Clinical therapeutic efficacy of mycophenolate mofetil in the treatment of SARDS in dogs – a prospective open-label pilot study.

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ABSTRACT

Objective. Sudden acquired retinal degeneration syndrome (SARDS) is a leading cause of irreversible blindness in dogs, yet no treatment has been objectively evaluated, or proven to be effective. Consensus of opinion is that SARDS is immune-mediated, although corticosteroid medications may exacerbate associated systemic signs. We examined the effect of sole-agent treatment with mycophenolate mofetil (MMF), a potent immunosuppressive medication unlikely to exacerbate associated systemic signs.

Animals Studied. Ten client-owned dogs with SARDS prospectively recruited within 6 weeks of vision loss.

Procedures. Clinical history, findings of systemic and ophthalmic examinations, blood parameters, visual navigation ability, electroretinography, and optical coherence tomography (OCT) were collected at baseline and at recheck after approximately 6 weeks of treatment with 10mg/kg q 12hr of oral MMF.

Results Twenty percent of dogs (2/10) experienced side effects (diarrhea, vomiting, lethargy), which resolved with reduction in dose to 8mg/kg q12hr. No significant changes in systemic signs, physical examination findings or laboratory test results were detected at the recheck examination. Compared with baseline, visual ability significantly declined at the recheck examination, and the amplitude of a slow onset negative waveform noted on dark-adapted electroretinography was reduced at the recheck examination. The outer retinal layers were significantly thinner at the recheck examination as measured by OCT.

Conclusions Mycophenolate mofetil as a sole agent has no measureable positive effect on physical health, vision, or retinal structure following a 6-week trial period. Further studies are needed to evaluate other treatment options for SARDS.

Key words: retina, vision, dog, SARDS, treatment, mycophenolate mofetil
INTRODUCTION

Sudden acquired retinal degeneration syndrome (SARDS) is a condition characterized by the sudden loss of sight that affects middle-aged dogs with a slight over-representation of females. (1-4) Although any breed can be affected, the most commonly reported breeds are Dachshunds, mixed-breeds, and miniature Schnauzers. (2, 4) In addition to acute vision loss, systemic manifestations including polydipsia, polyuria, polyphagia, and weight gain can contribute to decreased quality of life. (2, 5, 6) Dogs typically present for assessment of acute blindness that progresses over days to weeks. (2, 7) One study showed that 58% of cases had no funduscopic changes, while the remaining cases had varying degrees of tapetal hyper-reflectivity and vascular attenuation indicating retinal degeneration. (8) Retinal function as assessed by electroretinogram (ERG) shows extremely low, or absent, a- and b-wave amplitudes. (7, 9, 10)

There are a number of similarities between SARDS and human autoimmune retinopathy (AIR). AIR is characterized by rapid vision deterioration with decreased or extinguished electroretinogram (ERG) amplitudes in the absence of funduscopic changes, although in advanced cases, arteriolar attenuation, retinal pigment epithelium pigment changes, and optic disc pallor may occur. (11-14) Similar to dogs with SARDS, human AIR is more common in females than males, and the age of onset is typically middle-aged to older. (11-13) Human AIR is rare, accounting for less than 1% of cases seen at a tertiary ocular immunology clinic. (13) It is divided into paraneoplastic (cancer associated retinopathy; CAR) and non-paraneoplastic (npAIR) forms. (14-17) CAR has been associated with small cell lung cancer, breast cancer, Hodgkin’s lymphoma, and melanoma. (14, 16, 17) The same association with cancer has not been found in canine SARDS in a systematic evaluation for evidence of neoplasia using thoracic radiographs, abdominal ultrasound and computed tomography of the pituitary gland. (5)

Despite a lack of evidence for systemic neoplasia resulting in paraneoplastic AIR in SARDS patients, there is evidence of anti-retinal antibody development in canine SARDS, supporting similarities to human nonparaneoplastic AIR. (18, 19) AIR in humans has been associated with the retinal antigens recoverin, alpha-enolase, heat shock protein 70, and transient receptor potential cation channel, subfamily M, member 1. (16, 20-23) Circulating anti-recoverin and anti-alpha-enolase antibodies have been detected in dogs with SARDS, but
also in controls without SARDS. (5, 24) Bellhorn et al reported the presence of antiretinal antibodies in the serum of all 5 SARDS dogs examined. (18) A more recent study by Braus et al, demonstrated that 25% of SARDS patients had circulating antibodies to neuron-specific enolase, contrasting with 0% of control dogs. (19)

Immunomodulation is considered the optimal method of treatment for human AIR, including local and systemic corticosteroids, intravenous immunoglobulin therapy, plasmapheresis, and immunosuppressive medications including mycophenolate mofetil, cyclosporine, and azathioprine, although response to therapy is inconsistent. (13, 25) In a study of 24 people affected with npAIR, all were treated with at least 1 systemic immunosuppressive medication, and 62.5% had a positive response measured by improved visual acuity or field of vision. (26) SARDS is considered an untreatable, blinding disease by many veterinary ophthalmologists although some response to immunomodulatory therapy has been suggested. (10, 27) Many owners seek therapeutic options for their pets despite lack of an objective evidence of effect and an uncertain risk of side effects. Potential adverse effects of immunosuppressive therapy in SARDS patients include exacerbation of systemic clinical signs such as polyuria, polydipsia, and polyphagia, gastrointestinal injury, myelosuppression, hepatic damage, and drug reaction, which could be life threatening. (28) Therefore, there is an urgent need to prospectively evaluate potential treatments for SARDS cases, as outlined in a recent review paper. (3)

