Prospetive Echocardiographic Assessment of Cardiac Structure and Function in Chinese Persons Living With HIV

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Background. Persons living with human immunodeficiency virus (HIV) are at increased risk of developing cardiovascular disease. Few studies have focused on echocardiographic abnormalities in the HIV population. We performed an echocardiography substudy of 325 PLWH.

Methods. China AIDS Clinical Trial 0810 is a prospective, multicenter cohort study of persons living with HIV (PLWH). We examined the prevalence of left ventricular systolic dysfunction (LVSD), diastolic dysfunction (DD), pulmonary arterial hypertension (PAH), and increased left ventricular mass (ILVM) in antiretroviral therapy (ART)–naive PLWH at baseline and week 48 after initiation of ART.

Results. Compared with age- and sex-matched healthy controls, PLWH had a higher prevalence of DD (16.5% vs 7.2%, P < .027) and a marginally significant higher prevalence of LVSD (7.3% vs 2.1%, P = .056). The increase in the prevalence of DD from baseline to week 48 in PLWH was marginally significant (P = .056). No significant difference was observed in the prevalence of LVSD, PAH, or ILVM at baseline and week 48 in PLWH. In logistic regression analysis of all participants, age was significantly associated with LVSD; HIV infection, age, and hypertension were associated with DD whereas HIV infection and hypertension were associated with ILVM at baseline. Logistic regression analysis of PLWH showed that only age was significantly associated with LVSD and DD.

Conclusions. The prevalence of echocardiographic abnormalities was significantly higher in ART-naive PLWH than in controls. HIV infection was significantly associated with cardiac abnormalities. No significant change in echocardiographic abnormalities was observed after 48 weeks of ART. Longer-term prospective studies are warranted.

Keywords. HIV; cardiac abnormalities; echocardiography; ART.
As part of this large cohort study, resting ECG was performed prior to ART initiation and after 48 weeks of treatment. Clinical, behavioral, and metabolic assessments were completed and data were entered into an electronic database and analyzed at Peking Union Medical College Hospital (PUMCH).

METHODS

Study Population

CACT0810 was conducted in clinical trial units located in 8 Chinese cities: Beijing, Shanghai, Guangzhou, Shenzhen, Fuzhou, Kunming, Zhengzhou, and Xian. ART-naive HIV-infected participants were recruited between January and November 2009. Participants between the ages of 18 and 65 years with documented HIV type 1 infection and CD4 count <350 cells/µL for >1 month were eligible for inclusion. Exclusion criteria included (1) acute HIV infection; (2) an active AIDS-defining illness; (3) pregnancy or breastfeeding; (4) injection drug use; (5) alcohol abuse; (6) severe psychiatric or neurologic disease; (7) neutrophil count <1.0 × 10⁹/L, hemoglobin <90 g/L, or platelet count <75 × 10⁹/L; (8) aminotransferase, alkaline phosphatase, bilirubin, amylase, or creatine kinase levels 3 times higher than the upper limit of normal (ULN) and serum creatinine levels 1.5 times higher than the ULN.

For the ECG substudy, participants with a documented history of CVD, cardiac symptoms, or abnormal baseline 12-lead ECG were excluded. The general condition of most participants was good. After baseline assessment, participants were treated with 2 nucleoside reverse transcriptase inhibitors (NRTIs) plus nevirapine (NVP).

During the same period, 97 age- and sex-matched uninfected controls were recruited in clinical trial units located in Beijing and Zhengzhou. The controls had no clinical evidence of vascular, metabolic, neoplastic, or inflammatory disease by history, physical examination, or routine laboratory testing.

The study protocol was approved by an independent ethics committee and the institutional review board of PUMCH. The trial was carried out in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical Data Collection

Clinical evaluation included a complete history and physical examination. Physicians responsible for recruitment collected data on symptoms and medical history, including cardiovascular risk factors and medication use. Behavioral risk factors including smoking and alcohol intake were obtained from self-report on direct questioning. Anthropometrical data were collected, along with blood pressure and a resting 12-lead ECG. The standardized mercury sphygmomanometers were used for blood pressure measurement in the study. Following the 1999 World Health Organization (WHO) hypertension guideline, 2 consecutive readings of blood pressure were taken on the right arm by trained staff after the participants had rested for at least 5 minutes [13].

