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HDL-C as a potential protective target against anthracycline-induced subclinical cardiotoxicity in DLBCL patients - an observational prospective study

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Keywords: cardiotoxicity; DLBCL; anthracycline; HDL-C; GLS

ABSTRACT

Objectives Anthracycline-induced cardiotoxicity is a debilitating cardiac dysfunction for which there are no effective treatments, making early prevention of anthracycline-induced subclinical cardiotoxicity (AISC) crucial. High-density lipoprotein (HDL) is a cardio-protective lipoprotein, but its impact on AISC remains unclear. Our study aims to elucidate the protective capacity of HDL in AISC in patients with diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP.

Design Observational prospective study

Setting An institution in China from September 2020 to September 2022.

Participant 70 chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to receive the standard dose of R-CHOP chemotherapy regimen.

Primary outcome measures Serum biomarkers including HDL-cholesterol (HDL-C) and apoprotein A1, 2D speckle tracking echocardiography, and conventional echocardiography were

measured at baseline, at the end of the 3rd and 6th cycle of R-CHOP, and 6 and 12 months after the completion of chemotherapy.

Results During the follow-up period, 24 patients experienced AISC, while 10 did not. 36 patients were censored. Higher levels of HDL-C were associated with a significantly lower risk of AISC (unadjusted, HR=0.24, P=0.006; adjusted for age and sex, HR=0.28, P=0.018; adjusted for age, sex, hypertension, BMI and E, HR=0.27, P=0.017). Patients without AISC had a more stable and higher HDL-C level during the follow-up period. HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients (P=0.034), and particularly in the AISC group (P=0.003). The highest level of HDL-C was significantly higher in patients without AISC than in those with AISC (1.52±0.49 vs. 1.22±0.29, P=0.034).

Conclusions Our study suggests that higher levels of HDL-C may be associated with a lower risk of AISC in DLBCL patients treated with R-CHOP. HDL-C could be a cardio-protective target, but further research is needed to confirm its benefits and limitations.

Trial registration number ChiCTR2100054721

Strengths and limitations of this study

- This study is the first observational prospective study that investigated the association between HDL-C and AISC, providing an opportunity for investigators to develop a tool for early intervention and prevention of AISC
- The study used advanced imaging techniques (2D-STE) to assess the subclinical cardiac dysfunction in the patients, which can provide more sensitive and accurate results compared to traditional echocardiography.
- The study only included patients with DLBCL who received R-CHOP, which may limit the generalizability of the findings to patients with other types of cancer or chemotherapy regimens.
- Additional studies are necessary to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in anthracycline-treated cancer patients.

INTRODUCTION

The improved management of cancer has led to a significant increase in the survival rate of cancer survivors(1). However, anthracycline, one of the most effective chemotherapeutic agents used to treat various cancers, is associated with potentially life-threatening and severe cardiovascular diseases(2). Studies have shown a significant increase in mortality in cancer patients with cardiovascular disease(3, 4). As advances in cancer treatment and an aging population continue, the number of patients with both conditions is rising(5). As a result, the field of cardio-oncology has become increasingly important in recent years.

Non-Hodgkin's lymphoma (NHL) is the 7th most common cancer in the United States and the most frequent hematologic malignancy globally, accounting for about 3% of cancer cases and deaths(6). Among NHL, DLBCL is the most prevalent type, representing approximately one-third of all cases(7). The combination of cyclophosphamide, vincristine, doxorubicin, and prednisone

with rituximab (R-CHOP) is a standard first-line therapy that has substantially improved survival outcomes in DLBCL patients(8). Nonetheless, anthracycline-containing chemotherapy agents are associated with cardiotoxicity, a major long-term adverse effect that significantly affects the quality of life and survival of cancer survivors.

Anthracycline-induced cardiotoxicity (AIC) is a devastating consequence of successful cancer treatment, often leading to hypokinetic cardiomyopathy and ultimately heart failure. AIC is an irreversible form of cardiac dysfunction for which no guidelines or accepted therapies for cardioprotection currently exist(9, 10). Therefore, early prevention and detection of AIC are crucial for providing opportunities for early intervention. Anthracycline-induced subclinical cardiotoxicity (AISC) is an early stage of AIC, characterized by abnormal echocardiography index without clinical symptoms(11). Early intervention is recommended by the 2022 International Cardio-Oncology Society (IC-OS) consensus statement once AISC is detected(12). Global longitudinal peak systolic strain (GLS) measured by 2D speckle tracking echocardiography can reliably identify most early myocardial deformation variations. In our study, we used early measurement of GLS to identify AISC(13, 14).

High-density lipoprotein (HDL) is the sole lipoprotein with protective attributes among the five types of lipoproteins. Its salutary effects include antioxidant, anti-inflammatory, and anti-apoptotic properties. Numerous preclinical investigations have suggested that HDL may have direct and indirect protective effects against AIC(15-17). However, clinical data on the relationship between HDL and anthracycline-related cardiotoxicity are currently limited.

We undertook an observational prospective study to investigate the potential impact of HDL on AISC. Using 2D speckle tracking echocardiography, we identified AISC and sought to establish any correlation between HDL and AISC. Additionally, we assessed the fluctuations in HDL-cholesterol (HDL-C) levels during R-CHOP chemotherapy in chemotherapy-naïve patients recently diagnosed with DLBCL.

METHODS

Study population

We recruited chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to receive the standard dose of R-CHOP chemotherapy regimen at our institution from September 1st, 2020, to September 1st, 2022. Our inclusion criteria were as follows: newly diagnosed DLBCL, age between 18 and 80 years; both sex (male and female), Eastern Cooperative Oncology Group (ECoG) performance status $\leqslant$2, left ventricular ejection fraction (LVEF) $\geqslant$50%, and acceptable bone marrow, renal, and hepatic functions for chemotherapy. Conversely, our exclusion criteria were symptomatic heart failure, a history of myocardial ischemia, myocarditis, myocardial infarction, clinical or subclinical pericardial effusion, arrhythmia requiring medical intervention, a history of other cancers, under lipid-lowing treatment, and severe active infections such as syphilis, hepatitis, or human immunodeficiency virus (HIV) infection.

Treatment

Patients received a total of 6 cycles of standard R-CHOP (cyclophosphamide at 750 mg/m^2 on D1, doxorubicin at 50 mg/m^2 on D1, vincristine at 1.4 [maximum 2] mg/m^2 on D1, and 100 mg prednisone on D1-5, with rituximab at 375 mg/m^2 on D1 in each cycle), with or without 2 cycles of rituximab maintenance (rituximab at 375 mg/m^2 on D1 in each cycle). None of the patients in our study received lipid-lowering treatment during the follow-up period.

Definition of subclinical cardiotoxicity

According to the IC-OS consensus statement, the definition of subclinical cardiotoxicity was a relative GLS decrease from baseline [(baseline – current GLS)/baseline GLS] of >12%, but with a normal left ventricular ejection fraction (LVEF)(12).

Study protocol

This is an observational prospective study. The study was registered in the Chinese Clinical Trial (Approval NO. ChiCTR2100054721). The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (Approval NO. 2018-016). And all participating patients provided written informed consent.

At baseline, the end of the 3rd cycle of R-CHOP, the end of the 6th cycle of R-CHOP, and 6 and 12 months after chemotherapy completion, all enrolled patients underwent conventional echocardiography, 2D speckle tracking echocardiography, and blood sampling. Demographic data and clinical variables, including age, gender, body mass index (BMI), ECoG performance status, diabetes mellitus, hypertension, drinking history, and smoking history were collected at the time of enrollment. Left ventricular systolic dysfunction was measured by LVEF, fractional shortening (FS), left ventricular mass index (LVMI), left ventricular diastolic dimension (LVDd), E, e', E/e', and GLS. HDL-C, ApoA1, low-density lipoprotein cholesterol (LDL-C), cardiac troponin T (cTnT), high sensitivity C-reactive protein (hsCRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), total cholesterol (TC) and total triglyceride (TG) were measured. We used the baseline HDL-C level as a surrogate marker for HDL quantity. The patients were categorized into two groups based on the average HDL-C value for males and females in the modified criteria of the National Cholesterol Educated Program Adult Treatment Panel (NCEP ATP III)(18). High HDL-C was defined as a serum HDL-C≥1.16mmol/L, while low HDL-C was defined as a serum HDL-C<1.16mmol/L.

Statistical analysis

The study was conducted with two aims: firstly, to evaluate the relationship between HDL and AISC; and secondly, to conduct a preliminary exploration of the differences in HDL-C and the variability of HDL-C changes between patients with and without AISC during the follow-up period. Continuous variables were expressed as mean± standard deviation (SD) and compared using the t-test. Non-normally distributed variables were presented as median (Q1- Q3) and compared with the Wilcoxon Mann-Whitney test. Categorical variables were expressed as n (%) and compared using the Chi-square or Fisher's exact test, as appropriate. Correlation analysis was conducted to investigate the associations of change in HDL-C with change in GLS. Multiple hypothesis tests were performed using the Bonferroni adjustment. The probabilities of survival

were calculated using Kaplan-Meier methods and compared using Log-rank tests. Cox proportional-hazards regression models were conducted to assess the association between variables and AISC. Covariates for multivariable Cox regression models included age, sex, and variables that had a P-value of less than 0.15 in the univariable Cox regression analysis (GLS was excluded as it is the factor that defines AISC). Two multivariable Cox regression models were constructed: the first model included age and sex; and the second model included age, sex, hypertension, BMI, and E. Statistical analysis and visualization were performed using IBM SPSS V.22.0 and GraphPad Prism 8. X-Tile software was conducted to calculate the cut-off point of ApoA1(19), with the optimal cut-off value (the lowest P-value under the log-rank test) being 1.02g/L. All statistical tests were two-sided, with a P-value less than 0.05 being considered statically significant.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

RESULTS

Assessment of the association between HDL-C and AISC

Study population and baseline characteristics

This investigation enrolled a total of 70 patients with chemotherapy-naïve DLBCL and were planned to be treated with the standard R-CHOP regimen. Based on the baseline HDL-C level, we segregated the patients into two groups: the high-level group (HDL-C$\geqslant$1.16mmol/L, n=28) and the low-level group (HDL-C<1.16mmol/L, n=42). Patients with drinking history had a greater chance of having a high HDL-C level (P=0.034). The patients with high HDL-C showed substantially higher total cholesterol (P=0.011), lower total triglyceride (P=0.002), and higher ApoA1 (P<0.001). The baseline characteristics of the patients in both groups were well balanced (Table S1).

High HDL-C was an independent protective target of anthracycline-induced subclinical cardiotoxicity

The clinical endpoint was defined as the first detection of AISC, and the median survival time of the whole cohort was 16 months. The median survival time of patients with low HDL-C was 4 months, while that of patients with high HDL-C was not reached. The median follow-up time of the cohort was 10 months. During the follow-up period, 24 patients experienced AISC, while 10 did not. Approximately half of the patients (n=36) were censored. A flowchart detailing the patients enrolled in the study and the reasons for censored patients can be found in Figure S1.

The log-rank test revealed that patients with higher HDL-C were less likely to experience AISC (P=0.001, HR=0.26, 95%CI: 0.12-0.58) (Figure S2a). However, there were no significant differences in AISC occurrence between different ApoA1 levels (P=0.123, HR=0.54, 95%CI: 0.20-1.45) (Figure S2b).

According to the results of the univariable Cox regression analysis, variables that had a P-value of less than 0.15 including age, BMI, hypertension, GLS, E, and HDL-C group. Increasing age was significantly associated with a decreased HR of 0.97 (95%CI 0.943-0.998, P=0.034) per 1-year increase. BMI showed a HR of 1.09 (95% CI 0.97-1.22, P=0.139) per 1 kg/m2 increase. Similarly, hypertension had a HR of 0.22 (95% CI 0.03-1.62, P=0.136) for yes versus no. A lower GLS was significantly associated with an increased HR of 1.46 (95% CI 1.20-1.77, P<0.001) per -1% decrease. E velocity showed a HR of 1.03 (95% CI 1.00-1.06, P=0.075) per 1 cm/s increase. The HDL-C group (high versus low) had a significantly lower HR of 0.24 (95% CI 0.09-0.67, P=0.006). Further details about other variables can be found in Table S2.

The results of the multivariable Cox regression analysis showed that high HDL-C was significantly associated with a lower risk of AISC after adjusting for age and sex (model 1) (HR=0.28, 95%CI:0.10=0.84, P=0.018). Similarly, after adjusting for age, sex, and variables that P<0.15 in the univariable Cox regression analysis (excluding GLS as it defines AISC) (model 2), the same association was observed (HR=0.27, 95%CI: 0.09-0.79, P=0.017). In contrast, ApoA1 did not show any influence on AISC in either of the two regression models (P=0.401 and P=0.237). (Table 1)

Table 1. Outcomes of study participants.

	HR (95%CI) (unadjusted)	P values	HR (95%CI) (adjusted*)	P values	HR (95%CI) (adjusted#)	P values
Low HDL-C	Ref		Ref		Ref	
High HDL-C	0.24 (0.09-0.67)	**0.006**	0.28 (0.10-0.80)	**0.018**	0.27 (0.09- 0.79)	**0.017**
Low ApoA1	Ref		Ref		Ref	
High ApoA1	0.54 (0.22-1.29)	0.165	0.66 (0.26-1.73)	0.401	0.54 (0.19-1.50)	0.237

The endpoint was defined as the first detection of anthracycline-induced subclinical cardiotoxicity.

Low HDL-C: HDL-C<1.16mmol/L; High HDL-C: HDL-C≥1.16mmol/L. Low ApoA1: ApoA1<1.02g/L; High ApoA1: ApoA1≥1.02g/L. *Adjusted for age and sex. #Adjusted for age, sex, hypertension, body mass index, E. HR, hazard ratio; HDL-C, high-density lipoprotein-cholesterol, ApoA1, apolipoprotein A1.

Preliminary exploration of the difference of HDL-C between patients with AISC and without AISC

Study population and baseline characteristics

In this analysis, we selectively included 34 of the enrolled patients who remained uncensored. The patients who exhibited AISC at any time during the follow-up period were segregated into the AISC group (n=24), while those who did not demonstrate AISC were classified into the NO-AISC group (n=10). Patients within the AISC group were comparatively younger (50±12.45 vs. 59.7±9.67, P=0.035) and exhibited a higher baseline GLS [22.0 (21.0, 22.8) vs. 18.0 (17.0, 20.0), P<0.001]. More baseline information can be seen in Table S3.

Timeline of HDL-C level in patients with and without AISC

Figure 1 displays the timeline of HDL-C levels in patients with and without AISC. In Figure 1a, the patient population was categorized into four groups based on the time of AISC detection.

Among the groups, 12 patients were identified with AISC at the end of the 3rd cycle of chemotherapy, 7 patients at the end of the 6th cycle, 3 patients at 6 months after treatment completion, and 2 patients at 12 months after treatment completion. With the exception of the group in which patients detected AISC at the end of the 3rd cycle of chemotherapy, all other groups exhibited a reduction in HDL-C values from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy. Figure 1b portrays the HDL-C level in patients without AISC, indicating that the HDL-C level was more stable than in patients with AISC. Moreover, the overall HDL-C level was higher in patients without AISC than in patients with AISC throughout the follow-up period (Figure 2a). In Figure 2b, there was a significant decrease in GLS during the chemotherapy period (from 0-4 months), which remained stable after completion of chemotherapy (after 4 months) in patients with AISC.

Based on Figure 2, we observed that the fluctuations in HDL-C and GLS were most pronounced during the chemotherapy period. The fluctuations in HDL-C levels of patients with DLBCL during R-CHOP chemotherapy were presented in Figure 3. The levels of HDL-C significantly increased for all patients from baseline to the end of the 3rd cycle of chemotherapy (P=0.012) and significantly decreased from the end of the 3rd cycle to the end of the 6th cycle of chemotherapy (P=0.034) (Figure 3a). Patients with AISC showed a significant decrease in HDL-C levels during R-CHOP chemotherapy from the end of the 3rd cycle to the end of the 6th cycle (P=0.003) (Figure 3b). However, no significant difference was observed in HDL-C levels for patients without AISC during R-CHOP chemotherapy (Figure 3c). We conducted correlation analysis separately for the change in HDL-C and GLS from baseline to after 3 cycles of chemotherapy, from baseline to after 6 cycles of chemotherapy, and from after 3 cycles to after 6 cycles of chemotherapy. However, we found no statistically significant differences in the associations between changes in HDL-C and GLS (P=0.965, 0.087, 0.449).

Contrasting values of HDL-C parameters between patients with and without AISC

Figure 4 presents the contrasting values between patients with AISC and those without in terms of four parameters, namely the highest and lowest levels of HDL-C during chemotherapy, the increment and decline in HDL-C values from baseline. Patients without AISC showed significantly higher values in the highest level of HDL-C (1.52±0.49 vs. 1.22±0.29, P=0.034, Figure 4a). However, no significant differences were observed between the two groups in terms of HDL-C increment from baseline to the highest value (0.31±0.31 vs. 0.22±0.23, P=0.386, Figure 4b). While the lowest level of HDL-C was lower in patients with AISC, the difference was not statistically significant (0.84±0.16 vs. 1.03±0.41, P=0.182, Figure 4c). Furthermore, there were no significant differences in HDL-C decline between patients with AISC and those without (0.16±0.20 vs. 0.18±0.26, P=0.777, Figure 4d).

DISCUSSION

This prospective observational study investigated the relationship between HDL-C and incidence of AISC in 70 patients with DLBCL who were receiving anthracycline-containing chemotherapy. The study found that higher levels of HDL-C were associated with a lower incidence of AISC.

Moreover, patients without AISC had more stable and higher levels of HDL-C than those with AISC during the follow-up period. The results also showed that HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients, especially in the AISC group, indicating that anthracycline-containing chemotherapy has adverse effects on HDL-C levels. Notably, the highest level of HDL-C was significantly higher in patients without AISC compared to those with AISC. These findings suggest that HDL-C may have a protective role against AISC in DLBCL patients undergoing anthracycline-containing chemotherapy and maintaining a relatively high level of HDL-C may be more effective in managing cardio-protection than monitoring changes in HDL-C levels over time. The results of this study highlight the importance of early serum lipid management in these patients.