We selected the immunomodulatory medication mycophenolate mofetil as the therapeutic drug in this pilot clinical trial due to its rapid onset of action, and its published use as sole agent to treat immune-mediated diseases in veterinary patients. (29) Side effects in dogs include gastrointestinal upset, lethargy, and weight loss, however these are infrequent, generally mild, and commonly responsive to dose reduction. (28, 30) Mycophenolate mofetil is metabolized to mycophenolic acid, a potent, selective, and reversible inhibitor of inosine-5-monophosphate dehydrogenase (IMPDH), which is required for synthesis of guanosine nucleotides. Without de novo synthesis of guanosine nucleotides, lymphocytes are unable to differentiate, proliferate, or produce immunoglobulins. (28, 31, 32) Bioavailability from oral administration is reported as over 90% in humans. (32), and half-life of the drug in humans ranges from 9-17 hours; steady state concentration of the drug can be expected in 1.8-3.5 days. (31) Half-life following oral administration in dogs is approximately 8 hours, and robust IMPDH inhibition has been shown after a single oral dose of mycophenolate mofetil in dogs. (33)
We report the effect of treatment with oral mycophenolate mofetil on systemic health, visual outcome and retinal structure in 10 cases of canine SARDS in a prospective open-label clinical trial.

MATERIALS & METHODS

Study Design

This study was conducted with full institutional approval (Institutional Care and Use Committee approval and Institutional Review Board exemption) and client written informed consent. *A priori* power analysis established that 9 patients would be required to detect an ERG b-wave amplitude of at least 25µV at 3cds/m², based on historical data from the institution. Clients were enrolled by direct contact with the principal investigator, or through referral from another veterinarian. Clients underwent a telephone interview prior to enrollment to evaluate eligibility. Inclusion criteria included commitment to participation, vision loss of less than 6 weeks’ duration, a clearly visible fundus using ophthalmoscopy, and a confirmed diagnosis of SARDS (history, minimal fundus evidence of retinal degeneration with severe attenuation of ERG a- and b-wave amplitudes, consistent chromatic PLR findings). Exclusion criteria included systemic administration of immunosuppressive medications within the last 21 days, and any prior history of systemic or ocular neoplasia, uveitis, glaucoma, intraocular surgery or inherited retinal disorders. Clients presented their dogs for examination prior to beginning the medication (baseline) and a second time following a period of medication administration at home (recheck). Dogs were treated with 10mg/kg of mycophenolate mofetil oral suspension, compounded to a concentration that made dosing straightforward for the client (up to 160mg/mL; Stokes Pharmacy, NJ, USA), every 12 hours and owners were asked to return for recheck 4-6 weeks following treatment initiation.

Client Questionnaire

Each client was asked to fill in a questionnaire at baseline and recheck to assess the client’s perception of systemic and ocular health. A visual analogue scale was used for owners to grade vision and quality of life on a scale of poor to excellent and represented as a proportion of 100. The canine visual function instrument (CVFI) was also used as previously described. (34)
Physical and Ophthalmic Examination

Dogs underwent full physical examination. Baseline systolic blood pressure was measured using the Doppler ultrasound technique (Parks Medical Electronics; OR, USA); the measurement location, cuff size, and recumbency were recorded. Three readings were acquired and averaged.

Dogs underwent complete ophthalmic examination including menace response, dazzle and pupillary light reflexes (white light), tear production (Schirmer tear test strips; Merck Animal Health, NJ, USA), corneal fluorescein staining (Fluorescein strips, Akorn, IL, USA), tonometry (Tono-Vet, iCare, Finland), slit lamp biomicroscopy (SL-15, SI-17, Kowa Medical), and binocular indirect ophthalmoscopy with a 20-diopter lens (Keeler Instruments, PA, USA; Volk, OH, USA). Conjunctival hyperemia was scored from 0-3 (0=none, 1=mild, 2=moderate, 3=severe). Dogs also underwent subjective assessment of chromatic PLR (red and blue light 200cd/m² intensity; Melan 100, Biomed Vision, USA), and fundus photography (Retcam II, Clarity Medical Systems, CA, USA).

Laboratory Assessment

Complete blood count (CBC), serum biochemistry profile, and urinalysis were performed at both baseline and recheck. At the initial visit, adrenal function was assessed with an adrenocorticotropic hormone (ACTH) stimulation test by obtaining a baseline serum cortisol measurement, followed by intravenous administration of 5μg/kg of exogenous ACTH (Cortrosyn; Sandoz, NJ, USA), and a second serum cortisol measurement 1 hour later. Urinalysis was performed on a voided sample at the baseline visit, and on a cystocentesis sample at the recheck visit to facilitate aerobic urine culture.

Vision Testing

Objective vision testing was performed on a subset of dogs at both examinations prior to sedation using a vision-testing device previously described. (35) Briefly, a junction box with 4 tunnels was constructed. A light with an adjustable rheostat was placed at the exit of each tunnel. For all runs, one tunnel was open, while the
others were covered. Four light intensities were chosen to assess photopic and mesopic vision (maximal achievable room lighting, 5, 1, and 0.04 cd/m²) with 8 runs recorded for each light intensity. For each run, the open tunnel was chosen at random. The dog was placed in the central junction box and was timed until exiting the correct tunnel; the percentage of runs the correct initial tunnel choice was made was also recorded and used in statistical analysis.

