


BMJ Open 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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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 a role in cardioprotection, 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.

Participants 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 third and sixth cycles of R-CHOP and 6 and 12 months after 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 HR=0.24, 95% CI 0.09 to 0.67, p=0.006; adjusted HR=0.27, 95% CI 0.09 to 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 significantly decreased from the end of the third cycle of chemotherapy to the end of the sixth 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 cardioprotective target, but further research is needed to confirm its benefits and limitations.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This prospective observational study contributes to our understanding of the association between high-density lipoprotein cholesterol (HDL-C) and anthracycline-induced subclinical cardiotoxicity, offering a foundation for the development of early intervention and prevention strategies.
- ⇒ The study used advanced imaging techniques (2D speckle tracking echocardiography) to assess the subclinical cardiac dysfunction in the patients, which can provide more sensitive and accurate results compared with traditional echocardiography.
- ⇒ The study only included patients with diffuse large B-cell lymphoma who received R-CHOP (cyclophosphamide, vincristine, doxorubicin, prednisone and rituximab), which may limit the generalisability 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 generalisability 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 cardioprotective strategy in anthracycline-treated patients with cancer.

Study registration number Study registration number: ChiCTR2100054721

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 patients with cancer with cardiovascular disease.^{3 4} As advances in cancer treatment

and an ageing 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 seventh most common cancer in the USA and the most frequent haematological malignancy globally, accounting for about 3% of cancer cases and deaths.⁶ Among NHLs, diffuse large B-cell lymphoma (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, characterised 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 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 antiapoptotic 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 a 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 three cycles of (R)-CHOP.¹⁸ Both the case-control study and the 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 analysed 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 1 September 2020 to 1 September 2022. Our inclusion criteria were as follows: newly diagnosed DLBCL, age between 18 and 80 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 , left ventricular ejection fraction (LVEF) $\geq 50\%$, and acceptable bone marrow, renal and hepatic functions for chemotherapy. Conversely, our exclusion criteria were symptomatic heart failure, a history of myocardial ischaemia, myocarditis, myocardial infarction, clinical or subclinical pericardial effusion, arrhythmia requiring medical intervention, a history of other cancers, under lipid-lowering treatment and severe active infections such as syphilis, hepatitis or 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 six cycles of standard R-CHOP (cyclophosphamide at 750 mg/m² on D1, doxorubicin at 50 mg/m² on D1, vincristine at 1.4 (maximum 2) mg/m² on D1 and 100 mg prednisone on D1–5, with rituximab at 375 mg/m² on D1 in each cycle), with or without two cycles of rituximab maintenance (rituximab at 375 mg/m² 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 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 third cycle of R-CHOP, the end of the sixth 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 their lifetime, with each drink 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 enrolment. Left ventricular systolic dysfunction was measured by LVEF, fractional shortening, left ventricular mass index, left ventricular diastolic dimension, E, e', E/e' and GLS. HDL-C, low-density lipoprotein cholesterol, cardiac troponin T, high-sensitivity C reactive protein, N-terminal prohormone of brain natriuretic peptide, 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 categorised 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 III.¹⁹ High HDL-C was defined as a serum HDL-C ≥ 1.16 mmol/L, while low HDL-C was defined as a serum HDL-C < 1.16 mmol/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: first, to evaluate the relationship between HDL-C and AISC; and second, 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 \pm 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 χ^2 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 < 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

visualisation were performed using IBM SPSS V.22.0 and GraphPad Prism V.8. Statistical tests were two sided, with a p value < 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 ≥ 1.16 mmol/L, n=28) and the low-level group (HDL-C < 1.16 mmol/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 TC (p=0.011) and lower total TG (p=0.002). The baseline characteristics of the patients in both groups were well balanced (online supplemental table S1).

High HDL-C was an independent protective target of AISC

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 flow chart detailing the patients enrolled in the study can be found in online supplemental 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 to 0.58) (online supplemental figure S2).

According to the results of the univariable Cox regression analysis, variables had a p value < 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 to 0.998, p=0.034) per 1-year increase. BMI showed an HR of 1.09 (95% CI 0.97 to 1.22, p=0.139) per 1 kg/m² increase. Similarly, hypertension had an HR of 0.22 (95% CI 0.03 to 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 to 1.77, p < 0.001) per -1% decrease. E velocity showed an HR of 1.03 (95% CI 1.00 to 1.06, p=0.075) per 1 cm/s increase. The HDL-C group (high vs low) had a significantly lower HR of 0.24 (95% CI 0.09 to 0.67, p=0.006). Further details about other variables can be found in online supplemental 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 to 0.84, p=0.018). Similarly,

Table 1 Outcomes of study participants

	HR (95% CI) (Unadjusted)	P value	HR (95% CI) (Adjusted*)	P value	HR (95% CI) (Adjusted†)	P value
Low HDL-C	Ref		Ref		Ref	
High HDL-C	0.24 (0.09 to 0.67)	0.006	0.28 (0.10 to 0.80)	0.018	0.27 (0.09 to 0.79)	0.017

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

Low HDL-C: <1.16 mmol/L. High HDL-C: \geq 1.16 mmol/L.

