Veterinary Research Communications

# Clinical utility of urine kidney injury molecule-1 (KIM-1) and gamma-glutamyl transferase (GGT) in the diagnosis of canine acute kidney injury

--Manuscript Draft--

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| **Abstract:** | The aim of the present study was to evaluate the sensitivity and specificity of urine KIM-1 and urine GGT for the detection of naturally-occurring AKI, compared to healthy control dogs, dogs with stable chronic kidney disease (CKD), and dogs with lower urinary tract disorders (LUTD). The study included AKI grade 1 (n = 21), AKI grade 2 to 5 (n = 11), stable CKD (n = 11), LUTD (n = 15), and healthy dogs (n = 37). Urine KIM-1 (ng/mg) and GGT (U/l) were normalized to urine creatinine (uCr). Statistically significant difference in KIM/uCr ($p=0.0007$) and GGT/uCr ($p<0.0001$) was found among the study groups. Area under the curve (AUC) for KIM-1/uCr and GGT/uCr as predictors of non-azotemic AKI was 0.81 and 0.91 respectively. Values of KIM-1/uCr of 0.73 ng/mg and of GGT/uCr of 54.33 showed the best combination of sensitivity and specificity (75% and 75.6%; 85.7% and 89.1% respectively). A significant positive correlation ($p<0.0001$) between KIM-1/uCr and GGT/uCr was found. Both urine KIM-1/uCr and GGT/uCr seemed to be potentially good markers for the diagnosis of non-azotemic AKI. Dogs with AKI showed significantly higher levels of urine KIM-1/uCr and urine GGT/uCr, compared with healthy dogs. Caution should be used in the evaluation of elevated urine KIM-1/uCr and GGT/uCr in dogs with pre-existing CKD and/or LUTD. Urine KIM-1/uCr and GGT/uCr might have a significant clinical utility, as complementary test, particularly in diagnosis early, non-azotemic stages of AKI. |

Pisa, 14th November 2017

Dear Editor in Chief of the Veterinary Research Communications,

We here by submit a paper entitled "**Clinical utility of urine kidney injury molecule-1 (KIM-1) and gamma-glutamyl transferase (GGT) in the diagnosis of canine acute kidney injury** " to be considered for publication in your journal.

Diagnosis of acute kidney injury (AKI) commonly bases on finding of elevated serum creatinine and urea. However, serum creatinine is not a very sensitive and specific marker of AKI, and it is more accurate to assess renal function loss, rather then kidney injury. For this reason, during the last years the attention focused on the application of new urine and serum biomarkers, which may help clinicians to early diagnose AKI and prevent further progression of the disease

In the present paper we investigated the sensitivity and specificity of urine KIM-1 and urine GGT for the detection of naturally-occurring AKI, compared to healthy control dogs, dogs with stable chronic kidney disease (CKD), and dogs with lower urinary tract disorders (LUTD).

To the Authors' knowledge, this is the first report investigating the clinical utility of urine KIM-1 and urine GGT in diagnosing AKI in in dogs.

We declare that this manuscript has not been published before and is not currently being considered for publication elsewhere.

The research activity of this paper has been conducted in agreement and by the approval of the Ethical Committee of the University of Pisa.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

The manuscript has been revised and approved by all named authors.

We hope you may find our manuscript suitable for publication and look forward to hearing from you.

Sincerely,

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# Clinical utility of urine kidney injury molecule-1 (KIM-1) and gamma-glutamyl transferase (GGT) in the diagnosis of canine acute kidney injury

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*Abstract*

The aim of the present study was to evaluate the sensitivity and specificity of urine KIM-1 and urine GGT for the detection of naturally-occurring AKI, compared to healthy control dogs, dogs with stable chronic kidney disease (CKD), and dogs with lower urinary tract disorders (LUTD). The study included AKI grade 1 (n = 21), AKI grade 2 to 5 (n = 11), stable CKD (n = 11), LUTD (n = 15), and healthy dogs (n = 37). Urine KIM-1 (ng/mg) and GGT (U/l) were normalized to urine creatinine (uCr). Statistically significant difference in KIM/uCr ($p=0.0007$) and GGT/uCr ($p<0.0001$) was found among the study groups. Area under the curve (AUC) for KIM-1/uCr and GGT/uCr as predictors of non-azotemic AKI was 0.81 and 0.91 respectively. Values of KIM-1/uCr of 0.73 ng/mg and of GGT/uCr of 54.33 showed the best combination of sensitivity and specificity (75% and 75.6%; 85.7% and 89.1% respectively). A significant positive correlation ($p<0.0001$) between KIM-1/uCr and GGT/uCr was found. Both urine KIM-1/uCr and GGT/uCr seemed to be potentially good markers for the diagnosis of non-azotemic AKI. Dogs with AKI showed significantly higher levels of urine KIM-1/uCr and urine GGT/uCr, compared with healthy dogs. Caution should be used in the evaluation of elevated urine KIM-1/uCr and GGT/uCr in dogs with pre-existing CKD and/or LUTD. Urine KIM-1/uCr and

GGT/uCr might have a significant clinical utility, as complementary test, particularly in diagnosis early, non-azotemic stages of AKI.

