



Utility of arterial blood gas, CBC, biochemistry and cardiac hormones as evaluation parameters of cardiovascular disease in nonhuman primates

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ABSTRACT. Cardiovascular disease (CVD) has a tremendous impact on the quality of life of humans. While experimental animals are valuable to medical research as models of human diseases, cardiac systems differ widely across various animal species. Thus, we examined a CVD model in cynomolgus monkeys. Laboratory primates are precious resources, making it imperative that symptoms of diseases and disorders are detected as early as possible. Thus, in this study we comprehensively examined important indicators of CVD in cynomolgus monkeys, including arterial blood gas, complete blood count (CBC), biochemistry and cardiac hormones. The control group included 20 healthy macaques showing non-abnormal findings in screening tests, whereas the CVD group included 20 macaques with valvular disease and cardiomyopathy. An increase of red blood cell distribution width was observed in the CBC, indicating chronic inflammation related to CVD. An increase of HCO₃ was attributed to the correction of acidosis. Furthermore, development of the CVD model was supported by significant increases in natriuretic peptides. It is suggested that these results indicated a correlation between human CVD and the model in monkeys. Moreover, blood tests including arterial blood gas are non-invasive and can be performed more easily than other technical tests. CVD affected animals easily change their condition by anesthesia and surgical invasion. Pay attention to arterial blood gas and proper respond to their condition are important for research. This data may facilitate human research and aid in the management and veterinary care of nonhuman primates.

KEY WORDS: arterial blood gas, cardiac hormone, cardiovascular disease, complete blood count, nonhuman primate

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Cardiovascular disease (CVD) can be diagnosed using several blood exams, in addition to technical examinations such as echocardiography, X-ray, and electrocardiogram (ECG). Complete blood count (CBC), biochemical parameters, and arterial and venous blood gas measurements are major clinical diagnostic tools. Arterial blood gas is a particularly important parameter because the cardiovascular system is closely related to the respiratory system. In addition, the oxygenation of blood and the excretion of carbon dioxide and other gases can be determined directly from arterial blood gas. Diagnosis can be performed with each of the various exams; however, an accurate diagnosis can be made earlier when a combination of several exams is used. Arterial blood gas is the most sensitive diagnostic, and is often used during operative anesthesia. Since CVD often results in irreversible dysfunction, utilization of the arterial blood gas test to obtain earlier estimates of CVD parameters will add the utility of experimental animals for clinical and medical treatment.

In research of cardiovascular systems, nonhuman primates (NHPs) are especially appropriate for the development of the medical sciences [1, 2, 6, 26, 28]. The genetics, physiology and anatomy of NHPs are very similar to humans, and NHPs are susceptible to

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Table 1. Physical characteristics of the cynomolgus monkeys

	Sex	N	Age	Body weight
			Mean \pm SD	Mean \pm SD
Control	Male	3	28.67 \pm 4.03	6.12 \pm 1.61
	Female	17	19.76 \pm 6.49	4.02 \pm 0.70
DCM	Male	2	29.50 \pm 1.50	6.20 \pm 0.10
	Female	2	18.50 \pm 1.50	3.79 \pm 1.33
VD	Male	4	24.25 \pm 6.18	5.55 \pm 0.91
	Female	11	29.73 \pm 5.71	3.63 \pm 0.94

DCM, dilated cardiomyopathy; VD, valvular heart disease.

many of the same diseases [7, 12–15, 20, 22]. However, only partial information for many reference values is available for NHPs, thus medical researchers commonly rely on data from other animals (mice, rats, rabbits, dogs and cats) for use as reference values for humans in their studies.

Currently, common methods used to diagnose CVD include ECG, echocardiography and analysis of cardiac hormones. Echocardiography is a particularly sensitive technique to diagnose CVDs; however, it is highly technical and requires considerable skill. Laboratory primates are precious resources, thus, it is necessary to detect symptoms of diseases and disorders as early as possible. In medical research, a high skill level is required for observations and treatments, while simple and easy methods are important to ensure proper management of animals. This study aims to investigate the applicability of arterial blood gases and other blood exams as evaluation parameters. In the future, the increasingly common use of arterial blood gas as a measurement parameter will enable the management of animals with conditions such as CVDs. Additionally, clarification of the utility of the arterial blood gas test would allow its application to various studies such as stable surgical anesthesia.

