# AGREEMENT BETWEEN ESTIMATED APOLIPOPROTEIN B-100 CONCENTRATION AND APOLIPOPROTEIN B-100 CONCENTRATION MEASURED BY IMMUNOTURBIDIMETRIC METHOD

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## ABSTRAK

*Apo B-100 disintesis di hati dan hanya satu molekul apo B yang ada dalam setiap partikel LDL, IDL, dan VLDL, karena itu nilai apo B-100 ini mengindikasikan total jumlah lemak yang berpotensi aterogenik. Rumus estimasi apo B-100 mungkin dapat membantu klinisi di daerah dengan sumber daya dan dana yang terbatas untuk menentukan pasien yang beresiko menderita penyakit kardiovaskuler, agar dapat dilakukan penatalaksanaan yang sesuai. Penelitian ini bertujuan untuk mengetahui kesesuaian antara konsentrasi apolipoprotein B-100 yang dihitung berdasarkan rumus dengan konsentrasi apolipoprotein B-100 yang diukur dengan metode imunoturbidimetri. Jenis penelitian yang digunakan adalah analitik observasional cross sectional. Sampel serum diperoleh secara simple random sampling dan semua sampel diukur konsentrasi kolesterol-LDL, trigliserida, dan apolipoprotein B-100, serta dihitung konsentrasi apo B-100 dengan rumus ([Apo B-100= -33,12 + 0,675 x LDL + 11,95x ln (TG)]. Dari 30 sampel yang diperiksa, 29 sampel (96,67%) mempunyai konsentrasi apo B-100 hitung lebih tinggi daripada apo B-100 ukur. Perbedaan nilai rata-rata konsentrasi apo B-100 hitung dan ukur adalah 10,941 mg/dL, dimana perbedaan tersebut bermakna secara statistik (t= 9,134 (p < 0,0001)). Concordance correlation coefficient (CCC) antara konsentrasi apolipoprotein B-100 hitung dan ukur adalah 0,854 (<0,90= poor agreement). Kami menyimpulkan bahwa ada kesesuaian konsentrasi apo B-100 hitung dengan konsentrasi apo B-100 yang diukur dengan metode imunoturbidimetri kurang baik, dimana sebagian besar hasil penghitungan rumus lebih tinggi daripada konsentrasi apo B-100 ukur. Faktor yang mungkin menyebabkan hal ini adalah proses freeze-thawing sampel sebelum pengukuran apo B-100 dengan imunoturbidimetri dan adanya variabel trigliserida pada rumus estimasi apo B-100.**(FMI 2014;50:10-14)***

***Kata kunci:*** *Apo B-100, kolesterol-LDL, trigliserida, imunoturbidimetri*

## ABSTRACT

*Apo B-100 is synthesized in the liver and only one apo B molecule exists in every LDL, IDL and VLDL particle, therefore, the level of apo B-100 represents the total number of potentially atherogenic lipid. A formula estimating apo B-100 might help clinicians in areas with limited resources to identify patients with risk factors for cardiovascular disease, so proper management can be carried out. To evaluate an agreement between estimated apolipoprotein B-100 concentration and apolipoprotein B-100 concentration measured by immunoturbidimetric method. This study was an analytical observational cross sectional design. Serum samples were collected using simple random sampling method and all specimens were measured for LDL-cholesterol, triglyceride and apolipoproten B-100 concentration. Estimated apo B-100 concentrations were counted using a [Apo B-100= -33.12 + 0.675 x LDL + 11.95x ln (TG)] formula. Twenty nine of 30 samples (96.67%) showed estimated apo B-100 concentrations higher than measured apo B-100 concentrations. Difference of the mean of estimated and measured apo B-100 concentration was 10.941 mg/dL, and was statistically significant (t= 9.134 (p <0.0001)). Concordance correlation coefficient (CCC) between estimated and measured apolipoprotein B-100 concentration was 0.854 (<0.90= poor agreement). The agreement between estimated apo B-100 concentration and apo B-100 concentration measured with immunoturbidimetric method was poor. Factors that might have caused this result were freeze-thawing of samples prior to apo B-100 measurement using immunoturbidimetry and the use of triglyceride as one of the variables in the formula in estimating apo B-100.**(FMI 2014;50:10-14)***

***Keywords:*** *Apo B-100, LDL-cholesterol, triglyceride, immunoturbidimetry.*

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## INTRODUCTION

Concentration of serum total cholesterol is high, especially form Low Density Lipoprotein cholesterol (LDL), is a major risk factor for coronary heart disease (CHD) (Walldius & Jungner 2004, Orlova et al 1999). The recommendation of current management of dyslipidemia is largely based on a treatment to reduce the concentration of cholesterol – LDL (Cho et al 2012). However, some recent research suggests that the types

of lipoproteins are involved in the process of atherogenesis was not only LDL-cholesterol. A number of patients with atherosclerotic disease have LDL - cholesterol concentrations and total cholesterol in the recommended range. Some patients who have achieved a significant reduction in LDL - cholesterol concentrations with treatment can still have coronary heart disease. Increased concentrations of Intermediate Density Lipoprotein (IDL) and Very Low Density Lipoprotein (VLDL) is also associated with an increased risk of cardiovascular disease, as well as the concentration of low density lipoprotein (HDL) and high concentrations of triglycerides (TG) in serum (Walldius & Jungner 2004).

