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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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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 ≤ 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 ischemia, 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 human immunodeficiency virus (HIV) infection.

Treatment

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3 119 Patients received a total of 6 cycles of standard R-CHOP (cyclophosphamide at 750 mg/m² on D1,
4 120 doxorubicin at 50 mg/m² on D1, vincristine at 1.4 [maximum 2] mg/m² on D1, and 100 mg
5 121 prednisone on D1-5, with rituximab at 375 mg/m² on D1 in each cycle), with or without 2 cycles
6 122 of rituximab maintenance (rituximab at 375 mg/m² on D1 in each cycle). None of the patients in
7 123 our study received lipid-lowering treatment during the follow-up period.

124 **Definition of subclinical cardiotoxicity**

11 125 According to the IC-OS consensus statement, the definition of subclinical cardiotoxicity was a
12 126 relative GLS decrease from baseline [(baseline – current GLS)/baseline GLS] of >12%, but with
13 127 a normal left ventricular ejection fraction (LVEF)(12).

15 128 **Study protocol**

16 129 This is an observational prospective study. The study was registered in the Chinese Clinical Trial
17 130 (Approval NO. ChiCTR2100054721). The study was conducted in accordance with the
18 131 Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of
19 132 Chongqing Medical University (Approval NO. 2018-016). And all participating patients provided
20 133 written informed consent.

23 134 At baseline, the end of the 3rd cycle of R-CHOP, the end of the 6th cycle of R-CHOP, and 6 and
24 135 12 months after chemotherapy completion, all enrolled patients underwent conventional
25 136 echocardiography, 2D speckle tracking echocardiography, and blood sampling. Demographic data
26 137 and clinical variables, including age, gender, body mass index (BMI), ECoG performance status,
27 138 diabetes mellitus, hypertension, drinking history, and smoking history were collected at the time
28 139 of enrollment. Left ventricular systolic dysfunction was measured by LVEF, fractional shortening
29 140 (FS), left ventricular mass index (LVMI), left ventricular diastolic dimension (LVDd), E, e', E/e',
30 141 and GLS. HDL-C, ApoA1, low-density lipoprotein cholesterol (LDL-C), cardiac troponin T
31 142 (cTnT), high sensitivity C-reactive protein (hsCRP), N-terminal prohormone of brain natriuretic
32 143 peptide (NT-proBNP), total cholesterol (TC) and total triglyceride (TG) were measured. We used
33 144 the baseline HDL-C level as a surrogate marker for HDL quantity. The patients were categorized
34 145 into two groups based on the average HDL-C value for males and females in the modified criteria
35 146 of the National Cholesterol Educated Program Adult Treatment Panel (NCEP ATP III)(18). High
36 147 HDL-C was defined as a serum HDL-C \geq 1.16mmol/L, while low HDL-C was defined as a serum
37 148 HDL-C<1.16mmol/L.

43 149 **Statistical analysis**

44 150 The study was conducted with two aims: firstly, to evaluate the relationship between HDL and
45 151 AISC; and secondly, to conduct a preliminary exploration of the differences in HDL-C and the
46 152 variability of HDL-C changes between patients with and without AISC during the follow-up
47 153 period. Continuous variables were expressed as mean \pm standard deviation (SD) and compared
48 154 using the t-test. Non-normally distributed variables were presented as median (Q1- Q3) and
49 155 compared with the Wilcoxon Mann-Whitney test. Categorical variables were expressed as n (%)
50 156 and compared using the Chi-square or Fisher's exact test, as appropriate. Correlation analysis was
51 157 conducted to investigate the associations of change in HDL-C with change in GLS. Multiple
52 158 hypothesis tests were performed using the Bonferroni adjustment. The probabilities of survival

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3 159 were calculated using Kaplan-Meier methods and compared using Log-rank tests. Cox
4 160 proportional-hazards regression models were conducted to assess the association between
5 161 variables and AISC. Covariates for multivariable Cox regression models included age, sex, and
6 162 variables that had a P-value of less than 0.15 in the univariable Cox regression analysis (GLS was
7 163 excluded as it is the factor that defines AISC). Two multivariable Cox regression models were
8 164 constructed: the first model included age and sex; and the second model included age, sex,
9 165 hypertension, BMI, and E. Statistical analysis and visualization were performed using IBM SPSS
10 166 V.22.0 and GraphPad Prism 8. X-Tile software was conducted to calculate the cut-off point of
11 167 ApoA1(19), with the optimal cut-off value (the lowest P-value under the log-rank test) being
12 168 1.02g/L. All statistical tests were two-sided, with a P-value less than 0.05 being considered
13 169 statically significant.

170 **Patient and public involvement**

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

173 174 **RESULTS**

175 **Assessment of the association between HDL-C and AISC**

176 *Study population and baseline characteristics*

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

185 186 *High HDL-C was an independent protective target of anthracycline-induced subclinical 187 cardiotoxicity*

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

194 The log-rank test revealed that patients with higher HDL-C were less likely to experience AISC
195 (P=0.001, HR=0.26, 95%CI: 0.12-0.58) (Figure S2a). However, there were no significant
196 differences in AISC occurrence between different ApoA1 levels (P=0.123, HR=0.54, 95%CI:
197 0.20-1.45) (Figure S2b).

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

207 The results of the multivariable Cox regression analysis showed that high HDL-C was significantly
 208 associated with a lower risk of AISC after adjusting for age and sex (model 1) (HR=0.28,
 209 95%CI:0.10=0.84, P=0.018). Similarly, after adjusting for age, sex, and variables that P<0.15 in
 210 the univariable Cox regression analysis (excluding GLS as it defines AISC) (model 2), the same
 211 association was observed (HR=0.27, 95%CI: 0.09-0.79, P=0.017). In contrast, ApoA1 did not
 212 show any influence on AISC in either of the two regression models (P=0.401 and P=0.237). (Table
 213 1)

214 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

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

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

222 Preliminary exploration of the difference of HDL-C between patients with AISC and 223 without AISC

224 *Study population and baseline characteristics*

225 In this analysis, we selectively included 34 of the enrolled patients who remained uncensored. The
 226 patients who exhibited AISC at any time during the follow-up period were segregated into the
 227 AISC group (n=24), while those who did not demonstrate AISC were classified into the NO-AISC
 228 group (n=10). Patients within the AISC group were comparatively younger (50±12.45 vs.
 229 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),
 230 P<0.001]. More baseline information can be seen in Table S3.

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

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

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

245 Based on Figure 2, we observed that the fluctuations in HDL-C and GLS were most pronounced
246 during the chemotherapy period. The fluctuations in HDL-C levels of patients with DLBCL during
247 R-CHOP chemotherapy were presented in Figure 3. The levels of HDL-C significantly increased
248 for all patients from baseline to the end of the 3rd cycle of chemotherapy ($P=0.012$) and
249 significantly decreased from the end of the 3rd cycle to the end of the 6th cycle of chemotherapy
250 ($P=0.034$) (Figure 3a). Patients with AISC showed a significant decrease in HDL-C levels during
251 R-CHOP chemotherapy from the end of the 3rd cycle to the end of the 6th cycle ($P=0.003$) (Figure
252 3b). However, no significant difference was observed in HDL-C levels for patients without AISC
253 during R-CHOP chemotherapy (Figure 3c). We conducted correlation analysis separately for the
254 change in HDL-C and GLS from baseline to after 3 cycles of chemotherapy, from baseline to after
255 6 cycles of chemotherapy, and from after 3 cycles to after 6 cycles of chemotherapy. However, we
256 found no statistically significant differences in the associations between changes in HDL-C and
257 GLS ($P=0.965, 0.087, 0.449$).

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

259 Figure 4 presents the contrasting values between patients with AISC and those without in terms of
260 four parameters, namely the highest and lowest levels of HDL-C during chemotherapy, the
261 increment and decline in HDL-C values from baseline. Patients without AISC showed significantly
262 higher values in the highest level of HDL-C (1.52 ± 0.49 vs. 1.22 ± 0.29 , $P=0.034$, Figure 4a).
263 However, no significant differences were observed between the two groups in terms of HDL-C
264 increment from baseline to the highest value (0.31 ± 0.31 vs. 0.22 ± 0.23 , $P=0.386$, Figure 4b). While
265 the lowest level of HDL-C was lower in patients with AISC, the difference was not statistically
266 significant (0.84 ± 0.16 vs. 1.03 ± 0.41 , $P=0.182$, Figure 4c). Furthermore, there were no significant
267 differences in HDL-C decline between patients with AISC and those without (0.16 ± 0.20 vs.
268 0.18 ± 0.26 , $P=0.777$, Figure 4d).

269

270 **DISCUSSION**

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

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3 274 Moreover, patients without AISC had more stable and higher levels of HDL-C than those with
4 275 AISC during the follow-up period. The results also showed that HDL-C levels were significantly
5 276 decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of
6 277 chemotherapy in all patients, especially in the AISC group, indicating that anthracycline-
7 278 containing chemotherapy has adverse effects on HDL-C levels. Notably, the highest level of HDL-
8 279 C was significantly higher in patients without AISC compared to those with AISC. These findings
9 280 suggest that HDL-C may have a protective role against AISC in DLBCL patients undergoing
10 281 anthracycline-containing chemotherapy and maintaining a relatively high level of HDL-C may be
11 282 more effective in managing cardio-protection than monitoring changes in HDL-C levels over time.
12 283 The results of this study highlight the importance of early serum lipid management in these
13 284 patients.

14 285 Lipoproteins are classified into five categories, namely chylomicron, very-low-density lipoprotein
15 286 (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density
16 287 lipoprotein (HDL), based on their size, density, and lipid composition (cholesterol and
17 288 triglycerides)(20). Among these, HDL exhibits distinctive cytoprotective actions and triggers anti-
18 289 oxidative, anti-inflammatory, and anti-apoptotic effects. The protective roles of HDL in
19 290 cardiovascular disease have been controversial in recent years, and that the quality of HDL
20 291 (cholesterol efflux capacity, antioxidant activity, anti-inflammatory activity, endothelial function,
21 292 etc.) rather than the quantity of HDL has been proposed as the true cardioprotective effect. The
22 293 Framingham Heart Study, as early as 1988, reported a correlation between HDL-C and
23 294 cardiovascular mortality(21). Recent studies have challenged the HDL-C hypothesis by revealing
24 295 that HDL-C level is not inversely correlated with cardiovascular diseases(22, 23). In our study, we
25 296 used the baseline HDL-C level as a surrogate marker for HDL quantity, but we did not directly
26 297 measure the quality of HDL. Measuring the level of HDL-C in serum is a commonly used method
27 298 to assess the effect of HDL on cardiovascular health. HDL facilitates the transportation of
28 299 cholesterol from the body's tissues back to the liver, and higher levels of HDL-C are generally
29 300 associated with a lower risk of heart disease. Nevertheless, it's crucial to note that HDL-C levels
30 301 may not accurately reflect the functional properties of HDL. ApoA1, the most abundant protein in
31 302 HDL, is associated with several beneficial effects of HDL(15, 24). The function and abundance of
32 303 ApoA1 are reported to play a dominant role in HDL quality(25). In the context of AIC, several
33 304 studies have indicated that HDL can protect against anthracycline-induced cardiomyocyte
34 305 apoptosis and atrophy in isolated cardiomyocytes(26, 27) and animal models(16, 27). Based on
35 306 these earlier trials, HDL-C and ApoA1 could serve as protective factors against anthracycline-
36 307 related cardiovascular disease. However, our study results indicate that there was no significant
37 308 association observed between ApoA1 and AISC in patients with DLBCL who were treated with
38 309 R-CHOP. It's possible that factors other than HDL function, such as genetics or inflammation, may
39 310 affect ApoA1 levels. Furthermore, we must acknowledge that our study was observational in
40 311 nature and, therefore, cannot establish causality. Additionally, other unmeasured factors may have
41 312 contributed to the observed relationship between HDL-C levels and AISC risk.

