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ORIGINAL ARTICLE

Altered serum lipid levels are associated with prognosis of diffuse large B cell lymphoma and influenced by utility of rituximab

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Abstract

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, and the prognosis of the disease varied. This research aims to investigate the impact of serum lipid level on the outcome of DLBCL patients and their interaction with rituximab (RTX). Data of newly diagnosed DLBCL in the third affiliated hospital of Soochow University were retrospectively collected. Baseline serum lipid levels, clinical data, and survival information were simultaneously recorded. Data of healthy controls were collected with age matching. Serum lipid levels significantly differed for the patients. All were transformed into categorical variables for the analysis of survival. During a median follow-up of 58 months, 32.8% patients died. Univariate analysis revealed all serum lipid indicators were associated with overall survival (OS); all except for total cholesterol (TC) and apolipoprotein B (apoB) showed significant impact on progression-free survival (PFS). Multivariable analysis confirmed the adverse effect of triglyceride (TG) on PFS ($P = 0.013$) and favorable impact of high-density lipoprotein (HDL) on OS ($P = 0.003$). For cases treated without RTX, apolipoprotein A (apoA) had independent favorable effect on both PFS ($P = 0.004$) and OS ($P = 0.001$). Comparably, for patients who received RTX, HDL showed remarkably predictive value of PFS ($P = 0.011$) and OS ($P = 0.019$). In conclusion, the abnormal serum lipids occurred throughout the course of DLBCL, and the associations of serum lipids and the prognosis of the disease were interfered by RTX. Trial registration: 2022(科)CL033; June 26, 2022, retrospectively registered

Keywords Diffuse large B cell lymphoma · Serum lipids · High-density lipoprotein · Apolipoprotein A · Prognosis · Rituximab

Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults and accounts for 25~30% of non-Hodgkin lymphoma (NHL), with approximately 72,400 new cases in 2019 in the United States [1, 2].

Implementation of rituximab (RTX) as an adjuvant to standard chemotherapy CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) has significantly improved the outcome and survival of DLBCL patients [3], representing the standard of care for the majority of patients until now. However, 30–40% of the patients relapsed after initial response or exhibited primary refractory disease [4]. Patients who are ineligible for allogeneic stem cell transplantation (ASCT) or who fail after second-line therapy have a dismal prognosis [5]. For patients' refractory to first-line therapy, the objective response to the next line therapy was 26%, with only 6% of complete response and 6.3 months of the median overall survival (OS) [6]. The underlying mechanism of the clinical heterogeneity was still poorly understood. Considering ceaseless new drugs targeting specific gene or pathway, there is an urgent need for innovative strategies to distinguish the patients who cannot benefit from the empirical chemotherapy.

Fei Wang, Luo Lu, and HuiJuan Chen provided equal contribution to this work.

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To maintain the abnormal growth and survival, malignant cells frequently share the attribute of metabolic reprogramming, which is a hallmark of malignant disease processed by series of oncogenes and tumor suppressors [7]. The most understood "Warburg effect," shift of aerobic to anaerobic glycolysis in malignant cells, transfers glucose to carbon to build more molecules for survival instead of completely oxidizing them for energy supplement [8]. Lipid metabolism is also a vital alteration in cancer cells. Normal tissues ensure their lipid requirements mainly through the uptake of free fatty acids (FFAs) and lipoproteins from the blood serum [9], while cancer cells presented exacerbated de novo FA synthesis [10]. This biosynthesis is supported by increased glycolysis with both energy and substrate [11] and catalyzed by fatty acid synthase (FASN) [12]. Extremely high levels of FASN have been reported by immunohistochemistry in many cancers [13] and are associated with higher risk and death of carcinoma [14, 15]. Besides FASN, other genes involved in lipid metabolism, including stearoyl-coenzyme A desaturase 1 (SCD1) and fatty acid-binding protein 4 (FABP4), were also proved to play essential roles in many types of human cancers [16]. Increasing evidence showed specific alterations in different aspects of lipid metabolism in cancer, affecting numerous cellular processes, including cell growth, proliferation, differentiation, and motility [17]. In corporation with the above, alteration of metabolic profiles was also detected in malignant B cells, with upregulation of both aerobic glycolysis and fatty acid synthesis in a variety of B-NHL cell lines [18]. MYC is a master regulator in DLBCL pathogenesis [19], which also profoundly reprograms cellular metabolism and regulates nucleoside metabolism [20], glutamine metabolism [21], glucose uptake, and glycolysis as well as lipid biosynthesis [22, 23]. In DLBCL cells, cholesterol and cholesteryl esters were proved to maintain membrane-anchored pro-survival and pro-proliferative signaling pathways, such as B cell receptor signaling [24].