Lipoproteins are classified into five categories, namely chylomicron, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), based on their size, density, and lipid composition (cholesterol and triglycerides)(20). Among these, HDL exhibits distinctive cytoprotective actions and triggers anti-oxidative, anti-inflammatory, and anti-apoptotic effects. The protective roles of HDL in cardiovascular disease have been controversial in recent years, and that the quality of HDL (cholesterol efflux capacity, antioxidant activity, anti-inflammatory activity, endothelial function, etc.) rather than the quantity of HDL has been proposed as the true cardioprotective effect. The Framingham Heart Study, as early as 1988, reported a correlation between HDL-C and cardiovascular mortality(21). Recent studies have challenged the HDL-C hypothesis by revealing that HDL-C level is not inversely correlated with cardiovascular diseases(22, 23). In our study, we used the baseline HDL-C level as a surrogate marker for HDL quantity, but we did not directly measure the quality of HDL. Measuring the level of HDL-C in serum is a commonly used method to assess the effect of HDL on cardiovascular health. HDL facilitates the transportation of cholesterol from the body's tissues back to the liver, and higher levels of HDL-C are generally associated with a lower risk of heart disease. Nevertheless, it's crucial to note that HDL-C levels may not accurately reflect the functional properties of HDL. ApoA1, the most abundant protein in HDL, is associated with several beneficial effects of HDL(15, 24). The function and abundance of ApoA1 are reported to play a dominant role in HDL quality(25). In the context of AIC, several studies have indicated that HDL can protect against anthracycline-induced cardiomyocyte apoptosis and atrophy in isolated cardiomyocytes(26, 27) and animal models(16, 27). Based on these earlier trials, HDL-C and ApoA1 could serve as protective factors against anthracycline-related cardiovascular disease. However, our study results indicate that there was no significant association observed between ApoA1 and AISC in patients with DLBCL who were treated with R-CHOP. It's possible that factors other than HDL function, such as genetics or inflammation, may affect ApoA1 levels. Furthermore, we must acknowledge that our study was observational in nature and, therefore, cannot establish causality. Additionally, other unmeasured factors may have contributed to the observed relationship between HDL-C levels and AISC risk.

As far as we know, few clinical studies have investigated the association between HDL-C and AISC. This study is the first clinical trial that utilizes the IC-OS consensus statement(12) to define subclinical cardiotoxicity, with univariate and multivariable analyses being used to identify the influential factors of AISC in DLBCL patients in this cohort. Kaplan-Meier methods and Log-rank tests reveal that patients with high HDL-C levels were less likely to develop AISC. After subjecting it to univariate and multivariable Cox regression methods, high HDL-C levels still showed statistically significant differences. These results suggest that high HDL-C could be a potentially independent protective factor for AISC in DLBCL patients and provide an opportunity for investigators to develop a tool for early intervention and prevention of AISC. Further research is necessary to confirm our findings.

Several studies have demonstrated that serum lipid levels are altered during anthracycline-containing chemotherapy in cancer patients(28, 29). Huxley et al and Averina et al have shown that imbalanced serum lipid distribution is a risk factor for cardiovascular disease(30, 31). As a result, anthracycline-containing treatment can induce dyslipidemia and facilitate the occurrence and development of cardiovascular diseases in cancer patients. In a study of 394 breast cancer patients, Xin et al found that HDL-C levels after chemotherapy were significantly lower than those before chemotherapy(32). Similarly, Lu et al and Hana et al found that HDL-C levels were significantly decreased during anthracycline-containing chemotherapy in patients with breast cancer(33, 34). In our study, we specifically assessed the changes in HDL-C levels over time during follow-up. Except for the group of patients who experienced AISC at 12 months after treatment completion, HDL-C levels in all other groups increased from baseline to the 3rd cycle of chemotherapy. This phenomenon may be due to the fact that anti-tumor drugs require cholesterol to cross cell membranes(35). However, HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients, especially in the AISC group, which is consistent with previous research results(32-34), and further confirmed that anthracycline-contained chemotherapy has adverse effects on HDL-C levels in DLBCL patients. The HDL-C level in patients without AISC was more stable than that in patients with AISC. Therefore, anthracycline-containing chemotherapy may promote the occurrence and development of cardiotoxicity in DLBCL patients by inducing HDL-C turbulence. Besides, the findings of our study indicate a significant decrease in GLS during the chemotherapy period in patients with AISC. This result is consistent with previous research, which has reported that doxorubicin dose at the range of 100-150mg/m^2 can cause cardiotoxicity(36). Notably, we also observed that GLS remained stable after completion of chemotherapy, suggesting that the cardiac effects of anthracycline-based chemotherapy may be dose-related. These findings have important implications for the monitoring and management of cardiotoxicity in patients undergoing anthracycline-based chemotherapy, as early detection of cardiac dysfunction during treatment may improve patient outcomes. We investigated the associations of change in HDL-C with change in GLS, no statistically significant differences were found.

The analysis of HDL-C levels should not only consider the changes over time, but also the absolute values. In our study, patients without AISC had significantly higher absolute highest HDL-C levels

than those with AISC, while the absolute lowest HDL-C levels did not differ significantly between the two groups. The alterations from HDL-C extremes to baseline did not exhibit any variation between the groups either. This suggests that the highest absolute HDL-C value was a preferable indicator of AISC protection than the change in HDL-C from baseline to the extremum value. Maintaining a relatively high level of HDL-C may be more effective in managing the cardio-protection of anthracycline-treated cancer patients than monitoring changes in HDL-C levels over time.

In our investigation, we observed that among the four patients with pre-existing hypertension, only one patient experienced AISC during the follow-up (Table S3). Although there was no statistically significant difference, it appears that patients with pre-existing hypertension were less likely to experience AISC. Hypertension, a common risk factor for both cancer and cardiovascular diseases, was also recognized as a risk factor for cardiotoxicity. Studies have reported that pre-existing hypertension was associated with anthracycline-and trastuzumab induced left ventricular ejection fraction (LVEF) decline in a retrospective study(37), and early left ventricular systolic dysfunction in patients with lymphoma receiving (R)-CHOP in a prospective study(38). However, in our study, multivariable Cox regression analysis showed that hypertension had no influence on AISC ($P>0.05$). However, in our investigation, multivariable Cox regression analysis showed that hypertension did not have a significant impact on AISC ($P>0.05$). We noted that all patients with hypertension were under a single antihypertensive drug regimen (beta-blockers or ACEI/ARB) to manage their blood pressure. Two meta-analyses have demonstrated that beta-blockers and ACEI can prevent cardiotoxicity caused by chemotherapy (39, 40). We speculate that the protective effects of beta-blockers and ACEI/ARB may have contributed to the result observed in our study regarding the relationship between hypertension and AISC.

There are several limitations to our study that must be acknowledged. Firstly, while our study highlights the potential importance of HDL-C in managing AISC, additional studies are necessary to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in anthracycline-treated cancer patients. Secondly, this is a single-center observational prospective study with a medium sample size. To confirm our findings, a larger sample size study conducted at multiple centers is needed. Thirdly, previous studies have suggested that there may be a reversed U-shaped relationship between HDL-C levels and cardiovascular diseases(41). Due to the small sample size of this study, we didn't further investigate the influence of extremely high levels of HDL-C on cardiotoxicity, and further clinical studies should be done to verify it. However, due to the small sample size of our study, we did not investigate the potential impact of extremely high HDL-C levels on cardiotoxicity. Further clinical studies are required to explore this issue. Besides, the measurement of GLS was only taken at baseline and at several points throughout the chemotherapy treatment and follow-up period. It is crucial to extend the duration of follow-up in future research to obtain a more comprehensive understanding of the long-term effects of anthracycline treatment on cardiovascular health.

CONCLUSIONS

In conclusion, our observational prospective study suggests that higher levels of HDL-C may be associated with a lower risk of AISC in patients with DLBCL treated with R-CHOP chemotherapy. HDL-C levels remained stable and consistently higher in patients without AISC compared to those with AISC. Additionally, the highest absolute HDL-C value was found to be a preferable indicator of AISC protection. These findings suggest that HDL-C may be a potential cardio-protective target for managing AISC in this patient population. However, further research is needed to confirm and expand on these findings, including determining the optimal HDL-C level for cardio-protection and the potential benefits of early serum lipid management.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang; Data curation, Wenxin Ou, Tiantian Jiang, Yue Weng and Xi Zhou; Formal analysis, Wenxin Ou and Tiantian Jiang; Funding acquisi-tion, Wenxin Ou, Nan Zhang, Dong Wang and Xiaoqiong Tang; Investigation, Dong Wang, Qian Dong and Xiaoqiong Tang; Methodology, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang; Resources, Dong Wang, Qian Dong and Xiaoqiong Tang; Supervision, Qian Dong and Xiaoqiong Tang; Validation, Nan Zhang and Kai Lu; Visualization, Wenxin Ou and Tiantian Jiang; Writing – original draft, Wenxin Ou; Writing – review & editing, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang. Qian Dong and Xiaoqiong Tang contributed equally to this work and are considered as co-corresponding authors.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

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FIGURE LEGENS

Figure 1. (**a**) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after treatment completion. 2 patients were detected AISC at 12 months after treatment completion. (**b**) Timeline of HDL-C levels of patients without AISC.

Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (**a**) and global longitudinal strain (GLS) (**b**) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

Figure 3. (**a**) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (**b**) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (**c**) Changes of HDL-C in patients without AISC.

Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (**a**) Highest level of HDL-C during chemotherapy. (**b**)The HDL-C value increment from baseline to the highest value. (**c**) The lowest level of HDL-C during chemotherapy. (**d**) The HDL-C value declined from baseline to the lowest.

Figure S1. Flow diagram of patients enrolled in our observational research and reasons for censored patients.

Figure S2. Kaplan-Meier curves of the percentage of patients without AISC in patients stratified by HDL-C level (**a**) and ApoA1 level (**b**). High HDL-C: HDL-C≥1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L. High ApoA1: ApoA1≥1.02g/L. Low ApoA1: ApoA1<1.02g/L. AISC, anthracycline-induced cardiotoxicity; ApoA1, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio.

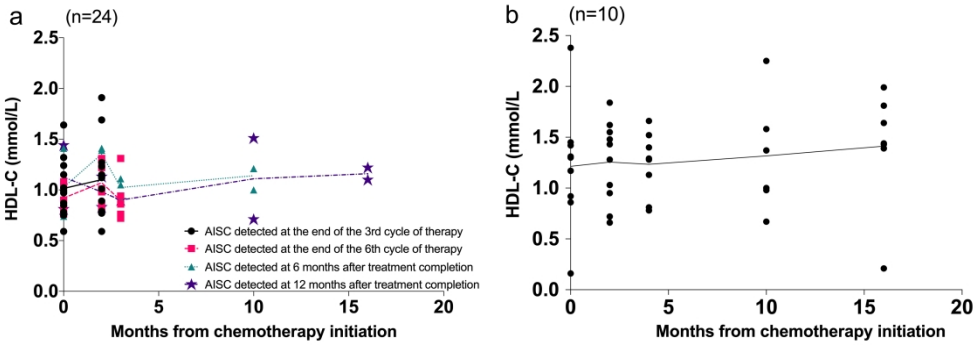

Figure 1. (a) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after treatment completion. 2 patients were detected AISC at 12 months after treatment completion. (b) Timeline of HDL-C levels of patients without AISC.

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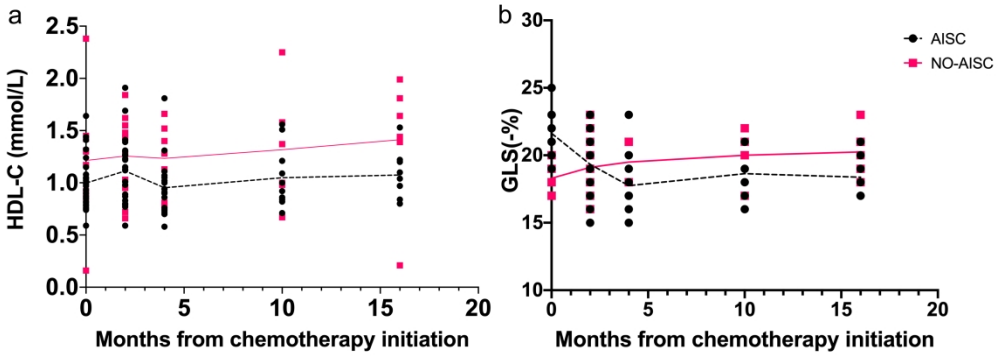

Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (a) and global longitudinal strain (GLS) (b) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

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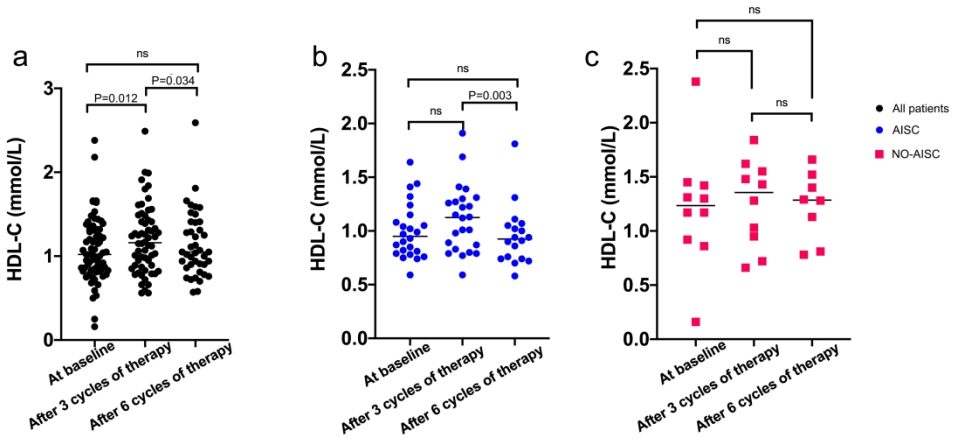

Figure 3. (a) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (b) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (c) Changes of HDL-C in patients without AISC.

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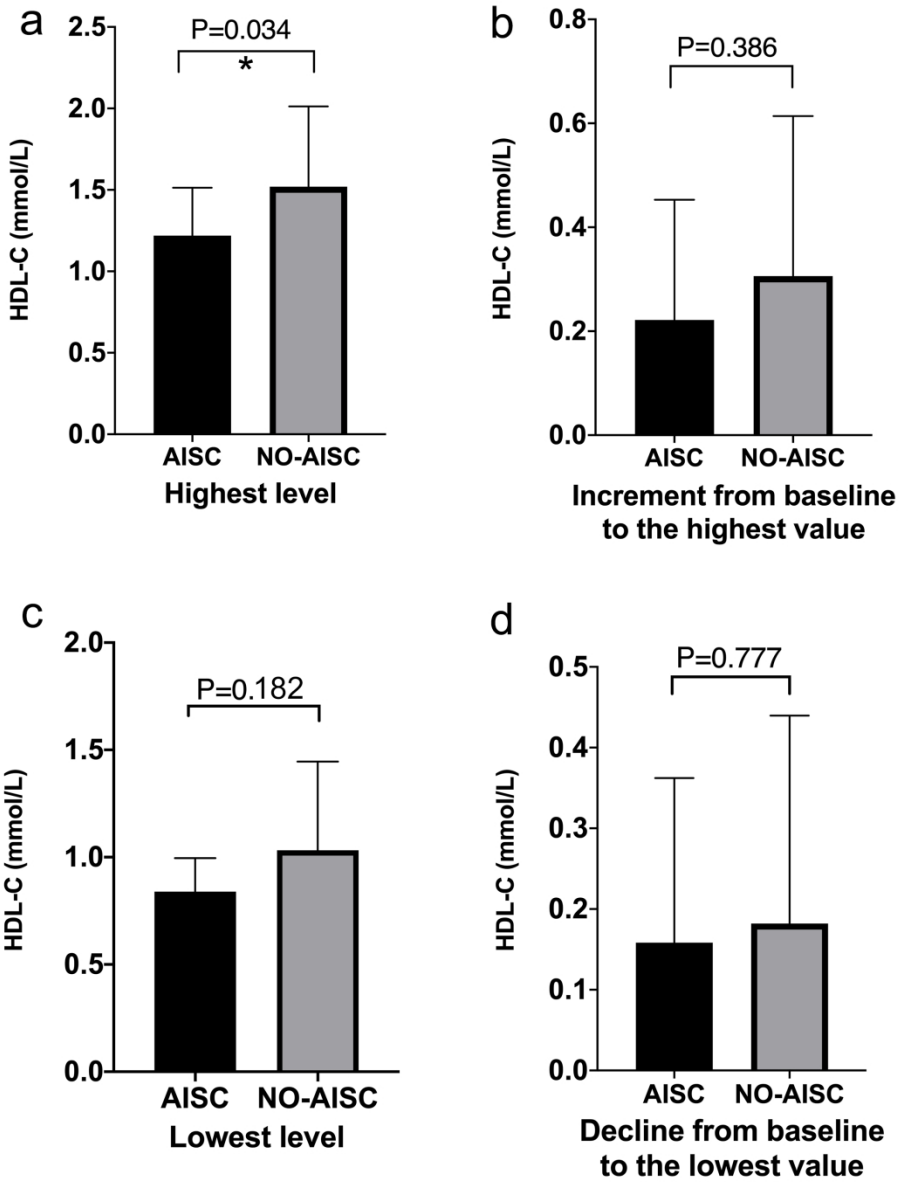

Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (a) Highest level of HDL-C during chemotherapy. (b)The HDL-C value increment from baseline to the highest value. (c) The lowest level of HDL-C during chemotherapy. (d) The HDL-C value declined from baseline to the lowest.