Laboratory Data

Blood samples were drawn from each participant after an overnight fast on the morning of the study visit. Total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and glucose were determined by enzymatic methods using commercial kits. CD4⁺ and CD8⁺ lymphocyte cell counts were analyzed by 3-color flow cytometry (Beckman-Coulter, Brea, California). Whole blood was incubated with monoclonal antibodies against CD3, CD4, CD8, CD38, and HLA-DR (Immunotech, Marseille, France). Plasma samples were immediately frozen at −80°C and transported to the PUMCH central laboratory for viral load measurements using the COBAS AmpliPrep/COBAS TaqMan real-time reverse transcription polymerase chain reaction assay (Roche Diagnostics, Indianapolis, Indiana).

ECG Examinations

Transthoracic ECG was performed at all sites following the American Society of Echocardiography (ASE) guidelines [14]. Four structural and functional cardiac outcomes were evaluated: left ventricular systolic dysfunction (LVSD), diastolic dysfunction (DD), pulmonary artery hypertension (PAH), and increased left ventricular mass (ILVM).

LVSD was defined using ejection fraction calculated as the difference between end diastolic and end systolic volume, measured using quantitative 2-D (biplane Simpson) method, divided by end diastolic volume. A normal ejection fraction was >55%, mildly decreased 45%–54%, moderately decreased 35%–44%, and severely decreased <35% [14].

DD was assessed on the basis of values of peak early (E) and peak late (A) Doppler velocities, E wave deceleration time (EDT), and tissue Doppler imaging–derived myocardial early diastolic (E) tissue velocities obtained from the apical 4-chamber view at the lateral and septal annulus. Using the average septal and lateral E,, the E/E, ratio was computed. Diastolic function was categorized as normal; mild DD with reduced E/A ratio <1.0 and EDT >240 ms; moderate DD with increased E/A ratio >1.0, increased E/E, ratio >10, and EDT >140 ms; and severe DD with increased E/A ratio >1.5, increased E/E, ratio >10, and reduced EDT <140 ms [15–17].

PAH was defined based on pulmonary artery systolic pressure (PASP). PASP was estimated by Doppler ECG from the systolic right ventricular to right atrial pressure gradient using the modified Bernoulli equation (4 × V², V = peak tricuspid regurgitant velocity). Right atrial pressure (RAP) was estimated based on ECG characteristics of the inferior vena cava and assigned a standardized value [18]. None of the subjects had
significant right ventricular outflow tract obstruction. PASP = \(4 \times V^2 + \text{RAP} \text{ mm Hg}\). PAH was categorized as normal (15–30 mm Hg), borderline (31–35 mm Hg), mild (36–40 mm Hg), moderate (41–50 mm Hg), and severe (>50 mm Hg) [19, 20].

ILVM was defined based on left ventricular (LV) mass calculated using a standard, validated formula. End-diastolic measurements of left ventricular chamber diameter (LVIDd), interventricular septum thickness (IVSTd), and posterior wall thickness (PWTd) were performed. LV mass (g) = \(0.80(1.04(\text{IVSTd} + \text{PWTd} + \text{LVIDd})^3 - (\text{LVIDd})^3) + 0.6 \text{ g}\) [14, 21]. LV mass was analyzed after indexation to body surface area. ILVM was determined using partition values of \(\geq 110 \text{ g/m}^2\) after body surface area indexation, for which the prognostic value has been validated in the general population [22–24].

At each site, ECG was performed and interpreted by a single experienced technician blinded to HIV status. These ECG data were recorded on the software and transferred to PUMCH. To maintain standardization and quality control of data, staff at PUMCH provided guidance to technician staff at participating sites.