With the underlying mechanism of altered lipid metabolism, serum lipids also presented clues of malignances. Circulating total cholesterol (TC) was proved to be associated with decreased cancer mortality [25], and high-density lipoprotein cholesterol (HDL) was observed as a protective factor against cancer [26, 27]. HDL and apolipoprotein A (apoA) were inversely associated with risk of colon cancer [27]. Pretreatment serum HDL levels were inversely related to survival of cancers, including breast cancer, lung cancer, and hepatocellular carcinoma [28]. In line with HDL, apoA, as the main component of HDL, has also been reported to be associated with prognosis of many cancers, including gastrointestinal cancer and breast cancer [29–31]. The association of hypocholesterolemia and lymphoma has long been recognized [32]. In follicular lymphoma, low HDL levels showed predictive value for 24 months of first-line chemoimmunotherapy (POD24) and worse prognosis [33]. As for DLBCL, limited retrospective or prospective studies have demonstrated inconsistent results. Studies showed the decreased total serum cholesterol, high-density lipoprotein, and low-density lipoprotein levels of lymphoma cases in the years prior to diagnosis [34]. But the prognostic value of the serum lipids and its impact on the treatment have been poorly understood.

In this study, we performed a comprehensive analysis of serum lipid profiles of DLBCL patients, compared with normal controls and combined with clinical prognostic factors and therapy regimens, to (1) assess whether DLBCL patients present differences in their serum lipids compared with normal condition, (2) investigate the relationship between serum lipids and clinical outcomes, and (3) explore the impact of serum lipids for different treatments, especially the application of RTX.

Patients and methods

Patients and controls

This retrospective study was approved by the Third Affiliated Hospital of Soochow University's institutional review board; the requirement for written patient consent was waived. To protect patient privacy, we removed all identifiers from our records after the completion of our analyses.

Between 2010 and 2018, 307 newly diagnosed DLBCL cases were included according to the morphological and immunohistological criteria of the World Health Organization classification. All special DLBCL subtypes including primary cutaneous B cell lymphoma, primary mediastinal large B cell lymphoma, primary mediastinal DLBCL, primary DLBCL of the central nervous system, and transformed DLBCL were excluded. Baseline clinical characteristics including age, gender, lactate dehydrogenase (LDH), B symptoms, ECOG PS and Ann Arbor stage, BMI, Ki-67, Hans classification, TC, triglyceride (TG), HDL, low-density lipoprotein (LDL), apoA, and apolipoprotein B (apoB) were considered during admission.

Among 1841 subjects that participated in health screenings in January 2019, 398 healthy controls were finally recruited matching in age and gender, with 1.26 males for every female and 1.3 controls for every patient within 6 groups of ten years (<40 and ≥ 80 defined as one group, respectively).

Follow-up and endpoints

Follow-up was conducted through making telephone calls and rechecking medical records. All patients were followed up until August 30, 2019, or the death of patients. Time from disease diagnosis to death of any cause or the final time of follow-up

was clarified as OS, and time from disease diagnosis to progression of lymphoma for any aspect or the time of final follow-up as progression-free survival (PFS). The survival status of all patients was confirmed with death records or a telephone call to next of kin (if patient died during the follow-up) or to the patients themselves.

Statistical analysis

All categorical variables were recorded as numbers (percentages) and all continuous variables as median (interquartile range, IQR). The χ^2 test was used to analyze the differences for clinical factors. All serum lipid indicators were transformed into a categorical variable by MaxStat analysis (titled as Maximally Selected Rank Statistics). The univariate association between PFS and OS was analyzed by Cox proportional hazard model. The Kaplan–Meier method was used to calculate survival curves. The variables with significance in univariable analysis were kept in the multivariate analysis. All the statistical tests were two-sided, with the statistical significance set at $P < 0.05$.

