A novel method for estimating LDC levels: total cholesterol and non-HDLC can be used to predict the normal LDLC level in apparently healthy population

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Authors' Contribution

1. Conception or design of the work;
2. Data acquisition;
3. Data analysis and interpretation;
4. Drafting the article;
5. Critically revising the article;
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ABSTRACT

Background: Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC) are usually

1 ordered together to test lipid metabolism in physical examination and clinical
2 application. LDLC is a component of TC and its change is closely correlated to TC.

3 **Objective:** We aim to predict the normal LDLC level by using TG, TC, HDLC and
4 non-HDLC (nonHDLC) in this study.

5 **Methods:** TG, TC, HDLC, and LDLC data were obtained from Laboratory Information
6 System (LIS) based on 4 years (Octo, 1, 2013-Sept, 30, 2017) period health check-up.
7 TG, TC, HDLC, and LDLC(direct clearance method) were measured using
8 TBA2000FR biochemical analyzer. The nonHDLC was calculated with TC minus
9 HDLC. Correlation between TG, TC, nonHDLC, and LDLC were analyzed using
10 Spearman's rank approach. Receiver operating characteristics curve analysis was
11 used to evaluate the predictive of TG, TC and nonHDLC for the normal LDLC
12 level(less than 130mg/dL).

13 **Results:** Both TC($r = 0.870$) and nonHDLC ($r = 0.893$) were significantly positively
14 correlated with LDLC. Area under curve of TC and nonHDLC can be used to predict
15 normal LDLC level. Optimal thresholds were 182.5 mg/dL(4.72 mmol/L) for TC and
16 135.3 mg/dL(3.50 mmol/L) for nonHDLC. Based on these optimal thresholds, less
17 than 0.5% and 0.4% of tests with elevated LDLC might be missed, but the missing
18 elevated LDLC is not too high(less than 147.3 mg/dL).

19 **Conclusion:** If the nonHDLC is less than 135.3mg/dL(3.50 mmol/L) and/or TC is less
20 than 182.5mg/dL(4.72 mmol/L) for the apparently healthy populations the LDLC level
21 will be less than 130mg/dL(3.36 mmol/L). Total cholesterol and non-HDLC can be
22 used to predict the normal LDLC level in apparently healthy population is a novel

1 method for estimating LDC level.

2 **Keywords:** Total cholesterol, high-density lipoprotein cholesterol, low-density
3 lipoprotein cholesterol, non-high-density lipoprotein cholesterol, predict.

4 INTRODUCTION

5 Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL),
6 and Low-density lipoprotein cholesterol (LDL) is commonly used in clinical practice
7 [1]. The TC test is a measure of the level of total amount of cholesterol for a person,
8 including LDL and high-density lipoprotein cholesterol (HDL) [2], as well as a
9 certain type of triglycerides. The test of cholesterol is very important because elevated
10 level of cholesterol can clog the arteries even lead to heart diseases and strokes [3].
11 Usually, serum TG, TC, HDL, and LDL are ordered together in the clinical
12 laboratory. Since LDL is a component of the TC and the change of LDL is closely
13 correlated to TC in most cases, we are wondering that LDL may not be necessarily
14 tested until the TC and nonHDL are lower than the normal level. Thus, we proposed
15 that serum normal LDL level could be predicted by using TC and non-HDL
16 (nonHDL) as reflex tests. Reflex testing was defined as automatically adding or
17 removing a test by the biochemical analyzer for saving costs of clinical laboratories.
18 For example, serum total bilirubin (TBIL) and conjugated bilirubin (CBIL) are highly
19 correlated and approximately 87% of CBIL test reduced from TBIL test [4]. Most labs
20 use automated technology for doing lipid profiles with automatic calculation of LDL-C
21 by Friedewald in Europe and America, and all LDL levels were measured using a
22 direct clearance method on auto biochemical analyzer in China. However, the

1 problem with Friedewald equation is that LDLC levels are commonly discordant with
 2 both direct clearance method, and the direct clearance method due to slightly higher
 3 costs. LDLC may not accurately reflect the true risk of LDL particles[3], the elevated
 4 LDLC should be measured by direct method. Hence, we search for a novel method for
 5 estimating the LDLC level. In this study, we analyzed the correlation between TG, TC,
 6 nonHDLc, and LDLC and concluded that the nonHDLc is under 135.3mg/dL(3.50
 7 mmol/L) and/or TC is under 182.5mg/dL(4.72 mmol/L), LDLC is not more than
 8 130mg/dL(3.36 mmol/L) for the apparently healthy population.