The Tsukuba Primate Research Center (National Institutes of Biomedical Innovation Health and Nutrition) maintains approximately 1,400 cynomolgus monkeys in a closed environment for biomedical research, and is the largest experimental colony in Japan. The macaques are used for numerous research projects at our institution, including vaccinology, infectious disease, gene therapy, regenerative medicine, and aging studies. Furthermore, this colony is being used in advanced clinical studies from around the world [1, 2, 6, 13, 20, 26, 28].

Especially, proper management that referred to various blood test is necessary to obtain ideal results in medical science research for the CVD affected monkeys. From the viewpoint of animal welfare, these proper management is indispensable in using the experimental animals. Although it was known that CVD affected animals have high risk for anesthesia and surgical invasion, but there has been not reported in cynomolgus monkeys. This study is the first report to comprehensively examine important indicators of CVD in cynomolgus monkeys, including arterial blood gas, CBC, biochemistry and cardiac hormones. Thus, it is the first report to investigate the method of management for CVD affected monkeys from the findings of various blood test in normal and CVD affected monkeys. These results could be highly applicable to the stable management and maintenance of NHPs.

MATERIALS AND METHODS

Animals

Forty cynomolgus monkeys (10 years and older) were used for this study, all of which were bred at the Tsukuba Primate Research Center and housed under a consistent environment. Specifically, the animals were individually housed in stainless steel cages at 23 to 27°C, 50 to 70% humidity, 12 air changes/hr, and a 12/12-hr light/dark cycle, and were fed 70 g of commercial monkey chow (CMK-2; CLEA Japan, Inc., Tokyo, Japan) and 200 g of fruit daily. The 20 healthy monkeys (3 males, 17 females) in the control group were from 11 to 35 years old (Table 1), and received medical examinations in our facility. The control animals did not have abnormalities in chest auscultation, ECG, chest X-ray examination and echocardiographic examination. Additionally, no underlying diseases or abnormalities in appetite and behavior were observed in the control group. The CVD group included 20 monkeys (6 male, 14 female) from 17 to 35 years old (Table 1). The animals in the CVD group appeared normal, with no obvious abnormalities in appetite or behavior, and medical treatment was not required. However, the CVD group exhibited abnormalities in many parameters, including chest auscultation, ECG, chest X-ray examination, and echocardiographic examination. The CVD animals included 16 cases of valvular heart disease (VD), which is characterized by regurgitation and stenosis of the aortic valve and pulmonary valve (Fig. 1A), and 4 cases of dilated cardiomyopathy (DCM; Fig. 1B). However, clinical symptoms were not observed, indicating that the CVD grade was in the early to middle period. Based on the cardiovascular and pathological diagnoses, CVD animals were further separated into the VD group and the DCM group (Fig. 1). Additionally, the control group was conducted as age-matched controls, because the CVD group did not contain young animals less than 17 years old. Moreover, because of the characteristics of the breeding colony at our facility, there were a limited number of male monkeys included in this study.

This study was conducted according to the Rules for Animal Care and Management of the Tsukuba Primate Center, the Guiding Principles for Animal Experiments Using Nonhuman Primates formulated by the Primate Society of Japan and the Institute for Laboratory Animal Research (ILAR) Guide for Care and Use of Laboratory Animals [8–10]. The Animal Welfare and Animal Care Committee of the National Institutes of Biomedical Innovation Health and Nutrition (NIBIOHN, Osaka, Japan) approved the experimental protocol.

