

## CORRELATION BETWEEN QUALITATIVE PROTEINURIA ASSESSED WITH SDS-AGE AND RENAL HISTOPATHOLOGIC FINDINGS IN DOGS

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### Introduction

The qualitative evaluation of urinary proteins assumes a central role in early diagnosis of glomerular pathologies. Sodium Dodecyl Sulphate-Agarose Gel Electrophoresis (SDS-AGE) permits the visualization of urinary proteins having molecular masses from 9 kDa ( $\beta_2$  microglobulin) to 900 kDa ( $\alpha_2$  macroglobulin). The method consists in the separation of single proteic fractions in agarose gel according to mass, independently of charge; interference on the migration of smaller proteins by larger proteins is eliminated.

In the absence of histopathologic lesions in dogs, qualitative evaluation of proteinuria does not permit the identification of individual protein bands nor does it permit the exclusive identification of selective glomerular proteinuria characterized by the sole presence of albumin (Fig. 1) (1).

The identification of non-selective glomerular proteinuria (albumin bands and concomitant presence of one or more bands having molecular masses greater than 69 kDa) in inert urine sediment is theoretically due to the presence of glomerular lesions (Fig. 1). Following the assessment of laboratory tests that may be indicative of glomerular histopathological lesions, the diagnostic methodology involves identification of the underlying lesion. For renal analysis, conventional histological examination is the method that provides the greatest sensitivity and specificity.

### Materials and Methods

Forty-nine dogs were subjected to echo-assisted cystocentesis in which 10 ml samples of urine were collected. All urine samples were subjected to chemical/physical examination within two hours after collection. Urine samples destined for quantitative and qualitative determination of proteinuria were conserved in sterile containers at 4° to 8° C after the addition of 1  $\mu$ l of 1% sodium azide per milliliter of urine.

Quantitative and qualitative assays of proteinuria by colorimetric methods using pyrogallol and by SDS-AGE, respectively, were performed within 7 days.

All the examinations were performed at a single laboratory. Qualitative evaluation of proteinuria by SDS-AGE is based on the electrophoretic separation of urinary proteins by mass, following a pretreatment that gives all proteins a negative charge proportional to their mass.

The limit of sensitivity for each individual fraction is 15 mg/L. The following proteins can be identified:  $\beta_2$  microglobulin, lysozyme, retinol binding protein,  $\kappa$  and  $\lambda$  light chains,  $\alpha_1$  microprotein, dimers of light chains, albumin, transferrin, IgG, IgA, haptoglobin, and  $\alpha_2$  macroglobulin. All dogs were subjected to ultrasound assisted renal biopsies for the identification of primary histopathological lesions.

All dogs were also subjected to preliminary hematological exams (complete blood count and hemocoagulative profile), abdominal ultrasound examination, and determination of systolic pressure by Doppler analysis (Ultrasonic Doppler Flow Detector Model 811-BTS; Parks Medical Electronics, Inc. Aloha, Oregon U.S.A.).

The following alterations in coagulation were considered as incompatible with biopsy: PT greater than 12 sec, aPTT greater than 19 sec, fibrinogen less than 100 mg/dl, inadequate platelet counts and/or bleeding time (1,2,3).

Hypertension was not considered incompatible with renal biopsy as long as the hemocoagulative profile was unaltered with normal bleeding times and adequate platelet counts. The eventual administration of aspirin or anti-inflammatory steroids was suspended at least 5 days prior to biopsy (3). Renal biopsies were carried out under ultrasonographic guidance; the animals were sedated after fasting for 12 hours, and placed in dorsal recumbency. The abdominal areas were sterilized according to surgical normatives.

In all cases biopsies were performed with a semi-automatic tru-cut apparatus (Temno Biopsy Device T 189, 18 Gauge x 9 cm needle). Samples were fixed in buffered formalin (10% with respect to sample volume/formalin volume). Each sample was stained with eosin-hematoxylin, PAS, Goldner's trichrome, methenamine, and upon request of the pathologist, with Congo Red.