*Adjusted for age and sex.

†Adjusted for age, sex, hypertension, body mass index and E.

HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio.

after adjusting for age, sex and variables with $p < 0.15$ 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 to 0.79, $p=0.017$) (table 1).

Preliminary exploration of the difference of HDL-C between patients with 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 online supplemental 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 categorised into four groups based on the time of AISC detection. Among the groups, 12 patients were identified with AISC at the end of the third cycle of

chemotherapy, 7 patients at the end of the sixth 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 third cycle of chemotherapy, all other groups exhibited a reduction in HDL-C values from the end of the third cycle of chemotherapy to the end of the sixth 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 to 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 third cycle of chemotherapy ($p=0.012$) and significantly decreased from the end of the third cycle to the end of the sixth cycle of chemotherapy ($p=0.034$)

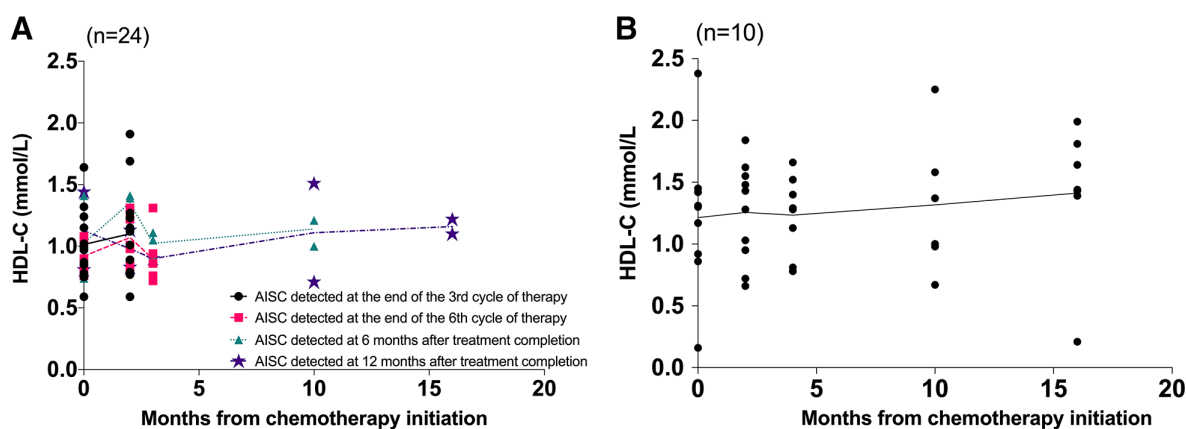


Figure 1 (A) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the third cycle of chemotherapy. 7 patients were detected AISC at the end of the sixth 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.

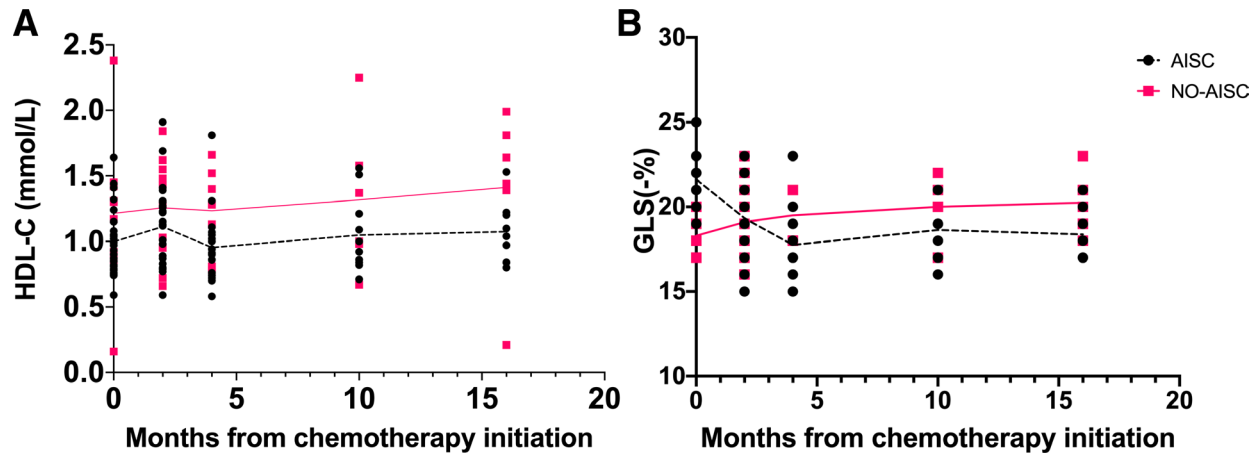


Figure 2 Timeline of high-density lipoprotein cholesterol (HDL-C) levels (A) and global longitudinal strain (GLS) (B) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