All data were calculated by IBM SPSS 21.0 (IBM Inc., Armonk, USA), R software (version 4.0.3; http://www.Rproject.org) and Stata version 15.0.

Results

Baseline characteristics of DLBCL patients

With a median follow-up of 58 months, 135 (43.9%) patients experienced progression across the course of the disease, and 101 (32.8%) of them ultimately succumbed to the disease. All clinical and demographic data and the association of serum lipid levels are presented in Table 1. Patients had a median age of 63 (ranging from 14 to 91); 168 (54.7%) patients were male; 176 (57.3%) cases were advanced Ann Arbor stage (III/IV) and 137 (44.6%) showed high LDH level (>250). Intermediate high or high risk of IPI at diagnosis accounted for 40.2% (120) of all the patients. All detected lipid indexes were significantly associated with IPI score, but only HDL presented significant association in all groups with poor prognosis features. Further analysis also verified association of HDL levels with aa-IPI ($P = 0.007$) and NCCN-IPI ($P < 0.001$). Data of COO evaluated by Hans method were available for 284 patients; 109 (38.3%) of which were GCB subtype with decreased TC and LDL levels. Expression of different markers including MYC, BCL2, and BCL6 had little to do with serum lipid index as continuous variables. Of the 298 patients who received treatment during the course of disease, 187 (62.7%) were RTX-contained regimen as first-line treatment, mainly RCHOP or RCHOP-like. No differences of serum lipid levels were observed between gender and therapeutic regimen (with or without RTX).

Aberrant serum lipid of DLBCL patients compared to healthy controls

Baseline characteristics were well balanced between the DLBCL patients and healthy controls. For all the healthy controls, only TC, TG, HDL, and LDL were detected. All

Table 1 Baseline characteristics and the association with serum lipid indicators

	Variables (*n*)	TC (mmol/L)	*P* value	TG (mmol/L)	*P* value	HDL (mmol/L)	*P* value	LDL (mmol/L)	*P* value
Gender	Male (168)	4.367 ± 1.065	0.425	1.677 ± 0.987	0.959	1.050 ± 0.373	0.887	2.233 ± 0.674	0.146
	Female (139)	4.318 ± 0.928		1.672 ± 1.113		1.098 ± 0.421		2.207 ± 0.639	
Age	≤60 (131)	4.397 ± 0.895	0.377	1.584 ± 0.954	0.201	1.130 ± 0.424	0.021	2.247 ± 0.569	0.509
	˃60 (176)	4.294 ± 1.087		1.738 ± 1.103		1.024 ± 0.370		2.197 ± 0.719	
Stage	I/II (131)	4.271 ± 0.990	0.025	1.731 ± 1.023	0.197	1.053 ± 0.434	<0.001	2.193 ± 0.630	0.103
	III/IV (176)	4.388 ± 1.023		1.628 ± 1.058		1.082 ± 0.366		2.237 ± 0.680	
ECOG	<2 (210)	4.411 ± 1.066	0.053	1.690 ± 1.011	0.086	1.050 ± 0.393	<0.001	2.278 ± 0.690	0.019
	≥2 (97)	4.180 ± 0.860		1.633 ± 1.115		1.113 ± 0.403		2.090 ± 0.566	
LDH	≤250 (170)	4.321 ± 0.987	0.742	1.700 ± 1.068	0.610	1.077 ± 0.429	0.720	2.198 ± 0.599	0.555
	˃250 (137)	4.359 ± 1.039		1.638 ± 1.014		1.060 ± 0.355		2.243 ± 0.727	
Extranodal sites	0–1 (192)	4.358 ± 1.055	0.210	1.690 ± 1.108	0.256	1.049 ± 0.315	<0.001	2.212 ± 0.689	0.588
	≥2 (113)	4.319 ± 0.924		1.650 ± 0.934		1.106 ± 0.503		2.238 ± 0.605	
IPI	0–2(178)	4.190 ± 0.982	0.002	1.681 ± 1.074	0.048	1.038 ± 0.349	<0.001	2.156 ± 0.657	0.004
	≥3 (120)	4.584 ± 1.023		1.662 ± 1.025		1.105 ± 0.454		2.341 ± 0.652	
Treatment	With RTX (187)	4.425 ± 0.853	0.879	1.780 ± 1.153	0.999	1.090 ± 0.466	0.658	2.262 ± 0.593	0.858
	Without RTX (111)	4.309 ± 1.095		1.584 ± 0.923		1.062 ± 0.350		2.214 ± 0.697	
COO	GCB (109)	4.226 ± 0.962	0.007	1.723 ± 1.144	0.143	1.050 ± 0.357	0.319	2.121 ± 0.705	0.026
	Non-GCB (175)	4.361 ± 1.035		1.589 ± 0.829		1.076 ± 0.430		2.250 ± 0.596	