Electroretinography

Following 20 minutes of dark adaptation, dogs were lightly sedated with intravenous dexmedetomidine hydrochloride (Zoetis, NJ, USA; 1.25 μg/kg) and butorphanol tartrate (Bayer Healthcare LLC, KS, USA; 0.1mg/kg). Dogs were reversed after the procedure with atipamazole hydrochloride (Zoetis, NJ, USA; 0.0625mg/kg). Pupils were dilated with topical tropicamide 1% ophthalmic solution (Akorn Pharmaceuticals, Lake Forest, IL). The head was placed in a Ganzfeld dome with a computer controlled LED light stimulator (Ganzfeld Q450SC and RETIport system; Roland Consult, Germany) and ERGs were recorded from both eyes simultaneously using contact lens electrodes (ERG-jet; Fabrinal, Switzerland) with reference electrodes (Grass platinum subdermal electrodes, Natus Neurology, WI) placed subcutaneously 3cm from the lateral canthus over the zygomatic arch bilaterally, and a ground electrode placed at the occiput. High- and low-pass filters were set at 0.2 and 300 Hertz (Hz), respectively. A dark-adapted intensity series in response to 9 different intensities of a 5-millisecond white flash (ranging from 0.001 to 10 cd-s/m²) was recorded. Based on the stimulus intensity, flash frequency ranged from 0.05 to 0.2 Hz, averaging 4 flashes with 10-70 seconds between flash series.

Following dark-adapted ERG, eyes were light-adapted with white light at 25 cd/m² for 10 minutes. Light-adapted response to white flash (3 cd-s/m²) was recorded. Stimulus frequency was 2 Hz. Cone flicker ERG was recorded using white flash stimulus at 3 cd-s/m² with an intensity of 33 Hz and cycle time of 0.03 seconds. Following 1 blind cycle, 30 tracings were acquired. All light adapted ERGs were recorded with a 5-millisecond white flash superimposed on a rod-suppressing white background of 25 cd/m². Any waveforms were assessed for amplitude and implicit time.
Spectral-Domain Optical Coherence Tomography

Imaging was obtained with a high-resolution handheld spectral domain-OCT (SD-OCT) with a 70° field of view general retina lens (840 nm laser; Envisu R2300; Leica Microsystems Inc., IL, USA). Rectangular volume scans (6 x 6 mm, 100 B-scans and 1000 A-scans per B-scan) were obtained in four separate quadrants, superior, inferior, temporal, and nasal to the optic nerve head. Three measurements of retinal layer thickness per region were made between 1.34 and 2.68mm from the edge of the optic nerve head using the Leica software InvivoVue Diver 3.0.8 (Leica Microsystems Inc., IL, USA) and image analysis software (Image J, National Institutes of Health, Bethesda, MD) by the same masked investigator; an average of the three measurements per region was used in analysis. In order to consistently measure the same location in each quadrant per eye, a plot with equally spaced points of reference was utilized. Layers measured included total retinal thickness (internal limiting membrane to RPE), nerve fiber layer, ganglion cells and inner plexiform layer (NFL+; internal limiting membrane to edge of inner nuclear layer) inner nuclear layer (INL), outer nuclear layer (ONL), receptor plus (REC+; outer nuclear layer and inner and outer segments) and photoreceptor inner and outer segment thickness. A representative image with layers delineated is shown in Supplementary Figure 3. A second masked observer also analyzed a subset of images (dorsal region) to verify the data and provide information on interobserver correlation.

Statistical Analysis

Analysis was performed in GraphPad Prism 5 for Mac (GraphPad Software Inc., LaJolla, CA). Results from the right and left eye were compared using a paired t-test, and if no difference was identified, the mean of the right and left eye was used in subsequent analysis. Baseline and recheck testing results were compared for each dog using a paired student’s t-test except for the following comparisons. One-way ANOVA was used to analyze vision testing data at the 4 different lighting intensities at baseline examination. Two-way repeated measures ANOVA was used to analyze the effect of treatment on OCT retinal thickness, considering region of
the eye as an additional variable, and to analyze the effect of treatment on visual navigation and ERG with light intensity used as an additional variable, followed by Bonferroni post-test. For all measured variables, the baseline/recheck matching was effective (P<0.05 or lower), confirming the appropriateness of using a repeated measures two-way ANOVA for statistical analysis of the effect of treatment. Spearman rank correlation was performed to examine correlations between OCT, vision testing, and ERG parameters at baseline and recheck examinations. Standard error is presented in graphs and significance was set at p<0.05.

RESULTS

Participants

Over an 11.5-month period, 14 dogs were enrolled in the study. Four dogs were disenrolled (1 male, 3 females); 1 dog was diagnosed with diabetes mellitus during treatment and treatment was discontinued, 2 were euthanized: one for poor quality of life (prior to starting medication) and the other for uncontrollable polyphagia, and 1 dog was lost to follow-up. The remaining 10 dogs completed the trial period and were included in data analysis. Table 1 summarizes the demographics of the cases enrolled – the most common breed was the Dachshund (3/10). Five cases were female (1 intact, 4 spayed) and 5 were castrated males. Median duration of vision loss prior to initial examination was 16 days (range 3-39 days) and median duration of vision loss prior to initiation of treatment was 25 days (range 11-46 days), accounting for delivery of the medication to the client from the compounding pharmacy. One dog (patient 4) had a greater than anticipated delay in starting medication, resulting in a shorter than anticipated treatment duration (23 days) at the time of recheck. All other dogs received medication for greater than 35 days. Two dogs had adverse effects from the medication (vomiting, diarrhea and lethargy in 1 dog, diarrhea in 1 dog) that resolved with 20% dose reduction of the medication. Both dogs completed the trial on 8mg/kg q12hr dosing. Median duration of treatment was 46 days (range 23-56).