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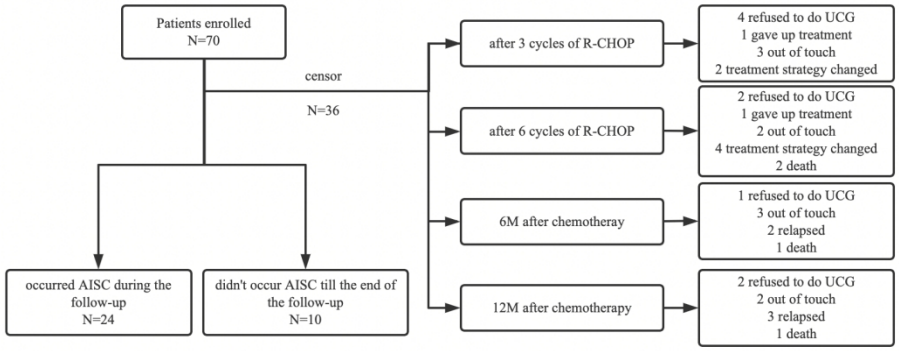

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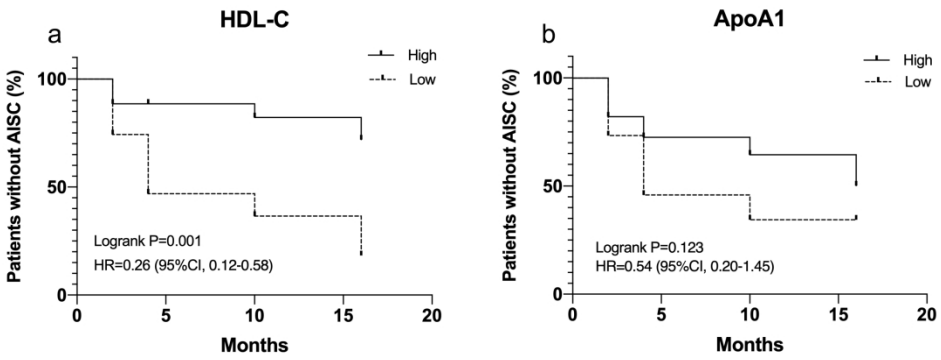

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Table S1. Baseline clinical characteristics of patients enrolled

	Total cohort n=70	Low HDL-C* n=42	High HDL-C* n=28	*P*-value
Age (year)	55.03±14.79	52.48±16.11	58.86±11.83	0.077
Male/Female (n)	34/36	22/20	12/16	0.435
BMI (kg/m²)	23.08±3.33	23.51±3.29	22.43±3.33	0.183
ECoG performance status				0.465
0 (%)	37 (52.86)	22 (52.38)	15 (53.57)	
1 (%)	24 (34.29%)	13 (30.95)	11 (39.28)	
2 (%)	9 (12.85)	7 (16.67)	2 (7.14)	
Hypertension (%)	9 (12.86)	5 (11.90)	4 (14.28)	1.000
Antihypertensive treatment	9 (12.86)	5 (11.90)	4 (14.28)	1.000
ACEI/ARB	5	2	3	
Beta-blockers	4	3	1	
Diabetes mellitus (%)	5 (7.14)	3 (7.14)	2 (7.14)	1.000
Smoking (%)	21 (30.00)	10 (23.81)	11 (39.28)	0.166
Drinking (%)	18 (25.71)	7 (16.67)	11 (39.28)	**0.034**
Heart rate	80.67±10.61	81.50±11.04	79.43±10.02	0.428
LVEF (%)	65.5 (63.0, 67.0)	65.0 (63.0, 67.0)	66.0 (63.2, 67.8)	0.522
FS (%)	35.67±2.10	35.60±2.14	35.79+2.08	0.713
GLS (-%)	20.0 (19.0, 22.0)	21.0 (19.0, 22.0)	20.0 (18.2, 21.0)	0.167
LVDd (mm)	46.30±3.50	46.93±3.44	45.36±3.43	0.065
LVMi (g/m²)	94.72±17.55	96.79±18.54	91.62±15.76	0.230
E (cm/s)	69.12±13.12	69.71±12.79	68.23±13.80	0.647
e'(cm/s)	7.60±2.15	7.60±2.07	7.59±2.30	0.987
E/e'	9.61±2.41	9.66±2.30	9.53±2.59	0.832
NT-proBNP (ng/L)	62.50 (31.25, 132.25)	51.00 (29.00, 128.75)	79.00 (41.75, 168.50)	0.171
cTnT (ng/mL)	0.004 (0.000, 0.006)	0.004 (0.002, 0.007)	0.004 (0.000, 0.005)	0.509
HsCRP	2.61 (1.00, 15.64)	4.04 (1.17, 20.00)	1.95 (0.76, 14.51)	0.161
TC (mmol/L)	4.09±0.93	3.86±0.97	4.44±0.76	**0.011**
TG (mmol/L)	1.40 (0.97, 1.68)	1.51 (1.19, 1.93)	1.10 (0.82, 1.50)	**0.002**
LDL (mmol/L)	2.55±0.77	2.42±0.80	2.75±0.69	0.084
HDL-C (mmol/L)	1.08±0.38	0.84±0.21	1.44±0.28	**<0.001**
ApoA1 (g/L)	1.17±0.34	1.01±0.25	1.40±0.32	**<0.001**

Values are expressed as mean±standard deviation, n (%), or median (Q1-Q3). Bold values indicate statistical significance.

* Low HDL-C: HDL-C<1.16mmol/L; High HDL-C: HDL-C≥1.16mmol/L. ApoA1 values were available in 63 patients. ApoA1: apolipoprotein A1; BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density

lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

Table S2. Univariable Cox regression analysis of enrolled patients.

Variables	Univariate analysis		P values
	HR	95%CI	
Age, per 1 year	0.97	0.943-0.998	**0.034**
Female vs Male	1.41	0.63-3.19	0.404
BMI, per 1 kg/m^2	1.09	0.97-1.22	0.139
ECoG, 0 or 1 vs status 2	0.63	0.30-1.32	0.220
Hypertension, yes vs no	0.22	0.03-1.62	0.136
Diabetes mellitus, yes vs no	0.35	0.05-2.61	0.307
Smoking, yes vs no	0.77	0.31-1.96	0.589
Drinking, yes vs no	0.84	0.31-2.26	0.728
Heart rate, per 1 bp	0.99	0.96-1.03	0.645
LVEF, per 1%	1.09	0.94-1.25	0.250
FS, per 1%	1.13	0.93-1.37	0.219
GLS, per -1%	1.46	1.20-1.77	**<0.001**
LVDd, per 1 mm	1.05	0.93-1.19	0.425
LVMi, per 1 g/m^2	1.00	0.97-1.02	0.762
E, per 1 cm/s	1.03	1.00-1.06	0.075
e', per 1 cm/s	1.14	0.94-1.40	0.189
E/e', per 1	0.97	0.81-1.16	0.752
NT-proBNP, per 1 ng/L	1.00	0.99-1.00	0.153
cTnT, per lg1 ng/mL	1.19	0.83-1.72	0.340
HsCRP, per 1	1.02	0.97-1.07	0.453
TC, per 1 mmol/L	0.78	0.51-1.20	0.259
TG, per 1 mmol/L	0.93	0.55-1.58	0.796
LDL-C, per 1 mmol/L	0.94	0.56-1.55	0.795
HDL-C group (high vs low)	0.24	0.09-0.67	**0.006**
ApoA1 group (high vs low)	0.54	0.22-1.29	0.165

High HDL-C group: HDL-C≥1.16mmol/L. Low HDL-C group: HDL-C<1.16mmol/L. High ApoA1 group: ApoA1≥1.02g/L. ApoA1: apolipoprotein A1; BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

Table S3. Baseline clinical characteristics of un-censored patients with or without AISC.

	Total cohort	NO-AISC	AISC	P-value
	n=34	n=10	n=24	
Age (year)	52.85±12.37	59.7±9.67	50.0±12.45	**0.035**
Male/Female (n)	19/15	5/5	14/10	0.947
BMI (kg/m^2)	24.01±3.21	23.67±3.83	24.15±3.00	0.698
ECoG performance status				1.000
0 (%)	23 (67.65)	7 (70.00)	16 (66.67)	
1 (%)	7 (20.59)	2 (20.00)	5 (20.83)	
2 (%)	4 (11.76)	1 (10.00)	3 (12.50)	
Hypertension (%)	4 (11.76)	3 (30.00)	1 (4.17)	0.122
Antihypertensive treatment	4 (11.76)	3 (30.00)	1 (4.17)	0.122
ACEI/ARB	3	2	1	
Beta-blockers	1	1	0	
Diabetes mellitus (%)	3 (8.82)	2 (20.00)	1 (4.17)	0.412
Smoking (%)	11 (32.35)	5 (50.00)	6 (25.00)	0.309
Drinking (%)	7 (20.59)	2 (20.00)	5 (20.83)	1.000
Heart rate	81.03±11.08	84.60±13.53	79.54±9.83	0.231
LVEF (%)	65.0 (63.0, 67.0)	64.0 (62.0, 67.3)	66.0 (64.0, 67.0)	0.270
FS (%)	35.74±2.12	35.10±2.28	36.0±2.04	0.266
GLS (-%)	21.0 (19.0, 22.0)	18.0 (17.0, 20.0)	22.0 (21.0, 22.8)	**<0.001**
LVDd (mm)	46.62±3.08	45.61±3.41	47.04±2.90	0.218
LVMi (g/m^2)	94.04±16.26	93.27±18.87	94.36±15.78	0.862
E (cm/s)	70.87±12.84	66.43±11.78	72.73±13.04	0.197
e'(cm/s)	7.59±1.77	6.90±1.88	7.88±1.68	0.144
E/e'	9.66±2.17	9.95±1.79	9.54±2.34	0.626
NT-proBNP (ng/L)	46.00 (28.50, 109.00)	78.50 (31.50, 133.25)	41.00 (28.00, 122.00)	0.308
cTnT (ng/mL)	0.004 (0.000, 0.007)	0.004 (0.000, 0.008)	0.004 (0.000, 0.007)	0.816
HsCRP	2.28 (0.95, 17.18)	1.62 (0.54, 13.79)	3.84 (1.21, 20.00)	0.216
TC (mmol/L)	4.07±0.95	4.38±1.31	3.95±0.75	0.219
TG (mmol/L)	1.40 (1.12, 1.63)	1.54 (1.67, 1.85)	1.38 (1.10, 1.56)	0.344
LDL-C (mmol/L)	2.59±0.83	2.73±1.15	2.53±0.68	0.530
HDL-C (mmol/L)	1.06±0.38	1.21±0.56	1.00±0.26	0.128
HDL-C group*				**0.019**
High (%)	12 (35.29)	7 (70.00)	5 (20.83)	
Low (%)	22 (64.71)	3 (30.00)	19 (79.17)	
ApoA1 (g/L)	1.19±0.34	1.35±0.44	1.12±0.26	0.068
ApoA1 group#				0.266
High (%)	23 (71.88)	9 (90.0)	14 (63.64)	
Low (%)	9 (28.12)	1 (10.0)	8 (36.36)	

*High HDL-C: HDL-C≥1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L. # High ApoA1: ApoA1≥1.02g/L. Low ApoA1: ApoA1<1.02g/L. ApoA1 values were available in 32 patients.

AISC: anthracycline-induced subclinical cardiotoxicity; ApoA1: apolipoprotein A1; AST: aspartate

transaminase; ALT: alanine aminotransferase; BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	3
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	4
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	4
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	4
Outcome data	15*	Report numbers of outcome events or summary measures over time	6

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6,7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7,8
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8,9,10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Role of HDL-C in anthracycline-induced subclinical cardiotoxicity: an observational prospective study in DLBCL patients

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Role of HDL-C in anthracycline-induced subclinical cardiotoxicity: an observational prospective study in DLBCL patients

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Keywords: cardiotoxicity; DLBCL; anthracycline; HDL-C; GLS

ABSTRACT

Objectives Anthracycline-induced cardiotoxicity is a debilitating cardiac dysfunction for which there are no effective treatments, making early prevention of anthracycline-induced subclinical cardiotoxicity (AISC) crucial. High-density lipoprotein cholesterol (HDL-C) plays role in cardio-protection, but its impact on AISC remains unclear. Our study aims to elucidate the protective capacity of HDL-C in AISC in patients with diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP.

Design Observational prospective study

Setting An institution in China from September 2020 to September 2022.

Participant 70 chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to receive the standard dose of R-CHOP (cyclophosphamide, vincristine, doxorubicin, prednisone, and rituximab).

Primary outcome measures Serum biomarkers including HDL-C, 2D speckle tracking echocardiography, and conventional echocardiography were measured at baseline, at the end of the 3rd and 6th cycle of R-CHOP, and 6 and 12 months after the completion of chemotherapy.
Results 24 patients experienced AISC, while 10 did not. 36 patients were lost to follow-up. Cox regression analysis showed that higher levels of HDL-C were associated with a significantly lower risk of AISC (unadjusted, hazard ratio [HR]=0.24, 95%CI: 0.09-0.67, P=0.006; adjusted, HR=0.27, 95%CI: 0.09-0.79, P=0.017). Patients without AISC had a more stable and higher HDL-C level during the follow-up period. HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients (P=0.034), and particularly in the AISC group (P=0.003). The highest level of HDL-C was significantly higher in patients without AISC than in those with AISC (1.52±0.49 vs. 1.22±0.29, P=0.034).
Conclusions Our study suggests that higher levels of HDL-C may be associated with a lower risk of AISC in DLBCL patients treated with R-CHOP. HDL-C could be a cardio-protective target, but further research is needed to confirm its benefits and limitations.
Study registration number ChiCTR2100054721

Strengths and limitations of this study

- This observational prospective study contributes to our understanding of the association between HDL-C and AISC, offering a foundation for the development of early intervention and prevention strategies.
- The study used advanced imaging techniques (2D-STE) to assess the subclinical cardiac dysfunction in the patients, which can provide more sensitive and accurate results compared to traditional echocardiography.
- The study only included patients with DLBCL who received R-CHOP, which may limit the generalizability of the findings to patients with other types of cancer or chemotherapy regimens.
- The relatively small sample size in this study may potentially impact the robustness and generalizability of our findings. Additional comprehensive studies, including both clinical and basic research, are necessary to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in anthracycline-treated cancer patients.

INTRODUCTION

The improved management of cancer has led to a significant increase in the survival rate of cancer survivors(1). However, anthracycline, one of the most effective chemotherapeutic agents used to treat various cancers, is associated with potentially life-threatening and severe cardiovascular diseases(2). Studies have shown a significant increase in mortality in cancer patients with cardiovascular disease(3, 4). As advances in cancer treatment and an aging population continue, the number of patients with both conditions is rising(5). As a result, the field of cardio-oncology has become increasingly important in recent years.

Non-Hodgkin's lymphoma (NHL) is the 7th most common cancer in the United States and the most frequent hematologic malignancy globally, accounting for about 3% of cancer cases and

deaths(6). Among NHL, DLBCL is the most prevalent type, representing approximately one-third of all cases(7). The combination of cyclophosphamide, vincristine, doxorubicin, and prednisone with rituximab (R-CHOP) is a standard first-line therapy that has substantially improved survival outcomes in DLBCL patients(8). Nonetheless, anthracycline-containing chemotherapy agents are associated with cardiotoxicity, a major long-term adverse effect that significantly affects the quality of life and survival of cancer survivors.

Anthracycline-induced cardiotoxicity (AIC) is a devastating consequence of successful cancer treatment, often leading to hypokinetic cardiomyopathy and ultimately heart failure. AIC is an irreversible form of cardiac dysfunction for which no guidelines or accepted therapies for cardioprotection currently exist(9, 10). Therefore, early prevention and detection of AIC are crucial for providing opportunities for early intervention. Anthracycline-induced subclinical cardiotoxicity (AISC) is an early stage of AIC, characterized by abnormal echocardiography index without clinical symptoms(11). Early intervention is recommended by the 2022 International Cardio-Oncology Society (IC-OS) consensus statement once AISC is detected(12). Global longitudinal peak systolic strain (GLS) measured by 2D speckle tracking echocardiography can reliably identify most early myocardial deformation variations. In our study, we used early measurement of GLS to identify AISC(13, 14).

High-density lipoprotein (HDL) is the sole lipoprotein with protective attributes among the five types of lipoproteins. Its salutary effects include antioxidant, anti-inflammatory, and anti-apoptotic properties. Numerous preclinical investigations have suggested that HDL may have direct and indirect protective effects against AIC(15-17). The roles of HDL-cholesterol (HDL-C) and apolipoprotein A1 (ApoA1) in providing cardiovascular protection of HDL have been the subject of recent debate. Our team recently conducted a case-control study, revealing that both HDL-C and ApoA1 serve as predictive factors in patients with DLBCL treated with 3 cycles of (R)-CHOP(18). Nonetheless, further investigation is warranted to explore the clinical data pertaining to the association between HDL and anthracycline-related cardiotoxicity.

We undertook an observational prospective study to investigate the potential impact of HDL-C on AISC. Using 2D speckle tracking echocardiography, we identified AISC and sought to establish any correlation between HDL-C and AISC. Additionally, we assessed the fluctuations in HDL-C levels during R-CHOP chemotherapy in chemotherapy-naïve patients recently diagnosed with DLBCL.

METHODS

Study population

We recruited chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to receive the standard dose of R-CHOP chemotherapy regimen at our institution from September 1st, 2020, to September 1st, 2022. Our inclusion criteria were as follows: newly diagnosed DLBCL, age between 18 and 80 years, Eastern Cooperative Oncology Group (ECoG) performance status $\leqslant 2$, left ventricular ejection fraction (LVEF) $\geqslant 50\%$, and acceptable bone marrow, renal, and hepatic functions for chemotherapy. Conversely, our exclusion criteria were symptomatic heart

failure, a history of myocardial ischemia, myocarditis, myocardial infarction, clinical or subclinical pericardial effusion, arrhythmia requiring medical intervention, a history of other cancers, under lipid-lowing treatment, and severe active infections such as syphilis, hepatitis, or human immunodeficiency virus (HIV) infection.

