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Association of Brain-Type Natriuretic Protein and Cardiac Troponin I with Incipient Cardiovascular Disease in Chimpanzees (*Pan troglodytes*)

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Cardiovascular disease (CVD) is the primary cause of morbidity and mortality in chimpanzees, but its etiology and clinical presentations remain poorly understood. The disease in chimpanzees differs sufficiently from that in humans that simple extrapolation from human findings are inadequate to guide clinical diagnoses. Nevertheless, the burden of disease posed by CVD made it important to attempt to identify specific chimpanzees at risk of developing CVD to allow clinical intervention prior to clinical presentation of advanced disease. We screened 4 CVD biomarkers used in human and veterinary medicine to identify markers with prognostic value in chimpanzees. Biomarkers included complete lipid panel, C-reactive protein, brain-type natriuretic protein, and cardiac troponin I. Serum levels of brain-type natriuretic protein differed between chimpanzees with CVD and heart-healthy controls. Cardiac troponin I gave mixed results. C-reactive protein and lipid panel values were not informative for cardiovascular disease, although total cholesterol, LDL-cholesterol, and triglycerides increased significantly with decade of life. Values of brain-type natriuretic protein exceeding 163 mg/mL had a specificity of 90.5% for CVD, whereas levels of cardiac troponin I above the threshold of detection (0.20 ng/mL) appeared to be clinically relevant. More extensive clinical studies are recommended to validate these specific values. We conclude that brain-type natriuretic protein and possibly cardiac troponin I are useful diagnostic biomarkers for incipient CVD processes in chimpanzees.

Abbreviations: BNP, brain-type natriuretic protein; CTnI, cardiac troponin I; CVD, cardiovascular disease; hsCRP, high-specificity C-reactive protein.

Cardiovascular disease (CVD) is the primary cause of chimpanzee morbidity and mortality.[50,51,65,75] All African apes seem to display a similar CVD pathology,[32,64] but CVD presents a somewhat different clinical picture in chimpanzees than in humans.[75] In chimpanzees, the most common pathology associated with CVD is similar to the structural remodeling seen in hypertensive human hearts. Little is known regarding the etiology of CVD in chimpanzees, and our understanding of its clinical manifestations and progression over time remain rudimentary. Cardiomyopathy (including left ventricular hypertrophy and dilated cardiomyopathy), valvular disease, and electrocardiographic abnormalities have been observed.[15,50] As in other species, the range of CVD that affects chimpanzees results in variable clinical signs and disease progression, which are dependent on the specific disease diagnosed. Moreover, determinants and risk factors for chimpanzee CVD remain poorly understood. The common pathologic similarity to human hypertensive disease suggests a potential role of blood pressure. Reliable reference values for normotensive and hypertensive blood pressure have been defined,[19] but the prevalence of hypertension and its long-term effects in chimpanzees remain unknown. Interestingly, although they have higher cholesterol levels than do humans,[18,19] chimpanzees rarely develop arteriosclerosis, only minor plaques have been observed, and myocardial infarction is rare.[49,75] Nevertheless, reliable lipid reference intervals are needed. Arrhythmias, specifically ventricular ectopy, appear to be a common clinical sign in the development of cardiomyopathy in chimpanzees.[15] In addition, cardiac murmurs are present with valvular heart disease.[15] Beyond these, there seem to be few clinical signs that reliably herald the development of cardiomyopathy or precede sudden cardiac death in chimpanzees.[50,51] Frequently postmortem chimpanzee hearts are characterized by varying degrees of myocardial fibrosis.[50,51,65,75]

Given the burden of disease posed by CVD and the lack of definitive signs preceding sudden cardiac death in chimpanzees, a method is needed that can identify subjects at risk of CVD or sudden cardiac death and allow focused monitoring and clinical intervention prior to the clinical presentation of advanced heart disease or death. Previous studies demonstrated that 2 biomarkers of fibrosis metabolism (procollagen III N-terminal protein and initial carboxyl-terminal telopeptide) in chimpanzee serum samples can be used to detect cardiovascular disease.[20] The objective of the present study was to assess the utility of several other CVD biomarkers in distinguishing chimpanzees with CVD from heart-healthy controls, for application in colony surveillance and clinical care.