1
2
3 313 As far as we know, few clinical studies have investigated the association between HDL-C and
4 314 AISC. This study is the first clinical trial that utilizes the IC-OS consensus statement(12) to define
5 315 subclinical cardiotoxicity, with univariate and multivariable analyses being used to identify the
6 316 influential factors of AISC in DLBCL patients in this cohort. Kaplan-Meier methods and Log-rank
7 317 tests reveal that patients with high HDL-C levels were less likely to develop AISC. After subjecting
8 318 it to univariate and multivariable Cox regression methods, high HDL-C levels still showed
9 319 statistically significant differences. These results suggest that high HDL-C could be a potentially
10 320 independent protective factor for AISC in DLBCL patients and provide an opportunity for
11 321 investigators to develop a tool for early intervention and prevention of AISC. Further research is
12 322 necessary to confirm our findings.

13 323 Several studies have demonstrated that serum lipid levels are altered during anthracycline-
14 324 containing chemotherapy in cancer patients(28, 29). Huxley et al and Averina et al have shown
15 325 that imbalanced serum lipid distribution is a risk factor for cardiovascular disease(30, 31). As a
16 326 result, anthracycline-containing treatment can induce dyslipidemia and facilitate the occurrence
17 327 and development of cardiovascular diseases in cancer patients. In a study of 394 breast cancer
18 328 patients, Xin et al found that HDL-C levels after chemotherapy were significantly lower than those
19 329 before chemotherapy(32). Similarly, Lu et al and Hana et al found that HDL-C levels were
20 330 significantly decreased during anthracycline-containing chemotherapy in patients with breast
21 331 cancer(33, 34). In our study, we specifically assessed the changes in HDL-C levels over time
22 332 during follow-up. Except for the group of patients who experienced AISC at 12 months after
23 333 treatment completion, HDL-C levels in all other groups increased from baseline to the 3rd cycle
24 334 of chemotherapy. This phenomenon may be due to the fact that anti-tumor drugs require
25 335 cholesterol to cross cell membranes(35). However, HDL-C levels were significantly decreased
26 336 from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all
27 337 patients, especially in the AISC group, which is consistent with previous research results(32-34),
28 338 and further confirmed that anthracycline-contained chemotherapy has adverse effects on HDL-C
29 339 levels in DLBCL patients. The HDL-C level in patients without AISC was more stable than that
30 340 in patients with AISC. Therefore, anthracycline-containing chemotherapy may promote the
31 341 occurrence and development of cardiotoxicity in DLBCL patients by inducing HDL-C turbulence.
32 342 Besides, the findings of our study indicate a significant decrease in GLS during the chemotherapy
33 343 period in patients with AISC. This result is consistent with previous research, which has reported
34 344 that doxorubicin dose at the range of 100-150mg/m² can cause cardiotoxicity(36). Notably, we
35 345 also observed that GLS remained stable after completion of chemotherapy, suggesting that the
36 346 cardiac effects of anthracycline-based chemotherapy may be dose-related. These findings have
37 347 important implications for the monitoring and management of cardiotoxicity in patients
38 348 undergoing anthracycline-based chemotherapy, as early detection of cardiac dysfunction during
39 349 treatment may improve patient outcomes. We investigated the associations of change in HDL-C
40 350 with change in GLS, no statistically significant differences were found.

41 351 The analysis of HDL-C levels should not only consider the changes over time, but also the absolute
42 352 values. In our study, patients without AISC had significantly higher absolute highest HDL-C levels

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3 353 than those with AISC, while the absolute lowest HDL-C levels did not differ significantly between
4 354 the two groups. The alterations from HDL-C extremes to baseline did not exhibit any variation
5 355 between the groups either. This suggests that the highest absolute HDL-C value was a preferable
6 356 indicator of AISC protection than the change in HDL-C from baseline to the extremum value.
7 357 Maintaining a relatively high level of HDL-C may be more effective in managing the cardio-
8 358 protection of anthracycline-treated cancer patients than monitoring changes in HDL-C levels over
9 359 time.

10 360 In our investigation, we observed that among the four patients with pre-existing hypertension, only
11 361 one patient experienced AISC during the follow-up (Table S3). Although there was no statistically
12 362 significant difference, it appears that patients with pre-existing hypertension were less likely to
13 363 experience AISC. Hypertension, a common risk factor for both cancer and cardiovascular diseases,
14 364 was also recognized as a risk factor for cardiotoxicity. Studies have reported that pre-existing
15 365 hypertension was associated with anthracycline-and trastuzumab induced left ventricular ejection
16 366 fraction (LVEF) decline in a retrospective study(37), and early left ventricular systolic dysfunction
17 367 in patients with lymphoma receiving (R)-CHOP in a prospective study(38). However, in our study,
18 368 multivariable Cox regression analysis showed that hypertension had no influence on AISC
19 369 ($P>0.05$). However, in our investigation, multivariable Cox regression analysis showed that
20 370 hypertension did not have a significant impact on AISC ($P>0.05$). We noted that all patients with
21 371 hypertension were under a single antihypertensive drug regimen (beta-blockers or ACEI/ARB) to
22 372 manage their blood pressure. Two meta-analyses have demonstrated that beta-blockers and ACEI
23 373 can prevent cardiotoxicity caused by chemotherapy (39, 40). We speculate that the protective
24 374 effects of beta-blockers and ACEI/ARB may have contributed to the result observed in our study
25 375 regarding the relationship between hypertension and AISC.

26 376 There are several limitations to our study that must be acknowledged. Firstly, while our study
27 377 highlights the potential importance of HDL-C in managing AISC, additional studies are necessary
28 378 to fully evaluate the benefits and limitations of HDL-C as a cardio-protective strategy in
29 379 anthracycline-treated cancer patients. Secondly, this is a single-center observational prospective
30 380 study with a medium sample size. To confirm our findings, a larger sample size study conducted
31 381 at multiple centers is needed. Thirdly, previous studies have suggested that there may be a reversed
32 382 U-shaped relationship between HDL-C levels and cardiovascular diseases(41). Due to the small
33 383 sample size of this study, we didn't further investigate the influence of extremely high levels of
34 384 HDL-C on cardiotoxicity, and further clinical studies should be done to verify it. However, due to
35 385 the small sample size of our study, we did not investigate the potential impact of extremely high
36 386 HDL-C levels on cardiotoxicity. Further clinical studies are required to explore this issue. Besides,
37 387 the measurement of GLS was only taken at baseline and at several points throughout the
38 388 chemotherapy treatment and follow-up period. It is crucial to extend the duration of follow-up in
39 389 future research to obtain a more comprehensive understanding of the long-term effects of
40 390 anthracycline treatment on cardiovascular health.

41 391

42 392 **CONCLUSIONS**

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

401

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404 assisted in this study.

405

406 **COMPETING INTERESTS**

407 The authors declare no conflict of interest.

408

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420

421 **AUTHOR CONTRIBUTIONS**

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

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DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

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30 558 **FIGURE LEGENS**

31 559 **Figure 1. (a)** Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients
32 560 detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients
33 561 were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC
34 562 at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after
35 563 treatment completion. 2 patients were detected AISC at 12 months after treatment completion.
36 564 **(b)** Timeline of HDL-C levels of patients without AISC.

37 565 **Figure 2.** Timeline of high-density lipoprotein cholesterol (HDL-C) levels **(a)** and global
38 566 longitudinal strain (GLS) **(b)** in patients with and without anthracycline-induced subclinical
39 567 cardiotoxicity (AISC) during the whole follow-up period.

40 568 **Figure 3. (a)** Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from
41 569 baseline to the end of the 6th cycle of chemotherapy. **(b)** Changes of HDL-C in patients with
42 570 anthracycline-induced subclinical cardiotoxicity (AISC). **(c)** Changes of HDL-C in patients
43 571 without AISC.

44 572 **Figure 4.** High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-
45 573 induced subclinical cardiotoxicity (AISC) and No-AISC. **(a)** Highest level of HDL-C during
46 574 chemotherapy. **(b)**The HDL-C value increment from baseline to the highest value. **(c)** The lowest
47 575 level of HDL-C during chemotherapy. **(d)** The HDL-C value declined from baseline to the
48 576 lowest.

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3 577 **Figure S1.** Flow diagram of patients enrolled in our observational research and reasons for
4 578 censored patients.

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6 579 **Figure S2.** Kaplan-Meier curves of the percentage of patients without AISC in patients stratified
7 580 by HDL-C level (**a**) and ApoA1 level (**b**). High HDL-C: HDL-C \geq 1.16mmol/L. Low HDL-C:
8 HDL-C < 1.16mmol/L. High ApoA1: ApoA1 \geq 1.02g/L. Low ApoA1: ApoA1 < 1.02g/L. AISC,
9 581 anthracycline-induced cardiotoxicity; ApoA1, apolipoprotein A1; HDL-C, high-density
10 582 lipoprotein cholesterol; HR, hazard ratio.
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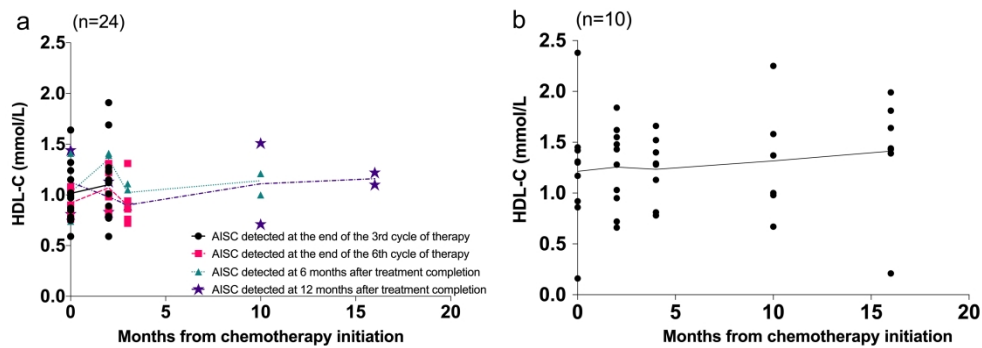


Figure 1. (a) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC at the end of the 6th 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.

420x144mm (300 x 300 DPI)

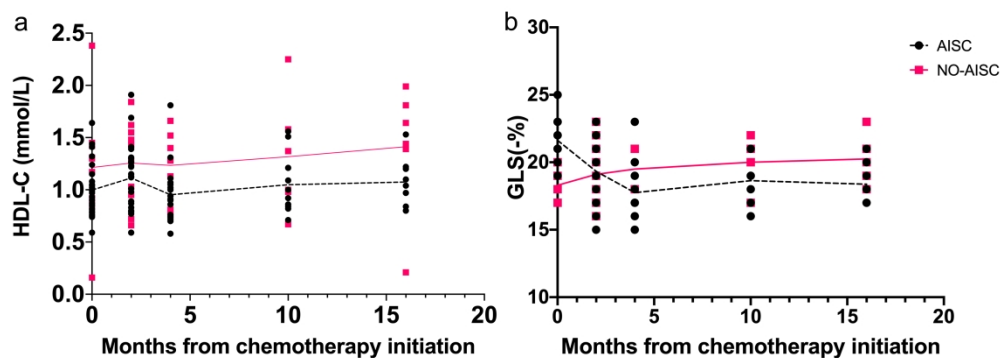


Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (a) and global longitudinal strain (GLS) (b) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

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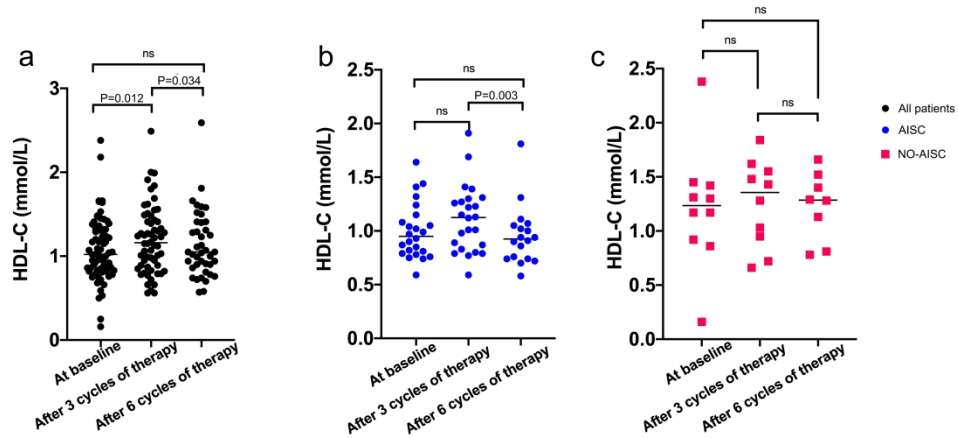


Figure 3. (a) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (b) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (c) Changes of HDL-C in patients without AISC.