Treatment

Patients received a total of 6 cycles of standard R-CHOP (cyclophosphamide at 750 mg/m^2 on D1, doxorubicin at 50 mg/m^2 on D1, vincristine at 1.4 [maximum 2] mg/m^2 on D1, and 100 mg prednisone on D1-5, with rituximab at 375 mg/m^2 on D1 in each cycle), with or without 2 cycles of rituximab maintenance (rituximab at 375 mg/m^2 on D1 in each cycle).

Definition of subclinical cardiotoxicity

According to the IC-OS consensus statement, the definition of subclinical cardiotoxicity was a relative GLS decrease from baseline [(baseline – current GLS)/baseline GLS] of >12%, but with a normal left ventricular ejection fraction (LVEF)(12).

Study protocol

We defined 'baseline' as the initial assessment conducted before the initiation of the first cycle of chemotherapy. At baseline, the end of the 3rd cycle of R-CHOP, the end of the 6th cycle of R-CHOP, and 6 and 12 months after chemotherapy completion, all enrolled patients underwent conventional echocardiography, 2D speckle tracking echocardiography, and blood sampling. Every patient received electrocardiography (ECG) examination before every cycle of chemotherapy to ensure the safety of the treatment. Demographic data and clinical variables, including age, gender, body mass index (BMI), ECoG performance status, diabetes mellitus, hypertension, drinking history (an adult who has consumed more than 20 drinks in lifetime, with each drink is considered to have an average alcohol content of 12 g), and smoking history (an adult who has smoked at least 100 cigarettes in their lifetime) were collected at the time of enrollment. Left ventricular systolic dysfunction was measured by LVEF, fractional shortening (FS), left ventricular mass index (LVMI), left ventricular diastolic dimension (LVDd), E, e', E/e', and GLS. HDL-C, low-density lipoprotein cholesterol (LDL-C), cardiac troponin T (cTnT), high sensitivity C-reactive protein (hsCRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), total cholesterol (TC) and total triglyceride (TG) were measured. We used the baseline HDL-C level as a surrogate marker for HDL quantity. The patients were categorized into two groups based on the average HDL-C value for males and females in the modified criteria of the National Cholesterol Educated Program Adult Treatment Panel (NCEP ATP III)(19). High HDL-C was defined as a serum HDL-C≥1.16mmol/L, while low HDL-C was defined as a serum HDL-C<1.16mmol/L. We determined the sample size using an online sample size calculator, which indicated a total requirement of 23 events(20).

Statistical analysis

The study was conducted with two aims: firstly, to evaluate the relationship between HDL and AISC; and secondly, to conduct a preliminary exploration of the differences in HDL-C and the variability of HDL-C changes between patients with and without AISC during the follow-up period. Continuous variables were expressed as mean± standard deviation (SD) and compared

using the t-test. Non-normally distributed variables were presented as median (Q1- Q3) and compared with the Wilcoxon Mann-Whitney test. Categorical variables were expressed as n (%) and compared using the Chi-square or Fisher's exact test, as appropriate. Correlation analysis was conducted to investigate the associations of change in HDL-C with change in GLS. The probabilities of survival were calculated using Kaplan-Meier methods and compared using Log-rank tests. Cox proportional-hazards regression models were conducted to assess the association between variables and AISC. Covariates for multivariable Cox regression models included age, sex, and variables that had a P-value of less than 0.15 in the univariable Cox regression analysis (GLS was excluded as it is the factor that defines AISC). Two multivariable Cox regression models were constructed: the first model included age and sex; and the second model included age, sex, hypertension, BMI, and E. Statistical analysis and visualization were performed using IBM SPSS V.22.0 and GraphPad Prism 8. Statistical tests were two-sided, with a P-value less than 0.05 being considered statically significant.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

RESULTS

Assessment of the association between HDL-C and AISC

Study population and baseline characteristics

This investigation enrolled a total of 70 patients with chemotherapy-naïve DLBCL and were planned to be treated with the standard R-CHOP regimen. Based on the baseline HDL-C level, we segregated the patients into two groups: the high-level group (HDL-C$\geqslant$1.16mmol/L, n=28) and the low-level group (HDL-C<1.16mmol/L, n=42). Patients with drinking history had a greater chance of having a high HDL-C level (P=0.034). The patients with high HDL-C showed substantially higher total cholesterol (P=0.011), and lower total triglyceride (P=0.002). The baseline characteristics of the patients in both groups were well balanced (Table S1).

High HDL-C was an independent protective target of anthracycline-induced subclinical cardiotoxicity

The clinical endpoint was defined as the first detection of AISC, and the median survival time of the whole cohort was 16 months. The median survival time of patients with low HDL-C was 4 months, while that of patients with high HDL-C was not reached. The median follow-up time of the cohort was 10 months. During the follow-up period, 24 patients experienced AISC, while 10 did not. Approximately half of the patients (n=36) were lost to follow-up. A flowchart detailing the patients enrolled in the study and the reasons for lost to follow-up patients can be found in Figure S1.

The log-rank test revealed that patients with higher HDL-C were less likely to experience AISC (P=0.001, HR=0.26, 95%CI: 0.12-0.58) (Figure S2).

According to the results of the univariable Cox regression analysis, variables that had a P-value of less than 0.15 including age, BMI, hypertension, GLS, E, and HDL-C group. Increasing age was significantly associated with a decreased HR of 0.97 (95%CI 0.943-0.998, P=0.034) per 1-year increase. BMI showed a HR of 1.09 (95% CI 0.97-1.22, P=0.139) per 1 kg/m2 increase. Similarly, hypertension had a HR of 0.22 (95% CI 0.03-1.62, P=0.136) for yes versus no. A lower GLS was significantly associated with an increased HR of 1.46 (95% CI 1.20-1.77, P<0.001) per -1% decrease. E velocity showed a HR of 1.03 (95% CI 1.00-1.06, P=0.075) per 1 cm/s increase. The HDL-C group (high versus low) had a significantly lower HR of 0.24 (95% CI 0.09-0.67, P=0.006). Further details about other variables can be found in Table S2.

The results of the multivariable Cox regression analysis showed that high HDL-C was significantly associated with a lower risk of AISC after adjusting for age and sex (model 1) (HR=0.28, 95%CI:0.10=0.84, P=0.018). Similarly, after adjusting for age, sex, and variables that P<0.15 in the univariable Cox regression analysis (excluding GLS as it defines AISC) (model 2), the same association was observed (HR=0.27, 95%CI: 0.09-0.79, P=0.017). (Table 1)

Table 1. Outcomes of study participants.

	HR (95%CI) (unadjusted)	P values	HR (95%CI) (adjusted*)	P values	HR (95%CI) (adjusted#)	P values
Low HDL-C	Ref		Ref		Ref	
High HDL-C	0.24 (0.09-0.67)	**0.006**	0.28 (0.10-0.80)	**0.018**	0.27 (0.09- 0.79)	**0.017**

The endpoint was defined as the first detection of anthracycline-induced subclinical cardiotoxicity.

Low HDL-C: HDL-C<1.16mmol/L; High HDL-C: HDL-C≥1.16mmol/L. *Adjusted for age and sex. #Adjusted for age, sex, hypertension, body mass index, E. HR, hazard ratio; HDL-C, high-density lipoprotein-cholesterol.

Preliminary exploration of the difference of HDL-C between patients with AISC and without AISC

Study population and baseline characteristics

In this analysis, we selectively included 34 of the enrolled patients who were not lost to follow-up. The patients who exhibited AISC at any time during the follow-up period were segregated into the AISC group (n=24), while those who did not demonstrate AISC were classified into the NO-AISC group (n=10). Patients within the AISC group were comparatively younger (50±12.45 vs. 59.7±9.67, P=0.035) and exhibited a higher baseline GLS [22.0 (21.0, 22.8) vs. 18.0 (17.0, 20.0), P<0.001]. More baseline information can be seen in Table S3.

Timeline of HDL-C level in patients with and without AISC

Figure 1 displays the timeline of HDL-C levels in patients with and without AISC. In Figure 1a, the patient population was categorized into four groups based on the time of AISC detection. Among the groups, 12 patients were identified with AISC at the end of the 3rd cycle of chemotherapy, 7 patients at the end of the 6th cycle, 3 patients at 6 months after treatment completion, and 2 patients at 12 months after treatment completion. With the exception of the group in which patients detected AISC at the end of the 3rd cycle of chemotherapy, all other groups exhibited a reduction in HDL-C values from the end of the 3rd cycle of chemotherapy to the end

of the 6th cycle of chemotherapy. Figure 1b portrays the HDL-C level in patients without AISC, indicating that the HDL-C level was more stable than in patients with AISC. Moreover, the overall HDL-C level was higher in patients without AISC than in patients with AISC throughout the follow-up period (Figure 2a). In Figure 2b, there was a significant decrease in GLS during the chemotherapy period (from 0-4 months), which remained stable after completion of chemotherapy (after 4 months) in patients with AISC.

Based on Figure 2, we observed that the fluctuations in HDL-C and GLS were most pronounced during the chemotherapy period. The fluctuations in HDL-C levels of patients with DLBCL during R-CHOP chemotherapy were presented in Figure 3. The levels of HDL-C significantly increased for all patients from baseline to the end of the 3rd cycle of chemotherapy (P=0.012) and significantly decreased from the end of the 3rd cycle to the end of the 6th cycle of chemotherapy (P=0.034) (Figure 3a). Patients with AISC showed a significant decrease in HDL-C levels during R-CHOP chemotherapy from the end of the 3rd cycle to the end of the 6th cycle (P=0.003) (Figure 3b). However, no significant difference was observed in HDL-C levels for patients without AISC during R-CHOP chemotherapy (Figure 3c). We conducted correlation analysis separately for the change in HDL-C and GLS from baseline to after 3 cycles of chemotherapy, from baseline to after 6 cycles of chemotherapy, and from after 3 cycles to after 6 cycles of chemotherapy. However, we found no statistically significant differences in the associations between changes in HDL-C and GLS (P=0.965, 0.087, 0.449).

Contrasting values of HDL-C parameters between patients with and without AISC

Figure 4 presents the contrasting values between patients with AISC and those without in terms of four parameters, namely the highest and lowest levels of HDL-C during chemotherapy, the increment and decline in HDL-C values from baseline. Patients without AISC showed significantly higher values in the highest level of HDL-C (1.52±0.49 vs. 1.22±0.29, P=0.034, Figure 4a). However, no significant differences were observed between the two groups in terms of HDL-C increment from baseline to the highest value (0.31±0.31 vs. 0.22±0.23, P=0.386, Figure 4b). While the lowest level of HDL-C was lower in patients with AISC, the difference was not statistically significant (0.84±0.16 vs. 1.03±0.41, P=0.182, Figure 4c). Furthermore, there were no significant differences in HDL-C decline between patients with AISC and those without (0.16±0.20 vs. 0.18±0.26, P=0.777, Figure 4d).

DISCUSSION

This prospective observational study investigated the relationship between HDL-C and incidence of AISC in 70 patients with DLBCL who were receiving anthracycline-containing chemotherapy. The study found that higher levels of HDL-C were associated with a lower incidence of AISC. Moreover, patients without AISC had more stable and higher levels of HDL-C than those with AISC during the follow-up period. The results also showed that HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients, especially in the AISC group, indicating that anthracycline-containing chemotherapy has adverse effects on HDL-C levels. Notably, the highest level of HDL-

C was significantly higher in patients without AISC compared to those with AISC. These findings suggest that HDL-C may have a protective role against AISC in DLBCL patients undergoing anthracycline-containing chemotherapy and maintaining a relatively high level of HDL-C may be more effective in managing cardio-protection than monitoring changes in HDL-C levels over time. The results of this study highlight the importance of early serum lipid management in these patients.

Lipoproteins are classified into five categories, namely chylomicron, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), based on their size, density, and lipid composition (cholesterol and triglycerides)(21). Among these, HDL exhibits distinctive cytoprotective actions and triggers anti-oxidative, anti-inflammatory, and anti-apoptotic effects. The protective roles of HDL in cardiovascular disease have been controversial in recent years, and that the quality of HDL (cholesterol efflux capacity, antioxidant activity, anti-inflammatory activity, endothelial function, etc.) rather than the quantity of HDL has been proposed as the true cardioprotective effect. The Framingham Heart Study, as early as 1988, reported a correlation between HDL-C and cardiovascular mortality(22). Recent studies have challenged the HDL-C hypothesis by revealing that HDL-C level is not inversely correlated with cardiovascular diseases(23, 24). In our study, we used the baseline HDL-C level as a surrogate marker for HDL quantity, but we did not directly measure the quality of HDL. Measuring the level of HDL-C in serum is a commonly used method to assess the effect of HDL on cardiovascular health. HDL facilitates the transportation of cholesterol from the body's tissues back to the liver, and higher levels of HDL-C are generally associated with a lower risk of heart disease. Nevertheless, it's crucial to note that HDL-C levels may not accurately reflect the functional properties of HDL. ApoA1, the most abundant protein in HDL, is associated with several beneficial effects of HDL(15, 25). The function and abundance of ApoA1 are reported to play a dominant role in HDL quality(26). In the context of AIC, several studies have indicated that HDL can protect against anthracycline-induced cardiomyocyte apoptosis and atrophy in isolated cardiomyocytes(27, 28) and animal models(16, 28). Our recent case-control study, revealing that both HDL-C and ApoA1 serve as predictive factors in patients treated with 3 cycles of anthracycline-contained chemotherapy(18). Based on these earlier trials, HDL-C and ApoA1 could serve as protective factors against anthracycline-related cardiovascular disease. However, our study didn't focus on the investigation of the impact of ApoA1 on AISC. Even when ApoA1 was included in the Cox regression model, no significant association with AISC in patients with DLBCL treated with R-CHOP was observed ($P>0.05$, data not shown), probably due to the number of events in our study was insufficient to support a robust ApoA1 analysis. Therefore, the role of ApoA1, the most abundant protein in HDL, in the context of AISC, warrants further investigation in future research.

As far as we know, few clinical studies have investigated the association between HDL-C and AISC. This study is the first clinical research that utilizes the IC-OS consensus statement(12) to define subclinical cardiotoxicity, with univariate and multivariable analyses being used to identify the influential factors of AISC in DLBCL patients in this cohort. Kaplan-Meier methods and Log-

rank tests reveal that patients with high HDL-C levels were less likely to develop AISC. After subjecting it to univariate and multivariable Cox regression methods, high HDL-C levels still showed statistically significant differences. These results suggest that high HDL-C could be a potentially independent protective factor for AISC in DLBCL patients and provide an opportunity for investigators to develop a tool for early intervention and prevention of AISC. Further research is necessary to confirm our findings.

Several studies have demonstrated that serum lipid levels are altered during anthracycline-containing chemotherapy in cancer patients(29, 30). Huxley et al and Averina et al have shown that imbalanced serum lipid distribution is a risk factor for cardiovascular disease(31, 32). As a result, anthracycline-containing treatment can induce dyslipidemia and facilitate the occurrence and development of cardiovascular diseases in cancer patients. In a study of 394 breast cancer patients, Xin et al found that HDL-C levels after chemotherapy were significantly lower than those before chemotherapy(33). Similarly, Lu et al and Hana et al found that HDL-C levels were significantly decreased during anthracycline-containing chemotherapy in patients with breast cancer(34, 35). In our study, we specifically assessed the changes in HDL-C levels over time during follow-up. Except for the group of patients who experienced AISC at 12 months after treatment completion, HDL-C levels in all other groups increased from baseline to the 3rd cycle of chemotherapy. This phenomenon may be due to the fact that anti-tumor drugs require cholesterol to cross cell membranes(36). However, HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients, especially in the AISC group, which is consistent with previous research results(33-35), and further confirmed that anthracycline-contained chemotherapy has adverse effects on HDL-C levels in DLBCL patients. The HDL-C level in patients without AISC was more stable than that in patients with AISC. Therefore, anthracycline-containing chemotherapy may promote the occurrence and development of cardiotoxicity in DLBCL patients by inducing HDL-C turbulence. Besides, the findings of our study indicate a significant decrease in GLS during the chemotherapy period in patients with AISC. This result is consistent with previous research, which has reported that doxorubicin dose at the range of 100-150mg/m^2 can cause cardiotoxicity(37). Notably, we also observed that GLS remained stable after completion of chemotherapy, suggesting that the cardiac effects of anthracycline-based chemotherapy may be dose-related. These findings have important implications for the monitoring and management of cardiotoxicity in patients undergoing anthracycline-based chemotherapy, as early detection of cardiac dysfunction during treatment may improve patient outcomes. We investigated the associations of change in HDL-C with change in GLS, no statistically significant differences were found.

The analysis of HDL-C levels should not only consider the changes over time, but also the absolute values. In our study, patients without AISC had significantly higher absolute highest HDL-C levels than those with AISC, while the absolute lowest HDL-C levels did not differ significantly between the two groups. The alterations from HDL-C extremes to baseline did not exhibit any variation between the groups either. This suggests that the highest absolute HDL-C value was a preferable indicator of AISC protection than the change in HDL-C from baseline to the extremum value.

Maintaining a relatively high level of HDL-C may be more effective in managing the cardio-protection of anthracycline-treated cancer patients than monitoring changes in HDL-C levels over time.

In our investigation, we observed that among the four patients with pre-existing hypertension, one patient experienced AISC during the follow-up (Table S3). Multivariable Cox regression analysis showed that hypertension did not have a significant impact on AISC ($P>0.05$). Hypertension, a common risk factor for both cancer and cardiovascular diseases, was also recognized as a risk factor for cardiotoxicity. Studies have reported that pre-existing hypertension was associated with anthracycline-and trastuzumab induced left ventricular ejection fraction (LVEF) decline in a retrospective study(38), and early left ventricular systolic dysfunction in patients with lymphoma receiving (R)-CHOP in a prospective study(39). We noted that all patients with hypertension in our study were under a single antihypertensive drug regimen (beta-blockers or ACEI/ARB) to manage their blood pressure. Two meta-analyses have demonstrated that beta-blockers and ACEI can prevent cardiotoxicity caused by chemotherapy (40, 41). We speculate that the protective effects of beta-blockers and ACEI/ARB may have contributed to the result observed in our study regarding the relationship between hypertension and AISC.

