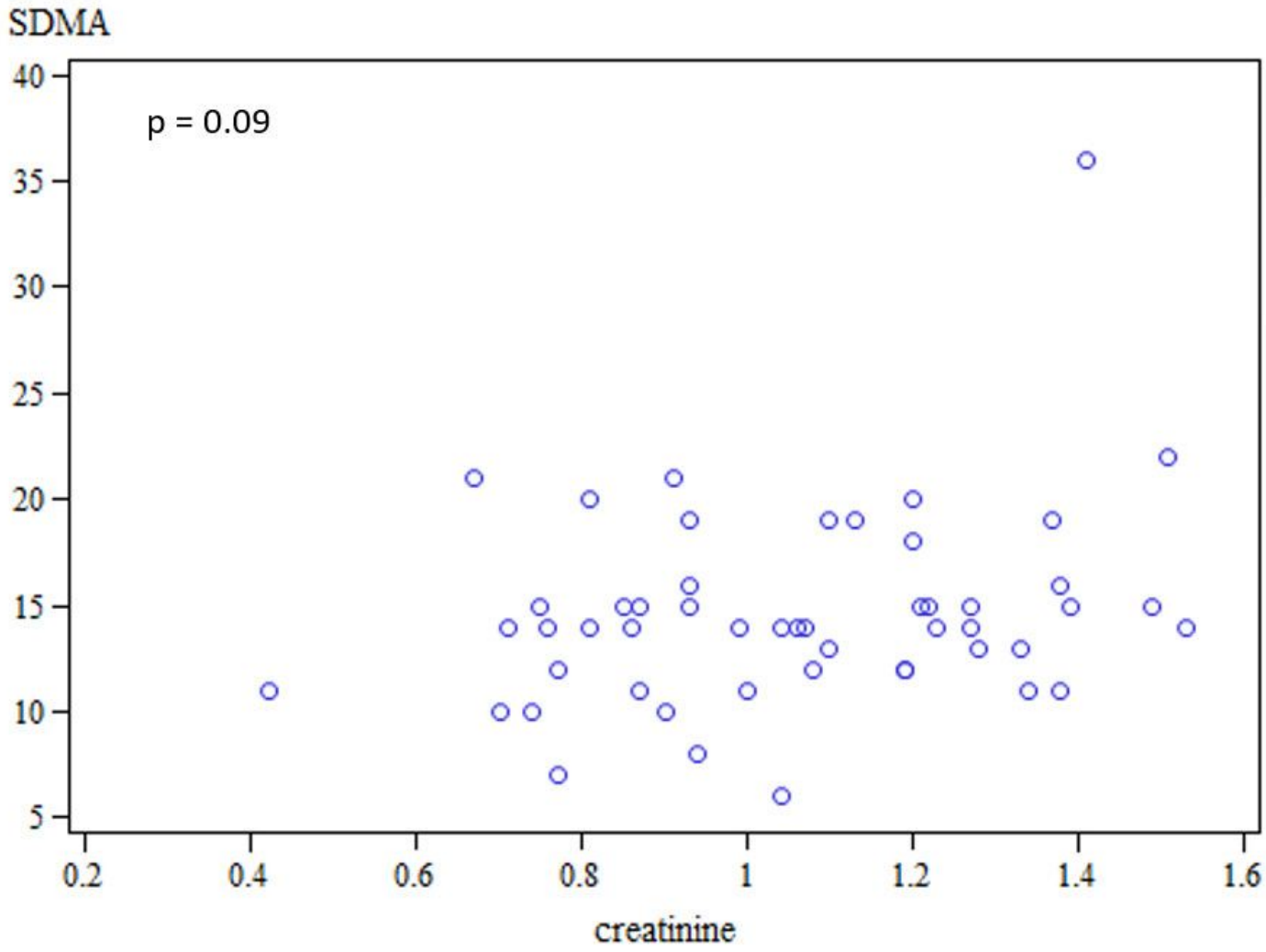

Figure 8

Scatter plot displaying the relation between measured creatinine and SDMA values. Most patients showed serum creatinine between 0.7 mg/dl and 1.4 mg/dl and SDMA between 10 µg/dl and 20 µg/dl. Creatinine and SDMA levels were not significantly correlated (p = 0.09).