365x166mm (300 x 300 DPI)

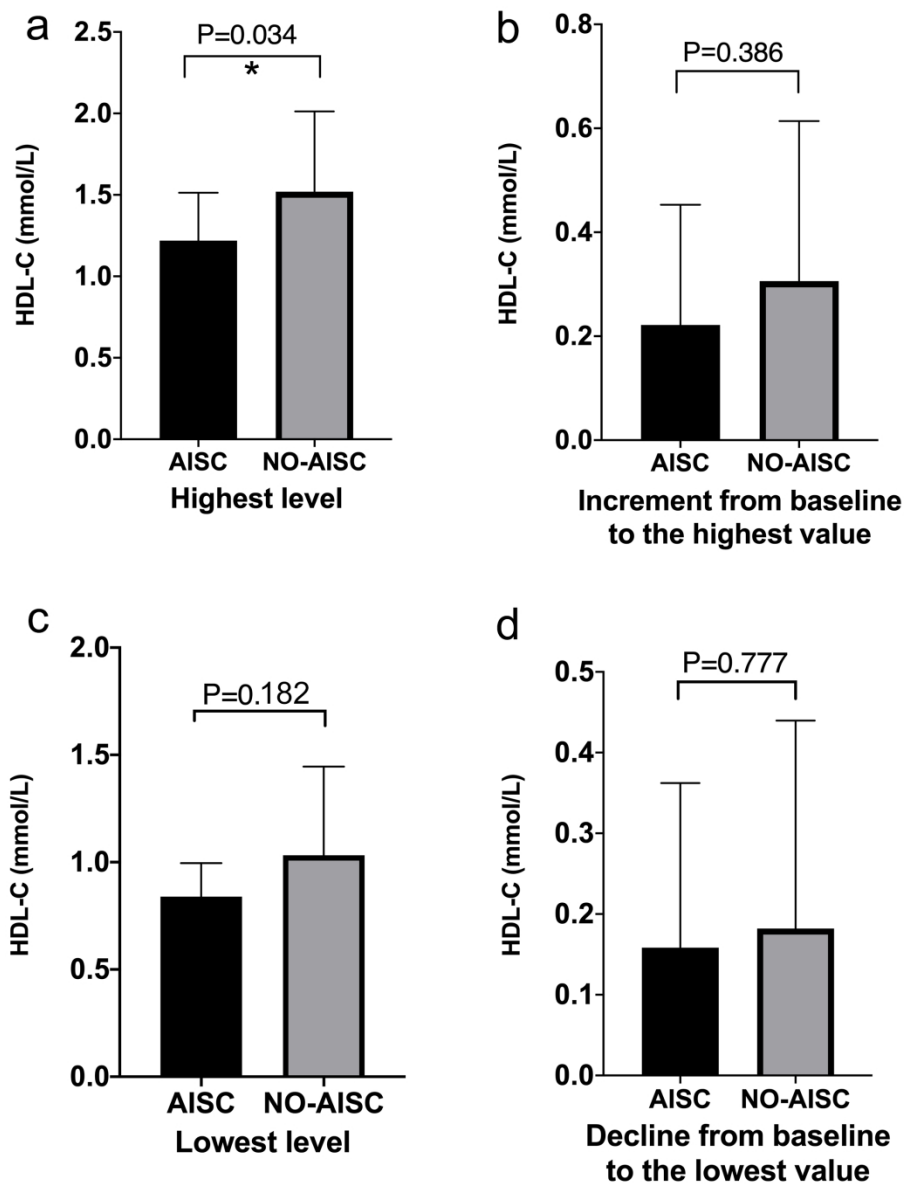
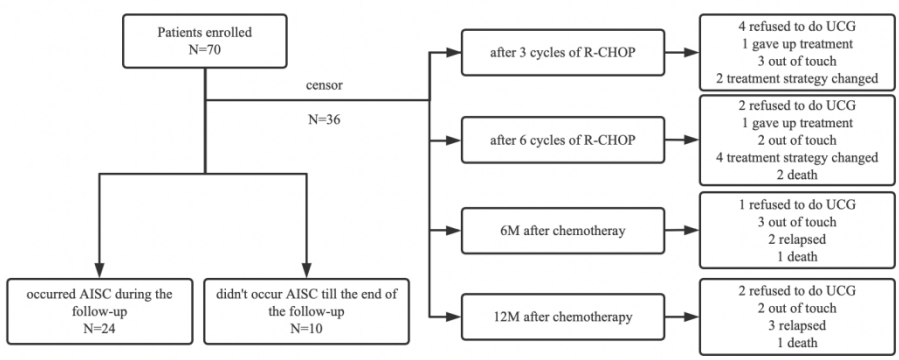


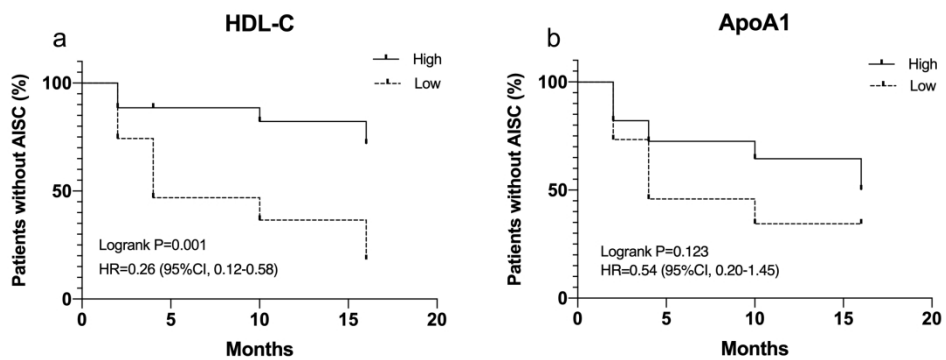
Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (a) Highest level of HDL-C during chemotherapy. (b) The HDL-C value increment from baseline to the highest value. (c) The lowest level of HDL-C during chemotherapy.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

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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
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

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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 \geq 1.16mmol/L. Low HDL-C group: HDL-C<1.16mmol/L. High ApoA1 group: ApoA1 \geq 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 \geq 1.16mmol/L. Low HDL-C: HDL-C $<$ 1.16mmol/L. # High ApoA1: ApoA1 \geq 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

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

1	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	6,7
2				
3			(b) Report category boundaries when continuous variables were categorized	
4			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
5	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7,8
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11	Discussion			
12	Key results	18	Summarise key results with reference to study objectives	8
13	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
14				
15	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
16				
17	Generalisability	21	Discuss the generalisability (external validity) of the study results	10
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20				
21	Other information			
22	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
23				
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*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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1 Role of HDL-C in anthracycline-induced subclinical 2 cardiotoxicity: an observational prospective study in 3 DLBCL patients

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

25 26 **ABSTRACT**

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

33 **Design** Observational prospective study

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

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

38 **Primary outcome measures** Serum biomarkers including HDL-C, 2D speckle tracking
39 echocardiography, and conventional echocardiography were measured at baseline, at the end of
40 the 3rd and 6th cycle of R-CHOP, and 6 and 12 months after the completion of chemotherapy.

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

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

52 **Study registration number** ChiCTR2100054721

54 **Strengths and limitations of this study**

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

69 **INTRODUCTION**

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

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

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

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

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

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

110

111 **METHODS**

112 **Study population**

113 We recruited chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to
114 receive the standard dose of R-CHOP chemotherapy regimen at our institution from September
115 1st, 2020, to September 1st, 2022. Our inclusion criteria were as follows: newly diagnosed DLBCL,
116 age between 18 and 80 years, Eastern Cooperative Oncology Group (ECOG) performance status
117 ≤ 2 , left ventricular ejection fraction (LVEF) $\geq 50\%$, and acceptable bone marrow, renal, and
118 hepatic functions for chemotherapy. Conversely, our exclusion criteria were symptomatic heart

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3 119 failure, a history of myocardial ischemia, myocarditis, myocardial infarction, clinical or subclinical
4 120 pericardial effusion, arrhythmia requiring medical intervention, a history of other cancers, under
5 121 lipid-lowering treatment, and severe active infections such as syphilis, hepatitis, or human
6 122 immunodeficiency virus (HIV) infection.

7 123 **Treatment**

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10 124 Patients received a total of 6 cycles of standard R-CHOP (cyclophosphamide at 750 mg/m² on D1,
11 125 doxorubicin at 50 mg/m² on D1, vincristine at 1.4 [maximum 2] mg/m² on D1, and 100 mg
12 126 prednisone on D1-5, with rituximab at 375 mg/m² on D1 in each cycle), with or without 2 cycles
13 127 of rituximab maintenance (rituximab at 375 mg/m² on D1 in each cycle).

14 128 **Definition of subclinical cardiotoxicity**

15
16 129 According to the IC-OS consensus statement, the definition of subclinical cardiotoxicity was a
17 130 relative GLS decrease from baseline [(baseline – current GLS)/baseline GLS] of >12%, but with
18 131 a normal left ventricular ejection fraction (LVEF)(12).

19 132 **Study protocol**

20
21 133 We defined 'baseline' as the initial assessment conducted before the initiation of the first cycle of
22 134 chemotherapy. At baseline, the end of the 3rd cycle of R-CHOP, the end of the 6th cycle of R-
23 135 CHOP, and 6 and 12 months after chemotherapy completion, all enrolled patients underwent
24 136 conventional echocardiography, 2D speckle tracking echocardiography, and blood sampling.
25
26 137 Every patient received electrocardiography (ECG) examination before every cycle of
27 138 chemotherapy to ensure the safety of the treatment. Demographic data and clinical variables,
28 139 including age, gender, body mass index (BMI), ECOG performance status, diabetes mellitus,
29 140 hypertension, drinking history (an adult who has consumed more than 20 drinks in lifetime, with
30 141 each drink is considered to have an average alcohol content of 12 g), and smoking history (an adult
31 142 who has smoked at least 100 cigarettes in their lifetime) were collected at the time of enrollment.
32 143 Left ventricular systolic dysfunction was measured by LVEF, fractional shortening (FS), left
33 144 ventricular mass index (LVMI), left ventricular diastolic dimension (LVDD), E, e', E/e', and GLS.
34 145 HDL-C, low-density lipoprotein cholesterol (LDL-C), cardiac troponin T (cTnT), high sensitivity
35 146 C-reactive protein (hsCRP), N-terminal pro-hormone of brain natriuretic peptide (NT-proBNP),
36 147 total cholesterol (TC) and total triglyceride (TG) were measured. We used the baseline HDL-C
37 148 level as a surrogate marker for HDL quantity. The patients were categorized into two groups based
38 149 on the average HDL-C value for males and females in the modified criteria of the National
39 150 Cholesterol Educated Program Adult Treatment Panel (NCEP ATP III)(19). High HDL-C was
40 151 defined as a serum HDL-C ≥ 1.16 mmol/L, while low HDL-C was defined as a serum HDL-
41 152 C < 1.16 mmol/L. We determined the sample size using an online sample size calculator, which
42 153 indicated a total requirement of 23 events(20).

43 154 **Statistical analysis**

44 155 The study was conducted with two aims: firstly, to evaluate the relationship between HDL and
45 156 AISC; and secondly, to conduct a preliminary exploration of the differences in HDL-C and the
46 157 variability of HDL-C changes between patients with and without AISC during the follow-up
47 158 period. Continuous variables were expressed as mean ± standard deviation (SD) and compared

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3 159 using the t-test. Non-normally distributed variables were presented as median (Q1- Q3) and
4 160 compared with the Wilcoxon Mann-Whitney test. Categorical variables were expressed as n (%)
5 161 and compared using the Chi-square or Fisher's exact test, as appropriate. Correlation analysis was
6 162 conducted to investigate the associations of change in HDL-C with change in GLS. The
7 163 probabilities of survival were calculated using Kaplan-Meier methods and compared using Log-
8 164 rank tests. Cox proportional-hazards regression models were conducted to assess the association
9 165 between variables and AISC. Covariates for multivariable Cox regression models included age,
10 166 sex, and variables that had a P-value of less than 0.15 in the univariable Cox regression analysis
11 167 (GLS was excluded as it is the factor that defines AISC). Two multivariable Cox regression models
12 168 were constructed: the first model included age and sex; and the second model included age, sex,
13 169 hypertension, BMI, and E. Statistical analysis and visualization were performed using IBM SPSS
14 170 V.22.0 and GraphPad Prism 8. Statistical tests were two-sided, with a P-value less than 0.05 being
15 171 considered statically significant.