There are several limitations to our study that must be acknowledged. Firstly, while our study highlights the potential importance of HDL-C in managing AISC, additional studies are necessary to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in anthracycline-treated cancer patients. Secondly, this is a single-center observational prospective study with a medium sample size. To confirm our findings, a larger sample size study conducted at multiple centers is needed. Thirdly, previous studies have suggested that there may be a reversed U-shaped relationship between HDL-C levels and cardiovascular diseases(42). Due to the small sample size of this study, we didn't further investigate the influence of extremely high levels of HDL-C on cardiotoxicity, and further clinical studies should be done to verify it. Besides, the measurement of GLS was only taken at baseline and at several points throughout the chemotherapy treatment and follow-up period. It is crucial to extend the duration of follow-up in future research to obtain a more comprehensive understanding of the long-term effects of anthracycline treatment on cardiovascular health.

CONCLUSIONS

In conclusion, our observational prospective study suggests that higher levels of HDL-C may be associated with a lower risk of AISC in patients with DLBCL treated with R-CHOP chemotherapy. HDL-C levels remained stable and consistently higher in patients without AISC compared to those with AISC. Additionally, the highest absolute HDL-C value was found to be a preferable indicator of AISC protection. These findings suggest that HDL-C may be a potential cardio-protective target for managing AISC in this patient population. However, further research is needed to confirm and expand on these findings, including determining the optimal HDL-C level for cardio-protection and the potential benefits of early serum lipid management.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang; Data curation, Wenxin Ou, Tiantian Jiang, Yue Weng and Xi Zhou; Formal analysis, Wenxin Ou and Tiantian Jiang; Funding acquisi-tion, Wenxin Ou, Nan Zhang, Dong Wang and Xiaoqiong Tang; Investigation, Dong Wang, Qian Dong and Xiaoqiong Tang; Methodology, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang; Resources, Dong Wang, Qian Dong and Xiaoqiong Tang; Supervision, Qian Dong and Xiaoqiong Tang; Validation, Nan Zhang and Kai Lu; Visualization, Wenxin Ou and Tiantian Jiang; Writing – original draft, Wenxin Ou; Writing – review & editing, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang. Qian Dong and Xiaoqiong Tang contributed equally to this work and are considered as co-corresponding authors.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

ETHICS APPROVAL

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (Approval NO. 2018-016). And all participating patients provided written informed consent.

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FIGURE LEGENS

Figure 1. (**a**) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after treatment completion. 2 patients were detected AISC at 12 months after treatment completion. (**b**) Timeline of HDL-C levels of patients without AISC.

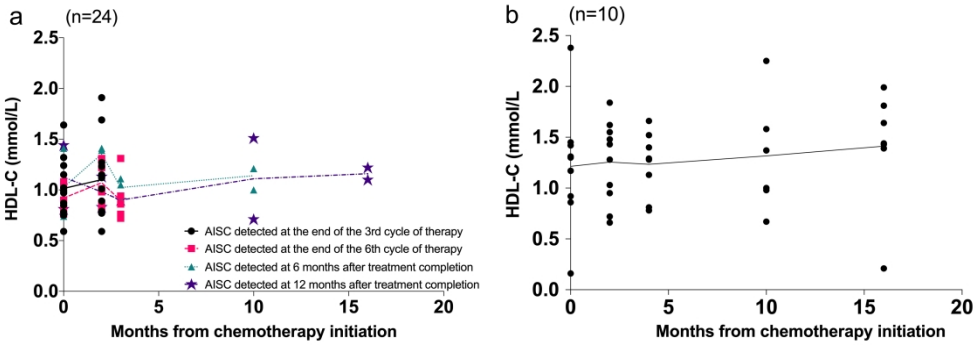
Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (**a**) and global longitudinal strain (GLS) (**b**) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

Figure 3. (**a**) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (**b**) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (**c**) Changes of HDL-C in patients without AISC.

Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (**a**) Highest level of HDL-C during chemotherapy. (**b**)The HDL-C value increment from baseline to the highest value. (**c**) The lowest level of HDL-C during chemotherapy. (**d**) The HDL-C value declined from baseline to the lowest.

Figure S1. Flow diagram of patients enrolled in our observational research and reasons for lost to follow-up.

Figure S2. Kaplan-Meier curves of the percentage of patients without AISC in patients stratified by HDL-C level. High HDL-C: HDL-C⩾1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L. AISC, anthracycline-induced cardiotoxicity; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio.



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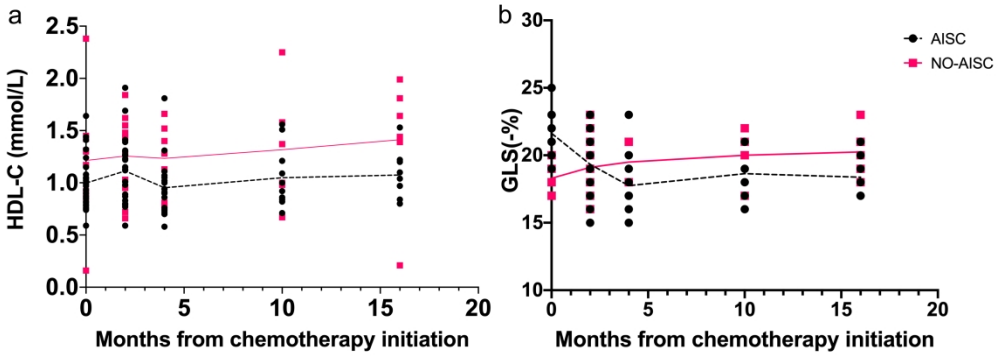

Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (a) and global longitudinal strain (GLS) (b) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

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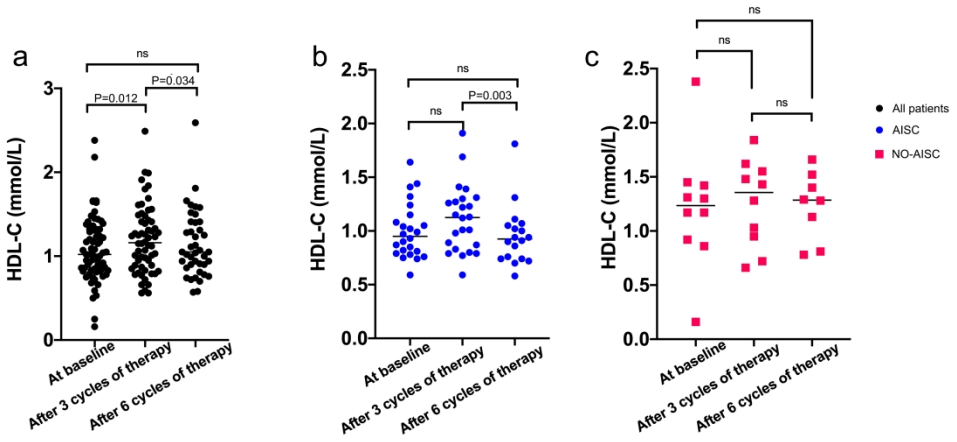

Figure 3. (a) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (b) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (c) Changes of HDL-C in patients without AISC.

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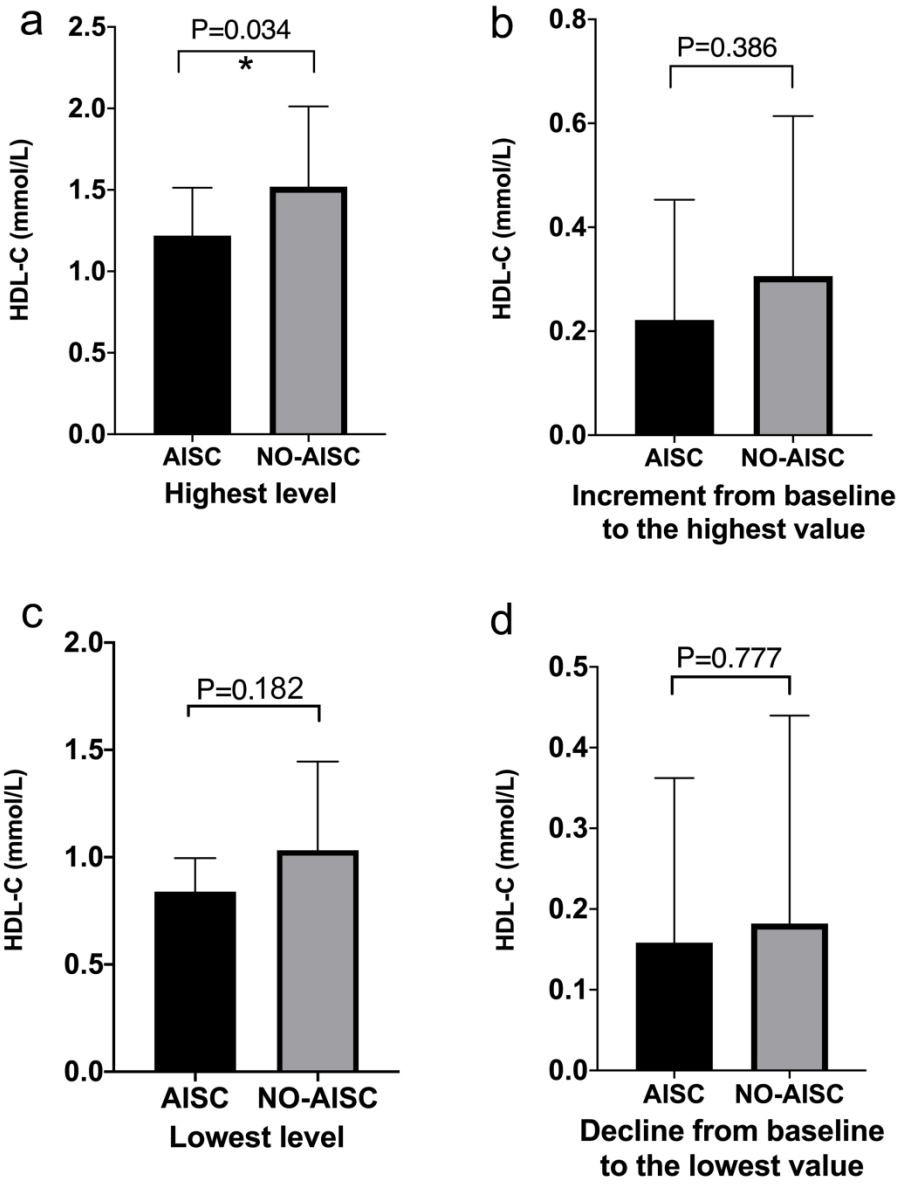

Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (a) Highest level of HDL-C during chemotherapy. (b)The HDL-C value increment from baseline to the highest value. (c) The lowest level of HDL-C during chemotherapy. (d) The HDL-C value declined from baseline to the lowest.

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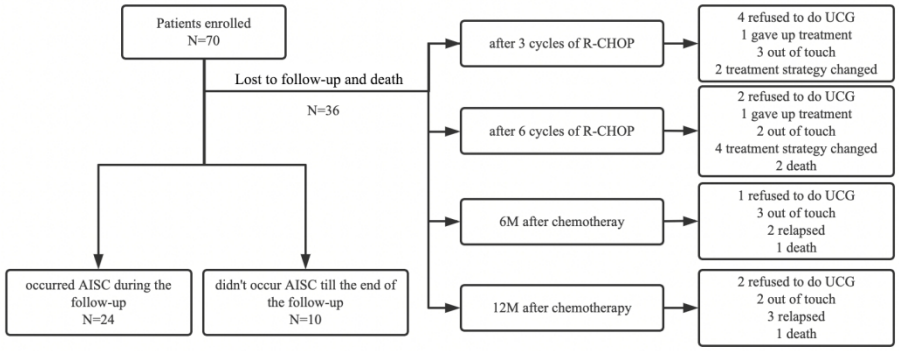

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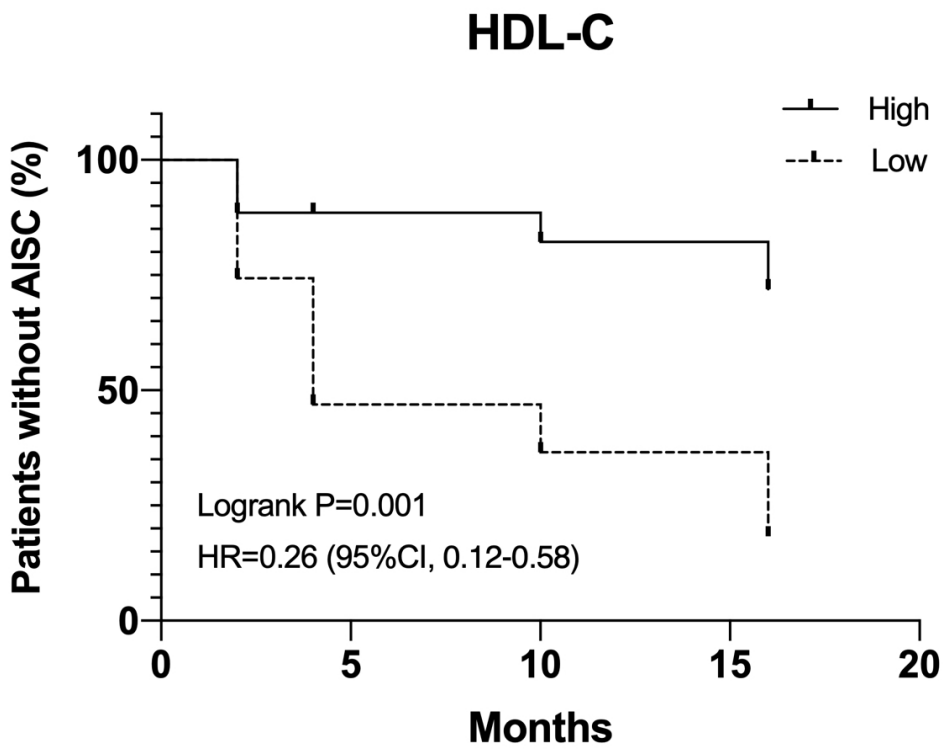

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Table S1. Baseline clinical characteristics of patients enrolled

	Total cohort n=70	Low HDL-C* n=42	High HDL-C* n=28	*P*-value
Age (year)	55.03±14.79	52.48±16.11	58.86±11.83	0.077
Male/Female (n)	34/36	22/20	12/16	0.435
BMI (kg/m^2)	23.08±3.33	23.51±3.29	22.43±3.33	0.183
ECoG performance status				0.465
0 (%)	37 (52.86)	22 (52.38)	15 (53.57)	
1 (%)	24 (34.29%)	13 (30.95)	11 (39.28)	
2 (%)	9 (12.85)	7 (16.67)	2 (7.14)	
Hypertension (%)	9 (12.86)	5 (11.90)	4 (14.28)	1.000
Antihypertensive treatment	9 (12.86)	5 (11.90)	4 (14.28)	1.000
ACEI/ARB	5	2	3	
Beta-blockers	4	3	1	
Diabetes mellitus (%)	5 (7.14)	3 (7.14)	2 (7.14)	1.000
Smoking (%)	21 (30.00)	10 (23.81)	11 (39.28)	0.166
Drinking (%)	18 (25.71)	7 (16.67)	11 (39.28)	**0.034**
Heart rate	80.67±10.61	81.50±11.04	79.43±10.02	0.428
LVEF (%)	65.5 (63.0, 67.0)	65.0 (63.0, 67.0)	66.0 (63.2, 67.8)	0.522
FS (%)	35.67±2.10	35.60±2.14	35.79+2.08	0.713
GLS （-%）	20.0 (19.0, 22.0)	21.0 (19.0, 22.0)	20.0 (18.2, 21.0)	0.167
LVDd (mm)	46.30±3.50	46.93±3.44	45.36±3.43	0.065
LVMi (g/m^2)	94.72±17.55	96.79±18.54	91.62±15.76	0.230
E (cm/s)	69.12±13.12	69.71±12.79	68.23±13.80	0.647
e'(cm/s)	7.60±2.15	7.60±2.07	7.59±2.30	0.987
E/e'	9.61±2.41	9.66±2.30	9.53±2.59	0.832
NT-proBNP (ng/L)	62.50 (31.25, 132.25)	51.00 (29.00, 128.75)	79.00 (41.75, 168.50)	0.171
cTnT （ng/mL）	0.004 (0.000, 0.006)	0.004 (0.002, 0.007)	0.004 (0.000, 0.005)	0.509
HsCRP	2.61 (1.00, 15.64)	4.04 (1.17, 20.00)	1.95 (0.76, 14.51)	0.161
TC (mmol/L)	4.09±0.93	3.86±0.97	4.44±0.76	**0.011**
TG (mmol/L)	1.40 (0.97, 1.68)	1.51 (1.19, 1.93)	1.10 (0.82, 1.50)	**0.002**
LDL (mmol/L)	2.55±0.77	2.42±0.80	2.75±0.69	0.084
HDL-C (mmol/L)	1.08±0.38	0.84±0.21	1.44±0.28	**<0.001**

Values are expressed as mean±standard deviation, n (%), or median (Q1-Q3). Bold values indicate statistical significance.

* Low HDL-C: HDL-C<1.16mmol/L; High HDL-C: HDL-C≥1.16mmol/L. BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

Table S2. Univariable Cox regression analysis of enrolled patients.