Client Questionnaire
In the baseline questionnaire, vision loss was reported as sudden in 6/10, subacute in 2/10 (1 day to 1 week), gradual in 2/10 (greater than 1 week). Before complete vision loss, 6/10 described a preceding period of intermittent vision changes, ranging from 2-6 weeks prior to complete vision loss; during this time, 2/6 clients commented that vision was worse in one eye than the other, and 3/6 clients commented that vision was worse at night/evening time than daytime. Clients self-reported the following clinical signs in their dogs at baseline (from most to least common): 8/10 reduced activity, 7/10 increased body weight, 6/10 increased drinking, 6/10 increased urination 5/10 increased appetite, 4/10 decline in coat condition (most reported as dry and flaky skin), 3/10 reduced sense of smell, 3/10 disorientation, 2/10 increased defecation, 2/10 increased panting, 2/10 irritated or red eyes, 1/10 reduced hearing. All clients reported at least one clinical sign.

At the time of recheck, clients reported that treatment had no overall effect on vision or clinical signs described at the baseline exam. However, vision was perceived to deteriorate in 3 dogs (cases 1, 5, 8) and improve in 1 dog (case 10, per the visual analogue scale, median score 47/100, range 20-52), and 2/4 clients that reported altered coat condition at baseline reported improvement on the medication. The majority of clients (7/9) reported a small non-significant improvement in quality of life at the recheck examination: visual analogue scale quality of life on a scale from poor to excellent, median 60/100 (range 31-77) at baseline, 71/100 (range 28-86) at recheck, paired t-test p = 0.07.

The canine visual function instrument (34) was completed by 6/10 clients at both baseline and recheck. All clients graded SARDS to most severely affect their dog’s ability to navigate unfamiliar surroundings, catch snacks thrown to them and track an object when thrown. There was no effect of treatment on any of the 16 vision-related questions in the canine visual function instrument (P>0.05, paired student’s t-test).

Anecdotally, two clients reported acute worsening of vision (within 1-3 days) in their dogs (both Dachshunds, patients 6 and 9) after abrupt discontinuation of the medication at the end of the trial period. Further clinical assessment of vision was not performed. Both dogs had been described by their owners to have dry flaky skin at baseline, which improved significantly with treatment. No other differences in history, clinical assessment, ERG or OCT parameters were noted between these dogs and the remainder of the group that showed no change in vision after discontinuation of the medication.
**Physical and ophthalmic examination**

Temperature, pulse and respiratory rate were within normal limits for all patients at baseline and recheck examinations. Body weight and body condition scores are presented in Table 1. Baseline mean systolic blood pressure was $147 \pm 9.7$mmHg and 2 dogs fit the criteria for hypertension (mean systolic blood pressure >160mmHg). In addition, 2 dogs had previously been placed on antihypertensive medications by their referring veterinarian prior to baseline examination, those 2 dogs had normal blood pressure at the time of examination. One of the 2 hypertensive dogs at baseline remained persistently hypertensive at recheck examination despite combination therapy with amlodipine and benazepril, the other dog’s blood pressure was normal on recheck examination and had received no therapy.

Ophthalmic examination findings are summarized in Table 2. At baseline, menace response was absent in both eyes in all cases, dazzle to bright white light was present bilaterally in 5/10 (cases 1-5), unilaterally in 2/10 (cases 6 and 9) and absent in 3/10. PLR to white light was present in all cases; there was a slight bilateral response to red light in 6/10, unilateral in 3/10, and absent in 1/10. There were strong bilateral pupil responses to blue light in all cases; no significant changes in any of these responses were detected at recheck. Mean Schirmer tear test value at baseline was $19.25 \pm 1.32$ mm/min and $19.85 \pm 1.26$ mm/min at recheck; one dog had marginally low production (14.5mm/min) which persisted at the recheck examination (13mm/min). One dog had a superficial corneal ulcer at baseline, which healed without complication with standard topical antibiotic therapy. No dogs had clinical evidence of uveitis at either examination. Mean IOP at baseline was $13.2 \pm 0.97$ mmHg compared with $16.2 \pm 0.88$mmHg at recheck, a difference that was significant (p<0.05, paired t-test). Conjunctival hyperemia was present in 8/10 at the baseline examination, mean score was $1.1\pm 0.2$ and 7/10 had conjunctival hyperemia at recheck, mean score was $1.2 \pm 0.3$ (p = 0.29, paired student’s t-test). Mucoid or serous ocular discharge was present in 5/10 dogs at baseline and 10/10 dogs at recheck. All dogs had some detectable change in fundus appearance at baseline examination: 6/10 had mild vascular attenuation, 4/10 had diffuse, mild hyper-reflectivity of the tapetal fundus, 4/10 had focal or multifocal areas of more significant tapetal hyper-reflectivity in one or both eyes. Focal or diffuse hyper-reflectivity was more prevalent in dogs in...
which vision loss preceded examination by greater than 14 days (11/12 eyes) compared with those in which vision loss was less than or equal to 14 days (2/8 eyes). Diffuse tapetal hyper-reflectivity subjectively increased between baseline and recheck examination in 3/4 dogs in which it was present at baseline, and was present in 3 additional dogs in which it was not apparent at baseline examination. Representative fundus images from all patients are presented in Supplementary Figure 1.