Particles of LDL is the major cholesterol carrier in the circulation and its physiological function is to bring cholesterol into the cell. LDL contains apolipoprotein B - 100 22%, 22% phospholipids, 8% cholesterol, cholesterol esters 42% and triglycerides 6% (Orlova et al 1999). Apolipoprotein B - 100 (apo B - 100) is an important component of the LDL particle. Apo B - 100 mediates the uptake of LDL by the liver and peripheral tissues via specific interactions with the receptor of LDL (Cho et al 2012). Apo B - 100 is synthesized in liver and present in LDL particles, IDL, and VLDL. In each of these lipoprotein particles there is only one molecule of apo B - 100, because the value of apo B - 100 is able to describe the amount of lipids that potentially aterogenik (Walldius & Jungner 2004, Davidson 2009, Hermans et al 2011). The addition of apo B concentrations examination on routine lipid profile of patients who has heart failure risk and able to improve the management of patients who received fat treatment (Contois et al 2009). The consensus of the American Diabetes Association and the American College of Cardiology foundation suggested targets for the treatment of patients with cardio - metabolic risk and lipoprotein abnormalities were the target of the treatment of patients at particularly high risk category (disease cardiovascular or DM + one/more major cardiovascular risk factors), namely LDL cholesterol <70 mg/dL, non-HDL cholesterol <100 mg/dL, ApoB <80 mg/dL and high risk categories (without diabetes or clinical cardiovascular disease, but with two/more additional major cardiovascular risk factors and diabetes mellitus, but without other major cardiovascular risk factors), namely LDL cholesterol <100 mg/dL, non-HDL cholesterol <130 mg/dL, ApoB <90 mg/dL (Brunzell et al 2008).

The method which widely used for the measurement of apo B-100 was immunoturbidimetry and immunonephelometry method (Dati & Tate 1992). In both these methods, apolipoprotein which contained in human serum samples would be incubated with antibodies anti apolipoprotein B-100, so then it formed immune complexes. Immune complexes which had been formed would cause the intensity of the light transmitted through the solution was decreased due to reflection, absorption or scatter of light on the immune complex particles. On imunoturbidimetry method, it would measure the decrease of light transmitted through the liquid (Tiffany 1999). While the method of immunonephelometry, nephelometer would measure the amount of light that was spread out by formed immune complexes. The intensity of light which was scattered was proportional to the concentration of apolipoprotein in the sampel (Tiffany 1999).

To predict cardiovascular disease risk factors, measurement of apo B-100 has several advantages if compared with measurements of direct LDL-cholesterol and LDL-cholesterol calculation with the Friedewald formula. In the measurement of apo B-100 should not used fasting serum, so it made patient will feel more pleasure (Davidson 2009, Chen et al 2010). In addition, the patients with high serum triglyceride concentrations, ie >400 mg/dL (4.5 mmol/L), cholesterol-LDL can not be precisely calculated using the Friedewald formula, because of the higher concentration of serum triglycerides is greater error which may occur in the calculation of the cholesterol-LDL (Sniderman et al 2001). Although there are clinical advantages measurement of apo B-100 concentrations which compared with conventional lipid measurements (measurement of total cholesterol, triglycerides, cholesterol-HDL & LDL) to assess the risk of cardiovascular disease, but the concentration of apo B-100 are not routinely measured because of cost considerations.

Some previous researchers developed a formula to determine the serum concentration of apolipoprotein B, one of them was Hermans et al (2011) conducted a research in 45 North Caucasian subjects with diabetes and developped an algorithm for estimating the apo B-100, namely [Apo B-100 = 6.3 + 0.65 x non-HDL-cholesterol]. Then Cho et al (2012) had research on 73.047 Koreans who underwent medical check-ups and develop a formula for determining the concentration of apolipoprotein B-100 based on the concentration of LDL-cholesterol and triglycerides, namely [Apo B-100 = -33.12 + 0.675 + xLDL 11, 95x ln (TG)].