172 **Patient and public involvement**

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

175 **RESULTS**

176 **Assessment of the association between HDL-C and AISC**

177 *Study population and baseline characteristics*

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

185 *High HDL-C was an independent protective target of anthracycline-induced subclinical 186 cardiotoxicity*

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

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

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

207 The results of the multivariable Cox regression analysis showed that high HDL-C was significantly
 208 associated with a lower risk of AISC after adjusting for age and sex (model 1) (HR=0.28,
 209 95%CI:0.10=0.84, P=0.018). Similarly, after adjusting for age, sex, and variables that P<0.15 in
 210 the univariable Cox regression analysis (excluding GLS as it defines AISC) (model 2), the same
 211 association was observed (HR=0.27, 95%CI: 0.09-0.79, P=0.017). (Table 1)

212 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

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

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

218 Preliminary exploration of the difference of HDL-C between patients with AISC and 219 without AISC

220 Study population and baseline characteristics

221 In this analysis, we selectively included 34 of the enrolled patients who were not lost to follow-
 222 up. The patients who exhibited AISC at any time during the follow-up period were segregated into
 223 the AISC group (n=24), while those who did not demonstrate AISC were classified into the NO-
 224 AISC group (n=10). Patients within the AISC group were comparatively younger (50±12.45 vs.
 225 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),
 226 P<0.001]. More baseline information can be seen in Table S3.

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

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

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3 276 C was significantly higher in patients without AISC compared to those with AISC. These findings
4 277 suggest that HDL-C may have a protective role against AISC in DLBCL patients undergoing
5 278 anthracycline-containing chemotherapy and maintaining a relatively high level of HDL-C may be
6 279 more effective in managing cardio-protection than monitoring changes in HDL-C levels over time.
7
8 280 The results of this study highlight the importance of early serum lipid management in these
9 281 patients.

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11 282 Lipoproteins are classified into five categories, namely chylomicron, very-low-density lipoprotein
12 283 (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density
13 284 lipoprotein (HDL), based on their size, density, and lipid composition (cholesterol and
14 285 triglycerides)(21). Among these, HDL exhibits distinctive cytoprotective actions and triggers anti-
15 286 oxidative, anti-inflammatory, and anti-apoptotic effects. The protective roles of HDL in
16 287 cardiovascular disease have been controversial in recent years, and that the quality of HDL
17 288 (cholesterol efflux capacity, antioxidant activity, anti-inflammatory activity, endothelial function,
18 289 etc.) rather than the quantity of HDL has been proposed as the true cardioprotective effect. The
19 290 Framingham Heart Study, as early as 1988, reported a correlation between HDL-C and
20 291 cardiovascular mortality(22). Recent studies have challenged the HDL-C hypothesis by revealing
21 292 that HDL-C level is not inversely correlated with cardiovascular diseases(23, 24). In our study, we
22 293 used the baseline HDL-C level as a surrogate marker for HDL quantity, but we did not directly
23 294 measure the quality of HDL. Measuring the level of HDL-C in serum is a commonly used method
24 295 to assess the effect of HDL on cardiovascular health. HDL facilitates the transportation of
25 296 cholesterol from the body's tissues back to the liver, and higher levels of HDL-C are generally
26 297 associated with a lower risk of heart disease. Nevertheless, it's crucial to note that HDL-C levels
27 298 may not accurately reflect the functional properties of HDL. ApoA1, the most abundant protein in
28 299 HDL, is associated with several beneficial effects of HDL(15, 25). The function and abundance of
29 300 ApoA1 are reported to play a dominant role in HDL quality(26). In the context of AIC, several
30 301 studies have indicated that HDL can protect against anthracycline-induced cardiomyocyte
31 302 apoptosis and atrophy in isolated cardiomyocytes(27, 28) and animal models(16, 28). Our recent
32 303 case-control study, revealing that both HDL-C and ApoA1 serve as predictive factors in patients
33 304 treated with 3 cycles of anthracycline-contained chemotherapy(18). Based on these earlier trials,
34 305 HDL-C and ApoA1 could serve as protective factors against anthracycline-related cardiovascular
35 306 disease. However, our study didn't focus on the investigation of the impact of ApoA1 on AISC.
36 307 Even when ApoA1 was included in the Cox regression model, no significant association with
37 308 AISC in patients with DLBCL treated with R-CHOP was observed ($P>0.05$, data not shown),
38 309 probably due to the number of events in our study was insufficient to support a robust ApoA1
39 310 analysis. Therefore, the role of ApoA1, the most abundant protein in HDL, in the context of AISC,
40 311 warrants further investigation in future research.

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51 312 As far as we know, few clinical studies have investigated the association between HDL-C and
52 313 AISC. This study is the first clinical research that utilizes the IC-OS consensus statement(12) to
53 314 define subclinical cardiotoxicity, with univariate and multivariable analyses being used to identify
54 315 the influential factors of AISC in DLBCL patients in this cohort. Kaplan-Meier methods and Log-

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3 316 rank tests reveal that patients with high HDL-C levels were less likely to develop AISC. After
4 317 subjecting it to univariate and multivariable Cox regression methods, high HDL-C levels still
5 318 showed statistically significant differences. These results suggest that high HDL-C could be a
6 319 potentially independent protective factor for AISC in DLBCL patients and provide an opportunity
7 320 for investigators to develop a tool for early intervention and prevention of AISC. Further research
8 321 is necessary to confirm our findings.

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10 322 Several studies have demonstrated that serum lipid levels are altered during anthracycline-
11 323 containing chemotherapy in cancer patients(29, 30). Huxley et al and Averina et al have shown
12 324 that imbalanced serum lipid distribution is a risk factor for cardiovascular disease(31, 32). As a
13 325 result, anthracycline-containing treatment can induce dyslipidemia and facilitate the occurrence
14 326 and development of cardiovascular diseases in cancer patients. In a study of 394 breast cancer
15 327 patients, Xin et al found that HDL-C levels after chemotherapy were significantly lower than those
16 328 before chemotherapy(33). Similarly, Lu et al and Hana et al found that HDL-C levels were
17 329 significantly decreased during anthracycline-containing chemotherapy in patients with breast
18 330 cancer(34, 35). In our study, we specifically assessed the changes in HDL-C levels over time
19 331 during follow-up. Except for the group of patients who experienced AISC at 12 months after
20 332 treatment completion, HDL-C levels in all other groups increased from baseline to the 3rd cycle
21 333 of chemotherapy. This phenomenon may be due to the fact that anti-tumor drugs require
22 334 cholesterol to cross cell membranes(36). However, HDL-C levels were significantly decreased
23 335 from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all
24 336 patients, especially in the AISC group, which is consistent with previous research results(33-35),
25 337 and further confirmed that anthracycline-contained chemotherapy has adverse effects on HDL-C
26 338 levels in DLBCL patients. The HDL-C level in patients without AISC was more stable than that
27 339 in patients with AISC. Therefore, anthracycline-containing chemotherapy may promote the
28 340 occurrence and development of cardiotoxicity in DLBCL patients by inducing HDL-C turbulence.
29 341 Besides, the findings of our study indicate a significant decrease in GLS during the chemotherapy
30 342 period in patients with AISC. This result is consistent with previous research, which has reported
31 343 that doxorubicin dose at the range of 100-150mg/m² can cause cardiotoxicity(37). Notably, we
32 344 also observed that GLS remained stable after completion of chemotherapy, suggesting that the
33 345 cardiac effects of anthracycline-based chemotherapy may be dose-related. These findings have
34 346 important implications for the monitoring and management of cardiotoxicity in patients
35 347 undergoing anthracycline-based chemotherapy, as early detection of cardiac dysfunction during
36 348 treatment may improve patient outcomes. We investigated the associations of change in HDL-C
37 349 with change in GLS, no statistically significant differences were found.

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39 350 The analysis of HDL-C levels should not only consider the changes over time, but also the absolute
40 351 values. In our study, patients without AISC had significantly higher absolute highest HDL-C levels
41 352 than those with AISC, while the absolute lowest HDL-C levels did not differ significantly between
42 353 the two groups. The alterations from HDL-C extremes to baseline did not exhibit any variation
43 354 between the groups either. This suggests that the highest absolute HDL-C value was a preferable
44 355 indicator of AISC protection than the change in HDL-C from baseline to the extremum value.

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

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

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

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386 CONCLUSIONS

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

395

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399

400 **COMPETING INTERESTS**

401 The authors declare no conflict of interest.

402

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414

415 **AUTHOR CONTRIBUTIONS**

416 Conceptualization, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong
417 Wang, Qian Dong and Xiaoqiong Tang; Data curation, Wenxin Ou, Tiantian Jiang, Yue Weng and
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419 Zhang, Dong Wang and Xiaoqiong Tang; Investigation, Dong Wang, Qian Dong and Xiaoqiong
420 Tang; Methodology, Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong
421 Wang, Qian Dong and Xiaoqiong Tang; Resources, Dong Wang, Qian Dong and Xiaoqiong Tang;
422 Supervision, Qian Dong and Xiaoqiong Tang; Validation, Nan Zhang and Kai Lu; Visualization,
423 Wenxin Ou and Tiantian Jiang; Writing – original draft, Wenxin Ou; Writing – review & editing,
424 Wenxin Ou, Tiantian Jiang, Nan Zhang, Kai Lu, Yue Weng, Xi Zhou, Dong Wang, Qian Dong
425 and Xiaoqiong Tang. Qian Dong and Xiaoqiong Tang contributed equally to this work and are
426 considered as co-corresponding authors.

427

428 **DATA AVAILABILITY STATEMENT**

429 Data are available upon reasonable request.

430

431 **ETHICS APPROVAL**

432 The study was conducted in accordance with the Declaration of Helsinki and approved by the
433 ethics committee of the First Affiliated Hospital of Chongqing Medical University (Approval NO.
434 2018-016). And all participating patients provided written informed consent.

435

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30 560 **FIGURE LEGENS**

31 561 **Figure 1. (a)** Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients
32 562 detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients
33 563 were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC
34 564 at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after
35 565 treatment completion. 2 patients were detected AISC at 12 months after treatment completion.
36 566 **(b)** Timeline of HDL-C levels of patients without AISC.

37 567 **Figure 2.** Timeline of high-density lipoprotein cholesterol (HDL-C) levels **(a)** and global
38 568 longitudinal strain (GLS) **(b)** in patients with and without anthracycline-induced subclinical
39 569 cardiotoxicity (AISC) during the whole follow-up period.

40 570 **Figure 3. (a)** Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from
41 571 baseline to the end of the 6th cycle of chemotherapy. **(b)** Changes of HDL-C in patients with
42 572 anthracycline-induced subclinical cardiotoxicity (AISC). **(c)** Changes of HDL-C in patients
43 573 without AISC.

44 574 **Figure 4.** High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-
45 575 induced subclinical cardiotoxicity (AISC) and No-AISC. **(a)** Highest level of HDL-C during
46 576 chemotherapy. **(b)**The HDL-C value increment from baseline to the highest value. **(c)** The lowest
47 577 level of HDL-C during chemotherapy. **(d)** The HDL-C value declined from baseline to the
48 578 lowest.

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3 579 **Figure S1.** Flow diagram of patients enrolled in our observational research and reasons for lost
4 580 to follow-up.

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6 581 **Figure S2.** Kaplan-Meier curves of the percentage of patients without AISC in patients stratified
7 582 by HDL-C level. High HDL-C: HDL-C \geq 1.16mmol/L. Low HDL-C: HDL-C < 1.16mmol/L.
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9 583 AISC, anthracycline-induced cardiotoxicity; HDL-C, high-density lipoprotein cholesterol; HR,
10 584 hazard ratio.

