Course name

Submitted to:

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Contents

Novel Biomarkers Enhance Early Detection of Renal Disease in Dogs

Clinical Question

In dogs presenting with renal disease symptoms, do the novel biomarkers, compared to conventional markers, offer superior diagnostic accuracy and early disease detection, potentially leading to improved treatment outcomes?

Search Strategy

Search Engine(s): PubMed, Google Scholar

Search Date: The search was conducted in September 2023.

Language Restrictions: Articles in English were included due to the availability of relevant literature in this language.

Cutoff Dates for Literature Inclusion: Articles published from January 2013 to August 2023 were considered.

Search Terms: The following search terms and Boolean operators were used:

1. ("Canine renal disease" OR "dog kidney disease" OR "canine nephropathy")
2. ("Novel biomarkers" OR "new biomarkers" OR "emerging biomarkers")
3. ("Diagnostic utility" OR "diagnostic accuracy" OR "early detection")
4. ("Conventional markers" OR "traditional markers")
5. ("Treatment outcomes" OR "patient management")

Focus and Exclusion Techniques

The search initially included all relevant studies without specific exclusions. However, not peer-reviewed studies (e.g., conference abstracts, in vitro studies) and those not directly addressing the diagnostic utility of novel biomarkers in canine renal disease were excluded.

Expansion Techniques

The bibliographies of the papers were combed through to find any more research that had been missed. This helped make sure that all of the necessary literature was covered.

Number and Types of Studies Identified

A total of 10 studies were identified after the initial search and exclusion of irrelevant articles. These studies include peer-reviewed research articles, systematic reviews, and meta-analyses, all contributing to synthesising evidence regarding the diagnostic utility of novel biomarkers in canine renal disease.

Main Results

A systematic analysis was conducted, analysing 10 studies that looked into biomarkers related to renal health in dogs to improve our ability to diagnose kidney injuries and disorders in canines. This analysis aimed to determine how relevant the studies' findings would be in clinical practice by analysing their methodological quality and other relevant factors.

The quality of the evidence and the methods used in the studies were the first factors considered. Review papers, observational studies, and prospective studies were all present in the publications. Reviews summarised current information without primary research data, whereas observational and prospective studies supplied direct clinical evidence, enhancing the strength of the evidence.

The second element, the representativeness of study participants, played a pivotal role in assessing the external validity of the findings. Some studies used specific breeds, such as Beagle dogs, raising questions about generalizability to other breeds. In contrast, studies focusing on particular conditions, like X-linked hereditary nephropathy (XLHN), had more limited applicability.

The third element emphasised inclusion/exclusion criteria. Well-defined criteria ensured that study participants were suitable for the research question. For example, the study involving dogs with chronic kidney disease (CKD) required stable CKD cases to reduce variation and maintain study integrity.

Group definitions and selection, the fourth element, determined the clarity and appropriateness of group definitions. Studies, such as the one involving Amphotericin B (AmpB) injury in Beagle dogs, established specific groups, enabling clear comparisons. Similarly, the XLHN study defined groups of dogs with XLHN and controls.

The fifth element, outcome measures and validity, focused on choosing outcome measures. Validated biomarkers, like symmetric dimethylarginine (SDMA) and urinary clusterin, bolstered the reliability of findings. In the CKD study, urinary neutrophil gelatinase-associated lipocalin (uNGAL) and kidney injury molecule-1 (uKIM-1) were assessed, and their coefficients of variation were calculated, enhancing the assessment of their stability as biomarkers.

Ensuring the equal scrutiny of study groups, the sixth element, was vital for maintaining internal validity. For example, the AmpB study concentrated on the sensitivity of urinary clusterin to AmpB-induced injury while concurrently examining other biomarkers.

The seventh element, consideration of confounders, involved accounting for potential confounding factors. The study of Canine leishmaniasis (CanL) considered confounders when assessing urinary biomarkers in dogs at different disease stages.

The eighth element, statistical power, emphasised the necessity of adequate sample sizes to detect significant differences. Some studies, such as the CKD observational study, had relatively small sample sizes, potentially limiting their ability to detect subtle effects.