The use of a formula to estimate the concentration of serum apolipoprotein B-100 in Indonesia has not been systematically evaluated. The formula was developed by Cho et al (2012) because th epopulation was obtained from studies in Asian, may be applied in Indonesia to assisted inland clinicians with limited resources and funds to determining the patients which

were has risk of suffering cardiovascular disease so it able to conducted appropriate management. Therefore, this study was conducted to evaluate the fit between the concentrations of apolipoprotein B-100 calculated based on the formula [Apo B-100 = -33.12 + 0.675 + 11.95 x xLDL ln (TG)] with apolipoprotein B-100 concentrations were measured by imunoturbidimetry method. The results of this study were expected to assist the inland clinician with limited resources and funds to identified patients who had a risk of suffering cardiovascular disease and for the treatment of dyslipidemia patients.

## MATERIALS AND METHODS

This research used diagnostic test with a cross-sectional observational study design. The samples in this study were serum specimens which had been examined its LDL-cholesterol concentrations and triglycerides in the Laboratory of Clinical Pathology Hospital Dr. Soetomo using a Dimension RXL Max Clinical Chemistry System (Siemens Healthcare Diagnostics). The examination of serum apolipoprotein B-100 performed in the laboratory Graha Amerta Hospital Surabaya. The sample size was 30, serum samples were obtained by simple random sampling on 20-21 May 2013.

This LDL-cholesterol tests used homogeneous method that directly measured the concentration of LDL-cholesterol in serum without any pretreatment required and measured offline with endpoint bicromatic techniques (λ 540, 700 nm). The examination of triglycerides used enzymatic procedures and measured by a combination of techniques endpoint bicromatic (λ 510, 700 nm).

The rest of the specimens that have been examined its concentration of LDL-cholesterol and triglycerides was stored at -20 °C for 13-14 days, then it was examined the concentration of apolipoprotein B-100. The examination of the concentration of apolipoprotein B-100 performed in the laboratory Graha Amrita Hospital Surabaya used imunoturbidimetry method by TMS System (Daiichi). Reference value for the concentration of apoB-100 was 69-105 mg/dL

Statistical analysis was conducted by using MedCalc version 12.6.1.0. The relationship between the concentration of serum apolipoprotein B-100 which was calculated based on the concentration of serum apolipoprotein B-100 which measured by imunoturbidimetry method was evaluated by analysis with concordance of coefficient correlation (CCC). The difference value of the concentration of serum apolipoprotein B-100 which was calculated as the concentration of serum apolipoprotein B-100 which measured by the imunoturbidimetry method had been evaluated by paired t-test. All of tests, p-value of <0.05 was considered significant.

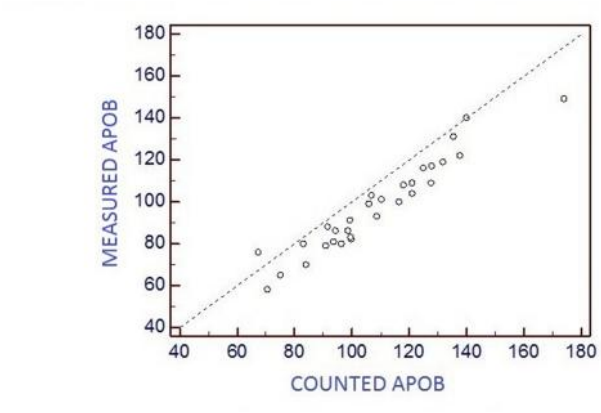
## RESULTS

The average age of the subjects were 60.23 ± 8.084 (Table 1) with an age range 41-74 years. Most (60%) of the study subjects were women. All subjects were examined its concentrations of triglycerides and LDL-cholesterol in serum and the average value for each parameter was as follows: 130 mg / dL, 125.97 mg / dL. From the 30 samples which had been tested, 29 samples (96.67%) had a concentration of apo B-100 count was higher concentrations than apo B-100 measurement, there was only one sample (3.33%) of the concentration of apo B-100 count was lower than apo B-100 concentrations measured. The average value of the concentration of serum apo B-100 were obtained from the calculation which used the higher formula than average concentration of apo B-100 were obtained from measurements with imunoturbidimetry method, ie 108.44 mg / dL to apo B-100 count and 97.5 mg / dL for apo B-100 concentrations measured. The difference average value was 10.941 mg / dL (SD = 6.541), and the difference was statistically significant t = 9.134 (p < 0.0001) .