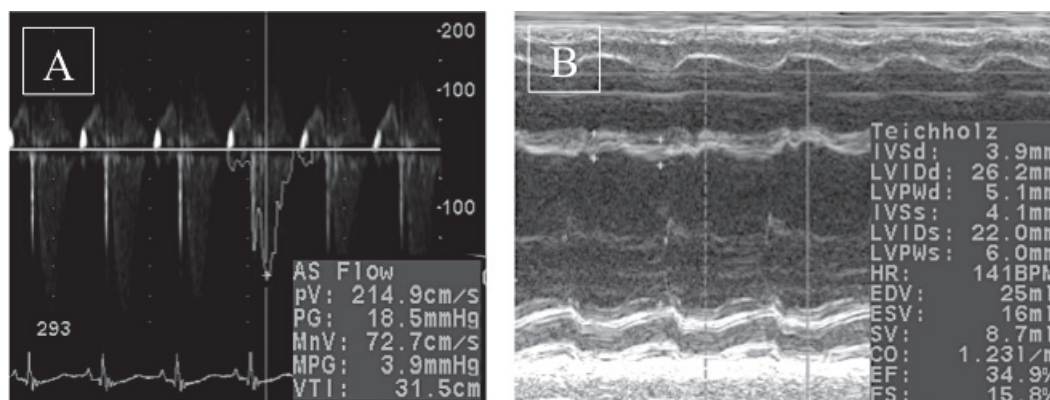


Fig. 1. Echocardiography of the nonhuman primate CVD model. Echocardiographs of aortic valve stenosis (A) and dilated cardiomyopathy (B) are shown. (A) The subject was 17 years old and weighed 2.11 kg. This animal was diagnosed with aortic stenosis because of the rapid turbulence in the aortic valve from stenosis. (B) The subject was 31 years old and weighed 6.1 kg. This animal was diagnosed with dilated cardiomyopathy, characterized by dilated ventricles and diffuse left ventricle hypokinesis. However, no abnormalities in behavior caused by CVD were observed in each animal. The macaque CVD model shows similar findings in echocardiography.

Blood sample collection

Blood samples were collected from the femoral or caudal artery while the monkeys were under ketamine sedation (5–10 mg/kg IM, Ketalar; Daiichi Sankyo Propharma, Tokyo, Japan). For blood sampling, the artery was identified by palpating the pulse and the needle was inserted into femoral artery. The collected blood was immediately examined for blood gases. SpO₂ values were used to confirm arterial blood gas and the blood gas values were recorded. CBC analysis samples were collected in K2-EDTA tubes. The human brain natriuretic peptide (hBNP) analysis was performed on chilled K2-EDTA, and human atrium natriuretic peptide (hANP) analysis was performed on aprotinin plasma. Biochemical analyzes were performed on serum samples.

Blood analysis

We analyzed arterial blood gas, CBC, biochemistry and cardiac hormones. An auto-analyzer (Rapid Lab 348; Siemens, Berlin, Germany) was used to evaluate blood gas parameters, including pH, pCO₂, pO₂, sodium ion (Na⁺), potassium ion (K⁺), calcium ion (Ca⁺⁺), HCO₃, base excess (BE), CO₂ concentration (ctCO₂), Ca⁺⁺(7.4) and O₂ saturation (SAT). The corrected HCO₃ (cHCO₃) and anion gap were calculated in reference to previous reports [15, 20]. CBC values were analyzed using a CBC auto-analyzer (KX-21; SYSMEX, Kobe, Japan). White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (Plt), red blood cell distribution width (RDW), platelet cell distribution width (PDW) and mean platelet volume (MPV) were determined. Biochemical parameters were analyzed using an auto-analyzer (AU480; Beckman Coulter, CA, U.S.A.). The biochemical parameters determined were total protein (TP), albumin (ALB), albumin-globulin ratio (A/G), blood urea nitrogen (BUN), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), C-reactive protein (CRP), serum creatinine (CRE), gamma-glutamyl transferase (GGT), chloride. The Na/Cl ratio was calculated from these values. The cardiac hormones evaluated were hANP and hBNP, which were analyzed using an auto-enzyme immunoassay device (AIA-360; Tosoh Corp., Tokyo, Japan).

Statistical analysis

A two-tailed Welch's *t*-test was employed to compare mean values obtained from the CVD and control groups. *P* values ≤0.05 were considered statistically significant. A one-tailed test was performed to compare the reactive items such as the cardiac hormone levels and CRP. A one-way analysis of variance (ANOVA) and Tukey's correction were used for analysis of three groups in this study. All statistical analyzes were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics [11].

RESULTS

The results of the blood analyzes of the control and CVD groups are presented in Tables 2–7.

Arterial blood gas

Several significant differences were observed in the disease group (Tables 2 and 3). Significantly higher pCO₂ levels were observed, and ctCO₂ showed a tendency to be increased. While HCO₃ was not significantly altered, cHCO₃ was significantly elevated. cHCO₃ has been used in human clinical medicine and is applied to the HCO₃ calculation formula. The electrolyte analysis

