Clin. Lab. 2018;64:XXX-XXX

ORIGINAL ARTICLE

Low Serum Levels of Pre-Surgical Total Cholesterol are Associated with Unfavorable Overall Survival in Patients with Operable Non-Small Cell Lung Cancer

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SUMMARY

Background: **Cholesterol is an essential building block of the cell membrane and an important molecule for cell signaling and function. The dysregulation of cholesterol metabolism has been linked to several diseases, including cancer. The aim of this study is to investigate whether serum cholesterol is associated with the survival outcomes of patients with non-small cell lung cancer (NSCLC).**

Methods: **The concentration of total cholesterol (TC) was measured in pre-operative serum samples of 637 NSCLC patients. The associations of TC with recurrence and overall survival were analyzed using a Cox proportional hazard regression model. Kaplan-Meier survival curves were calculated for overall survival analysis.**

Results: **Our analyses showed that low serum levels of TC were associated with an increased risk of death. The association between TC and overall survival remained significant after patient age at diagnosis, gender, disease stage, histotype, tumor grade, body mass index (BMI), and smoking status were adjusted in the analysis. The patients with low serum TC had a 61% (95% confidence interval: 1.18 - 2.19) higher risk of death compared to those with normal TC. A Kaplan-Meier survival analysis showed similar results. No association was found between TC and recurrence in NSCLC.**

Conclusions: **Our study suggests that the pre-surgical serum level of TC may be an independent prognostic indicator for NSCLC overall survival.**

(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.170823)

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Manuscript accepted October 21, 2017

KEY WORDS

non-small cell lung cancer, total cholesterol, prognosis

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most malignant neoplasm in the world. Less than 15% of patients diagnosed with the disease survive more than 5 years [1]. The disease mortality is even higher in elderly patients [2]. Surgery remains the treatment of choice for patients with early stage NSCLC. However, the prognosis after surgery varies widely from patient to patient due to substantial cellular and molecular differences in the disease [3]. Thus, finding valuable biomarkers is important in the clinical management of NSCLC patients [4].

Lipids, important secondary messengers and crucial molecules for hormone production and key cell membrane components, play pivotal roles in cell composition and signal transduction. Cholesterol is a neutral lipid molecule, a precursor for bile acids and steroid hormones, and essential material for cell structure [5]. Under healthy homeostatic conditions, circulating levels of cholesterol are well regulated by a complex mechanism that controls cellular synthesis, influx and efflux [6]. The disruption of this homeostatic state is often accompanied by many pathogenic processes, including tumorigenesis [7]. There are a number of studies that have examined the associations of circulating cholesterol with cancer mortalities or risk [8-10]. Hepatocellular carcinoma, pancreatic cancer, esophageal carcinoma, prostate cancer, and renal cell carcinoma were all found to be linked to hypocholesterolemia [11-16] while high serum cholesterol was associated with colon cancer [17], prostate cancer [18], and breast cancer [19]. Therefore, total cholesterol concentrations may be closely related to cancer mortality.

Few studies, however, have evaluated the circulating levels of cholesterol in association with cancer survival. There have only been three previous studies that reported that low pre-operative serum cholesterol was associated with poor prognosis of patients with clear cell renal carcinoma [13,20] or Hodgkin's lymphoma [21]. One study also indicated that NSCLC patients with low pre-operative serum cholesterol had a poor prognosis, but the difference did not reach statistical significance [22]. Another study on NSCLC observed a similar insignificant association [23]. Thus far limited evidence suggests that serum cholesterol levels may be useful for prediction of cancer prognosis.

In order to further evaluate the role of cholesterol in NSCLC survival, we recruited 637 NSCLC patients and analyzed their survival outcomes in association with cholesterol levels in pre-operative blood.