(figure 3A). Patients with AISC showed a significant decrease in HDL-C levels during R-CHOP chemotherapy from the end of the third cycle to the end of the sixth 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 three cycles of chemotherapy, from baseline to after six cycles of chemotherapy and from after three cycles to after six 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

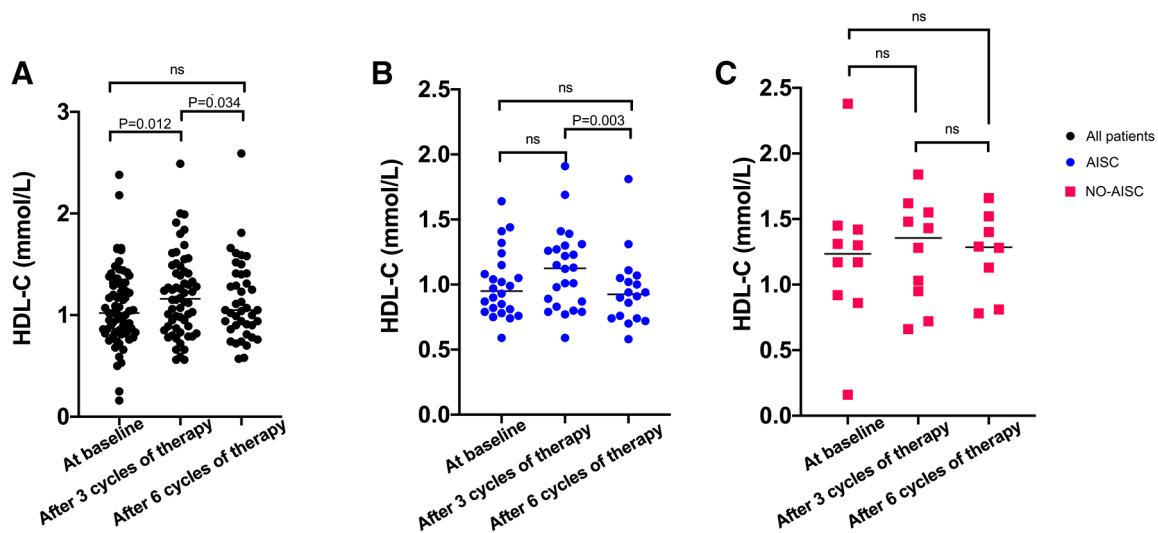


Figure 3 (A) Changes in high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the sixth cycle of chemotherapy. (B) Changes in HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (C) Changes in HDL-C in patients without AISC.