the baseline characteristics of DLBCL patients and controls are shown in Table 2. The median age was 63 years and 62 years of DLBCL patients and healthy controls, respectively. The Kolmogorov-Smirnov test showed that only LDL obeys normal distribution, while TC, TG, and HDL were all skewed distribution. Serum TC level was significantly lower in DLBCL patients, which remains in males but disappear in female groups. The mean TC levels were 4.338 ± 0.058 in DLBCL patients and were 4.481 ± 0.027 in the healthy controls, showing a significant decrease in the patient cohort ($P = 0.001$), and similar differences were also observed in the comparison of HDL and LDL. On the contrary, TG levels were significantly higher than the healthy controls. Meanwhile, for all the tested four indicators, the differences between DLBCL patients and healthy controls remained in the younger (<60) and elder group (≥60).

The optimal cut-off value for serum lipid levels based on MaxStat

According to the maximal chi-square method, 3.69 mmol/L, 2.48 mmol/L, 0.95 mmol/L, 1.62 mmol/L, 1.03 g/L, and 1.18 g/L were the optimal cut-off value, respectively, for the serum TC, TG, HDL, LDL, apoA, and apoB levels that distinguished between different prognostic groups most effectively (Table 3, Fig. 1, only shows HDL). All serum lipid indexes are divided into two groups as for those greater than the cut-off values refined as high group. The analysis besides were all based on the new definition.

Univariable and multivariable analysis of DLBCL patients

A univariable analysis revealed that all serum lipid indicators were associated with OS, but TC and apoB showed no significant relationship with PFS. Consistently to previous results, low TG was a favorable factor for PFS and OS, while low HDL, LDL, and apoA show adverse impact on PFS and OS. Additionally, age, stage, B symptom, ECOG status, involvement of more than one extranodal site, serum LDH group (>250 U/L), and application of RTX also appeared to be strong predictors for PFS and OS for all patients. Multivariable analysis only confirmed the positive effect of TG ($P = 0.013$) on PFS and adverse impact of low HDL ($P = 0.003$) on OS. Age, stage, and utility of RTX were all independent prognostic factors for PFS and OS across the whole cohort, while ECOG status only affected the PFS not OS, B symptom and LDH group only for OS but not PFS (Table 4).

Impact of RTX on the prognostic values of HDL

Considering the treatment strategies, we divided the whole cohort according to the first-line therapy with or without RTX. For the cases treated without RTX, multivariable analysis showed only that low apoA had an adverse effect on both PFS and OS (Table 5). Meanwhile, age and stage were the other only two independent factors for OS among this group of patients (Table 5). For the other patients that received RTX at first-line treatment, HDL showed remarkably predictive value; PFS and OS were all superior for the HDL-high group. TG was an independent factor for PFS but not OS (Table 6). KM curve confirmed the superior OS and PFS for high-HDL patients treated without RTX (Fig. 2A, B). And for patients treated with RTX, high-TG group showed inferior OS (Fig. 2E) and PFS (Fig. 2F), while HDL also affected both OS (Fig. 2C) and PFS (Fig. 2D) in this group of patients.