Evaluating the consistency with conclusions, the ninth element, ensured that the authors' conclusions aligned with the presented data. In studies like CKD and CKD-CanL, authors concluded that certain biomarkers held promise for early detection, supported by their findings.

Lastly, the clinical relevance of findings, the tenth element, considered the practical implications of observed differences. For instance, in the AmpB study, urinary clusterin's high sensitivity held clinical relevance for early detection of kidney injury in dogs exposed to AmpB.

Table 1: Literature Review Findings

Study Design	Participants	Intervention	Key Results	Limitations
Review	Not specified	Acute kidney injury (AKI) in dogs: a review of urine biomarkers as diagnostic tools.	• Serum creatinine (also known as SCr) and the blood urea nitrogen (also known as BUN) lack the sensitivity and the specificity for timely kidney dysfunction diagnosis. • Urinary biomarkers, especially in urine, may be useful for studying nephropathies, including AKI. • Standardisation of biomarker assays is needed for better validation.	Not based on primary research, lacks specific intervention details, standardisation issues (De Loor *et al*., 2013)
Prospective Study	Dogs with XLHN and controls	Proteomic analysis of dog urine for the biomarkers of timely tubulointerstitial	• Urinary RBP is a promising biomarker for early detection of tubulointerstitial damage and progression to end-	Small sample size, specific to XLHN, proteomic techniques, and lack of

		damage in chronic kidney disease	stage renal disease in dogs with XLHN. • Several known and promising new biomarkers were identified. • Clinical data and histologic evaluation supported the findings.	intervention details (Nabity *et al.*, 2013)
Review	Not Specified	CKD and AKI linked; SDMA better early diagnosis.	• Chronic renal disease and kidney damage are linked, increasing the likelihood of the other. • For accurate and sensitive diagnoses, it is necessary to use species- and organ-specific biomarkers. • Combining diagnostics assessing kidney function and active injury enhances patient management and outcomes. • Symmetric dimethylarginine (SDMA) is an earlier and more reliable CKD marker than creatinine. • Kidney-specific clusterin, inosine and cystatin B are effective biomarkers for active kidney damage dogs.	Species-specific renal biomarkers, SDMA for CKD, Diagnostic challenges, Ongoing assay development. (Yerramilli *et al.*, 2016)
Review	Nonclinical safety studies (duration >3 months)	Symmetrical dimethyl arginine, cystatin C, and dickkopf homolog 3 are three novel biomarkers.	• It may be possible to anticipate modifications to GFR in people because novel biomarkers indicate chronic renal damage in animals and serve as	Review, no specific study design or participants (Obert et al., 2021)

			substitutes for GFR.	
Observational Study	Dogs with and without renal diseases	L-FABP in the urine, relative to the creatinine level (uCre)	• Increases in serum creatinine, urine specific gravity, urea nitrogen, and the urine protein/creatinine ratio were all linked with increased urinary L-FABP in dogs with renal illness.	Limited to dogs, single-species study (Takashima *et al.*, 2021)
Observational Study	Dogs with CKD and healthy controls	Serum uromodulin concentration	• Serum uromodulin concentrations were lower in the CKD group, negatively correlated with conventional renal markers, and had higher AUC than SDMA for CKD diagnosis.	Limited to dogs, no specific treatment interventions (Seo *et al.*, 2022).
Review	Not specified	Biomarkers for nephrotoxicity and kidney failure	• Discusses the need for sensitive and specific biomarkers for the early diagnosis and the prognosis of the AKI and nephrotoxicity	Review, no specific study design or participants (Kavitha *et al*., 2022)
Observational Study	Dogs suffering from Canine leishmaniasis (CanL) at various stages and healthy dogs	Urinary cystatin C (uCysC), urine specific gravity (USG), N-acetyl-beta-D-glucosaminidase (uNAG), urine protein to creatinine ratio (UPC)	• uCysCc and uNAGc significantly increased starting from group LI compared to healthy controls. • Cutoff values for uNAGc (2.25 IU/g) and uCysCc (258.85 µg/g) were identified for LI dogs.	Observational study, limited to dogs, no specific treatment interventions (Ruiz *et al.*, 2023)
Prospective, observational study	Twenty-five dogs having stable chronic kidney disease (also known as	kidney injury molecule-1 (uKIM-1) and Urinary neutrophil gelatinase-associated	• Median coefficient of variation (CV) for uNGAL: 42% (ranging from 7% to 127%) • Median coefficient of variation for uKIM-	- Small sample size (25 dogs) - Limited to stable CKD cases - Short-term study period (14 days)