9 **MATERIALS AND METHODS**

10 **Study cohort and data extraction**

11 The data of TG, TC, HDLC and LDLC were obtained from laboratory information
 12 system (LIS). There are 34270 subjects (21651 males and 12619 females) (Table 1.)
 13 for health check-ups from October 2013 to September 2017 in Shuyang People's
 14 Hospital were examined. Due to no complaint for visiting Shuyang People's Hospital,
 15 we regard them as apparently healthy individuals. Age, gender, fasting serum TG
 16 (GPO-PAP no correction), TC (Cholesterol Oxidase method), HDLC (Direct Clearance
 17 Method) and LDLC (Direct Clearance Method) were extracted from LIS. TG, TC,
 18 HDLC, and LDLC were measured using TBA2000FR biochemical analyzer (Toshiba
 19 Co., Ltd., Japan). Regular quality control procedures is conducted every day in the
 20 laboratory medicine of Shuyang People's Hospital. The external quality assessment
 21 scheme of Jiangsu Center for Clinical Laboratories were performed twice a year to
 22 validate the quality of TC, HDLC, and LDLC results. The ethics committee of Shuyang

1 People's Hospital approved this study.

2 **Statistical Analysis and Calculation**

3 The nonHDLc was calculated from TC minus HDLC [1] and their relationship was
4 analyzed using Spearman's approach. For normal LDLc level, receiver operating
5 characteristics curve analysis was used to evaluate the predictive accuracy of TG, TC,
6 HDLC, and nonHDLc. Normal LDLc level were less than 130 mg/dL (3.36 mmol/L) [5,
7 6]. All statistical analyses were performed using EXCEL2007 (Microsoft Corporation,
8 Beijing, China) and MedCalc 15.2.2 (MedCalc Software, Ostend, Belgium). A p value
9 less than 0.05 was considered statistically significant.

10 **RESULTS**

11 **The characteristics of the subjects**

12 The data of 34270 pairs of cholesterols and triglycerides tests between October 1,
13 2013, and September 30, 2017 were extracted from the health examination center of
14 Shuyang People's Hospital, including 21651 males and 12619 females (Table 1).

15 **Correlation between TG, TC, HDLC, and nonHDLc and LDLc**

16 Both TC ($r = 0.870$, $P < 0.0001$) and nonHDLc ($r = 0.893$, $P < 0.0001$) were
17 significantly positively correlated with LDLc (**Figure 1**).

18 **The optimal threshold of TC and nonHDLc in predicting normal LDLc level**

19 **Figure 2** shows the ROC curves of TG, TC, HDLC, and nonHDLc for predicting
20 normal LDLc level (less than 130mg/dL). Areas under curve (AUC) were 0.675, 0.950,
21 0.541, and 0.957 for TG, TC, HDLC, and nonHDLc, respectively. As list in **Table 2**,
22 nonHDLc was notably better than TC for predicting of normal LDLc level in terms of

1 diagnostic performance and leakage in different TG levels. At these thresholds of TC
2 and nonHDLc, less than 2.6% and 1.8% of tests with elevated LDLc might be missed,
3 but the missing elevated LDLc is lower (less than 147.3 mg/dL=3.81 mmol/L). As list in
4 **Table 3**, when the nonHDLc is less than 135.3mg/dL(3.50 mmol/L) and/or TC is less
5 than 182.5mg/dL(4.72 mmol/L), the LDLc will be less than 130mg/dL for the all the
6 populations. If the nonHDLc is under 139.2mg/dL(3.60 mmol/L) and/or TC is under
7 182.5mg/dL(4.72 mmol/L), the LDLc will be low 130mg/dL for the populations (The
8 TG is less than 400mg/dL).

9 **DISCUSSION**

10 In this study, we analyzed serum TG, TC, nonHDLc, LDLc and their correlation in a
11 large cohort of apparently healthy subjects, and we found that TC and nonHDLc were
12 positively correlated with LDLc. Subjects have low TC and nonHDLc levels usually
13 accompany with a low LDLc levels and vice versa. The results of ROC for predicting
14 normal LDLc level has supported our hypothesis. The AUC was more than 0.95 for
15 both TC and nonHDLc, indicating that TC and nonHDLc have accuracy for predicting
16 normal LDLc level. Therefore, serum lower LDLc levels (less than 130mg/dL) could
17 be predicted by using TC and non-HDLc. Given the diagnostic performance and the
18 proportion of elevated LDLc, nonHDLc is notably better than TC for predicting normal
19 LDLc level. Two optimal thresholds of TC and nonHDLc for predicting normal LDLc
20 level were 182.5 mg/dL(4.72 mmol/L) and 139.2 mg/dL(3.60 mmol/L) (TG is less than
21 400mg/dL). If TC is less than 182.5 mg/dL and/or nonHDLc is less than 139.2 mg/dL,
22 there will be not more than 130 mg/dL(3.36 mmol/L) for LDLc levels. According to

