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# A Novel Method for Estimating Low-Density Lipoprotein (LDL) Levels: Total Cholesterol and Non-High-Density Lipoprotein (HDL) Can Be Used to Predict Abnormal LDL Level in an Apparently Healthy Population

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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**Background:** We aimed to predict the abnormal LDL level by using TG, TC, HDL, and non-HDL in this study.

**Material/Methods:** Triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) data were obtained from the Laboratory Information System (LIS) for 4 years (Oct 1, 2013 to Sept 30, 2017) from among 34 270 healthy Chinese patients at Shuyang People's Hospital. TG, TC, HDL, and LDL (direct clearance method) were measured using a TBA2000FR biochemical analyzer. The non-HDL was calculated as TC minus HDL. Correlations between TG, TC, non-HDL, and LDL were analyzed using Spearman's rank correlation. Receiver operating characteristics (ROC) curve analysis was used to evaluate the predictive utility of TG, TC, and non-HDL for the abnormal LDL level (<130 mg/dL).

**Results:** Both TC (r=0.870) and non-HDL (r=0.893) were significantly positively correlated with LDL. The area under curve of TC and non-HDL can be used to predict abnormal LDL levels. Optimal thresholds were 182.5 mg/Dl (4.72 mmol/L) for TC and 135.3 mg/Dl (3.50 mmol/L) for non-HDL. Based on these optimal thresholds, less than 0.5% and 0.4% of tests with elevated LDL were missed using TC and non-HDL, respectively, but the value of these missed LDL levels was not very high (<147.3 mg/dL).

**Conclusions:** If the value of non-HDL is less than 135.3 mg/Dl (3.50 mmol/L) and/or TC is less than 182.5 mg/Dl (4.72 mmol/L) for the apparently healthy populations, the LDL level will be less than 130 mg/Dl (3.36 mmol/L). TC and non-HDL can be used to predict the abnormal LDL level in apparently healthy populations.

**MeSH Keywords:** Cholesterol, HDL • Cholesterol, LDL • Embolism, Cholesterol

**Abbreviations:** **TC** – total cholesterol; **non-HDL** – non-high-density lipoprotein cholesterol; **LDL** – low-density lipoprotein cholesterol; **HDL** – high-density lipoprotein cholesterol

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## Background

Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) are commonly used in clinical practice as a part of routine lipid evaluation [1]. Testing of cholesterol is vital in patients with diabetes, stroke, and heart diseases [3]. Normally, tests for serum TG, TC, HDL, and LDL are ordered together in the clinical laboratory. Since LDL is a component of the TC and the change of LDL is closely correlated to TC in most cases, we hypothesized that LDL testing might not be necessary, especially if both TC and non-HDL are within normal limits. Thus, we proposed that serum abnormal LDL level could be predicted by using TC and non-HDL as reflex tests. Reflex testing was defined as automatically adding or removing a test by the biochemical analyzer for saving clinical laboratory costs. For example, serum total bilirubin (TBIL) and conjugated bilirubin (CBIL) are highly correlated and approximately 87% of CBIL test results are lower than TBIL test results [4]. Most labs in Europe and the United States use automated technology to perform lipid profiles with automatic calculation of LDL by Friedewald method; however, all LDL levels are measured using a direct clearance method on auto biochemical analyzers in China. The results of LDL by Friedewald equation are often discordant with the direct clearance method results, and the cost of the direct clearance method is higher than for the indirect method. LDL may not accurately reflect the true level of LDL [3] and the elevated LDL should be measured by direct method. Hence, we searched for a novel method for estimating LDL levels. In this study, we analyzed the correlation between TG, TC, non-HDL, and LDL.