MATERIALS AND METHODS

NSCLC patients

A retrospective hospital cohort was assembled from 697 NSCLC patients who were treated at Zhejiang Cancer Hospital in China between October 2006 and December 2009. All of the patients in the study underwent surgery, either lobectomy or pneumonectomy, depending on the location and size of the tumor, together with regional lymph node dissection. Other inclusion criteria included no history of previous anti-cancer therapy, no history of other malignancies, and no concurrent conditions that may influence the serum levels of lipids, such as major chronic hepatitis, chronic heart failure, hyperlipidemia, diabetes that are likely to alter lipid metabolism [24], or lipid-lowering medications. In total, 637 people were eligible for the study. The diagnosis of NSCLC was based on the combination of bronchoscopy, biopsy, peribronchial needle biopsy, pulmonary function tests and computed tomography. Tumor differentiation and histological type were determined following the World Health Organization (WHO) criteria. Disease stage was classified according to the criteria recommended by the International Association for the Study of Lung Cancer (IASLC). Body mass index (BMI) was classified into low (< 18.5 kg/m^2), normal (18.5 - 23.0 kg/m^2), and high (> 23.0 kg/m^2) groups. This study was approved by the institutional review board of Zhejiang Cancer Hospital.

The patients were followed every three months for the first two years after surgery, every six months during the third year and yearly thereafter if there were no signs or symptoms of disease recurrence or metastasis. The criterium of recurrence is that new lesions or distant metastases appears. The follow-up time for these patients ranged between 1 and 71 months, and the median was 48 months. Patients were followed up until February, 2013. Disease-free survival was defined as the time interval between the date of surgery and the date of local recurrence, distant metastasis, death, or the last follow-up (censored). Overall survival was the time interval from surgery to death or being censored.

Measurement of serum total cholesterol

Each patient provided a 3 mL pre-operative blood sample. The blood was centrifuged for 5 minutes at 3,500 rpm within 30 minutes of collection. The serum levels of lipid markers were analyzed with commercial kits (Roche Diagnostics, Mannheim, Germany) on the Cobas Mira automated analyzer (Roche Diagnostics). The concentration of total cholesterol was reported, and the levels of the concentrations were classified into low (< 3.60 mmol/L), normal (3.60 - 6.50 mmol/L), and high (> 6.50 mmol/L) groups based on the assay protocols.

Statistical analysis

The level of cholesterol was analyzed as categorical variables (normal, low, and high categories). A chi-

Table 1. Association of total cholesterol level with clinicopathological factors in patients with NSCLC.

Factors	Patients n (%)	TC			p
		Normal	Low	High	
Gender					
Male	486 (76.3)	357 (73.5)	123 (25.3)	6 (1.2)	**0.003**
Female	151 (23.7)	128 (84.8)	19 (12.6)	4 (2.6)	
Age					
< 65	458 (71.9)	356 (77.7)	94 (20.5)	8 (1.7)	0.207
≥ 65	179 (28.1)	129 (72.1)	48 (26.8)	2 (1.1)	
Disease stage					
II – III	433 (68.0)	319 (73.7)	105 (24.2)	9 (2.1)	0.064
III	203 (31.9)	165 (81.3)	37 (18.2)	1 (0.5)	
Histology [2]					
Squamous cell carcinoma	319 (50.1)	231 (72.4)	82 (25.7)	6 (1.9)	0.272
Adenocarcinoma	305 (47.9)	243 (79.7)	58 (19.0)	4 (1.3)	
Others	13 (2.0)	11 (84.6)	2 (15.4)	0 (0)	
Differentiation [2]					
Well-Moderate	306 (48.0)	217 (70.9)	84 (27.5)	5 (1.6)	**0.042**
Poor	273 (42.9)	218 (79.9)	51 (18.7)	4 (1.5)	
Smoking [3]					
Never	187 (29.4)	155 (82.9)	29 (15.5)	3 (1.6)	**0.027**
Ever/Current	426 (66.9)	312 (73.2)	108 (25.4)	6 (1.4)	
BMI [4]					
Normal	343 (53.8)	256 (74.6)	83 (24.2)	4 (1.2)	0.113
Low	48 (7.5)	31 (64.6)	16 (33.3)	1 (2.1)	
High	202 (31.7)	163 (80.7)	35 (17.3)	4 (2.0)	

[1] Number of unknown = 1 (0.2%).
[2] Number of unknown = 58 (9.1%).
[3] Number of unknown = 24 (3.8%).
[4] Number of unknown = 44 (6.9%).