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Materials and Methods

Animals. All chimpanzees at the study facility (Alamogordo Primate Facility, Holloman Air Force Base, New Mexico) are maintained in accordance with the *Guide for the Care and Use of Animals*.[41] The facility and its program are fully AAALAC-accredited. At the beginning of the study, the facility housed 229 chimpanzees. Subspecies affiliations were not entirely known, but previously uncharacterized African-born founders were determined to be *Pan troglodytes verus*, as expected.[19] Animals were maintained in same-sex group housing to comply with the NIH breeding moratorium.[42] As many as 6 chimpanzees were kept in each indoor den (180 ft^2, 9.5 ft high) with radiant heated floor and air conditioning, 24-h access to outdoor areas (242 ft^2, 12 ft high), and access to outdoor play yards (802 ft^2). All chimpanzees were observed every 2 h by veterinarians or experienced, AALAS-certified technicians for general health, activity levels, elimination, exercise tolerance, and recovery rates. Chimpanzees were fed a commercial primate diet (Monkey Diet Jumbo 5LR2, Purina Lab, Colombia, MO). The enrichment program involved daily fruits and vegetables, foraging opportunities, and provision of novelty devices and activities designed to simulate naturalistic conditions and promote species-typical behavior.

Clinical and cardiac assessment. All chimpanzees were sedated (2.5 mg/kg; Telazol, Wyeth, Ft Dodge, IA) and annually received a complete physical examination, including CBC and serum chemistry (Vetscan, Abaxis, Union City, CA), body weight, abdominal ultrasonography (Prosound 5000, Aloka, Tokyo, Japan), tuberculosis testing, and dental prophylaxis. Core body temperature, heart rate, oxygen saturation, blood pressure assessment, and electrocardiography were assessed by using a handheld data acquisition device (Datascope Passport 2, Soma Technology, Bloomfield, CT). Animals previously identified with structural cardiac abnormalities or suspected by our clinical veterinarians of developing CVD received cardiac examinations by a board-certified veterinary cardiologist. The cardiac exam included echocardiography (Prosound 5000, Aloka) using a 2.5-mHz sector transducer. Complete (including Doppler) serial echocardiography included assessment of diastolic left-ventricular wall thickness, diastolic and systolic left-ventricular internal diameters, aortic and left atrial diameters, and Doppler interrogation (color and spectral) of all valves. At the time of the study, 28 (12.2%) of all chimpanzees at the facility had clinically been identified as having structural cardiac abnormalities, including various forms of cardiomyopathy ($n = 8$), cardiac arrhythmias ($n = 10$), systemic hypertension ($n = 9$), and valvular heart disease ($n = 1$).

Experimental design. The current study had 2 components, the first of which was a clinically based analysis of severity of cardiovascular conditions. The clinical severity study involved assessment of 17 chimpanzees suspected by our clinical veterinarians as having CVD and scheduled for cardiac examination during a single 2-d visit by our consulting board-certified veterinary cardiologist (MMS). After evaluation, the cardiologist ordered animals by severity, as having normal cardiac structure, mild cardiac remodeling, or moderately severe cardiac remodeling. All animals were assayed for 2 biomarkers: high-specificity C-reactive protein (hsCRP) and cardiac troponin I (CTnI).

The second component was a case–control study, which was designed as a follow-up to the clinical severity study and expanded to include both a larger sample size and an expanded panel of biomarkers including brain-type natriuretic protein (BNP), hsCRP, CTnI, and complete lipid panel. The design included all 28 known CVD cases (22 male, 6 female) as well as 57 age-, sex-, and infectious-disease–matched controls ($n = 85$). All controls were selected from the group of chimpanzees that lacked any clinical signs of CVD and were presumed to be heart-healthy. These animals were assessed for 4 biomarkers: hsCRP, CTnI, BNP, and complete lipid panel.