Table 2 Baseline characteristics of DLBCL patients and healthy controls

	DLBCL patients ($n = 307$)	Healthy controls ($n = 398$)	P value
Age, years	60.82 ± 13.94	61.36 ± 13.80	0.812
Sex (male)	168 (54.7)	218 (54.8)	0.973
Lipid profiles			
TC	4.338 ± 0.058	4.481 ± 0.027	0.001
TG	1.672 ± 0.060	1.173 ± 0.016	<0.001
HDL	1.069 ± 0.023	1.266 ± 0.014	<0.001
LDL	2.218 ± 0.038	2.493 ± 0.027	<0.001

Table 3 Optimal cut-off value for the serum lipid levels

MaxStat	TC	TG	HDL	LDL	apoA	apoB
Chi-square value	2.142	2.691	4.967	2.739	4.378	1.396
Optimal cut-off	3.69	2.48	0.95	1.62	1.03	1.18
P value	0.027	0.005	<0.001	0.004	<0.001	0.140

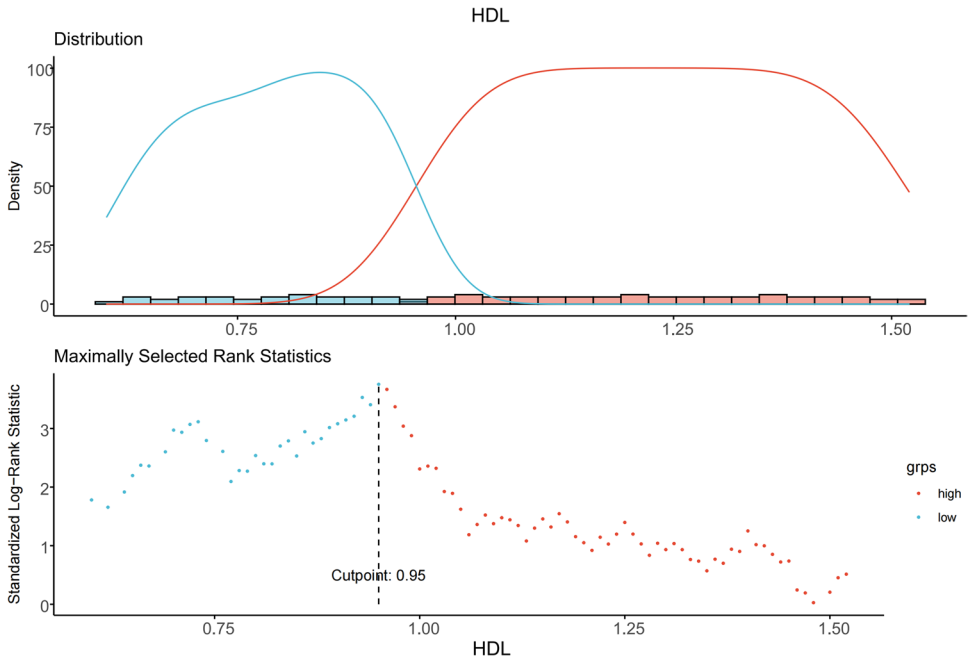

Fig. 1 Cut-off point of HDL defined by using maximally selected log-rank statistics. The estimated optimal cut-off point of HDL was 0.95 mmol/L

Table 4 Multivariable analysis of PFS and OS for DLBCL patients

Variables	PFS			Variables	OS		
	HR	95% CI	*P* value		HR	95% CI	*P* value
TG	1.882	1.143–3.096	0.013	HDL	0.521	0.339–0.799	0.003
Age	1.505	1.026–2.208	0.036	Age	3.162	2.030–4.925	<0.001
RTX	0.491	0.338–0.713	<0.001	RTX	0.550	0.364–0.833	0.005
Stage	1.770	1.164–2.692	0.008	Stage	2.091	1.251–3.498	0.005
ECOG	1.895	1.270–2.828	0.017	B symptom	1.654	1.016–2.693	0.043
LDH ˃ 250	1.449	0.966–2.172	0.073	LDH ˃ 250	1.738	1.111–2.717	0.015

Table 5 Multiple analysis of PFS and OS for patients treated without RTX

Variables	PFS			Variables	OS		
	HR	95% CI	*P* value		HR	95% CI	*P* value
ApoA	0.436	0.249–0.764	0.004	ApoA	0.359	0.195–0.659	0.001
Age	1.753	0.994–3.092	0.053	Age	3.744	1.963–7.143	<0.001
ECOG	1.548	0.859–2.782	0.146	Stage	2.194	1.102–4.369	0.025
LDH > 250	1.616	0.936–2.791	0.085	LDH > 250	1.752	0.959–3.198	0.068

