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Case Reports

EFFECTS OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN TWO POODLE DOGS WITH PULMONARY HYPETENSION SECONDARY TO MYXOMATOUS MITRAL VALVE DISEASE

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SUMMARY

A 13-year-old neutered female Poodle dog (Case 1) developed right heart failure due to pulmonary hypertension (PH) secondary to stage C myxomatous mitral valve disease (MMVD). The owner consented to adipose-derived mesenchymal stem cell (AD-MSC) treatment, which consisted of 2 injections, to alleviate the PH. Following the treatments, improvement in clinical signs, right hemodynamics, and resolution of ascites were observed without remarkable adverse effects. The dog survived for 475 days without recurrence. A 12-year-old neutered female Poodle dog (Case 2), presenting with stage D MMVD and concurrent PH, received 3 injections of AD-MSC. After the treatment, coughing was significantly reduced, and left hemodynamics were improved. There were no evident changes in the blood test post-treatment. The dog survived at the time of writing. This paper describes the beneficial therapeutic effects of AD-MSC treatments in two dogs with PH secondary to MMVD.

Keywords: adipose-derived mesenchymal stem cell, pulmonary hypertension, myxomatous mitral valve disease

INTRODUCTION

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs, accounting for more than 70% of all canine heart disease (Parker and Kilroy-Glynn, 2012). A common sequela of MMVD is the development of pulmonary hypertension (PH), where chronic elevated pulmonary capillary wedge pressure and pulmonary venous congestion result in progressive vascular remodeling. In this context, the pulmonary vascular walls undergo progressive muscularization and lumen narrowing due to smooth muscle hypertrophy and hyperplasia (Leong *et al.,* 2018a) and inhibition of apoptosis in the pulmonary vascular wall (Grzeczka *et al.,* 2024). Currently, phosphodiesterase (PDE)-5 inhibitors such as sildenafil and tadalafil are primarily used in PH treatment. However, there is a potential risk that these PDE-5 inhibitors may worsen MMVD by dilating pulmonary blood vessels (Reinero *et al.,* 2020). Therefore, treatment options for PH secondary to MMVD are limited and carefully performed, especially when a dog has left heart failure or impending heart failure. Additionally, a lack of targeted therapies aimed at inhibiting pulmonary vascular remodeling results in a poor prognosis for survival in affected dogs, although anti-remodeling properties of tyrosine kinase inhibitor such as low-dose imatinib on PH in rats and dogs (Leong *et al.,* 2018) have been reported. Furthermore, coughing can be caused by concurrent lower airway disease, such as chronic bronchitis and bronchomalacia, compression of the left mainstem bronchus by the enlarged left atrium in the presence of the aforementioned airway disease (Ferasin *et al.,* 2013) and possibly pulmonary fibrosis (Uray *et al.,* 2020). It remains a frustrating condition for both dog owners and their pets, despite the use of cough suppressants.

Adipose-derived mesenchymal stem cells (AD-MSCs) are known for their pulmonary tropism (Kraitchman *et al.,* 2005), anti-inflammatory effects, tissue regeneration, and anti-fibrotic properties (Zhao *et al.,* 2023). In murine studies, MSCs were observed to localize in the lungs after intravenous administration and exert a long-lasting immunomodulatory role in lung tissue by promoting immune tolerance through Treg proliferation (Nemeth *et al.,* 2010) and normalizing the Th1/Th2 balance (Kavanagh and Mahon, 2011). Additionally, MSCs were shown to ameliorate PH in rats (Zhao *et al.,* 2023; Wang *et al.,* 2019). Regarding AD-MSCs' anti-inflammatory effects in the lung, intravenously administered AD-MSCs were demonstrated to reduce airway inflammation, airway hyper-responsiveness, and remodeling in cats experimentally induced with asthma (Trzil *et al.,* 2016). Given that PH is characterized by inflammation and remodeling processes and considering the promising effects of AD-MSCs on airway inflammation and lung disease due to their unique pulmonary tropism, our study aimed to evaluate the therapeutic potential of AD-MSCs in dogs with PH secondary to MMVD. We used AD-MSCs from VetCell Therapeutics Asia Limited, Hong Kong, which are commercially available and sourced from Beagle dogs (Product name: ReGen OA+; Lot number: 10017). These cells, licensed for the treatment of canine osteoarthritis, were evaluated for their clinical effects on left and right cardiac hemodynamics in two Poodle dogs. The AD-MSCs were thawed and removed from the cryopreservation medium through dilution, centrifugation, and resuspension