Statistical Analysis
Statistical analysis was performed using SPSS version 11.5 (SPSS Inc, Chicago, Illinois). Continuous variables were presented as mean and standard deviation (SD) or median. Viral load data were logarithmically transformed to obtain near-normality before analysis. Some data were not normally distributed, as determined with the Kolmogorov-Smirnov test. The Wilcoxon rank-sum test was used to compare variables, and \(\chi^2\) tests were used to compare categorical variables. For all analysis, each cardiac outcome was transformed into a dichotomous variable divided into “normal” and “abnormal” results. To investigate the impact of HIV and CVD factors on the prevalence of ECG abnormalities, logistic regression was performed. In univariate analysis, \(\chi^2\) test was used for categorical variables and Student \(t\) test was used for continuous variables. Significant variables \((P < .10)\) in univariate analysis were included in multivariate logistic regression models. We performed an additional logistic regression analysis only for PLWH to evaluate the association between HIV-specific factors, CVD factors, and ECG abnormalities. The model was built using the following variables: sex, age, current smokers, diabetes, hypertension, TC, LDL-C, HDL-C, history of opportunistic infections, CD4 cell count, HIV RNA, and percentage of CD8⁺CD38⁺ cells and CD8⁺HLA-DR⁺ cells. A \(P\) value of .05 was used to test for statistical significance and all tests were 2 tailed.

RESULTS
Participant Characteristics
Of the 745 participants referred to the CACT0810 study, 517 eligible participants were enrolled. Eighty-seven were excluded from the ECG substudy due to CVD history, cardiac symptoms, or an abnormal baseline ECG. Of the eligible 430 participants for the ECG substudy, 325 (75.6%) had ECG data available for analysis (Figure 1).
The characteristics of the PLWH and controls are shown in Table 1. The frequency of diabetes and current smoking was similar between the 2 groups. Hypertension was less prevalent in PLWH than in controls ($P < .001$). PLWH also had a lower body mass index ($P < .001$), possibly related to a long pre-ART disease history and malnutrition. TC, LDL-C, and HDL-C levels in the PLWH were lower compared with controls ($P < .05$). The mean log-transformed HIV RNA at baseline was 4.56.

Two hundred two PLWH were treated with stavudine + lamivudine + NVP, and 123 were treated with zidovudine + lamivudine + NVP. As expected, HIV RNA levels decreased after 48 weeks of ART. Only 6% of participants had HIV RNA >400 copies/mL at 48 weeks. CD4 counts increased from a median of 190 cells/µL (interquartile range [IQR], 106–259 cells/µL) to 312 cells/µL (IQR, 220–426 cells/µL).

Additionally, after 48 weeks of ART, the percentage of CD8$^+$ CD38$^+$ T cells and CD8$^+$HLA-DR$^+$ cells decreased significantly ($P < .05$).

### Prevalence of ECG Abnormalities in PLWH Compared With Controls

Twenty-four of 325 PLWH (7.3%) had LVSD. Most had mild LVSD (23 [7.0%]), and none had severe LVSD. The prevalence of LVSD in PLWH trended toward being higher than in controls (7.3% vs 2.1%, $P = .056$).

Of the 279 PLWH who underwent complete baseline DD evaluation, 46 (16.5%) met criteria for DD. Mild DD was detected in 29 participants (10.4%) and moderate DD in 14 participants (5%). Three had severe DD (1.1%). The prevalence of DD in PLWH was significantly higher than in controls (16.5% vs 7.2%, $P = .027$).
Because of limited access to accurate measurement of PASP, only 73 PLWH were included in the analysis. Two (2.7%) had borderline PAH, whereas no participants had moderate or severe PAH. The prevalence of PAH in PLWH was similar to that in uninfected controls (2.7% vs 7.3%, $P = .197$).

The prevalence of ILVM in the 298 PLWH who underwent baseline LV mass measurements was higher than in the control group, but the difference did not reach statistical significance (11.1% vs 5.1%, $P = .086$).

### Comparison of Baseline ECG Abnormalities With Data After ART
Comparison of baseline ECG abnormalities with data after 48 weeks of ART is depicted in Figure 2. The prevalence of LVSD in PLWH at week 48 was 9.8%. Most had mild LVSD (7.3%), 5 (2.1%) had moderate LVSD, and only 1 (0.42%) had severe LVSD. No significant difference was observed between the prevalence of LVSD at baseline and at week 48 (9.8% vs 7.3%, $P = .263$).