Table 2. Results of the arterial blood gas analysis

Unit	CVD			Control			Significant difference	
	Mean	Median	SD	Mean	Median	SD		
pH	7.40	7.40	0.06	7.41	7.41	0.04		
pCO ₂	mmHg	39.16	39.85	5.04	34.32	34.40	4.58	a)
pO ₂	mmHg	95.22	91.60	12.86	94.54	96.40	10.36	
Na ⁺	mmol/l	147.30	148.00	3.59	146.76	148.00	3.62	
K ⁺	mmol/l	4.01	3.93	0.54	3.67	3.65	0.45	a)
Cl	mmol/l	105.90	106.50	3.00	107.94	108.00	2.21	a)
Ca ⁺⁺	mmol/l	1.22	1.23	0.08	1.18	1.19	0.06	
HCO ₃	mmol/l	23.74	24.25	3.23	22.42	22.30	2.79	
BE	mmol/l	-0.78	0.05	4.11	-2.56	-2.60	3.42	
ctCO ₂	mmol/l	24.96	25.20	3.98	22.34	22.90	3.63	0.05
Ca ⁺⁺ (7.4)	mmol/l	1.22	1.21	0.07	1.19	1.19	0.06	
AG		17.67	16.45	4.44	16.40	17.40	3.73	
O ₂ SAT	%	97.03	97.00	1.09	97.18	97.20	0.77	
Na/Cl		1.39	1.40	0.03	1.36	1.36	0.03	a)
cHCO ₃	mmol/l	25.40	26.00	2.99	22.82	23.00	2.73	a)

a) $P < 0.05$.

Table 3. Results of the arterial blood gas analysis

Unit	DCM			VD			
	Mean	Median	SD	Mean	Median	SD	
pH	7.41	7.42	0.06	7.40	7.41	0.05	
pCO ₂	mmHg	37.30	35.25	6.61	39.57	40.30	4.59
pO ₂	mmHg	102.68	103.80	6.37	94.61	90.00	12.90
Na ⁺	mmol/l	141.75	141.50	1.48	148.33	148.00	2.09
K ⁺	mmol/l	4.20	4.51	0.54	3.90	3.77	0.47
Cl	mmol/l	104.00	104.00	2.55	106.13	107.00	2.85
Ca ⁺⁺	mmol/l	1.20	1.19	0.10	1.22	1.22	0.07
HCO ₃	mmol/l	23.50	23.90	2.66	24.13	24.90	3.19
BE	mmol/l	-1.30	-0.75	3.20	-0.23	0.80	4.11
ctCO ₂	mmol/l	24.13	23.15	3.53	25.49	25.70	3.99
Ca ⁺⁺ (7.4)	mmol/l	1.20	1.20	0.07	1.23	1.21	0.07
AG		14.25	14.70	1.09	18.07	17.10	4.35
O ₂ SAT	%	97.65	97.90	0.65	97.10	97.00	0.73
Na/Cl		1.36	1.36	0.04	1.40	1.40	0.03
cHCO ₃	mmol/l	21.75	21.50	3.03	26.20	26.00	2.20

indicated significant increases of potassium, chloride and the Na/Cl ratio. Additionally, there were significant increases of cHCO₃, pCO₂ and the Na/Cl ratio in the VD group (ANOVA). In particular, cHCO₃ showed clear differences (Fig. 2).

CBC

The results of the CBC analysis indicated that a few parameters were significantly altered in the disease group (Tables 4 and 5). Specifically, significantly increased RDW and a tendency for decreased MCHC were observed. In addition, ANOVA revealed significant differences in RDW (Fig. 3A), MCV (Fig. 3B), MCH (Fig. 3C) and MCHC (Fig. 3D). Further, there were significant increases in MCV, MCH and MCHC in the DCM group (Fig. 3B–D).

Biochemistry and cardiac hormones

The CRP value was increased in the CVD group (Tables 6 and 7). Analysis of cardiac hormones indicated that hANP was significantly increased in the CVD group. Although hBNP did not significantly differ, it showed a tendency to increase. Furthermore, in the results of the statistical analysis for the three group, the DCM group showed a significant increase in potassium, hANP and hBNP (Fig. 4A and 4B).