Biopsies were considered adequate having at least five glomeruli per section, with the exception of positivity with Congo Red, where individuation of a single glomerulus was considered adequate for correct diagnosis.

Tubulointerstitial lesions were subdivided in five groups according to histopathologic results: (0) absence of lesions, (+) focal lymphoplasmacytic infiltration, (++) focal lymphoplasmacytic infiltration and corresponding tubular ectasia or atrophy, (+++) focal lymphoplasmacytic infiltration and corresponding tubular ectasia or atrophy and focal fibrosis, (++++) vascular lesions and/or acute tubular necrosis and/or lesion observed in (++) or (+++) but with diffuse pattern. Validity of SDS-AGE analysis as a diagnostic test was assessed using specificity and sensitivity in accordance to Barber and Elliot, 1998 (4).

## **Results**

Histopathology disclosed glomerular diseases in 8 dogs, tubulointerstitial lesions in 5 dogs and mixed disorders in 36 dogs (Tab. 1). Considering the overall forty-nine dogs, the sensitivity of SDS-AGE in detecting glomerular and tubulointerstitial involvement was 100% and 92.7%, with a specificity of 40.0% and 62.5%, respectively. The validity of SDS-AGE was also calculated for mixed and isolated lesions. Sensitivity for diagnosis of mixed lesions was 91.7%, with a specificity of 53.8%, while sensitivity for isolated glomerular and tubulointerstitial diseases was 62.5% and 40.0%, with a specificity of 92.7% and 100%, respectively.

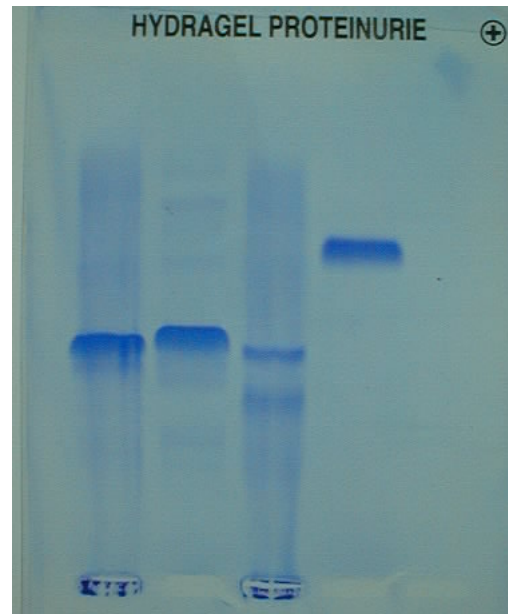
## **Discussion**

Our results confirm the finding of other Authors (5) regarding the high sensibility and specificity of the biopsy technique approaching renal pathology in dogs. The SDS-AGE analysis appears to be an useful tool highly sensitive in the early detection of renal tissue damage of glomerular, tubulointerstitial, or mixed origin, subsequently validated by histologic examination. The low sensitivity observed leads us to further investigate the noninvasive SDS-AGE method as a screening procedure in patients wherein an organ lesion is suspected. The electrophoretic method applied in nephrology along with echo-assisted biopsy actually represents the technique of choice for identification of renal pathologies.

Further studies are in progress to evaluate the specificity and sensitivity of SDS-AGE in the recognition of tubulointerstitial disorders in order to exclude from statistical analysis light chain monomers as they may be present in urine of patients with systemic diseases not necessary involving the renal tubulointerstitial compartment (i.e. multiple mieloma).

## References

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**Fig. 1** Sodium Dodecyl Sulfate-Agar Gel Electrophoresis. Selective glomerular proteinuria characterized by the sole presence of Albumin (1<sup>st</sup> and 2<sup>nd</sup> to the left) and non selective glomerular proteinuria (3<sup>rd</sup> to the right).