Table 2. Associations of total cholesterol concentration and patient survival [a].

		Crude HR	95% CI [b]	p-value	Adjusted HR	95% CI	p-value
Disease-Free Survival [c]							
TC	normal	1			1		
	low	1.22	0.89 - 1.67	0.208	1.30	0.91 - 1.84	0.152
	high	1.01	0.32 - 3.17	0.983	1.48	0.36 - 6.15	0.592
Overall Survival [d]							
TC	normal	1			1		
	low	1.48	1.12 - 1.95	**0.005**	1.61	1.18 - 2.19	**0.002**
	high	0.79	0.25 - 2.46	0.680	0.93	0.23 - 3.78	0.921

[a] Associations determined by Cox proportional hazards regression and adjusted for sex, age, disease stage, histology, differentiation, smoking status and BMI. Significance level of p = 0.05.
[b] 95% Confidence Interval.
[c] Hazard ratio (HR) for relapse with respect to normal lipid concentration.
[d] Hazard ratio (HR) for death with respect to normal lipid concentration.

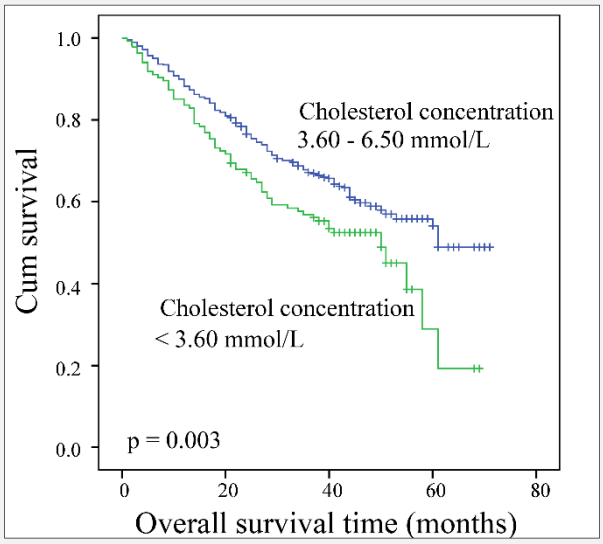

Figure 1. A low serum cholesterol level predicts inferior outcomes in NSCLC patients.

Overall survival data were analyzed and plotted using the Kaplan-Meier method. The patients were classified into low (< 3.60 mmol/L) and normal (3.60 - 6.50 mmol/L) groups.

square test was performed to evaluate the associations of cholesterol with clinical and pathological features of lung cancer. Kaplan-Meier survival curves and the Cox proportional hazard regression model were used to determine the associations of cholesterol with survival outcomes, including disease-free and overall survival. Comparison of survival curves was carried out using the log-rank test. The survival analysis was performed at both univariate and multivariate levels. A multivariate Cox proportional hazards regression model was applied to estimate the adjusted hazard ratio (HR) and 95% confidence interval (CI) and to identify independent prognostic factors. The Cox proportional hazard regression model was adjusted for gender, age, disease stage, histology, differentiation, smoking status, and BMI. The intrinsic stability of the Cox model was tested by the bootstrap analysis. Statistical software SPSS version 18.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Two-sided p-values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the NSCLC patients

The patients enrolled in the study included 486 males (76.3%) and 151 females (23.7%). The median age of the patients at surgery was 60 years, ranging from 30 to 82 years. Patient clinical and pathological characteristics are listed in Table 1. According to the IASLC staging system and classification criteria, 433 patients (68.0%) had stage I or II disease and 203 (31.9%) had stage III. Of the patients, 319 (50.1%) had squamous cell carcinoma, 305 (47.9%) had adenocarcinoma, and 13 (2.0%) had other histological types. Three hundred and six patients (48.0%) had well differentiated tumors, whereas 273 (42.9%) had poorly differentiated ones. A total of 426 patients (66.9%) had a history of smoking. According to the WHO criteria, 343 patients (53.8%) had a normal BMI, 48 (7.5%) had a high BMI and 202 (31.7%) had a low BMI.