## Material and Methods

### Study cohort and data extraction

TG, TC, HDL, and LDL data were obtained from the Laboratory Information System (LIS). There were 34 270 subjects (21 651 males and 12 619 females) (Table 1) who came for routine health check-ups from October 2013 to September 2017 in Shuyang People's Hospital. Since these individuals denied any complaints and did not have any medical history, we considered them as apparently healthy individuals. Data on age, sex, fasting serum TG (GPO-PAP no correction), TC (cholesterol oxidase method), HDL (direct clearance method), and LDL (direct clearance method) were extracted from the LIS. TG, TC, HDL, and LDL were measured using a TBA2000FR biochemical analyzer (Toshiba® Co., Ltd., Japan). Regular quality control procedures are conducted daily in the Laboratory Medicine Unit of Shuyang People's Hospital. The external quality assessment scheme of Jiangsu Center for Clinical Laboratories is performed twice a year to validate the quality of these results.

**Table 1.** The characteristics of the participants.

| Parameters | Female | Male |
|---|---|---|
| | 12619 | 21651 |
| Age | 35<br>(30–48) | 43<br>(31–55) |
| Total cholesterol (mmol/L) | 172.9<br>(152.0–197.2) | 179.0<br>(156.6–203.0) |
| Triglycerides (mmol/L) | 89.4<br>(64.6–131.9) | 123.1<br>(86.8–179.8) |
| LDL (mmol/L) | 98.6<br>(80.8–119.1) | 106.7<br>(88.6–126.1) |
| NonHDL (mmol/L) | 120.7<br>(100.2–144.6) | 133.0<br>(111.4–156.2) |
| HDL (mmol/L) | 50.3<br>(43.3–58.0) | 44.5<br>(38.7–51.8) |

All data is represented bymedian and interquartile range.

The Ethics Committee of Shuyang People's Hospital approved this study on Feb 15, 2017.

### Statistical analysis and calculation

Non-HDL was calculated as TC minus HDL [1] and their relationship was analyzed using Spearman's correlation. For abnormal LDL level, receiver operating characteristics (ROC) curve analysis was used to evaluate the predictive accuracy of TG, TC, HDL, and non-HDL. LDL was considered to be abnormal if >130 mg/dL (3.36 mmol/L) [5,6]. All statistical analyses were performed using EXCEL®2007 (Microsoft Corporation, Beijing, China) and MedCalc® 15.2.2 (MedCalc Software, Ostend, Belgium). A $p$ value less than 0.05 was considered statistically significant.

## Results

### The characteristics of the subjects

The data on 34 270 pairs of cholesterol and triglyceride tests between October 1, 2013, and September 30, 2017 were extracted from the Health Examination Center of Shuyang People's Hospital, including 21 651 males and 12 619 females (Table 1).

### Correlation among TG, TC, HDL, and non-HDL, and LDL

Both TC (r=0.870, $p$<0.0001) and non-HDL (r=0.893, $p$<0.0001) were significantly positively correlated with LDL (Figure 1).