Variables	Univariate analysis		P values
	HR	95%CI	
Age, per 1 year	0.97	0.943-0.998	**0.034**
Female vs Male	1.41	0.63-3.19	0.404
BMI, per 1 kg/m^2	1.09	0.97-1.22	0.139
ECoG, 0 or 1 vs status 2	0.63	0.30-1.32	0.220
Hypertension, yes vs no	0.22	0.03-1.62	0.136
Diabetes mellitus, yes vs no	0.35	0.05-2.61	0.307
Smoking, yes vs no	0.77	0.31-1.96	0.589
Drinking, yes vs no	0.84	0.31-2.26	0.728
Heart rate, per 1 bp	0.99	0.96-1.03	0.645
LVEF, per 1%	1.09	0.94-1.25	0.250
FS, per 1%	1.13	0.93-1.37	0.219
GLS, per -1%	1.46	1.20-1.77	**<0.001**
LVDd, per 1 mm	1.05	0.93-1.19	0.425
LVMi, per 1 g/m^2	1.00	0.97-1.02	0.762
E, per 1 cm/s	1.03	1.00-1.06	0.075
e', per 1 cm/s	1.14	0.94-1.40	0.189
E/e', per 1	0.97	0.81-1.16	0.752
NT-proBNP, per 1 ng/L	1.00	0.99-1.00	0.153
cTnT, per lg1 ng/mL	1.19	0.83-1.72	0.340
HsCRP, per 1	1.02	0.97-1.07	0.453
TC, per 1 mmol/L	0.78	0.51-1.20	0.259
TG, per 1 mmol/L	0.93	0.55-1.58	0.796
LDL-C, per 1 mmol/L	0.94	0.56-1.55	0.795
HDL-C group (high vs low)	0.24	0.09-0.67	**0.006**

High HDL-C group: HDL-C≥1.16mmol/L. Low HDL-C group: HDL-C<1.16mmol/L. BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

Table S3. Baseline clinical characteristics of un-censored patients with or without AISC.

	Total cohort	NO-AISC	AISC	P-value
	n=34	n=10	n=24	
Age (year)	52.85±12.37	59.7±9.67	50.0±12.45	**0.035**
Male/Female (n)	19/15	5/5	14/10	0.947
BMI (kg/m^2)	24.01±3.21	23.67±3.83	24.15±3.00	0.698
ECoG performance status				1.000
0 (%)	23 (67.65)	7 (70.00)	16 (66.67)	
1 (%)	7 (20.59)	2 (20.00)	5 (20.83)	
2 (%)	4 (11.76)	1 (10.00)	3 (12.50)	
Hypertension (%)	4 (11.76)	3 (30.00)	1 (4.17)	0.122
Antihypertensive treatment	4 (11.76)	3 (30.00)	1 (4.17)	0.122
ACEI/ARB	3	2	1	
Beta-blockers	1	1	0	
Diabetes mellitus (%)	3 (8.82)	2 (20.00)	1 (4.17)	0.412
Smoking (%)	11 (32.35)	5 (50.00)	6 (25.00)	0.309
Drinking (%)	7 (20.59)	2 (20.00)	5 (20.83)	1.000
Heart rate	81.03±11.08	84.60±13.53	79.54±9.83	0.231
LVEF (%)	65.0 (63.0, 67.0)	64.0 (62.0, 67.3)	66.0 (64.0, 67.0)	0.270
FS (%)	35.74±2.12	35.10±2.28	36.0±2.04	0.266
GLS（-%）	21.0 (19.0, 22.0)	18.0 (17.0, 20.0)	22.0 (21.0, 22.8)	**<0.001**
LVDd (mm)	46.62±3.08	45.61±3.41	47.04±2.90	0.218
LVMi (g/m^2)	94.04±16.26	93.27±18.87	94.36±15.78	0.862
E (cm/s)	70.87±12.84	66.43±11.78	72.73±13.04	0.197
e'(cm/s)	7.59±1.77	6.90±1.88	7.88±1.68	0.144
E/e'	9.66±2.17	9.95±1.79	9.54±2.34	0.626
NT-proBNP (ng/L)	46.00 (28.50, 109.00)	78.50 (31.50, 133.25)	41.00 (28.00, 122.00)	0.308
cTnT（ng/mL）	0.004 (0.000, 0.007)	0.004 (0.000, 0.008)	0.004 (0.000, 0.007)	0.816
HsCRP	2.28 (0.95, 17.18)	1.62 (0.54, 13.79)	3.84 (1.21, 20.00)	0.216
TC (mmol/L)	4.07±0.95	4.38±1.31	3.95±0.75	0.219
TG (mmol/L)	1.40 (1.12, 1.63)	1.54 (1.67, 1.85)	1.38 (1.10, 1.56)	0.344
LDL-C (mmol/L)	2.59±0.83	2.73±1.15	2.53±0.68	0.530
HDL-C (mmol/L)	1.06±0.38	1.21±0.56	1.00±0.26	0.128
HDL-C group*				**0.019**
High (%)	12 (35.29)	7 (70.00)	5 (20.83)	
Low (%)	22 (64.71)	3 (30.00)	19 (79.17)	

*High HDL-C: HDL-C≥1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L.

AISC: anthracycline-induced subclinical cardiotoxicity; AST: aspartate transaminase; ALT: alanine aminotransferase; BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total

cholesterol; TG: total triglyceride.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	3-4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	3
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	5-6
Outcome data	15*	Report numbers of outcome events or summary measures over time	5-6

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-7
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Role of HDL cholesterol in anthracycline-induced subclinical cardiotoxicity: a prospective observational study in patients with diffuse large B-cell lymphoma treated with R-CHOP

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Role of HDL cholesterol in anthracycline-induced subclinical cardiotoxicity: a prospective observational study in patients with diffuse large B-cell lymphoma treated with R-CHOP

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Keywords: cardiotoxicity; DLBCL; anthracycline; HDL-C; GLS

ABSTRACT

Objectives: Anthracycline-induced cardiotoxicity is a debilitating cardiac dysfunction for which there are no effective treatments, making early prevention of anthracycline-induced subclinical cardiotoxicity (AISC) crucial. High-density lipoprotein cholesterol (HDL-C) plays role in cardio-protection, but its impact on AISC remains unclear. Our study aims to elucidate the protective capacity of HDL-C in AISC in patients with diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP (cyclophosphamide, vincristine, doxorubicin, prednisone, and rituximab).
Design: Prospective observational study
Setting: Conducted in China from September 2020 to September 2022.
Participant: 70 chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to receive the standard dose of R-CHOP; 60 participants included in a case-control study (DOI: 10.1186/s12885-022-10085-6).

Primary outcome measures: Serum biomarkers, 2D speckle tracking echocardiography, and conventional echocardiography were measured at baseline, at the end of the 3rd and 6th cycle of R-CHOP, and 6 and 12 months post-chemotherapy.

Results: 24 patients experienced AISC, while 10 did not. 36 patients were lost to follow-up and death. Cox regression analysis showed that higher levels of HDL-C were associated with a significantly lower risk of AISC (unadjusted, hazard ratio [HR]=0.24, 95%CI: 0.09-0.67, P=0.006; adjusted, HR=0.27, 95%CI: 0.09-0.79, P=0.017). Patients without AISC had a more stable and higher HDL-C level during the follow-up period. HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients (P=0.034), and particularly in the AISC group (P=0.003). The highest level of HDL-C was significantly higher in patients without AISC than in those with AISC (1.52±0.49 vs. 1.22±0.29, P=0.034).

Conclusions: Our study suggests that higher HDL-C levels may associate with lower AISC risk in patients with DLBCL treated with R-CHOP. HDL-C could be a cardio-protective target, but further research is needed to confirm its benefits and limitations.

Study registration number: ChiCTR2100054721

Strengths and limitations of this study

- This prospective observational study contributes to our understanding of the association between HDL-C and AISC, offering a foundation for the development of early intervention and prevention strategies.
- The study used advanced imaging techniques (2D-STE) to assess the subclinical cardiac dysfunction in the patients, which can provide more sensitive and accurate results compared to traditional echocardiography.
- The study only included patients with DLBCL who received R-CHOP, which may limit the generalizability of the findings to patients with other types of cancer or chemotherapy regimens.
- The relatively small sample size in this study may potentially impact the robustness and generalizability of our findings. Additional comprehensive studies, including both clinical and basic research, are necessary to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in anthracycline-treated cancer patients.

INTRODUCTION

The improved management of cancer has led to a significant increase in the survival rate of cancer survivors(1). However, anthracycline, one of the most effective chemotherapeutic agents used to treat various cancers, is associated with potentially life-threatening and severe cardiovascular diseases(2). Studies have shown a significant increase in mortality in cancer patients with cardiovascular disease(3, 4). As advances in cancer treatment and an aging population continue, the number of patients with both conditions is rising(5). As a result, the field of cardio-oncology has become increasingly important in recent years.

Non-Hodgkin's lymphoma (NHL) is the 7th most common cancer in the United States and the most frequent hematologic malignancy globally, accounting for about 3% of cancer cases and deaths(6). Among NHL, DLBCL is the most prevalent type, representing approximately one-third of all cases(7). The combination of cyclophosphamide, vincristine, doxorubicin, and prednisone with rituximab (R-CHOP) is a standard first-line therapy that has substantially improved survival outcomes in patients with DLBCL(8). Nonetheless, anthracycline-containing chemotherapy agents are associated with cardiotoxicity, a major long-term adverse effect that significantly affects the quality of life and survival of cancer survivors.

Anthracycline-induced cardiotoxicity (AIC) is a devastating consequence of successful cancer treatment, often leading to hypokinetic cardiomyopathy and ultimately heart failure. AIC is an irreversible form of cardiac dysfunction for which no guidelines or accepted therapies for cardioprotection currently exist(9, 10). Therefore, early prevention and detection of AIC are crucial for providing opportunities for early intervention. Anthracycline-induced subclinical cardiotoxicity (AISC) is an early stage of AIC, characterized by abnormal echocardiography index without clinical symptoms(11). Early intervention is recommended by the 2022 International Cardio-Oncology Society (IC-OS) consensus statement once AISC is detected(12). Global longitudinal peak systolic strain (GLS) measured by 2D speckle tracking echocardiography can reliably identify most early myocardial deformation variations. In our study, we used early measurement of GLS to identify AISC(13, 14).

High-density lipoprotein (HDL) is the sole lipoprotein with protective attributes among the five types of lipoproteins. Its salutary effects include antioxidant, anti-inflammatory, and anti-apoptotic properties. Numerous preclinical investigations have suggested that HDL may have direct and indirect protective effects against AIC(15-17). The roles of HDL-cholesterol (HDL-C) and apolipoprotein A1 (ApoA1) in providing cardiovascular protection of HDL have been the subject of recent debate. Therefore, further investigation is warranted to explore the clinical data pertaining to the association between HDL and anthracycline-related cardiotoxicity.

We undertook an prospective observational study to investigate the potential impact of HDL-C on AISC. Using 2D speckle tracking echocardiography, we identified AISC and sought to establish any correlation between HDL-C and AISC. Additionally, we assessed the fluctuations in HDL-C levels during R-CHOP chemotherapy in chemotherapy-naïve patients recently diagnosed with DLBCL. Subsequently, our team conducted a case-control study, revealing that HDL-C serves as a predictive factor for AISC in patients with DLBCL treated with 3 cycles of (R)-CHOP(18). Both the case-control study and present study are from the same database of the registered study 'Study of Antineoplastic Drugs Induced Early Cardiotoxicity in Patients with Lymphoma'(ChiCTR2100054721). Even though this case-control study was analyzed with data from different parts of the same database for different objectives, the result underscores the significance of further investigating the relationship between HDL-C and AISC.

METHODS

Study population

We recruited chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to receive the standard dose of R-CHOP chemotherapy regimen at our institution from September 1st, 2020, to September 1st, 2022. Our inclusion criteria were as follows: newly diagnosed DLBCL, age between 18 and 80 years, Eastern Cooperative Oncology Group (ECoG) performance status $\leqslant 2$, left ventricular ejection fraction (LVEF) $\geqslant 50\%$, and acceptable bone marrow, renal, and hepatic functions for chemotherapy. Conversely, our exclusion criteria were symptomatic heart failure, a history of myocardial ischemia, myocarditis, myocardial infarction, clinical or subclinical pericardial effusion, arrhythmia requiring medical intervention, a history of other cancers, under lipid-lowing treatment, and severe active infections such as syphilis, hepatitis, or human immunodeficiency virus (HIV) infection. This study shares its database with a case-control study previously conducted by our group, as referenced earlier(18). The patients enrolled in the two study were not identical because of a few differences in specific objectives, inclusion criteria, and exclusion criteria. In brief, the case-control study specifically included patients with DLBCL who received the standard dose of (R)-CHOP chemotherapy regimen (CHOP with or without rituximab combination), and patients undergoing lipid-lowering therapy were not excluded. In the current study, 10 of the 70 enrolled participants were not enrolled in the case-control study. More details can be seen in this case-control study(18).

Treatment

Patients received a total of 6 cycles of standard R-CHOP (cyclophosphamide at 750 mg/m^2 on D1, doxorubicin at 50 mg/m^2 on D1, vincristine at 1.4 [maximum 2] mg/m^2 on D1, and 100 mg prednisone on D1-5, with rituximab at 375 mg/m^2 on D1 in each cycle), with or without 2 cycles of rituximab maintenance (rituximab at 375 mg/m^2 on D1 in each cycle).

Definition of subclinical cardiotoxicity

According to the IC-OS consensus statement, the definition of subclinical cardiotoxicity was a relative GLS decrease from baseline [(baseline – current GLS)/baseline GLS] of >12%, but with a normal left ventricular ejection fraction (LVEF)(12).

Study protocol

We defined 'baseline' as the initial assessment conducted before the initiation of the first cycle of chemotherapy. At baseline, the end of the 3rd cycle of R-CHOP, the end of the 6th cycle of R-CHOP, and 6 and 12 months after chemotherapy completion, all enrolled patients underwent conventional echocardiography, 2D speckle tracking echocardiography, and blood sampling. Every patient received electrocardiography (ECG) examination before every cycle of chemotherapy to ensure the safety of the treatment. Demographic data and clinical variables, including age, gender, body mass index (BMI), ECoG performance status, diabetes mellitus, hypertension, drinking history (an adult who has consumed more than 20 drinks in lifetime, with each drink is considered to have an average alcohol content of 12 g), and smoking history (an adult who has smoked at least 100 cigarettes in their lifetime) were collected at the time of enrollment. Left ventricular systolic dysfunction was measured by LVEF, fractional shortening (FS), left ventricular mass index (LVMI), left ventricular diastolic dimension (LVDd), E, e', E/e', and GLS. HDL-C, low-density lipoprotein cholesterol (LDL-C), cardiac troponin T (cTnT), high sensitivity

C-reactive protein (hsCRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), total cholesterol (TC) and total triglyceride (TG) were measured. We used the baseline HDL-C level as a surrogate marker for HDL quantity. The patients were categorized into two groups based on the average HDL-C value for males and females in the modified criteria of the National Cholesterol Educated Program Adult Treatment Panel (NCEP ATP III)(19). High HDL-C was defined as a serum HDL-C≥1.16mmol/L, while low HDL-C was defined as a serum HDL-C<1.16mmol/L. We determined the sample size using an online sample size calculator, which indicated a total requirement of 23 events(20).

Statistical analysis

The study was conducted with two aims: firstly, to evaluate the relationship between HDL-C and AISC; and secondly, to conduct a preliminary exploration of the differences in HDL-C and the variability of HDL-C changes between patients with and without AISC during the follow-up period. Continuous variables were expressed as mean± standard deviation (SD) and compared using the t-test. Non-normally distributed variables were presented as median (Q1- Q3) and compared with the Wilcoxon Mann-Whitney test. Categorical variables were expressed as n (%) and compared using the Chi-square or Fisher's exact test, as appropriate. Correlation analysis was conducted to investigate the associations of change in HDL-C with change in GLS. The probabilities of survival were calculated using Kaplan-Meier methods and compared using Log-rank tests. Cox proportional-hazards regression models were conducted to assess the association between variables and AISC. Covariates for multivariable Cox regression models included age, sex, and variables that had a P-value of less than 0.15 in the univariable Cox regression analysis (GLS was excluded as it is the factor that defines AISC). Two multivariable Cox regression models were constructed: the first model included age and sex; and the second model included age, sex, hypertension, BMI, and E. Statistical analysis and visualization were performed using IBM SPSS V.22.0 and GraphPad Prism 8. Statistical tests were two-sided, with a P-value less than 0.05 being considered statically significant.

Patient and public involvement

None.

RESULTS

Assessment of the association between HDL-C and AISC

Study population and baseline characteristics

This investigation enrolled a total of 70 patients with chemotherapy-naïve DLBCL and were planned to be treated with the standard R-CHOP regimen. Based on the baseline HDL-C level, we segregated the patients into two groups: the high-level group (HDL-C⩾1.16mmol/L, n=28) and the low-level group (HDL-C<1.16mmol/L, n=42). Patients with drinking history had a greater chance of having a high HDL-C level (P=0.034). The patients with high HDL-C showed substantially higher total cholesterol (P=0.011), and lower total triglyceride (P=0.002). The baseline characteristics of the patients in both groups were well balanced (Table S1).

High HDL-C was an independent protective target of anthracycline-induced subclinical cardiotoxicity

The clinical endpoint was defined as the first detection of AISC, and the median survival time of the whole cohort was 16 months. The median survival time of patients with low HDL-C was 4 months, while that of patients with high HDL-C was not reached. The median follow-up time of the cohort was 10 months. During the follow-up period, 24 patients experienced AISC, while 10 did not. Approximately half of the patients (n=36) were lost to follow-up and death. A flowchart detailing the patients enrolled in the study can be found in Figure S1.

The log-rank test revealed that patients with higher HDL-C were less likely to experience AISC (P=0.001, HR=0.26, 95%CI: 0.12-0.58) (Figure S2).

According to the results of the univariable Cox regression analysis, variables that had a P-value of less than 0.15 including age, BMI, hypertension, GLS, E, and HDL-C group. Increasing age was significantly associated with a decreased HR of 0.97 (95%CI 0.943-0.998, P=0.034) per 1-year increase. BMI showed a HR of 1.09 (95% CI 0.97-1.22, P=0.139) per 1 kg/m^2 increase. Similarly, hypertension had a HR of 0.22 (95% CI 0.03-1.62, P=0.136) for yes versus no. A lower GLS was significantly associated with an increased HR of 1.46 (95% CI 1.20-1.77, P<0.001) per -1% decrease. E velocity showed a HR of 1.03 (95% CI 1.00-1.06, P=0.075) per 1 cm/s increase. The HDL-C group (high versus low) had a significantly lower HR of 0.24 (95% CI 0.09-0.67, P=0.006). Further details about other variables can be found in Table S2.

