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Elevated angiotensin-converting enzyme 2 (ACE2) expression in cats with hypertrophic cardiomyopathy

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https://doi.org/10.1016/j.rvsc.2022.09.024
Received 10 June 2022; Received in revised form 12 September 2022; Accepted 22 September 2022
Available online 27 September 2022

ABSTRACT

Angiotensin-converting enzyme 2 (ACE2) is an enzyme within the renin-angiotensin-aldosterone system that plays a role in regulating blood pressure. However, it is also a cellular receptor for infection with SARS coronaviruses. Although most cats develop subclinical or mild disease following infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) acquired from human patients, a previous study has suggested hypertrophic cardiomyopathy (HCM) is a potential risk factor for the development of severe disease in the cat. Herein we investigate the ACE2 protein expression in the lung, heart, and kidney from a small subset of cats with (n = 10) and without HCM (n = 10) by immunohistochemistry. The abundance and intensity of ACE2 expression is slightly elevated in alveoli (p = 0.09; 0.07, respectively) and bronchioles (p = 0.095; 0.37, respectively). However, statistically elevated abundance and intensity of ACE-2 expression was only evident in the heart of cats with HCM (p = 0.032; p = 0.011, respectively). Further investigation did not demonstrate a statistical correlation between the ACE2 expression in the heart in relation to the heart weight to body weight ratio, and the ventricular wall ratio. Current findings suggest an overexpression of ACE2 in HCM cases but follow up study is warranted to understand the pathophysiological process.

1. Introduction

The causative agent for Coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in a sustained pandemic since the end of 2019. Throughout the period, numerous spill over events from humans to cats have been documented (Adler et al., 2022; Barroso-Arévalo et al., 2022; Barrs et al., 2020; Jairak et al., 2021; Schulz et al., 2021; van der Leij et al., 2021) and in some rare circumstances from other infected animals to cats or among cats (van Aart et al., 2021). Most of these cases do not present clinical signs or only display with mild upper respiratory clinical signs (Barroso-Arévalo et al., 2022; Hosie et al., 2021; Klaus et al., 2021). Generally, the susceptibility of cats to SARS-CoV-2 infection is thought to be related to the expression of cognate viral receptor ACE2 in the upper respiratory tract (Färbler et al., 2022; Gerhards et al., 2021; Krüger et al., 2021; Lean et al., 2021). Similar observations have also been made from in vivo modelling of SARS-CoV-2 infection in cats (Bosco-Lauth et al., 2020; Gaudreault et al., 2020).

Feline hypertrophic cardiomyopathy (HCM), a disease condition commonly reported in cats and characterised by increased thickness of the left ventricular wall and/or interventricular septum, has been reported as a comorbidity in some cats found to have SARS-CoV-2 infection (Carpenter et al., 2021; Carvallo et al., 2021; Segales et al., 2020). In several cases, respiratory distress has been reported and was attributed to congestive heart failure from underlying HCM and not related to SARS-CoV-2 infection (Barrs et al., 2020; Carpenter et al., 2021; Rotstein et al., 2022; Segales et al., 2020). However, there is one case which has demonstrated viral infection in situ and association with viral-induced pneumonia and myocardial degeneration (Carvallo et al., 2021). Current in vivo studies have only utilised young and healthy cats (Bosco-Lauth et al., 2020; Gaudreault et al., 2020). Therefore, the role of underlying cardiac comorbidity contributing to severe COVID outcome in cats is not well understood.

Apart from the respiratory tract, ACE2 is also expressed in the heart and kidney of mammalian species (Hikmet et al., 2020; Lean et al., 2021) and plays an endocrinological function within the renin-
angiotensin-aldosterone system (RAAS) (Bekassy et al., 2021). In humans, underlying cardiovascular disease is associated with poor disease outcome during COVID-19, with evidence suggesting a relationship with an elevated ACE2 expression in the heart (Matsushita et al., 2020; Vukusic et al., 2022). Currently, there is a lack of knowledge regarding the expression of ACE2 in the lung, heart, and kidney in clinically healthy or HCM cats. Here we present the first association between HCM and ACE2 expression in the lung and heart, in the absence of SARS-CoV-2 infection.

2. Materials and methods

2.1. Case materials

Formalin-fixed and paraffin-embedded (FFPE) feline tissues were selected from the pathology archive of the Department of Pathobiology & Population Sciences of the Royal Veterinary College. Hypertrophic cardiomyopathy cases were defined as cats with cardiomegaly (total heart weight > 20 g and/or > 1% body weight) and with cardiac ventricular wall ratio (right ventricle: interventricular septum: left ventricle) of > 1:3:3 at necropsy, and with compatible histopathological findings of myofiber disarray and/or degeneration and fibrous replacement. Cats with normal heart and absence of clinical cardiac issues were selected as non-HCM control animals.

2.2. Immunohistochemistry

ACE2 immunolabelling was performed as described previously (Lean et al., 2021). Briefly, this involved antigen retrieval of FFPE sections in pH 6 buffer, immunolabelling with rabbit polyclonal antibody against ACE2 (Abcam) and Envision flex (Dako) and visualisation with DAB chromogen.