*Keywords*: KIM-1, GGT, AKI, dog,

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*Keywords*: KIM-1, GGT, AKI, dog,

*Introduction*

Acute kidney injury (AKI) is characterized by a sudden onset of renal injury, caused by pre-renal, intrinsic parenchymal, post-renal damage, or a combination of them. Diagnosis of AKI commonly bases on finding of elevated serum creatinine and urea. However, early stages of the disease may be undetected, when kidney function is assessed through these markers (Palm CA et al., 2016). Serum creatinine is not a very sensitive and specific marker of AKI, and it is more accurate to assess renal function loss, rather then kidney injury (Huang Y. and Wauchope A,C,D., 2011). For this reason, during the last years the attention focused on the application of new urine and serum biomarkers (Lee YJ et al, 2012; Palm CA et al, 2016; Bruchim Y et al, 2017; Nivy R et al, 2017). Early diagnosis of AKI may help clinicians to intervene timely and to prevent further progression of the disease (Yerramilli M et al., 2016).

Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein, which is primarly expressed on the surface of T cells. In normal kidneys, KIM-1 expression is low, but it increases significantly in proximal tubule cells, following kidney injury (Jin Y et al, 2017). In human AKI patients, urine KIM-1 was seen to increase by 2 hours from kidney injury, and it lasted elevated up to 48 hours after injury. KIM-1 increased significantly in human AKI patients, compared to non AKI patients, showing an excellent diagnostic performance (Huang Y and Wauchope A,C,D, 2011). Particularly, KIM-1 showed a good potential in prediction of AKI in patients within 24 hours of cardiac surgery. In these patients, a two-fold increase in urine KIM-1 at 2 hour post surgery increased the odds of developing AKI by 1.96 fold (Lianghos O et al, 2009). Although KIM-1 showed elevated in AKI patients with different aetiologies, its levels were higher in patients with acute tubular necrosis, compared to patients with contrast induce nephropathy, nephrotoxins or other causes (Huang Y and Wauchope A,C,D, 2011).

Gamma-glutamyl transferase (GGT) is a brush border enzyme, which is mainly located in the metabolically active proximal tubule. As the high molecular weight, GGT and other urinary enzymes cannot cross the glomerular barrier. Therefore, its urine level is primarily due to tubular rather then glomerular injury (Clemo FA, 1998; Cobrin AR et al, 2013). In a preliminary study in dogs, urine GGT showed relatively low discriminatory power for the diagnosis of AKI (Nivy R et al, 2017).

The aim of the present study was to evaluate the sensitivity and specificity of urine KIM-1 and urine GGT for the detection of naturally-occurring AKI, compared to healthy control dogs, dogs with stable chronic kidney disease (CKD), and dogs with lower urinary tract disorders (LUTD).

*Methods and materials*

The study was conducted at the Department of Veterinary Science of University of Pisa (Italy). Client-owned dogs were prospectively enrolled (Ethics Committee approval number 9778), and divided into four groups: (1) AKI grade 1; (2) AKI grades 2-5; (3) stable CKD dogs; and (4) dogs with LUTD. Controls included dogs presented for annual check and clinically healthy on the basis of history, physical examination and complete blood work and urinalysis. Diagnosis of LUTD based on clinical, urinary and imaging findings. CKD and AKI were diagnosed on the basis of the International Renal Interest Society (IRIS) guidelines and grading system.

Urine KIM-1 concentrations were measured in duplicate by using a commercially available ELISA kit (ab205084-Dog KIM-1 ELISA Kit, abcam®, UK). The determination of urinary GGT, generally intended for the determination of GGT in human serum or plasma, was used for the quantitative in vitro determination of γ-glutamyl transferase. A Liasys© AMS Assel spectrophotometer (for enzymatic chemical type immunoturbidimetric and colorimetric analysis) was used on refrigerated samples (+4°C) within 24 hours of collection (Mancinelli E et al, 2012).