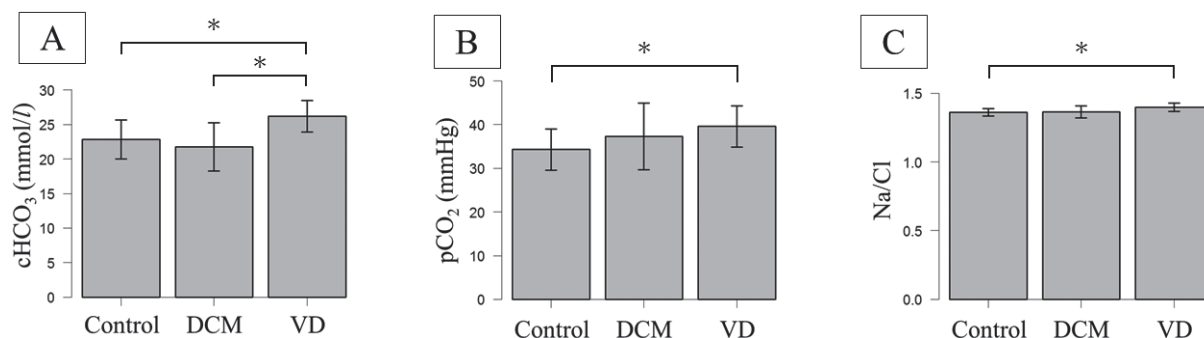


Fig. 2. Analysis of arterial blood gas parameters in CVD subgroups. cHCO₃⁻ and the Na/Cl ratio were especially elevated in valvular heart disease (A, B). Among heart disease groups, valvular heart disease is known to affect metabolism in particular. **P*<0.05, one-way ANOVA and Tukey's correction.

Table 4. Results of CBC analysis

Unit	CVD			Control			Significant difference
	Mean	Median	SD	Mean	Median	SD	
WBC 10 ² /μl	92.21	96.00	30.91	86.73	91.00	27.24	
RBC 10 ² /μl	687.53	674.00	89.62	673.55	673.50	93.04	
HGB g/dl	13.17	12.80	1.91	13.28	13.25	1.78	
HCT %	46.36	45.40	6.60	45.55	46.65	5.72	
MCV fl	67.59	67.00	5.59	67.83	67.75	2.86	
MCH pg	19.23	18.70	2.07	19.78	19.45	1.34	
MCHC g/dl	28.42	27.80	1.22	29.14	29.10	1.18	0.07
PLT 10 ⁴ /μl	33.25	31.70	8.50	33.53	30.95	13.16	
RDW fl	35.68	35.50	3.47	31.55	33.50	5.34	a)
PDW fl	11.72	11.60	1.52	11.82	11.00	2.03	
MPV fl	9.82	9.60	1.01	10.00	10.00	1.08	

a) *P*<0.05. WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; RDW, red blood cell distribution width; PDW, platelet cell distribution width; MPV, mean platelet volume.

Table 5. Results of CBC analysis

Unit	DCM			VD		
	Mean	Median	SD	Mean	Median	SD
WBC 10 ² /μl	108.25	109.50	37.50	85.64	95.50	26.89
RBC 10 ² /μl	594.25	626.00	80.31	715.14	711.00	76.12
HGB g/dl	13.10	14.00	2.58	13.23	12.80	1.73
HCT %	43.80	45.85	6.92	47.16	45.55	6.54
MCV fl	73.45	73.55	2.33	65.93	65.05	5.31
MCH pg	21.85	22.30	1.49	18.51	18.25	1.64
MCHC g/dl	29.73	30.45	1.47	28.09	27.80	0.89
PLT 10 ⁴ /μl	37.60	31.55	10.80	31.09	31.75	6.55
RDW fl	34.93	36.00	2.93	35.89	34.95	3.70
PDW fl	12.45	12.15	0.87	11.50	11.25	1.65
MPV fl	10.63	10.45	0.83	9.56	9.40	0.96

WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; RDW, red blood cell distribution width; PDW, platelet cell distribution width; MPV, mean platelet volume.