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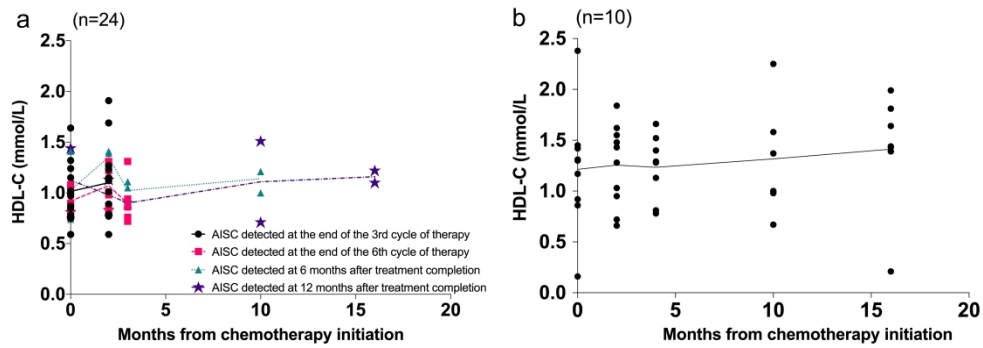


Figure 1. (a) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC at the end of the 6th 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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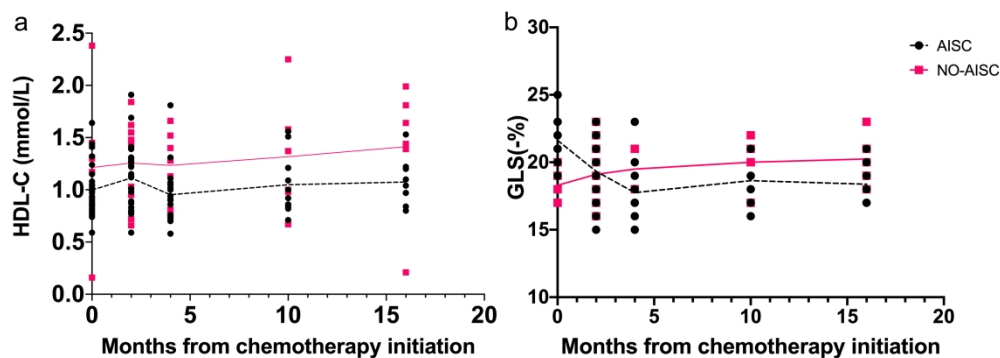


Figure 2. Timeline of high-density lipoprotein cholesterol (HDL-C) levels (a) and global longitudinal strain (GLS) (b) in patients with and without anthracycline-induced subclinical cardiotoxicity (AISC) during the whole follow-up period.

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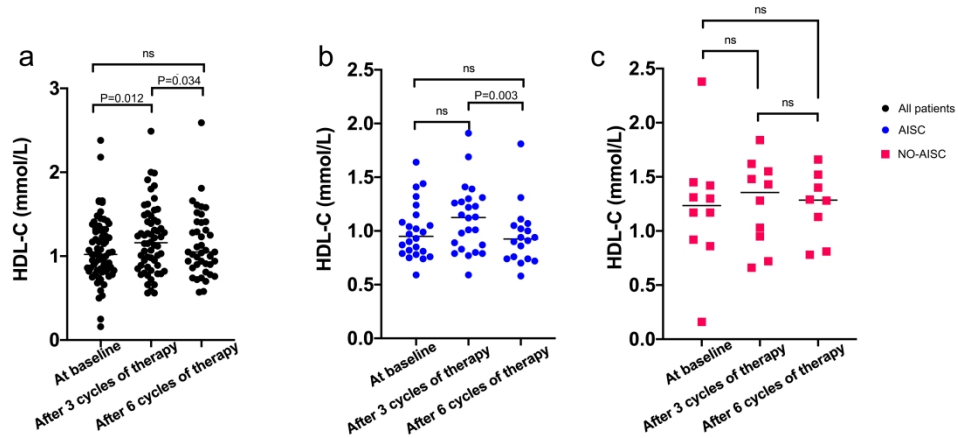


Figure 3. (a) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from baseline to the end of the 6th cycle of chemotherapy. (b) Changes of HDL-C in patients with anthracycline-induced subclinical cardiotoxicity (AISC). (c) Changes of HDL-C in patients without AISC.

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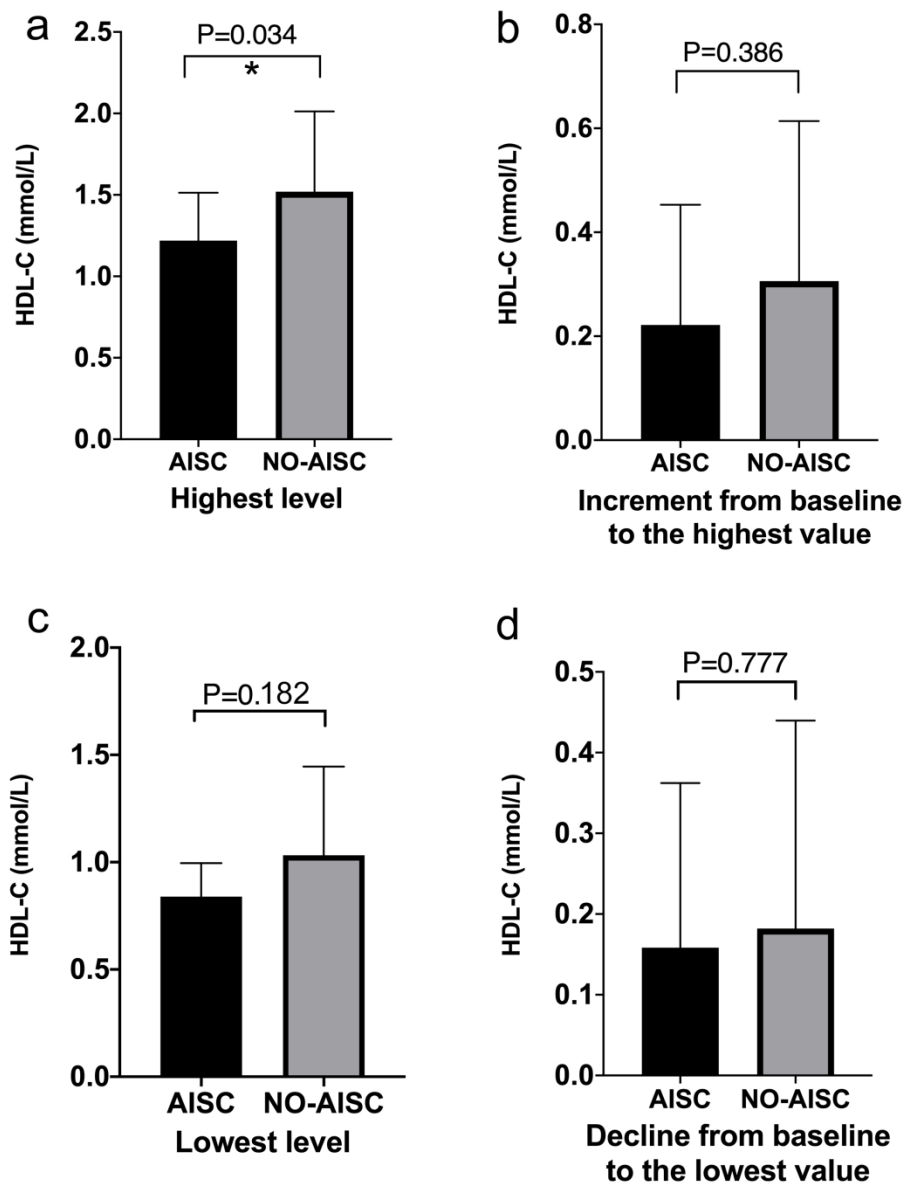
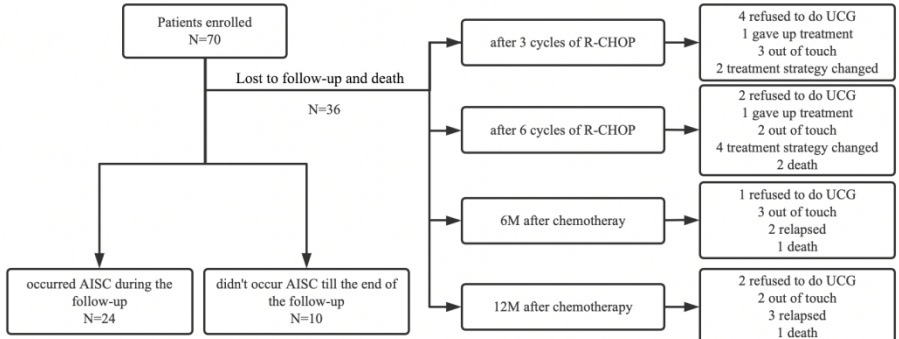


Figure 4. High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-induced subclinical cardiotoxicity (AISC) and No-AISC. (a) Highest level of HDL-C during chemotherapy. (b) The HDL-C value increment from baseline to the highest value. (c) The lowest level of HDL-C during chemotherapy.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

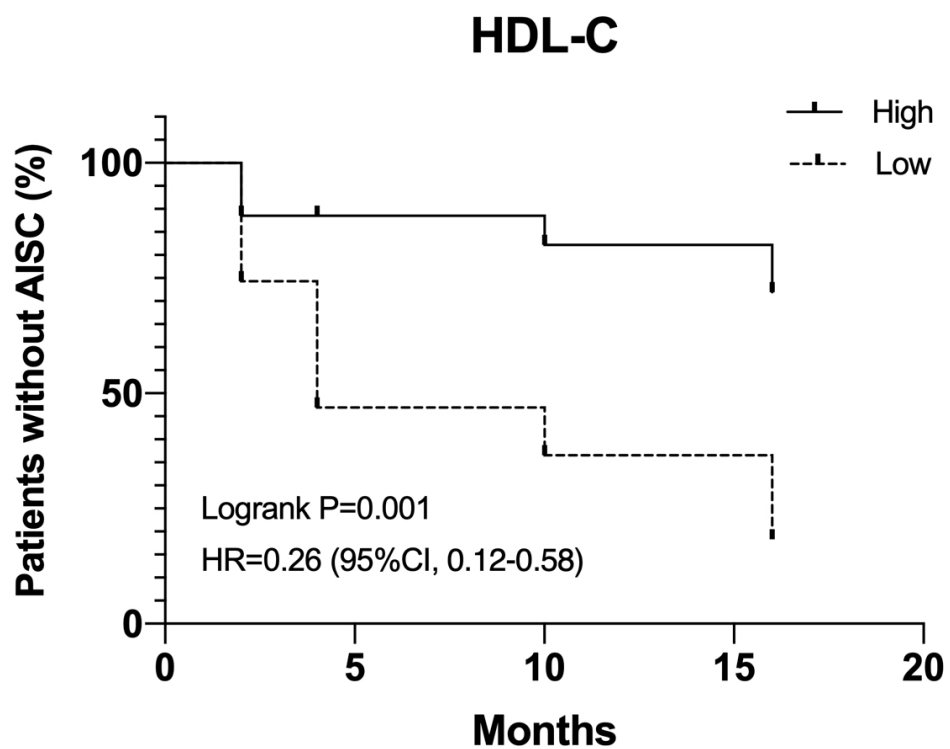
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Table S1. Baseline clinical characteristics of patients enrolled

	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 \geq 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 \geq 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

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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

1	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	6-7
2			(b) Report category boundaries when continuous variables were categorized	
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
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9	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-7
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11	Discussion			
12				
13	Key results	18	Summarise key results with reference to study objectives	7-8
14	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
15				
16	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
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18				
19	Generalisability	21	Discuss the generalisability (external validity) of the study results	10
20				
21	Other information			
22	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
23				
24				

*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>.

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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Secondary Subject Heading:	Cardiovascular medicine, Medical management
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1 Role of HDL cholesterol in anthracycline-induced subclinical 2 cardiotoxicity: a prospective observational study in patients 3 with diffuse large B-cell lymphoma treated with R-CHOP

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23
24 **Keywords:** cardiotoxicity; DLBCL; anthracycline; HDL-C; GLS

26 ABSTRACT

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

33 **Design:** Prospective observational study

34 **Setting:** Conducted in China from September 2020 to September 2022.

35 **Participant:** 70 chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled
36 to receive the standard dose of R-CHOP; 60 participants included in a case-control study (DOI:
37 10.1186/s12885-022-10085-6).

