Prevalence of Selected Cardiotropic Pathogens in the Myocardium of Adult Dogs with Unexplained Myocardial and Rhythm Disorders

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Current state of knowledge on etiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases

Update on Myocarditis

ACUTE/SUBACUTE MYOCARDITIS
- RHYTHM DISTURBANCES:
  - Atrioventricular and intraventricular block
  - Ventricular arrhythmias
  - Frequent Ventricular ectopic beats
  - Monomorphic ventricular tachycardia
  - Supraventricular arrhythmias
  - Atrial fibrillation
  - Focal atrial tachycardia
  - Atrial flutter
- DCM PHENOTYPE (ACUTE ONSET)
- SUDDEN DEATH

High Prevalence of Viral Genomes and Multiple Viral Infections in the Myocardium of Adults With “Dilatopathy” Left Ventricular Dysfunction

ARVC in Boxer

DCM in Boxer
CHRONIC MYOCARDITIS

- RHYTHM DISTURBANCES
  - Atrioventricular and intraventricular block
  - Ventricular arrhythmias
  - Supraventricular arrhythmias

- DCM PHENOTYPE
  - Non familiar
  - Post-PM implantation

Elevations of Cardiac Tropon I Associated With Myocarditis
Experimental and Clinical Correlates
Stacy C. Smith, MD; Jack H. Ladenson, PhD; Jay M. Mason, MD; Allan S. Jaffe, MD

Sensitivity 34% - Specificity 89% - Positive Predictive 82%

Techniques for Right and Left Ventricular Endomyocardial Biopsy
Diagnostic Yield – 70-89%

Myocarditis in Dogs

<table>
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<tr>
<th>RNA VIRUS</th>
<th>DNA VIRUS</th>
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<tr>
<th>BACTERIA</th>
<th>PROTOZOA</th>
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<td>Bartonella spp (1990, others)</td>
<td>Babesia C. (1994, others)</td>
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Virus serology in patients with suspected myocarditis severity or efficacy?
Ark P. M., Blauvelt R. M., Stenmark P. O., Hennig M., Perneger T., Rolinski J., and Sigurdsson H.

Sensitivity 9% – Specificity 77%
Polymerase chain reaction analysis for viruses in paraffin-embedded myocardium from dogs with dilated cardiomyopathy or myocarditis

Adenovirus type 1

7/11 Dilated cardiomyopathy

4/11 Ventricular arrhythmias

6/11 + Borrelia B.

1/11 + Staphylococcus A.

6/11 SCD

Bartonella-associated inflammatory cardiomyopathy in a dog

PCR 16S-23S intergenic sequences POSITIVE Bartonella spp.

7/25 – DCM, 2/25 – ARVC

5 dog with DCM had PCR positive for cardiotrophic viruses:

- Parvovirus, Herpes Virus and Canine Coronavirus

- No complication noted

Conventional and quantitative PCR and (CPV2)

30% of young dogs with myocardial necrosis 5% in control dogs

47% myocarditis cases – 53% control group
Prevalence of selected cardiotoxic pathogens in the myocardium of adult dogs with unexplained myocardial and rhythm disorders or with congenital heart disease

Roberta A. Svasti, et al.

AIM OF THE STUDY
- To describe the feasibility of EMB in dogs
- To investigate a possible role of myocarditis in case of:
  - Non familial dilated cardiomyopathy (DCM) phenotypes,
  - New onset
  - High-grade AVBs,
  - Supraventricular arrhythmias
  - Ventricular arrhythmias.

MATERIAL AND METHODS
- 43 dogs of different breeds,
- Gender:
  - 30 males (5 in CHD group) and 17 females (5 in CHD group)
- Median age:
  - 4 years (range 0.5 - 11 years) – UMRD group
  - 2.5 years (range 0.7 - 6 years) – CHD group
- Median body weight:
  - 32 Kg (range 11.7 - 64.0 Kg) – UMRD group
  - 29.7 Kg (range 15.0 - 51.0 Kg) – CHD group

Histopathology
- Light microscopy
  - Paraffin-embedded myocardial and blood samples were processed by routine methods, embedded in paraffin wax, and sectioned at a thickness of 5 μm.
  - Twenty-four serial sections were prepared for each EMB sample; 6 were evaluated histologically stained with H&E stain for morphological evaluation (slides 1, 6, 11, 16, 21, and 24).
  - 6 were evaluated histologically stained with Masson trichrome stain to detect collagen deposition.
  - Each EMB sample was assessed by a single individual (MT) for evidence of myocyte hypertrophy, sarcoplasm vacuolization, fibrosis, and lymphocytic inflammation.

Immunohistochemical analysis
- 12 were used for immunohistochemical analysis by use of the standard avidin-biotin-peroxidase complex method.
  - Sections were incubated for 60 minutes with the primary antibody:
    - Polyclonal rabbit anti-human CD3 antibody [1:50 dilution] to detect T lymphocytes
    - Monoclonal mouse anti-human CD79 antibody [clone JCB117; 1:25 dilution] to detect B lymphocytes

Morphometric analysis
- Sections stained with H&E stain were evaluated to quantify the extent of lymphocytic inflammation (calculated as the number of lymphocytes per mm²).
- Commercial photo-editing software was used.