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in bovine serum. After counting, the cell dose was washed with bovine serum, centrifuged again, and resuspended in 0.7 mL of 0.9% sodium chloride. The cells were then loaded into a sterile, capped syringe, transported in a cooled container, and administered to the dogs within 4 hours of preparation (Maki *et al.,* 2020).

CASE REPORT

Case 1

A 4 kg, 13-year-old, neutered female Poodle dog was referred for a cardiology examination due to ascites. The dog was on regular heartworm prevention and had been treated for stage C myxomatous mitral valve disease (MMVD) with furosemide (5 mg/kg, every 12 hours, per os (PO)), pimobendan (0.31 mg/kg, every 12 hours, PO), and benazepril (0.31 mg/kg, every 12 hours, PO) for the past 7 months. On physical examination, the dog was bright and alert with a pale-pink mucous membrane, a distended abdomen, and exertional coughs. Chest auscultation revealed a left systolic heart murmur of grade 4/6, a right systolic murmur of grade 3/6, and harsh lung sounds. Hematology revealed unremarkable findings, whereas serum biochemistry showed mildly increased alanine transaminase (ALT) (194 U/L, reference interval (RI): 10-125 U/L) and alkaline phosphatase (ALP) (330 U/L; RI: 23-212 U/L). Serum N-terminal B-type natriuretic peptide (NT-proBNP) was severely increased (>10000 ρmol/L, RI<900 ρmol/L) (Table 1).

Table 1. Selective serum biochemistry pre- and post-AD-MSC treatment in dog 1.

Parameter	Unit	RI	R-CHF	AD-MSC 1	Day 73 post AD-MSC 1	Day 226 post AD-MSC 2
N-terminal pro-b-type natriuretic peptide	ρmol/L	<900	>10000	4000	1673.4	559.3
Troponin-I	ng/mL	<0.18	-	0.51	0.56	0.13
Symmetric dimethylarginine	µg/dL	0-14	10	-	15	10
Creatinine	µmol/L	44-159	36	-	161	110
Blood urea nitrogen	mmol/L	2.5-9.6	11.8	-	25.1	25.4
Alanine aminotransaminase	U/L	10-125	194	-	-	144
Alkaline phosphatase	U/L	23-212	330	-	-	264
Sodium	mmol/L	144-160	145	157	-	150
Chloride	mmol/L	109-122	106	117	-	106
Potassium	mmol/L	3.5-5.8	4.7	3.5	-	4.6

RI, reference interval; R-CHF, right congestive heart failure; AD-MSC, adipose-derived mesenchymal stem cell.

Chest radiography showed generalized heart enlargement (vertebral heart score (VHS): 13, RI<10.5) and loss of abdominal serosal details consistent with ascites (Figure 1). Abdominocentesis was performed to remove 200 mL of serosanguineous modified transudate. Echocardiography (Mylab 7 Esoate; P2-9) showed moderate dilation of left cardiac dimensions (Table 2). The maximal velocity of tricuspid regurgitation (TRmax) was 4.35 m/s, giving rise to an estimated systolic pulmonary arterial pressure (SPAP) of 75.7 mmHg (Figure 1) using simplified Bernoulli's equation (4× TRmax2). Therefore, it was concluded that the ascites was attributable to right-sided heart failure due to pulmonary hypertension (group 2) secondary to stage C MMVD. The dog was treated with torasemide (0.19 mg/kg in the morning and 0.10 mg/kg in the evening, PO), pimobendan (0.31 mg/kg, every 12 hours, PO), spironolactone (1.56 mg/kg, every 24 hours, PO), and sildenafil (3.1 mg/kg in the morning and 1.56 mg/kg in the evening, PO). After 2 months, the dog owner consented to adipose-derived mesenchymal stem cell (AD-MSC) therapy, and 2 injections were given intravenously, 3 months apart.

