Cardiopulmonary and inflammatory biomarkers in the assessment of the severity of canine dirofilariosis

E. Carretón [a],[*], R. Morchón [b], F. Simón [b], M.C. Juste [a], J.C. Méndez [a], J.A. Montoya-Alonso [a]

[a] Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, Las Palmas, Spain
[b] Laboratory of Parasitology, Faculty of Pharmacy and Institute of Biomedical Research of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain

ARTICLE INFO

ABSTRACT

The prognosis and success of adulticide treatment depends on the baseline severity of infection with *Dirofilaria immitis* in dogs and can influence the therapeutic protocol to choose. A study was conducted to assess the utility of the cardiopulmonary biomarkers N-terminal of the prohormone brain natriuretic peptide (NT-proBNP), cardiac troponin I (cTnI), myoglobin, and D-dimer, as well as the biomarker of inflammation C-reactive protein (CRP) to evaluate the severity of canine heartworm disease in different stages. Serum samples were collected from 20 heartworm-infected dogs for measurement of these biomarkers. Dogs were assigned to four groups ($n = 5$/group) according to the severity of the disease: Class I and Class II dogs showed concentrations of biomarkers generally within normal ranges for healthy dogs, except CRP in dogs classified as Class II, which showed values slightly above laboratory reference values (19.46 ± 5.69 mg/l). Dogs of Class III showed elevated levels of NT-proBNP (1220.12 ± 465.18 pmol/l) ($p < 0.05$), cTnI (0.99 ± 0.39 ng/ml), CRP (33.98 ± 7.99 mg/l) ($p < 0.05$), and 40% of these dogs (2/5) presented pathological values of D-dimer and myoglobin ($p < 0.05$). Dogs of Class IV (caval syndrome) presented elevated levels of NT-proBNP (>2530.8 pmol/l), cTnI (1.99 ± 0.32 ng/ml), and CRP (80.24 ± 47.69 mg/l); all Class IV dogs showed pathological elevations of D-dimer (0.81 ± 0.46 ng/ml), and 60% (3/5) of these dogs showed pathological elevations of myoglobin (significant elevations [$p < 0.05$] for all biomarkers with respect to reference values and values for dogs in Classes I, II, and III). In canine heartworm disease, chronic presence of *D. immitis* causes a proliferative endoarteritis, thromboembolisms, pulmonary hypertension, and right-sided congestive heart failure. As the severity of the disease worsens, serum values of cTnI, myoglobin, and NT-proBNP increase, indicating significant cardiac damage. The finding of pathological concentrations of D-dimer suggests the presence of thromboembolism and/or disseminated intravascular coagulation. CRP increases according to the severity of the disease, indicating inflammatory processes that could contribute to the progression of the disease. These preliminary results demonstrate the utility of cardiopulmonary and inflammation biomarkers to assist in the establishment of the severity of canine heartworm disease.

© 2014 Elsevier B.V. All rights reserved.

* Correspondence to: Internal Medicine, Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, 35413 Arucas, Las Palmas, Spain. Tel.: +34 636024279; fax: +34 928451114.

E-mail address: ecarreton@becarios.ulpgc.es (E. Carretón).

1. Introduction

Heartworm disease is caused by the filarial nematode *Dirofilaria immitis*, which resides in pulmonary arteries and the right side of the heart of the infected animals, being a potentially life-threatening disease (Simón et al., 2012). The prognosis and success of adulticide treatment depends on the baseline severity of the infected animal and can influence the therapeutic protocol to choose.

A biomarker is defined as a biological parameter that can be objectively measured and quantified as an indicator of pathogenic processes or response to a therapeutic intervention (Atkinson et al., 2001). The use of the cardiorespiratory biomarkers is relatively recent; over the past 50 years, their contribution to the management of cardiac diseases has become increasingly sophisticated, and currently, they are routinely used in the diagnosis, prognosis, and monitoring of cardiorespiratory diseases in humans (Dolci and Panteghini, 2006). In veterinary medicine, these biomarkers have been shown to be useful tools in the diagnosis and evaluation of several cardiorespiratory pathologies (Boswood, 2009).