1 these thresholds, less than 0.5% and 0.4% of elevated LDLC could be missed, and
 2 the missing elevated LDLC is lower (Table 2 and Table 3). In this study, approximately
 3 56% of LDLC test or calculation could be reduced. The price of a LDLC testing is
 4 approximately 4RMB (0.65 USD) [4] in China, thus, the cost for LDLC test would be
 5 greatly saved. If nonHDLc is used as a reflex test for LDLC (when elevated LDLC is
 6 more than 130 mg/dL), approximately 11.3 million LDLC test would be reduced and
 7 7.2 million USD would be saved annually. The direct LDLC test will automatically be
 8 performed when the predicting LDLC level is not normal level. This saves time for
 9 the doctor by not needing to order the direct LDLC test, saves time for the patient by
 10 not needing to draw another blood sample, and speeds up the time to obtain the
 11 accurate LDLC results. Compared with the Friedewald formula [7] and the formula is
 12 considered inaccurate (TG level is more than 220mg/dL)[8], in this study we take into
 13 account the cost savings but also screen out the normal LDLC level, and it was not
 14 affected by triglyceride levels.

15 There are some limitations in this study. First, this is a single-center study. Second,
 16 the subjects in this study come from health check-ups. Third, if the LDLC level is less
 17 than 130 mg/dL, there will be less than 0.5% for missing elevated LDLC.

18 The results of present study indicate that a large number of normal LDLC level can be
 19 predicted by using TC and/or nonHDLc. To our knowledge, this study is the first to
 20 predict LDLC level by using receiver operating characteristics curve analysis. The
 21 approach of this study may be suited for other subjects. However, because of
 22 difference of the detection system and subjects, they should get the optimal threshold

1 based on local data for using TC and/or nonHDLc to predict normal LDLc level.

2 **Disclosures**

3 The authors declare no conflict of interest.

4 **Acknowledgements**

5 None

6 **References**

- 7 [1] Martin Seth S, Blaha Michael J, Elshazly Mohamed B et al. Comparison of a novel method vs the Friedewald equation for
8 estimating low-density lipoprotein cholesterol levels from the standard lipid profile. JAMA, 2013, 310(19): 2061-8..
9 [2] <https://medlineplus.gov/magazine/issues/summer12/articles/summer12pg6-7.html>/Accessed Mar 18. 2017.
- 10 [3] John D. Brunzell, FACP, Michael Davidson, FACC, Curt D. Furberg et al. Lipoprotein Management in Patients With
11 Cardiometabolic Risk, J Am Coll Cardiol, 2008; 51:1512-1524..
- 12 [4] Zhang Guo-Ming, Hu Zhi-De. Conjugated bilirubin as a reflex test for increased total bilirubin in apparently healthy population. J
13 Clin Lab Anal. 2017. DOI: 10.1002/jcla.22233.
- 14 [5] <https://labtestsonline.org/understanding/analytes/lipid/tab/test/>Accessed Mar 18. 2017.
- 15 [6] Chinese Joint Committee of prevention and treatment of dyslipidemia of Revision of guidelines. Guidelines for prevention and
16 treatment of dyslipidemia in Chinese adults (revised 2016). Chinese Journal of Cardiology. 2016 44(10):833-853.
17 DOI:10.3760/cma.j.issn.0253-3758.2016.10.005
- 18 [7] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma,
19 without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun; 18(6):499-502.
- 20 [8] Sniderman AD, Blank D, Zakarian R, Bergeron J, Frohlich J (October 2003). "Triglycerides and small dense LDL: the twin
21 Achilles heels of the Friedewald formula". *Clinical Biochemistry*. **36**(7): 499–504.

1 Table 1.The characteristics of the participants

Parameters	female	male	2
	12619	21651	3
Age	35	43	
	(30-48)	(31-55)	
Total Cholesterol(mmol/L)	172.9 (152.0-197.2)	179.0 (156.6-203.0)	
Triglycerides(mmol/L)	89.4 (64.6-131.9)	123.1 (86.8-179.8)	
LDLC(mmol/L)	98.6 (80.8-119.1)	106.7 (88.6-126.1)	
nonHDLc(mmol/L)	120.7 (100.2-144.6)	133.0 (111.4-156.2	
HDLc(mmol/L)	50.3 (43.3-58.0)	44.5 (38.7-51.8)	

4 All data is represented bymedian and interquartile range.

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1 Table 2. The optimal threshold and accuracy of total cholesterol and non-high density lipoprotein cholesterol, and its performance in predicting normal
2 low-density lipoprotein cholesterol