The distribution of continuous variables was assessed using the D'agostino Pearson omnibus normality test. Based on data distribution, non-parametric tests were used. Kruskal-Wallis test (followed by Dunn's multiple comparison test) was used to compare urine KIM-1 to urinary creatinine ratio (KIM-1/uCr), and urine GGT to urinary creatinine ratio (uGGT/uCr) among the study groups. The receiver operator characteristic (ROC) analysis, with its area under the curve (AUC) and 95% confidence interval (CI), was used to assess uKIM-1/uCr and uGGT/uCr as predictors of AKI. Correlation between KIM-1/uCr and uGGT/uCr was assessed by Spearman's correlation test. For all tests, $P$ value < 0.05 was considered to be significant. Statistical analyses were performed using Graphpad prism for Mac.

*Results*

The study included 95 dogs, which were divided into AKI grade 1 (n = 21), AKI grade 2 to 5 (n = 11), stable CKD (n = 11), LUTD (n = 15), and healthy dogs (n = 37). Median age was 4 years (1-13 years)

in healthy dogs, 7 years (1-15 years) in AKI grade 1, 7 years (1-14 years) in AKI 2 to 5, 11 years (1-13 years) in CKD, and 7 years (2-13 years) in LUTD.

Median values of serum creatinine, UPC, urine GGT, KIM-1/uCr, and uGGT/uCr, and mean values of urine KIM-1 of healthy dogs, AKI-1, AKI 2 to 5, CKD and LUTD dogs are reported in table 1.

Table 1 Median values of serum creatinine, urine GGT, KIM-1/uCr, and uGGT/uCr, and mean urine KIM-1 of healthy dogs, AKI-1, AKI 2 to 5, CKD and LUTD dogs

Kruskal-Wallis test showed a statistically significant difference in urine KIM-1/uCr among the study groups. Statistical significance was of $p=0.0004$ when all grades of AKI were considered as a single group, and of $p=0.0007$ when AKI grade 1 was separated from AKI grade 2 to 5.

**Fig. 1a and 1b** Kruskal-Wallis test of median urine values of KIM-/uCr (ng/mg) among the study groups.

Kruskal-Wallis test showed a statistically significant difference ($p<0.0001$) in urine GGT/uCr (U/l) among the study groups

**Fig.2a and 2b** Kruskal-Wallis test of median urine values of urine GGT/uCr (U/g) among the study groups

Spearman test showed a statistically significant ($p<0.0001$) linear positive correlation between urine KIM-1/uCr and urine GGT/uCr

**Fig.3** Spearman's correlation test between urine KIM-1/uCr and urine GGT/uCr ($p<0.0001$; $r=0.52$)

ROC analysis for urine KIM-1/uCr and AKI, for the AKI and healthy dogs, showed an area under the curve (AUC) of 0.76 (95% confidence interval 0.64-0.88). ROC analysis for urine KIM-1/uCr and AKI, for the AKI-1 and healthy dogs, showed an area under the curve (AUC) of 0.81 (95% confidence interval 0.68-0.93).

**Fig.4a and 4b** ROC curve for urine KIM-1/uCr and AKI, for the AKI and healthy dogs

ROC analysis for urine GGT/uCr and AKI, for the AKI and healthy dogs, showed an area under the curve (AUC) of 0.87 (95% confidence interval 0.78-0.96). ROC analysis for urine GGT/uCr and AKI, for the AKI-1 and healthy dogs, showed an area under the curve (AUC) of 0.91 (95% confidence interval 0.82-0.99)

**Fig.5a and 5b** ROC curve for urine GGT/uCr and AKI, for the AKI and healthy dogs

Table 1 Median values of serum creatinine, UPC, urine GGT, KIM-1/uCr, and GGT/uCr, and mean values of urine KIM-1 of healthy dogs, AKI-1, AKI 2 to 5, CKD and LUTD dogs.

| | **Healthy** | **AKI-1** | **AKI 2-5** | **CKD** | **LUTD** |
|---|---|---|---|---|---|
| **KIM-1 (ng/ml)** | 0.71 (± 0.16) | 0.72 (± 0.29) | 0.38 (± 0.27) | 0.47 (± 0.23) | 0.66 (± 0.16) |
| **KIM-1/uCr (ng/mg)** | 0.48 (0.11-1.15) | 1.03 (0.1-2.92) | 0.79 (0.04-2.15) | 0.89 (0.46-1.72) | 0.74 (0.25-3.32) |
| **GGT (U/L)** | 42.5 (10-227) | 82 (18-615) | 30 (3-253) | 39 (10-172) | 33.5 (7-114) |
| **GGT/uCr (U/g)** | 31.5 (6.9-105.6) | 111.8 (20.9-698.9) | 78.9 (16.6-384.1) | 88.2 (12.7-260) | 32.2 (6-182.8) |
| **sCr (mg/dl)** | 0.9 (0.3-1.2) | 1 (0.5-1.3) | 5.5 (2.4-13.7) | 2.7 (1.6-6.2) | 0.7 (0.5-1.3) |
| **UPC** | 0.11 (0.02-0.2) | 0.62 (0.05-3.39) | 1.68 (0.2-1.9) | 1.44 (0.12-5.93) | 0.18 (0.05-2.83) |