The pre-AD-MSC treatment evaluation showed TRmax and SPAP of 4.28 m/s and 72.3 mmHg, respectively (Figure 2). The serum NT-proBNP and cardiac troponin-I (cTnI) levels were 4000 ρmol/L and 0.51 ng/mL (RI < 0.18 ng/mL), respectively (Table 1). On day 73, after the first AD-MSC injection, the dog was re-examined. Physical examination revealed an increased lean body weight (4.4 kg) and resolved ascites. Although the blood test showed azotemia from the mildly increased SDMA (15 µg/dL, RI: 0-14 µg/dL), creatinine (161 µmol/L, RI: 44-159 µmol/L), and blood urea nitrogen (25.1 mmol/L) levels (Table 1), the dog had a good appetite and did not show signs attributable to the azotemia. The serum NT-proBNP level was remarkably reduced (1673.4 ρmol/L), whereas the cTnI level was comparable to the previous pre-treatment reading (0.56 ng/mL) (Table 1). Besides, the cardiac silhouette was slightly reduced (VHS: 12.25) on chest radiography.

Consistently, echocardiography also demonstrated a reduced left cardiac dimension and improvement in left hemodynamics, as indicated by decreased trans-mitral E velocity and a lower ratio of mitral valve E wave velocity to E' wave velocity (E/E' values). Further, reduced pulmonary hypertension was observed, based on lower TRmax (3.64 m/s) and SPAP (53 mmHg) (Figure 2). On day 80 after the second AD-MSC injection, the dog showed normalized NT-proBNP (559.3 ρmol/L) and troponin-I levels (0.13 ng/mL) levels (Table 1), consistent with echocardiographic indicators of left ventricular filling pressure. The TRmax and SPAP also further decreased to 3.38 m/s and 45.7 mmHg, respectively (Figure 2).

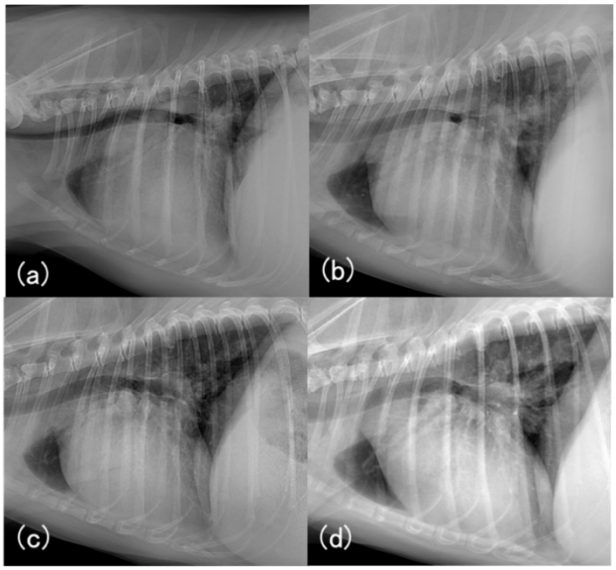

Figure 1. Right lateral view chest radiography in dog 1, taken (a) when right heart failure was diagnosed, (b) on day 73 post-AD-MSC 1, (c) on day 63 post-AD-MSC 2, and (d) on day 281 post-AD-MSC 2.
AD-MSC, adipose-derived mesenchymal stem cell

Day 281 after the second AD-MSC injection, the repeat Day 281 after the second AD-MSC injection, the repeat chest radiograph showed a reduced heart size (VHS: 11.75). Medications were not adjusted after the AD-MSC injections. The dog survived 475 days after the right-sided heart failure was diagnosed and passed away due to pancreatitis.