C-reactive protein (CRP) is an acute phase protein, synthesized by the liver in response to tissue damage by inflammation (from infection) or trauma. In veterinary medicine, several researchers have identified serum CRP as a useful acute phase marker in dogs, and its utility has been evaluated in several inflammatory pathologies (Cerón et al., 2005; Eckersall and Bell, 2010).

Previous studies have demonstrated the utility of the biomarkers cardiac troponin I (cTnI) and myoglobin as markers for cardiac damage, and D-dimer as a marker supportive for thromboembolism in dogs with heartworm disease (Carretón et al., 2011, 2012, 2013a), so our next aim was to establish its usefulness to correctly determine the clinical class of a canine patient based on the severity of the infection.

Therefore, the objective of this study was to assess the utility of the cardiopulmonary biomarkers NT-proBNP, cTnI, myoglobin, and D-dimer, as well as the biomarker of inflammation CRP to evaluate the severity of the disease in the different stages of canine heartworm disease.

2. Materials and methods

2.1. Study animals

Serum samples from 20 heartworm-infected dogs were used in this study. All were client-owned dogs brought to the Veterinary Medicine Service of Las Palmas de Gran Canary University. All owners gave their consent for their dogs to participate in this study. Heartworm infections were diagnosed using a commercial ELISA test kit to detect circulating antigens (Uranotest Dirofilaria®, Urano Vet SL, Barcelona, Spain) following manufacturer instructions. The dogs were further evaluated for the presence of microfilariae using a modified Knott test.

Dogs were divided into four groups (n = 5 per group) based on the severity of the disease, according to classifications described by Di Sacco and Vezzoni (1992). To determine the appropriate classification for each dog, physical examination, radiographic, and echocardiographic findings were evaluated.

Briefly, dogs included in Class I had no abnormal physical examination, radiographic, or echocardiographic findings. Class II included animals with mild clinical signs, such as an occasional cough with or without exercise intolerance. Thoracic radiographs showed mild pulmonary artery enlargement with mixed alveolar-interstitial lesions. Class III dogs were those presented with persistent coughing, severe exercise intolerance, weight loss, cachexia, and often right-sided congestive heart failure. Thoracic radiographs showed severe pulmonary artery enlargement, right ventricular enlargement, and diffuse pulmonary infiltrates. Class IV dogs included animals with caval syndrome. They were presented with ascites, anorexia, dyspnea, tachypnea, tachycardia, pale mucous membranes, faint pulse, jugular distension, weakness, and often collapse. Laboratory tests for Class IV dogs showed anemia, hemoglobinemia, and hepatic and renal dysfunction in some cases. Echocardiographic examination showed worms in the right atrium and right ventricle, and worms were also often visualized in the venae cavae.

2.2. Laboratory evaluations

Blood samples were drawn from the cephalic vein of each animal for measurements of concentrations of cTnI, myoglobin, and D-dimer as previously described by Carretón et al. (2011). Reference ranges of cTnI, myoglobin, and D-dimer for healthy dogs were established as 0.71 ± 0.25 ng/ml, <74 ng/ml, and <0.2 μg/ml, respectively (Burgener et al., 2006; Bauer and Moritz, 2009; Carretón et al., 2013b).

Values of high-sensitivity CRP were measured by latex turbidimetric method on a chemistry autoanalyzer Clima Plus® RA133000 (RAL S.A., Barcelona, Spain) using goat anti-human CRP antibodies (Gernon®, Germany). Reference ranges for CRP in our laboratory for healthy dogs were established at <16 mg/l. NT-proBNP values in the study dogs were determined by a contracted laboratory (Cardiopet proBNP Test-Canine, IDEXX Laboratories, Barcelona, Spain). Reference ranges for healthy dogs established by the laboratory were <900 pmol/l.

2.3. Statistical analysis

For data statistical analysis, the IBM® SPSS® Statistics (version 19) was used throughout. Descriptive analyses of the variables were carried out using tests of proportions for qualitative variables; measurements of central tendency (mean) and dispersion (standard deviation; SD) were used for analysis of quantitative variables. For continuous variables, the Kolmogorov–Smirnov test was used to verify the variables which were normally distributed. Comparisons of absolute means between groups were made with Student's t test for normally distributed variables. Bivariate analyses of proportionality of distribution of categorical variables were estimated using the χ^2 test. A p value <0.05 determined statistical significance.