Serum total cholesterol concentration in NSCLC patients

Table 1 also shows the relationships of cholesterol with patient clinical and pathological characteristics. Total cholesterol (TC) concentrations in pre-operative sera were significantly different by gender (low in men, $p = 0.003$), tumor differentiation (low in well or moderately differentiated tumors, $p = 0.042$), and smoking status (low in current smokers, $p = 0.027$).

Association of serum total cholesterol concentration with patient survival

A total of 485 (76.1%) patients had normal levels of total cholesterol (3.60 - 6.50 mmol/L) and 142 (22.3%) patients had low TC (< 3.60 mmol/L). Only 10 (1.6%) patients had high TC (> 6.50 mmol/L). The 3- and 5-

year survival rates were 67.1% and 54.1% for patients with normal levels of TC, but 56.1% and 19.3% for those with low TC, respectively. Kaplan-Meier survival curves indicated that patients with low total cholesterol had worse overall survival than patients with normal TC (Figure 1).

A Cox regression analysis showed that serum TC was an independent prognostic indicator in operable NSCLC (Table 2). Patients with hypocholesterolemia had an increased risk of death compared to those with normal TC levels. The hazards ratio for death was 1.61 (95% CI: 1.18 - 2.19) between patients with low and normal total cholesterol. The trend between low TC levels and high risk was statistically significant for overall survival ($p < 0.005$). No associations, however, were found between TC and disease-free survival. The variables that were adjusted in the regression model included age, gender, disease stage, histotype, tumor differentiation, BMI, and smoking status. As a sensitivity test, we performed bootstrap analysis and found the results of survival analysis did not change substantially.

DISCUSSION

In this retrospective study, we found that low serum total cholesterol was associated with unfavorable overall survival of patients with operable NSCLC. Furthermore, our finding of the association was independent of several key clinical and pathological features of NSCLC, suggesting that total cholesterol may serve as an independent prognostic indicator for patients with operable NSCLC. Our observation was consistent with the results of a previous study that showed that pre-operative hypocholesterolemia was associated with poorer survival outcomes in NSCLC patients [22]. Our results were also supported by another study although that study used a dichotomous variable (high versus low) to analyze the association of total cholesterol and survival in NSCLC [23]. Additionally, the relationship between a low concentration of pre-operative serum TC and poor prognosis was found in patients with clear cell renal carcinoma [13,20] or Hodgkin's lymphoma [21].

Cholesterol is an essential lipid with many important functions, including maintaining cell membrane integrity, transmitting cell signaling, and regulating cellular activities. All of these require cholesterol to be well regulated in our system [5]. Either excess or shortage of this essential lipid may cause or reflect some pathogenic processes [6], for example, cholesterol lowering therapies especially lowering the LDL-cholesterol level were shown to have a survival benefit in coronary heart patients [25,26], and tumor development may also be affected by the concentration of circulating cholesterol [27]. Although there is no direct evidence to explain how decreased cholesterol can promote tumor progression, some observational studies do suggest that cholesterol may be involved in tumor progression. Cancer cells have more lipid rafts, which are dynamic assemblies with in cell membranes that contain highly enriched cholesterol and sphingolipids along with signaling receptors [28] such as Fas receptor (FasR) and TNF-related apoptosis-inducing ligand (TRAIL) receptors 1 and 2. More receptors are translocated into lipid rafts to deregulate apoptosis when cholesterol is depleted. Depletion of cholesterol may also inhibit the loss of mitochondrial transmembrane potential, caspase activation and apoptosis, such as TRAIL-induced cell death and caspase-8 cleavage [29,30].