*sCr* serum creatinine

Table 2. Receiver operating characteristic (ROC) analyses for KIM-1/uCr and GGT/uCr as predictors of naturally occurring AKI grade 1 in dogs.

| | **Cut off** | **Sensistivity (%)** | **CI (%)** | **Specificity (%)** | **CI (%)** | **AUC** | **CI (%)** |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **KIM-1/uCr** | 0.628<br>**0.739**<br>0.893 | 85<br>**75**<br>65 | 62.11-96.79<br>**50.90-91.34**<br>40.78-84.61 | 64.86<br>**75.68**<br>86.49 | 47.46-79.79<br>**58.82-88.23**<br>71.23-95.46 | **0.81** | **0.58-0.93** |
| **GGT/uCr** | 39.950<br>**54.330**<br>73.890 | 90.4<br>**85.7**<br>71.4 | 69.62-98.83<br>**63.66-96.95**<br>47.82-88.72 | 65.57<br>**89.19**<br>97.30 | 50.21-81.99<br>**74.58-96.97**<br>85.84-99.93 | **0.91** | **0.82-0.99** |

*Discussion*

In our study, both urine KIM-1/uCr and GGT/uCr seemed to be potentially good markers for the diagnosis of AKI. Dogs with AKI showed significantly higher levels of urine KIM-1/uCr and urine GGT/uCr, compared with healthy dogs. KIM-1/uCr showed elevated in both AKI group (grade 1 to 5) and stable CKD. However, when AKI grade 1 dogs were analysed as an individual group, no significant difference in KIM-1/uCr was found between healthy dogs and AKI 2 to 5. In this case, urine levels of KIM-1/uCr were significantly higher in AKI-1 compared with healthy dogs, while they did not differ significantly among healthy dogs and the other study groups. Urine KIM-1/uCr seemed to elevate in early, non-azotemic AKI, rather than in more advanced grades of AKI. This finding was in agreement with previously found in human medicine (Liangos O et al, 2009), where urine KIM-1 increased very quickly, by 2 hours from kidney injury, and lasted elevated up to 48 hours. The increase in KIM-1 did not match the increase in serum creatinine, which started to rise between 12 and 24 hours from injury (Liangos O et al, 2009). The discrepancy between the rise in urine KIM-1 levels and serum creatinine might explain the higher levels of urine KIM-1/uCr, which we found in non-azotemic (AKI-1), compared with azotemic AKI dogs (AKI 2-5). In our study, KIM-1/uCr showed an accurate predicting marker of AKI. ROC analysis of urine KIM-1/uCr as a predictor of AKI showed an AUC of 0.76 (95% confidence interval between 0.64 and 0.88; Figure 4a). When non-azotemic AKI dogs were considered as an individual group, ROC analysis showed an AUC of 0.81 (95% confidence interval between 0.68 and 0.93; Figure 4b). A cut off point for KIM-1/uCr of 0.73 ng/mg was considered the best combination of sensitivity (75%) and specificity (75.6%). This finding seemed to reflect what found in human medicine, where KIM-1 showed an accurate predictor of AKI within 24 hours from

renal injury, with an AUC between 0.78 and 0.91 (Liangos O et al, 2009). Similarly to our results, the predicting ability of KIM-1 reduced over time, with an AUC between 0.52 and 0.84 within 72 hours from injury (Liangos O et al, 2009). The relatively lower urine levels of KIM-1 in azotemic AKI dogs, compared with non-azotemic AKI dogs might also reflect a tubular enzyme depletion with progression of tubular damage and time, as previously reported in a murine model of AKI (Malyusz M and Braun D, 1981). Different elevation in urine KIM-1/uCr in AKI dogs might also be influenced by the kind of tubular damage. In human patients, urine KIM-1 levels were higher in acute tubular necrosis, than in contrast induced nephropathy or nephrotoxins (Huang Y and Wauchope ACD, 2011). Unfortunately, no histopathology was available in our study for dogs of the AKI group.