Laboratory methods. Prior to each physical examination, 1 mL whole blood was drawn into an EDTA tube, shipped on ice by overnight courier to a nearby clinical reference laboratory (Tricore, Albuquerque, NM), and assessed for circulating levels of the 4 biomarkers using standard assays (CTnI: Immunolite DPC 2000, Siemens Medical Solutions, Malvern, PA; BNP: Axsym, Abbot Labs, Abbot Park, IL; hsCRP: Immulite 2000, Siemens Medical Solutions, Malvern, PA; lipid panel: Vitros CHOL, dHDL, and TRIG; Ortho-Clinical Diagnostics, Rochester, NY). LDL-cholesterol was estimated by the Freidewald equation.[26] Specific biomarkers were selected on the basis of their utility in the diagnosis and management of human CVD. Lipids were selected because they are classic risk factors for ischemic heart disease, and the ratio of total cholesterol to HDL-cholesterol is efficient in predicting heart disease in humans.[47] hsCRP was selected because it is an inflammatory protein accepted as one of the best biomarkers for estimating risk of ischemic heart disease in humans.[77] BNP was selected because it is released secondary to increased cardiac wall stress and frequently is elevated in diseases such as systemic hypertension,[55] valvular disease, rhythm abnormalities, and pulmonary hypertension;[6] BNP also been used successfully to identify the presence and severity of mitral valve disease and dilated cardiomyopathy in dogs.[61] CTnI was selected because it is a biomarker of myocardial necrosis and is released in association with cardiac arrhythmias in laboratory animals[60] and numerous other cardiac and extracardiac diseases.[2,78] Furthermore, both BNP and CTnI have been recommended for the study of the left ventricular remodeling stage of acute coronary syndromes associated with fibrogenic remodeling in humans.[76] CVD conditions expected to result in elevated levels of CTnI (cardiomyopathy and valve disease, but not hypertension or arrhythmias) were based on our cardiologists' experience and on epidemiologic associations gleaned from the human and veterinary literature.[10,30,36,40,54,58,59,69]

Statistical methods. All statistical modeling and testing were conducted with SYSTAT version 11(SYSTAT Software, Inc., Richmond,CA). Statistical significance was set at $P < 0.05$. Biomarker level data were evaluated for fit to normal (Gaussian) distribution by using the Shapiro–Wilks goodness-of-fit test ($P < 0.05$).[13,33,38] Box–Cox power series transformation [$x' = (x^{\lambda} - 1)/\lambda$] was used to normalize the data, as recommended,[11,33,38,66] by using a maximum-likelihood estimation procedure[9,33] on the full case–control data to estimate λ. Transformation efficiency was verified by the Anderson–Darling test and by coefficient-based tests.[33,38,52] Outliers were identified by the interquartile method[73] and Studentized residuals.[17] Many CTnI values (53% of the severity dataset, 62% of the case–control dataset) were below the assay's analytical threshold of detection (0.20 ng/mL). This situation is a special case of missing observations, which are common in datasets from a wide variety of disciplines including astronomy,[23] environmental toxicology,[53] failure time models,[45] hydrology,[12] immunology,[74] health care,[62] occupational health,[24] psychology,[31] public health,[46,81] and medical studies.[7] Datasets that include missing observations below a detection limit are called 'left-censored,'

whereas the missing values are referred to as 'nondetects.'[34] Analytical methods for handling left-censored data include the case deletion method, substitution methods, and data imputation.[66] The case deletion method simply excludes nondetects by restricting analysis to complete cases but is inefficient, results in biased parameter estimates, and has reduced power.[16,39,80] Substitution methods replace all nondetects with a fabricated constant (for example, 0, the mean, the detection limit, or half the detection limit) but introduce large amounts of bias into the resulting parameter estimates.[29,35,37] Data imputation is a Monte Carlo technique that uses characteristics of the observed data to generate plausible simulated values for missing data,[16,63] which are simply treated as random variability.[14,39,63,80] Data imputation methods are highly reliable, even with moderate amounts (50% to 60%) of missing data and violations of normality.[14,46,66,80] Censored data methods are reliable for as few as 10 to 20 observations and as much as 70% to 80% of nondetects.[3,29,44] An maximum-likelihood estimation procedure was used to produce robust unbiased estimates of the mean and standard deviation of the left-censored CTnI population distribution,[25] as recommended.[28,29,37,44,46,66] Imputed data generated from a log-normal distribution[67] having the estimated maximum-likelihood parameters were substituted randomly for all nondetects.[67] The number of imputed datasets, m, needed to yield an expected bias less than 5% was estimated as m = 6 by using the Schafer[63] formula for relative efficiency. Imputed datasets were evaluated for statistical significance by averaging.[21,44,63] Statistical analyses included ANOVA with the omnibus F-test, the nonparametric Kolmogorov–Smirnov 2-way test and Kruskal–Wallis one-way test, logistic regression for continuous data, and contingency table methods for categorical data (above or below the detection limit).[1,13,34,68] Statistical modeling included age and sex as covariates, because they are important determinants of the distribution of human and chimpanzee health and disease.[20,27] Biomarker sensitivity and specificity were estimated by using standard methods.[71]