Cholesterol is also a precursor of bile acids and steroid hormones, reinforcing the role of cholesterol in cell signaling [6]. Cholesterol is synthesized via a cascade of enzymatic reactions known as the mevalonate pathway. Hydroxyl-methyl glutaryl-coenzyme A (HMG-CoA), an important regulatory step in cholesterol synthesis, catalyzes mevalonate formation [31]. The activity of HMG-CoA reductase may be reduced due to decreased transcriptional regulation, which is mediated by sterol-regulatory element binding proteins (SREBPs). Cancer cell proliferation is highly dependent on sterol biosynthesis [32]. SREBPs, master regulators of lipid homeostasis, are required for long-term cell proliferation [33]. The dysregulation of this pathway is observed in cancer cells, which may promote tumor progression and drug resistance.

Furthermore, hypocholesterolemia may impair the function of the immune system. The oxidation of cholesterol produces oxysterols that act as critical signaling molecules in the regulation of the immune function [34]. Cellular levels of cholesterol are largely maintained through the activity of two transcription factors, SREBPs and the liver X receptors (LXRs). Oxysterols, natural ligands for LXRs, are generated when cholesterol levels are high. When excess oxysterols accumulate in the cell, they can directly bind to and activate LXRs, attenuating inflammation in the immune cells through their ability to directly repress the expression of inflammatory genes [35]. The role of oxidized cholesterol in promoting inflammation responses can facilitate tumor progression [36].

This study has a number of strengths. First, our observation was based on a reasonable sample size. Second, the diagnosis and treatment of the patients were relatively consistent because they all came from a single institution. Third, we included several key clinical and pathological variables in our study, and the survival analysis was adjusted for these clinical and pathological factors.

Our study also had a few limitations. First, this is a retrospective study, and our findings need to be confirmed by large independent studies, especially prospective investigations. Second, the study lacks information on post-operative therapies, which may influence survival. Although our results were adjusted for BMI, we cannot rule out the possibility about that weight change before diagnosis may affect the cholesterol levels in patients. This possible influence can be better addressed in patients whose blood samples are collected pre-diagnostically. Third, serum level of cholesterol was measured on-

ly once in a cross-sectional manner, and no specimens were available for assessing changes in serum cholesterol, especially post-operative alterations. Fourth, we did not have data or information on and therefore were unable to control for other confounding factors which could potentially affect both the cholesterol levels and disease outcomes, such as tumor volume and inflammatory cytokines.

The biological mechanism underlying the effects of serum cholesterol on lung cancer survival remains unclear. More studies are needed not only to confirm our observation but also to elucidate why low cholesterol is associated with worse overall survival in NSCLC patients. Furthermore, our finding of low cholesterol being associated with overall survival, but not disease-free survival, indicates that low cholesterol may be associated with not only lung cancer mortality, but also all-cause mortality. Previous studies have reported inverse correlations between cholesterol levels and all-cause mortality [37,38]. To determine if cholesterol is related to lung cancer specific or all cause-mortality, cause-specific mortality needs to be analyzed in future studies.

CONCLUSION

In summary, our study showed that NSCLC patients with low total cholesterol in pre-operative serum had poor overall survival compared to those with normal levels of total cholesterol. This association was independent of several existing prognostic indictors of NSCLC, suggesting that pre-operative serum levels of total cholesterol may be used as a prognostic indicator for NSCLC. Further research is needed to confirm this finding and to elucidate the mechanism of cholesterol in NSCLC progression.

Acknowledgement:
This study was supported by grants from the National Nature Science Foundation of China (No. 81472203 and No. 81602615), the Public Welfare Technology Foundation of Zhejiang Province of China (No. 2017 C34001), the Major Science and Technology Project of Medical and Health of Zhejiang Province of China (No. WKJ-ZJ-1403), the Major Science and Technology Project of Zhejiang Province of China (No. 2014C 03029), the Natural Science Foundation of Zhejiang Province of China (No. LY13H160028), and the Zhejiang high-level innovative talent program and the 1022 program of Zhejiang Cancer Hospital.

Declaration of Interest:
No conflicts.