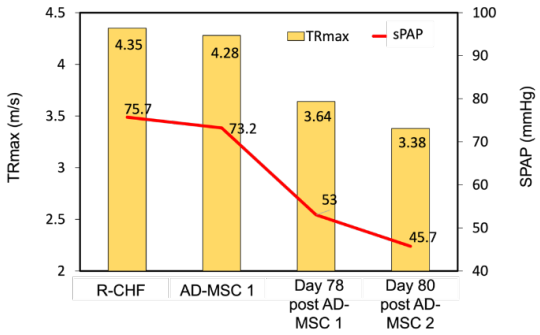

Figure 2. Graph which shows tricuspid regurgitation maximal velocity (TRmax) and estimated systolic pulmonary arterial pressure (SPAP) during right congestive heart failure (R-CHF) and pre- and post-adipose-derived mesenchymal stem cell (AD-MSC) treatment.

Table 2. Body weight, blood pressures, and echocardiography parameters pre- and post-AD-MSC treatment in dog 1.

Parameter	Unit	R-CHF	AD-MSC 1	Day 78post AD-MSC 1	Day 80 post AD-MSC 2
Body weight	kg	4.0	4.1	4.4	4.4
SBP	mmHg	-	125	152	158
DBP	mmHg	-	90	132	117
MAP	mmHg	-	104	137	120
IVSd	mm	7.6	7.5	7.6	8.3
LVIDD	mm	30.9	26.8	23.0	23.0
LVPWd	mm	5.1	7.2	7.0	8.0
IVSs	mm	12.0	11.2	8.3	9.6
LVIDS	mm	10.4	9.3	9.6	10.3
LVPWs	mm	10.0	11.8	9.5	10.9
FS	%	66	65	58	55
EF	%	94	94	90	88
LA/AO	-	1.98	1.59	1.58	1.64
MV E	m/s	0.99	0.67	0.66	0.56
MV A	m/s	1.15	1.13	0.99	1.23
MV E/A	-	0.86	0.59	0.67	0.45
DecT	ms	113	130	114	128
E/IVRT		2.2	2.3	1.5	1.4
E/e' lat	-	9.0	-	9.20	8.38
E/e’ sep	-	35.0	10.58	15.96	14.49
E/E’ avg	-	22.32	10.49	13.78	13.92
MRmax	m/s	5.08	5.70	5.70	5.80

R-CHF, right congestive heart failure; AD-MSC, adipose-derived mesenchymal stem cell; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; IVSd, interventricular septal thickness in diastole; LVIDD, left ventricular internal dimension in diastole; LVPWd, left ventricular posterior wall thickness in diastole; LVIDS, left ventricular internal dimension in systole; IVSs, interventricular septal thickness in systole; LVPWs, left ventricular posterior wall thickness in systole; FS, fractional shortening; EF, ejection fraction; LA/AO, left atrial to aortic root ratio; MV E, mitral valve E wave velocity; MV A, mitral valve A wave velocity; MV E/A, ratio of mitral valve E to A wave velocities; DecT, deceleration time; E/IVRT, ratio of E wave to isovolumic relaxation time; E/e' lat, ratio of mitral valve E wave velocity to lateral E' wave velocity; E/E' sep, ratio of mitral valve E wave velocity to septal E' wave velocity; E/E' avg, average ratio of mitral valve E wave velocity to the sum of lateral and septal E' wave velocities; MRmax, maximal mitral regurgitation velocity.

Case 2

A 5.5 kg, 12-year-old spayed female dog, previously treated for stage C MMVD, showed progression to stage D MMVD with concurrent PH and worsened coughing. It was treated with a high dose torasemide (0.27 mg/kg, every 12 hours, PO), pimobendan (0.45 mg/kg, every 12 hours, PO), spironolactone (1.14 mg/kg, every 24 hours, PO), sildenafil (1.8 mg/kg, every 12 hours, PO), and theophylline (5.7 mg/kg, every 12 hours, PO). On echocardiography, the TRmax and SPAP were 3.84 m/s and 59 mmHg, respectively, indicating moderate pulmonary hypertension. Besides, the left cardiac dimension was also severely dilated, and the estimated left ventricular filling pressure was increased (Table 3). The chest radiography (Figure 3) taken after resolution of pulmonary edema showed moderately increased cardiac silhouette (VHS: 12.25; vertebral left atrial score (VLAS): 3.0) and bronchial lung pattern, suggestive of chronic bronchitis. Hematology and serum biochemistry findings were unremarkable (Table 4). Given the disease severity, which suggested a poor prognosis for the dog, the owner agreed to AD-MSC treatment, consisting of 3 injections. The second injection was given 1 month after the first, whereas the third injection was given 3 months after the second.