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

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

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

53 **Study registration number:** ChiCTR2100054721

54 55 **Strengths and limitations of this study**

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

69 70 **INTRODUCTION**

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

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3 78 Non-Hodgkin's lymphoma (NHL) is the 7th most common cancer in the United States and the
4 79 most frequent hematologic malignancy globally, accounting for about 3% of cancer cases and
5 80 deaths(6). Among NHL, DLBCL is the most prevalent type, representing approximately one-third
6 81 of all cases(7). The combination of cyclophosphamide, vincristine, doxorubicin, and prednisone
7 82 with rituximab (R-CHOP) is a standard first-line therapy that has substantially improved survival
8 83 outcomes in patients with DLBCL(8). Nonetheless, anthracycline-containing chemotherapy agents
9 84 are associated with cardiotoxicity, a major long-term adverse effect that significantly affects the
10 85 quality of life and survival of cancer survivors.

11 86 Anthracycline-induced cardiotoxicity (AIC) is a devastating consequence of successful cancer
12 87 treatment, often leading to hypokinetic cardiomyopathy and ultimately heart failure. AIC is an
13 88 irreversible form of cardiac dysfunction for which no guidelines or accepted therapies for
14 89 cardioprotection currently exist(9, 10). Therefore, early prevention and detection of AIC are
15 90 crucial for providing opportunities for early intervention. Anthracycline-induced subclinical
16 91 cardiotoxicity (AISC) is an early stage of AIC, characterized by abnormal echocardiography index
17 92 without clinical symptoms(11). Early intervention is recommended by the 2022 International
18 93 Cardio-Oncology Society (IC-OS) consensus statement once AISC is detected(12). Global
19 94 longitudinal peak systolic strain (GLS) measured by 2D speckle tracking echocardiography can
20 95 reliably identify most early myocardial deformation variations. In our study, we used early
21 96 measurement of GLS to identify AISC(13, 14).

22 97 High-density lipoprotein (HDL) is the sole lipoprotein with protective attributes among the five
23 98 types of lipoproteins. Its salutary effects include antioxidant, anti-inflammatory, and anti-apoptotic
24 99 properties. Numerous preclinical investigations have suggested that HDL may have direct and
25 100 indirect protective effects against AIC(15-17). The roles of HDL-cholesterol (HDL-C) and
26 101 apolipoprotein A1 (ApoA1) in providing cardiovascular protection of HDL have been the subject
27 102 of recent debate. Therefore, further investigation is warranted to explore the clinical data pertaining
28 103 to the association between HDL and anthracycline-related cardiotoxicity.

29 104 We undertook an prospective observational study to investigate the potential impact of HDL-C on
30 105 AISC. Using 2D speckle tracking echocardiography, we identified AISC and sought to establish
31 106 any correlation between HDL-C and AISC. Additionally, we assessed the fluctuations in HDL-C
32 107 levels during R-CHOP chemotherapy in chemotherapy-naïve patients recently diagnosed with
33 108 DLBCL. Subsequently, our team conducted a case-control study, revealing that HDL-C serves as
34 109 a predictive factor for AISC in patients with DLBCL treated with 3 cycles of (R)-CHOP(18). Both
35 110 the case-control study and present study are from the same database of the registered study 'Study
36 111 of Antineoplastic Drugs Induced Early Cardiotoxicity in Patients with
37 112 Lymphoma'(ChiCTR2100054721). Even though this case-control study was analyzed with data
38 113 from different parts of the same database for different objectives, the result underscores the
39 114 significance of further investigating the relationship between HDL-C and AISC.

40 115

41 116 **METHODS**

42 117 **Study population**

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3 118 We recruited chemotherapy-naïve patients newly diagnosed with DLBCL who were scheduled to
4 119 receive the standard dose of R-CHOP chemotherapy regimen at our institution from September
5 120 1st, 2020, to September 1st, 2022. Our inclusion criteria were as follows: newly diagnosed DLBCL,
6 121 age between 18 and 80 years, Eastern Cooperative Oncology Group (ECOG) performance status
7 122 ≤ 2 , left ventricular ejection fraction (LVEF) $\geq 50\%$, and acceptable bone marrow, renal, and
8 123 hepatic functions for chemotherapy. Conversely, our exclusion criteria were symptomatic heart
9 124 failure, a history of myocardial ischemia, myocarditis, myocardial infarction, clinical or subclinical
10 125 pericardial effusion, arrhythmia requiring medical intervention, a history of other cancers, under
11 126 lipid-lowering treatment, and severe active infections such as syphilis, hepatitis, or human
12 127 immunodeficiency virus (HIV) infection. This study shares its database with a case-control study
13 128 previously conducted by our group, as referenced earlier(18). The patients enrolled in the two
14 129 study were not identical because of a few differences in specific objectives, inclusion criteria, and
15 130 exclusion criteria. In brief, the case-control study specifically included patients with DLBCL who
16 131 received the standard dose of (R)-CHOP chemotherapy regimen (CHOP with or without rituximab
17 132 combination), and patients undergoing lipid-lowering therapy were not excluded. In the current
18 133 study, 10 of the 70 enrolled participants were not enrolled in the case-control study. More details
19 134 can be seen in this case-control study(18).

135 **Treatment**

20 136 Patients received a total of 6 cycles of standard R-CHOP (cyclophosphamide at 750 mg/m² on D1,
21 137 doxorubicin at 50 mg/m² on D1, vincristine at 1.4 [maximum 2] mg/m² on D1, and 100 mg
22 138 prednisone on D1-5, with rituximab at 375 mg/m² on D1 in each cycle), with or without 2 cycles
23 139 of rituximab maintenance (rituximab at 375 mg/m² on D1 in each cycle).

24 140 **Definition of subclinical cardiotoxicity**

25 141 According to the IC-OS consensus statement, the definition of subclinical cardiotoxicity was a
26 142 relative GLS decrease from baseline [(baseline – current GLS)/baseline GLS] of $>12\%$, but with
27 143 a normal left ventricular ejection fraction (LVEF)(12).

28 144 **Study protocol**

29 145 We defined 'baseline' as the initial assessment conducted before the initiation of the first cycle of
30 146 chemotherapy. At baseline, the end of the 3rd cycle of R-CHOP, the end of the 6th cycle of R-
31 147 CHOP, and 6 and 12 months after chemotherapy completion, all enrolled patients underwent
32 148 conventional echocardiography, 2D speckle tracking echocardiography, and blood sampling.
33 149 Every patient received electrocardiography (ECG) examination before every cycle of
34 150 chemotherapy to ensure the safety of the treatment. Demographic data and clinical variables,
35 151 including age, gender, body mass index (BMI), ECOG performance status, diabetes mellitus,
36 152 hypertension, drinking history (an adult who has consumed more than 20 drinks in lifetime, with
37 153 each drink is considered to have an average alcohol content of 12 g), and smoking history (an adult
38 154 who has smoked at least 100 cigarettes in their lifetime) were collected at the time of enrollment.
39 155 Left ventricular systolic dysfunction was measured by LVEF, fractional shortening (FS), left
40 156 ventricular mass index (LVMI), left ventricular diastolic dimension (LVDd), E, e', E/e', and GLS.
41 157 HDL-C, low-density lipoprotein cholesterol (LDL-C), cardiac troponin T (cTnT), high sensitivity

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3 158 C-reactive protein (hsCRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP),
4 159 total cholesterol (TC) and total triglyceride (TG) were measured. We used the baseline HDL-C
5 160 level as a surrogate marker for HDL quantity. The patients were categorized into two groups based
6 161 on the average HDL-C value for males and females in the modified criteria of the National
7 162 Cholesterol Educated Program Adult Treatment Panel (NCEP ATP III)(19). High HDL-C was
8 163 defined as a serum HDL-C \geq 1.16mmol/L, while low HDL-C was defined as a serum HDL-
9 164 C $<$ 1.16mmol/L. We determined the sample size using an online sample size calculator, which
10 165 indicated a total requirement of 23 events(20).

14 166 **Statistical analysis**

15 167 The study was conducted with two aims: firstly, to evaluate the relationship between HDL-C and
16 168 AISC; and secondly, to conduct a preliminary exploration of the differences in HDL-C and the
17 169 variability of HDL-C changes between patients with and without AISC during the follow-up
18 170 period. Continuous variables were expressed as mean \pm standard deviation (SD) and compared
19 171 using the t-test. Non-normally distributed variables were presented as median (Q1- Q3) and
20 172 compared with the Wilcoxon Mann-Whitney test. Categorical variables were expressed as n (%)
21 173 and compared using the Chi-square or Fisher's exact test, as appropriate. Correlation analysis was
22 174 conducted to investigate the associations of change in HDL-C with change in GLS. The
23 175 probabilities of survival were calculated using Kaplan-Meier methods and compared using Log-
24 176 rank tests. Cox proportional-hazards regression models were conducted to assess the association
25 177 between variables and AISC. Covariates for multivariable Cox regression models included age,
26 178 sex, and variables that had a P-value of less than 0.15 in the univariable Cox regression analysis
27 179 (GLS was excluded as it is the factor that defines AISC). Two multivariable Cox regression models
28 180 were constructed: the first model included age and sex; and the second model included age, sex,
29 181 hypertension, BMI, and E. Statistical analysis and visualization were performed using IBM SPSS
30 182 V.22.0 and GraphPad Prism 8. Statistical tests were two-sided, with a P-value less than 0.05 being
31 183 considered statically significant.

34 184 **Patient and public involvement**

35 185 None.

40 186

41 187 **RESULTS**

42 188 **Assessment of the association between HDL-C and AISC**

43 189 *Study population and baseline characteristics*

44 190 This investigation enrolled a total of 70 patients with chemotherapy-naïve DLBCL and were
45 191 planned to be treated with the standard R-CHOP regimen. Based on the baseline HDL-C level, we
46 192 segregated the patients into two groups: the high-level group (HDL-C \geq 1.16mmol/L, n=28) and
47 193 the low-level group (HDL-C $<$ 1.16mmol/L, n=42). Patients with drinking history had a greater
48 194 chance of having a high HDL-C level (P=0.034). The patients with high HDL-C showed
49 195 substantially higher total cholesterol (P=0.011), and lower total triglyceride (P=0.002). The
50 196 baseline characteristics of the patients in both groups were well balanced (Table S1).

51 197

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

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

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

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

217 The results of the multivariable Cox regression analysis showed that high HDL-C was significantly
 218 associated with a lower risk of AISC after adjusting for age and sex (model 1) (HR=0.28,
 219 95%CI:0.10=0.84, P=0.018). Similarly, after adjusting for age, sex, and variables that P<0.15 in
 220 the univariable Cox regression analysis (excluding GLS as it defines AISC) (model 2), the same
 221 association was observed (HR=0.27, 95%CI: 0.09-0.79, P=0.017). (Table 1)

222 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

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

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

228
 229 **Preliminary exploration of the difference of HDL-C between patients with AISC and**
 230 **without AISC**

231 *Study population and baseline characteristics*

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

236 (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
237 (17.0, 20.0), P<0.001]. More baseline information can be seen in Table S3.

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

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

252 Based on Figure 2, we observed that the fluctuations in HDL-C and GLS were most pronounced
253 during the chemotherapy period. The fluctuations in HDL-C levels of patients with DLBCL during
254 R-CHOP chemotherapy were presented in Figure 3. The levels of HDL-C significantly increased
255 for all patients from baseline to the end of the 3rd cycle of chemotherapy (P=0.012) and
256 significantly decreased from the end of the 3rd cycle to the end of the 6th cycle of chemotherapy
257 (P=0.034) (Figure 3a). Patients with AISC showed a significant decrease in HDL-C levels during
258 R-CHOP chemotherapy from the end of the 3rd cycle to the end of the 6th cycle (P=0.003) (Figure
259 3b). However, no significant difference was observed in HDL-C levels for patients without AISC
260 during R-CHOP chemotherapy (Figure 3c). We conducted correlation analysis separately for the
261 change in HDL-C and GLS from baseline to after 3 cycles of chemotherapy, from baseline to after
262 6 cycles of chemotherapy, and from after 3 cycles to after 6 cycles of chemotherapy. However, we
263 found no statistically significant differences in the associations between changes in HDL-C and
264 GLS (P=0.965, 0.087, 0.449).