Amplified Genomic Sequence for PCR
- To preserve nucleic acids myocardial and blood stored at -80°C in RNA later solution
- Nucleic acids extraction
  - Phenol-chloroform technique (Qiagen, Courtaulds) adapted to small sample size
- ATPase used to assess integrity of RNA extracted
- GAPDH used to assess integrity of DNA extracted
Amplified Genomic Sequence for PCR

Proposed Classification
- **Non-specific Cardiomyopathy**: mild hypertrophy with fixed interstitial and replacement fibrosis and/or mildly degeneration with negative PCR.
- **Primary rhythm disturbances**: presence of AF, RT, AVD with no echocardiographic changes or mild hypertrophy without interstitial or endocardial fibrosis with negative PCR.
- **Acute viral, lymphocytic myocarditis (LM)**: focal lymphocytic infiltrates (>14 leukocytes/mm²), with absence of neutrophils, eosinophils, and mononuclear cells, no replacement or myocarditis, with positive or negative PCR.
- **Borderline myocardiitis**: focal lymphocytic infiltrates (<14 leukocytes/mm²) without evidence of necrosis and myocyte degeneration, no replacement or myocarditis, with positive or negative PCR.
- **Chronic immune-mediated myocarditis/inflammatory cardiomyopathy**: focal lymphocytic infiltrates (>14 leukocytes/mm²) with replacement or myocarditis and positivity PCR;
- **Chronic infective myocardial disease (CMD)**: mild hypertrophy with fixed replacement or endocardial fibrosis and/or mildly degeneration with or without focal lymphocytic or thrombotic (intramyocardial, perivascular), and positive PCR.

Statistical Analysis
- Minimum sample size was calculated to be 21 dogs with UMD and dogs with CMD.
- Means were compared using Student’s t-test for normally distributed continuous variables and the Wilcoxon-Mann-Whitney test for nonparametric data.
- Differences between dogs with UMD and dogs with CMD regarding sex, age, and body weight were determined with a Fisher’s exact test for categorical variables.
- Logistic regression models were performed to identify factors associated with the outcomes (e.g., detection of nucleic acid or detection of viral pathogens by PCR assay).
- Multivariable logistic regression models were used to identify continuous variables (e.g., age, sex) that affected the outcome of interest.
- Variables with a p-value of ≤ 0.10 were included in the multivariable regression model.
- Odds ratios and 95% CI were used to assess the likelihood of the association between the predictor and the outcome.
- All analyses were performed with commercially available software. Two-sided values of P < 0.05 were considered significant.

RESULTS

Blood samples were available from 18 of the 21 dogs with positive PCR.

Histopathology

Feasibility

113 samples (range 1-7/dog)

- 3/47 dogs had complications associated with the EMB
  - 1 dog perforation of the RV wall and self-limiting myocardial hemorrhage without cardiac tamponade
  - 2 dogs self-limiting polyomaviral-like exanthema during the procedure

Histopathology

Feasibility

113 samples (range 1-7/dog)

Diagnosis

Chronic immune-mediated myocarditis/inflammatory cardiomyopathy
- 10/10 (ARVC)
- 1 Chronic Myocarditis
- 4 Normal

Acute/subacute myocardial infarction (AMI)

Borderline Myocarditis

Non-specific Cardiomyopathy (LAWM)

2/10 (51%)

Acute/myocarditis (LM)

Non-specific Cardiomyopathy

Borderline Myocarditis

Acute/myocarditis (LM)

Chronic immune-mediated myocarditis/inflammatory cardiomyopathy

Acute/subacute myocardial infarction (AMI)

Borderline Myocarditis

Non-specific Cardiomyopathy (LAWM)

Acute/myocarditis (LM)

Chronic immune-mediated myocarditis/inflammatory cardiomyopathy
**Feasibility**

47/47 dogs, 84 samples (range 1-3/day)

**Diagnosis**

15 positive single pathogens
6 positive >1 pathogens
16 negative

- Canine Distemper
- CPV-2
- Bartonella
- Canine Coronavirus
- Adenovirus
- 4 positive bacteria
- 1 positive protozoa
- 4 positive RNA viruses
- 8 positive DNA viruses

**Conclusion**

- Despite evident limitation due minimal sampling of the right ventricle, and myocardial biopsy should be considered as a promising diagnostic tool in patient with:
  - Non-familial DCM phenotypes,
  - New onset AVB particularly in young dogs,
  - Supraventricular or ventricular arrhythmias in patients with no cardiostructural diseases.
- Viral myocarditis can be common in this subset of dogs and can induce different forms of hypokinetic heart. In such a cases EMB is crucial to guide early treatment and prevent end-stage disease.

**Limitations**

- Small sample’s size and number due to in-vivo diagnosis
- Negative results of Immunohistochemistry
- Negative results in case of acute myocarditis
- Limited number of cardiotoxic pathogen tested
- inability to test blood samples in all patient with myocardial PCR positive
- Impossibility to correlate if the pathogen isolated from the heart was causing the disease
- Lack of titer for tick-transmitted diseases, Neospora, toxoplasma, and CTnI in all dogs.
In vivo evaluation of pathogens in dogs with myocarditis: from endomyocardial bioptic tissue to Next Generation Sequencing.

Investigators:
Roberto Santilli DMV, ECVIM-CA (Cardiology), PhD; Antonio Di Loria DMV, PhD; Paolo Ciaramella DMV, Claudia Kohl.

Future Directions
The VIPER Study
Randomized Single-Blinded Study on the Efficacy of Recombinant Feline Interferon-Omega Therapy in Dogs with Viral Persistence Inflammatory Cardiomyopathy.

Investigators:
Roberto Santilli DMV, PhD, ECVIM-CA (Cardiology), Manuela Perego DMV, ECVIM-CA (Cardiology).

Chronic Infectious Myocardial Disease (Distemper)
T0 – CHF EF 21%  
T360 Post-Interferon-Omega – EF 33%  
Dr Manuela Perego – DECVIM-CA (Cardiology)  
Dr Massimiliano Tursi group for histological examination  
Dr Elena Grego group for genomic PCR analysis  
Referring veterinarians and owners of the dogs

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