Table 6 Multiple analysis of PFS and OS for patients treated with RTX

Variables	PFS			Variables	OS		
	HR	95% CI	*P* value		HR	95 %CI	*P* value
HDL	0.453	0.246–0.833	0.011	HDL	0.450	0.231–0.875	0.019
TG	2.245	1.168–4.315	0.015	Age	3.841	1.938–7.612	<0.001
Stage	1.777	0.936–3.375	0.079	Stage	2.758	1.224–6.219	0.014
B symptom	1.726	0.917–3.249	0.091	B symptom	2.695	1.281–5.669	0.009
LDH > 250	1.537	0.880–2.683	0.131	LDH > 250	1.746	0.898–3.398	0.101

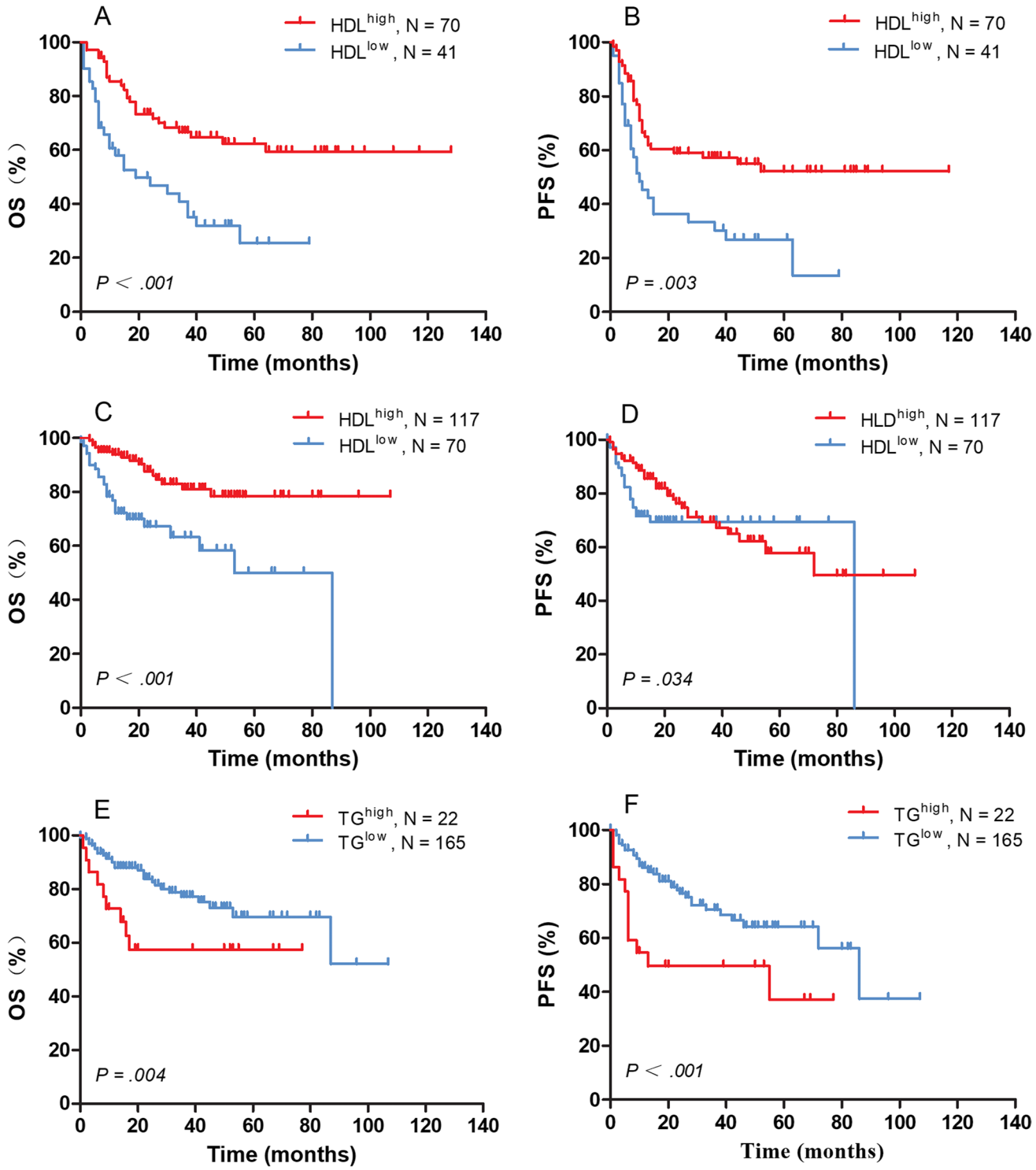

Fig. 2 Outcomes according to serum lipid levels between different treatment groups. High HDL level was associated with superior OS and PFS for patients treated without RTX (**A**, **B**). For patients treated with RTX, high HDL showed association with OS (**C**) and PFS (**D**). Low TG levels were associated with favorable OS (**E**) and PFS (**F**)

Discussion

The primary aim of this study was to establish the role that plasma lipids, particularly cholesterol, contribute to the occurrence and progression of DLBCL. To our knowledge, this is the first study investigating the serum lipid profiles during the occurrence and development of DLBCL, as well as its role in the treatment application of the disease. Although

the elaborate regulated mechanism of lipid metabolism in DLBCL is far from clear, these results might provide clues for further investigation.

Besides the function as the nutritional and essential requirement and membrane synthesis of malignant cells for growth and progression, lipids also play a key role in intracellular signal transduction [35]. Mainly synthesized in the liver, they are transported to cells across the body through bloodstream. Based on these, the potential relevance between abnormal serum cholesterol levels and risk of certain types of cancer has long been recognized. Strasak and colleagues confirmed the short-term associations of serum TC and overall cancer incidence in a prospective study which enrolled 172,210 Austrian adults with a follow-up for a median of 13.0 years [36]. Another research found that an increase of 10 mg/dL in cholesterol was associated with a 9% prostate cancer recurrence increase [37]. Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, powerful cholesterol-lowering medications) were claimed to contribute to reduction of cancer-specific mortality including colorectal cancer and breast cancer [38, 39].