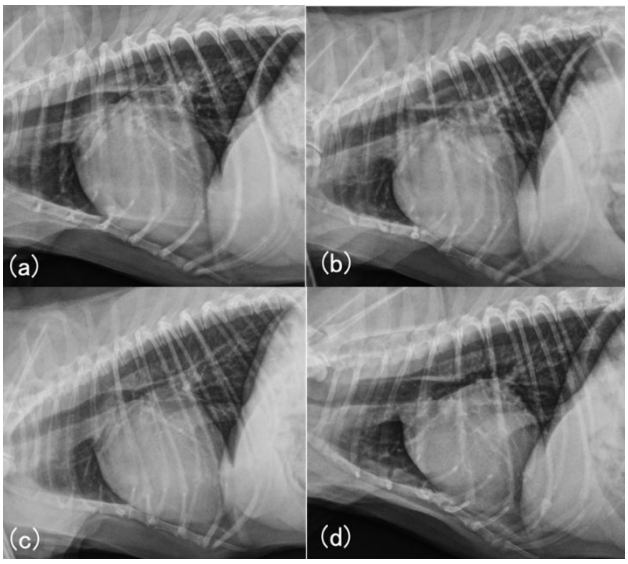

Figure 3. Right lateral view chest radiography in dog 2, taken (a) when stage D heart failure was diagnosed, (b) on day 28 post-adipose-derived mesenchymal stem cell (AD-MSC) 1, (c) on day 163 post-AD-MSC 2, and (d) on day 157 post-AD-MSC 3.

Table 3. Body weight and echocardiography parameters pre- and post-AD-MSC treatment in dog 2.

Parameter	Unit	Stage D MMVD	Day 20 Post-AD-MSC 1	Day 84 Post-AD-MSC 2	Day 63 Post-AD-MSC 3
Body weight	kg	5.9	5.8	6.3	6.3
LA/AO	-	2.73	2.32	2.21	2.08
IVSd	mm	7.0	7.4	6.4	6.3
LVIDD	mm	33.4	32.4	29.3	29.5
LVPWd	mm	7.4	7.2	7.0	6.4
IVSs	mm	11.4	11.6	9.1	9.8
LVIDS	mm	16.9	16.4	13.9	14.9
LVPWs	mm	10.8	10.2	11.2	10.0
FS	%	49	50	52	50
EF	%	82	82	85	82
MV E	m/s	1.28	1.14	1.01	0.93
MV A	m/s	0.63	0.69	0.9	0.8
MV E/A	-	2.2	1.66	1.11	1.15
DecT	ms	83	110	109	121
IVRT	ms	58	50	68	64
E/IVRT	-	2.2	2.28	1.48	1.44
MRmax	m/s	5.08	5.7	5.7	5.8
E/E’ lat	-	16.38	10.42	12.13	13.39
E/E’ sept	-	35	10.58	15.96	14.49
E/E’ avg	-	22.32	10.49	13.78	13.92

AD-MSC, adipose-derived mesenchymal stem cell; LA/AO, left atrial to aortic root ratio; IVSd, interventricular septal thickness in diastole; LVIDD, left ventricular internal dimension in diastole; LVPWd, left ventricular posterior wall thickness in diastole; LVIDS, left ventricular internal dimension in systole; IVSs, interventricular septal thickness in systole; LVPWs, left ventricular posterior wall thickness in systole; FS, fractional shortening; EF, ejection fraction; MV E, mitral valve E wave velocity; MV A, mitral valve A wave velocity; MV E/A, ratio of mitral valve E to A wave velocities; DecT, deceleration time; MRmax, maximal mitral regurgitation velocity; E/e' lat, ratio of mitral valve E wave velocity to lateral E' wave velocity; E/E' sept, ratio of mitral valve E wave velocity to septal E' wave velocity; E/E' avg, average ratio of mitral valve E wave velocity to both lateral and septal E' wave velocities.