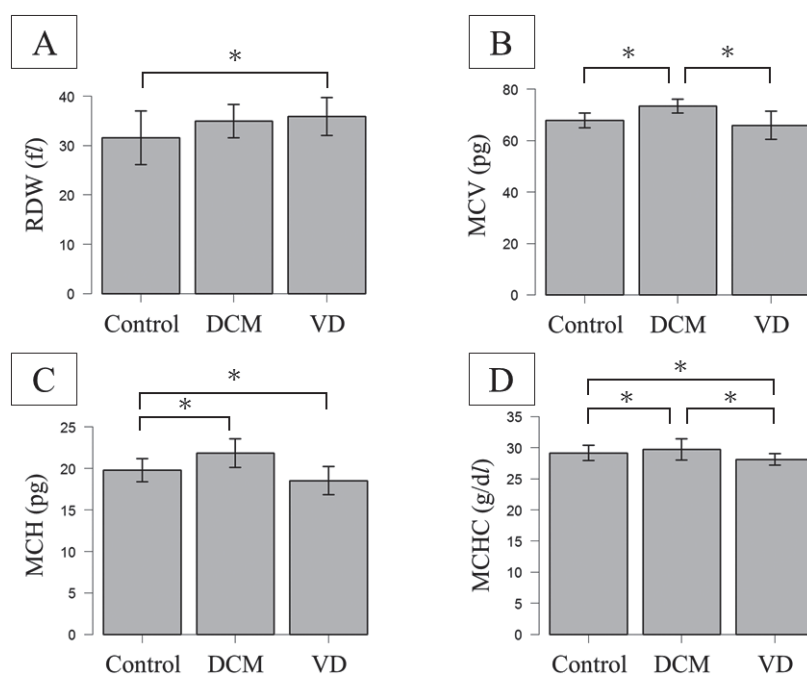


Fig. 3. Analysis of CBC parameters in CVD subgroups. Both CVD subgroups showed similar increases in RDW (A). MCHC was decreased in the VD group (D), and MCV and MCH were increased in the DCM group (B, C). * $P < 0.05$, one-way ANOVA and Tukey's correction.

Table 6. Results of biochemical analyses

Unit	CVD			Control			Significant difference
	Mean	Median	SD	Mean	Median	SD	
TP g/dl	7.30	7.40	0.58	7.35	7.25	0.53	
ALB g/dl	3.77	3.90	0.60	3.99	3.95	0.23	
A/G	1.12	1.10	0.33	1.22	1.22	0.19	
BUN mg/dl	16.18	15.75	3.72	16.40	15.65	2.98	
GLU mg/dl	70.30	64.50	26.72	60.25	60.00	19.18	
AST IU/l	49.65	49.50	14.18	46.65	44.00	14.02	
ALT IU/l	76.85	68.00	48.53	89.40	62.50	70.14	
CPK IU/l	449.40	185.00	685.89	240.45	219.50	111.13	
LDH IU/l	1,032.05	1,069.50	465.95	879.50	843.00	375.65	
CRP mg/dl	0.32	0.14	0.53	0.08	0.08	0.04	a)
CRE mg/dl	0.55	0.50	0.20	0.65	0.60	0.18	
GGT IU/l	73.00	70.00	30.27	60.33	56.00	19.92	
hANP pg/ml	78.24	58.15	104.70	27.12	24.40	9.34	a)
hBNP pg/ml	20.23	4.36	41.43	4.95	4.00	2.88	0.06

a) $P < 0.05$. TP, total protein; ALB, albumin; A/G, albumin-globulin ratio; BUN, blood urea nitrogen; GLU, glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatinine phosphokinase; LDH, lactate dehydrogenase; CRP, C-reactive protein; CRE, serum creatinine; GGT, gamma-glutamyl transferase; hANP, human atrium natriuretic peptide; hBNP, human brain natriuretic peptide.

DISCUSSION

Arterial blood gas

None of the subjects in this study exhibited clinical symptoms accompanying CVD, nor were signs of respiratory dysfunction observed. However, $p\text{CO}_2$ and ctCO_2 increased in the CVD group (Table 2). It is reasonable to think that the CO_2 levels were abnormal because the observed elevated values are also higher than the reported standard values [20]. We speculate that this might have been caused by pulmonary circulatory insufficiency, although this is not a clinical symptom, or the accumulation of liquid in the lung, or other unknown mechanisms. In addition, the increase of cHCO_3 likely indicates metabolic acidosis (Fig. 2A). In human medicine, cHCO_3 values have been used to accurately assess ionic dynamics when the anion gap is open [12, 25]. In this study,

Table 7. Results of biochemical analyses

Unit	DCM			VD		
	Mean	Median	SD	Mean	Median	SD
TP g/dl	7.00	7.10	0.81	7.35	7.40	0.49
ALB g/dl	3.25	3.20	0.65	3.87	3.90	0.50
A/G	0.95	0.94	0.35	1.15	1.11	0.32
BUN mg/dl	18.00	17.65	3.07	15.41	14.80	3.64
GLU mg/dl	86.75	91.50	28.70	64.53	62.00	24.45
AST IU/l	53.00	51.00	13.66	49.73	50.00	14.18
ALT IU/l	73.00	80.00	33.30	74.53	61.00	51.65
CPK IU/l	781.00	361.00	847.31	377.13	166.00	630.36
LDH IU/l	870.00	793.50	345.96	1,106.60	1,219.00	479.86
CRP mg/dl	0.69	0.23	0.87	0.24	0.14	0.36
CRE mg/dl	0.55	0.65	0.21	0.55	0.50	0.20
GGT IU/l	55.00	55.00	15.00	75.53	70.00	31.93
hANP pg/ml	215.63	172.80	166.92	41.73	44.40	28.61
hBNP pg/ml	68.67	28.25	70.93	4.95	4.00	2.12