Results

Clinical severity study. We investigated the association between 2 biomarkers, hsCRP and CTnI, and the severity of cardiovascular disease (normal, mild, moderate) in 17 chimpanzees clinically assessed for CVD abnormalities. ANOVA to test for differences in biomarker levels between severity groups indicated that hsCRP was not associated with disease severity ($F_{2,8} = 1.402$, $P = 0.301$). Furthermore, group means were inconsistent with the expected trend for hsCRP to increase with disease severity. Specifically, mild cases had relatively high median levels of hsCRP (1.1 mg/dL), whereas moderate cases (0.3 mg/dL) and healthy individuals (0.4 mg/dL) had lower (and nearly equal) levels of hsCRP.

For CTnI, analysis of imputed data showed no effect of either age ($F_{1,11} = 0.325$, $P = 0.580$) or severity ($F_{2,11} = 1.917$, $P = 0.223$). However there was a nonsignificant tendency ($0.05 < P < 0.10$) for median CTnI levels to be about equal between normal (0.12 mg/dL) and mild severity (0.13 mg/dL), and for both to be less that moderate severity (0.26 mg/dL). We cross-classified the CTnI data according to whether CTnI levels were below or above the threshold of detection, by using a disease severity dichotomy (normal or mild severity compared with moderate severity). Results indicated the presence of a significant association ($G^2_1 = 4.43$, $P = 0.035$) between disease severity and CTnI detection. Moderate CVD cases were 4 times more likely to have CTnI values above the threshold of detection, compared with mild cases or healthy animals.

Case–control study. Given the promising results of the clinical severity study, all 28 chimpanzees with known cardiovascular conditions and 57 control animals were evaluated for all 4 biomarkers (lipid panel, BNP, hsCRP, and CTnI). Results varied by biomarker, with some biomarkers showing a significant difference between cases and controls, others clearly not, and some intermediate (Table 1).

hsCRP showed no differences by sex ($F_{1,79} = 0.271$, $P = 0.604$) or decade of life ($F_{1,79} = 0.527$, $P = 0.592$). Case–control status verged on statistical significance ($F_{1,79} = 3.656$, $P = 0.059$) but was opposite to the expected direction, with CVD cases having lower hsCRP levels (0.6 mg/dL) than did heart-healthy controls (1.0 mg/dL).

Cases and controls showed no differences for any component of lipid panel. For total cholesterol, there was no effect of sex ($F_{1,81} = 1.806$, $P = 0.183$) or case–control status ($F_{1,80} = 0.057$, $P = 0.812$). Total cholesterol showed a highly significant effect of age ($F_{2,80} = 5.105$, $P = 0.008$), increasing linearly by decade of life, from the teens (192 mg/dL), to the 20s (207 mg/dL), to animals 30 or more years of age (229 gm/dL). For HDL cholesterol, there was no effect of age ($F_{2,80} = 0.133$, $P = 0.876$), sex ($F_{1,80} = 0.623$, $P = 0.432$), or case–control status ($F_{1,80} = 0.006$, $P = 0.941$). For LDL cholesterol, there was no effect of sex ($F_{1,80} = 0.594$, $P = 0.443$) or case–control status ($F_{1,80} = 0.098$, $P = 0.755$), but age was significant ($F_{2,80} = 4.063$, $P = 0.021$). As with total cholesterol, mean LDL cholesterol increased linearly by decade, from the teens (106 mg/dL), to the 20s (118 mg/dL), to animals 30 or more years of age (131 gm/dL). For triglycerides, there was no effect of case–control status ($F_{1,80} = 0.020$, $P = 0.889$), but both age ($F_{2,80} = 9.940$, $P < 0.000$) and sex ($F_{1,80} = 4.909$, $P = 0.030$) were significant. Female chimpanzees had significantly higher levels of triglycerides (103 mg/dL) than did male (82 mg/dL). As with total and LDL-cholesterol, there was a significant trend for mean triglycerides to increase linearly by decade, from the teens (70 mg/dL), to the 20s (80 mg/dL), to animals 30 or more years of age (101 gm/dL). For analysis of the ratio of total: HDL cholesterol, we deleted a single outlier ($z = 3.680$, $P = 0.00042$), due to the well-known relationship between chronic hepatitis infection and reduced levels of total and HDL cholesterol.[57,70] Subsequently, neither sex ($F_{1,79} = 0.094$, $P = 0.760$) nor case–control status ($F_{1,79} = 0.012$, $P = 0.914$) were significant. However, age was significant ($F_{1,79} = 3.422$, $P = 0.034$), and the ratio showed the same trend to increase across decade, from the teens (2.71), to the 20s (2.87), to animals 30 or more years of age (3.12).

