

# MiR-146a as a potential biomarker for left ventricular remodelling in dogs with myxomatous mitral valve disease - a comparison with plasma NT-proBNP

Ana Reis-Ferreira<sup>1,2</sup>, José Portugal<sup>3</sup>, Joana Santos-Gomes<sup>4</sup>, Carmen Brás-Silva<sup>4</sup>, Ana Patrícia Fontes-Sousa<sup>2,5,6</sup>, Luís Lobo<sup>1,7,8</sup>

<sup>1</sup>Porto Veterinary Hospital, Porto, Portugal.

<sup>2</sup>Abel Salazar Institute of Biomedical Sciences of the University of Porto (ICBAS-UP), Porto, Portugal.

<sup>3</sup>Faculty of Veterinary Medicine, Lusófona University, Lisbon University Center, Lisbon, Portugal.

<sup>4</sup>UnIC@RISE, Department of Surgery and Physiology, Faculty of Medicine, University of Porto, Porto, Portugal.

<sup>5</sup>Department of Immuno-Physiology and Pharmacology, Center for Pharmacological Research and Drug Innovation (MedInUP); Porto, Portugal.

<sup>6</sup>University of Porto Veterinary Hospital (UPVET), Porto, Portugal.

<sup>7</sup>Research in Veterinary Medicine (I-MVET), Faculty of Veterinary Medicine, Lusófona University, Lisbon University Centre, Portugal.

<sup>8</sup>Center for Animal Science Studies, University of Porto, Porto, Portugal.

**Objectives:** Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease in dogs. Elevated levels of natriuretic peptide are observed in MMVD and secondary heart failure, but new biomarkers like circulating microRNAs (e.g., miR-146a), could further enhance our understanding on its biomolecular mechanisms. This study aimed to (1) assess potential associations between plasma miR-146a and echocardiographic variables of left cardiac remodelling (left atrial to aorta diameter, LA/Ao; left ventricular internal diameter in diastole normalized to body weight, LVIDdN), and (2) to compare plasma miR-146a concentrations with plasma NT-proBNP concentrations in canine MMVD.

**Material and Methods:** Thirty dogs of both genders were enrolled in this study and categorized into groups based on ACVIM MMVD staging (control,  $n=7$ ; B1,  $n=7$ ; B2,  $n=8$ ; or C,  $n=8$ ). Total RNA was extracted from plasma samples, followed by reverse transcription and qRT-PCR to quantify relative levels of miR-146a. Plasma samples were also analysed for NT-proBNP levels using the CardioPet NT-proBNP assay at the regional IDEXX reference laboratory.

**Results:** Both biomarkers correlated with ACVIM stages, but neither has demonstrated the ability to distinguish between different stages. MiR-146a was found to correlate with the LA/Ao ratio but not with LVIDdN. In contrast, NT-proBNP correlated with both LVIDdN and the LA/Ao ratio. Statistical tests indicated that while both biomarkers correlate with the LA/Ao ratio, NT-proBNP exhibited a stronger correlation, as evidenced by its lower  $p$  value compared to miR-146a.

**Conclusion:** These preliminary results suggest that miR-146a plays a role in left cardiac remodelling in canine MMVD. Further validation is essential to confirm its diagnostic potential in dogs with cardiac remodelling caused by MMVD.

**Keywords:** Myxomatous mitral valve disease, Biomarkers, NT-proBNP, MiR-146a.

**Funding:** This work received financial support from the Portuguese Foundation for Science and Technology (UIDB/04308/2020, UID/IC/00051/2013, and Project IMPAcT - PTDC/MED - FSL/31719/2017 (COMPETE2020, Portugal 2020,

FEDER)) and from a scholarship for the integrated master's in veterinary medicine at Lusófona University.