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

266 Figure 4 presents the contrasting values between patients with AISC and those without in terms of
267 four parameters, namely the highest and lowest levels of HDL-C during chemotherapy, the
268 increment and decline in HDL-C values from baseline. Patients without AISC showed significantly
269 higher values in the highest level of HDL-C (1.52±0.49 vs. 1.22±0.29, P=0.034, Figure 4a).
270 However, no significant differences were observed between the two groups in terms of HDL-C
271 increment from baseline to the highest value (0.31±0.31 vs. 0.22±0.23, P=0.386, Figure 4b). While
272 the lowest level of HDL-C was lower in patients with AISC, the difference was not statistically
273 significant (0.84±0.16 vs. 1.03±0.41, P=0.182, Figure 4c). Furthermore, there were no significant
274 differences in HDL-C decline between patients with AISC and those without (0.16±0.20 vs.
275 0.18±0.26, P=0.777, Figure 4d).

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3 2764 277 **DISCUSSION**

6 278 This prospective observational study investigated the relationship between HDL-C and incidence
7 279 of AISC in 70 patients with DLBCL who were receiving anthracycline-containing chemotherapy.
8 280 The study found that higher levels of HDL-C were associated with a lower incidence of AISC.
9 281 Moreover, patients without AISC had more stable and higher levels of HDL-C than those with
10 282 AISC during the follow-up period. The results also showed that HDL-C levels were significantly
11 283 decreased from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of
12 284 chemotherapy in all patients, especially in the AISC group, indicating that anthracycline-
13 285 containing chemotherapy has adverse effects on HDL-C levels. Notably, the highest level of HDL-
14 286 C was significantly higher in patients without AISC compared to those with AISC. These findings
15 287 suggest that HDL-C may have a protective role against AISC in patients with DLBCL undergoing
16 288 anthracycline-containing chemotherapy and maintaining a relatively high level of HDL-C may be
17 289 more effective in managing cardio-protection than monitoring changes in HDL-C levels over time.
18 290 The results of this study highlight the importance of early serum lipid management in these
19 291 patients.

20 292 Lipoproteins are classified into five categories, namely chylomicron, very-low-density lipoprotein
21 293 (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density
22 294 lipoprotein (HDL), based on their size, density, and lipid composition (cholesterol and
23 295 triglycerides)(21). Among these, HDL exhibits distinctive cytoprotective actions and triggers anti-
24 296 oxidative, anti-inflammatory, and anti-apoptotic effects. The protective roles of HDL in
25 297 cardiovascular disease have been controversial in recent years, and that the quality of HDL
26 298 (cholesterol efflux capacity, antioxidant activity, anti-inflammatory activity, endothelial function,
27 299 etc.) rather than the quantity of HDL has been proposed as the true cardioprotective effect. The
28 300 Framingham Heart Study, as early as 1988, reported a correlation between HDL-C and
29 301 cardiovascular mortality(22). Recent studies have challenged the HDL-C hypothesis by revealing
30 302 that HDL-C level is not inversely correlated with cardiovascular diseases(23, 24). In our study, we
31 303 used the baseline HDL-C level as a surrogate marker for HDL quantity, but we did not directly
32 304 measure the quality of HDL. Measuring the level of HDL-C in serum is a commonly used method
33 305 to assess the effect of HDL on cardiovascular health. HDL facilitates the transportation of
34 306 cholesterol from the body's tissues back to the liver, and higher levels of HDL-C are generally
35 307 associated with a lower risk of heart disease. Nevertheless, it's crucial to note that HDL-C levels
36 308 may not accurately reflect the functional properties of HDL. ApoA1, the most abundant protein in
37 309 HDL, is associated with several beneficial effects of HDL(15, 25). The function and abundance of
38 310 ApoA1 are reported to play a dominant role in HDL quality(26). In the context of AIC, several
39 311 studies have indicated that HDL can protect against anthracycline-induced cardiomyocyte
40 312 apoptosis and atrophy in isolated cardiomyocytes(27, 28) and animal models(16, 28). Based on
41 313 these earlier trials, HDL-C and ApoA1 could serve as protective factors against anthracycline-
42 314 related cardiovascular disease. The case-control study conducted by our team, utilizing the same
43 315 database, demonstrated that both HDL-C and ApoA1 act as predictive factors in patients

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3 316 undergoing three cycles of anthracycline-containing chemotherapy(18). Both the present study and
4 317 the case-control study are derived from a registered research (ChiCTR2100054721) as mentioned
5 318 before. The objective of this registered study was to explore the correlation between cardiotoxicity
6 319 occurrence and lymphoma, antitumor drugs, and cardiovascular risk factors. This registered study
7 320 included patients with clinically diagnosed lymphoma who were evaluated by a hematologist as
8 321 requiring chemotherapy. The study collected the demographic data and clinical variables of
9 322 enrolled patients. Enrolled patients underwent conventional echocardiography, 2D speckle
10 323 tracking echocardiography, and blood sampling at baseline, the end of the 3rd cycle of
11 324 chemotherapy, the end of the 6th cycle of chemotherapy, and 6 and 12 months after completing
12 325 chemotherapy. In our case-control study, according to the changes of GLS at baseline and after the
13 326 3rd cycle of chemotherapy, patients were divided into the AISC and No-AISC groups. Then
14 327 demographic data, clinical variables, and biochemical variables were compared between the two
15 328 groups. In contrast to the current study, this case-control study specifically included patients with
16 329 DLBCL who received the standard dose of (R)-CHOP chemotherapy regimen (CHOP with or
17 330 without rituximab combination), and patients undergoing lipid-lowering therapy were not
18 331 excluded. The aim of this case-control study was to analyze the influencing factors of AISC in
19 332 patients with DLBCL treated with 3 cycles of (R)-CHOP chemotherapy regimen, and results
20 333 indicated that both HDL-C and ApoA1 act as predictive factors against AISC. However, our
21 334 present study didn't focus on the investigation of the impact of ApoA1 on AISC. Even when
22 335 ApoA1 was included in the Cox regression model, no significant association with AISC in patients
23 336 with DLBCL treated with R-CHOP was observed ($P>0.05$, data not shown), probably due to the
24 337 number of events in our study was insufficient to support a robust ApoA1 analysis. Therefore, the
25 338 role of ApoA1, the most abundant protein in HDL, in the context of AISC, warrants further
26 339 investigation in future research.

27 340 As far as we know, few clinical studies have investigated the association between HDL-C and
28 341 AISC. This study is the first clinical research that utilizes the IC-OS consensus statement(12) to
29 342 define subclinical cardiotoxicity, with univariate and multivariable analyses being used to identify
30 343 the influential factors of AISC in patients with DLBCL in this cohort. Kaplan-Meier methods and
31 344 Log-rank tests reveal that patients with high HDL-C levels were less likely to develop AISC. After
32 345 subjecting it to univariate and multivariable Cox regression methods, high HDL-C levels still
33 346 showed statistically significant differences. These results suggest that high HDL-C could be a
34 347 potentially independent protective factor for AISC in patients with DLBCL and provide an
35 348 opportunity for investigators to develop a tool for early intervention and prevention of AISC.
36 349 Further research is necessary to confirm our findings.

37 350 Several studies have demonstrated that serum lipid levels are altered during anthracycline-
38 351 containing chemotherapy in cancer patients(29, 30). Huxley et al and Averina et al have shown
39 352 that imbalanced serum lipid distribution is a risk factor for cardiovascular disease(31, 32). As a
40 353 result, anthracycline-containing treatment can induce dyslipidemia and facilitate the occurrence
41 354 and development of cardiovascular diseases in cancer patients. In a study of 394 breast cancer
42 355 patients, Xin et al found that HDL-C levels after chemotherapy were significantly lower than those

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3 356 before chemotherapy(33). Similarly, Lu et al and Hana et al found that HDL-C levels were
4 357 significantly decreased during anthracycline-containing chemotherapy in patients with breast
5 358 cancer(34, 35). In our study, we specifically assessed the changes in HDL-C levels over time
6 359 during follow-up. Except for the group of patients who experienced AISC at 12 months after
7 360 treatment completion, HDL-C levels in all other groups increased from baseline to the 3rd cycle
8 361 of chemotherapy. This phenomenon may be due to the fact that anti-tumor drugs require
9 362 cholesterol to cross cell membranes(36). However, HDL-C levels were significantly decreased
10 363 from the end of the 3rd cycle of chemotherapy to the end of the 6th cycle of chemotherapy in all
11 364 patients, especially in the AISC group, which is consistent with previous research results(33-35),
12 365 and further confirmed that anthracycline-contained chemotherapy has adverse effects on HDL-C
13 366 levels in patients with DLBCL. The HDL-C level in patients without AISC was more stable than
14 367 that in patients with AISC. Therefore, anthracycline-containing chemotherapy may promote the
15 368 occurrence and development of cardiotoxicity in patients with DLBCL by inducing HDL-C
16 369 turbulence.

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22 370 Besides, the findings of our study indicate a significant decrease in GLS during the chemotherapy
23 371 period in patients with AISC. This result is consistent with previous research, which has reported
24 372 that doxorubicin dose at the range of 100-150mg/m² can cause cardiotoxicity(37). Notably, we
25 373 also observed that GLS remained stable after completion of chemotherapy, suggesting that the
26 374 cardiac effects of anthracycline-based chemotherapy may be dose-related. These findings have
27 375 important implications for the monitoring and management of cardiotoxicity in patients
28 376 undergoing anthracycline-based chemotherapy, as early detection of cardiac dysfunction during
29 377 treatment may improve patient outcomes. We investigated the associations of change in HDL-C
30 378 with change in GLS, no statistically significant differences were found.

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34 379 The analysis of HDL-C levels should not only consider the changes over time, but also the absolute
35 380 values. In our study, patients without AISC had significantly higher absolute highest HDL-C levels
36 381 than those with AISC, while the absolute lowest HDL-C levels did not differ significantly between
37 382 the two groups. The alterations from HDL-C extremes to baseline did not exhibit any variation
38 383 between the groups either. This suggests that the highest absolute HDL-C value was a preferable
39 384 indicator of AISC protection than the change in HDL-C from baseline to the extremum value.
40 385 Maintaining a relatively high level of HDL-C may be more effective in managing the cardio-
41 386 protection of anthracycline-treated cancer patients than monitoring changes in HDL-C levels over
42 387 time.

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45 388 In our investigation, we observed that among the four patients with pre-existing hypertension, one
46 389 patient experienced AISC during the follow-up (Table S3). Multivariable Cox regression analysis
47 390 showed that hypertension did not have a significant impact on AISC (P>0.05). Hypertension, a
48 391 common risk factor for both cancer and cardiovascular diseases, was also recognized as a risk
49 392 factor for cardiotoxicity. Studies have reported that pre-existing hypertension was associated with
50 393 anthracycline-and trastuzumab induced left ventricular ejection fraction (LVEF) decline in a
51 394 retrospective study(38), and early left ventricular systolic dysfunction in patients with lymphoma
52 395 receiving (R)-CHOP in a prospective study(39). We noted that all patients with hypertension in
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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.

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415 **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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428

429 **COMPETING INTERESTS**

The authors declare no conflict of interest.

431

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3 436 doxorubicin promoting atherosclerosis in lymphoma patients through NF- κ B/miR-33 signaling
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12 443

13 444 **CONTRIBUTORS**

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21 452 Wenxin Ou and Tiantian Jiang; Writing – original draft, Wenxin Ou; Writing – review & editing,
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23 454 and Xiaoqiong Tang. Qian Dong and Xiaoqiong Tang contributed equally to this work and are
24 455 considered as co-corresponding authors.

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30 457 **DATA AVAILABILITY STATEMENT**

31 458 Data are available upon reasonable request.

33 459

34 460 **ETHICS APPROVAL**

35 461 The study was conducted in accordance with the Declaration of Helsinki and approved by the
36 462 ethics committee of the First Affiliated Hospital of Chongqing Medical University (Approval NO.
37 463 2018-016). And all participating patients provided written informed consent.

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589 FIGURE LEGENDS

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18 590 **Figure 1.** (a) Timeline of high-density lipoprotein cholesterol (HDL-C) levels in patients
19 591 detected anthracycline-induced subclinical cardiotoxicity (AISC) at four time points. 12 patients
20 592 were detected AISC at the end of the 3rd cycle of chemotherapy. 7 patients were detected AISC
21 593 at the end of the 6th cycle of chemotherapy. 3 patients were detected AISC at 6 months after
22 594 treatment completion. 2 patients were detected AISC at 12 months after treatment completion.