Table 4. Selective serum biochemistry pre- and post-AD-MSC treatment in dog 2.

Parameter	Unit	RI	Stage D MMVD	Day 30 Post-AD-MSC 1	Day 84 Post-AD-MSC 1	Day 145 Post-AD-MSC 2	Day 157 Post-AD-MSC 3
Symmetric Dimethylarginine	µg/dL	0-14	12	10	16	13	14
Creatinine	µmol/L	44-159	117	145	117	114	142
Blood urea nitrogen	mmol/L	2.5-9.6	10.4	15	-	9.8	19.3
Alanine aminotransferase	U/L	10-125	56	49	-	78	52
Alkaline phosphatase	U/L	23-212	103	63	-	48	59

RI, reference interval; AD-MSC, adipose-derived mesenchymal stem cell.

After the first treatment, the dog showed significantly reduced coughing, an increased appetite, and an increased body weight (5.8 kg). A repeat examination on day 20 after the first AD-MSC injection showed no remarkable changes in hematology and serum biochemistry. The chest radiography showed mildly reduced cardiac silhouette (VHS 12.0; VLAS 3.0). On echocardiography, the left cardiac dimension was noticeably reduced. Improved left hemodynamics, particularly in left ventricular filling pressure and diastolic function, was indicated by reduced trans-mitral E wave velocity (E), a lower ratio of E wave to isovolumic relaxation time, and a decreased E/E' ratio. Additionally, the TRmax (3.47 m/s) and SPAP (48 mmHg) were mildly reduced. However, for 4 days after the second AD-MSC injection, the dog showed reduced appetite. On day 84 after the second AD-MSC injection, the dog's weight increased to 6.3 kg. The SDMA level was mildly increased (16 µg/dL) but the creatinine level (117 µmol/L) was within normal limits, and the dog was eating well. On echocardiography, the left cardiac dimension and hemodynamic showed further improvements, while the pulmonary pressure showed no remarkable changes (TRmax: 3.35 m/s; SPAP: 44.9 mmHg).

At the time of writing (644 days after the diagnosis of stage D MMVD), the dog was bright and alert and treated with a reduced dosage of torasemide (0.14 mg/kg in the morning, 0.20 mg/kg in the evening, PO), pimobendan (0.45 mg/kg, every 12 hours, PO), spironolactone (1.14 mg/kg, every 24 hours, PO), sildenafil (1.8 mg/kg, every 12 hours, PO), and theophylline (5.7 mg/kg, every 12 hours, PO). The latest blood test showed no remarkable changes on renal and liver parameters, whereas the cardiac silhouette was within normal limits (VHS: 10.5; VLAS: 2.0).

DISCUSSION

This study reports the long-term effects of AD-MSC therapy on two poodle dogs diagnosed with left heart failure due to MMVD. Despite standard polypharmacy, the dogs showed the development of PH, defined by an increase in estimated SPAP greater than 30 mmHg on echocardiography. In Case 1, the dog eventually succumbed to right heart failure, while in Case 2, the dog's MMVD further deteriorated to a refractory stage, necessitating a higher diuretic dosage to maintain vital signs, making it difficult to increase the sildenafil dosage. Besides, both dogs were also coughing, suspected to be attributable to concurrent bronchitis and/or progressive pulmonary fibrosis. Further evaluation for coughing with a chest CT scan was not performed due to the high risk associated with general anesthesia, and the results would not have made a difference in the outcome. Considering the AD-MSCs' properties of pulmonary tropism, anti-inflammatory, and anti-fibrotic properties (Kraitchman *et al.,* 2005; Zhao *et al.,* 2023; Wang *et al.,* 2019), AD-MSC therapy was recommended to the dog owners.