	CKD)	lipocalin (uNGAL)	1: 29% (range: 16%-91%) CV 90th percentiles: uNGAL - 97%, uKIM-1 - 56% • Normalisation of urinary creatinine (uCr) was also assessed	- No comparison to healthy controls - No discussion of clinical implications (Chen *et al.*, 2023).
Prospective Study	Beagle dogs with AmpB injury	Identification of AmpB-induced kidney injury: evaluation of urine biomarkers.	• Amphotericin B (AmpB) causes kidney damage in dogs, and urinary clusterin strongly indicates this. • Other biomarkers analysed were less sensitive than the blood urea nitrogen (BUN) and the serum creatinine. • Tubular alterations in AmpB-related injury confirmed urinary clusterin's sensitivity.	Small sample size, specific to AmpB-induced injury, limited literature on utility in dogs (Adedeji *et al.*, 2023).

In summary, the strength and quality of evidence across the ten reviewed articles varied due to differences in study design, sample size, and specificity to certain conditions. Nonetheless, these studies collectively underscored the potential of urinary biomarkers, including SDMA, clusterin, and cystatin B, to enhance the early diagnosis and monitoring of kidney injuries and diseases in dogs. Despite limitations, such as small sample sizes and specificity, these findings provided valuable insights for veterinary practitioners. Further research is needed to develop and broaden these diagnostic techniques to improve clinical treatment and results in veterinary nephrology.

Comments

Several factors, such as research design, generalizability, sample size, and clinical relevance, must be considered when assessing the quality and strength of the evidence from the 10 papers on renal biomarkers in dogs. Different articles add to our overall knowledge of kidney biomarkers and how they are utilised in veterinary medicine in unique ways.

Yerramilli *et al.* (2016) reviewed the problems with using blood tests to diagnose kidney disease in dogs and explored the advantages of using urine tests instead. The evidence quality is moderate since it doesn't depend on primary research and lacks specifics about the interventions.

Adedeji *et al.* (2023) prospective research on urine biomarkers for early diagnosis of renal impairment in Beagle dogs undergoing Amphotericin B (AmpB) damage is very helpful. However, its small number of samples and sensitivity to AmpB-induced damage hinders the evidence's generalizability to larger clinical situations.

De Loor *et al*. (2013) review highlights the promise of urine biomarkers for researching nephropathies in dogs and the limits of established renal biomarkers. It has a moderate level of evidence, however, due to methodological flaws and issues with standardisation.

Nabity *et al*. (2013) conducted a prospective research that sheds light on the early diagnosis of kidney impairment by analysing urine biomarkers of dogs having X-linked hereditary nephropathy (XLHN). Nevertheless, the small sample size, specificity to XLHN, and reliance on proteomic techniques affect its generalizability and clinical applicability.

A review by Obert *et al*. (2021) discusses the need for sensitive and specific biomarkers in diagnosing kidney injury and nephrotoxicity, highlighting the importance of species-specific tests. Lacking details about the study's methodology and subjects, the evidence is of limited quality.

Urinary liver-type fat acid-binding protein (L-FABP) is being studied as a potential biomarker for canine renal disease in an observational study by Takashima *et al.* (2021). While this study provides useful information, it is limited to dogs and cannot be applied to other species.

Seo *et al*. (2022) conducted an observational study on the plasma uromodulin levels of dogs with CKD. Due to its small sample size and lack of standardised treatment protocols, this study provides only moderate-quality data.

A review by Kavitha *et al.* (2022) discusses the need for sensitive and specific biomarkers to diagnose acute kidney injury (AKI) and nephrotoxicity. However, it lacks a specific study design or participant information, resulting in moderate evidence quality.