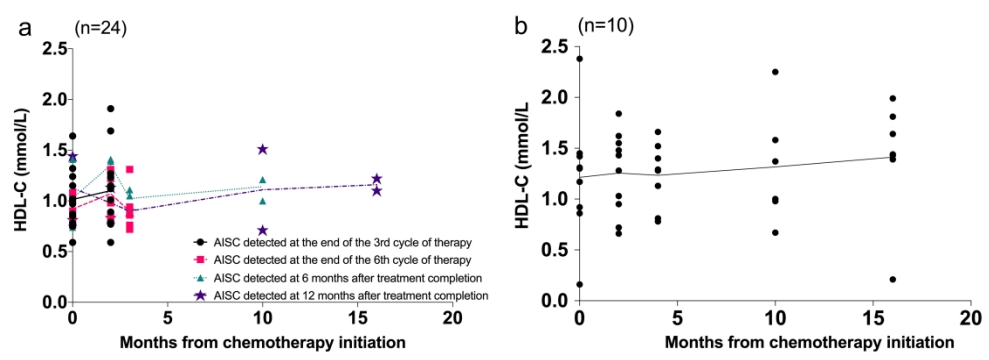
23 595 (b) Timeline of HDL-C levels of patients without AISC.

24 596 **Figure 2.** Timeline of high-density lipoprotein cholesterol (HDL-C) levels (a) and global
25 597 longitudinal strain (GLS) (b) in patients with and without anthracycline-induced subclinical
26 598 cardiotoxicity (AISC) during the whole follow-up period.

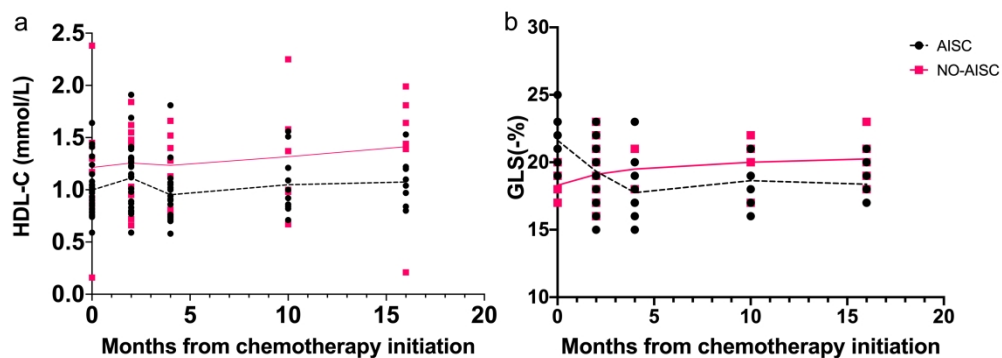
27 599 **Figure 3.** (a) Changes of high-density lipoprotein cholesterol (HDL-C) in all patients from
28 600 baseline to the end of the 6th cycle of chemotherapy. (b) Changes of HDL-C in patients with
29 601 anthracycline-induced subclinical cardiotoxicity (AISC). (c) Changes of HDL-C in patients
30 602 without AISC.

31 603 **Figure 4.** High-density lipoprotein cholesterol (HDL-C) differences between anthracycline-
32 604 induced subclinical cardiotoxicity (AISC) and No-AISC. (a) Highest level of HDL-C during
33 605 chemotherapy. (b) The HDL-C value increment from baseline to the highest value. (c) The lowest
34 606 level of HDL-C during chemotherapy. (d) The HDL-C value declined from baseline to the
35 607 lowest.
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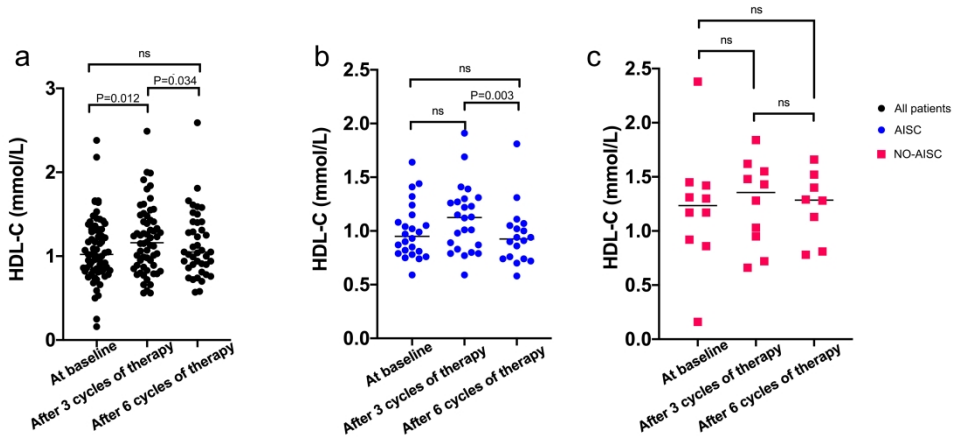


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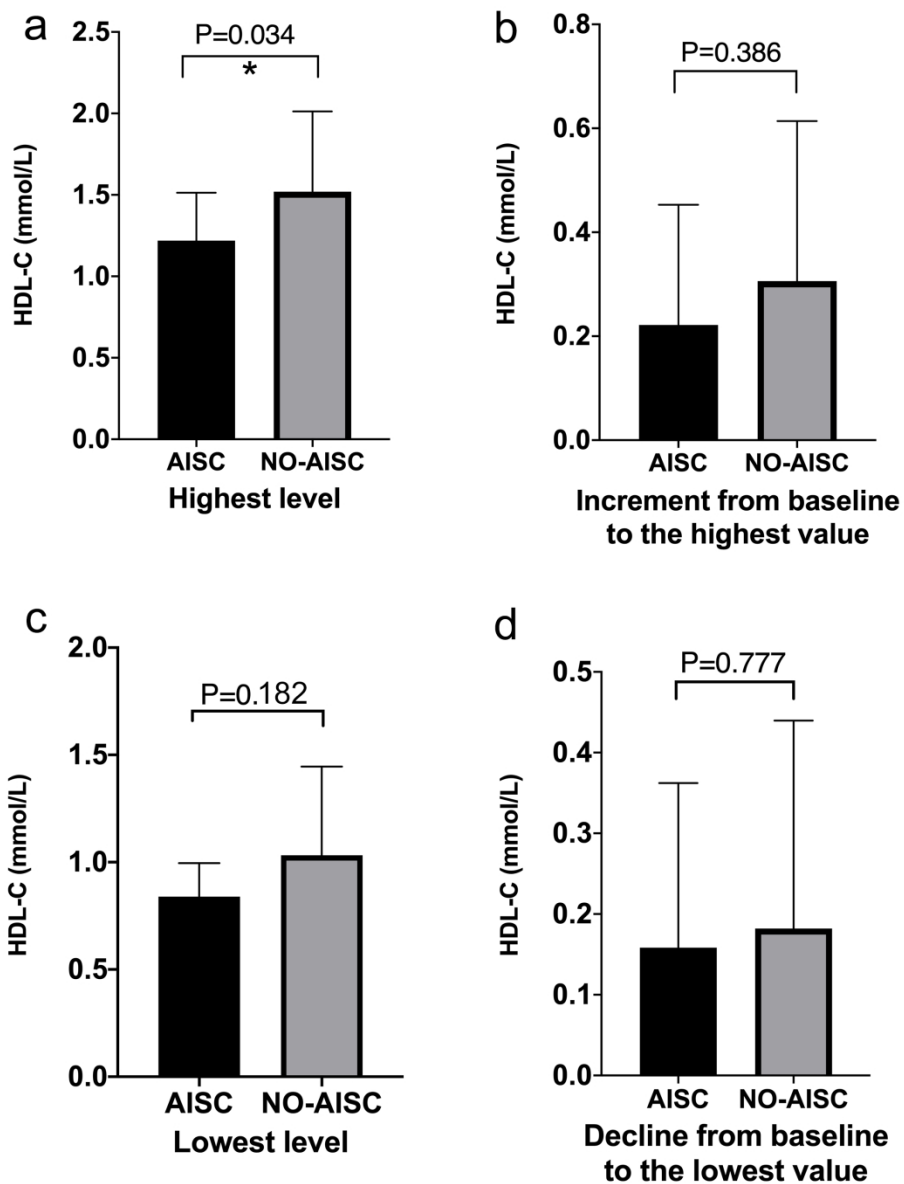


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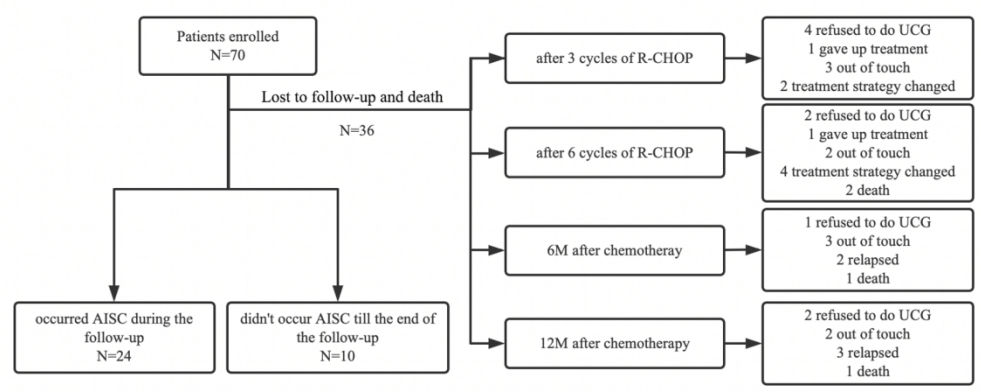


Figure S1. Flow diagram of patients enrolled in the prospective observational study.

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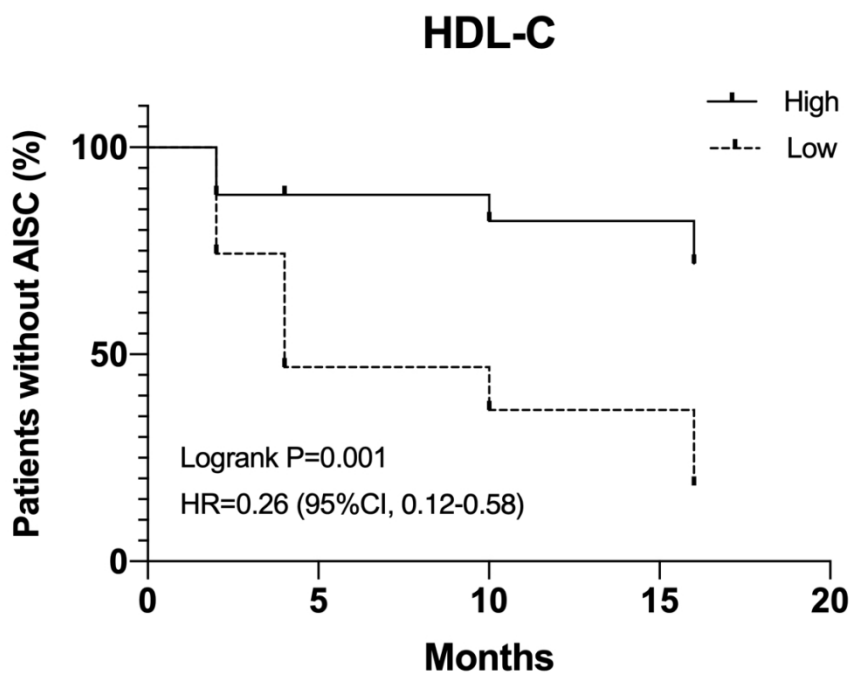


Figure S2. Kaplan-Meier curves of the percentage of patients without AISC in patients stratified by HDL-C level. High HDL-C: HDL-C \geq 1.16mmol/L. Low HDL-C: HDL-C<1.16mmol/L. AISC, anthracycline-induced cardiotoxicity; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio.

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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 \geq 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 \geq 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

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3 cholesterol; TG: total triglyceride.
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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

1	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	6-7
2			(b) Report category boundaries when continuous variables were categorized	
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
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9	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-7
10				
11	Discussion			
12				
13	Key results	18	Summarise key results with reference to study objectives	7-8
14	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
15				
16	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
17				
18				
19	Generalisability	21	Discuss the generalisability (external validity) of the study results	10
20				
21	Other information			
22	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
23				
24				

*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>.