After 48 weeks of ART, 23.3% of PLWH had DD. The increase in the prevalence of DD from baseline to week 48 was marginally significant (23.3% vs 16.5%, $P = .056$).

At week 48, of 72 PLWH who completed PASP measurement, 3 (4.2%) had borderline PAH and 2 (2.7%) had mild PAH. The prevalence of PAH in PLWH at week 48 after ART did not differ significantly from baseline (6.9% vs 2.7%, $P = .238$).

After 48 weeks of ART, 15.6% of PLWH had ILVM. No significant difference was observed between the prevalence of ILVM at baseline and at week 48 (15.6% vs 11.1%, $P = .116$).

### Factors Associated With ECG Abnormalities at Baseline
In univariate analysis, factors associated ($P < .10$) with LVSD were age and HIV infection. Factors associated with DD ($P < .10$) were age, hypertension, glucose, and HIV infection.

### Table 2. Logistic Regression Analysis of Factors of Echocardiographic Abnormalities Among Study Participants and Controls

<table>
<thead>
<tr>
<th>Echocardiographic Abnormality</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.051</td>
<td>1.012–1.092</td>
<td>.011</td>
</tr>
<tr>
<td>DD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection</td>
<td>3.651</td>
<td>1.336–9.978</td>
<td>.012</td>
</tr>
<tr>
<td>Age</td>
<td>1.083</td>
<td>1.048–1.119</td>
<td>.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5.317</td>
<td>1.307–21.629</td>
<td>.02</td>
</tr>
<tr>
<td>ILVM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection</td>
<td>3.764</td>
<td>1.130–12.534</td>
<td>.031</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9.606</td>
<td>2.598–35.524</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; DD, diastolic dysfunction; HIV, human immunodeficiency virus; ILVM, increased left ventricular mass; LVSD, left ventricular systolic dysfunction; OR, odds ratio.
Factors associated with ILVM ($P < .10$) were age, hypertension, glucose, TC, and HIV infection.

As shown in Table 2, in multivariate analysis, age was the only risk factor associated with LVSD ($P = .011$). For DD, HIV infection, age, and hypertension were significant risk factors ($P < .05$), whereas HIV infection and hypertension were significantly associated with ILVM ($P < .05$).

We performed multiple logistic regression analysis in PLWH to evaluate the association between HIV and immune-specific variables and ECG abnormalities. Age was the only risk factor associated with LVSD (odds ratio [OR], 1.077; 95% confidence interval [CI], 1.026–1.131; $P < .01$). For DD, age was a significant risk factor (OR, 1.13; 95% CI, 1.081–1.181; $P < .001$). We did not find a significant association between hypertension and DD in PLWH; neither did we find significant association between hypertension and ILVM. No significant association was found between HIV RNA, CD4 count, percentage of CD8$, \text{CD38}^+$ T cells, or percentage of CD8$, \text{HLA-DR}^+$ cells and ECG abnormalities.

**DISCUSSION**

This study is the first large prospective multicenter cohort study evaluating the prevalence of cardiac abnormalities diagnosed by ECG in PLWH before and after initiation of ART.

ECG abnormalities associated with HIV have been described previously. In the pre–combination ART (cART) era, a large ECG study involving 952 asymptomatic PLWH found that 8% of subjects had dilated cardiomyopathy [25]. In the cART era, one of the largest ECG studies is the SUN study (Study to Understand the Natural History of HIV and AIDS in the Era of Effective Therapy), which enrolled 656 HIV-infected adults. In this study, the prevalence of cardiac abnormalities was greater than expected for age. However, because no suitable uninfected controls were available, the study used data reported in the literature for the general population as comparison group [6]. We were able to compare PLWH with age- and sex-matched uninfected controls. Moreover, the SUN study was cross-sectional and only performed ECG at one time point.