Table 1. Characteristics of research subject

| Characteristics | Result |
|---|---|
| Age (year) | 60.23 ± 8.084 |
| Gender (male/female) | 12/18 |
| Triglyceride (mg/dL) | 130.00 ± 67.299 |
| LDL-Cholesterol (mg/dL) | 125.97 ± 28.592 |
| Measured Apo B-100 (mg/dL) | 97.50 ± 21.785 |
| Counted Apo B-100 (mg/dL) | 108.44 ± 23.40 |

All data above was expressed in mean ± SD, except gender which expressed in its each amounts.Figure 1 showed a scatter plot for the relationship between apo B-100 measuring and arithmetic. In this picture, it appeared that the value of the concentration of apo B-100 count tended to be higher than the value of apo B-100 concentrations measured. Concordance correlation coefficient (CCC) between the concentration of serum apolipoprotein B-100 which was calculated based the formula with concentration of serum apolipoprotein B-100 which measured by the imunoturbidimetry method was amounted to 0.854 (95% CI [.753 to .916]), which means that the fit between the concentration of apo B-100 was a bad measurement and count (CCC value <0.90 = poor agreement) (McBride 2005).




----- The perfect concordance line

Figure 1. Scatter Plot of Concentration value of apo B-100 count and measurement.

## DISCUSSION

The concentration measurement of apo B-100 might indicate the total number of potentially atherogenic lipoprotein particles, so the addition of apo B concentration parameters on routine lipid profile could correct the determination of patients at risk for cardiovascular disease and improve the management of patients receiving fat lowering treatment (Walldius & Jungner 2004, Davidson 2009, Hermans et al 2011, Contois et al 2009). But to date, the examination of apo B-100 is not always available because it is costly and takes time (time consuming) (Cho et al 2012). So that the existence of a formula for estimating the concentration of apo B-100 can assist inland clinicians with limited resources and funding.

In this study, the concentration of apo B - 100 were calculated based on the formula [Apo B-100= -33,12 + 0,675xLDL + 11,95x ln (TG)] did not show good agreement with the concentrations of apo B - 100 which were measured by the imunoturbidimetry method. From 29 of 30 samples (96.67%) showed the concentration of apo B - 100 count higher than the concentration of apo B - 100 measurement. This could be caused by preanalitic factors of measuring apo B - 100 or factor of the formula which was used in this study. Preanalitic factors that might cause the concentration of apo B - 100 count higher than the concentration of apo B - 100 was a measurement of the existence of freeze -thawing process of the sample before the measurement of the concentration of apo B - 100 carried out, which ledded the concentration of apo B - 100 decreases. The research by Brown et al (1990) stated that the results of measurements of apo B concentrations will decline as much as 6.8% after one cycle of freeze-thawing which was caused by a decrease of imunoreaktivitas in apo B (Brown et al 1990). In our study, the concentration of LDL-cholesterol and triglycerides were measured before the samples were frozen, while the concentration apo B-100 were measured after the samples were frozen and thawed one time, so there might be a decrease in the concentration of apo B-100 measuring, then caused the lower measurement of apo B-100 than the apo B-100 count. The next factor was the use of variables in this formula triglycerides. Triglycerides are the core of all lipoproteins, but the greatest concentration of triglyceride molecules present in chylomicrons which rich of triglyceride and VLDL, whereas triglycerides in LDL is low. so that when triglycerides were included in the formula, its results of measurement could occur the higher apo B-100 than apo B-100 measurement. Maybe when used non-HDL cholesterol (total cholesterol-HDL cholesterol) instead of the variable in the formula will provide better conformity with apo B-100 concentrations measured. Especially non-HDL cholesterol included LDL-cholesterol, IDL, VLDL. Some studies suggested that non-HDL cholesterol had a strong correlation with apolipoprotein B and could be used as a candidate biometrikal equivalent to apo B (Hermans et al 2011, Lu et al 2003, Li et al 2011).

The limitedness of this study was bit number of the samples, so we need a larger study with more number of samples to validate whether the estimation formula apo B-100 is able to obtain accordance results of apo B-100 concentrations which were measured by the imunoturbidimetry method on Indonesia population. Another limitedness of this research was the process of freeze-thawing specimens before measuring the concentration of apo B-100 with imunoturbidimetry method which caused a decrease in the concentration of apo B-100 measuring, so for subsequent research should use fresh specimens (fresh specimens) or specimens are stored at a temperature 2-8 °C for the measurement of the concentration of apo B-100.

## CONCLUSION

The results showed that there were correspondence between the value of the concentration of apo B-100 measuring & count were poor (CCC = 0.854), where the mostly (96.67%) the results of the estimated formula calculation of apolipoprotein B-100 concentration was higher than the concentration of apo B-100 measured by the imunoturbidimetry method. The factors that might cause this was the process of freeze-thawing of samples before measurement of the apo B-100 concentration with imunoturbidimetry method, so that obtained the lower concentration of apo B-100 measured. Another factor was triglycerides variable in estimation formula

of apo B-100 led the higher apo B-100 concentrations count.

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