Semiquantitative and qualitative assessment were performed by analysing 5 high-power fields at 200× magnification per tissue from each case by veterinary pathologists on a conventional light microscope. The level of protein expression was determined through the abundance and intensity of immunolabelling. The abundance of ACE2 immunolabelling was scored as 0 (no positive staining), 1 (0 to < 25% positive cells), 2 (≥ 25% to < 50% positive cells), 3 (≥ 50% to < 75% positive cells) and 4 (≥ 75% to 100% positive cells) through estimation of the number of positive cells. The immunolabelling intensity was recorded as 0 (negative), 1 (weak, faint brown), 2 (moderate, brown), 3 (strong, dark brown and saturated). Slides were blind reviewed by two veterinary pathologists, followed by case discussion to achieve consensus results including standardisation of relative intensity.

Bronchiole and alveoli were assessed separately, with the bronchiolar epithelium evaluated for bronchiole, whereas the alveoli included type 1 and 2 pneumocytes and septal vessels. Left and right cardiac ventricular walls were evaluated. In the kidney, the cortical renal

Fig. 1. ACE2 immunohistochemical analysis of the cat lung, heart, and kidneys from hypertrophic cardiomyopathy (HCM) and non-HCM cases. (a) Total score of abundance and intensity of ACE2 immunolabelling, expressed as sum of bronchiole, alveoli, heart, and kidney scores for each animal, were compared between the two groups. (b, c) Semi-quantitative and qualitative assessment were made for the abundance or intensity of ACE2, respectively. Score as follow: abundance - 0 (no positive staining), 1 (0 to < 25% positive cells), 2 (≥ 25% to < 50%), 3 (≥ 50% to < 75%) and 4 (≥ 75% to 100%); intensity 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Ten cases were reviewed for both HCM and non-HCM. Height of bars (a, b, c) represent the mean value and the error bars represent standard deviation. Mann-Whitney test. *p < 0.05.
tubules were assessed.

2.3. Statistical analysis

Data were analysed and graphs generated using GraphPad Prism 8. Two-tailed Mann-Whitney test was used for comparing distribution of populations and the Spearman test for assessing correlation between cardiac parameters.

3. Results

HCM histology cases were derived from cats aged between 2.8 and 13.8 years old, and between 1 month old to 16 years old of non-HCM cats. Generally, there was increased abundance (\( p = 0.090 \)) and intensity (\( p = 0.011 \)) of ACE2 immunolabelling in HCM cats compared to non-HCM cats when scores were assessed at the host level (defined as sum of scores of bronchiole, alveoli, heart and kidney) (Fig. 1a). All kidney samples showed similar levels of ACE2 in the renal tubular epithelium and did not differ between HCM and non-HCM cases. Heart tissue from both groups expressed ACE2. However, the abundance (\( p = 0.032 \)) and intensity (\( p = 0.011 \)) of ACE2 immunolabelling in the heart were statistically greater in the HCM than that from non-HCM cats (Fig. 1b and c). The immunolabelling morphology was suggestive of endothelium of the capillary and medium-sized arteriole (Fig. 2a and b) and occasionally within tunica media of arteriole. Cardiomyocyte labelling was however not detected in either group.

In the lung, ACE2 expression in healthy cats was limited to type I (Fig. 2c; 5 out 10 cats, 50%) and rarely in type II pneumocytes (1 out of 10 cats, 10%). In addition, there was infrequent immunolabelling of the bronchial submucosal glandular cells (5 out of 10 cats, 50%) and absent in bronchiolar epithelium. In contrast, the intensity (\( p = 0.07 \)) and abundance (\( p = 0.09 \)) of ACE2 immunolabelling was greater within the alveoli of the HCM group (Fig. 1b and c), commonly in type I pneumocytes (Fig. 2d; 10 out of 10 cats, 100%) and infrequently in type II pneumocytes (3 out of 10 cats, 30%) and in alveolar septa with morphology suggestive of capillaries (2 out of 10 cats, 20%). Immunolabelling in the bronchioles was limited to the mucosal epithelium and often weak (\( p = 0.37 \)) and relatively infrequent (\( p = 0.095 \)). Mild to moderate immunopositive labelling levels were also detected within the medium-sized arteries of lung sections from HCM cats (9 out of 10 cats, 90%). This was, however, less frequent and with weaker immunolabelling in the non-HCM lung (7 out of 10 cats, 70%).

Further analysis of the ACE2 expression in conjunction with cardiac parameters showed weak correlation between heart to body weight ratio with ACE2 abundance (rs = 0.15, \( p = 0.53 \)) or intensity (rs = 0.25, \( p = 0.29 \)), and between ventricular wall ratio with ACE2 abundance (rs =
over the outcome of COVID-19. Nevertheless, further investigation of ACE2 profiles in a wider cat population, as well as incorporation of other molecular techniques for protein (eg. western blot) and genomic (eg. quantitative polymerase chain reaction) detection on fresh tissues, will be needed to better understand the risk factor associated with ACE2-mediated viral infection.

**Ethical statement**

No ethical approval was required as tissue blocks were derived from histology archives of the Dept Pathobiology & Population Sciences of the Royal Veterinary College.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflict of interest**

Authors declare no conflict of interest.

**Acknowledgements**

This work was supported by the European Joint Programme One Health EJP COVIR project funded under the European Union’s Horizon 2020 Research and Innovation Programme https://onehealthjp.eu/jicp-covir/ [Grant Number 773830]. The authors would like to thank the pathology scientist at APHA for their laboratory work and support and Dr. Colín Birch from the Department of Epidemiological Sciences for biostatistical consultation.

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