

The urine aldosterone to creatinine ratio (UAldo:C) determined by enzyme-linked immunosorbent assay (ELISA) in healthy dogs and dogs with myxomatous mitral valve disease

Galizzi A.1, Bagardi M.1, Savarese A.1, Locatelli C.1, Zanaboni A.2, Borromeo V.1, Brambilla P.G1

1 Department of Veterinary Medicine, University of Milan, Italy;

2 Department of Computer Science, University of Milan, Italy

The urinary aldosterone to creatinine ratio (UAldo:C) from a single urine sample is comparable to 24h urinary aldosterone excretion in dogs, and it represents a meaningful index of general renin-angiotensin-aldosterone system (RAAS) activation. Periodic evaluation of the UAldo:C could help optimize the therapeutic approach to the progression of heart disease and identify the best time to initiate the mineralocorticoid receptor blockers therapy. To the authors' knowledge, urinary aldosterone (UAldo) has always been measured with a radioimmunoassay (RIA) method in dogs, and literature reports that the UAldo:C rarely exceeds 1 $\mu\text{g/g}$ in healthy dogs. However, the RIA method couldn't be used in any clinical scenario. The aim of this study was firstly to evaluate the performances in dogs of a species-independent ELISA kit available for measurement of UAldo and, secondly, to determine the UAldo:C in healthy dogs and dogs affected only by myxomatous mitral valve disease (MMVD) in ACVIM stage B1. All urine samples were collected by spontaneous micturition. Seventy-four dogs were included in this prospective study, 29 healthy (11 males and 18 females; median age 5 IQR 4-8 years; median weight 15 IQR 5.65-25 kg) and 45 affected by MMVD stage B1 (21 males and 24 females; mean age 9.64 ± 3.47 years; median weight 8.7 IQR 5.85-14.75 kg). The ELISA kit was found to be sensitive (linearity: from 3.9 pg/mL to 250 pg/mL; $R^2=0.98$), accurate (parallelism test; average recovery: 84%) and precise (intra-assay coefficient of variation 12.2%-17.5%) for the determination of canine UAldo after acid hydrolysis. The median (IQR) of UAldo:C was 2.65 $\mu\text{g/gr}$ (0.89-4.28) in healthy dogs and 2.16 $\mu\text{g/gr}$ (1.01-4.24) in dogs with B1 MMVD. The median UAldo:C value was not significantly different between healthy and B1 dogs ($p=0.922$). As a result of statistical analysis on all included patients, UAldo:C showed a moderate negative correlation with age (Pearson's coefficient $\rho=-0.418$) and a moderate positive correlation with Urine Specific Gravity ($\rho=0.418$). The UAldo:C values obtained in this study were higher than those reported in literature; thus, could be related to differences in the assay (ELISA vs RIA) and/or included population. Moreover, MMVD in stage B1 doesn't appear to be correlated with an increase in aldosterone levels. The determination of UAldo with ELISA technique could be an advantage for research and clinical practice, as it is quicker, cheaper, doesn't require specialized

technical figures or radiation protection measures and then, unlike the RIA, can be performed by most laboratories.