The results of the multivariable Cox regression analysis showed that high HDL-C was significantly associated with a lower risk of AISC after adjusting for age and sex (model 1) (HR=0.28, 95%CI:0.10=0.84, P=0.018). Similarly, after adjusting for age, sex, and variables that P<0.15 in the univariable Cox regression analysis (excluding GLS as it defines AISC) (model 2), the same association was observed (HR=0.27, 95%CI: 0.09-0.79, P=0.017). (Table 1)

Table 1. Outcomes of study participants.

	HR (95%CI) (unadjusted)	P values	HR (95%CI) (adjusted*)	P values	HR (95%CI) (adjusted#)	P values
Low HDL-C	Ref		Ref		Ref	
High HDL-C	0.24 (0.09-0.67)	**0.006**	0.28 (0.10-0.80)	**0.018**	0.27 (0.09- 0.79)	**0.017**

The endpoint was defined as the first detection of anthracycline-induced subclinical cardiotoxicity.

Low HDL-C: HDL-C<1.16mmol/L; High HDL-C: HDL-C≥1.16mmol/L. *Adjusted for age and sex. #Adjusted for age, sex, hypertension, body mass index, E. HR, hazard ratio; HDL-C, high-density lipoprotein-cholesterol.

Preliminary exploration of the difference of HDL-C between patients with AISC and without AISC

Study population and baseline characteristics

In this analysis, we selectively included 34 of the enrolled patients who were not lost to follow-up and death. The patients who exhibited AISC at any time during the follow-up period were segregated into the AISC group (n=24), while those who did not demonstrate AISC were classified into the NO-AISC group (n=10). Patients within the AISC group were comparatively younger

(50±12.45 vs. 59.7±9.67, P=0.035) and exhibited a higher baseline GLS [22.0 (21.0, 22.8) vs. 18.0 (17.0, 20.0), P<0.001]. More baseline information can be seen in Table S3.

Timeline of HDL-C level in patients with and without AISC

Figure 1 displays the timeline of HDL-C levels in patients with and without AISC. In Figure 1a, the patient population was categorized into four groups based on the time of AISC detection. Among the groups, 12 patients were identified with AISC at the end of the 3rd cycle of chemotherapy, 7 patients at the end of the 6th cycle, 3 patients at 6 months after treatment completion, and 2 patients at 12 months after treatment completion. With the exception of the group in which patients detected AISC at the end of the 3rd cycle of chemotherapy, all other groups exhibited a reduction in HDL-C values from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy. Figure 1b portrays the HDL-C level in patients without AISC, indicating that the HDL-C level was more stable than in patients with AISC. Moreover, the overall HDL-C level was higher in patients without AISC than in patients with AISC throughout the follow-up period (Figure 2a). In Figure 2b, there was a significant decrease in GLS during the chemotherapy period (from 0-4 months), which remained stable after completion of chemotherapy (after 4 months) in patients with AISC.

Based on Figure 2, we observed that the fluctuations in HDL-C and GLS were most pronounced during the chemotherapy period. The fluctuations in HDL-C levels of patients with DLBCL during R-CHOP chemotherapy were presented in Figure 3. The levels of HDL-C significantly increased for all patients from baseline to the end of the 3rd cycle of chemotherapy (P=0.012) and significantly decreased from the end of the 3rd cycle to the end of the 6th cycle of chemotherapy (P=0.034) (Figure 3a). Patients with AISC showed a significant decrease in HDL-C levels during R-CHOP chemotherapy from the end of the 3rd cycle to the end of the 6th cycle (P=0.003) (Figure 3b). However, no significant difference was observed in HDL-C levels for patients without AISC during R-CHOP chemotherapy (Figure 3c). We conducted correlation analysis separately for the change in HDL-C and GLS from baseline to after 3 cycles of chemotherapy, from baseline to after 6 cycles of chemotherapy, and from after 3 cycles to after 6 cycles of chemotherapy. However, we found no statistically significant differences in the associations between changes in HDL-C and GLS (P=0.965, 0.087, 0.449).

Contrasting values of HDL-C parameters between patients with and without AISC

Figure 4 presents the contrasting values between patients with AISC and those without in terms of four parameters, namely the highest and lowest levels of HDL-C during chemotherapy, the increment and decline in HDL-C values from baseline. Patients without AISC showed significantly higher values in the highest level of HDL-C (1.52±0.49 vs. 1.22±0.29, P=0.034, Figure 4a). However, no significant differences were observed between the two groups in terms of HDL-C increment from baseline to the highest value (0.31±0.31 vs. 0.22±0.23, P=0.386, Figure 4b). While the lowest level of HDL-C was lower in patients with AISC, the difference was not statistically significant (0.84±0.16 vs. 1.03±0.41, P=0.182, Figure 4c). Furthermore, there were no significant differences in HDL-C decline between patients with AISC and those without (0.16±0.20 vs. 0.18±0.26, P=0.777, Figure 4d).

DISCUSSION

This prospective observational study investigated the relationship between HDL-C and incidence of AISC in 70 patients with DLBCL who were receiving anthracycline-containing chemotherapy. The study found that higher levels of HDL-C were associated with a lower incidence of AISC. Moreover, patients without AISC had more stable and higher levels of HDL-C than those with AISC during the follow-up period. The results also showed that HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients, especially in the AISC group, indicating that anthracycline-containing chemotherapy has adverse effects on HDL-C levels. Notably, the highest level of HDL-C was significantly higher in patients without AISC compared to those with AISC. These findings suggest that HDL-C may have a protective role against AISC in patients with DLBCL undergoing anthracycline-containing chemotherapy and maintaining a relatively high level of HDL-C may be more effective in managing cardio-protection than monitoring changes in HDL-C levels over time. The results of this study highlight the importance of early serum lipid management in these patients.

Lipoproteins are classified into five categories, namely chylomicron, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), based on their size, density, and lipid composition (cholesterol and triglycerides)(21). Among these, HDL exhibits distinctive cytoprotective actions and triggers anti-oxidative, anti-inflammatory, and anti-apoptotic effects. The protective roles of HDL in cardiovascular disease have been controversial in recent years, and that the quality of HDL (cholesterol efflux capacity, antioxidant activity, anti-inflammatory activity, endothelial function, etc.) rather than the quantity of HDL has been proposed as the true cardioprotective effect. The Framingham Heart Study, as early as 1988, reported a correlation between HDL-C and cardiovascular mortality(22). Recent studies have challenged the HDL-C hypothesis by revealing that HDL-C level is not inversely correlated with cardiovascular diseases(23, 24). In our study, we used the baseline HDL-C level as a surrogate marker for HDL quantity, but we did not directly measure the quality of HDL. Measuring the level of HDL-C in serum is a commonly used method to assess the effect of HDL on cardiovascular health. HDL facilitates the transportation of cholesterol from the body's tissues back to the liver, and higher levels of HDL-C are generally associated with a lower risk of heart disease. Nevertheless, it's crucial to note that HDL-C levels may not accurately reflect the functional properties of HDL. ApoA1, the most abundant protein in HDL, is associated with several beneficial effects of HDL(15, 25). The function and abundance of ApoA1 are reported to play a dominant role in HDL quality(26). In the context of AIC, several studies have indicated that HDL can protect against anthracycline-induced cardiomyocyte apoptosis and atrophy in isolated cardiomyocytes(27, 28) and animal models(16, 28). Based on these earlier trials, HDL-C and ApoA1 could serve as protective factors against anthracycline-related cardiovascular disease. The case-control study conducted by our team, utilizing the same database, demonstrated that both HDL-C and ApoA1 act as predictive factors in patients

undergoing three cycles of anthracycline-containing chemotherapy(18). Both the present study and the case-control study are derived from a registered research (ChiCTR2100054721) as mentioned before. The objective of this registered study was to explore the correlation between cardiotoxicity occurrence and lymphoma, antitumor drugs, and cardiovascular risk factors. This registered study included patients with clinically diagnosed lymphoma who were evaluated by a hematologist as requiring chemotherapy. The study collected the demographic data and clinical variables of enrolled patients. Enrolled patients underwent conventional echocardiography, 2D speckle tracking echocardiography, and blood sampling at baseline, the end of the 3rd cycle of chemotherapy, the end of the 6th cycle of chemotherapy, and 6 and 12 months after completing chemotherapy. In our case-control study, according to the changes of GLS at baseline and after the 3rd cycle of chemotherapy, patients were divided into the AISC and No-AISC groups. Then demographic data, clinical variables, and biochemical variables were compared between the two groups. In contrast to the current study, this case-control study specifically included patients with DLBCL who received the standard dose of (R)-CHOP chemotherapy regimen (CHOP with or without rituximab combination), and patients undergoing lipid-lowering therapy were not excluded. The aim of this case-control study was to analyze the influencing factors of AISC in patients with DLBCL treated with 3 cycles of (R)-CHOP chemotherapy regimen, and results indicated that both HDL-C and ApoA1 act as predictive factors against AISC. However, our present study didn't focus on the investigation of the impact of ApoA1 on AISC. Even when ApoA1 was included in the Cox regression model, no significant association with AISC in patients with DLBCL treated with R-CHOP was observed ($P>0.05$, data not shown), probably due to the number of events in our study was insufficient to support a robust ApoA1 analysis. Therefore, the role of ApoA1, the most abundant protein in HDL, in the context of AISC, warrants further investigation in future research.

As far as we know, few clinical studies have investigated the association between HDL-C and AISC. This study is the first clinical research that utilizes the IC-OS consensus statement(12) to define subclinical cardiotoxicity, with univariate and multivariable analyses being used to identify the influential factors of AISC in patients with DLBCL in this cohort. Kaplan-Meier methods and Log-rank tests reveal that patients with high HDL-C levels were less likely to develop AISC. After subjecting it to univariate and multivariable Cox regression methods, high HDL-C levels still showed statistically significant differences. These results suggest that high HDL-C could be a potentially independent protective factor for AISC in patients with DLBCL and provide an opportunity for investigators to develop a tool for early intervention and prevention of AISC. Further research is necessary to confirm our findings.

Several studies have demonstrated that serum lipid levels are altered during anthracycline-containing chemotherapy in cancer patients(29, 30). Huxley et al and Averina et al have shown that imbalanced serum lipid distribution is a risk factor for cardiovascular disease(31, 32). As a result, anthracycline-containing treatment can induce dyslipidemia and facilitate the occurrence and development of cardiovascular diseases in cancer patients. In a study of 394 breast cancer patients, Xin et al found that HDL-C levels after chemotherapy were significantly lower than those

before chemotherapy(33). Similarly, Lu et al and Hana et al found that HDL-C levels were significantly decreased during anthracycline-containing chemotherapy in patients with breast cancer(34, 35). In our study, we specifically assessed the changes in HDL-C levels over time during follow-up. Except for the group of patients who experienced AISC at 12 months after treatment completion, HDL-C levels in all other groups increased from baseline to the 3rd cycle of chemotherapy. This phenomenon may be due to the fact that anti-tumor drugs require cholesterol to cross cell membranes(36). However, HDL-C levels were significantly decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all patients, especially in the AISC group, which is consistent with previous research results(33-35), and further confirmed that anthracycline-contained chemotherapy has adverse effects on HDL-C levels in patients with DLBCL. The HDL-C level in patients without AISC was more stable than that in patients with AISC. Therefore, anthracycline-containing chemotherapy may promote the occurrence and development of cardiotoxicity in patients with DLBCL by inducing HDL-C turbulence.

Besides, the findings of our study indicate a significant decrease in GLS during the chemotherapy period in patients with AISC. This result is consistent with previous research, which has reported that doxorubicin dose at the range of 100-150mg/m^2 can cause cardiotoxicity(37). Notably, we also observed that GLS remained stable after completion of chemotherapy, suggesting that the cardiac effects of anthracycline-based chemotherapy may be dose-related. These findings have important implications for the monitoring and management of cardiotoxicity in patients undergoing anthracycline-based chemotherapy, as early detection of cardiac dysfunction during treatment may improve patient outcomes. We investigated the associations of change in HDL-C with change in GLS, no statistically significant differences were found.

The analysis of HDL-C levels should not only consider the changes over time, but also the absolute values. In our study, patients without AISC had significantly higher absolute highest HDL-C levels than those with AISC, while the absolute lowest HDL-C levels did not differ significantly between the two groups. The alterations from HDL-C extremes to baseline did not exhibit any variation between the groups either. This suggests that the highest absolute HDL-C value was a preferable indicator of AISC protection than the change in HDL-C from baseline to the extremum value. Maintaining a relatively high level of HDL-C may be more effective in managing the cardio-protection of anthracycline-treated cancer patients than monitoring changes in HDL-C levels over time.

In our investigation, we observed that among the four patients with pre-existing hypertension, one patient experienced AISC during the follow-up (Table S3). Multivariable Cox regression analysis showed that hypertension did not have a significant impact on AISC ($P>0.05$). Hypertension, a common risk factor for both cancer and cardiovascular diseases, was also recognized as a risk factor for cardiotoxicity. Studies have reported that pre-existing hypertension was associated with anthracycline-and trastuzumab induced left ventricular ejection fraction (LVEF) decline in a retrospective study(38), and early left ventricular systolic dysfunction in patients with lymphoma receiving (R)-CHOP in a prospective study(39). We noted that all patients with hypertension in

our study were under a single antihypertensive drug regimen (beta-blockers or ACEI/ARB) to manage their blood pressure. Two meta-analyses have demonstrated that beta-blockers and ACEI can prevent cardiotoxicity caused by chemotherapy (40, 41). We speculate that the protective effects of beta-blockers and ACEI/ARB may have contributed to the result observed in our study regarding the relationship between hypertension and AISC.

There are several limitations to our study that must be acknowledged. Firstly, while our study highlights the potential importance of HDL-C in managing AISC, additional studies are necessary to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in anthracycline-treated cancer patients. Secondly, this is a single-center prospective observational study with a medium sample size. To confirm our findings, a larger sample size study conducted at multiple centers is needed. Thirdly, previous studies have suggested that there may be a reversed U-shaped relationship between HDL-C levels and cardiovascular diseases(42). Due to the small sample size of this study, we didn't further investigate the influence of extremely high levels of HDL-C on cardiotoxicity, and further clinical studies should be done to verify it. Besides, the measurement of GLS was only taken at baseline and at several points throughout the chemotherapy treatment and follow-up period. It is crucial to extend the duration of follow-up in future research to obtain a more comprehensive understanding of the long-term effects of anthracycline treatment on cardiovascular health.

CONCLUSIONS

In conclusion, our prospective observational study suggests that higher levels of HDL-C may be associated with a lower risk of AISC in patients with DLBCL treated with R-CHOP chemotherapy. HDL-C levels remained stable and consistently higher in patients without AISC compared to those with AISC. Additionally, the highest absolute HDL-C value was found to be a preferable indicator of AISC protection. These findings suggest that HDL-C may be a potential cardio-protective target for managing AISC in this patient population. However, further research is needed to confirm and expand on these findings, including determining the optimal HDL-C level for cardio-protection and the potential benefits of early serum lipid management.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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CONTRIBUTORS

Conceptualization, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang; Data curation, Wenxin Ou, Tiantian Jiang, Yue Weng and Xi Zhou; Formal analysis, Wenxin Ou and Tiantian Jiang; Funding acquisi-tion, Wenxin Ou, Nan Zhang, Dong Wang and Xiaoqiong Tang; Investigation, Dong Wang, Qian Dong and Xiaoqiong Tang; Methodology, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang; Resources, Dong Wang, Qian Dong and Xiaoqiong Tang; Supervision, Qian Dong and Xiaoqiong Tang; Validation, Nan Zhang and Kai Lu; Visualization, Wenxin Ou and Tiantian Jiang; Writing – original draft, Wenxin Ou; Writing – review & editing, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong and Xiaoqiong Tang. Qian Dong and Xiaoqiong Tang contributed equally to this work and are considered as co-corresponding authors.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

ETHICS APPROVAL

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (Approval NO. 2018-016). And all participating patients provided written informed consent.

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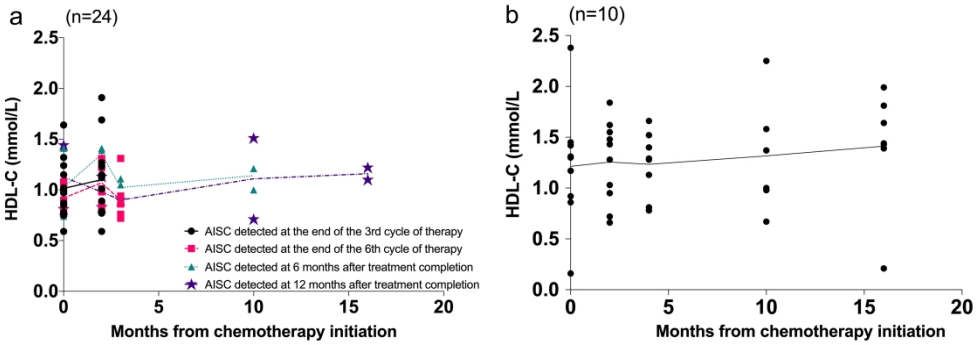
FIGURE LEGENDS

Figure 1. (**a**) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after treatment completion. 2 patients were detected AISC at 12 months after treatment completion. (**b**) Timeline of HDL-C levels of patients without AISC.

Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (**a**) and global longitudinal strain (GLS) (**b**) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

Figure 3. (**a**) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (**b**) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (**c**) Changes of HDL-C in patients without AISC.

Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (**a**) Highest level of HDL-C during chemotherapy. (**b**)The HDL-C value increment from baseline to the highest value. (**c**) The lowest level of HDL-C during chemotherapy. (**d**) The HDL-C value declined from baseline to the lowest.