References:

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11-30 (PMID: 23335087).
2. Subramanian J, Morgensztern D, Goodgame B, et al. Distinctive characteristics of non-small cell lung cancer (NSCLC) in the young: a surveillance, epidemiology, and end results (SEER) analysis. J Thorac Oncol. 2010;5(1):23-8 (PMID: 19934774).
3. Brundage MD, Davies D, Mackillop WJ. Prognostic factors in non-small cell lung cancer: a decade of progress. Chest. 2002; 122(3):1037-57 (PMID: 12226051).
4. Altman DG. Systematic reviews of evaluations of prognostic variables. BMJ. 2001;323(7306):224-8 (PMID: 11473921).
5. Brown AJ. Cholesterol, statins and cancer. Clin Exp Pharmacol Physiol. 2007;34(3):135-41 (PMID: 17250629).
6. Simons K, Ikonen E. How cells handle cholesterol. Science. 2000;290(5497):1721-6 (PMID: 11099405).
7. Silvente-Poirot S, Poirot M. Cholesterol metabolism and cancer: the good, the bad and the ugly. Curr Opin Pharmacol. 2012;12(6): 673-6 (PMID: 23103112).
8. Smith GD, Shipley MJ, Marmot MG, Rose G. Plasma cholesterol concentration and mortality. The Whitehall Study. JAMA. 1992; 267(1):70-6 (PMID: 1727199).
9. Isles CG, Hole DJ, Gillis CR, Hawthorne VM, Lever AF. Plasma cholesterol, coronary heart disease, and cancer in the Renfrew and Paisley survey. BMJ. 1989;298(6678):920-4 (PMID: 24978 58).
10. Chang AK, Barrett-Connor E, Edelstein S. Low plasma cholesterol predicts an increased risk of lung cancer in elderly women. Prev Med. 1995;24(6):557-62 (PMID: 8610078).
11. Neaton JD, Blackburn H, Jacobs D, et al. Serum cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. Multiple Risk Factor Intervention Trial Research Group. Arch Intern Med. 1992;152(7):1490-500 (PMID: 1627030).
12. Iso H, Ikeda A, Inoue M, Sato S, Tsugane S; JPHC Study Group. Serum cholesterol levels in relation to the incidence of cancer: the JPHC study cohorts. Int J Cancer. 2009;125(11):2679-86 (PMID: 19544528).
13. Ohno Y, Nakashima J, Nakagami Y, et al. Clinical implications of preoperative serum total cholesterol in patients with clear cell renal cell carcinoma. Urology. 2014;83(1):154-8 (PMID: 241491 06).
14. Nago N, Ishikawa S, Goto T, Kayaba K. Low cholesterol is associated with mortality from stroke, heart disease, and cancer: the Jichi Medical School Cohort Study. J Epidemiol. 2011;21(1):67-74 (PMID: 21160131).
15. Faintuch J, Cabraitz R, Martín Nieto AR, et al. [The prognostic value of cholesterol levels in malnourished patients with esophageal carcinoma]. Nutr Hosp. 1993;8(6):352-7 (PMID: 8373878).
16. Cvetkovic Z, Cvetkovic B, Petrovic M, et al. Lipid profile as a prognostic factor in cancer patients. J BUON. 2009;14(3):501-6 (PMID: 19810145).
17. Brown SR, Hossain MB, Forrester IT. Associations between cholesterol, colon cancer screening, behavior, and diet. Am J Health Behav. 2013;37(3):360-8 (PMID: 23985183).

18. Solomon KR, Freeman MR. The complex interplay between cholesterol and prostate malignancy. Urol Clin North Am. 2011; 38(3):243-59 (PMID: 21798387).

19. Kitahara CM, Berrington de González A, Freedman ND, et al. Total cholesterol and cancer risk in a large prospective study in Korea. J Clin Oncol. 2011;29(12):1592-8 (PMID: 21422422).

20. de Martino M, Leitner CV, Seemann C, et al. Preoperative serum cholesterol is an independent prognostic factor for patients with renal cell carcinoma (RCC). BJU Int. 2015 Mar;115(3):397-404 (PMID: 24698164).