BNP showed no significant differences by decade of life ($F_{1,80} = 0.671$, $P = 0.514$) or sex ($F_{1,80} = 0.107$, $P = 0.745$). In contrast, the case–control difference was highly significant ($F_{1,83} = 14.028$, $P < 0.000$) and explained 14.5% of the variance in serum BNP levels. Cases had an expected average of 100 pg/mL BNP (95% confidence interval, 87 to 114 pg/mL), or nearly twice the expected average of 55 pg/mL (95% confidence interval, 51 to 60 pg/mL) among controls (Figure 1).

Results were mixed for CTnI. ANOVA using the case-deletion method showed that neither age ($F_{1,29} = 0.196$, $P = 0.662$) nor case–control status ($F_{1,29} = 2.250$, $P = 0.144$) was significant. Analysis of the simulant datasets indicated that age was not significant ($F_{1,81} = 1.382$, $P = 0.243$). Case–control status approached but did not achieve significance ($F_{1,81} = 2.954$, $P = 0.090$), with CVD cases having slightly higher mean CTnI levels (0.15 ng/mL) than did

Table 1. Descriptive statistics (mean [95% confidence interval]) for CVD-related biomarkers by case–control status

	C-reactive protein	Brain natriuretic protein	Total cholesterol	Triglyceride	HDL cholesterol	LDL cholesterol	Total:HDL cholesterol	Cardiac troponin I
Cases	0.63 (0.42, 0.97)	100 (75, 134)	218 (200, 236)	90 (79, 104)	73 (67, 79)	122 (111, 135)	3.0 (2.8, 3.2)	0.18 (0.14, 0.23)
Controls	1.04 (0.70, 1.53)	61 (49, 77)	213 (197, 229)	91 (80, 107)	71 (62, 79)	119 (108, 132)	3.0 (2.7, 3.4)	0.14 (0.12, 0.17)

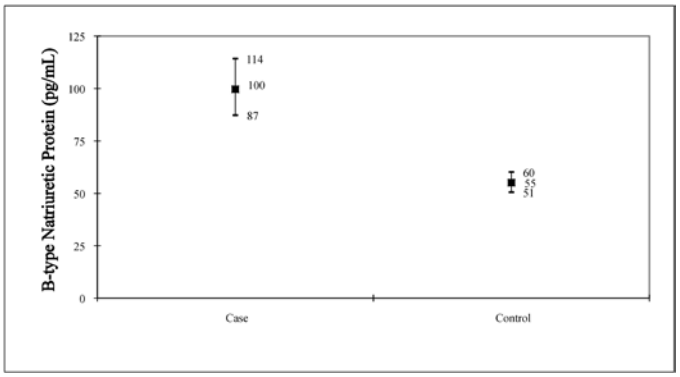

Figure 1. Estimated level (mean, 95% confidence interval) of brain natriuretic protein by case–control status.