Currently, there are no established guidelines for the use of AD-MSC treatment for PH, particularly regarding dosage, frequency of injections, and intervals between injections. In this present study, injections containing approximately 5-million cells per kg of body weight, equivalent to 20 to 27.5 × 106 cells per total dose, were administered. The AD-MSC was given every 1 to 3 months, depending on the clinical response of the dogs, as MSCs can survive in the host body for durations ranging from a few hours to 90 days (Guest *et al.,* 2010). The administration interval was shortened if the dog exhibited increased coughing. In a recent study for the treatment of canine osteoarthritis, intra-articular injections containing 12 × 106 cells per dose were used (Kriston-Pál *et al.,* 2020). To promote AD-MSC homing and accommodate the larger size of the lungs compared to the joints, the concentration of the AD-MSC cells was increased in the dogs.

After the AD-MSC therapy, the dogs did not receive any immunosuppressive medications such as corticosteroids, as these drugs can be cytotoxic to the MSC in a dose-dependent manner (Wyles *et al.,* 2015). Although migration and localization of the AD-MSC post-injections were not tracked, to ensure that the beneficial clinical effects were truly attributable to the AD-MSC treatments, the background therapy for both dogs remained unchanged, except for reducing the diuretic dosage, which was deemed not to contribute to hemodynamic or clinical improvement. Therefore, the clinical improvement observed in the two dogs was believed to be directly attributable to the beneficial effects of the AD-MSC.

Serial examinations were performed to evaluate the dogs' health status and hemodynamics. In both cases, there was no exacerbation but improvement in PH. The TRmax and SPAP remained lower than the pre-treatment levels, with a more notable reduction in dog 1 compared to dog 2. Furthermore, the dogs' vital signs remained stable, and adjustment of sildenafil medication was not needed. Moreover, the dog in case 1 survived 475 days after the

onset of right heart failure without recurrence, whereas the dog in case 2 is alive at present, suggesting that AD-MSC treatment may be effective in controlling PH.

Surprisingly, improvements in left hemodynamic parameters, including LVIDD, LVIDS, E, E/E', and E/IVRT values, were observed in the dogs, independent of diuretic up-titration. These improvements indicated that AD-MSC treatment ameliorated left ventricular dimensions, contractility, and diastolic function. Both dogs, particularly Dog 2, demonstrated reduced left cardiac dilation and left ventricular filling pressure, which was further supported by a concurrent decrease in plasma NT-proBNP levels. Given that heart failure is associated with vascular damage and mesenchymal stem cells (MSCs) have been shown to have inhibitory effects on vascular damage, the improved left hemodynamics observed in the dogs could be attributed to the amelioration of endothelial dysfunction through the inhibitory effects of MSC therapy on vascular damage (Premer *et al.,* 2015). Additionally, a decrease in coughing in the dogs was noted post-treatment, probably due to the anti-inflammatory and immune-modulatory effects of the AD-MSCs (Zhao *et al.,* 2023).

With respect to side effects, both dogs showed self-limiting inappetence and mild fatigue after the second and third injections. However, from the blood tests, no remarkable changes were observed throughout the observational period, suggesting that the AD-MSC therapy is safe. Although not observed in the study, concerns of neoplasm after stem cell therapy (Castro-Oropeza *et al.,* 2020) have also been reported. This may require further investigation in a larger sample size. Nonetheless, an in-depth discussion about benefits versus risks with the pet owners is recommended before performing the treatment.

CONCLUSION

In this pilot study involving two client-owned poodle dogs diagnosed with PH secondary to hemodynamically significant MMVD, the AD-MSC treatment was found to be effective and safe. This therapy may be considered as a last resort when polypharmacy fails to stabilize vital signs, and neoplastic conditions have been ruled out. The findings of this study may offer valuable insights for designing further research with a larger sample size.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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