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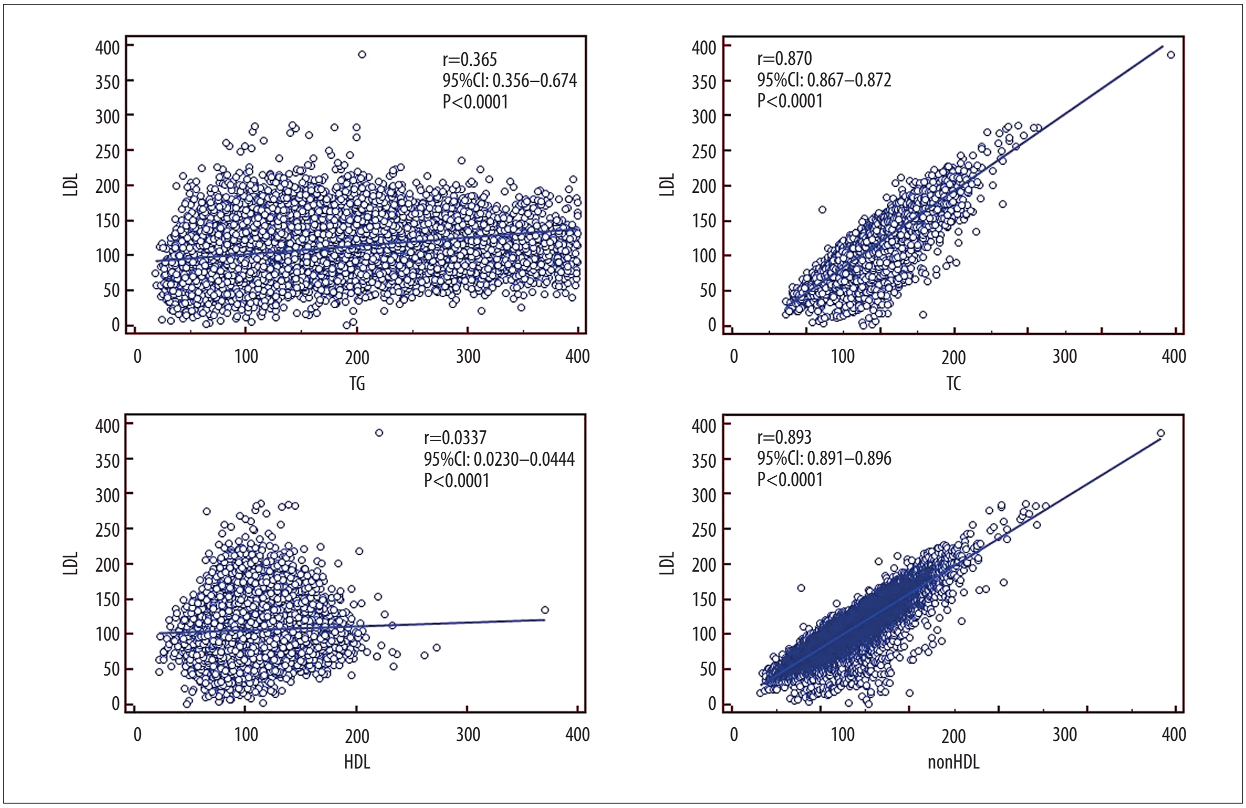


**Figure 1.** Scatter plots for low-density lipoprotein cholesterol (LDLC) and Triglycerides(TG), LDLC and total cholesterol(TC), LDLC and high-density lipoprotein cholesterol (HDLC) LDLC and non-density lipoprotein cholesterol (nonHDLC). Their relationship was analyzed using Spearman's approach.

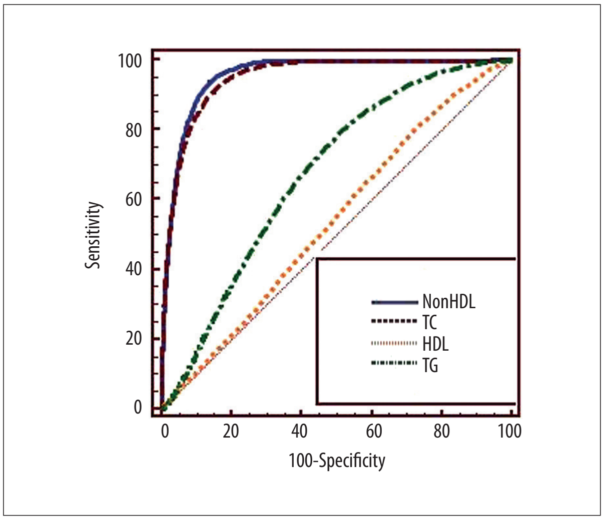


**Figure 2.** Receiver operating characteristics curves of Triglycerides(TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC) for predicting abnormal low-density lipoprotein cholesterol(LDLC).

### The optimal threshold of TC and non-HDL in predicting abnormal LDL level

Figure 2 shows the ROC curves of TG, TC, HDL, and non-HDL for predicting abnormal LDL level (<130 mg/dL). Areas under the curve (AUC) were 0.675, 0.950, 0.541, and 0.957 for TG, TC, HDL, and non-HDL, respectively. As listed in Table 2, non-HDL was markedly better than TC for predicting abnormal LDL level in terms of diagnostic performance and leakage in different TG levels. At these threshold values of TC and non-HDL, <2.6% and 1.8% of tests with elevated LDL would have been missed, but the missing elevated LDL was not very high (<147.3 mg/dL=3.81 mmol/L). As listed in Table 3, when the non-HDL is <135.3 mg/Dl (3.50 mmol/L) and/or TC is <182.5 mg/dL (4.72 mmol/L), the LDL will be <130 mg/dL for the all the population. If the non-HDL is under 139.2 mg/dLl (3.60 mmol/L) and/or TC is under 182.5 mg/dL (4.72 mmol/L), the LDL will be low 130 mg/dL for the population (the TG is less than 400 mg/dL).