TP, total protein; ALB, albumin; A/G, albumin-globulin ratio; BUN, blood urea nitrogen; GLU, glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatinine phosphokinase; LDH, lactate dehydrogenase; CRP, C-reactive protein; CRE, serum creatinine; GGT, gamma-glutamyl transferase; hANP, human atrium natriuretic peptide; hBNP, human brain natriuretic peptide.

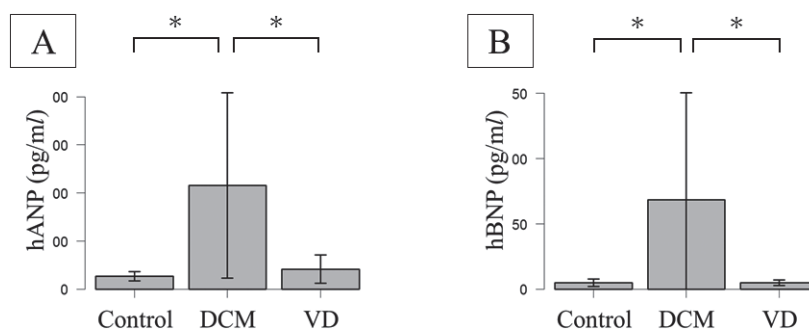


Fig. 4. Analysis of biochemical parameters in CVD subgroups. Compared with VD, DCM showed a clear increase in the hANP (A) and hBNP (B). This is related to the mechanism of cardiomyopathy, in which the disorder specifically occurs in the cardiac muscle. $*P < 0.05$, one-way ANOVA and Tukey's correction.

corrected HCO_3^- values were calculated for all subjects. Furthermore, the increase in the Na/Cl ratio (Fig. 2C) suggests activation of the renin-angiotensin-aldosterone system (Fig. 2C) due to deterioration of circulations, which is associated with cHCO_3^- increases.

CBC

RDW in the CVD group was significantly increased in this study (Table 4). This result suggests that the decay and regeneration of erythrocytes occurred due to decreases in the deterioration of erythrocyte membrane stability resultant from chronic circulation reduction. RDW has been reported in reference to anemia; however, it is also known that RDW increases in association with CVDs, and its relevance and influence on prognosis has been discussed in recent human clinical reports [4, 5, 16, 23]. This study also suggested that RDW was significantly increased in the CVD group, reflecting the same pathological conditions as humans. MCHC showed a tendency to decrease in the CVD group (Fig. 3D). Since RDW was increased (Fig. 3A), it was thought that the observed decreases in MCV, MCH, MCHC were also caused by the collapse and regeneration of erythrocytes as a result of a chronic increase in erythrocyte load (Fig. 3B–D) [5].

Biochemistry and cardiac hormones

We observed several significant differences in biochemical parameters and cardiac hormones. In the CVD group, the potassium value in the electrolytes increased (Table 6). This is the same result obtained from blood gas analysis, and is related to cardiac load [23]. A few nonspecific effects, such as increased CRP were confirmed in the CVD group. These results suggest that the CVD group was prone to infection. However, it remains difficult to determine whether or not this is due to CVD. This also suggests that the cause of these changes is related to the observed increase in RDW. In other words, underlying chronic inflammation may have

increased RDW, which signifies the presence of continuous intravascular inflammation [4, 16, 23]. Research on the relationship between RDW and CVD has been sparse, and the causal mechanism remains unknown. Thus, further research is necessary to elucidate the regulation of RDW in CVD [5]. In addition, the increase of CRP is suspected to involve other diseases such as myocardial damage. In humans and dogs, diagnostic applications to CRP of CVD were reported as high sensitive CRP in previous studies [19, 24]. These reports suggest that the findings of this study show the similar mechanism.