The pathogenesis of HIV-associated cardiac abnormalities is likely multifactorial. First, HIV may impair cardiac function by directly affecting both cardiomyocytes and myofibrils [26, 27]. Poznan et al [26] showed cardiac alterations in 83% of untreated HIV-infected patients’ necropsies. They detected cardiomyocyte apoptosis and ultrastructural damage, such as mitochondrialosis with increased dense bodies and reduction and disarray of myocardial myofibrils. Second, immune activation and inflammation due to HIV may lead to cardiac dysfunction. PLWH have high level of inflammation biomarkers, which may predispose them to cardiac dysfunction [28]. Monsuez et al [29] reported cardiac involvement with cardiomyocyte apoptosis in HIV infection primarily due to proinflammatory cytokines. Third, several studies suggest that cardiac dysfunction in PLWH is associated with ART [30, 31]. Fourth, traditional cardiovascular risk factors may contribute to cardiac dysfunction in PLWH. Currier et al showed that the prevalence of CVD in PLWH was increased by increasing age, diabetes, smoking, and hypertension [32, 33].

In this cohort of PLWH with low prevalence of traditional cardiovascular risk factors, the prevalence of ECG abnormalities is significantly higher than in uninfected controls. Multivariate logistic analysis show HIV is the significant risk factor of DD and ILVM. Thus, our results suggest that HIV may trigger mechanisms leading to cardiac dysfunction.

Immune activation due to HIV has been postulated as a contributing factor of CVD, although this remains controversial. However, we did not detect an association between immune activation markers, percentage of CD8$, \text{CD38}^+$ and CD8$, \text{HLA-DR}^+$ cells, with any ECG abnormality. Other HIV-specific factors including CD4 count and HIV RNA were also not associated with any ECG abnormality. The role of immune activation and control of HIV on cardiac abnormalities is unclear. Additional studies are needed to evaluate this further.

The role of ART in the development of CVD in PLWH remains unclear. Some studies suggest that ART may accelerate the onset of CVD in PLWH [30, 34–36]. NRTIs have been linked to adverse effects on cardiac function, possibly from mitochondrial toxicity [35]. On the other hand, other studies have shown that ART substantially decreases the degree of immune activation and inflammation caused by HIV and decreases the incidence of CVD [37]. In our study, the ART regimen consisted of 2 NRTIs plus NVP. No significant difference was observed between prevalence of LVSD and ILVM at baseline and at week 48 after ART. We found a trend toward an increase in the prevalence of DD after ART. However, no conclusion can be drawn regarding the role of ART on the development of ECG abnormalities from our study due to the relatively short duration of follow-up.

Traditional cardiovascular factors have been reported to contribute to CVD in PLWH, and dyslipidemia and diabetes have been regarded as CVD risk factors in general population. In our study, multiple logistic regression analysis in PLWH shows that only age was a risk factor for LVSD and that other CVD risk factors, such as hypertension, dyslipidemia, and diabetes, are not associated with ECG abnormalities. The reason might be that the PLWH in the study had low prevalence of CVD risk factors. The possible reason for low prevalence of dyslipidemia at baseline might be that many PLWH in this study had advanced HIV infection and low CD4 count. They usually had lower LDL-C concentrations, which is similar to findings from the other study [38].

There are some limitations to our study. One limitation might be the small sample size of PAH assessment. Even though
operators were trained and measurements were made in accordance with ASE guidelines, variability existed with capabilities between units. Several units could not accurately measure PASP and we did not have PASP data from these units for analysis. The sample size of PAH assessment was relatively small, limiting our ability to detect a difference between 2 groups. In addition, the prevalence of traditional CVD risk factors in PLWH was lower compared with other studies. The possible reason might be the exclusion of subjects with CVD history, cardiac symptoms, or abnormal ECG, who usually had higher prevalence of CVD risk factors. This may have actually resulted in an underestimation of cardiac abnormalities in PLWH. Furthermore, given that many adverse effects of ART may develop over a long period of time, the relatively short duration of follow-up may have impaired our ability to identify changes in cardiac structure and function. Longer-term prospective studies are needed. Finally, the cART regimen used in this study contained zidovudine or stavudine, both of which cause mitochondrial toxicity, and was not the first-line regimen recommended by WHO.

In conclusion, our data support the hypothesis that HIV can play a role in the genesis of cardiac dysfunction. No significant change in ECG abnormalities was noted after 48 weeks of ART, and longer prospective studies are warranted.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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