## Discussion

In this study, we analyzed serum TG, TC, non-HDL, LDL and their correlations in a large Chinese cohort of apparently healthy



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

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

* Compare with TC and nonHDL groups; TC – total cholesterol; nonHDL – non- high density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol.

subjects, and we found that TC and non-HDL were positively correlated with LDL. Healthy individuals who have low TC and non-HDL levels usually also have low LDLC levels and vice versa. The results of ROC for predicting abnormal LDLC level has supported our hypothesis. The AUC was >0.95 for both TC and non-HDL, indicating that TC and non-HDL have high accuracy for predicting abnormal LDL level. Therefore, normal LDL levels (<130 mg/dL) were easily be predicted using TC and non-HDL. Given the diagnostic performance and the proportion of elevated LDL, non-HDL is notably better than TC for predicting abnormal LDL levels. Two optimal thresholds of TC and non-HDL for predicting abnormal LDLC level were 182.5 mg/dL (4.72 mmol/L) and 139.2 mg/dL (3.60 mmol/L) (given TG is <400 mg/dL). If TC is <182.5 mg/dL and/or non-HDL





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**Table 3.** The optimal threshold total cholesterol and non- high density lipoprotein cholesterol, and their performance in predicting abnormal 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 pridicting abnormal LDLC level (less than 130 mg/dL) | 56.0% 19175/ 34270 | 61.7% 21138/ 34270 | 57.3% 19175/ 33486 | 62.7% 20995/ 33486 | 40.7% 1884/ 4624 | 44.2% 2043/ 4624 | 59.4% 17132/ 28862 | 66.5% 19206/ 28862 |
| Elevated LDLC (≥130 mg/dL) | 0.5% 87/19175 | 0.4% 83/21138 | 0.5 87/19175 | 0.4% 83/20995 | 0.6 11/1884 | 0.5% 11/2043 | 0.4 71/17132 | 0.3% 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.

is <139.2 mg/dL, the LDL will be <130 mg/dL (3.36 mmol/L). According to these thresholds, only <0.5% and 0.4% of elevated LDL (>130 mg/dL) would have been missed, and these missed values were not very high (Tables 2, 3).

In this study, we found that approximately 56% of direct LDL tests or calculations could have been avoided. The price of a LDL test is approximately 4RMB (0.65 USD) [4] in China, thus, the expense of an LDL test would be greatly reduced. If non-HDL is used as a reflex test for LDL (when elevated LDL is >130 mg/dL), approximately 11.3 million LDL tests would have been avoided, which corresponds to savings of almost 7.2 million USD. The direct LDL test can be automatically performed when the predicting LDL level is not at a normal level. This saves physician time by not needing to order the direct LDL test, saves patient time by not needing to draw another blood sample, and expedites obtaining accurate LDL results. In contrast to the Friedewald formula [7], which is considered inaccurate (TG level is more than 220 mg/dL) [8], in this study we took into account the cost savings but also screened out the abnormal LDL level, and it was not affected by triglyceride levels.

## Conclusions

There are some limitations to this study. First, this was a single-center study on individuals who came from routine health check-ups. Second, some individuals (<0.5%) with abnormal LDL levels can still be missed.

The results of present study indicate that almost all abnormal LDL levels can be predicted by using TC and/or non-HDL. To the best of our knowledge, this study is the first to predict LDL level by using ROC curve analysis. The approach of this study may be suited for other subjects. However, because of differences in the detection system and subjects, optimal threshold should be based on local data for using TC and/or non-HDL to predict abnormal LDL level.

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