Lipid metabolic processes account for a significant proportion in the hematological malignancy energy metabolism, including leukemia and lymphoma [40]. Cholesterol, particularly HDL, was found to be decreased in different kinds of hematological cancers including acute leukemia, lymphoma, multiple myeloma, and myeloproliferative syndrome [41]. High cholesterol levels were inversely associated with incidence of myeloid neoplasms in women and low-grade B cell lymphoma in men [42]. Here, we assessed serum lipid levels measured by routing exams in DLBCL patients, demonstrating the extensive decrease of serum cholesterol, particularly HDL, of the DLBCL patients compared to normal controls. These findings were in line with the previous epidemiological studies suggesting a decrease of serum cholesterol level before the incidence of lymphoma [34, 43, 44]. HDL was the most significantly decreased one, consistent with previous study which ensured its role with risk of hematologic malignancy [34, 43]. In a median follow-up of 8.3 years of 9,596,145 subjects in health screening from 2009, 15,864 cases of hematologic cancers occurred; among which individuals in the lowest quartile of HDL had the highest risk of overall hematologic cancers compared to those in the highest HDL quartile [43]. In this cohort, lower HDL level also associated with lower TC and higher TG levels, similar with our results that DLBCL patients have higher TC and lower TG levels compared with healthy controls. Another research confirmed the association of HDL with the risk of NHL among 27,072 healthy male smokers aged from 50 to 69 years, showing that each 5 mg/dL increase in HDL was associated with a 15% reduction in risk of NHL during the first 10 years of the follow-up [44].