**Laboratory Assessment**

All dogs underwent laboratory testing at baseline and recheck (summarized in Table 3). Baseline complete blood count results were consistent with expected changes in SARDS patients: 3/10 dogs had mild leukocytosis, 4/10 had mild neutrophilia, 2/10 had mild lymphopenia. No dogs were anemic or thrombocytopenic at baseline. Treatment with mycophenolate mofetil did not induce any changes in complete blood count, and no dog had neutropenia, thrombocytopenia or anemia at recheck. Plasma protein was elevated in 7/8 dogs at baseline, and 4/10 had increased serum Alkaline Phosphatase, 5/10 had increased Alanine Aminotransferase and 4/10 had increased serum cholesterol, consistent with that expected in SARDS patients; no significant changes were observed at the recheck examination. No changes in blood urea nitrogen were observed at either baseline or recheck, although serum creatinine was low in 2/10 dogs at baseline and 5/10 dogs at recheck; creatinine values at the recheck were significantly lower than those at baseline (p = 0.04, paired t-test). ACTH stimulation testing mean baseline resting cortisol value was 3.96 ± 0.55 μg/dL (reference range 1-4.5μg/dL); mean 1 hour post-ACTH cortisol value was 17.18 ± 1.07 (reference range 7.0-20.0μg/dL), 1/10 dogs had an elevated post-ACTH cortisol (23.4 μg/dL). Urine specific gravity was below 1.015 in 2/10 dogs at baseline examination and 3/10 dogs at recheck, a difference that was not significant. Urine culture was positive in 1/10 dogs (coagulase negative *Staphylococcus* spp.) at the recheck examination, which was treated with oral antibiotics by the referring veterinarian.

**Vision Testing**
Both baseline and recheck vision testing was performed in 7/10 dogs. Mean light intensity for each of the 4 test intensities was 146.19 ± 42.84 cd/m², 5.02 ± 0.017 cd/m², 1.011 ± 0.006 cd/m², and 0.0399 ± 0.0011 cd/m². At baseline examination, dogs navigated significantly more quickly in bright light than dim light (brightest vs. dimmest light intensity, one-way ANOVA, p < 0.01), there were no significant differences in the percentage of correct initial tunnel choice made between different light intensities at baseline. At the recheck examination, vision had significantly declined. There were no significant differences between baseline and recheck for the dimmest and brightest light intensities tested, but at the recheck examination, dogs were significantly slower at 1cd/m² (p<0.05) and 5cd/m² intensities (p<0.01, two-way ANOVA with treatment and intensity as the 2 variables, Fig. 1A). At the recheck examination, dogs made a significantly smaller percentage of correct first tunnel choices at the 5cd/m² intensity compared with baseline (p<0.05, two-way ANOVA, treatment variable was significant at p<0.01, Fig. 1B)

Electroretinography

All dogs completed baseline and recheck examinations. No a- or b-waves were identified in any dog at either baseline or recheck examination, at any light intensity (dark- and light-adapted conditions). However, a dark-adapted delayed onset negative waveform (mean implicit time 91.2 milliseconds) was identified in 6/10 dogs, 12/20 eyes (at 3cd-s/m² intensity) and 9/10 dogs, 16/20 eyes (at 10cd-s/m² intensity) at baseline, which increased in amplitude between the 3cd-s/m² intensity and the 10cd-s/m² intensity. Fig. 2 shows a representative intensity series of one eye of one dog at baseline and recheck (right eye, subject 4; Fig. 2A, B). At the recheck examination, 4/10 dogs, 5/20 eyes (3cd-s/m²) and 7/10 dogs, 9/20 eyes (10cd-s/m²) had this response present. The implicit time of the peak response did not change with treatment (Fig. 2C) but amplitude of this response at both 3 and 10cd-s/m² (Fig. 2D) decreased significantly at the recheck examination (p<0.05, two-way ANOVA treatment variable was significant at p<0.001). ERG waveforms from a single eye from all dogs are shown in Supplementary Figure 2.

Spearman rank correlation analysis showed correlations between ERG waveform amplitudes and vision testing performance; significant positive correlations were present between baseline delayed onset negative
waveform amplitude at 3cd-s/m$^2$ and the vision testing percent correct choice at 1cd/m$^2$ ($r = 0.82$, $p<0.05$) and 5cd/m$^2$ ($r = 0.83$, $p<0.05$). No significant correlations of the baseline waveform amplitude and baseline visual navigation at 0.04 or 10cd-s/m$^2$ or with any parameter at the recheck examination were identified. Duration of blindness at the time of presentation or initiation of treatment did not correlate with baseline or recheck ERG waveform amplitude.