In our study urine GGT/uCr levels were significantly higher in AKI dogs compared with healthy dogs, both in case AKI-1 was considered as an individual group, than as part of AKI. AKI-1 dogs showed median urine levels of GGT/uCr significantly higher than healthy dogs and LUTD. Although urine GGT/uCr has been shown to increase in dogs with experimentally induced AKI (Rivers BJ et al, 1996), Nivy R and Colleagues reported an unsatisfactory predicting power of GGT/uCr for diagnosing AKI in dogs with naturally acquired AKI (Nivy R et al, 2017). In the study of Nivy R and Colleagues, the ROC analysis for urine GGT/uCr as a marker of AKI showed an AUC of 0.65. In our study ROC analysis of urine GGT/uCr as a predictor of AKI showed an AUC of 0.87 (95% confidence interval between 0.78 and 0.96; Figure 5a). The accuracy of urine GGT/uCr in predicting AKI showed excellent when AKI-1 dogs were analysed as an individual group. In this case ROC analysis showed an AUC of 0.91 (95% confidence interval between 0.82 and 0.99; Figure 5b). A cut off point for GGT/uCr of 54.3 U/l was considered the best combination of sensitivity (85.7%) and specificity (89.1%).

Both urine KIM/uCr and GGT/uCr showed a poor ability to discriminate between AKI and CKD. Urine KIM-1/uCr and GGT/uCr were elevated in both AKI and CKD group. The power to discriminate did not increase, when AKI-1 dogs were analysed as an individual group. This finding seems to be in agreement with the study of Nivy R and Colleagues, in which a significant inter-group overlapping in GGT/uCr was found (Nivy R et al, 2017). It is also possible that the overlapping in urine GGT/uCr between AKI and CKD patients may be secondary to proteinuria. The finding of proteinuria, particularly of tubular origin, has been associated with an elevation in urine levels of GGT/uCr in dogs affected by Leishmania Infantum (Ibba F et al, 2016). Although no urine electrophoresis was available in our study, it is plausible that proteinuric CKD dogs might experience increase in urine GGT/uCr.

This finding may represent a significant limitation in the ability of urine GGT/uCr to discriminate between stable CKD and active injury in CKD (AKI on CKD). Proteinuric CKD dogs may show elevated urine GGT/uCr, despite a condition of stable CKD. Similar results have been found for urine KIM-1 in human medicine, where elevations in KIM-1 have been associated with albuminuria. In human CKD patients, urine KIM-1 resulted elevated. The increase in urine KIM-1 during CKD has been considered as the result of local hypoxia and nephrotoxic effects of mediators of kidney injury. In the same patients, urine KIM-1 tended to reduce with the progression of CKD, probably as a consequence of a lower production, due to diminished kidney tubular mass (Waikar SS et al, 2016).

Although a moderate overlapping was present for urine KIM-1/uCR between AKI and LUTD group, no significant overlapping was found for urine GGT. Despite clinical signs of LUTD, such as pyuria and haematuria have been reported to interfere with urinary GGT measurement (Clemo FA, 1998), our results showed a good ability of urine GGT/uCr to discriminate between AKI and LUTD.

The present study has a number of limitations. First of all, the aetiology of AKI was not always known and histopathology was not performed in none of the AKI dogs. As a consequence, it was not possible to interpreter urine KIM-1/uCr levels according to different kinds of renal injury. The second limitation is the lack of a short term and long term follow up for the majority of these patients. Therefore no evaluation regarding the potential prognostic role of urine KIM-1/uCr and GGT/uCr was performed. The third limitation was represented by the inclusion of stable CKD patients only. It would be interesting to include also CKD patients with active AKI and end-stage renal disease.

In conclusion, urine KIM-1/uCr and GGT/uCr showed respectively a moderately good to excellent performance in diagnosing AKI in canine patients. Both markers were relatively easy to measure and rapidly available for the clinician, although the disadvantage of urine GGT to be measured on fresh urine sample. Urine KIM-1/uCr and GGT/uCr may be easily assessed as a bed-side test, especially in hospitalized dogs at risk of developing AKI. However, the measurement of these markers cannot replace clinical and laboratory parameters in the diagnosis of AKI. Caution should be used in the evaluation of elevated urine KIM-1/uCr and GGT/uCr in dogs with pre-existing CKD and/or LUTD. Urine KIM-1/uCr and GGT/uCr might have a significant clinical utility, as complementary test, particularly in diagnosing early, non-azotemic stages of AKI.

*Conflict of interest*

The Authors declare no conflict of interest. This paper was not supported by grants.

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