TG levels	less than 400 mg/dL		less than 300 mg/dL		200-400 mg/dL		less than 200 mg/dL		less than 150 mg/dL		less than 100 mg/dL	
	TC	nonHDL	TC	nonHDL	TC	nonHDL	TC	nonHDL	TC	nonHDL	TC	nonHDL
AUC	0.954	0.963	0.955	0.966	0.940	0.946	0.956	0.969	0.957	0.973	0.965	0.980
	0.951-0.956	0.961-0.965	0.953-0.957	0.964-0.968	0.933-0.947	0.939-0.952	0.953-0.958	0.967-0.971	0.954-0.959	0.971-0.975	0.962-0.968	0.978-0.982
Thresholds(mg/dL)	196.8	148.1	196.8	147.3	205.7	162	194.1	145	192.2	145	192.2	140.8
Sensitivity	87.1	87.7	87.7	87.9	84.9	84.6	86.4	87.8	86.1	90.5	88.6	91.4
	86.7-87.5	87.3-88.1	87.3-88.1	87.5-88.3	83.6-86.1	83.3-85.9	86.0-86.8	87.4-88.3	85.6-86.6	90.1-90.9	88.1-89.2	90.9-91.9
Specificity	90.0	93.2	89.7	93.6	88.2	89.7	91.3	94.8	92.2	93.8	92.0	95.9
	89.3-90.8	92.5-93.8	88.9-90.4	93.0-94.2	86.4-89.8	88.0-91.2	90.5-92.1	94.2-95.4	91.4-93.2	92.9-94.5	90.4-93.4	94.7-96.9
the percentage of predicting normal												
LDLC level	72.2%	71.9%	73.0%	72.3%	62.2%	61.5%	72.7%	73.4%	74.2%	77.9%	81.2%	83.5%
(less than 130 mg/dL)	24173/33486	24090/33486	23666/32427	23458/32427	2875/4624	2843/4624	20993/28862	21178/28862	17946/24181	18840/24181	11922/14674	12253/14674
Elevated LDLC(≥ 130 mg/dL)	2.6%	1.7%	2.6%	1.6%	5.7%	5.0%	2.0%	1.2%	1.5%	1.1%	0.8%	0.4%
95% double-side(mg/dL)	616/24173	407/24090	611/23666	368/23458	165/2875	143/2843	409/20993	245/21178	262/17946	213/18840	100/11922	53/12253
	130.3-147.3	130.3-145.3	130.3-147.2	130.3-145.3	130.3-153.1	130.3-152.9	130.3-146.8	130.3-144.5	130.3-145.7	130.3-145.1	130.3-146.9	130.3-142.8
Z value	12.3		15.6		17.9		18		18.2		12.3	
p-value	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001	

3 *Compare with TC and nonHDL groups,

4 TC: total cholesterol ; nonHDL: non- high density lipoprotein cholesterol; LDLC: low-density lipoprotein cholesterol

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Table 3 The optimal threshold total cholesterol and non- high density lipoprotein cholesterol, and their performance in predicting normal low-density lipoprotein cholesterol

TG levels	total		less than 400 mg/dL		200-400 mg/dL		less than 200 mg/dL	
	TC	nonHDLc	TC	nonHDLc	TC	nonHDLc	TC	nonHDLc
The optimal thresholds	182.5	135.3	182.5	139.2	186.8	147.7	181.4	138.4
The percentage of predicting normal LDLc level (less than 130 mg/dL)	56.0%	61.7%	57.3%	62.7%	40.7%	44.2%	59.4%	66.5%
	19175/34270	21138/34270	19175/33486	20995/33486	1884/4624	2043/4624	17132/28862	19206/28862
Elevated LDLc($\geq$ 130 mg/dL)	0.5%	0.4%	0.5	0.4%	0.6	0.5%	0.4	0.3%
	87/19175	83/21138	87/19175	83/20995	11/1884	11/2043	71/17132	65/19206
95% double-side(mg/dL)	130.3-147.3	130.3-145.3	130.3-147.3	130.3-145.3	130.3-139.6	130.3-138.8	130.3-147.3	130.3-145.3

TC: total cholesterol ; nonHDLc: non- high density lipoprotein cholesterol; LDLc: low-density lipoprotein cholesterol

Figure legends

Figure 1. Scatter plots for low-density lipoprotein cholesterol (LDLC) and Triglycerides(TG), LDLC and total cholesterol(TC), LDLC and high-density lipoprotein cholesterol (HDL) LDLC and non-density lipoprotein cholesterol (nonHDL). Their relationship was analyzed using Spearman's approach

1 **Figure 2.** Receiver operating characteristics curves of Triglycerides(TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density
2 lipoprotein cholesterol (LDL) for predicting normal low-density lipoprotein cholesterol(LDL).

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Sensitivity