Besides all the previous research above, our study further confirmed the metabolic dysfunction of cholesterol in DLBCL occurrence. In our research, there were significant associations of patient IPI scores and serum lipid indicators, further affirming the potential role of lipid dysfunction during the disease process of DLBCL. Serum TG have negative correlations with IPI scores, while all cholesterol compositions, including TC, HDL, and LDL, were reversely related. Triglycerides and cholesterol are two forms of lipid, necessary for cell viability. Malignant cells experienced altered lipid metabolism to support its energy and membrane production for its demand of rapid proliferating [17]. DLBCL cells treated with imidazole ketone erastin (IKE), an inducer of ferroptosis, showed a significant decrease of TG, and when co-treated with Fer-1, an inhibitor of ferroptosis, the inverse increase of TG in DLBCL cells further confirmed the role of TG as a buffer against oxidative damage [45]. Different evidences all implicated the protective role of TG for cell survival both in malignant and nonmalignant cells, but whether it has any correlation and influence with serum TG has not been further investigated. High TG level was an independent unfavorable prognostic factor for PFS of the entire cohort, but not for OS, which might be interrupted by other disease associated with high TG. The effect of TG on PFS remained in patients treated with RTX. Serum TG levels showed no independent prognostic value for patients treated without RTX. Precise underline mechanism still needs more investigation. In rheumatoid arthritis, depletion of B cell with RTX induced modest but significant increase of TG [46], indicating the interaction of RTX with TG. Considering the association of serum TG with individual diet as well as other metabolic disease, more validation is needed for this result.

Serum cholesterol significantly decreased in DLBCL patients, and further decline was observed in patients with poor prognosis. Different from the pattern of noncancerous human cells using circulating lipids for synthesis of structural compounds, malignant cells presented enhanced de novo fatty acid synthesis [47]. This might partially explain the upregulated cholesterol levels of DLBCL patients and their adverse effects on disease prognosis. Consistent with previous results, HDL presented the most significant correlation with IPI scores, accompanied with its significant correlation with all five factors of IPI score system. BCR signaling has been proved to directly promote cholesterol biosynthesis as a feed forward mechanism of maintaining the integrity of BCRs in lipid rafts in DLBCL [48].

Although all the serum lipid indicators were not independent prognostic factor for the entire DLBCL cohort, subgroup analysis presents possible influence of RTX for the results, which hints the participation of RTX in the

malignant lipid metabolism of DLBCL. To our knowledge, this is the first study to discover the potential effect of RTX in the lipid metabolic pathway of DLBCL. Until now, the definite antitumor mechanism of RTX has been poorly understood. Three different mechanisms have been proposed for the elimination of B cells by rituximab including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and stimulation of the apoptotic pathway. Cholesterol-lowering agents effectively reduce total cellular cholesterol levels and significantly promote CD20 surface expression in CLL cell line MEC-2, thereby enhancing cell chemo-immunosensitivity [49]. The association of hyper-crosslinked CD20 with detergent-insoluble rafts was prevented by cholesterol depletion, which attenuated calcium mobilization and apoptosis induced by RTX [50]. RTX-mediated CDC required plasma membrane cholesterol, thereby statins were found to significantly decrease rituximab-mediated CDC and ADCC of B lymphoma cells [51]. In xenograft models, specific lipid and metabolic profiles of higher presence of phosphatidylinositol and sphingomyelin fragments have been found for R-CHOP-resistant DLBCL. All uncovered the interaction of RTX and lipid metabolism. These may partly explain our findings of the different prognostic values for different groups divided by the utility of RTX in first-line therapy. Low HDL group was independently associated with shortened PFS and OS for patients who received RTX, while ApoA, as the main protein component of HDL, served as the same role among patients without RTX. Our results prompted that in DLBCL patients, utility of RTX also influences the serum lipid levels and lipid metabolism should have participated in the mechanism of RTX. These results reflected the complex interaction between tumor lipid alteration and effect of CD20 antibody. Other ingredients of cholesterol other than apolipoprotein may ultimately contribute to the sensitivity of RTX.

Several limitations should be acknowledged in our research. As a retrospective study based on one clinical center data, potential bias was unavoidable and the results therefore need to be confirmed in a larger prospective clinical trial. The relatively small size of population and short follow-up time of our research are all disadvantages. The long span of collected patient data and economic capability in RTX selection may lead to the different population background and result in some bias of the subgroup analysis. All of the results only provide some superficial details of lipid metabolism of the disease, and more experiments are needed to explore the underlying mechanism.

In conclusion, DLBCL patients presented aberrant serum lipid levels, associated with the prognosis of the disease, especially for HDL. Meanwhile, treatment of RTX also influences the prognostic value of HDL among the patients. Our research reflected the abnormal serum lipids throughout the course of DLBCL, which provided a clue for further research for the mechanism of changes in lipid metabolism of DLBCL.

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Declarations

Ethics approval This study was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University

Competing interests The authors declare no competing interests.

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