Cardiac hormone tests showed significant increases of hANP, while hBNP showed a tendency to increase in the CVD group (Table 6). It is known that these values increase in humans and other animals with CVD [18, 21], and these results support the applicability of these parameters in the health management of nonhuman primates. Since this study confirmed the results in the previous reports, the clinical value of hANP and hBNP levels as indicators of CVD was reaffirmed.

Insights derived from combining blood analyzes with pathological findings of CVD

The blood parameters evaluated in this study were analyzed according to the pathological condition of CVD (Tables 3, 5 and 7). The CVD group included subjects with valvular dysfunction (aorta valve regurgitation, pulmonary valve regurgitation, mitral valve regurgitation, tricuspid valve regurgitation, aorta valve stenosis, pulmonary valve stenosis, mitral valve stenosis, tricuspid valve stenosis) and cardiomyopathy such as DCM. As mentioned above, these animals did not exhibit behavior abnormalities and the grade of CVD was considered mild. However, pathological findings, echocardiography, X-ray examination and ECG revealed severe fibrosis and disruption of the myocardium (Fig. 1).

There were significant increases of cHCO_3 , pCO_2 and the Na/Cl ratio in the VD group (Fig. 2A–C). VD is commonly observed in elderly monkeys as well as in humans. These results suggest that metabolic and respiratory acidosis is associated with VD, and these symptoms have been reported in human VD [17]. This clearly demonstrates the effects on arterial blood gas in CVD, and the results of this study are highly applicable to disease diagnosis. Specifically, there are no reports of cHCO_3 increases related to CVD in macaques, thus, this result will be important for the management and veterinary care of nonhuman primates.

Analysis of the CBC parameters indicated differences of several erythrocyte indices among the CVD subgroups. Significant differences in RDW were observed between the control and VD groups (Fig. 3A). Additionally, there were significant increases in RDW and a variety of erythrocyte indices. MCV, MCH and MCHC were increased in the DCM group, which suggested the presence of macrocytosis. Additionally, there are significant decreases of MCHC in the VD group. The MCHC decreases indicate decline in HGB, suggesting that the underlying cause of the symptoms is chronic inflammation [23].

In examinations of biochemical parameters and cardiac hormones, there were significant differences in cardiac hormone levels between the VD and DCM groups although SD value was extremely broad. Significant increases were observed in hANP and hBNP (Fig. 4A and 4B), and increases in these hormones due to cardiomyopathy have been confirmed [18]. These findings are likely to relate to the physiological mechanism of these hormones, in which myocardial injury stimulates their production and release from the myocardium. Thus, cardiac hormone levels are expected to be elevated in cardiomyopathy as a result of the occurrence of myocardial damage. Furthermore, it is known that these cardiac hormones are increased in severe valvular heart disease [21, 27]. However, since the subjects used in this study had mild CVD, it is not unexpected that the cardiac hormone values did not fluctuate greatly. Scoring of the abnormal level is preferable to investigate, because the VD and DCM group showed bipolar data. In humans and dogs, methods of scoring the grade of CVD based on clinical symptoms and clinical laboratory findings have already been proposed and used in the classification by the New York Heart Association (NYHA) and International Small Animal Cardiac Health Council (ISACHC) [3, 19]. However, it was difficult to evaluate exercise intolerance, because all monkeys used in this study were kept in the cage, and clinical symptoms were not observed. Also, there are no reports that scoring the abnormal level of CVD in cynomolgus monkeys. Therefore, it will be important to consider each stage of CVD in future research.

In conclusion, the use of several blood tests in combination revealed a wealth of information regarding CVD in nonhuman primates relative to behavioral observations alone. Animals with CVD easily change their condition by anesthesia and surgical invasion. In this study, CVD group showed a drastic change in arterial blood gas and various blood biochemical tests. It was suggested that the combination of arterial blood gas and other biochemical tests are useful for CVD affected monkeys in laboratory. Careful handling is necessary to management of CVD affected monkeys as an animal welfare. Pay attention to arterial blood gas and proper respond to their condition are especially important for science research because it reflect the circulatory dynamics and respiratory conditions. Proper care makes it possible to carry out research that is safer and less invasive, and consequence better information will be given. Laboratory primates are precious resources, thus, the early detection of symptoms of diseases and disorders is imperative. The use of a combination of blood tests, including arterial blood gas, allowed the comprehensive management of animals to the same level as a technical exam. This study reports that specific blood test results were attributable to CVD. The results thus obtained will serve as the foundation for disease control in developing and breeding monkeys, as well as having utility as a substitute for human medicine and further development of human medical care.

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