21. Muller CP, Trilling B, Steinke B. The prognostic significance of total serum cholesterol in patients with Hodgkin's disease. Cancer. 1992;69(4):1042-6 (PMID: 1735071).

22. Tomita, M., T. Ayabe, T. Shimizu, and K. Nakamura. Preoperative Total Serum Cholesterol and Patients' Survival in Resected Nonsmall Cell Lung Cancer. Lung Cancer Int. 2012;2012: 463520.

23. Sok M, Ravnik J, Ravnik M. Preoperative total serum cholesterol as a prognostic factor for survival in patients with resectable non-small-cell lung cancer. Wien Klin Wochenschr. 2009;121(9-10): 314-7 (PMID: 19562293).

24. Rauchhaus M, Clark AL, Doehner W, et al. The relationship between cholesterol and survival in patients with chronic heart failure. J Am Coll Cardiol. 2003;42(11):1933-40 (PMID: 14662255).

25. Arsenault BJ, Rana JS, Stroes ES, et al. Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women. J Am Coll Cardiol. 2009;55(1):35-41 (PMID: 20117361).

26. Vallejo-Vaz AJ, Robertson M, Catapano AL, et al. LDL-Cholesterol Lowering for the Primary Prevention of Cardiovascular Disease Among Men with Primary Elevations of LDL-Cholesterol Levels of 190 mg/dL or Above: Analyses from the WOSCOPS 5-year Randomised Trial and 20-year Observational Follow-Up. Circulation. 2017 Nov 14;136(20):1878-91 (PMID: 28877913).

27. Ohvo-Rekilä H, Ramstedt B, Leppimäki P, Slotte JP. Cholesterol interactions with phospholipids in membranes. Prog Lipid Res. 2002;41(1):66-97 (PMID: 11694269).

28. Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997;387(6633):569-72 (PMID: 9177342).

29. Gajate C, Gonzalez-Camacho F, Mollinedo F. Lipid raft connection between extrinsic and intrinsic apoptotic pathways. Biochem Biophys Res Commun. 2009;380(4):780-4 (PMID: 19338752).

30. Song JH, Tse MC, Bellail A, et al. Lipid rafts and nonrafts mediate tumor necrosis factor related apoptosis-inducing ligand induced apoptotic and nonapoptotic signals in non small cell lung carcinoma cells. Cancer Res. 2007;67(14):6946-55 (PMID: 17638906).

31. Yachnin S, Toub DB, Mannickarottu V. Divergence in cholesterol biosynthetic rates and 3-hydroxy-3-methylglutaryl-CoA reductase activity as a consequence of granulocyte versus monocyte-macrophage differentiation in HL-60 cells. Proc Natl Acad Sci U S A. 1984;81(3):894-7 (PMID: 6583685).

32. Gabitova L, Gorin A, Astsaturov I. Molecular pathways: sterols and receptor signaling in cancer. Clin Cancer Res. 2014;20(1):28-34 (PMID: 24158702).

33. Goldstein JL, Rawson RB, Brown MS. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. Arch Biochem Biophys. 2002;397(2):139-48 (PMID: 11795864).

34. Liu C, Yang XV, Wu J, et al. Oxysterols direct B-cell migration through EBI2. Nature. 2011;475(7357):519-23 (PMID: 21796211).

35. Bensinger SJ, Bradley MN, Joseph SB, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. Cell. 2008;134(1):97-111 (PMID: 18614014).

36. Pelton K, Freeman MR, Solomon KR. Cholesterol and prostate cancer. Curr Opin Pharmacol. 2012;12(6):751-9 (PMID: 22824430).

37. Schatz IJ, Masaki K, Yano K, Chen R, Rodriguez BL, Curb JD. Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study. Lancet. 2001; 358(9279):351-5 (PMID: 11502313).

38. Jacobs D, Blackburn H, Higgins M, et al. Report of the Conference on Low Blood Cholesterol: Mortality Associations. Circulation. 1992;86(3):1046-60 (PMID: 1355411).