healthy controls (0.11 ng/mL). Sex was highly significant ($F_{1,81}$ = 5.115, P = 0.026), with male chimpanzees having higher CTnI levels (0.17 ng/mL) than did female (0.10 ng/mL). Nonparametric analysis gave similarly nonsignificant results for case–control status (Kolmogorov–Smirnov T = 0.274, P = 0.109; Mann–Whitney U = 942.5, P = 0.177), although the trend was in the expected direction, with levels in CVD cases (0.20 ng/mL) higher than those in heart-healthy controls (0.14 ng/mL). Cross-classification of the CTnI data as above or below detection threshold revealed a highly significant association with case–control status (G^2_1 = 4.45, P = 0.035). Chimpanzees with detectable CTnI levels were 2.7 times more likely to be CVD cases rather than healthy controls, closely replicating results of the severity study. Furthermore, cross-classification of CTnI results by the CVD trichotomy defined by the expected effect in raising CTnI levels (healthy controls, arrhythmias or hypertension, cardiomyopathy or valvular disease) was even more highly significant (G^2_2 = 7.72, P = 0.021). Specifically, CVD cases with cardiomyopathy or valve disease were 4.8 times more likely to be above the threshold of detection for CTnI compared with CVD cases of hypertension and arrhythmias and 8.2 times more likely to exceed the threshold of detection than were healthy controls. But the equivalent ANOVA model on imputed data showed that the CTnI trichotomy was not significant ($F_{2,81}$ = 1.471, P = 0.284). The relatively small differences among group means (healthy, 0.11 ng/mL; hypertension and arrhythmias, 0.15 ng/mL; cardiomyopathy and valvular disease, 0.15 ng/mL) indicated a small effect size. These mixed results suggested that a real effect involving CTnI may have been obscured by unquantified subthreshold variation.

ANOVA was useful for detecting between-group differences but it effectively conceptualizes causation in reverse, because group membership (CVD cases, heart-healthy controls) was already known, whereas the goal was to identify biomarkers able to identify CVD status before it presents clinically. Therefore, logistic regression was used to predict disease status (CVD case, heart-healthy control) by using the 3 most promising markers (BNP, CTnI, hsCRP). Neither CTnI (t = –0.734, P = 0.463) nor hsCRP (t = 1.095, P = 0.274) were significant predictors of CVD status, but BNP was (t = –2.11, P = 0.034). This result corroborated the earlier ANOVA finding of elevated levels of BNP in CVD cases compared with controls.

Discussion

Despite chimpanzees' close phylogenetic and physiologic similarities to humans, cardiomyopathy presents clinically as a different type of disease in chimpanzees and may have different underlying disease etiology. Such clinical differences, as well as genetic variation between species, can make the search for informative CVD biomarkers in chimpanzee medicine unpredictable purely on the basis of overall phylogenetic similarity to humans.[4] Clinically, human heart disease is predominantly ischemic. In chimpanzees, CVD most commonly manifests as sudden cardiac death, with postmortem cardiac histopathology characterized by variable amounts of interstitial myocardial fibrosis.[50,51,65,75] Some of the chimpanzees in our CVD group were diagnosed with this form of CVD; other forms of heart disease were also represented. Neither CRP nor lipid panel significantly predicted the various forms of CVD. Results for CTnI were equivocal. Neither the clinical severity study nor the case–control study showed a significant quantitative effect, yet both showed a very strong categorical effect. BNP was unequivocally significant. This result was not surprising, given that circulating BNP levels increase secondary to increased cardiac wall tension and heart enlargement, and individual cases of heart disease with heart enlargement typically are defined as more severe than are cases without heart enlargement. Moreover, BNP is an antifibrotic peptide that, through its interaction with connective tissue growth factor, is related to both myocardial fibrosis and hypertension.[8,48] The missing link in the etiology of chimpanzee CVD may well be the lack of reliable reference values for defining hypertension.[19]

Methodologically, identification of informative CVD biomarkers in the case–control study benefited from a relatively large sample size. The ANOVA approach to between-group comparison allowed identification of BNP's usefulness in discriminating CVD cases from heart-healthy controls. The ANOVA results for BNP were confirmed by logistic regression modeling to predict case–control status. Contingency table modeling demonstrated that CTnI significantly distinguished CVD cases from heart-healthy controls. In-depth analysis revealed that specific forms of heart disease (cardiomyopathy and valve disease, but not hypertension) were associated with elevated levels of CTnI, as expected. Furthermore, ANOVA using multiple imputation methods to correct for missing CTnI data was marginally significant.