**Optical Coherence Tomography**

All dogs underwent baseline and recheck optical coherence tomography imaging. Overall, total retinal thickness declined significantly between baseline and recheck examinations (Fig. 3A, B; $P<0.0001$), although the only individual region that had a statistically significant decline in total thickness was the dorsal retina ($P<0.01$, Fig. 3C). The thickness of the NFL+ and INL did not change significantly between baseline and recheck examinations, and no regional effects of treatment were identified (data not shown; $P>0.05$). Receptor plus (REC+; outer nuclear layer and photoreceptor inner/outer segments) significantly decreased overall between baseline and recheck examinations ($P<0.0001$), and there were significant differences at the region-level in all 4 regions (Fig. 3D). Although overall, both outer nuclear layer thickness ($P<0.0001$, Fig. 3E) and photoreceptor inner/outer segment thickness ($P<0.0001$, Fig. 3F) significantly declined between baseline and recheck examinations, significant effects at the region-level were only detected in outer nuclear layer thickness. An independent masked observer verified the findings in the dorsal region of total retinal thickness, outer nuclear layer thickness and REC+ (Supplementary figure 3), and interobserver correlation was significant ($r^2$ total thickness: 0.68, ONL thickness: 0.74, REC+ thickness: 0.72, all $P<0.0001$).

Spearman correlation analysis showed no significant correlation between either REC+ value or ONL value at either baseline and the following parameters: recheck examination and ERG waveform amplitude (3 and 10cd-s/m$^2$), vision testing time to exit (any light intensity) or percent correct choice (any light intensity), except for a moderate positive correlation ($r = 0.82$) between ONL thickness at baseline and time to exit at full lighting intensity ($p<0.05$). Duration of blindness at the time of treatment did not correlate with ONL or REC+ values.
DISCUSSION

This study prospectively demonstrates a lack of beneficial effect of single agent treatment with therapeutic doses of mycophenolate mofetil on vision in 10 cases of SARDS. We found a diminished retinal response to light, visual navigation ability and outer retinal layer thickness following an average period of 6 weeks of treatment, and no significant systemic adverse effects of mycophenolate mofetil were detected.

Our SARDS patient population consisted of middle-aged, overweight dogs, with the Dachshund breed over-represented, and with symptoms of polyuria, polydipsia, polyphagia, and laboratory evidence of leukocyte, liver enzyme, and cholesterol increases, mirroring cases described in other studies. (1-4, 6-8). Although our enrolled population contained a slight female majority (57%) similar to other studies, (1, 2, 4) the 10 dogs that completed the trial were equally spread between males and females. Fundus changes were discovered at a higher rate than previously described, (8) although remained mild compared with the extent of vision disturbance and ERG changes. Mycophenolate mofetil treatment resulted in 2 dogs experiencing minor adverse effects including lethargy, vomiting and diarrhea, similar to the 20-67% incidence of similar adverse effects reported in other studies. (29, 30, 36) Adverse effects were ameliorated by dose reduction, as also noted in another study. (36) No life-threatening systemic side effects were identified, and no significant effects on blood or urine parameters were detected. However, no patient showed consistent improvement in vision on treatment, and significant decline in retinal structure and function was identified. In human patients with nonparaneoplastic AIR, approximately 62% show improvement in visual acuity or visual field with a variety of immunosuppressive medications. (13) One potential explanation for the difference in response between humans with nonparaneoplastic AIR and dogs with SARDS is the timing of initiation of therapy, as is likely that human patients would report visual disturbance at an earlier stage of disease than that present in dogs with SARDS presenting to a veterinary ophthalmologist. It is possible that a very small protective effect of mycophenolate mofetil treatment occurred, due to the anecdotal report of 2 dogs becoming acutely more blind after treatment discontinuation. Reasons for this phenomenon remain unknown, but possibilities include bias of the owner due to open-label nature of study, partially effective immunomodulation resulting in very slight protection of the
retina during treatment, or treatment administered at too late a stage of disease to be more effective. There is evidence of inflammatory cell invasion into the retina in SARDS cases, including plasma cells (10) and macrophages. (37, 38) Whether these cells contribute to retinal damage, are recruited secondary to retinal damage, or whether immune complex deposition mediates pathology, remains unclear. An alternative etiology of SARDS other than immune-mediated remains a possibility based on the results of this study, as retinal inflammation has been shown to be present secondary to non-immune-mediated retinal degenerative disorders in dogs, (39) which might explain the possible slight treatment effect of mycophenolate mofetil in this study.

Our electroretinography studies confirm the findings of others of absent dark and light adapted a- and b-wave responses in dogs with SARDS. (2, 6, 7) However, we identified in dogs with SARDS, a negative waveform with slow kinetics in the dark-adapted state, most detectable at very bright light intensities, highly similar in appearance to a scotopic threshold response (STR). The STR is described to be present in dogs as a response to very low light intensity flashes in the dark-adapted retina, and has been described to have an implicit time of 75-96ms, similar to the implicit time of the 91ms timing of the response observed in dogs with SARDS. (40) The STR is considered to be rod photoreceptor derived, and represents a signal from the proximal retina in response to rod input, (41-43) We hypothesize that this response seen in dogs with SARDS represents a very elevated rod photoreceptor threshold to light, as its amplitude increases in the dark-adapted state with increasing light intensity, and because the response correlates with visual ability at the baseline examination, it may represent a measure of residual visual ability in early SARDS. The response amplitude declined within the study timeframe, in concert with other measures of visual ability and retinal structure.