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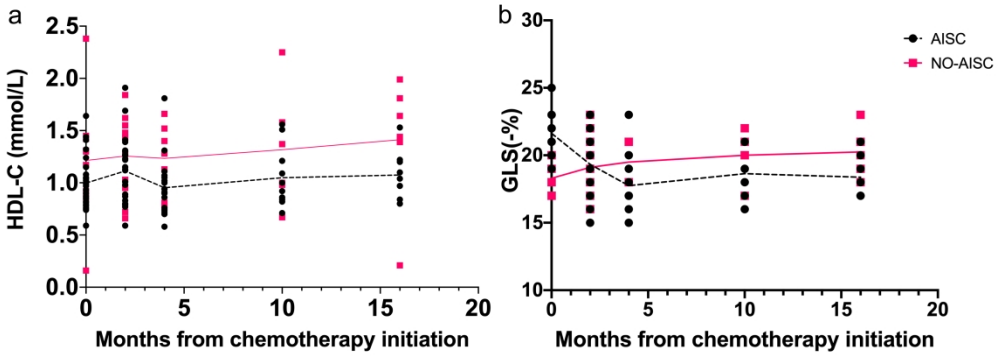

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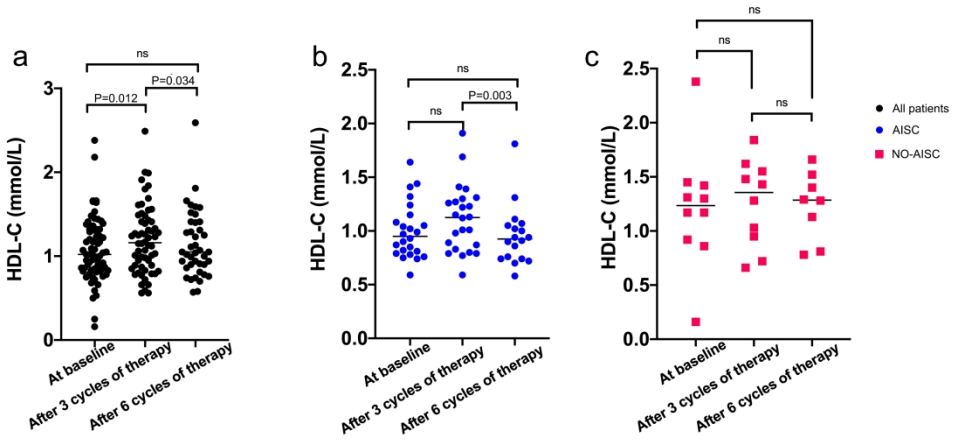

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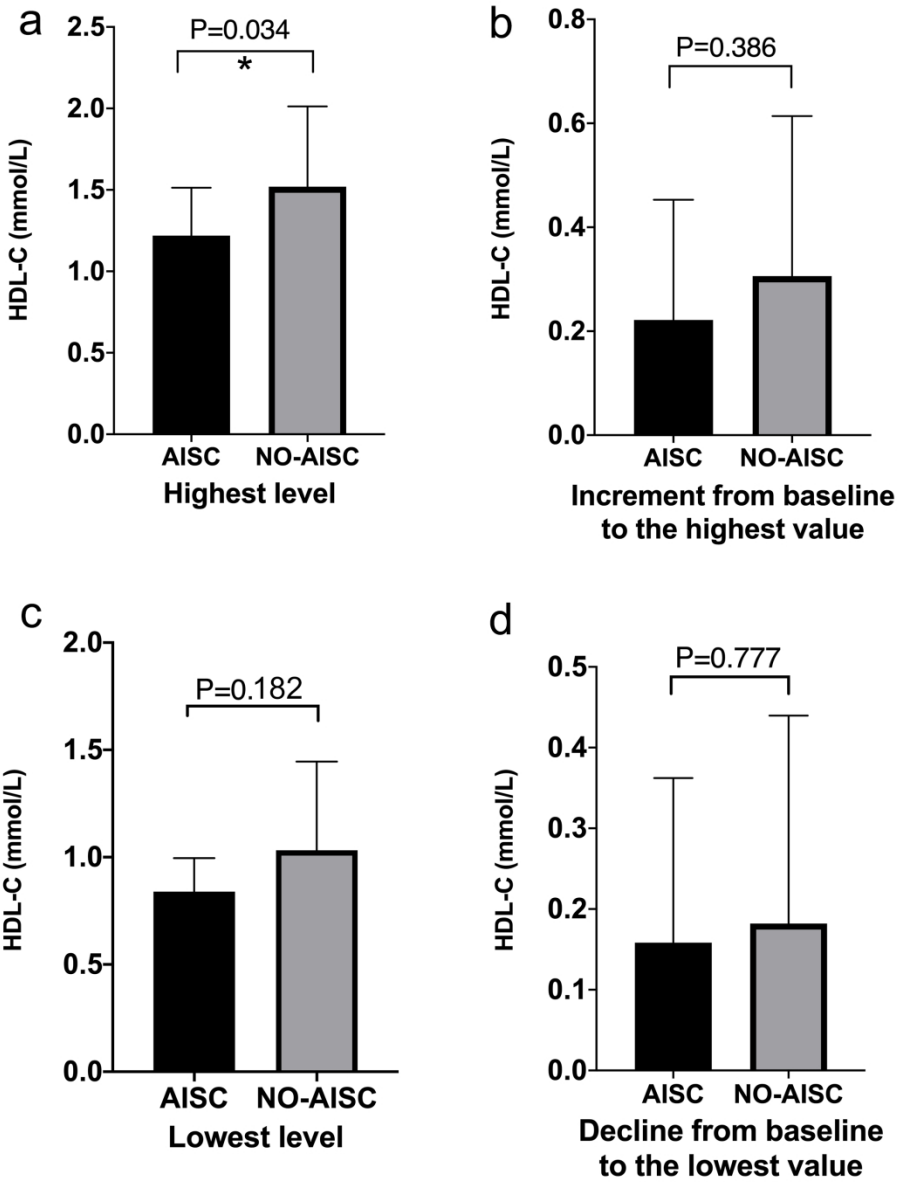

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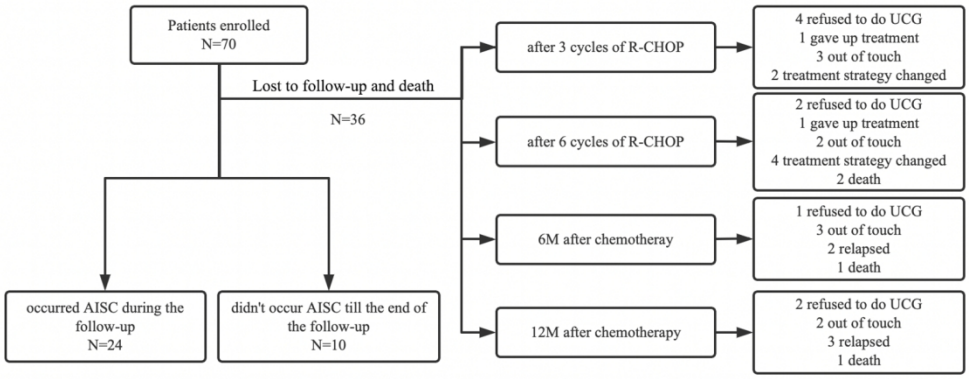

Figure S1. Flow diagram of patients enrolled in the prospective observational study.

148x68mm (300 x 300 DPI)

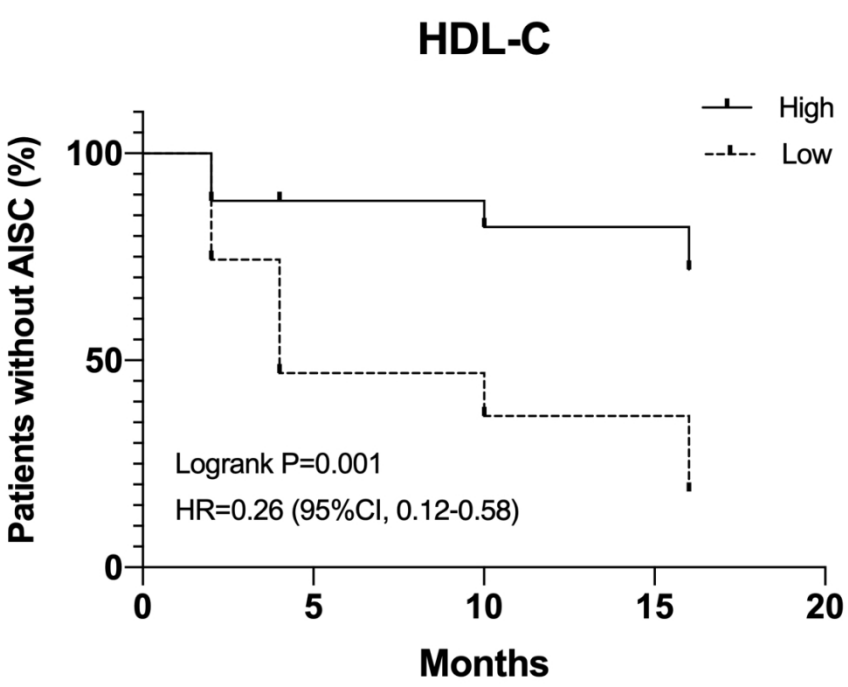

Figure S2. Kaplan-Meier curves of the percentage of patients without AISC in patients stratified by HDL-C level. High HDL-C: HDL-C≥1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L. AISC, anthracycline-induced cardiotoxicity; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio.

112x92mm (300 x 300 DPI)

Table S1. Baseline clinical characteristics of patients enrolled

	Total cohort n=70	Low HDL-C* n=42	High HDL-C* n=28	*P*-value
Age (year)	55.03±14.79	52.48±16.11	58.86±11.83	0.077
Male/Female (n)	34/36	22/20	12/16	0.435
BMI (kg/m^2)	23.08±3.33	23.51±3.29	22.43±3.33	0.183
ECoG performance status				0.465
0 (%)	37 (52.86)	22 (52.38)	15 (53.57)	
1 (%)	24 (34.29%)	13 (30.95)	11 (39.28)	
2 (%)	9 (12.85)	7 (16.67)	2 (7.14)	
Hypertension (%)	9 (12.86)	5 (11.90)	4 (14.28)	1.000
Antihypertensive treatment	9 (12.86)	5 (11.90)	4 (14.28)	1.000
ACEI/ARB	5	2	3	
Beta-blockers	4	3	1	
Diabetes mellitus (%)	5 (7.14)	3 (7.14)	2 (7.14)	1.000
Smoking (%)	21 (30.00)	10 (23.81)	11 (39.28)	0.166
Drinking (%)	18 (25.71)	7 (16.67)	11 (39.28)	**0.034**
Heart rate	80.67±10.61	81.50±11.04	79.43±10.02	0.428
LVEF (%)	65.5 (63.0, 67.0)	65.0 (63.0, 67.0)	66.0 (63.2, 67.8)	0.522
FS (%)	35.67±2.10	35.60±2.14	35.79+2.08	0.713
GLS （-%）	20.0 (19.0, 22.0)	21.0 (19.0, 22.0)	20.0 (18.2, 21.0)	0.167
LVDd (mm)	46.30±3.50	46.93±3.44	45.36±3.43	0.065
LVMi (g/m^2)	94.72±17.55	96.79±18.54	91.62±15.76	0.230
E (cm/s)	69.12±13.12	69.71±12.79	68.23±13.80	0.647
e'(cm/s)	7.60±2.15	7.60±2.07	7.59±2.30	0.987
E/e'	9.61±2.41	9.66±2.30	9.53±2.59	0.832
NT-proBNP (ng/L)	62.50 (31.25, 132.25)	51.00 (29.00, 128.75)	79.00 (41.75, 168.50)	0.171
cTnT （ng/mL）	0.004 (0.000, 0.006)	0.004 (0.002, 0.007)	0.004 (0.000, 0.005)	0.509
HsCRP	2.61 (1.00, 15.64)	4.04 (1.17, 20.00)	1.95 (0.76, 14.51)	0.161
TC (mmol/L)	4.09±0.93	3.86±0.97	4.44±0.76	**0.011**
TG (mmol/L)	1.40 (0.97, 1.68)	1.51 (1.19, 1.93)	1.10 (0.82, 1.50)	**0.002**
LDL (mmol/L)	2.55±0.77	2.42±0.80	2.75±0.69	0.084
HDL-C (mmol/L)	1.08±0.38	0.84±0.21	1.44±0.28	**<0.001**

Values are expressed as mean±standard deviation, n (%), or median (Q1-Q3). Bold values indicate statistical significance.

* Low HDL-C: HDL-C<1.16mmol/L; High HDL-C: HDL-C≥1.16mmol/L. BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

Table S2. Univariable Cox regression analysis of enrolled patients.

Variables	Univariate analysis		P values
	HR	95%CI	
Age, per 1 year	0.97	0.943-0.998	**0.034**
Female vs Male	1.41	0.63-3.19	0.404
BMI, per 1 kg/m^2	1.09	0.97-1.22	0.139
ECoG, 0 or 1 vs status 2	0.63	0.30-1.32	0.220
Hypertension, yes vs no	0.22	0.03-1.62	0.136
Diabetes mellitus, yes vs no	0.35	0.05-2.61	0.307
Smoking, yes vs no	0.77	0.31-1.96	0.589
Drinking, yes vs no	0.84	0.31-2.26	0.728
Heart rate, per 1 bp	0.99	0.96-1.03	0.645
LVEF, per 1%	1.09	0.94-1.25	0.250
FS, per 1%	1.13	0.93-1.37	0.219
GLS, per -1%	1.46	1.20-1.77	**<0.001**
LVDd, per 1 mm	1.05	0.93-1.19	0.425
LVMi, per 1 g/m^2	1.00	0.97-1.02	0.762
E, per 1 cm/s	1.03	1.00-1.06	0.075
e', per 1 cm/s	1.14	0.94-1.40	0.189
E/e', per 1	0.97	0.81-1.16	0.752
NT-proBNP, per 1 ng/L	1.00	0.99-1.00	0.153
cTnT, per lg1 ng/mL	1.19	0.83-1.72	0.340
HsCRP, per 1	1.02	0.97-1.07	0.453
TC, per 1 mmol/L	0.78	0.51-1.20	0.259
TG, per 1 mmol/L	0.93	0.55-1.58	0.796
LDL-C, per 1 mmol/L	0.94	0.56-1.55	0.795
HDL-C group (high vs low)	0.24	0.09-0.67	**0.006**

High HDL-C group: HDL-C≥1.16mmol/L. Low HDL-C group: HDL-C<1.16mmol/L. BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total cholesterol; TG: total triglyceride.

Table S3. Baseline clinical characteristics of un-censored patients with or without AISC.

	Total cohort	NO-AISC	AISC	P-value
	n=34	n=10	n=24	
Age (year)	52.85±12.37	59.7±9.67	50.0±12.45	**0.035**
Male/Female (n)	19/15	5/5	14/10	0.947
BMI (kg/m^2)	24.01±3.21	23.67±3.83	24.15±3.00	0.698
ECoG performance status				1.000
0 (%)	23 (67.65)	7 (70.00)	16 (66.67)	
1 (%)	7 (20.59)	2 (20.00)	5 (20.83)	
2 (%)	4 (11.76)	1 (10.00)	3 (12.50)	
Hypertension (%)	4 (11.76)	3 (30.00)	1 (4.17)	0.122
Antihypertensive treatment	4 (11.76)	3 (30.00)	1 (4.17)	0.122
ACEI/ARB	3	2	1	
Beta-blockers	1	1	0	
Diabetes mellitus (%)	3 (8.82)	2 (20.00)	1 (4.17)	0.412
Smoking (%)	11 (32.35)	5 (50.00)	6 (25.00)	0.309
Drinking (%)	7 (20.59)	2 (20.00)	5 (20.83)	1.000
Heart rate	81.03±11.08	84.60±13.53	79.54±9.83	0.231
LVEF (%)	65.0 (63.0, 67.0)	64.0 (62.0, 67.3)	66.0 (64.0, 67.0)	0.270
FS (%)	35.74±2.12	35.10±2.28	36.0±2.04	0.266
GLS（-%）	21.0 (19.0, 22.0)	18.0 (17.0, 20.0)	22.0 (21.0, 22.8)	**<0.001**
LVDd (mm)	46.62±3.08	45.61±3.41	47.04±2.90	0.218
LVMi (g/m^2)	94.04±16.26	93.27±18.87	94.36±15.78	0.862
E (cm/s)	70.87±12.84	66.43±11.78	72.73±13.04	0.197
e'(cm/s)	7.59±1.77	6.90±1.88	7.88±1.68	0.144
E/e'	9.66±2.17	9.95±1.79	9.54±2.34	0.626
NT-proBNP (ng/L)	46.00 (28.50, 109.00)	78.50 (31.50, 133.25)	41.00 (28.00, 122.00)	0.308
cTnT（ng/mL）	0.004 (0.000, 0.007)	0.004 (0.000, 0.008)	0.004 (0.000, 0.007)	0.816
HsCRP	2.28 (0.95, 17.18)	1.62 (0.54, 13.79)	3.84 (1.21, 20.00)	0.216
TC (mmol/L)	4.07±0.95	4.38±1.31	3.95±0.75	0.219
TG (mmol/L)	1.40 (1.12, 1.63)	1.54 (1.67, 1.85)	1.38 (1.10, 1.56)	0.344
LDL-C (mmol/L)	2.59±0.83	2.73±1.15	2.53±0.68	0.530
HDL-C (mmol/L)	1.06±0.38	1.21±0.56	1.00±0.26	0.128
HDL-C group*				**0.019**
High (%)	12 (35.29)	7 (70.00)	5 (20.83)	
Low (%)	22 (64.71)	3 (30.00)	19 (79.17)	

*High HDL-C: HDL-C≥1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L.

AISC: anthracycline-induced subclinical cardiotoxicity; AST: aspartate transaminase; ALT: alanine aminotransferase; BMI: body mass index; cTnT: cardiac troponin T; ECoG: Eastern Cooperative Oncology Group; FS: fractional shortening; GLS: global longitudinal peak systolic strain; HDL-C: high-density lipoprotein cholesterol; HsCRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVDd: left ventricular diastolic dimension; LVMi: left ventricular mass index; LDL-C: low-density lipoprotein cholesterol; NT-proBNP: N terminal-pro brain natriuretic peptide; TC: total

cholesterol; TG: total triglyceride.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	3-4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	5-6
Outcome data	15*	Report numbers of outcome events or summary measures over time	5-6

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-7
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11-12

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.