There is a pressing need to develop reliable reference intervals for BNP for clinical decision-making. The Harrell–Davis bootstrap method[79] gave a preliminary 90% reference interval for BNP

in healthy animals of 23 to 163 pg/mL. We tentatively suggest a value of 163 pg/mL (95th percentile) as the upper limit for heart-healthy animals. Higher BNP values would suggest the presence of CVD and the need for clinical evaluation by a cardiologist. At this level, sensitivity for CVD is relatively low (25.7%), but the specificity is quite high (90.5%). A cut-off at the 75th percentile (117 pg/mL) would give approximately the same specificity (92.9%) but would increase sensitivity to 42.9%. Regarding CTnI, we presently recommend that any observed value of CTnI above the detection threshold of the assay used here (0.20 ng/mL) be treated as an indicator of potential CVD in chimpanzees, as in humans.[5,22] Furthermore, given that the high threshold of detection for CTnI was problematic, it would be prudent to select an assay with greater analytical sensitivity, to avoid problems of missing subthreshold data.[37]

Several limitations of this study should be noted. The CTnI assay used had an analytical sensitivity above the threshold for humans and veterinary species in which it has been studied.[78] Therefore, some chimpanzees with heart disease may have had CTnI levels above the range for heart-healthy animals, but these elevations could not be detected with the CTnI assay used. Moreover, although we included 28 animals diagnosed with CVD, those cases had various underlying forms of CVD (including variable forms of cardiomyopathy, systemic hypertension, and valve disease). Because numbers were small (for example, one case of valve disease), these results might not be robust. CTnI is released secondary to myocardial cell necrosis, and elevation may be likely with a disease like myocarditis but may be unlikely with systemic hypertension.[60,76] Therefore, circulating CTnI levels are more likely to be diagnosis-dependent than is BNP, which is expected to be elevated with any disease leading to heart enlargement. Other important clinical risk factors for CVD, including advanced age (≥ 30 y old), male sex, and abnormal electrocardiographic patterns, require further study. CVD incidence can reasonably be expected to increase as chimpanzee populations age.

Finally, this study relied upon surrogate clinical endpoints, rather than on actual mortality and postmortem histologic confirmation of myocardial lesions. The 'gold standard' for assessing myocardial cellular damage is postmortem examination and histopathology, but these results were not available for comparison with biomarker findings. Mortality and postmortem histopathology are necessary to confirm that circulating levels of BNP, CTnI, or any other biomarker are reliable surrogate endpoints for CVD-related mortality.[43] Chimpanzees are a long-lived species, with an average age at death of 27.3 y, and only 3 of the 28 CVD cases (10.7%) have died since study inception. The high burden of CVD morbidity plus the species's longevity makes it unfeasible to simply wait to accumulate an adequate number of deaths for postmortem analysis; we believe that our interim data are worthwhile. Meanwhile, a larger prospective cohort study using surrogate end-points of clinically defined morbidity to validate these preliminary results and study other CVD biomarkers from the human epidemiologic and biomedical literature is in progress.

In summary, serum levels of BNP and CTnI are informative and potentially useful as biomarkers of incipient CVD in chimpanzees. We suggest routine analysis of BNP for chimpanzees suspected of developing CVD by using the suggested cut-off (163 pg/mL) as a clinical guideline. In addition, CTnI is likely to prove useful for animals with myocardial cellular damage. However, the assay used must be sufficiently sensitive to detect mild elevations at low levels of circulating CTnI.[56] Moreover, development of accurate reference intervals for healthy chimpanzees by using a current-generation, more sensitive CTnI assay is needed.[72] An ongoing prospective cohort study using a larger sample and additional biomarkers likely will yield more precise recommendations for the noninvasive diagnosis and prognosis of CVD in chimpanzees. Our long-term goal is to follow the natural course of the various heart diseases naturally occurring in chimpanzees and, by including postmortem analyses, ultimately obtain the most useful and accurate data to predict cardiovascular morbidity.

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