The results from visual navigation testing suggest that many SARDS patients retain some bright light vision despite apparent blindness and a lack of menace response. Bright light vision may be better maintained than dim light vision at more advanced stages of disease, as navigation in the brightest light intensity achievable in the testing system was unaffected in the 6-week duration of the trial. A protective effect of mycophenolate mofetil on this parameter cannot be excluded due to the open label nature of this study, and further work is needed to confirm the timecourse of visual perception in dogs with SARDS. However, from the owner questionnaire, 3/10 owners described that preceding complete blindness, night vision was impaired more than
daylight vision. The measurable STR-like response in the dark-adapted state suggest that the bright light vision might be rod-mediated, although the absence of detectable cone responses may reflect the relatively low density of cones in the canine retina, (44) making detection of a small amount of residual cone activity challenging with electroretinography.

Our OCT findings of progressive photoreceptor nuclear layer thinning combined with more moderate photoreceptor inner and outer segment shortening within the 6 week timeframe of this study supports the findings of others that early SARDS affects the photoreceptors (37, 38) and results in apoptosis of the photoreceptor outer nuclear layer. (9) However, we did not identify any progressive changes in inner retinal thickness, despite a previous study finding significant thinning of the nerve fiber layer without evidence of an effect on the retinal photoreceptors in 6 dogs with early SARDS compared with controls. (10) The reason for this discrepancy is unclear, although a negative effect of mycophenolate mofetil on outer retinal structure and a trophic effect on inner retinal structure cannot be excluded due to the nature of the pilot study design precluding recruitment of dogs receiving no treatment or placebo. A case-control study by the same authors is ongoing to compare OCT findings in age- and breed-matched controls. The absence of correlation of outer retinal thickness decline and the reduction in retinal responses and vision suggests the decline in retinal structure may be temporally disconnected from an earlier decline in vision. This has been shown in inherited canine retinal degeneration due to a mutation in Rpe65, which results in slow rod death, despite very early rod dysfunction.(35, 45, 46)

Future projects building on the findings of this pilot study might involve examining the efficacy of combination immunosuppressive therapy, as has been shown to be effective in other canine immune-mediated disease. (30, 47, 48) Further work, however, is necessary to definitively identify an immune-mediated etiology of SARDS, as the absence of any response to a potent immunosuppressive medication such as mycophenolate mofetil might indicate an alternative etiology such as retinal toxicity, as has been previously proposed. (49) Identification of early biomarkers of SARDS might allow a prompter diagnosis, potentially allowing therapy to be instituted before retinal degeneration and permanent dysfunction is inevitable.
Acknowledgements

Funded by an ACVO Vision for Animals Resident grant to Whitney Young (2016-1642/VAF2016-03), an ACVO Vision for Animals grant to Freya Mowat (2016-0459/VAFSARDS2015) and Stokes Pharmacy who provided the compounded study medication. The authors gratefully acknowledge the contributions of all the participating dogs and their owners, referring veterinarians and veterinary ophthalmologists, and Samantha Newton at Stokes Pharmacy.
REFERENCES


<table>
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<tr>
<th>Patient Number</th>
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<th>Weight (kg)</th>
<th>Body Condition Score (x/9)</th>
<th>Doppler BP (mmHg)</th>
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Table 2. Ophthalmic examination findings *p<0.05 compared with pre (paired student’s t test)

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<tr>
<th>Patient Number</th>
<th>Conjunctival hyperemia score</th>
<th>Chromatic PLR</th>
<th>Fundus findings</th>
<th>Mean STT OU (mm/min)</th>
<th>Mean IOP OU (mmHg)</th>
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<tbody>
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<td>1</td>
<td>Baseline Recheck Baseline Recheck</td>
<td>Baseline Recheck Baseline Recheck</td>
<td>Baseline Recheck Baseline Recheck</td>
<td>Baseline Recheck Baseline Recheck</td>
<td>Baseline Recheck Baseline Recheck</td>
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<tr>
<td>1</td>
<td>1 2 Red: slight + OU Blue: + OU Red: very slight + OU Blue: + OU</td>
<td>Mild diffuse hyper-reflectivity OU, focal hyper-reflectivity OD</td>
<td>Moderate diffuse hyper-reflectivity OU, focal hyper-reflectivity OD</td>
<td>16</td>
<td>19.5</td>
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<td>2 2 Red: very slight + OU Blue: + OU Red: slight + OU Blue: + OU</td>
<td>Mild diffuse hyper-reflectivity, arteriolar attenuation OU</td>
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<td>16.5</td>
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<td>Mild diffuse hyper-reflectivity OU</td>
<td>14.5</td>
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<td>Unchanged OU</td>
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<td>Mild arteriolar attenuation OU Multiple focal areas of hyper-reflectivity OS</td>
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<td>21.5</td>
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<td>Small focal areas of hyper-reflectivity OU</td>
<td>Mild diffuse hyper-reflectivity, mild arteriolar attenuation OU</td>
<td>28.5</td>
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<td>Unchanged OU</td>
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<td>17</td>
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<td>10</td>
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<td>Unchanged OU</td>
<td>18</td>
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### Table 3. Laboratory assessment parameters