The inter- and intra-individual variance of urine biomarkers in dogs having persistent chronic kidney disease, also called CK, is evaluated in a prospective observational research by Chen et al. (2023). While it provides valuable insights into the variation of these biomarkers, it is limited by a small sample size, specificity to CKD cases, and a short study period. The absence

of a healthy control group and a discussion of the clinical implications of variation impacts its overall evidence quality.

In an observational study by Ruiz *et al*. (2023), urinary biomarkers for renal damage in dogs with Canine leishmaniasis (CanL) are explored. While it identifies potential early renal damage biomarkers, it is limited to dogs and specific to CanL, and no specific treatment interventions were applied.

Overall Assessment

The evidence presented across these ten articles varies in quality and strength. None of the studies can be considered excellent in quality, as they all have limitations, such as small sample sizes, specificity to certain conditions or interventions, and a lack of healthy control groups. Additionally, some articles are review papers, which inherently rely on existing literature rather than primary research.

The strong point of evidence ranges from moderate to weak, with the most robust evidence coming from the prospective studies (Nabity *et al.*, 2013; Adeyemi *et al*., 2023; Ruiz *et al*., 2023). These studies offer valuable data on specific biomarkers and their applicability in certain clinical scenarios. However, their limited sample sizes and specificity reduce their generalizability.

The clinical relevance of these studies is evident in their exploration of novel biomarkers for kidney injury in dogs, addressing the need for early diagnosis and prognosis. However, to translate these findings into practical clinical applications, further research with larger and more diverse populations of dogs is necessary.

In summary, while these studies provide valuable insights into renal biomarkers, their limitations impact their overall quality and strength of evidence. Nevertheless, they contribute to our understanding of potential biomarkers for kidney damage in dogs and underscore the importance of future research in this area to improve clinical practice in veterinary medicine.

Clinical bottom line

- Current evidence suggests that urinary biomarkers can improve dogs' early diagnosis and monitoring of kidney injuries and diseases.
- Among the biomarkers evaluated, symmetric dimethylarginine (SDMA) emerges as a favourable early marker intended for chronic kidney disease (CKD) in both cats and dogs, allowing for earlier interventions.

- Despite limitations in study designs and sample sizes, urinary biomarkers like urinary clusterin, cystatin B, and inosine have shown effectiveness in diagnosing active kidney injury, particularly in specific clinical contexts.
- Future research must focus on larger and more diverse populations of dogs, including healthy controls, to improve the generalizability of findings and clinical applicability.
- Species-specific diagnostic tests for veterinary patients are essential to ensure accurate measurements and clinical utility.
- Veterinary practitioners should consider incorporating these biomarkers, particularly SDMA, into their diagnostic toolkit for more comprehensive patient management and improved outcomes in cases of kidney diseases and injuries.
- Continued research in this field is necessary to refine and expand our understanding of renal biomarkers in dogs and their specific applications in different clinical scenarios.

Conclusion

This systematic analysis of 10 research on renal biomarkers for dogs has contributed significantly to our understanding of how to detect and track kidney injuries and disorders in dogs at an early stage. Urinary biomarkers show promise in improving diagnosis accuracy for canine renal diseases, however the amount and quality of evidence differed between studies due to variances in sample size, design, and specificity. For both cats and dogs, symmetric dimethylarginine (SDMA) has emerged as a promising biomarker for early diagnosis of chronic kidney disease (CKD), highlighting the need of intervening as soon as possible. These findings provide helpful insight to veterinary practitioners and highlight the need to conduct further research to strengthen diagnostic techniques and enhance outcomes for patients in veterinary nephrology, despite certain limitations, such as small sample sizes and accuracy to particular clinical contexts. More research is needed with a wider range of participants, including healthy controls, to improve the generalizability for findings and their therapeutic application. To guarantee precise measurements and clinical value, it is crucial to provide species-specific diagnostic tests for veterinary patients. Veterinarians are strongly advised to think about adding urine biomarkers, and specifically SDMA, to their diagnostic toolkits, as this might lead to more thorough patient treatment and improved outcomes for animals suffering from kidney disorders and accidents. We need more studies like this one to learn more about renal biomarkers for dogs and how they might be used in different clinical settings to improve veterinary practice.

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