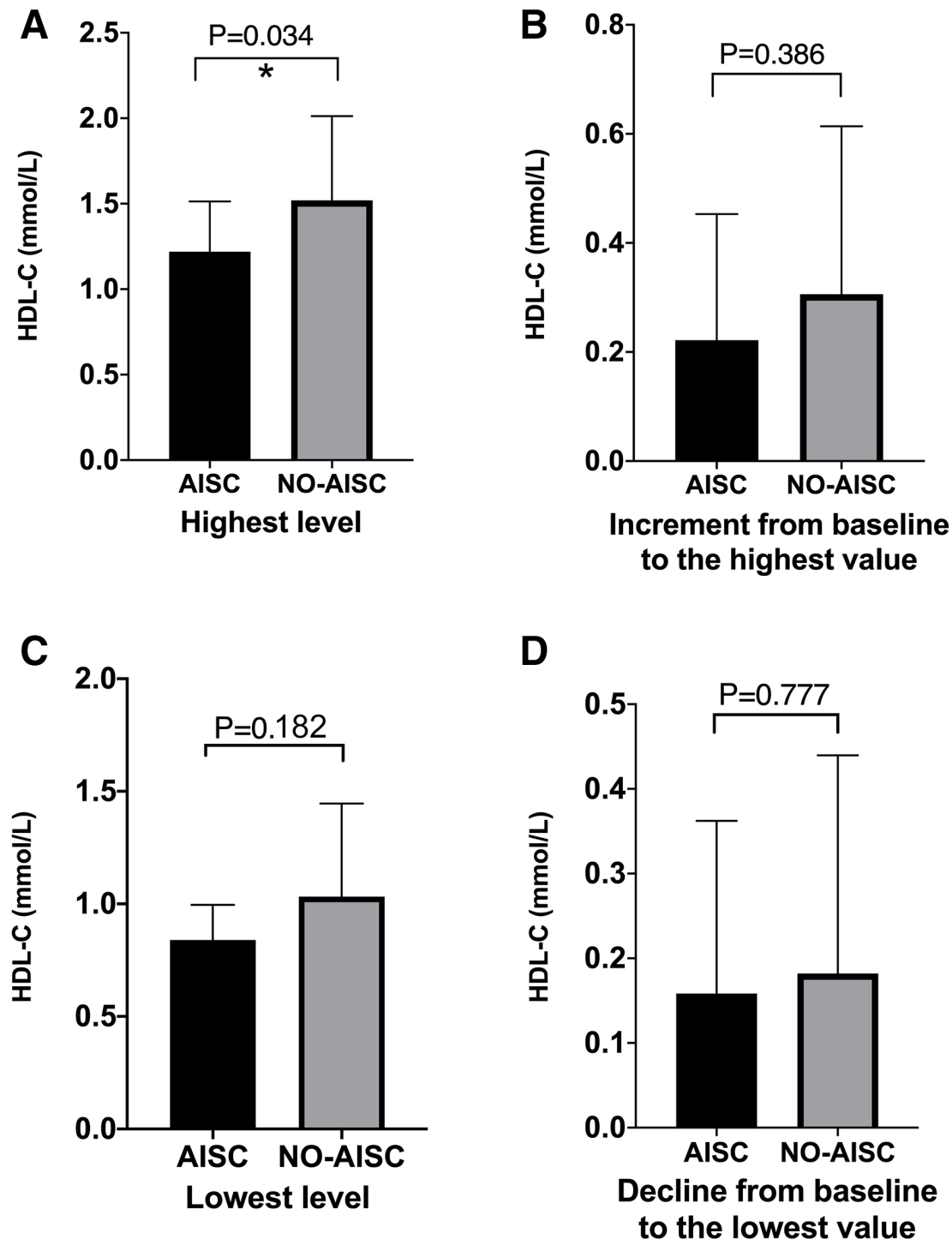


Figure 4 High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (A) Highest level of HDL-C during chemotherapy. (B) The HDL-C value increment from baseline to the highest value. (C) The lowest level of HDL-C during chemotherapy.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

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 significantly decreased from the end of the third cycle of chemotherapy to the end of the sixth 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 with 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 cardioprotection 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, intermediate-density lipoprotein, low-density lipoprotein and HDL, based on their size, density and lipid composition (cholesterol and TG).²¹ Among these, HDL exhibits distinctive cytoprotective actions and triggers antioxidative, anti-inflammatory and antiapoptotic 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 tissues back to the liver, and higher levels of HDL-C are generally associated with a lower risk of heart disease. Nevertheless, it is 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, using 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, antitumour drugs and cardiovascular risk factors. This registered study included patients with clinically diagnosed lymphoma who were evaluated by a haematologist 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 third cycle of chemotherapy, the end of the sixth cycle of chemotherapy and 6 and 12 months after completing chemotherapy. In our case-control study, according to

the changes in GLS at baseline and after the third 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 analyse the influencing factors of AISC in patients with DLBCL treated with three 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 did not 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 because 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 uses the IC-OS consensus statement¹² 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 patients with cancer.^{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 dyslipidaemia and facilitate the occurrence and development of cardiovascular diseases in patients with cancer. In a study of 394 patients with breast cancer, Li *et al* found that HDL-C levels after chemotherapy were significantly lower than those before chemotherapy.³³ Similarly, Lu *et al* and Kalábová *et al* found that HDL-C levels 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 third cycle of chemotherapy. This phenomenon may be due to the fact that antitumour drugs require cholesterol to cross cell membranes.³⁶ However, HDL-C levels significantly decreased from the end of the third cycle of chemotherapy to the end of the sixth 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-containing 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 100–150 mg/m² 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 consider the changes over time and the absolute values. In our study, patients without AISC had significantly higher absolute HDL-C levels than those with AISC, while the lowest absolute 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 cardioprotection of anthracycline-treated patients with cancer 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 (online supplemental table 3). 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 recognised as a risk factor for cardiotoxicity. Studies have reported that pre-existing hypertension was associated with anthracycline and trastuzumab-induced LVEF decline in a retrospective study³⁸ 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 ACE inhibitor/angiotensin receptor blocker (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. First, 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 cardioprotective strategy in anthracycline-treated patients with cancer. Second, this is a single-centre prospective observational study with a medium sample size. To confirm our findings, a larger sample size study conducted at multiple centres is needed. Third, 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 did not 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 with 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 cardioprotective 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 cardioprotection and the potential benefits of early serum lipid management.

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