<table>
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<tr>
<th>Parameter</th>
<th>Reference range (units)</th>
<th>Baseline mean (number outside reference range)</th>
<th>Recheck mean (number outside reference range)</th>
<th>P value (paired t-test)</th>
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<tr>
<td><strong>White Blood Cell Count</strong></td>
<td>4.39-11.61 (x10^3/µl)</td>
<td>9.54 (3/10)</td>
<td>9.02 (1/10)</td>
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<tr>
<td><strong>Segmented Neutrophil Count</strong></td>
<td>2.841-9.11 (x10^3/µl)</td>
<td>7.640 (4/10)</td>
<td>7.037 (3/10)</td>
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<tr>
<td><strong>Band Neutrophil Count</strong></td>
<td>0.03-1.264 (x10^3/µl)</td>
<td>0.051 (0/10)</td>
<td>0.127 (0/10)</td>
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<td><strong>Lymphocyte Count</strong></td>
<td>0.594-3.305 (x10^3/µl)</td>
<td>1.007 (2/10)</td>
<td>0.881 (2/10)</td>
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<tr>
<td><strong>Red Blood Cell Count</strong></td>
<td>5.0-8.01 (x10^6/µl)</td>
<td>6.87 (1/10)</td>
<td>6.77 (0/10)</td>
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<td><strong>Platelet Count</strong></td>
<td>190-468 (x10^3/µl)</td>
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<td>396 (2/10)</td>
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<td><strong>Plasma Protein</strong></td>
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<td><strong>Alkaline Phosphatase (ALP)</strong></td>
<td>16-140 (IU/mL)</td>
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<tr>
<td><strong>Alanine Aminotransferase (ALT)</strong></td>
<td>12-54 (IU/mL)</td>
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<td><strong>Blood urea nitrogen (BUN)</strong></td>
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<tr>
<td><strong>Serum creatinine</strong></td>
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<tr>
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<td>1.015-1.045</td>
<td>1.030 (2/10)</td>
<td>1.024 (3/10)</td>
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</table>

*some samples clumped, hemolyzed or lipemic
Figure 1. Visual navigation ability in dogs with SARDS at baseline and recheck examinations. Dogs navigated significantly more quickly at baseline than at recheck at the 1 and 5 cd/m² light intensity, but there was no difference in navigation speed at either 0.04 cd/m² or maximum room lighting (A). Dogs chose the correct exit tunnel a higher percentage of runs at baseline than at recheck at the 5 cd/m² light intensity, but there was no significant difference at any other light intensity (B).

*p<0.05, **p<0.01, two-way ANOVA.
Figure 2. ERG findings in dogs with SARDS at baseline and recheck examinations. In the dark-adapted intensity response series, a slow onset negative waveform was identified in the majority of dogs at either the 3cd-s/m² or 10cd-s/m² light intensity, which increased in amplitude between the two intensities (right eye of patient 4 at baseline shown in A). The response appeared to decline at recheck (right eye of patient 4 at recheck shown in B). The ERG waveforms of a normal age- and breed-matched control dog are shown in C for comparison (note the difference in scale of these waveforms). Implicit time of the negative response in SARDS patients did not differ between baseline and recheck examinations (3cd-s/m² n = 6 baseline, 4 recheck, 10cd-s/m² n = 9 baseline, 7 recheck; D), but mean amplitude significantly declined at both 3 (n = 6 dogs) and 10cd-s/m² (n = 9 dogs) intensities (E). *p<0.05, **p<0.01 two-way ANOVA.
Figure 3. OCT findings in dogs with SARDS at baseline and recheck examinations. In order to maintain consistency in the region evaluated, 6x6 mm scans were taken around the optic nerve head, using the optic nerve head for orientation (A). A representative scan at low magnification (A) and high magnification (B) are shown (patient 1, temporal scan, OS) illustrating evident thinning of the outer nuclear layer (ONL) and photoreceptor inner and outer segments (IS/OS). No obvious change in nerve fiber layer (NFL) was observed. Overall, when considering all retinal regions together, total retinal thickness, REC+, ONL and IS/OS thickness significantly declined between baseline and recheck examinations (p<0.05 for all). At the region level, total retinal thickness was significantly smaller at recheck examination in the dorsal retina (C), REC+ (D) and ONL (E) were significantly thinner at recheck in all quadrants of the retina, and IS/OS thickness did not differ at the regional level (F). Scale bar in A represents 100µm, in B represents 50µm *p<0.05, **p<0.01, ***p<0.001, two-way ANOVA, n = 10 dogs at each timepoint, average of both eyes for analysis.
Supplementary figure 1: Fundus Images. A single eye of each patient is shown at both baseline and recheck examination timepoints. The eye is denoted on the top left of the image, open arrowheads delineate focal areas of tapetal hyperreflectivity.
Supplementary Figure 2. ERG tracings from all participants. Tracings from 3cd-s/m2 (top trace) and 10cd-s/m2 (bottom trace) are presented from a single eye from each dog. Presence and location of the negative-type waveform used in subsequent analysis is represented with an arrowhead.
Supplementary figure 3: OCT analysis methodology, analysis by a second observer and interobserver correlation. Retinal layer delineation is shown in A, where 1 = internal limiting membrane, 2 = inner plexiform layer, 3 = outer plexiform layer, 4 = inner edge of ONL, 5 = outer edge of ONL, 6 = inner edge of choroid. Total retinal thickness was measured from 1-6, NFL+ measured from 1-2, ONL from 4-5 and REC+ from 4-6. A second observer measured a subset of the OCT images, to verify the small but significant differences found between baseline and recheck examinations. The dorsal region (both eyes, baseline and recheck examinations) was measured in a masked fashion, using the same scans as the initial observer. The comparisons of total thickness, ONL thickness and REC+ thickness between baseline and recheck examinations were statistically significant using a paired student’s t-test (B). Interobserver correlation was high (P<0.0001) for total retinal thickness (C), ONL (D) and REC+ (E).